

Neuroimaging to Assess Neonatal Brain Development and Brain Injury in Neonatal Encephalopathy

by

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Declaration

I declare that this thesis has not been submitted as an exercise for a degree at this or any other university and it is entirely my own work.

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Full and informed consent was obtained from the parent/guardian on behalf of all infant subjects.

A handwritten signature in grey ink that reads "Megan Dibble". The signature is written in a cursive, flowing style.

Megan Dibble

Dedicated in Loving Memory to

John Dibble

(who couldn't wait to have a doctor in the family... we did it Bampi)

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Abbreviations

AD	Axial Diffusivity
ADC	Apparent Diffusion Coefficient
ADHD	Attention-Deficit/Hyperactivity Disorder
ALFF	Amplitude of Low-Frequency Fluctuation
ALIC	Anterior Limb of the Internal Capsule
ASD	Autism Spectrum Disorder
BE	Base Excess
BGT	Basal Ganglia-Thalamic
BN	Basal Nuclei
B ₀	Net External Magnetic Field
BOLD	Blood Oxygenation Level Dependent
BSID	Bayley Scales of Infant Development
CC	Corpus Callosum
CGM	Cortical Grey Matter
Cing.	Cingulum
CP	Cerebral Palsy
CR	Corona Radiata
CRDI	Clinical Research Development Ireland
CS	Centrum Semiovale
CSF	Cerebrospinal Fluid
CST	Corticospinal Tract
CSVt	Cerebral Sinovenous Thrombosis
CVI	Cortical Visual Impairment
DGM	Deep (Sub-Cortical) Grey Matter
dHCP	Developing Human Connectome Project

DOF	Degrees of Freedom
DQ	Development Quotient
Draw-EM Maximisation	Developing Region Annotation with Expectation
DTI	Diffusion Tensor Imaging
DWI	Diffusion-Weighted Imaging
EC	External Capsule
EEG	Electroencephalography
ELISA	Enzyme-Linked Immunosorbent Assay
EM	Expectation Maximisation
EPO	Erythropoietin
FA	Fractional Anisotropy
fALFF	Fractional Amplitude of Low-Frequency Fluctuation
FDR	False Discovery Rate
FFE	Fast Field Echo
FLAIR	Fluid-Attenuated Inversion Recovery
FOV	Field of View
fMRI	Functional Magnetic Resonance Imaging
FW	Free Water Fraction
GA	Gestational Age
G-CSF	Granulocyte-Colony Stimulating Factor
GFAP	Glial Fibrillary Acidic Protein
GM	Grey Matter
GM-CSF	Granulocyte Macrophage-Colony Stimulating Factor
GMDS	Griffiths Mental Development Scales
GMFCS	Gross Motor Function Classification System
HIE	Hypoxic-Ischaemic Encephalopathy
IFN	Interferon
IFO	Inferior Fronto-Occipital Fasciculus

Inst.	Instrumental Delivery
IL	Interleukin
ILF	Inferior Longitudinal Fasciculus
IVH	Intraventricular Haemorrhage
LMIC	Low- and Middle-Income Countries
LSCS	Lower Segment Caesarean Section
M	Net Longitudinal Magnetisation
mALFF	Mean Amplitude of Low-Frequency Fluctuation
MAP	Mean Arterial Blood Pressure
MD	Mean Diffusivity
MDI	Mental Development Index
MODE	Multi-Organ Dysfunction and EEG Study
MOOSE	Meta-Analysis of Observational Studies in Epidemiology
MPS	Measurement, Phase and Slice Encoding
MRI	Magnetic Resonance Imaging
MRV	Magnetic Resonance Venography
MV	MultiVane
NAA	N-acetylaspartate
NBCI	Neonatal and Children's Brain Consortium Ireland
NBNA	Neonatal Behavioural Neurological Assessment
NDD	Neurodevelopmental Disorder
NDI	Neurite Density Index
NE	Neonatal Encephalopathy
NEBULA	Neonatal Brain Injury: Understanding Systemic Inflammation & Immunomodulation
NEON	Neonatal Neuroimaging
NEPTuNE	Neonatal Encephalopathy PhD Training Network
NICHD NRN	National Institute of Child Health and Human Development Neonatal Research Network

NICU	Neonatal Intensive Care Unit
NNNS	NICU Network Neurobehavioural Scale
NODDI	Neurite Orientation Dispersion and Density Imaging
ODI	Orientation Dispersion Index
OR	Optic Radiations
P	Peduncles
PDI	Psychomotor Developmental Index
PFC	Prefrontal Cortex
PLIC	Posterior Limb of the Internal Capsule
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PWML	Punctate White Matter Lesions
RD	Radial Diffusivity
RF	Radio Frequency
ROI	Region of Interest
rs-fMRI	Resting State Functional Magnetic Resonance Imaging
SDH	Subdural Haemorrhage
SENSE	Sensitivity Encoding
SI	Signal Intensity
SLF	Superior Longitudinal Fasciculus
Splen	Splenium
SNR	Signal to Noise Ratio
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
SVD	Spontaneous Vaginal Delivery
SWI	Susceptibility Weighted Imaging
TBI	Traumatic Brain Injury
TBSS	Tract-Based Spatial Statistics
TE	Echo Time

TI	Inversion Time
TNF	Tumour Necrosis Factor
TR	Repetition Time
VEGF	Vascular Endothelial Growth Factor
V_{ic}	Intracellular Volume Fraction
V_{iso}	Isotropic (free water) Diffusion
VISTA	Volume Isotropic Turbo Spin Echo Acquisition
WM	White Matter
WS	Watershed

Summary

Neonatal encephalopathy (NE) is a clinical syndrome describing perinatal brain injury and neurological dysfunction in the term-born infant. Magnetic resonance imaging (MRI) is a widely used tool in the assessment of NE, as it provides key information about the pattern and severity of the injury, helps guide treatment decision making, and can be used as a biomarker to predict neurodevelopmental outcome. Diffusion tensor imaging (DTI) is a particularly useful MRI tool, as diffusion abnormalities can be detected in the critical period shortly after birth. Other biomarkers of NE include inflammatory markers and placental pathology, and evidence suggests that these clinical factors may be linked to the pattern of injury seen on MRI in infants with NE. The goal of the present research was to first examine the microstructure of the typically developing neonatal brain, to better understand the effects that adverse neonatal events such as NE have on the brain. Secondly, we aimed to determine if there is an association between placental histology and inflammatory markers, and pattern of brain injury seen on MRI in NE. Finally, we aimed to determine whether these factors can be linked to neurodevelopmental outcome in NE.

We first outline typical brain development from conception to adulthood, as well as an overview of neuroimaging methods. We then examine NE in greater detail, and how neuroimaging is used as a tool in NE. We then present four studies – one systematic review and three empirical studies. The systematic review examines DTI studies in NE and identifies the corpus callosum and posterior limb of the internal capsule as two key white matter tracts that are significantly altered in infants with NE and that are predictive of adverse neurodevelopmental outcomes.

Empirical study 1 employs multi-sequence neuroimaging techniques to demonstrate microstructural changes and maturational trajectories of white and grey matter in the brain of the typically developing neonate. We found that DTI, NODDI, and myelin measures were strongly associated with gestational age throughout the brain, but also showed regional variation between metrics, with the strongest associations in the white and deep grey matter. We also identify fALFF as an fMRI measure that is sensitive to gestational age. We show a

relationship between MRI metrics and myelination, however, unexpectedly, DTI, NODDI, and myelin measures were not directly associated with fMRI measures.

Empirical studies 2 and 3 are retrospective analyses of prospective cohort studies that enrolled infants with NE admitted to the NICU at the National Maternity Hospital, Dublin. Study 2 examined the association between inflammatory markers (EPO, GM-CSF, IL-8, VEGF), placental histology, and MRI brain scores using Barkovich, NICHD NRN and Weeke scoring systems. We identified a positive linear relationship between GM-CSF and MRI brain scores assessed by the Weeke grey matter scoring system, however, no other cytokine or placental associations were found. Study 3 looked at whether the addition of placental histology and inflammatory markers may improve the ability of MRI to predict long-term neurodevelopmental outcome in infants with NE. We found that GM-CSF was predictive of cognitive outcomes (Bayley-III composite scores) when combined with almost all scoring systems: Barkovich, NICHD NRN, Weeke (white matter, cerebellum, and overall, but not grey matter), as well as for normal/abnormal MRI. IL-8 was predictive of both cognitive and motor outcomes when combined with the Weeke grey matter scoring system. No placental associations were found.

This thesis provides important information both for MRI researchers and clinicians, into the details of early brain development and injury. We first advocate for the use of multi-sequence MRI techniques to assess the developmental trajectories of structural and functional architecture in the healthy neonatal brain. Secondly, this thesis encourages the use of MRI brain scores and the inflammatory cytokine GM-CSF as a combined biomarker for the assessment of injury and the prediction of cognitive outcome in infants with NE.

Outputs

Peer Reviewed Publications

Dibble M, O'Dea MI, Hurley T, Byrne A, Colleran G, Molloy EJ, Bokde AL. Diffusion tensor imaging in neonatal encephalopathy: a systematic review. *Arch Dis Child Fetal Neonatal Ed*. 2020.

doi: <http://dx.doi.org/10.1136/archdischild-2019-318025> (This paper is found in chapter 5.3)

Dibble M, Ang JZ, Mariga L, Molloy EJ, Bokde AL. Diffusion tensor imaging in very preterm, moderate-late preterm and term-born neonates: a systematic review. *J Pediatr*. 2021.

doi: <https://doi.org/10.1016/j.jpeds.2021.01.008> (This paper is found in the appendix)

Dibble M, Molloy EJ, Bokde AL. Microstructural grey and white matter differences across the neonatal brain assessed by structural and functional imaging.

Submission in progress.

Conference Abstracts

Dibble M, O'Dea MI, Hurley T, Byrne A, Colleran G, Molloy EJ, Bokde AL. Diffusion tensor imaging in neonatal encephalopathy: a systematic review. Presented at: *The Irish Paediatric Association Conference; Athlone, Ireland, 2019.*

Hurley T, O'Dea MI, Branagan A, Ni Bhroín M, **Dibble M**, Bokde AL, Molloy EJ. Comparison of the prognostic value of different MRI techniques in neonatal encephalopathy: a systematic review and meta-analysis. Presented at: *The Pediatric Academic Societies Meeting; Denver, CO, USA; 2022.*

Chapter 1: Introduction

“At no other time in human life are the clinical manifestations of neurological disease as subtle as they are in the newborn infant”.

- *Dr Joseph J Volpe*

1.1 Neonatal Encephalopathy: An Introduction

Neonatal encephalopathy (NE) is a clinical syndrome in the term-born neonate that occurs at or shortly after birth. It is a form of brain injury that affects neurological functioning and can have serious developmental consequences in terms of cognition, behaviour, and mobility. Characterised by “subnormal level of consciousness or seizures, and often accompanied by difficulty with initiating and maintaining respiration and depression of tone and reflexes”¹, NE is one of the leading causes of infant morbidity and mortality. Presently, therapeutic hypothermia (TH) is the only treatment for NE, which must be started within six hours of birth. TH is a neuroprotective intervention that lowers the neonate’s total body temperature to 33-34°C over 72 hours in order to minimize damage to the brain and slow down the progression of NE.

1.2 NBCI and NEPTuNE

Ireland is currently at the forefront of research in neonatal brain injury, and the Neonatal and Children’s Brain Consortium Ireland (NBCI) was established with the goal of furthering and promoting understanding and awareness of NE in Ireland. With its members based across the major universities and hospitals in Ireland and with multidisciplinary expertise in fields such as neonatology, paediatrics, neurodevelopment, family-centred care, and neuroimaging, the consortium is working to bring together all aspects of neonatal and paediatric brain research.

With this in mind, the NBCI formed a major collaborative structured PhD research programme along with Clinical Research Development Ireland (CRDI) that is training five

candidates in the newest and most effective techniques in caring for NE neonates. The NEPTuNE (Neonatal Encephalopathy PhD Training Network) programme is a collaboration between Trinity College Dublin, University College Cork, and NUI Galway, providing a holistic patient-focused education in NE research through regular study days and training sessions for the candidates in five disciplines: psychology, neuroimaging, electroencephalography, circadian rhythm, and core outcomes.

1.3 The NEON Project

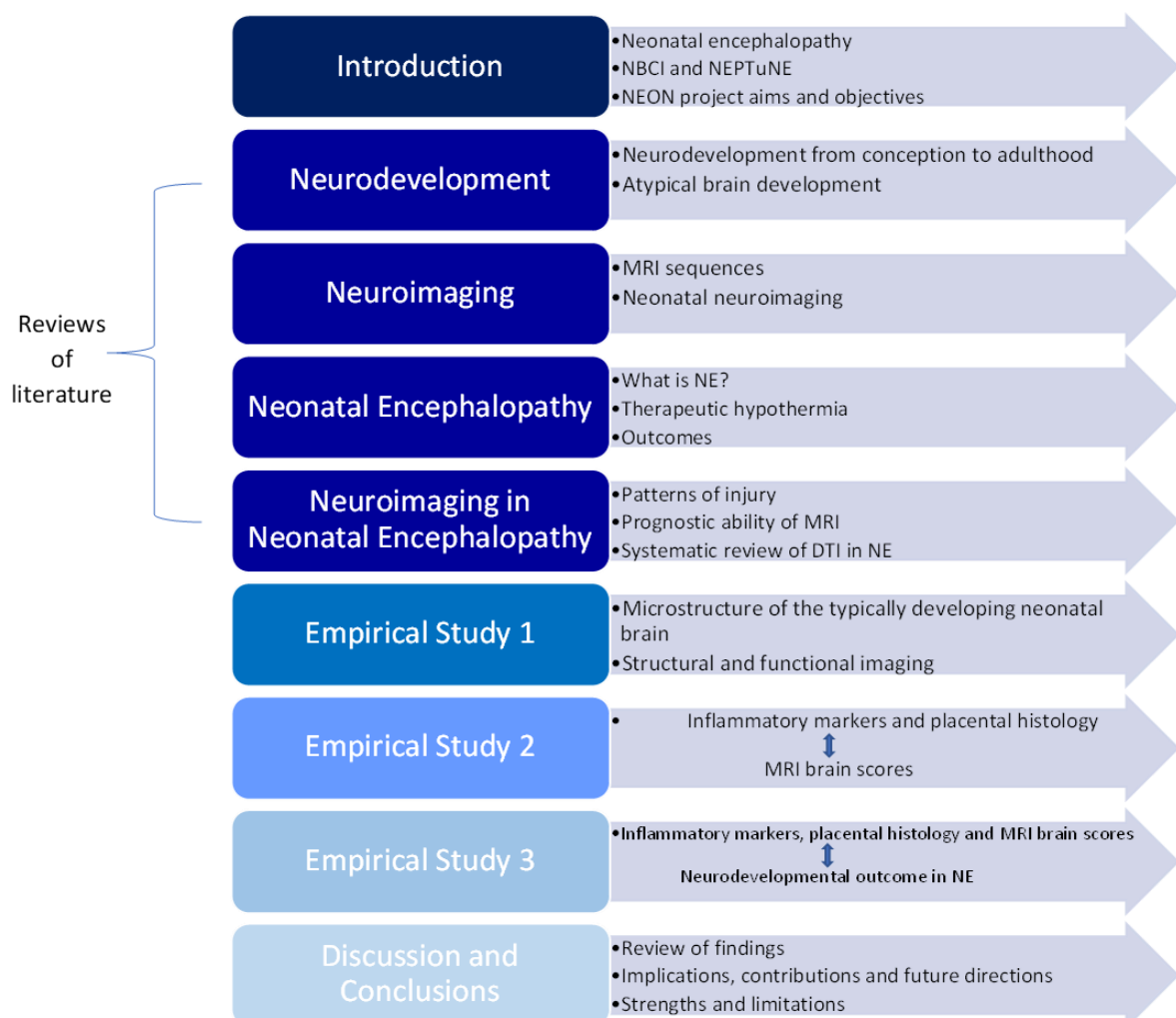
The NEPTuNE research project in which I am involved is Neonatal Neuroimaging (NEON) and its primary focus is using neuroimaging techniques to assess patterns and severity of brain injury in NE and to predict neurodevelopmental outcome. Magnetic resonance imaging (MRI) is a widely used neuroimaging tool alongside clinical evaluation in assessing NE as it can provide key information about the nature of the injury, effectiveness of treatment, and potentially be used as a biomarker to predict long-term outcome. Diffusion tensor imaging (DTI) during the first few days of life is an MRI technique with good prognostic ability in NE¹⁶⁷ and was the primary imaging focus of NEON. DTI is able to examine the structural and functional integrity of the brain by measuring the rate of water diffusion in different tissues. The advantage of DTI in NE is that diffusion abnormalities can be seen within the first few hours after birth, meaning neonates can be scanned early during the critical period where decisions are to be made regarding treatment and redirection of care. The use of MRI - and specifically DTI – as a way to assess brain development and brain injury in the newborn was the primary focus of the NEON project. Literature reviews, retrospective image processing, statistical and theoretical analysis of data were conducted by myself (Megan Dibble) for this project.

1.4 Aims and Objectives

The NEON project aimed to investigate the nature of brain injury in infants with NE, in order to facilitate treatment and predict neurodevelopmental outcome. The first objective of the NEON project was to use MRI to examine the microstructure of the typically developing neonatal brain, because in understanding the “normal”, it is then possible to define the

“abnormal”. NE has a multifactorial aetiology, and whilst MRI has already been widely investigated in NE, there is potential to combine MRI with other clinical biomarkers in order to improve its diagnostic and prognostic efficacy. The appearance of brain injury in infants with NE is a consequence of impaired cerebral autoregulation, and placental histology and inflammatory markers can provide insight into the events leading to the brain injury. The second objective was to determine if there is an association between placental histology and inflammatory markers to predict the pattern of brain injury seen on MRI. Additionally, it has been shown that the location of brain injury is associated with neurodevelopmental outcomes¹. The third objective of the NEON project was to examine whether the addition of placental histology and inflammatory markers may improve the ability of MRI to predict outcome measures in infants with NE.

The structure of the thesis is as follows:



Chapter 2: Neurodevelopment; From Conception to Adulthood

“The human brain has 100 billion neurons, each neuron connected to 10 thousand other neurons. Sitting on your shoulders is the most complicated object in the known universe”.

- *Dr Michio Kaku*

Brain development is a remarkably complex, dynamic, and adaptive process. Drastic changes occur within the brain during the time of development, which begins from around two weeks post-conception and continues into young adulthood². An infant is born with more than 99% of the neurons it will ever have³, yet the neonatal brain is only approximately 35% of the size of the adult brain⁴. During the first year of life the brain doubles in size, and by age six it reaches approximately 90% of its adult volume^{4,5}. The many changes that the brain undergoes during development are driven by a range of genetic, molecular, and environmental factors, and are accompanied by the development of numerous cognitive and motor functions. In understanding how the brain develops across the lifespan, it is possible to examine the effects that adverse neonatal events such as NE have on the brain, and how this may affect the infant's outcomes as they continue to grow and develop. This chapter will outline the processes that take place during neurodevelopment from the prenatal period right through to adulthood and will examine how atypical brain development may result in a wide variety of disorders and impairments.

2.1 Prenatal

The vast majority of the ‘work’ involved in brain development happens prenatally. The very beginnings of neural development occur from about 18 days post-conception. During the embryonic period (extending until eight weeks post-conception), the foundational neural changes begin in which there is a rapid emergence of new features⁵. By the end of the embryonic period, the elementary structures of the brain have been laid down, and during the foetal period that follows there is extensive elaboration of cortical and sub-cortical features^{5,6}.

At the end of gestation, although it is early in the developmental timeline, the neonate is born with most of the features that are recognisable in the adult brain – a cerebral cortex with left and right hemispheres, separated into four lobes (frontal, temporal, parietal, and occipital), as well as the cerebellum and brainstem (figure 1).

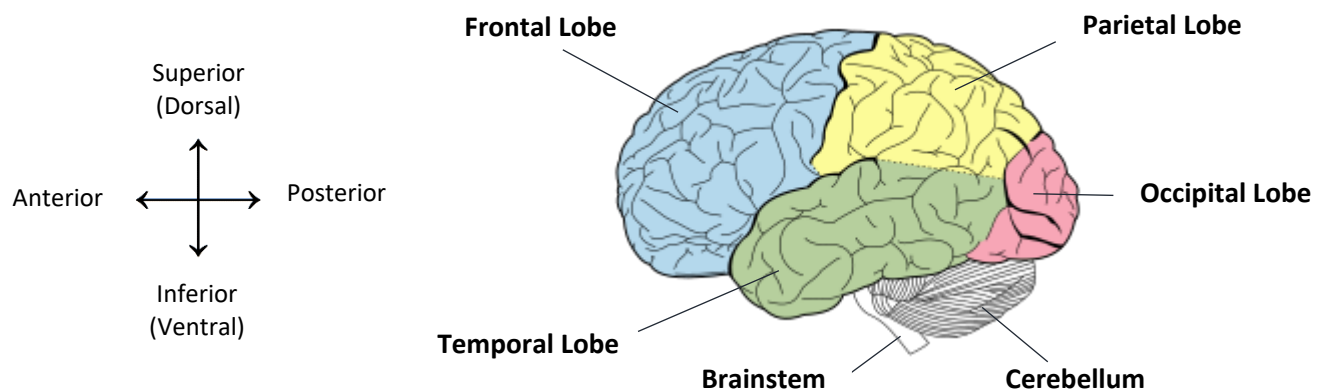


Figure 1. *Basic anatomy of the adult human brain (adapted from Gray's Anatomy of the Human Body).*

2.1.1 Gastrulation and Neurulation

The development of the nervous system begins with gastrulation at around the third week of embryonic development⁷. During gastrulation, the developing embryo reorganises itself into three germ layers - the endoderm, mesoderm, and ectoderm – which will all go on to form different parts of the body. It is the ectoderm that will eventually give rise to the central and peripheral nervous systems⁷. It begins with the forming of the neural plate, a layer of flattened cells which then begins to fold inward on itself, forming the neural groove. The sides of the neural groove then gradually fuse together and seal themselves into a neural tube, which closes by week four - this is neurulation^{2,7}. Different regions of the neural tube will become different brain and spinal cord structures. It first divides into three primary brain vesicles: the anterior part of the neural tube forms the prosencephalon (forebrain), the middle forms the mesencephalon (midbrain), and the posterior forms the rhombencephalon (hindbrain)⁵. By the seventh week, these then further divide into five brain vesicles; the

prosencephalon becomes the telencephalon (cerebral cortex) and the diencephalon (thalamus and hypothalamus), the rhombencephalon becomes the metencephalon (pons and cerebellum) and the myelencephalon (medulla), and the mesencephalon does not further divide (figure 2).

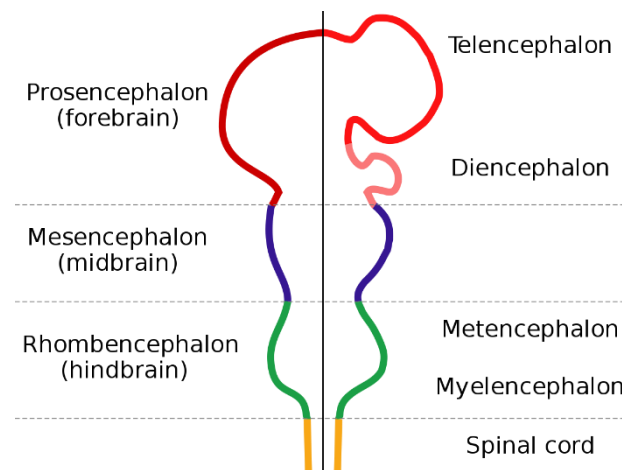


Figure 2. *Development of the neural tube (from I, Nrets).*

2.1.2 Proliferation and Migration

Once this regional structure of the neural tube has been established, there follows a rapid and extensive production of neurons. This rate of proliferation is staggering, with over 100 billion neurons being created from a mere 3-millimetre neural tube (approximately 250,000 neurons per minute), most of which happens by the middle of gestation⁸. The first step of proliferation involves the multiplication of neural progenitor cells, which divide and multiply symmetrically through mitosis. The second step is a shift to asymmetrical cell division, in which two different types of cells are produced – one new progenitor cell, and one neuron⁵. Whilst the progenitor cells remain in place within the proliferative zone to continue dividing, the neurons migrate away towards a more peripheral location. With the help of guiding glial cells, the neurons arrive at their final destinations to form the structure of the developing cerebral cortex. Migration happens in an inside-out pattern to form a six-layered cortex, with the first migrating neurons forming the innermost cortical structures, and the later migrating neurons forming the outer layers². Curiously, there is an overproduction of neurons in the

neonatal brain, meaning that we are born with far more neurons than we eventually have as an adult. This is balanced out by apoptosis, which is programmed cell death. Once proliferation stops, no new neurons are created. Proliferation peaks during the third and fourth months of gestation, and migration peaks between the third and fifth months of gestation⁹.

2.1.3 Differentiation

Once a young neuron has reached its intended destination in the cortex, it will then either differentiate into a mature neuron or it will face apoptosis. Differentiating neurons will develop axons and dendrites to allow them to become part of an interconnected neural network. Each neuron consists of one axon, a nerve fibre that sends signals away from the neuron; and numerous dendrites, branches that extend from the cell body and receive inputs from other neurons^{2,5} (figure 3). Axons develop extremely rapidly, at a rate of approximately 1 millimetre per day¹⁰. Together, fibre bundles of axons are what make up the white matter of the brain. Axons may be short, as in the case of interneurons, or they may be of considerable length, such as callosal and corticospinal fibres. Dendrites, however, develop more slowly during the first two trimesters, followed by an acceleration during the third trimester and even through to the first postnatal year¹¹. It is the cell bodies and their dendrites that make up the grey matter of the brain. As dendrites mature, they become increasingly complex, with an increasing number and density of branches. On the other hand, up to 50% of young neurons do not differentiate, and instead are subject to naturally occurring cell death⁵.

2.2 Infancy

At birth, the foundations and structure of the brain are set, and although proliferation and migration have largely occurred prenatally, differentiation and maturation continue during the postnatal period through infancy⁵. The connections and interactions that form between neurons help to develop the functional organisation of the neonatal brain, which evolves along with the developing cognitive and behavioural functioning of the infant.

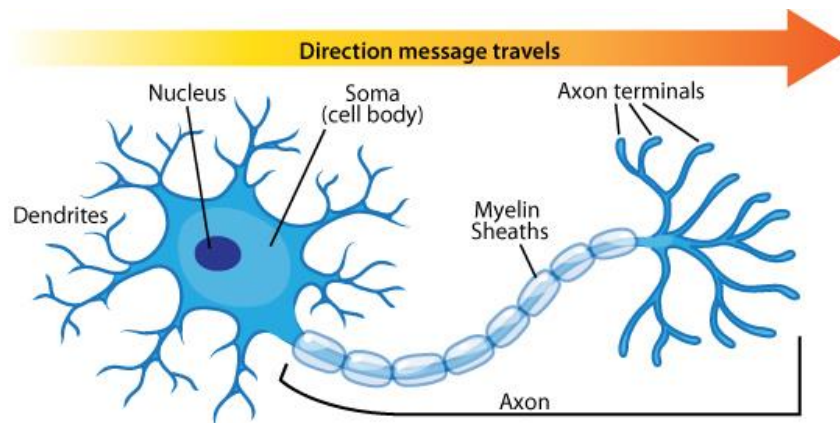


Figure 3. *Anatomy of a neuron (from Brett Szymik. (2011, May 03). Neuron Anatomy. ASU - Ask A Biologist, <https://askabiologist.asu.edu/neuron-anatomy>).*

2.2.1 Synaptogenesis and Pruning

A synapse is the point of connection, or junction between two neurons. One neuron communicates with another by sending an electrical or chemical signal to a receiving neuron, and so synapses are the key to the functional connectivity of the brain. The formation of new synapses is called synaptogenesis, and the very nature of a synapse means that the brain becomes a set of interconnected neural networks. Although the first synapses appear in the developing brain at around 23 weeks gestation, the peak in production occurs during the first year after birth². The peak of synapse production occurs at different time points for different areas of the brain. Sensory areas such as the visual cortex will reach peak synapse production between four and eight months postnatally, whereas higher-order functioning areas such as the prefrontal cortex peak around 15 months after birth².

As with neurogenesis, an exuberance of connections is formed during synaptogenesis, far more than is needed in the adult brain. Therefore, synaptogenesis is followed by a pruning back of the overabundance of synapses. The survival of synapses is determined by the Hebbian principle, which is often summarised as “neurons that fire together, wire together”¹². Hebb posits that for two neurons that repeatedly fire together, their synapse will be strengthened, however inefficient unused synapses will be eliminated. The number of synapses in the brain falls to approximately 50% by the age of two, however, pruning does continue at a lower rate

through adolescence until adult levels are reached¹⁰. The timing of pruning similarly depends upon in which region of the brain it occurs, for example, it continues up until about the age of six in the visual cortex. Synaptogenesis and pruning are also both influenced by experience-expectant and experience-dependent mechanisms. Experience-expectant refers to the development of synapses depending upon the presence of sensory experiences, whilst experience-dependent refers to the unique way in which the infant responds to their environment¹⁰.

2.2.2 Myelination

The final stage of brain development is myelination – the process in which the axons of neurons are surrounded by an insulating fatty sheath called myelin. Myelination is associated with more efficient neural connectivity and communication, as myelin dramatically increases axonal conduction velocity⁵. This means that white matter tracts made up of myelinated axons are able to transmit information about 10-100 times faster than those with unmyelinated axons¹³. It is generally assumed that a tract is not fully functional until it is myelinated, and myelination progresses in conjunction with increasing neurodevelopmental ability. Although myelination in the brain begins during the second trimester and continues through to adulthood, its most rapid development and peak is in the first year of life¹¹. Additionally, myelination occurs in several predictable neuroanatomical sequences: from central to peripheral, from posterior to anterior, and from caudal to rostral (figure 4⁹). Therefore, the brainstem and cerebellum myelinate before the cerebrum, myelination in the cerebrum begins from the central sulcus and moves outwards, and occipital poles myelinate before the frontal and temporal poles. Additionally, projection tracts myelinate before association tracts, and sensory tracts myelinate before motor tracts¹⁴. At birth, myelination can be seen in areas such as the central corona radiata (CR), the cerebellar peduncles, and the posterior limb of the internal capsule (PLIC). Between six months and one year of age, myelination has extended to the anterior limb of the internal capsule (ALIC), and the genu and splenium of the corpus callosum (CC). By two years of age, myelination can be seen in the central white matter of the four cerebral lobes¹⁵.

2.2.3 Developmental Skills and Milestones

When an infant is born, their brain is prepared for experience and learning, and the first few years of life are marked by the rapid development of sensory, motor, cognitive, language and emotional skills. A nurturing environment, appropriate experiences and positive relationships with caregivers are all crucial to brain development and the learning of these skills, which set the foundations for later life. The skills and milestones described below pertain to healthy, typically developing infants.

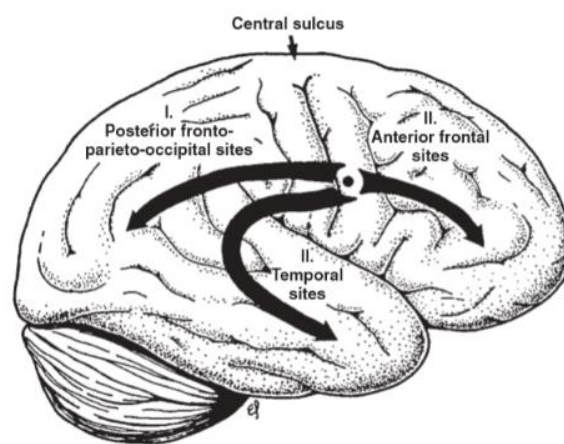


Figure 4. *The progression of myelination in the infant brain (from Volpe 2008⁹).*

Sensory and motor skills generally follow a typical developmental sequence in most infants. At birth, an infant already possesses a number of innate reflexes such as the sucking, rooting, grasping, and Moro reflexes¹⁰. By three months, the infant can control their head and respond to objects presented via sight or touch. By six months, the infant is able to roll over and develops a sense of depth perception, and by nine months, they are able to sit unsupported and pull themselves to standing. At approximately one year, the infant learns to walk, develops the pincer grasp, and can now see as well as an adult. During the next few years, the infant becomes increasingly mobile and agile, learning to do things such as run, climb stairs, feed and dress themselves, stack objects, and scribble with a pencil¹⁰.

Cognition and language similarly follow a developmental trajectory in the typically developing infant. Cognitive development during the early months is mostly perceptually based, with the infant learning to make eye contact with the caregiver and recognise their face and voice, to spontaneously smile and produce sounds, distinguish between and imitate facial expressions, and discriminate sounds from any language². Between six and twelve months, the infant will learn to laugh, imitate sounds made by others, to make social gestures, begin to understand object permanence, and start to specialise in discriminating sounds from their native language while losing this ability for non-native languages. The first evidence of word comprehension and word production also appears towards the end of this stage (8-10 months and 11-13 months respectively)¹⁶. Word comprehension initially occurs in response to contextually supported words (such as responding to “no” or their own name) but is soon followed by a rapid spurt in receptive vocabulary. Word production begins in a similar manner, usually by associating a word with an object or activity, and the year-old infant may have a vocabulary of about 5-10 words. This doubles over the next six months or so, and by the end of the second year their vocabulary may consist of roughly 300 words, and word combinations begin to emerge. At this time, infants are also able to categorically sort objects, respond to simple directions and play games. By 36 months, the infant will experience a burst of grammatical development, will have a vocabulary of roughly 1000 words, and be able to construct simple sentences^{16,17}.

2.3 Childhood

The structural and functional changes undergone in the brain during primary school years (age 4-11) reflect the refinement and specialisation of brain networks that are responsible for increasingly complex cognition, emotion, and behaviour. The brain adapts and develops both in response to the child’s learning environment, at school and at home, as well as building upon the synaptogenesis and pruning that began in infancy.

2.3.1 The Plastic Brain

A key feature of the brain during childhood is its plasticity; a remarkable adaptability which underlies a great deal of the learning that happens during this time². Brain plasticity is defined as “the ability of the nervous system to change its activity in response to intrinsic or extrinsic stimuli by reorganizing its structure, functions, or connections”¹⁸. Brain development is therefore particularly sensitive to experience and environmental influences during these childhood years, much more so than in later life. Input, experience, and practice all help to establish the mature organisation and connectivity of the brain seen in later life¹⁹. Referring back to Hebb’s rule of “neurons that fire together, wire together”¹²; if a child repeatedly engages in an activity (i.e., practices), for example learning to ride a bike, the neural pathways responsible for that activity will be strengthened, the child will learn the skill and will eventually be able to perform it effortlessly. This not only applies to the learning of motor skills but is also true for functions such as memory and language. For example, when new memories are formed, they must be mentally rehearsed or practiced, otherwise, they will be subject to pruning and forgotten¹⁹.

There are both critical and sensitive periods during childhood that influence brain development. A critical period is a short, strict window of time in which experience is essential for development, and without exposure to appropriate experiences during this time, that part of the brain and its subsequent behaviour may not develop properly, if at all. A sensitive period, on the other hand, is a broader term referring to a gradual window of time during which experiences have a particularly heightened effect on the brain and behaviour^{20,21}. The critical periods for certain sensory functions such as visual and auditory functioning are generally well defined. For example, Hubel and Wiesel’s²² famous animal studies have shown that occluding vision in one eye during a critical early postnatal period will irreversibly alter neural activity in the primary visual cortex pertaining to both the closed eye and the unaffected eye. The critical and sensitive periods for higher-order cognitive functions such as language are broader and more complex and are still an ongoing debate. These windows of time vary across the different aspects of language (syntax, morphology, semantics etc.) as well as for native language and second language acquisition. Lenneberg²³ first proposed the critical period hypothesis for language, suggesting that if language acquisition does not occur during the childhood years before puberty, subsequent language learning is limited and cannot be

mastered fully. It is also well known that children are able to acquire a second language much more easily and efficiently than adults, as their brain plasticity is far more conducive to learning than the adult brain. However, this is more likely a sensitive period rather than a critical period, as adults are still able to acquire a second language, although to a lesser extent.

2.3.2 Grey and White Matter Changes

The microstructural changes to brain networks due to plasticity can be seen on a macrostructural level as changes in grey and white matter volume. Changes to white matter during childhood are linear, increasing with age through to early adulthood. Grey matter changes, on the other hand, are non-linear and regionally specific. Grey matter volume increases in phases during childhood, until it peaks and then gradually decreases in volume from adolescence/young adulthood. Consequently, the brain during childhood is considerably larger than the adolescent/young adult brain. Developmental trajectories for grey matter are also different across different areas of the brain. Evidence suggests that the frontal, parietal, and temporal lobes follow an inverted U-shaped trajectory, with volume in the frontal and parietal lobes peaking at around age twelve, and at around age sixteen for the temporal lobe. The occipital lobe, however, increases linearly with age right up until young adulthood. Given the increase in white matter compared to the relative decrease in grey matter volume, the ratio of grey to white matter decreases with age^{5,19,24-26}.

2.3.3 Developmental Skills and Milestones

When a child begins school, they encounter a whole new learning environment which greatly develops the way they think about themselves, others, and the world around them. Motor skills are reinforced, communication and language are explored, and executive functions emerge. Not all children progress and develop at the same rate, due to their differing personalities, abilities, and environments. However, there are certain milestones that most typically developing children reach during their primary school years.

Competence in motor activities is achieved during childhood. Both gross and fine motor skills are developed and refined through engagement in physical activities, including hand-eye coordination, balance, and control. By six to seven years of age, a child learns to master fundamental movement patterns such as jumping, skipping, kicking, throwing and catching, as well as more refined skills such as tying shoelaces, learning to swim or riding a bike²⁷. This is also the time a child will learn to write and draw. By nine to twelve years of age, writing speed increases and handwriting becomes more fluid, a child can participate in team sports or learn to play an instrument, and will show an increased self-perception of their own body and abilities²⁷.

Cognitive development during childhood shows great variability, both in terms of individual differences as well as the developmental trajectories of different cognitive domains. For example, some basic cognitive abilities such as processing speed develop linearly at the same rate throughout childhood. On the other hand, most cognitive abilities develop non-linearly, improving steeply between the ages of six and ten and levelling off at approximately ten to twelve years of age²⁸. These include domains such as reading/verbal comprehension and working memory and suggest that adult levels of proficiency are reached in pre-adolescence. Some higher-level cognitive abilities show protracted development and continue well past childhood, such as inhibitory control²⁸.

Nevertheless, based on the work of Jean Piaget, there is evidence of some stage-like characteristics in cognitive development, in terms of large-scale cognitive reorganisations. The first occurs at approximately four years of age and is characterised by representation – a child acquires the ability to perform tasks in which they can coordinate two or more ideas, such as recognising others' viewpoints relative to their own and considering objects not present in their immediate experience²⁹. The second occurs between six and seven years of age and is characterised by concrete operations – the application of logical thinking to objects and events and increasingly complex social representations. The third emerges between ten and twelve years of age and is characterised by formal operations – a child is able to generalise, think abstractly and reason hypothetically²⁹.

2.4 Adolescence to Adulthood

Adolescence is a stage of transition where a child becomes an adult and is usually considered to span the ages of 10-24 years³⁰. It is an important period of emotional, social, intellectual, and physical development, during which the brain is still busy strengthening and pruning its synapses. Not only is the developing adolescent brain influenced by genetic and environmental factors, but it is now also under the influence of sex hormones. It has been shown that hormones such as oestrogen, progesterone, and testosterone play a key role in myelination and functional connectivity³¹. Changes are particularly significant in brain regions that relate to the heightened behaviours that are characteristic of teenagers, such as emotion regulation, self-control, and risk-taking.

2.4.1 Prefrontal Cortex

The prefrontal cortex (PFC) is a highly interconnected region of the foremost part of the frontal lobe and comprises almost a third of the cerebral cortex³². The orbital and medial areas of the PFC play a key role in emotional behaviour and controlling basic drives. The lateral region is involved in top-down cognitive control and is thought to be responsible for executive functions such as focusing attention, planning and strategizing, inhibition and impulse control, problem-solving, rational decision making, and modality integration. The PFC is one of the last brain regions to reach maturity, which continues to myelinate and prune away synapses through adolescence up until the age of 25 years. This may explain why adolescents typically exhibit behavioural immaturities, such as impulsivity, excessive risk-taking and novelty-seeking. The protracted maturation and plasticity of the PFC mean that adolescence is a critical period of both opportunity and vulnerability. Teenagers can maximise their emotional and cognitive development by engaging in positive learning activities and social interactions, however, they are also extremely vulnerable to adverse events and are susceptible to mental illnesses such as depression, anxiety, schizophrenia and addiction^{30,32-35}.

2.4.2 Limbic System

The limbic system is a complex network of deep cerebral structures consisting of the hippocampus, amygdala, and hypothalamus. Whilst the PFC is involved in top-down processes, the limbic system is a bottom-up system. It is involved in the expression and control of emotions, particularly fear, aggression, and the “fight or flight” response, as well as long-term and emotional memory and social cognition. It also regulates autonomic functions, sleep, appetite, and sexual behaviours. The limbic system develops earlier and on a steeper curve than the PFC, showing its most dramatic changes between the ages of ten and twelve. Therefore, whilst the executive functioning of the PFC is still under development, the adolescent brain is much more reliant on the functions of the limbic system. It is this mismatch in maturation (and not simply protracted PFC development alone) that is likely the reason adolescents are more likely to engage in risky behaviour and be propelled by their emotions and social relationships^{30,33,35,36}.

2.4.3 Corpus Callosum

The largest white matter structure in the brain, the CC is a commissural fibre tract that connects the left and right cerebral hemispheres. It is implicated in the communication and integration of sensory information and the mediation of complex behaviours. White matter growth is influenced by sex hormones, and the CC undergoes robust development during adolescence, continuing to mature into adulthood. Although the number of axons in the CC is fairly stabilised from an early postnatal period, CC area and thickness have been shown to increase with age and sexual maturity, which may be attributed to changes in myelination and axonal size³⁷⁻³⁹. These changes occur in conjunction with the development of more complex cognitive functions, and so the CC has been dubbed “part of the highest order-latest maturing neural network of the brain”⁴⁰.

2.4.4 Sex Differences

It is well known that there are female/male differences in the development of cognition, behaviour, and emotions, as well as differences in the prevalence of neuropsychiatric disorders. Sexual dimorphism also extends to human brain structure, although these differences are far from clear-cut and conclusive. Studies have shown that total brain volume is larger in males than females by approximately 9-10%, whilst women have a greater grey-to-white matter volume ratio⁴¹⁻⁴⁴. Differences in volume are relatively uniform across the brain except for the basal ganglia, where it has been found that females have greater volumes in the caudate and males have greater volumes in the globus pallidus⁴¹. It has also been found that females have proportionately greater frontal grey matter than males⁴². Total brain volume tends to peak one to two years earlier in females than in males, and males show greater reductions in grey matter during adolescence⁴³. Total white matter volume increases over adolescence for both sexes, but larger increases are seen in males in overall white matter and CC volumes⁴⁴. Amygdala and hippocampus volumes also increase with age for both sexes, but a larger increase is seen in the amygdala for males and in the hippocampus for females⁴¹. These differences should not be interpreted as functionally advantageous/disadvantageous, however, they may – along with other genetic and environmental factors – contribute to neuropsychiatric differences seen between males and females.

2.5 Atypical Brain Development

Understanding how the brain grows and changes across each of these critical periods of development provides us with a clearer picture of how the healthy adult brain comes to be. However, it is also critical in understanding what happens when brain development goes wrong. There are many brain disorders that may develop during any of these different stages of development and distinguishing how atypical development deviates from the typically developing brain is key in assessing and treating these disorders.

2.5.1 Congenital Malformations

Congenital brain malformations are a result of atypical brain development in utero, and consequently are present in the neonate at birth. Congenital malformations may be caused by genetic defects in the foetus, or in some cases by maternal exposure to infection, drugs, or alcohol. Different malformations arise from each of the prenatal processes involved in brain development, each with its own clinical presentation. Impairments may be mild to severe, and whilst some are obvious from birth, others may not be diagnosed until later in life. Neural tube defects arise due to the improper closure of the neural tube and represent the most serious of congenital malformations. The most common neural tube defects are anencephaly (which is mostly fatal) and spina bifida. Disorders of cortical development may also arise due to atypical proliferation, migration, or differentiation. This is a heterogeneous group of disorders that may lead to developmental delay or epilepsy, and it includes microcephaly, lissencephaly, schizencephaly and agenesis of the CC^{7,9,45}.

2.5.2 Neurodevelopmental Disorders

Manifesting early in the development period, usually during the early childhood years, neurodevelopmental disorders (NDDs) are a multifactorial group of conditions associated with impairments in communication, cognition, or behaviour. Influenced by both genetic and environmental factors, NDDs are associated with atypical neurodevelopmental trajectories. Several NDDs show an overlap in clinical symptoms, and often children are affected by more than one NDD. The two most common NDDs are autism spectrum disorder (ASD) and attention-deficit/hyperactivity disorder (ADHD). ASD is characterised by deficits in social communication and repetitive and restrictive behaviours, ranging from very mild to very severe, and is usually diagnosed by three to four years of age. ASD has been associated with brain overgrowth during infancy and childhood, followed by accelerated cortical thinning during adolescence. ADHD is a disruptive behaviour disorder characterised by inattention and hyperactivity and is usually diagnosed in school-aged children. ADHD has been associated with a thinner cortex and a delayed peak of cortical thickness, especially in prefrontal areas. Other NDDs include intellectual and learning disabilities, and communication and motor disorders, all of which tend to have lifelong effects⁴⁶⁻⁵².

2.5.3 Acquired Brain Injury

Acquired brain injury refers to injury acquired at any point after birth (as opposed to congenital disorders, or perinatal brain injury, which will be the primary research focus of this thesis) and may be classified as either traumatic or non-traumatic. Traumatic brain injury (TBI) refers to a sudden external physical blow to the brain, whereas non-TBI may be the result of illness such as infection, hypoxia or stroke⁵³. Neurodevelopmental outcomes vary significantly depending upon the nature of the injury, but may include impairments in memory and attention, executive functioning, academic ability, social skills, and motor incoordination⁵⁴. Because brain injury may be acquired at any point during development, impairments and recovery of skills may depend upon what is already established in the brain at the time of the injury - skills that are in a rapid stage of development at the time of the injury may be most disrupted. For example, it has been found that acquiring brain injury before the onset of formal reading instruction is a risk factor for reading difficulties, and IQ scores have been found to be lower in young children who have acquired brain injury compared to school-age children and adolescents, who will be experiencing some degree of formal education⁵⁵. However, the best evidence of functional plasticity and recovery in infants and children following acquired brain injury seems to be in the domain of language, with this ability potentially lasting up to ten years of age¹⁰.

2.5.4 Mental Illness/Psychiatric Disorders

Mental illnesses, or psychiatric disorders, are defined in the *Diagnostic and Statistical Manual of Mental Disorders* as “a syndrome characterized by clinically significant disturbance in an individual's cognition, emotion regulation, or behaviour that reflects a dysfunction in the psychological, biological, or developmental processes underlying mental functioning”⁵⁶. Typically, the onset of mental illness first emerges during adolescence – a time of considerable neurological and behavioural change which leaves the adolescent vulnerable to psychopathology^{57,58}. There are many classes of mental illness, each with its own symptoms and pathophysiology, which together are a significant global burden of disease. The most prevalent of these are anxiety disorders (which include generalised anxiety disorder, panic disorder and social anxiety disorder), and mood disorders (which include major depressive

disorder and bipolar disorder). It has been suggested that these disorders may be associated with abnormal engagement in brain regions related to emotional processing such as the prefrontal cortex and the amygdala. It has also been suggested that hormonal changes during adolescence may account for some of the risks for anxiety and mood disorders, due to the female:male prevalence changing from being equal during childhood to 2:1 after puberty⁵⁸. Other categories of mental illness include obsessive-compulsive disorders, trauma- and stressor-related disorders, dissociative disorders, personality disorders, impulse-control disorders, feeding and eating disorders, sleep-wake disorders, and addictive disorders. Thought to be due to a complex interplay between genetic predisposition and social/emotional stressors, mental illness is primarily treated and managed through some form of therapy, such as psychotherapy, counselling or cognitive-behavioural therapy, and/or psychiatric medication, such as antidepressants or antipsychotics.

2.5.5 Neurodegenerative Disorders

Neurodegeneration is the progressive structural and functional degeneration of the nervous system, attributed to pathological processes such as neuronal death, demyelination, and axonal regeneration failure⁵⁹. Neurodegenerative disorders are therefore associated with the progressive deterioration of motor and cognitive abilities, and although symptoms may be treated, they cannot be cured. The majority of these disorders are associated with ageing, and the two most prevalent are Alzheimer's and Parkinson's diseases⁶⁰. Alzheimer's disease is the most common cause of dementia, and is characterised by neuronal loss, primarily in the hippocampus but followed by widespread atrophy. Parkinson's disease is a movement disorder characterised by the loss of dopaminergic neurons in the substantia nigra. Huntington's disease is another age-related neurodegenerative disorder, characterised by both dementia and movement disorder, due to cell death in the basal ganglia. On the other hand, there is a neurodegenerative disorder that develops in young people and affects the developing brain. Multiple sclerosis is a chronic inflammatory disorder, characterised by extensive demyelination and axonal injury throughout the central nervous system, which disrupts neuronal communication. As such, multiple sclerosis may present with almost any type of neurological symptom which may involve periods of both relapse and remission^{59,60}.

Chapter 3: Neuroimaging; an Overview

“Brain imaging gives us the hope of opening up the black box”.

- *Prof Brian Knutson*

Information in this chapter regarding MRI physics and sequences was obtained from a series of lectures given by Professor Arun Bokde from 30th January – 11th February 2019 at Trinity College Dublin for the following module: PR7908-A-SEM202-201819: NEUROIMAGING (IMG).

3.1 Magnetic Resonance Imaging

MRI is a safe and non-invasive medical imaging technique that can provide highly detailed information about the living brain. Using magnetic fields and radio waves, MRI is able to examine anatomy, connectivity, neural activity, and pathology through a variety of different sequences. This chapter will briefly describe the basics of MRI physics and signal generation, and then outline the three main sequences used in neuroimaging research. Finally, the application of MRI to the neonatal brain will be discussed.

3.1.1 Basic Principles

MRI relies upon the properties of hydrogen nuclei within water molecules, which are abundant in the human brain. Consisting of a single positively charged proton, the hydrogen nucleus rotates about its own axis with a non-zero net spin. Behaving like a tiny rotating bar magnet, this spin motion produces angular momentum and magnetic moment. In the absence of a magnetic field, the axes of these hydrogen protons are pointing in random directions. However, in the presence of a strong magnetic field such as that produced by the large superconducting coil of an MRI scanner, hydrogen protons will slowly align in the direction of this net external magnetic field (B_0). Most protons will align themselves parallel to B_0 in a low

energy state, but a much smaller amount will align themselves antiparallel to B_0 in a high energy state, and it is these protons that contribute to the MR signal.

A key property of these spinning hydrogen protons is that they precess around the B_0 axis in a manner akin to a spinning top (figure 5). The frequency at which this precession occurs (f) depends upon the strength of the B_0 magnetic field and the molecule's unique gyromagnetic ratio (γ), and is calculated as follows:

$$f = \gamma * B_0$$

This frequency, at which the protons absorb a lot of energy and begin to precess, is called the resonance frequency or the Larmor frequency. When protons precess at the resonance frequency, it gives rise to a small secondary magnetic field, or magnetisation. At equilibrium, the net longitudinal axis of the protons is aligned to B_0 . This is called net longitudinal magnetisation (M), and although it is difficult to measure directly due to being very small relative to B_0 , changes in M can be detected and externally manipulated in order to obtain the MR signal.

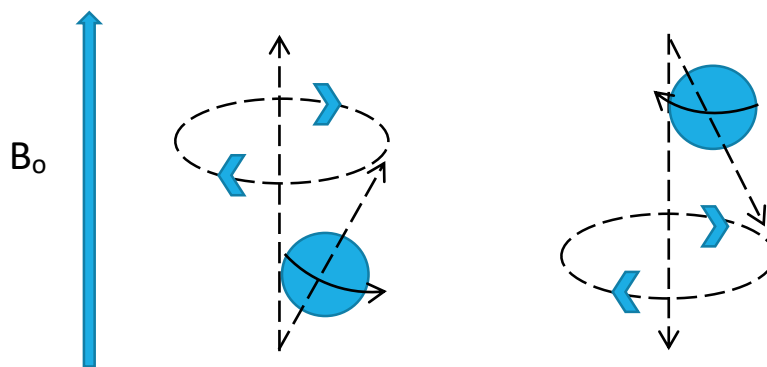


Figure 5. *Precessing protons in an external magnetic field, aligning in a parallel low energy state (left) and an antiparallel high energy state (right).*

In order to measure a change in M , a radio frequency (RF) pulse is applied at the resonance frequency of the hydrogen protons in the brain, exciting them and causing the resonance phenomenon. This means that the protons are able to absorb enough energy from

the RF pulse to cause their axes - and consequently, M - to rotate away from B_0 in the transverse direction. When the RF pulse is turned off, this energy is then released by the protons until they realign back to B_0 . The time it takes for the protons to realign in this longitudinal direction is called T_1 relaxation. T_1 relaxation depends upon the rate of energy transfer to surrounding tissue in the brain, and so is also referred to as “spin-lattice” relaxation.

The RF pulse also has another effect on the protons; it causes them to move in phase with each other in the transverse direction. When the RF pulse is turned off, the protons start to fall out of phase with each other. The time it takes for the protons to de-phase in this transverse direction is called T_2 relaxation. T_2 relaxation depends upon the rate of energy transfer to surrounding protons, and so is also referred to as “spin-spin” relaxation. It is these two mechanisms of relaxation that contribute to the MR signal.

In order to limit the resonance phenomenon to a specific location, i.e., the brain for neuroimaging, a supplemental gradient is applied to the specific slice of interest. This means that when the RF pulse is applied, it will excite only the protons within this slice. An additional frequency-encoding gradient is applied during measurement in order to localise the MR signal either within a slice or between slices, and a phase-encoding gradient is applied in order to map location based on the phase of the MR signal.

The acquisition of an MR image results in a vast amount of raw data stored as a function of frequency and phase. This data is then transformed into a 3D image (or volume) using a mathematical process known as the Fourier transformation⁶¹. The image is made up of 3D pixels which are called voxels, and it can be viewed in axial, sagittal and coronal planes.

3.1.2 Structural Imaging

In structural MRI, the primary interest is determining the shape and size of anatomical structures in the brain. It does this by taking advantage of the fact that both T_1 and T_2 relaxation times are different for different tissues in the brain due to their differing fat and water content – namely, grey matter, white matter, and cerebrospinal fluid (CSF). This is what produces the different contrasts between tissues, allowing for the differentiation of brain structures. Two different types of structural images can be generated depending upon the type of relaxation,

which is determined by changing the sequence of the RF pulse that is applied by the scanner. Repetition time (TR) is the amount of time between successive RF pulses and echo time (TE) is the amount of time between delivering an RF pulse and receiving the echo.

A T_1 -weighted image is produced using a short TR and TE and is useful in examining structures with high fat content. This makes it effective in differentiating between grey and white matter, with grey matter appearing dark grey and white matter appearing brighter. A T_2 -weighted image is produced using a long TR and TE and is useful in examining areas rich in water. This makes it effective in differentiating between brain tissue and CSF, with grey and white matter appearing as varying shades of grey and CSF appearing very bright. A T_1 -weighted image has higher spatial resolution, whereas a T_2 -weighted image has higher tissue contrast.

3.1.3 Functional Imaging

Functional MRI (fMRI) provides an indirect measure of neural activity by examining patterns of activation in the brain. More specifically, it is sensitive to the haemodynamic changes in the blood that accompany changes in neural firing, as oxygenated and deoxygenated blood respond differently to each other when in the presence of a magnetic field. The measure of this effect is known as the blood oxygenation level dependent (BOLD) signal, and it is this magnetic susceptibility that can be detected by fMRI⁶².

An increase in neural activity in the brain causes an increased demand for oxygen from the blood, meaning that areas of the brain that are active require higher levels of oxygen as a source of energy. To compensate for this, the body's vascular system increases blood flow to these areas, but in order to minimise the risk of starving the neurons of energy, much more oxygenated blood is delivered than is consumed. This, therefore, increases the amount of oxygenated haemoglobin relative to deoxygenated haemoglobin. The oxygenated blood is diamagnetic, meaning that the presence of an external magnetic field will create a secondary weak, repulsive magnetic field. The deoxygenated blood however is paramagnetic, meaning that it is attracted to an external magnetic field, which causes faster spin de-phasing and transverse decay. T_1 and T_2 effects are enhanced by paramagnetic substances and decreased by diamagnetic substances, and so during neural activation, when more oxygenated

haemoglobin is present, there is an increase in the local MR signal. This signal represents T_2 relaxation plus relaxation to inhomogeneities in the magnetic field, and as such it is known as T_2^* .

There are two primary research interests in fMRI; activity related to a task and functional connectivity at rest. Task fMRI involves an individual performing a specific task - such as a sensory, cognitive, or motor task - whilst inside the scanner. By measuring the BOLD signal at baseline and then during a task, it is possible to infer which regions of the brain are activated when that particular process is engaged. On the other hand, in resting state fMRI (rs-fMRI) the BOLD signal is measured in the absence of a task, where an individual is asked to simply sit in the scanner with their eyes open and asked to not think about anything. This measurement provides information about spontaneous brain activity and functional connectivity.

3.1.4 Diffusion Imaging

Diffusion-weighted imaging (DWI) is a routine MRI sequence that utilizes the motion of water in the brain to examine its cellular architecture and anatomical connectivity. Its primary focus is the integrity of white matter, which consists of fibre bundles of myelinated axons that connect the cortical and subcortical grey matter.

The principle which forms the basis of DWI is that of Brownian motion⁶³. Based upon the observations of Robert Brown, it describes the random motion of particles suspended in a fluid (such as water) resulting from their collision with the quick-moving molecules of that fluid. Albert Einstein later proved that Brownian motion is driven by thermal energy, with motion occurring in all directions if within a homogenous medium with no barriers. This molecular transport process is what is known as diffusion, which can be seen as a macroscopic demonstration of Brownian motion on a microscopic level. Einstein's diffusion equation can be stated as follows:

$$\langle r^2 \rangle = 6Dt$$

in which r^2 is the mean squared displacement of particles, D is the diffusion coefficient for a particular medium and t is the observation time⁶⁴. In addition to applying B_0 , a number of different gradient fields are also applied by the MRI scanner to the brain, in order to detect the direction of the moving water.

What makes this useful for measuring the diffusion of water in the brain is that diffusion differs depending on the type of tissue. In CSF, water is unrestricted and free to diffuse in any direction. This is called isotropic diffusion. However, in white matter, which mostly consists of axonal fibres pointing in a specific direction, diffusion is restricted and will occur along the same direction as the fibres, and so is anisotropic. In grey matter, which mostly consists of dendrites with a randomly oriented microstructure, diffusion may appear isotropic, although it is in fact anisotropic – this appearance of isotropy is due to limitations in the DWI sequences. DWI therefore is primarily used to examine white matter.

The mathematical representation of this diffusion in the brain is called diffusion tensor imaging (DTI), and it is used to visually map the direction of white matter tracts in the brain. The tensor model is a 3x3 matrix that quantifies diffusion in each direction. It is composed of three eigenvalues (λ_1 , λ_2 , and λ_3) which represent the amount of diffusion, and three corresponding eigenvectors (ϵ_1 , ϵ_2 , and ϵ_3) which represent the direction of diffusion. The first eigenvalue (λ_1) represents diffusion along the strongest direction - axial diffusivity (AD). The average of the remaining eigenvalues represents diffusion perpendicular to the strongest direction - radial diffusivity (RD). Mean diffusivity (MD) is the average of all three eigenvalues. However, the most widely used measure in DTI is fractional anisotropy (FA). FA measures the degree of anisotropy in a voxel, and is calculated as follows:

$$FA = \sqrt{\frac{(\lambda_1 - MD)^2 + (\lambda_2 - MD)^2 + (\lambda_3 - MD)^2}{2(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}}$$

FA has a value of between 0 and 1, with 0 being isotropic diffusion and 1 being anisotropic diffusion. The visualisation of diffusion in each voxel is represented by a tensor - an ellipsoid whose shape represents the degree of anisotropy (figure 6), and colour represents the direction (red is left-right, green is anterior-posterior, and blue is superior-inferior).

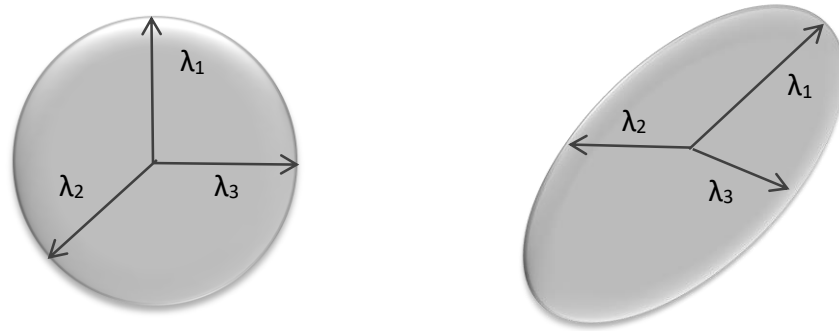


Figure 6. Examples of isotropic (left) and anisotropic (right) diffusion ellipsoids.

The process of examining the anatomical connectivity of the brain by following and visualising the trajectories of anisotropic streamlines is called tractography. Tractography can be either deterministic or probabilistic. Deterministic tractography assumes that each voxel contains a single orientation, whereas probabilistic tractography assumes that there is a distribution of orientations within a voxel.

A recent practical diffusion technique that can be used to further examine the morphology of axons and dendrites (collectively known as neurites) is Neurite Orientation Dispersion and Density Imaging (NODDI)⁶⁵. It provides estimations of three metrics: intracellular, extracellular, and free water. The intracellular volume fraction (v_{ic}) is a quantitative estimation of neurite density (also known as the Neurite Density Index, NDI), whilst the isotropic (or free water) fraction (v_{iso}) is attributed to CSF. The orientation dispersion index (ODI) estimates the angular variation/fanning of neurites. NODDI presents the possibility of extending the study of the brain's microstructure from white matter to grey matter, and of broadening investigations into brain connectivity, development, and pathologies such as neurodegenerative and demyelination disorders. Study 1 (Chapter 6) provides a more in-depth look at NODDI and how it is modelled, applying it to a dataset for analysis.

3.2 Neonatal Neuroimaging

MRI systems were designed with the adult brain in mind. However, recent advances in neuroimaging methods allow for the scanning of the neonatal brain, which requires careful

adaptation of MRI sequences and protocols. This must be done for several reasons, which include differences in size, myelination, and tissue contrast between the adult and neonatal brain, as well as issues of motion and monitoring⁶⁶. Nevertheless, MRI is a highly valuable tool in the assessment of the patient neonate within the clinical setting, and in examining maturational processes in the typically developing neonate brain within the research setting.

3.2.1 Scanning the Neonate

MRI is extremely sensitive to motion, and this poses a challenge when imaging the neonate as they are unable to follow instructions to remain still. There are two solutions to this problem – feed and wrap, and sedation. The ideal method is feed and wrap, where the parents are asked to keep their baby awake and to refrain from feeding them in the few hours prior to scanning. Upon arrival to the MRI department, the baby will then be fed, swaddled in a blanket, and allowed to fall into natural sleep before being placed in the scanner. Alternatively, a vacuum bag immobiliser may be used to comfortably stabilise the neonate. If the feed and wrap method does not work, the neonate may require sedation, however, this is usually avoided, when possible, in order to avoid possible complications. The most widely used drug for sedation of the neonate is chloral hydrate⁶⁷ and similarly is administered on an empty stomach.

Another consideration is that the noise of an MRI scanner can be fairly loud, and so in order to prevent the neonate from waking during the scan, ear protection is normally given in the form of specially designed earmuffs or earplugs. Additionally, all babies undergoing MRI must be continuously monitored throughout the scan for oxygen saturation, heart rate and temperature, and critically ill neonates coming from the neonatal intensive care unit (NICU) may require additional monitoring equipment and MRI-compatible transport.

3.2.2 Neonatal Structural Imaging

The most obvious structural difference between the neonatal and the adult brain is the size. At birth, brain volume is approximately one-third of the size of an adult⁶⁸, and so a higher

resolution is required when imaging. This means that the use of an adult-sized head coil will produce poorer signal-to-noise ratio (SNR), and so a smaller neonatal head coil is preferred when available. However, as discussed in chapter 2, the neonatal brain is by no means simply a smaller version of the adult brain and there are other morphological differences that will affect T_1 and T_2 relaxation. This means that neonatal T_1 and T_2 images look quite different to those of an adult.

The water content of the neonate brain is much higher than that of an adult. This is particularly true for the white matter, which isn't yet fully myelinated at birth. This results in a reversal of the adult contrasts seen on T_1 - and T_2 -weighted images. For the neonate, on T_1 -weighted images, white matter intensity is lower than that of grey matter, and on T_2 -weighted images, white matter intensity is higher than that of grey matter. Whereas a T_1 -weighted image is preferred for examining the structure of the adult brain, a T_2 -weighted image is preferred when examining the structure of the neonate brain. However, because some areas of the brain are already myelinated at birth whilst others are not, this means that tissue contrast between grey and white matter can be poor in some areas⁶⁹. For example, the internal capsule is usually myelinated at term and presents as hypointensity on a T_2 -weighted image in the healthy term infant.

Because of these differences, the standard MRI sequence parameters must be adapted for the neonate. T_1 and T_2 relaxation times are longer in the neonatal brain due to its higher water content and lower content of protein and lipid, and as a result, the TR should be increased in order to optimise SNR and tissue contrast⁷⁰.

3.2.3 Neonatal Diffusion Imaging

DWI and DTI have an advantage over structural imaging in the neonate because they can reveal microstructural information about the pre-myelinated brain. They, therefore, have the potential to examine the maturation of white matter in the developing neonate. Alongside the decreasing brain water content of the developing neonate, white matter also becomes more organised in terms of its axonal bundles, limiting the diffusion of water molecules to specific directions⁷¹. Therefore, for white matter, FA increases rapidly from birth as maturation

progresses, and high levels of FA in a specific tract may suggest that that tract is more finely and compactly organised and may be further along in the myelination process. Conversely, MD in white matter decreases with maturation. On the other hand, FA and MD in the grey matter of the cortex show a slightly different pattern, with both metrics being very high during foetal and preterm development and decreasing rapidly from birth⁷¹. DWI is also more sensitive than structural MRI in identifying patterns of acute brain injury in the very early neonate⁷², with increases in diffusion restriction presenting as an increase in signal on a diffusion-weighted image, indicative of injury in that area.

In DWI, a b-value is the amount of diffusion weighting that is applied. Because of the higher water content of the neonate brain, it has been suggested that using a lower b-value may be optimal for imaging this population. It is also recommended that a higher number of gradient directions be used due to the higher possibility of motion in neonates⁷³.

NODDI also has important applications to the neonate populations. The technique has been successfully used to demonstrate the microstructural properties of early maturing, partially mature, and immature white matter tracts in the neonate brain⁷⁴. Early maturing tracts contained projection fibres that were tightly packed and myelinated, such as the corticospinal tract (CST). Partially mature tracts contained tightly packed unmyelinated fibres, such as the corpus callosum (CC). Immature tracts contained fanning fibres that were loosely packed and subcortical U-fibres. In terms of NODDI metrics, this was demonstrated by the highest NDI values in the main white matter tracts of the neonate (such as the CC and the posterior limb of the internal capsule, PLIC), and lowest values in the grey matter; whilst low ODI values revealed the main white matter tracts to have very low dispersion⁷⁴.

3.2.4 Neonatal Functional Imaging

Although task-fMRI is challenging to perform in the pre-verbal neonate, it is possible to examine the functional connectivity and architecture of the neonate through rs-fMRI. Indeed, the past decade has seen a marked increase in the use of this technique to explore the emergence of resting-state functional networks in the newborn. In chapter 6, we examine neonatal fMRI in greater detail, applying fMRI analysis to a dataset from the Developing Human

Connectome Project (dHCP, <http://www.developingconnectome.org>) in order to see how functional architecture develops with gestational age. We employ quantitative voxel-level metrics that measure the amplitude of low frequency (<0.1 Hz) fluctuations in the BOLD signal whilst at rest^{185,186} to infer local brain activity during the neonatal period.

Chapter 4: Neonatal Encephalopathy; a Closer Look

“You have all these plans for bringing your baby home. As bad as it is, just enjoy your baby, even when they’re in there, because it’s so different and it’s so hard and you can’t touch them. And everything is beeping and you don’t know if it’s your baby’s monitor or somebody else’s baby’s monitor that’s beeping, and you don’t know if it’s beeping because something fell off, or it’s beeping because something is really wrong. As stressful as it is, try and enjoy your baby as much as you can. Even if you’re not doing this as planned”.

- *Parent of infant with NE (from Lemmon et al, 2017⁷⁵).*

4.1 What is Neonatal Encephalopathy?

Neonatal Encephalopathy (NE) is a heterogeneous clinical condition of disordered neurological function caused by an insult to the brain at birth or shortly following birth. It is a condition that affects babies born at term (or late preterm), classified as beyond 35 weeks gestation, and can lead to a broad range of outcomes requiring different levels of care. This chapter will first describe the clinical features and presentation of NE. It will then discuss how NE is treated and what that treatment currently looks like here in Ireland. Finally, the potential developmental outcomes of NE will be discussed.

4.1.1 Phenomenology

NE is clinically defined as “subnormal level of consciousness or seizures, and often accompanied by difficulty with initiating and maintaining respiration and depression of tone and reflexes”¹. Signs and symptoms will generally vary according to the severity of the injury and may include the following^{1,76}:

- (i) Low Apgar scores – a score of 0-3 beyond 5 minutes.

- (ii) Metabolic acidosis in foetal umbilical cord arterial blood – pH<7.0, base deficit $\geq$ 12.0mmol/L.
- (iii) Multisystem organ failure within 72 hours of birth.
- (iv) Loss of foetal heart rate variability or sudden sustained bradycardia.
- (v) Neuroimaging evidence of acute non-focal brain injury.
- (vi) Breathing difficulties and/or need for resuscitation/ventilation.
- (vii) Weak or absent cry at birth.
- (viii) Poor suck and Moro reflexes.
- (ix) Hyperalert or lethargic/coma.
- (x) Hypertonia or hypotonia.
- (xi) Feeding problems.
- (xii) Skin discolouration.
- (xiii) Abnormal EEG and/or seizures.
- (xiv) Exclusion of other identifiable contributing factors.

The key feature indicative of NE is the disturbance in the quality of consciousness; other features may occur alongside this but are not essential for diagnosis⁷⁷.

4.1.2 Aetiology

NE can arise due to a variety of adverse perinatal issues and as such, the term NE does not refer to any one specific aetiology. The term hypoxic-ischaemic encephalopathy (HIE) is widely used in the literature, often interchangeably with NE, to describe cases in which NE was apparently caused by perinatal asphyxia⁵. The terms perinatal asphyxia and birth asphyxia have also been used to indicate cases of HIE. It is important however, to understand the relationship and the differences between NE and HIE. Not all cases of NE are caused by HIE. HIE is in fact a subgroup of NE, and although it plays a role in a large proportion of cases of NE – possibly as high as 50-80%⁷⁸ – it is not the only possible cause. Additionally, it is often difficult to define a single hypoxic-ischaemic event and in many cases, HIE is a secondary event and not the sole cause of the encephalopathy⁷⁹. NE is therefore an umbrella term that refers to the condition of neurological dysfunction – regardless of the cause – and is now the preferred

term of use as it takes into account the multifactorial aetiology. HIE should only be used when hypoxia-ischemia is definitively known as the cause of the encephalopathy.

HIE refers to NE caused by a sentinel hypoxic-ischaemic event that happens immediately before or during labour; 'hypoxia' referring to a shortage of oxygen in the blood and 'ischaemia' referring to decreased blood flow to the brain. This results in two stages of energy failure. The primary energy failure is the immediate result of the hypoxic-ischaemic event and leads to a reduction in ATP, the failure of sodium/potassium pumps, an accumulation of lactate, and in severe cases, necrosis (cell death). This is followed by a 6–48-hour latent period of reperfusion and reoxygenation, which then gives way to the second energy failure, characterised by excitotoxicity, oxidative stress and mitochondrial dysfunction^{80,81}. Risk factors for HIE include antepartum and intrapartum factors, such as gestation ≥ 41 weeks, prolonged membrane rupture, shoulder dystocia, tight nuchal cord, failed vacuum and placental pathology^{82,83}.

As previously noted, HIE is not the only form of NE, and there are several other causes attributable to the condition. These include things such as metabolic disorders, perinatal infection, placental pathology, neonatal stroke/thrombophilia, congenital abnormalities, and genetic disorders. Familial and antenatal maternal risk factors have also been implicated in NE and may include family history of seizures or neurological disorders, socioeconomic status, fertility treatment, maternal hypertension, diabetes, thyroid disease, haemorrhage, or preeclampsia⁸³.

4.1.3 Epidemiology

The reported incidence of NE may vary depending on how the condition is defined, but most studies estimate that it is between 2.0 to 6.0 in every 1000 live births⁷⁶. In an epidemiological review of NE, Kurinczuk et al⁸⁴ suggest figures of 3.0 in every 1000 live births (95%CI 2.7 to 3.3) for NE and 1.5 for HIE (95%CI 1.3 to 1.7). There is no doubt, however, that NE is one of the leading causes of infant morbidity and mortality, and data from 2010 suggest that approximately 1.15 million neonates globally were affected annually by NE, with 287,000

neonates dying from their condition, 233,000 surviving with moderate or severe neurodevelopmental impairment, and 181,000 surviving with mild impairment⁸⁵.

Incidence of NE is reportedly higher for low- and middle-income countries (LIMCs) where antenatal and neonatal intensive care resources may be limited. LIMCs may in fact account for over 95% of cases of NE, with almost three-quarters of all cases of NE occurring in sub-Saharan Africa and South Asia⁸⁵. The proportion of cases of NE due to HIE is also estimated to be higher for LIMCs than in developed countries; approximately 60% of cases compared to 30%⁸⁴. It is suggested that there is a higher incidence of NE amongst nulliparous mothers than mothers who have previously given birth, possibly due to the higher risk of a longer labour⁷⁶. Curiously, NE also seems to affect more males than females, possibly due to sex-specific differences in hormones, apoptosis, and foetal size^{85,86}.

4.1.4 Classifications

The most widely used method of classifying the severity of NE is the Sarnat score⁸⁷. It categorises NE into mild, moderate, and severe based upon a neurological exam of the neonate completed every hour for the first six hours of life. It includes six clinical parameters; four neurological parameters, one autonomic nervous system parameter, and one parameter related to the presence or absence of seizures (see table 1). It is an important standardised clinical tool in evaluating and tracking the neurological status of the neonate and may be used to correlate with MRI findings and developmental outcomes⁸⁸.

	Stage 1 – Mild	Stage 2 – Moderate	Stage 3 - Severe
Level of Consciousness	Hyperalert	Lethargic / Obtunded	Stupor or Coma
Activity	Normal	Decreased	Absent
Neuromuscular Control:			
Muscle Tone	Normal	Mild hypo-/hypertonia	Flaccid/rigid
Posture	Mild distal flexion	Strong distal flexion	Intermittent decerebration
Tendon Reflexes	Overactive	Overactive	Decreased/absent
Complex Reflexes:			

Suck	Weak	Weak/absent	Absent
Moro	Strong, low threshold	Weak, incomplete, high threshold	Absent
Tonic Neck	Slight	Strong	Weak/Absent
Autonomic Nervous System:			
Pupils	Dilated	Constricted	Variable: often unequal, poor light reflex, fixed, dilated
Heart Rate	Tachycardia	Bradycardia	Variable
Respiratory Rate	Regular	Periodic Breathing	Apnoea
Seizure	None	Common; focal/multifocal	Uncommon (excluding decerebration)

Table 1. *The Modified Sarnat Staging for Neonatal Encephalopathy^{87,88}.*

The neonate with mild NE is hyperalert and may show transient behavioural irregularities such as poor feeding, excessive crying, or agitation. In mild NE, the neonate will require minimal support, symptoms typically resolve within 24 hours, and a neurological exam at 3-4 days is often normal^{89,90}. In the mildest cases of NE, symptoms may even go undetected. Children with mild NE tend to be at low risk for adverse outcomes and are often indistinguishable from their typically developing peers in school, however, there is increasing evidence to suggest that this is not always the case, and these children may present with less obvious or visible neurodevelopmental problems⁹¹. The PRIME (Prospective Research in Infants with Mild Encephalopathy) multicentre cohort study²⁷² found that 16% of infants with mild NE had disability at 18–22 months of age, and 40% had a Bayley-III score <85 (mostly affecting the language component), which highlights the need for long-term follow-up of this population.

The neonate with moderate NE is lethargic and hypotonic, with sluggish reflexes, and seizures may occur within the first 24 hours. They will be significantly more unwell than neonates with mild NE and will require more support. It is possible that an initial period of relative well-being may be followed by a sudden deterioration into a more moderate

encephalopathy^{89,90}. These neonates are a more heterogeneous group than the mild neonates and have a less certain prognosis. Although recovery is possible, so is the possibility of significant neurodevelopmental dysfunction or disability.

The neonate with severe NE is noticeably and critically unwell, often unresponsive to physical stimuli or in a coma, with absent reflexes and poor ocular motion. These neonates usually require resuscitation and/or ventilation as well as tube feeding. The mortality rate in severe NE is thought to be around 25-50%, with most deaths occurring within the first few days of life, typically due to multi-organ failure or redirection/withholding of care due to quality-of-life considerations. Those neonates that do survive develop serious long-term complications and/or disabilities⁸⁹.

Another classification system that is also often used is the Thompson score⁹². It is a derivation of the Sarnat score and consists of a neurological examination of nine signs: tone, level of consciousness, fits, posture, Moro, grasp, suck, respiratory pattern, and fontanelle tension. Each sign is given a score of 0-3 on days one, two, and three of life which are then totalled. A score of 1-10 indicates mild NE, a score of 11-14 indicates moderate NE, and a score of 15-22 indicates severe NE. It is argued that this method of scoring provides a more precise description of the encephalopathic neonate than the Sarnat score, as it recognises the possibility of mixed signs within the three categories⁹³. A cut-off score of 7 is used to start therapeutic hypothermia – a treatment used in NE, which is discussed below.

4.2 Therapeutic Hypothermia

Presently, therapeutic hypothermia (TH), or cooling, is the only treatment for NE (due to HIE), which must be started within six hours of birth. TH is a neuroprotective intervention for neonates with moderate or severe NE and has been shown to reduce rates of death and disability in this condition⁹⁴. Although every hospital will have its own neonatal intensive care guidelines, TH is considered the gold standard of care for these neonates, and it involves a specific set of principles that must be followed in order to be effective.

4.2.1 What is Therapeutic Hypothermia?

As mentioned previously, in between the primary and secondary stages of energy failure of HIE, there is a 6–48-hour latent period in which reperfusion and reoxygenation occur. This latent period of recovery provides a therapeutic window of opportunity in which the neonate's metabolic rate can be lowered in order to aid cell recovery, delay cell death, and ameliorate the impact of the second stage of energy failure⁹⁵. TH administered during this time helps to minimize damage to the brain and slows down the progression of NE. It involves lowering the neonate's total body temperature to between 33°C and 34°C over a 72-hour period beginning within six hours of birth, followed by re-warming the neonate to a normothermic temperature over a 6–12-hour period⁷⁶. Cooling is achieved by one of two methods: whole body cooling or selective head cooling. A cooling blanket or cooling cap is fitted around the neonate's body or head that circulates cold water, and the neonate's temperature and vital signs are closely monitored. Re-warming is achieved by gradually increasing the temperature of the cooling device by 0.5°C per hour until normothermia is reached (36.5-37°C)⁹⁴.

Major randomized trials investigating TH have shown that it is effective in reducing mortality and neurodevelopmental delay. One trial showed a reduction in a composite primary outcome of death or major sensorineural disability at two years of age by 15%⁹⁶. Others have shown that cooled infants have an increased rate of survival without neurologic abnormality, reduced risk of cerebral palsy, and improved scores on the Bayley Scales of Infant Development (BSID) and Gross Motor Function Classification System (GMFCS)^{97,98}. It is clear that the benefits of TH outweigh any short-term adverse effects that may occur in neonates with moderate and severe NE, which may include changes in haemodynamic status, fluid and electrolyte balance, and blood gases⁹⁹. However, there is currently little evidence to support the benefits outweighing the risk in cases of mild NE.

4.2.2 Inclusion Criteria

TH is generally administered to neonates with moderate and severe NE who fit the inclusion criteria, however, it has been shown that TH is increasingly being offered to neonates

with mild NE, despite there being little evidence to support this implementation¹⁰⁰. Nevertheless, the inclusion criteria are as follows⁷⁶:

- (i) Term (or late preterm) neonates born at or above 36 weeks gestation and weighing 1800g or above.
- (ii) Acidosis ($\text{pH} < 7.0$) in the umbilical cord or blood sample taken within 60 minutes of birth.
- (iii) Base deficit ≥ -16 mmol/L in the umbilical cord or blood sample taken within 60 minutes of birth.
- (iv) Evidence of an acute perinatal event (e.g., cord prolapse, placental abruption, uterine rupture).
- (v) Apgar score ≤ 5 at 10 minutes OR a minimum of 10 minutes positive-pressure ventilation.
- (vi) Evidence of moderate or severe encephalopathy, demonstrated by the presence of seizures OR a minimum of one sign in three or more of the six categories in the Modified Sarnat Table (see table 1).

Neonates not eligible for TH include those born prematurely or with very low birth weight, neonates with coagulopathy, life-threatening cardiovascular or respiratory abnormalities, and major congenital or genetic abnormalities. Additionally, TH should not be administered if it is unable to be initiated within six hours of birth or if the neonate is moribund¹⁰¹.

4.2.3 Therapeutic Hypothermia in Ireland

In their aggregate report 'Neonatal Therapeutic Hypothermia in Ireland', the National Clinical Programme for Paediatrics and Neonatology in collaboration with the National Perinatal Epidemiology Centre documented that during 2016-2018, 209 neonates were treated with TH (29% severe, 58% moderate, 14% mild)⁷⁶. This equates to approximately 1 in every 900 neonates born in the Republic of Ireland. However, TH is currently only administered in four Irish hospitals: three in Dublin (National Maternity Hospital, Rotunda Hospital, and Coombe Women and Infants University Hospital) and one in Cork (Cork University Maternity Hospital). 40% of neonates requiring TH during this time were born outside of these hospitals

and needed transportation to one of these four centres via the National Neonatal Transport Program. The report also noted an 89% rate of survival amongst the treated neonates (24 deaths). It is crucial, therefore, that NE is quickly and effectively diagnosed, in order to be able to transport the neonate and initiate treatment – all within the six-hour window.

4.3 Neurodevelopmental Outcomes

The adverse neurodevelopmental outcomes seen in NE children at follow-up vary broadly depending on the severity of NE and whether or not the neonate was treated with TH. Some mild cases of NE show normal neurodevelopment at follow-up, whereas the most severe cases may sadly end in the death of the infant or child. For those cases in between, however, there are a broad range of impairments seen that can be cognitive, motor, and behavioural in nature. Some deficits may be detected during the neonatal period, such as severe motor and sensory impairments, however other impairments may take some time until they become apparent. For example, a low development quotient may be detected during the second year, gross and fine motor impairments between two and four years of age, cognitive dysfunction between four and seven years of age, and learning disabilities between seven and nine years of age¹⁰². Generally, all neonates with moderate or severe NE should be assigned to a follow-up programme. Assessments at four to eight months focus on general health, growth, and motor development, and at 12-24 months psychometric assessments are employed to examine cognitive and language development as well as to confirm any motor deficits. Finally, preschool and primary school assessments are also recommended in order to identify children who may require referral to early education programmes⁷⁷.

4.3.1 Sensory

The impact of the brain injury in NE may result in visual or hearing impairments. Damage to the posterior visual pathway may cause cortical visual impairment (CVI). In CVI, the eyes themselves may be unaffected and it is the atypical way the brain processes visual information that causes the visual deficit. Symptoms may include reduced visual acuity, reduced contrast sensitivity, strabismus, abnormal fixation, smooth pursuit and saccadic

movements, refractive errors, and visual field deficits¹⁰³. It is possible, however, that CVI can improve with time and/or intervention. It is estimated that approximately half of NE survivors will experience some form of visual impairment¹⁰⁴. Infants with NE are also at risk of developing sensorineural hearing loss, however, this is more likely to be a transient loss rather than a permanent impairment. It has been suggested that approximately one-third of neonates with NE will experience transient hearing loss, but by one month of age, only about 10% of these infants will show signs of a persisting impairment¹⁰⁵.

4.3.2 Seizures

Seizures are a common feature of NE, and it is estimated up to 65% of neonates with moderate to severe NE will have neonatal seizures¹⁰⁶. They generally occur on the first day of life and can be difficult to control with anticonvulsants⁷⁷. Occasionally, neonatal seizures may be visibly detected in the form of jerking movements, stiff limbs, pedalling movements, or facial movements. However, it is very often the case that neonatal seizures in NE are subclinical, subtle, and show no observable clinical signs¹⁰⁷. The gold standard for detecting neonatal seizures, therefore, is continuous electroencephalography (EEG) monitoring, which measures the electrical activity of the brain. Approximately 10% of all infants with NE and 25% of those that have neonatal seizures will go on to develop epilepsy as children; a neurological disorder of recurrent seizures¹⁰⁸. It has also been found that seizure activity increases during rewarming, which is associated with a higher risk of death or disability at 2 year follow-up²⁷³.

4.3.3 Motor

The most common motor dysfunction that develops as a result of NE is cerebral palsy (CP), which develops in approximately 13% of encephalopathic infants⁷⁷. It is estimated that one-quarter of cases of CP in term-born children can be attributable to NE^{109,110}. CP is the name given for a group of lifelong movement disorders that affect coordination, posture, muscle tone, fine motor skills, gait, and speech. CP is an extremely diverse condition, with the severity of symptoms and subsequent quality of life varying widely amongst individuals with the condition. Although there is no cure for CP, supportive treatments can help to manage the

condition. The Gross Motor Function Classification System (GMFCS)¹¹¹ is a standardised classification system for CP often used in follow-up assessments of children with NE. It consists of five levels of increasing severity that are used to assess the child's motor function and to determine what mobility aids they may require. Children at level I can walk independently without limitations, but speed, balance, and coordination are somewhat limited. Children at levels II and III can walk with physical assistance, and children at level IV require powered mobility in most settings. Children at level V are the most severely impaired and require a wheelchair in all settings.

Most individuals with CP also have at least one other comorbidity (about 95%), be it medical, neurological, or mental/behavioural, and about a third of individuals have at least one comorbidity within all three categories¹¹². Evidence does suggest, however, that children who develop CP as a result of NE have a greater burden of disability than those who have CP that is not a result of NE¹¹³.

4.3.4 Cognition and Language

Cognitive and language outcomes vary broadly with the severity of NE. A large proportion of neonates with mild NE will go on to show normal infant development, and age-appropriate levels of intellectual ability and educational performance at school age, comparable to their healthy peers. On the other end of the scale, almost all neonates with severe NE or with CP due to NE will likely go on to show impairment in every cognitive domain, with low levels of functioning and low school readiness¹¹⁴. It is the children with moderate NE without CP that show a very heterogeneous outcome and it is estimated that between 25% and 63% of these infants will go on to demonstrate impairment in their general cognitive abilities¹¹⁵. In general, children with moderate NE have significantly lower intelligence than children with mild NE and their healthy peers, however, scores are still generally in the average range. Additionally, moderate NE children may be equally school-ready at 5.5 years, however they perform less well in domains such as reading, spelling, arithmetic, receptive vocabulary, and some sub-domains of memory and learning such as narrative memory and sentence repetition¹¹⁴. It is possible, therefore, that these children fall behind in school but are not identified on standardised testing as requiring educational assistance.

There are several standardised assessments that may be used to measure the development of cognition and language in infants and children. The standard follow-up assessment for infants with NE at 18-24 months here in Ireland is the Bayley Scales of Infant and Toddler Development (BSID)¹¹⁶, which has been validated in the Irish population²⁷⁴. The BSID is a clinical assessment that examines the developmental functioning of infants and toddlers in five categories: cognitive, language, motor, socio-emotional, and adaptive behaviour. The first three categories are scored through direct observation of the infant, who is presented with a series of play-based test materials and an examiner scores the infant based on their response/behaviour. The cognitive scale assesses skills such as sensorimotor, exploration, manipulation, object constancy and relatedness, and memory, whilst the language and motor scales are subdivided into receptive and expressive communication, and fine and gross motor skills, respectively. The latter two categories are scored through a questionnaire completed by the infant's primary caregiver, which assesses the development of the infant's relationships and interactions, and how the infant applies their skills to daily living. Other assessments that may be used include the Griffiths Mental Development Scales (GMDS)¹¹⁷; a similar standardised test to the BSID that is used for children up to eight years of age, and the Wechsler Intelligence Scales for preschool and school-age children^{118,119}. These tests all take approximately one hour to administer, and the scores obtained may then be compared to a standardised norm from typically developing children.

4.3.5 Behavioural

Research into the behavioural sequelae of NE is far more limited, and it may often be difficult to establish whether a behavioural problem is a direct result of NE or if it stems from the limitations and complications that NE gives rise to. For example, it is estimated that about a third of children with CP also struggle with behavioural difficulties¹²⁰. Nevertheless, it has been suggested that school-age children with NE show higher rates of problematic behaviour such as poor social integration, anxious/depressive behaviours, inattention, and hyperactivity¹²¹. One study has even reported unexpectedly high rates of autism spectrum disorder among children who had experienced NE compared to healthy controls¹²², however, there is very little other evidence to support this.

Chapter 5: Neuroimaging in Neonatal Encephalopathy

“This important group of patients deserve the extra effort and time involved in a technology which continues to evolve and offers diagnostic information not available with any other imaging techniques, and most importantly, without known biological hazard”.

- *Jacqueline M Pennock*¹²³

5.1 Typical Imaging Findings in Neonatal Encephalopathy

MRI is widely used in the assessment of NE in order to provide information about the nature, location, timing, and severity of the injury, and to guide clinical decision making. One study reported that over 90% of term infants with NE had evidence of perinatally acquired lesions on their MRI¹²⁴. MRI is also used to examine the effects of any treatment intervention and is an effective prognostic tool in predicting developmental outcome based on the type of injury. Although brain injury in NE is complex, injury can nevertheless be distinguished into two primary patterns of injury: the basal ganglia-thalamic (BGT) and the watershed (WS) patterns of injury. Different MRI sequences may be at their most effective in visualising these injuries at different times, with conventional MRI showing abnormalities after the first few days, and DWI even within the first few hours¹²⁵. This chapter will focus on these patterns of injury as seen on T₁-, T₂-weighted, and diffusion imaging, and how these injuries can be quantified using standardised scoring systems. The prognostic ability of MRI will be evaluated, and finally, the first study of this thesis is presented: a systematic review of DTI in NE.

5.1.1 Basal Ganglia-Thalamic

The BGT pattern of injury is most often seen in acute, severe NE¹²⁶. The first structures to sustain injury are the bilateral central deep grey nuclei of the basal ganglia and the thalamus (particularly the ventrolateral thalami and posterior putamina). At birth, these structures are actively myelinating and are highly susceptible to injury due to their high metabolic rate and

demand for energy. This is followed by injury to the perirolandic cortex, and occasionally the hippocampus and brainstem are also affected^{72,127}. On a T₁-weighted image, an abnormally increased signal intensity can be seen in the basal ganglia and the thalamus. Abnormal signal intensity in the posterior limb of the internal capsule (PLIC) is also a good indicator of a BGT injury. The PLIC is a white matter tract that separates the thalamus and the lenticular nucleus of the basal ganglia. On a T₁-weighted image of a healthy neonate, there is an increased signal intensity in the PLIC, and a corresponding decreased signal intensity on a T₂-weighted image, which reflects normal myelination. In the NE neonate with a BGT injury, however, there is a loss of this signal intensity on a T₁-weighted image and an absence of low signal intensity on a T₂-weighted image¹²³. It is important to note that T₁- and T₂-weighted images obtained on the first day of life often appear normal, and it is usually not until the end of the first week that these images are most diagnostically useful¹²⁸. Diffusion abnormalities, however, can usually be seen within the first 24 hours and can appear overtly abnormal even when structural images appear only mildly abnormal or even normal¹²⁹. Restricted diffusion is generally maximal at around the third day of life and can be seen as increased signal intensity on DWI in the BGT region⁷². More information about the injury may be obtained through apparent diffusion coefficient (ADC) and FA measurements. Evidence suggests that reduced ADC values can be seen in some neonates with severe BGT injury, and reduced FA values in both moderate and severe BGT injury¹³⁰.

5.1.2 Watershed

The other pattern of injury seen in NE is the WS distribution, which is associated with less severe and more prolonged injury, and hypotension. The parasagittal vascular zones between the major cerebral arteries are affected, primarily in the subcortical white matter, but cortical grey matter may also be affected in more severe cases (figure 7). WS injury may be bilateral or unilateral, and anterior and/or posterior¹²⁷. WS abnormalities can be quite subtle on T₁- and T₂-weighted images, however, there are two types of abnormality that may be seen on conventional MRI. The first is cortical highlighting, with abnormal areas of the cortex showing increased signal intensity seen on a T₁-weighted image and decreased signal intensity on a T₂-weighted image. The second is a loss of differentiation between grey and

white matter¹²³. The WS pattern of injury, however, is best seen on DWI, which highlights the areas of restricted diffusion as increased signal intensity. Similarly to BGT injury, evidence suggests that reduced ADC values can be seen in neonates with severe WS injury, and reduced FA values in both moderate and severe WS injury, with a low FA possibly reflecting a breakdown in white matter organisation¹³⁰.

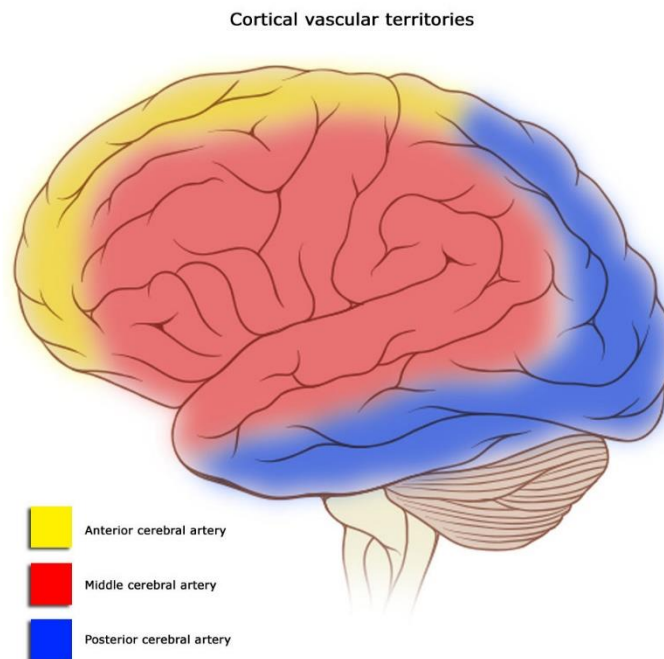


Figure 7. *The cortical vascular territories supplied by the anterior, middle, and posterior cerebral arteries that may be affected in watershed injury (derivative work: Frank Gaillard (talk) Brain_stem_normal_human.svg: Patrick J. Lynch, medical illustrator).*

5.1.3 Pseudonormalisation

DWI is the most sensitive MRI sequence when imaging the NE neonate within the first few days following the injury, as T_1 - and T_2 -weighted images may produce false negatives during this period. However, the phenomenon of pseudonormalisation is an extremely important factor when considering the optimal timing of DWI, which occurs at approximately one week of age for normothermic infants and between approximately 11-12 days for infants treated with TH^{127,131}. During this time, the extent of the injury may be underestimated by DWI. Within the first few hours, ADC is initially decreased in NE neonates, but by the end of

the first week of life, ADC increases to reach apparently normal levels. However, this is a false negative that does not reflect a resolution to the brain injury. Abnormally low FA values persist during this period of pseudonormalisation and may even continue to decrease¹³⁰. Ideally, neonates should be imaged between days 3-7 of life, for diffusion changes to be fully assessed in NE.

5.1.4 MRI Scoring Systems in Neonatal Encephalopathy

Quantifying the nature and severity of brain injury in NE is crucial in order to objectively assess the condition of the neonate, direct clinical care, and predict outcome. There are several validated MRI scoring systems including those of Barkovich et al¹³², Rutherford et al¹³³, the National Institute of Child Health and Human Development Neonatal Research Network (NICHD NRN)¹³⁴, and Weeke et al¹³⁵. The most established and widely used is the Barkovich scoring system¹³², which uses T₁- and T₂-weighted images to classify injury in terms of BGT and WS injury. Four sets of scores are provided based on these injuries. The first relates to BGT injury, in which a score is given from 0-4 indicating increasingly extensive damage to the basal ganglia and thalami. Similarly, WS injury is given a score from 0-5, with lower scores indicating less extensive damage and higher score indicating more extensive damage. The severity of the injury is scored as mild when the damage is focal and small and is scored more severely when the damage extends to multiple regions. The third and fourth set of scores are two different combination scores, one score that includes components of both injuries and another simple summation of the BGT and WS scores. A fifth score is also given to indicate the degree of enhancement seen on the post-contrast images. The complete Barkovich scoring system can be seen in table 2.

Score	Finding
Basal Ganglia (BG)	
0 =	Normal or isolated focal cortical infarct
1 =	Abnormal signal in thalamus
2 =	Abnormal signal in thalamus and lentiform nucleus
3 =	Abnormal signal in thalamus, lentiform nucleus, and perirolandic cortex
4 =	More extensive involvement
Watershed	

0 =	Normal
1 =	Single focal infarction
2 =	Abnormal signal in anterior or posterior watershed white matter
3 =	Abnormal signal in anterior or posterior watershed cortex and white matter
4 =	Abnormal signal in both anterior and posterior watershed zones
5 =	More extensive cortical involvement
Basal Ganglia/Watershed (BG/W)	
0 =	Normal
1 =	Abnormal signal in basal ganglia or thalamus
2 =	Abnormal signal in cortex
3 =	Abnormal signal in cortex and basal nuclei (basal ganglia or thalami)
4 =	Abnormal signal in entire cortex and basal nuclei
Summation (S)	
Arithmetic sum of BG and W	
Enhancement (E)	
0 =	No enhancement
1 =	Enhancement in white matter only
2 =	Enhancement in deep grey matter nuclei
3 =	Enhancement in cerebral cortex
4 =	Enhancement in cortex and deep grey matter or white matter

Table 2. *The Barkovich Scoring System (from Barkovich et al¹³²).*

The Rutherford scoring system¹³³ is very similar to the above, however, unlike Barkovich, it recognises the importance of the signal intensity in the PLIC. In this scoring system, each neonate is given a numerical score of increasingly extensive injury in four categories. The PLIC is scored from 0-2 based on abnormal signal intensity unilaterally or bilaterally. The BGT is scored from 0-3 based on increasingly widespread damage. Whereas Barkovich used a single WS score, Rutherford's final two categories separate this into a white matter score and a cortical involvement score, which are scored from 0-3 based upon increasingly exaggerated T_1 and T_2 , and abnormal signal intensity/cortical highlighting, respectively. Similarly, the NICHD NRN¹³⁴ uses conventional MRI to assess injury in the BGT, WS and PLIC, with scores described as following: 0 – normal; 1A – minimal cerebral lesions only; 1B – more extensive cerebral lesions without other involvement; 2A – basal ganglia, thalamic, internal capsule lesions only; 2B - basal ganglia, thalamic, internal capsule, and cerebral lesions; 3 – cerebral hemispheric devastation.

The most recent, and comprehensive, scoring system is that of Weeke and colleagues¹³⁵. Importantly, unlike the other two methods, Weeke also includes DWI as part of the assessment, acknowledging that it is the most reliable MRI sequence in assessing neonates with NE and can be used earlier than conventional MRI. Scores from 0-2 are obtained in 3 areas: grey matter, white matter/cortex, and the cerebellum. Scores are based on whether the injury is focal or extensive, and unilateral or bilateral. Six regions of grey matter are assessed: the thalamus, basal ganglia, PLIC (this is a white matter structure but indicates damage to the deep grey structures in cases of NE), brainstem, perirolandic cortex, and hippocampus. White matter/cortex is also assessed in six areas: the cortex, cerebral white matter, optic radiations, corpus callosum, punctate white matter lesions, and parenchymal haemorrhage. In addition to conventional MRI and DWI, Weeke also includes MR spectroscopy to score N-acetyl aspartate (NAA) and lactate. The complete Weeke scoring system can be seen in table 3.

	Items	Sequence used to assess injury	Degree		
Grey matter			0	1	2
1	Thalamus abnormal SI or diffusion restriction	T1/T2 DWI	No	Focal (<50%)	Extensive (≥50%)
	Specify location			Unilateral	Bilateral
2	Basal ganglia abnormal SI or diffusion restriction	T1/T2 DWI	No	Focal (<50%)	Extensive (≥50%)
	Specify location			Unilateral	Bilateral
3	PLIC myelination or diffusion restriction	T1/T2 DWI	Normal or no diffusion restriction	Equivocal/partially myelinated or partial (<50%) diffusion restriction	Absent myelination or extensive (≥50%) diffusion restriction
	Specify location			Unilateral	Bilateral
4	Brainstem (peduncles) abnormal SI or diffusion restriction	T1/T2 DWI	No	Focal (<50%)	Extensive (≥50%)
	Specify location			Unilateral	Bilateral
5	Perirolandic cortex diffusion restriction	DWI	No	Mild	Clear

	Specify location			Unilateral	Bilateral
6	Hippocampus diffusion restriction	DWI	No	Yes	
	Specify location			Unilateral	Bilateral
Grey matter subscore					
	Basal ganglia NAA	1H-MRS	Normal	Reduced	
	Basal ganglia lactate	1H-MRS	Absent	Increased	
Grey matter subscore (including 1H-MRS)					
White matter/cortex			0	1	2
1	Cortex abnormal SI or diffusion restriction not being perirolandic cortex	T1/T2 DWI	No	Focal (1 lobe)	Extensive (>1 lobe)
	Specify location			Unilateral	Bilateral
2	White matter increased SI or diffusion restriction not being PWML	T1/T2 DWI	No	Focal (1 lobe)	Extensive (>1 lobe)
	Specify location			Unilateral	Bilateral
3	PWML	T1/T2, DWI, SWI	No	<6	≥6
	Specify location			Unilateral	Bilateral
4	Haemorrhage not being PWML	T1/T2, SWI	No	Single haemorrhage <1.5 cm	≥1.5 cm or multiple haemorrhages
	Specify location			Unilateral	Bilateral
5	Optic radiation diffusion restriction	DWI	No	Mild	Clear
	Specify location			Unilateral	Bilateral
6	Corpus callosum diffusion restriction	DWI	No	Yes	
White matter subscore					
Cerebellum			0	1	2
1	Cerebellum abnormal SI or diffusion restriction	T1/T2 DWI	No	Focal (<0.5 cm)	Extensive (≥0.5 cm or multiple lesions)
	Specify location			Unilateral	Bilateral

2	Cerebellar haemorrhage	T1/T2, SWI	No	Single haemorrhage <0.5 cm	≥0.5 cm or multiple haemorrhages
	Specify location			Unilateral	Bilateral
Cerebellum subscore					
Additional			0	1	2
1	IVH	T1/T2, SWI	No	Yes	
2	SDH	T1/T2	No	Yes	
3	CSVT	T1/T2, MRV	No	Yes	
Additional subscore					
Total score (grey matter + white matter + cerebellum + additional score)					

Table 3. *The Weeke Scoring System (from Weeke et al¹³⁵). Abbreviations: CSVT, cerebral sinovenous thrombosis; IVH, intraventricular haemorrhage; MRV, magnetic resonance venography; PLIC, posterior limb of the internal capsule; PWML, punctate white matter lesions; SDH, subdural haemorrhage; SI, signal intensity; SWI, susceptibility weighted imaging.*

5.2 Prognostic Ability of Neuroimaging in Neonatal Encephalopathy

An early MRI scan has proven to be the single best predictor of outcome in infants with NE^{104,133,135-137} and different patterns of injury are associated with different neurodevelopmental outcomes. Generally, BGT injury is associated with more severely impaired motor and cognitive outcomes, while WS injury is more often associated with less severe cognitive impairment without motor deficits¹²⁶. A normal MRI is also often predictive of normal outcome¹³⁴. Additionally, TH has been associated with fewer grey and white matter abnormalities, and higher rates of normal MRI scans have been seen in treated neonates than in those who were not cooled. Critically, the accuracy of MRI to predict neurological impairment does not seem to be affected by TH, therefore MRI can be performed at 3-7 days of life, following cooling and rewarming¹³³.

5.2.1 Basal Ganglia-Thalamic

The basal ganglia are a set of interconnected subcortical grey matter nuclei within the forebrain, which include the globus pallidus, caudate, putamen, substantia nigra, and the subthalamic nucleus. Lateral to the basal ganglia is the thalamus, which contains the ventral and dorsal thalami (figure 8¹³⁸). These structures all play a key role in motor control, cognitive function, and motivational and emotional behaviour^{139,140}. BGT damage in NE frequently leads to severe disability, particularly CP, and sometimes death. A study of infants with BGT injury showed that early MRI can be used to predict the severity of motor outcomes and death in these infants, with the severity of BGT lesions being associated with the severity of motor outcome¹⁴¹. In this study of 175 infants, 49 died (28%) and of the 126 that survived, 89 developed CP (71%). Another study by the same group found that while 40% of children with mild BGT injury showed no speech and language difficulties, over 90% of those with severe BGT injury had some degree of impairment in this area, with most unable to communicate by speech¹⁰⁴. They also found that 10%, 50% and 90% of infants with mild, moderate, and severe BGT injury, respectively, had feeding problems, and 30-40% of all survivors developed epilepsy during childhood. Recent studies in radiomics have also used MRI-based texture analysis of the basal ganglia and thalami to accurately diagnose moderate-to-severe NE in neonates²⁷⁵. Radiomics is a relatively new approach that is being used more widely to identify microstructural changes that may be invisible to the naked eye and may therefore be a promising new tool in the diagnosis of BGT injury.

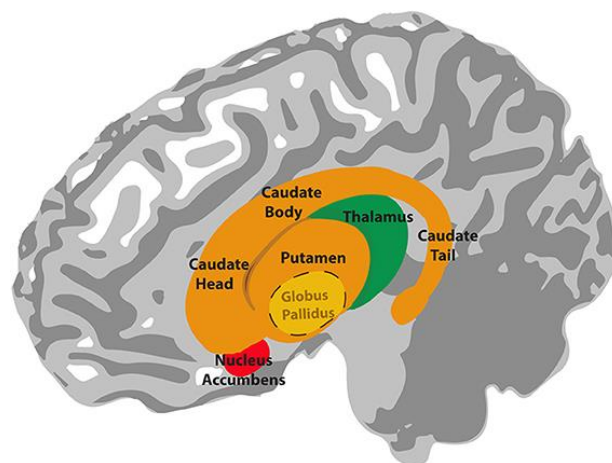


Figure 8. *The structures of the basal ganglia and thalamus (from Lim et al, 2014¹³⁸).*

An accurate predictor of neurodevelopmental outcome in BGT injury is abnormal signal intensity in the PLIC. One study of 73 neonates found that all of those who showed abnormal PLIC intensity had adverse neurodevelopmental outcome, whereas 88% of those with normal signal intensity had a normal outcome¹³⁶. Another study found that an abnormal PLIC intensity was the best predictor of an inability to walk independently by two years of age¹⁴¹. All children with normal PLIC intensity were able to walk independently at this age, but only 12% of those with an abnormal PLIC intensity were able to do so, most of whom started walking after 18 months. Finally, a study examining diffusion measures in the PLIC found that ADC values in the PLIC were associated with survival and neuromotor outcome¹⁴².

5.2.2 Watershed

Severe disability, especially motor impairments, is less common in WS injury, but other neurodevelopmental impairments may be seen. In a study of 84 neonates with selective WM/cortical injury, only 6% developed CP, all of whom could walk independently, there were no infants with severe motor impairments, and no infants died¹⁴³. However, there was a significant prevalence of global developmental delay, communication and behavioural problems, seizures, and visual impairment in these infants at two years of age, which were more prevalent the more severe the WS injury.

It is often the case that infants with WS injury are seen to have a normal outcome at a 12–18-month assessment, however, evidence suggests that these infants often show cognitive impairment at 30 months that may previously have been undetected. It was found that 18% of infants with WS injury had a mental development index (MDI) of less than 70 at 30 months, and MDI was also significantly lower in those infants when assessed at 30 months compared to 12 months¹²⁶. WS injury also seems to be associated with language impairments, and further studies by the same group found that WS brain injury correlates with verbal IQ at four years of age¹⁴⁴, and lower overall cognitive ability which may underlie early language difficulties²⁷⁶. Some suggest, however, that outcome for infants with a predominately WS injury depends upon the extent to which the basal ganglia and thalami are involved, rather than the severity of the WS injury. In a study of 22 neonates whose DWI abnormalities showed

a WS pattern of injury, it was found that 90% of infants with isolated WS injury had favourable developmental outcomes, however poor head growth was observed in these infants¹⁴⁵.

5.4 Systematic Review: Diffusion Tensor Imaging in Neonatal Encephalopathy

The content of this section is part of a systematic review that was published in *Archives of Disease in Childhood* (Dibble et al, 2020), and is the first study presented in this thesis.

5.4.1 Abstract

Background and Objective

Diffusion tensor imaging (DTI) during the first few days of life can be used to assess brain injury in neonates with neonatal encephalopathy for outcome prediction. The goal of this review was to identify specific white matter tracts of interest that can be quantified by DTI as being altered in neonates with this condition, and to investigate its potential prognostic ability.

Methods

Searches of Medline and the Cochrane Database of Systematic Reviews were conducted to identify studies with diffusion data collected in term-born neonates with neonatal encephalopathy.

Results

Nineteen studies were included which described restricted diffusion in encephalopathic neonates as compared to healthy controls, with the posterior limb of the internal capsule and the genu and splenium of the corpus callosum identified as particular regions of interest. Restricted diffusion was related to adverse outcomes in the studies that conducted a follow-up of these infants.

Discussion

Obtaining diffusion measures in these key white matter tracts early in life before pseudonormalization can occur can not only identify the extent of the damage but can be used to examine the effectiveness of treatment and to predict neurodevelopmental outcome.

5.4.2 Background and Objective

Neonatal encephalopathy (NE) is a heterogeneous clinical syndrome of disordered neurological function in the term-born neonate. It is characterised by “subnormal level of consciousness or seizures, and often accompanied by difficulty with initiating and maintaining respiration and depression of tone and reflexes”¹. Affecting approximately two to six in every 1000 live births⁷⁶, NE is one of the leading causes of infant morbidity and mortality in late preterm and term neonates. The behavioural, motor, and cognitive consequences of NE are generally associated with the severity of the brain injury, and can vary significantly between cases of mild, moderate, and severe NE. Severity of NE is most frequently graded using the Sarnat staging⁸⁷. Moderate-severe NE is associated with adverse outcomes (long-term morbidity and mortality) at a rate of approximately 50%. Survivors of NE are at risk of several long-term neurodevelopmental impairments, which may include cerebral palsy, intellectual disability, impairment of language skills or working memory, and problematic behaviour¹⁴⁶. Previously it was thought that there were minimal sequelae for neonates with mild NE, however recent studies suggest that mild NE is also associated with abnormal MRI findings and abnormal neurodevelopmental outcome¹⁴⁷.

NE can arise due to a variety of adverse perinatal issues, such as hypoxia-ischaemia, metabolic infectious and genetic disorders. As such, the term NE does not refer to any one specific aetiology. The term hypoxic-ischaemic encephalopathy (HIE) has often been used in the literature to describe cases in which NE was apparently caused by perinatal asphyxia¹⁴⁷. NE is now the preferred term of use as it takes into account the multifactorial aetiology and will be used for this review - which will focus on the predictive value of DTI in NE due to perinatal asphyxia – however, studies describing the condition as HIE have also been taken into account.

Magnetic Resonance Imaging (MRI) is a widely used tool in the assessment of NE, providing information about the nature, location, timing, and severity of the injury, and guiding clinical decision making. Several standardised scoring systems have been developed for the assessment of NE. The most widely known and used is the Barkovich scoring system¹³², in which T₁- and T₂-weighted images are assessed to grade the damage to the brain in infants with NE in terms of basal ganglia/thalamus injury, and watershed cortical injury. Recent MRI scoring systems, such as that of Weeke and colleagues¹³⁵, now also include diffusion-weighted imaging (DWI) as part of their assessment, which has been shown to be an effective predictor of outcome in NE¹⁴⁸. DWI examines the integrity of white matter in the brain by utilizing the anisotropy of water diffusion, and the three-dimensional shape of this diffusion is analysed through diffusion tensor imaging (DTI). DWI and DTI have an advantage over T₁- and T₂-weighted imaging, which may underestimate or not yet show any abnormalities in the first week of life¹⁴⁹, as diffusion abnormalities can be seen within the first few hours of life and extend further over the next few days¹²⁵, and so infants can be scanned early, in the critical period where decisions are to be made regarding treatment, and in certain situations redirection of care. At present, the only intervention for infants with NE is therapeutic hypothermia, a neuroprotective intervention that needs to be started within six hours of birth to have proven benefit, with a number needed to treat of seven to eight to improve one infant's outcome¹⁵⁰.

The purpose of this review was to examine current DTI findings in infants with NE, to determine which regions of white matter are most commonly affected, and how this type of injury may relate to neurodevelopmental outcome.

5.4.3 Methods

I performed a systematic review of the literature using the guidelines from the Meta-analysis of Observational Studies in Epidemiology (MOOSE)¹⁵¹ and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statements¹⁵². Medline (PubMed) and the Cochrane Database of Systematic Reviews were searched using combinations of the terms (neonatal encephalopathy), (hypoxic ischaemic encephalopathy) and (diffusion tensor imaging). References of included studies were also examined, and duplicates were eliminated. Figure 9 shows the literature search and selection process using the PRISMA flowchart.

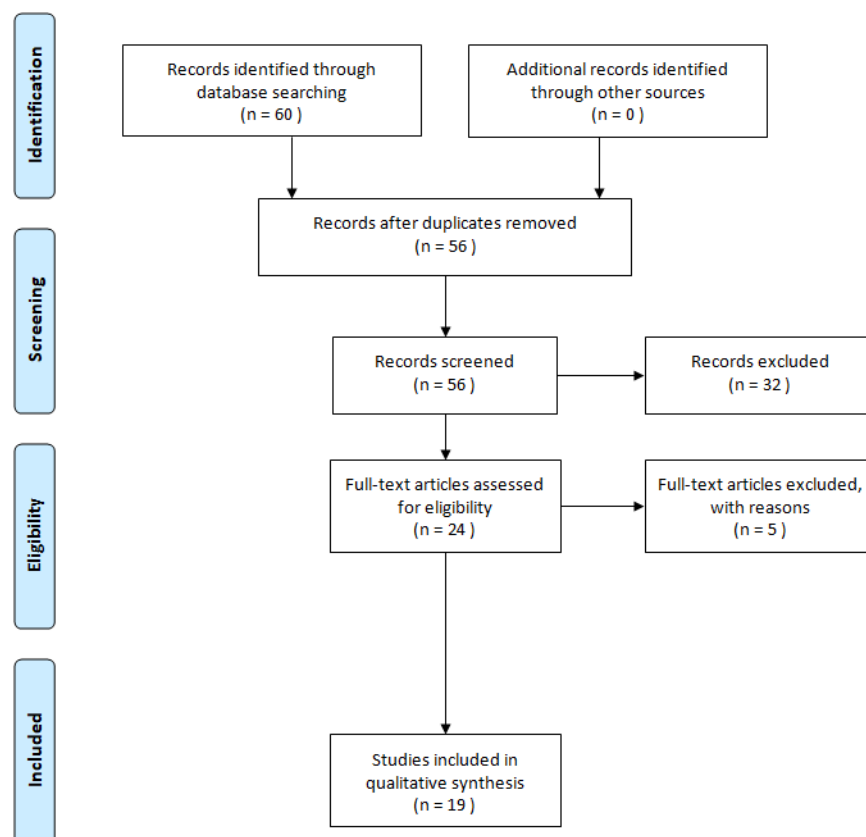


Figure 9. PRISMA flowchart of the search and selection process.

Studies were included based on the following inclusion criteria: (1) neonates with clinically diagnosed neonatal hypoxic-ischaemic encephalopathy or neonatal encephalopathy, (2) neonates born near or at term (≥ 35 weeks gestation), and (3) diffusion-tensor imaging was obtained along with the conventional MRI. Studies were excluded if there was insufficient detail/information, the neonate was born prematurely (< 35 weeks gestation), neonates were

scanned after one month of life, animal models were used, or if grey matter alone was studied. The review included studies with infants with mild, moderate, or severe NE, and that were either untreated or treated with TH. Studies were also assessed for quality and bias with the application of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement¹⁵³ to selected articles.

Data extracted from the studies included population characteristics, imaging protocols, definition of NE, results of any conventional MRI findings or scoring criteria, diffusion metrics for white matter, and any recorded outcome assessments. These variables were then compiled into a summarising table (table 4).

We aimed to define and quantify the specific white matter tracts, or regions of interest (ROIs), that seem to be implicated in NE. By using the JHU-ICBM DTI-81 white matter labels¹⁵⁴ as a starting point, we calculated the number of times each of these ROIs was mentioned in the results section of these studies as having altered diffusion measures. We then visualised the results by creating a greyscale image using FSLeves (<http://www.fmrib.ox.ac.uk/fsl>). Using FSL's FMRIB58 FA image as a template, we placed the JHU-ICBM DTI-81 white matter labels atlas as an overlay, selecting the relevant tracts and removing the tracts that were not part of the results in the literature review. The relative weighting of the tracts were a function of the number of times they appeared in the literature review.

Authors	Definition / Criteria	Groups	Age at Scan	N = Neonates with DWI Scan	Metrics	Conventional MRI Findings / Scoring Criteria	Outcomes	Whole Brain / ROI	Reported Findings
Porter et al (2010)	- Born at 36 weeks GA or after. - Apgar score of 5 or less or continued need for resuscitation within 10 mins of birth OR acidosis within 60 mins after birth. - Moderate to severe encephalopathy (lethargy, stupor or coma). - One or more of: hypotonia, abnormal reflexes, absent/weak suck, clinical seizures. - Abnormal EEG activity of at least 30 mins.	Untreated HIE & Cooled HIE & Healthy Term	1-21 days	10 / 10 / 8	FA	-	-	Whole Brain – TBSS	- Compared with the control group FA was significantly reduced not only in several white matter tracts in the noncooled infants but also in the internal capsule in the cooled group. - Noncooled infants had significantly lower FA than the cooled treated infants, indicating more extensive damage, in the anterior and posterior limbs of the internal capsule, the corpus callosum, and optic radiations.

Ly et al (2015)	-	HIE & Healthy Term	2-10 days	12 / 11	FA	-	-	Whole Brain – TBSS & ROI – CCGenu, CCSplen, CSTs, left and right temporal and periventricular areas.	- FA values from manual ROI and TBSS were strongly correlated. Both methods found decreased FA in 7 ROIs for HIE infants - left and right periventricular regions, splenium, genu, left and right CSTs, left temporal tract, and right temporal tract.
Lally et al (2014)	- Born at 36 weeks GA or after. - Resuscitation at birth and/or Apgar score of 5 or less within 5 mins of birth, and Thompson encephalopathy score of less than 6 within 6 hours of birth.	NE (Sarnat graded)	Within 3 weeks	31	FA	- 40 had WM injury. - 31 had cortex abnormalities. - 12 had BGT injury. - 5 had absence of high signal in the PLIC.	- 38 seen for follow-up. - Abnormal outcome in 16/38 (33% of mild, 50% of moderate, 100% of severe). - 3 had cerebral palsy. - 4 had Bayley-III <85. - 1 had abnormal vision. - 9 had a fall in occipital-frontal circumference. - 3 had ongoing need for anti-epileptics.	Whole Brain – TBSS	- Abnormalities were frequently observed in white matter (n=40, 91%) and cortex (n=31, 70%) while only 12 (27%) had abnormal basal ganglia/thalami. - Those infants with moderate/severe encephalopathy had globally reduced FA compared with the rest of the cohort - Reduced white matter FA was associated with Sarnat stage, deep grey nuclear injury, and MR spectroscopy N-acetylaspartate/choline, but not early Thompson scores. - Of the surviving babies, 42% had adverse neurodevelopmental outcomes.

Tusor et al (2012)	- Born at 36 weeks GA or after. - Apgar score of 5 or less or continued need for resuscitation within 10 mins of birth OR acidosis within 60 mins after birth. - Moderate to severe encephalopathy (lethargy, stupor or coma). - One or more of: hypotonia, abnormal reflexes, absent or weak suck, clinical seizures. - Abnormal EEG background for min 30 mins.	HIE (fulfilling criteria for therapeutic hypothermia)	Within 3 weeks	43	FA	- 27 had BGT injury (12 severe). - 20 had PLIC abnormalities. - WM abnormalities present in all but 2. - 21 had cortex abnormalities.	- 18 had unfavourable outcomes, including 9 who died. - 6 had GMDS-R (DQ) scores < 2 SD below the mean; 2 of which developed cerebral palsy (GMFCS level III-IV) and 2 were diagnosed with cerebral palsy (GMFCS level V). - Lower FA in infants that went on to have unfavourable outcomes. - Correlation between locomotor function and FA values in the CC and CSTs. - Correlation between DQ scores subscales and FA values in the fornix, cingulum and uncinate fasciculus.	Whole Brain – TBSS	- Significantly lower FA values were seen in infants who went on to have unfavourable outcomes as compared with those who had favourable outcomes. - These lower values of FA were seen in several regions of the brain, including the right centrum semiovale, splenium, isthmus and genu of the corpus callosum (CC), anterior limb of the internal capsule, PLIC, external capsules, optic radiations, cerebral peduncles, fornix, cingulum, and inferior longitudinal fasciculus. - The GMDS-R showed a significant linear correlation between FA values and developmental quotient (DQ) and all its component subscale scores.
Seo et al (2017)	- Apgar score less than 5 at both 1 and 5 mins after birth, or umbilical cord, arterial or capillary pH less than 7.0, or base deficit of at least 16mM within 1 hour after birth.	HIE & Healthy Term	36-44 weeks PCA	27 / 35	FA, AD, RD	- 20 had normal structural MRI. - 2 had BGT injury. - 2 had watershed without BGT injury. - 3 had WM abnormalities with hypo-intensity in the CC genu and splen.	- 24 seen for follow-up. - 18 normal. - 6 had an abnormal tone, motor problems, or developmental delay (GMDS-R).	Whole Brain – TBSS	- A group difference in the FA and RD between the normal and HIE was seen in the AC, ALIC, CS, GCC, IFO, right ILF, PLIC, SLF and SCC.

Gao et al (2012)	- Evidence of foetal distress (heart rate abnormalities or meconium-stained amniotic fluid) or neonatal distress (umbilical cord pH less than 7.2 and/or Apgar score less than 7 within 1 min of birth). - Clinical signs of NE in first 72 hours after delivery (abnormal consciousness, abnormal muscular tension, weak/absent primary reflexes, abnormal MR).	Mild HIE & Healthy Term	Within 28 days	24 / 17	FA, λ_1 , λ_2 , λ_3	- Mild HIE injuries were presented as punctate WM injury with hyperintensity in T1WIs and hypointensity in T2WIs and watershed WM injury with hypointensity in T1WIs and hyperintensity in T2WIs.	-	Whole Brain – TBSS	- We found decreased FA in CP, PLIC and CR in all injured neonates. - Decreased FA and increased λ_2, λ_3 in CST, GCC, EC and SCC in mild HI neonates. - Regions with FA decreases were accompanied by increased λ_2 and λ_3 (i.e., increased radial diffusivity), while the λ_1, which contributes to axial diffusivity and reflects the number and diameter of axons, exhibit no obvious changes. - These findings suggested that such mild HIE white matter injury mainly led to deficits or delays in myelination, instead of damage on axons.
Kline-Fath et al (2018)	- Metabolic acidosis (pH less than 7.1 and/or base deficit less than 15). - Apgar score of 5 or less within 5 mins of birth. - Prolonged resuscitation. - Neurologic exam consistent with HIE. - Group with and group without	HIE with seizures & HIE without seizures	Within 2 weeks	25 / 23	FA	- MRI scans were graded for injury in watershed areas, BGT, and PLIC. - We additionally graded for injury in brainstem, cerebellum, WM, parenchymal cortex, and hippocampus.	-	ROI – CCGenu, CCSplen, PLIC, ALIC.	- The seizure group had significantly more injury within white matter, basal ganglia, posterior limb of internal capsule, and watershed areas compared to the group without seizures. - The severity of injury in all measured areas increased with increasing seizure severity. - The seizure group also had

	clinical seizures. - Abnormal EEG background.								lower FA values in posterior limb of the internal capsule and the splenium of corpus callosum.
Carrasco et al (2018)	- Born at 35 weeks GA or after. - Diagnosis of moderate to severe HIE.	Cooled HIE	Within 10 days	25	MD	- NRN scoring system. (0): normal. (1A): minimal cerebral lesions only, without BGT / PLIC / ALIC / watershed. (1B): more extensive cerebral lesions, without BGT / PLIC / ALIC / watershed. (2A): BGT / PLIC / ALIC / watershed abnormalities without cerebral lesions. (2B): BGT / PLIC / ALIC / watershed abnormalities with cerebral lesions. (3): cerebral hemispheric devastation.	-	ROI – thalamus, basal ganglia, PLIC, antCS, postCS, cerebellar WM, pons.	- Neonates with blood pressure measurements within optimal MAP during rewarming had less brain injury by NRN score. - Longer duration of MAP within optimal MAP during hypothermia correlated with higher mean diffusivity in the anterior centrum semiovale and pons. - Blood pressure deviation below optimal MAP was associated with lower mean diffusivity in cerebellar white matter. - Higher optimal MAP values related to lower mean diffusivity in the basal ganglia, the thalamus, the posterior limb of the internal capsule, the posterior centrum semiovale, and the cerebellar white matter.

Li et al (2017)	- Diagnosis and inclusion criteria were in accordance with HIE diagnostic criteria and clinical classification of practical neonatal HIE.	Moderate HIE & Severe HIE & Healthy Term	10-14 days	14 / 8 / 10	ADC, FA	-	- NBNA at 15 days (behavioural ability, active and passive muscle tension, primitive reflex and evaluation of general condition) - scores significantly different among the three groups, lowest scores in severe HIE, and correlation coefficient highest for the PLIC.	ROI - 20 including the PLIC, ALIC, bilateral parietal cortex, deep WM of frontal lobe, CCGenu, CCSplen, head of caudate nucleus, lenticular nucleus, thalamus, pons.	- FA values in the PLICs and in the thalami showed statistically significant differences between the moderate and severe groups, and were significantly different between the control and moderate groups - Voxel volumes in the SLFs, PLICs and ALICs showed statistically significant differences between the moderate and severe HIE groups. - The fibre numbers in the PLICs, cingulate gyri, SLFs and IFOFs were significantly different between the moderate and severe HIE groups. - NBNA scores were significantly different among the three groups, with the severe HIE group having the lowest scores.
Lemmon et al (2017)	- pH of 7.0 or less or base deficit of 16 or more within 1 hour of birth. - Acute perinatal event. - Apgar score of 5 or less within 10 mins of birth or assisted ventilation at birth	Cooled HIE & Healthy Term	1-28 days	57 / 12	FA, MD	- Barkovich scoring: (1) normal (2) mild: injury of periventricular WM (T2-hyperintense signal or restricted diffusion) (3) moderate: injury of BGT or cortex (4) severe: injury of BGT and cortex.	-	ROI - bilateral thalami, superior, middle, and inferior CPs, and the dentate nucleus.	- FA values of the middle cerebellar peduncles and MD values of the superior cerebellar peduncles were significantly lower in patients with evidence of moderate or severe injury on MRI than in controls. - In patients, cerebellar DTI scalars correlated positively

	for at least 10 mins. - Required hypothermia without discontinuation before 72h.								with DTI scalars within the thalami.
Massaro et al (2015)	- Born at 36 weeks GA or after. - Weigh 1800g or greater at birth. - Metabolic acidosis and/or low Apgar scores. - Signs of moderate/severe NE.	Cooled HIE	7-10 days	45	FA, MD, AD, RD	- Barkovich scoring: BG & WS. - Miller scoring for WM: mild, moderate, severe.	- NICU network neurobehavioral scale (NNNS): motor performance and cognitive/behavioural functioning - infants with severe MRI injury obtained worse scores compared to those without. - BSID-II at min 21 months.	ROI – cerebral peduncle, PLIC, CS, CC.	- Significant associations were observed between DTI corticospinal tract integrity and NNNS neuromotor performance in HIE newborns. Neonatal neuromotor performance was also related to later early childhood motor outcomes.
Massaro et al (2014)	- Born at 36 weeks GA or after. - >1800g at birth. - Metabolic acidosis and/or low Apgar scores. - Signs of moderate to severe clinical encephalopathy.	Cooled HIE	7-10 days	50	FA, MD, AD, RD	- Barkovich scoring - BG/W score ranging from 0 to 4.	- Bayley-II - association between CST FA and Psychomotor Developmental Index (PDI), association between CC FA and Mental Developmental Index (MDI).	ROI - CSTs in the CP, PLIC, CS, CC.	- Lower CC and CST fractional anisotropy were associated with lower MDI and PDI respectively. - There was also a direct relationship between CC axial diffusivity and MDI, while CST radial diffusivity was inversely related to PDI.

Ancora et al (2013)	- Born at term. - Moderate or severe HIE (Sarnat).	Cooled HIE	4-16 days	15	FA, MD, λ_1 , λ_2	- Barkovich scoring - abnormal signal in deep grey matter nuclei detected in 7 infants, and changes in the watershed regions also present in 4.	- 6 showed a poor outcome. - 1 with severe HIE died. - 4 developed cerebral palsy, one of whom died at 18m. - 1 developed severe hearing loss. - Normal psychomotor outcome in remaining patients (GMDS).	ROI - supratentorial region, posterior cranial fossa, CCGenu, CCSplen, caudate, PLIC, thalamus, lenticular nucleus, optic radiation, occipital WM, frontal WM, fronto-parietal WM.	- MD showed significantly decreased values in many regions of white and grey matter, axial diffusivity showed the best predictive value in the genu of corpus callosum, and radial diffusivity was significantly decreased in fronto white matter and fronto parietal WM. - The decrement of FA showed the best AUC in the FPWM.
Gano et al (2013)	- Born at 36 weeks GA or after. - Clinically recognizable encephalopathy. - One or more of: foetal distress at delivery, requirement for resuscitation at birth, Apgar score ≤ 5 at 5 min, and metabolic acidosis (umbilical artery pH < 7.1 or base deficit > 10).	HIE	3 days	19	FA, MD	- Barkovich scoring - normal, WS, BN, total, and focal-multifocal injury.	- Therapeutic hypothermia associated with an attenuated reduction of MD in GM and WM from day 1 to 3. - Hypothermia also associated with increased neuronal metabolism and WM integrity, as well as reduced oxidative metabolism.	ROI - ant and post WS white matter, CSTs in the CS, CC, PLIC, ORs, caudate, putamen, ventrolateral thalami, calcarine region, and hippocampi.	- In grey matter, MD and metabolite ratios measured on day 1 were predictive of values on day 3. - In white matter, MD, FA and (NAA)/choline on days 1 and 3 were strongly related. - Hypothermia appeared to attenuate the severity and progression of brain injury in the six treated newborns.

Ward et al (2006)	- Born at 36 weeks GA or after. - Abnormal tone patterns. - Feeding difficulties. - Altered alertness. - At least 3 of: (1) late decelerations on foetal monitoring or meconium staining, (2) delayed onset of respiration, (3) pH <7.1, (4) Apgar scores <7 at 5 mins or (5) multiorgan failure.	HIE & Healthy Term	Within 3 weeks	20 / 7	ADC, FA, RA	- BGT grading: normal, mild (lesions small and focal with normal myelination in the PLIC), moderate (lesions multifocal with equivocal or abnormal PLIC) or severe (complete BGT abnormality with abnormal PLIC). - WM also graded normal, moderate or severe	-	ROI - CS (central), BGT, lateral lentiform nuclei, medial thalamus, ventrolateral nuclei of thalamus, PLIC, brainstem, cerebellum.	- During the first week, FA values were decreased with both severe and moderate WM and BGT injury, whereas ADC values were reduced only in severe WM injury and some severe BGT injury. - Abnormal ADC values pseudonormalized during the second week, whereas FA values continued to decrease.
van Pul et al (2005)	- Clinical symptoms of NE present in first days of life together with two or more of: foetal heart rate abnormalities, umbilical artery pH less than 7.10, meconium stained fluid, APGAR score after 5 minutes of less than 7	HIE	Within 10 days	22	ADC, FA, AD	- 11 neonates had focal white matter injury	Follow up MRI at 3m: - In 6 infants (severe) tissue loss or signal intensity abnormalities observed in the region of lesion, with abnormalities in the basal ganglia in 2, and mildly to moderately delayed myelination in 4 and normal myelination in 2. In both infants without abnormalities in the region of the infarct, myelination was delayed. - Severe neuromotor and mental delay in 3 children, cerebral palsy in 2, and visual impairment in 1	ROI - CC ad WM.	- Changes in lesions were characterized by a large decrease (40%) in all eigenvalues, with a stronger decrease in the direction perpendicular to the fibres, resulting in increased anisotropy indexes. - The relative change in anisotropy decreased linearly with time, with the strongest trend in anisotropy index AD.

							child. . Normal development observed in 2 children at 1Y, and in 1 at 6Y.		
Al Amrani et al (2017)	- Born at 36 weeks GA or after. - Evidence of foetal distress, e.g., history of acute perinatal event, cord pH ≤ 7.0 or base deficit ≤ 16, or continued need for ventilation initiated at birth for at least 10 mins. - Moderate or severe HIE.	Cooled HIE	10 days	29	ADC, FA	- Barkovich scoring - 0 for "no brain injury," 1 for "basal ganglia injury pattern," 2 for "watershed injury pattern," and 3 to 4 for "near-total injury pattern."	- Adverse outcomes - Cerebral Palsy, Global Developmental Delay, Epilepsy. - In neonates who developed an adverse outcome, ADC values significantly decreased on days 2-3 of life in the thalamus, PLIC, and lentiform nucleus. - Then, ADC values increased (pseudonormalization) and FA decreased around day 10 in the same ROIs.	ROI - thalamus, PLIC, lentiform nucleus, front and post WM, cortical GM.	- Among the newborns developing adverse outcome, ADC values were significantly decreased on days two to three of life and increased around day ten of life in the thalamus, PLIC, and the lentiform nucleus. - FA values decreased in the same regions around day 30 of life. - These newborns also had increased ADC around days 10 and 30 of life, and decreased FA around day 30 in the anterior and posterior WM.
Kansagra et al (2016)	- Born at 36 weeks GA or after. - Moderate or severe HIE. - Apgar score less than 5 within 10 mins of birth, prolonged resuscitation at birth, pH less than 7.0 within 60 mins of birth, or base deficit greater than 12.	Newborn NE & 6 Months NE	0 months & 6 months (excluded from this review)	12	FA, NDI, ODI, FW (NODDI)	-	- Infants assigned a validated neuromotor score — 0 for normal development, 1 for abnormal tone or reflexes, 2 for abnormal tone and reflexes, 3 for functional deficit of power, 4 for cranial nerve involvement with motor abnormality, and 5 for spastic quadriparesis. - All infants had scores of 0	ROI - PLIC, ALIC, OR, CCgenu, CCsplen.	- At birth, the CC collectively demonstrated significantly higher FA than the ALIC or optic radiations. - Moreover, the CC demonstrated significantly lower ODI and higher FW than the PLIC, ALIC, or optic radiations at birth. - Measured FA was significantly higher at 6m than at birth within all measured white matter tracts. In contrast, NDI was

							or 1 at an average 318 days.		significantly higher only within the PLIC, ALIC, OR, and splenium. ODI was significantly higher only within the ALIC and optic radiations. - Measured FW was significantly lower at 6m than at birth in the OR, genu, and splenium.
Lee et al (2012)	- Retrospective diagnosis of NE, all cooled.	NE (normal MRI) & NE (abnormal MRI) & Healthy Preterm at Term Equivalent	5-18 days	12 / 24 / 12	ADC, FA	- 11 had predominantly BGT injury without WM injury. - 4 had predominantly diffuse cortical injuries with underlying WM but no deep GM changes. - 6 had only WM injuries in the watershed distribution. - 3 had global mixed GM and WM injuries. - 12 of these neonates also had CC signal changes.	-	ROI - PLIC, temporal, occipital, parietal and frontal WM, CCGenu, CCSplen.	- We found significant reductions in measured FA in white matter in the ANE neonates compared to the control group. - There were, however, no significant differences in measured FA in white matter between the NNE and the control group. - The FA of CCGenu and CCSplen was the lowest in the global pattern, followed by the cortical pattern, the watershed pattern and the basal ganglia pattern.

Table 4. Data extracted from the literature for the main variables of interest.

5.4.4 Results

Nineteen studies were included in our final list for review, which included DTI data from 557 infants with NE and 112 healthy controls¹⁵⁵⁻¹⁷³. These studies all met the guidelines outlined in the STROBE statement¹⁵³ in regards to quality and bias. Follow-up/outcomes were available in 11 of these studies^{157-159,163,165-168,170-172}. On the whole, these studies described decreased levels of fractional anisotropy (FA) in infants with NE compared to healthy controls, with the severity of decreases in FA being associated with the severity of the NE and, when applicable, the severity of outcomes. One study also showed significantly lower FA in non-cooled neonates than neonates treated with therapeutic hypothermia¹⁵⁵.

We calculated the frequency of ROIs, the results of which can be seen in table 5.

Region of Interest (ROI)	n = ROI mentioned in results
PLIC	13
CCGenu	13
CCSplen	13
ALIC	5
Cing., IFOF/ILF, CerebralP	4
CCBody, SLF	3
CR, Fornix, OR, CerebellarP, CST	2
EC	1
Global WM	4

Table 5. A summarising table of ROIs implicated in NE from the literature and how often they were mentioned (ROIs chosen based on the JHU-ICBM DTI-81 white matter atlas). Abbreviations: PLIC, posterior limb of the internal capsule; CCGenu, genu of the corpus callosum; CCSplen, splenium of the corpus callosum; ALIC, anterior limb of the internal capsule; Cing., cingulum; IFOF, inferior fronto-occipital fasciculus; ILF, inferior longitudinal fasciculus; CerebralP, cerebral peduncles; CCBODY, body of the corpus callosum; SLF, superior longitudinal

fasciculus; CR, corona radiata (superior); Fornix; OR, optic radiations; CerebellarP, cerebellar peduncles; CST, corticospinal tract; EC, external capsule.

Three key ROIs were identified – the posterior limb of the internal capsule (PLIC), and the genu and splenium of the corpus callosum (CC). As we included studies that used a whole brain approach as well as an ROI approach, we also noted the number of times a significant change in global white matter was found. These frequencies were then converted to a greyscale image, in which the tracts with the most mentions (PLIC, CCGenu, CCSplen), are shown as the brightest (white), and the tracts with the least mentions are shown as the darkest (black), with various levels of grey in between (figure 10).

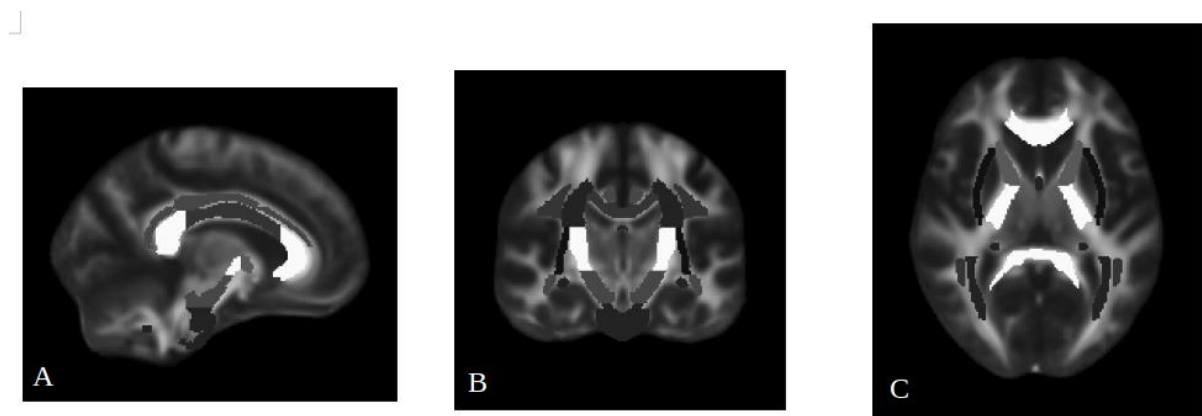


Figure 10. *Tract ROIs as defined by JHU-ICBM DTI-81 white matter atlas, shown on the FMRIB58-FA template, in a sagittal (A), coronal (B), and axial (C) view. Tracts shown are those that arose as areas of interest in the literature search, with colour representing the relative amount of times that ROI was mentioned; ROIs in white being the most mentioned and ROIs in black being the least. The PLIC, genu and splenium of the corpus callosum (bright white) are the 3 ROIs that most appeared in the search.*

For the 11 studies that reported on follow-up/outcomes, infants were assessed at time points ranging from 3 days to 3.5 years. Seven of these studies described four clinical assessment tests of global development; Bayley Scales of Infant Development (BSID), Griffiths Mental Development Scales (GMDS), Neonatal Behavioural Neurological Assessment (NBNA), NICU Network Neurobehavioural Scale (NNNS), and one clinical test applicable only to those

neonates who developed cerebral palsy; Gross Motor Function Classification System (GMFCS), (see table 6)^{157-159,163,165-167}. The remaining 4 studies described other clinical tests including measurements of neuronal metabolism, follow-up MRI scans, and other arbitrary scoring systems^{168, 170-172}. Six of these studies were able to show an association between adverse outcomes and FA measurements in the PLIC and CC^{158,163,165-167,171}. Lower FA values in the PLIC were associated with more adverse outcomes, defined by poorer scores on various subsets of these clinical tests including the GMDS (development quotient), NBNA, NNNS (total motor score), BSID (psychomotor developmental index) and global developmental delay, as well as the development of cerebral palsy and/or epilepsy. Lower FA values in the CC were associated with more adverse outcomes, defined by poorer scores on the GMDS (development quotient) and BSID (mental developmental index). Not all infants were reported to have adverse outcomes, with several children with milder cases of NE showing normal development at follow-up.

Clinical Assessment	n = Included in Outcomes
GMDS	4
BSID	3
GMFCS	2
NBNA	1
NNNS	1

Table 6. A summarising table of clinical assessments used to assess outcomes. Abbreviations: BSID, Bayley Scales of Infant Development; GMDS, Griffiths Mental Development Scales; GMFCS, Gross Motor Function Classification System; NBNA, Neonatal Behavioural Neurological Assessment; NNNS, NICU Network Neurobehavioural Scale.

5.4.5 Discussion

The present review identifies three main white matter tracts of interest that appear to show altered levels of diffusion in infants with NE; the PLIC and the genu and splenium of the CC. Previously established scoring systems such as that of Barkovich¹³² classify NE injury in terms of damage to the basal ganglia/thalamus and watershed cortical injury. Our identification of the PLIC as a region of interest in NE fits in well with the basal ganglia/thalamus classification of injury, as it contains ascending and descending fibres that pass through the basal ganglia and separate the thalamus and the lenticular nucleus. Indeed, expanding upon the work of Barkovich, Rutherford describes the presence of high signal intensity in the PLIC on a conventional T₁-weighted MRI image, or the absence of low signal intensity in the PLIC on a conventional T₂-weighted MRI image as an additional criteria for diagnosing NE¹³³. Usually myelinated at term, the PLIC can be seen as a low-intensity signal on a T₂-weighted scan in the healthy term infant. Loss of low signal or increased T₂ signal within the PLIC may indicate evidence of neonatal encephalopathy, and in fact, several of the studies reviewed included this as part of their diagnostic criteria^{157,158,161,162,169}.

Although not included as a classification of injury on the Barkovich¹³² and Rutherford¹³³ scoring systems, the CC has recently been included in a novel scoring system for NE that also utilises DWI, developed by Weeke and colleagues¹³⁵. They assessed brain injury in terms of deep grey matter (including the basal ganglia, thalamus and PLIC), cerebral white matter (including the cortex, optic radiations, and the CC), and the cerebellum. A large commissural fibre tract connecting the left and right hemispheres, the CC is thought to play a key role in cognitive¹⁷⁴ and even motor¹⁷⁵ functioning, deficits which are often seen in infants with NE later in life. It has in fact been shown that low ADC values in the CC are associated with adverse outcome in infants with NE at 18 months, and this association persisted (although to a lesser extent) in infants treated with therapeutic hypothermia¹⁷⁶.

The clinical criterion for defining NE in the studies under review was fairly homogeneous, with most studies describing the NE infant as being born at term, with an Apgar score of five or less, metabolic acidosis (as defined by pH levels or base deficit) and/or a continued need for ventilation. All neonates included in the study were also all scanned within the first month of life. Despite this consistency, however, DWI has been shown to vary

considerably even over the first few days and weeks of life¹⁷⁷. DWI is the most sensitive MRI sequence for imaging the encephalopathic neonate within the first few days following the hypoxic-ischaemic event, as T₁- and T₂-weighted images can produce false negatives at this time. However, at approximately one week of age for normothermic infants and between approximately 11-12 days for infants treated with therapeutic hypothermia, DWI may underestimate the extent of the brain injury due to the phenomenon of pseudonormalization^{131,178}. Whilst the apparent diffusion coefficient (ADC) is initially decreased in infants with NE within the first few hours following an acute brain injury, by the end of the first week of life, the ADC increases to reach apparently normal levels. It is therefore key that we take into account the careful timing of DWI in our prospective study going forward, in order to produce the most accurate representation of the damage. Ideally, neonates should be imaged between days 3 and 7 of life, for diffusion changes to be fully assessed in NE.

There was heterogeneity in the study populations under review, due mainly to the small number of studies conducted in this area, which meant the inclusion of infants with varying severity of NE (mild, moderate, severe) and infants that had and had not been treated with therapeutic hypothermia. This is likely to have added bias to the study due to the possibility that infants treated with therapeutic hypothermia and infants managed at normothermia may show differing diffusion measures. It will therefore be critical to consider and identify any possible subgroups in a prospective study, including the severity of NE and treatment interventions. This will be especially crucial when attempting to relate diffusion measures to neurodevelopmental outcome. Outcome variables were only obtained for thirteen of the reviewed studies; however, it is only through the long-term follow-up of these infants that we can discern the prognostic value of DWI and DTI in NE.

It should also be noted that, although this review focused solely on diffusion changes in the white matter tracts of the brain, DWI can also demonstrate detectable changes in the deep grey matter, in particular, the basal ganglia and the thalamus. Reduced ADC in these regions has been found in infants with severe NE, and these values may be informative of adverse neurodevelopmental outcome and the development of dyskinetic cerebral palsy¹²⁷. A prospective study should therefore not only examine diffusion measures in the white matter tracts, but also the deep grey matter structures of the NE infant brain.

5.4.6 Conclusions

The addition of DTI to the scanning protocol within the first week of life can be an excellent method to assess brain injury in neonates with neonatal encephalopathy. In particular, specific attention should be placed on obtaining fractional anisotropy measures in areas such as the PLIC and the genu and splenium of the CC. This data may then be used to assess the impact of therapeutic hypothermia on the injury, and as a prognostic tool to predict possible neurodevelopmental outcomes.

Chapter 6: Empirical Study 1 - Microstructural Grey and White Matter Differences Across the Neonatal Brain Assessed by Structural and Functional Imaging

“MRI gives superb detail of the developing brain. It is essential to have a thorough knowledge of the normal appearances at different ages in order to define the abnormal”.

- *Prof Frances M Cowan*¹²³

6.1 Introduction

At birth, the foundational microstructure of the brain is largely set, following rapid prenatal development. However, the differentiation, maturation, and myelination of neurons continue during the early postnatal period through infancy⁵. Indeed, an infant is born with more than 99% of the neurons it will ever have, yet the neonatal brain is only approximately 35% of the size of the adult brain. During the first year of life the brain doubles in size, and by age six it reaches approximately 90% of its adult volume³⁻⁵. Although there are widespread changes across the brain during this time, grey and white matter follow different developmental trajectories^{4,180}. Growth is far more robust in cortical grey matter compared to white matter, accounting for the majority of hemispheric growth. Grey matter has been shown to increase by 149% during the first year, as opposed to only an 11% increase for white matter⁴. Additionally, grey matter growth is characterised by posterior to anterior regional specificity¹⁸⁰. In contrast, white matter development during the first year is characterised by the rapid maturation and myelination of existing structures¹⁸¹, occurring in several predictable neuroanatomical sequences: from central to peripheral, from posterior to anterior, and from caudal to rostral⁹.

Magnetic Resonance Imaging (MRI) is a highly valuable tool in examining maturational processes in the typically developing neonate brain within the research setting. In particular, Diffusion Tensor Imaging (DTI) – an advanced MRI technique that utilizes the direction and

degree of water diffusion – allows for the quantitative analysis of the microstructure of the developing brain in vivo¹⁸². DTI examines properties such as the molecular diffusion rate (mean diffusivity, MD), the directional preference of diffusion (fractional anisotropy, FA), the axial (diffusion rate along the main axis of diffusion, AD), and radial (rate of diffusion in the transverse direction, RD) diffusivity, and is typically used for examining the white matter of the brain. DTI has an advantage over conventional MRI as it can reveal microstructural information about the pre-myelinated brain, and therefore has the potential to examine the maturation of white matter in the developing neonate. The decreasing water content of the developing neonate means that white matter becomes more organised in terms of its axonal bundles, limiting the diffusion of water molecules to specific directions⁷¹. Therefore, for white matter, FA increases rapidly from birth as maturation progresses, and high levels of FA in a specific tract may suggest that that tract is more finely and compactly organised and may be further along in the myelination process. Conversely, MD in white matter decreases with maturation. On the other hand, FA and MD in the grey matter of the cortex show a slightly different pattern, with both metrics being very high during foetal and preterm development and decreasing rapidly from birth⁷¹.

Neurite Orientation Dispersion and Density Imaging (NODDI) is a relatively new diffusion MRI technique that provides more detailed information on the morphology of axons and dendrites than DTI⁶⁵. It provides estimations of three metrics: restricted intracellular, hindered extracellular, and free water. The intracellular volume fraction (v_{ic}) is a quantitative estimation of neurite density (neurites being the collective term for axons and dendrites) – also known as the neurite density index, NDI – and is modelled as sticks. The extracellular component refers to everything except neurites and CSF, such as microglia, astrocytes, oligodendrocytes, and neuronal cell bodies, and is modelled by Gaussian anisotropic diffusion. The free water fraction (v_{iso}) is attributed to CSF and is represented by isotropic diffusion. The orientation dispersion index (ODI) estimates the angular variation/fanning of neurites (values range from 0-1: 0 for straight, perfectly aligned fibres; 1 for fully isotropic). NODDI is therefore able to extend the study of the brain's microstructure from white matter (primarily axons) to grey matter (primarily cell bodies and dendrites)¹⁸³. For a visual representation of the NODDI model, see figure 11.

Neurite morphology is a valuable marker of brain development⁶⁵ and NODDI can be used to identify the differing microstructural components of early maturing regions which are myelinated and tightly packed (characterised by higher NDI), and immature regions which are unmyelinated, less coherently oriented, less tightly packed and surrounded by more extra-cellular space (characterised by higher ODI)⁷⁴. Whilst NODDI has been successfully applied to the neonatal population in terms of white matter⁷⁴, there is little research using NODDI to examine the microstructural development of neonatal grey matter. DTI and NODDI are two different but complementary techniques to examine the microstructure of the brain, with DTI being a simple linear model accounting for the Gaussian diffusion of water, whilst NODDI explicitly models non-linear neurite properties using multishell data⁶⁵. The first aim of the present study was to use both DTI and NODDI to examine the different developmental trajectories of both the white matter and the cortical and sub-cortical (deep) grey matter of the typically developing full-term neonate, and how this is variable across the different regions of the brain.

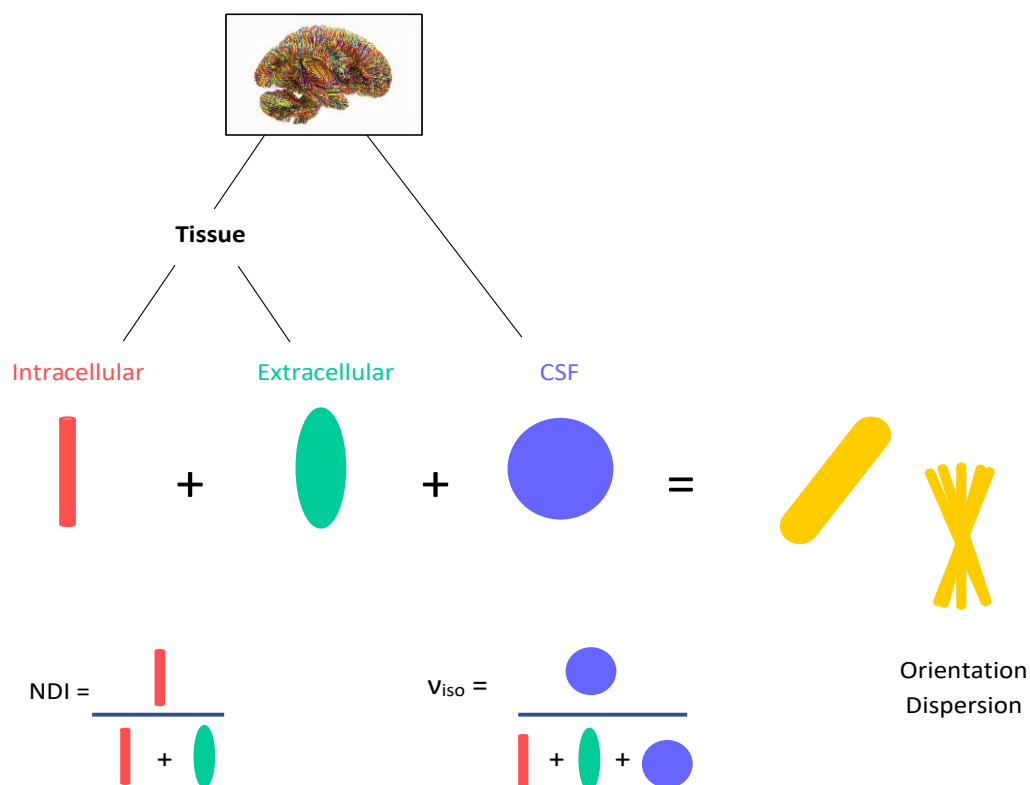


Figure 11. Schematic illustration of the NODDI model (adapted from Kamiya et al¹⁸⁴).

The past decade has also seen the emergence of resting-state functional MRI (rs-fMRI) as a technique to examine functional architecture in the developing neonate. By measuring the amplitude of low frequency (<0.1 Hz) fluctuations (ALFF) in the blood oxygenation level dependent (BOLD) signal whilst at rest, rs-fMRI is able to investigate the regional intensity of spontaneous neural activity^{185,186}. ALFF, fractional ALFF (fALFF; the ratio of the power spectrum of low frequency to the entire frequency range) and global mean ALFF (mALFF) are all quantitative voxel-level metrics deemed to reflect local brain function¹⁸⁶. The existing literature on neonatal brain function primarily consists of connectivity-based studies, investigating the emergence of resting state networks in the neonatal brain in conjunction with neural and behavioural development¹⁸⁷⁻¹⁸⁹. For instance, primary networks related to sensorimotor and visual functioning have been identified as being present from birth, followed by the emergence of attentional and default mode networks during the first year, and finally higher-order networks related to more complex cognitive functions such as decision making and working memory continue to develop through adolescence and adulthood¹⁸⁷⁻¹⁸⁹. However, very few studies have used ALFF to infer local brain activity during the neonatal period. Therefore, the second aim of the present study was to explore how regional brain activation (as measured by fMRI) may be associated with the microstructural changes seen in the typically developing full-term neonatal brain (as measured by DTI and NODDI), and how these measures change with gestational age.

Whilst DTI and NODDI analyses have been investigated in the white matter of preterm and term neonates, there are very few quantitative studies exploring the cortical and sub-cortical grey matter in the term-born population, and fewer still linking these microstructural measures to functional architecture through fMRI. By examining the maturational trajectory of DTI, NODDI, myelin and fMRI measures, and how these measures are linked to each other, it is possible to infer the nature of the complex microstructural processes that occur during early development as well as the corresponding development of functional brain activity. Our study will therefore contribute further to our understanding of the developing neonatal brain, using multi-sequence imaging techniques to examine the microstructural substrates for MRI changes seen during the first weeks of postnatal development.

6.2 Methods

6.2.1 Subjects

This study was a retrospective analysis of a subset of neonates from the second data release of the Developing Human Connectome Project (dHCP) (<http://www.developingconnectome.org>), who were recruited and imaged at the Evelina Newborn Imaging Centre in St Thomas' Hospital, London, UK. The dHCP study was approved by the National Research Ethics Committee and informed written consent was given by the parents of all participants. Our subset consisted of 126 term-born neonates (67 female, 59 male), with an average age at birth of 40.02 weeks gestation (36.29-42.29) and an average age at scan of 41.17 weeks gestation (36.57-44.71). We excluded any neonates that were born preterm (<36 weeks gestation), neonates with any incidental findings (radiology score of ≥ 3) or poor-quality anatomical data, or neonates that were sedated before scanning. Radiology and quality scores were given by a specialist perinatal neuroradiologist based upon the following criteria: 1=normal appearance for age; 2=incidental findings with unlikely significance for clinical outcome or analysis; 3=incidental findings with unlikely clinical significance but possible analysis significance; 4=incidental findings with possible clinical significance, unlikely analysis significance; 5=incidental finding with possible/likely significance for both clinical and imaging analysis; Q=poor quality anatomical data. Images were visually inspected for quality at 5 stages of the dHCP analysis: scanning notes; reconstruction; structural pipeline; fMRI pipeline; dMRI pipeline; and were given a pass or fail at each stage. Full criteria can be found in the processing pipelines for each sequence¹⁹⁷⁻¹⁹⁹.

Numerical DTI and NODDI data were available for all 126 infants, and myelin data for 122 infants. Of the 122, numerical fMRI data was available for 99 of these infants. Subjects with a total framewise displacement >800 were excluded (n=6), resulting in a total of 93 infants (48 female, 45 male) used for fMRI analyses, with an average age at scan of 41.11 (37.43-44.71).

6.2.2 Image Acquisition

Imaging was carried out on a 3T Philips Achieva MR scanner using a dedicated neonatal imaging system which included a neonatal 32 channel phased array head coil¹⁹⁰. Anatomical images (T_1w and T_2w), diffusion, and resting state functional (rs-fMRI) acquisitions were acquired in a total examination time of 63 minutes, using the optimized dHCP protocol for neonates¹⁹¹⁻¹⁹⁶. T_2w (TR=12000ms; TE=156ms; SENSE factor: axial=2.11, sagittal=2.58) and inversion recovery T_1w (TR=4795ms; TI=1740ms; TE=8.7ms; SENSE factor: axial=2.26, sagittal=2.66) multi-slice fast spin-echo images were acquired with an in-plane resolution of $0.8 \times 0.8 \text{ mm}^2$ and 1.6mm slices overlapped by 0.8mm. Multishell diffusion images (TR=3800ms; TE=90ms; SENSE factor=1.2; multiband factor=4) were acquired with four diffusion gradient encoding directions at four b-values: $b=0 \text{ s/mm}^2$ (n=20), $b=400 \text{ s/mm}^2$ (n=64), $b=1000 \text{ s/mm}^2$ (n=88), $b=2600 \text{ s/mm}^2$ (n=128), with an in-plane resolution of $1.5 \times 1.5 \text{ mm}^2$ and 3mm slices overlapped by 1.5mm. High temporal resolution rs-fMRI images (TE=38ms; TR=392ms, multiband factor=9) were acquired using accelerated echo-planar imaging, with an isotropic in-plane resolution of 2.15mm.

6.2.3 Image Processing

All images were pre-processed by the dHCP automated processing pipelines for each sequence¹⁹⁷⁻¹⁹⁹. All calculations were made using the IT Research computer resources (Kelvin) at TCD. The data analysis protocol was developed by my supervisor, Professor Arun Bokde, and analyses were performed by myself. Firstly, we fit each subject's diffusion data to a diffusion tensor model using FSL's²⁰⁰ DTIFit, for each individual b-value ($b=400 \text{ s/mm}^2$, $b=1000 \text{ s/mm}^2$, $b=2600 \text{ s/mm}^2$), and for all shells together, to generate FA (fractional anisotropy), MD (mean diffusivity), AD (axial diffusivity) and RD (radial diffusivity) maps. We then implemented the NODDI MATLAB toolbox⁶⁵, using the Watson distribution, to calculate NDI and ODI maps for each subject. Using a spatio-temporal neonatal brain atlas constructed by the dHCP team²⁰¹, we registered (linear followed by non-linear) each subject's T_2w image to the correct age MRI template (37-44 weeks gestation), using the age at scan. The MRI templates were also transformed into T_2w native space. The DTI and NODDI images were interpolated to 0.5mm isotropic voxel size, and native T_2w images were transformed to the FA

images at $b=1000\text{s/mm}^2$ (rigid body, 6 DOF). Using an extension of the Draw-EM algorithm²⁰², the DTI and NODDI images were segmented into white matter (WM), cortical grey matter (CGM), deep grey matter (DGM), cerebrospinal fluid (CSF), cerebellum and brainstem, which altogether comprised a total 87 regions of interest (ROIs). Draw-EM is a state-of-the-art segmentation framework designed specifically for neonatal brain MR images, and so is far more robust than manual segmentation. Finally, ROI values were calculated for each DTI and NODDI image. These values were transformed into weighted averages based on the size of the ROI. These segmentation and ROI calculation steps were also carried out for each subject's myelin map, which were estimated by the dHCP pre-processing pipeline using a T_1/T_2 ratio¹⁹⁷.

For the fMRI data, we cut each subject's time series into 1600 continuous volumes (from 2400 volumes) with the smallest amount of motion, using tools implemented in FSL²⁰⁰, and removed subjects with high framewise displacement (>0.5 mm) in more than 5% of the cropped dataset were excluded entirely from analysis (i.e. 80 volumes of the 1600 total volumes). From this, a mean data set was created, and the image was binarized to create a mask. Using AFNI's 3dRSFC tool²⁰³, rs-fMRI parameters (ALFF, fALFF, mALFF) were calculated with a frequency band of 0.01-0.1Hz. These output images were registered to the subject's FA at 1000s/mm^2 image using FSL²⁰⁰ (rigid body, 6 DOF). The fMRI images were then interpolated to the voxel size of the structural MRI (0.5mm isotropic). Finally, in the same manner as the DTI and NODDI data, the rs-fMRI images were segmented into the 87 ROIs and weighted average values were calculated.

6.2.4 Statistical Analyses

Statistical analyses and visualization were carried out by myself using iNZight (v.4.2.0) (The University of Auckland, New Zealand). General linear regression was performed to assess the association between DTI (FA, MD), NODDI (NDI, ODI), myelin and fMRI (ALFF, fALFF, mALFF) measures, with gestational age (at scan). This was performed separately for each ROI (as well as globally – all ROIs together), controlling for sex and brain volume (brain volume was region dependent, grouped into CGM, DGM, WM, cerebellum, and brainstem). For the fMRI data, motion was also controlled for, using the total framewise displacement for each infant, and analysis was focused to regions of grey matter only. The Bonferroni method was used to

correct for multiple comparisons, resulting in a probability value of <0.0025 taken as statistically significant. These associations were visualized as scatterplot graphs.

We also examined the relationship between DTI (FA, MD), NODDI (NDI, ODI) and myelin measures, and fMRI (ALFF, fALFF, mALFF) measures. General linear regression was performed separately for each metric (as well as globally), controlling for sex, brain volume by region, gestational age at scan and motion. This analysis was focused to regions of grey matter only, and so the Bonferroni correction for multiple comparisons resulted in a probability value of <0.0042 taken as statistically significant. This meant that the global measures included all the CGM ROIs together. These associations were visualized as scatterplot graphs.

Finally, we looked at the relationship between myelin and all MRI metrics, independent of gestational age. We performed an orthogonal regression on the myelin measures to remove the effects of gestational age, then used this new orthogonalized myelin to perform a general linear regression separately for each measure, controlling for sex, brain volume (region dependent), gestational age at scan and motion. For the DTI, NODDI and myelin measures, this was done for every ROI (both GM and WM), therefore the Bonferroni correction for multiple comparisons resulted in a probability value of <0.0025 taken as statistically significant. For the fMRI measures, we again only focused on the grey matter, thus taking a probability value of <0.0042 as statistically significant after correction for multiple comparisons. These associations were visualized as scatterplot graphs.

6.3 Results – DTI, NODDI and Myelin Measures vs Gestational Age

Globally (all ROIs added together), linear regression revealed all measures to be associated with increasing gestational age at scan. Scan age was positively associated with FA ($p<0.001$), NDI ($p<0.001$), ODI ($p=0.002$), and myelin ($p<0.001$), and negatively with MD ($p<0.001$) (figure 12).

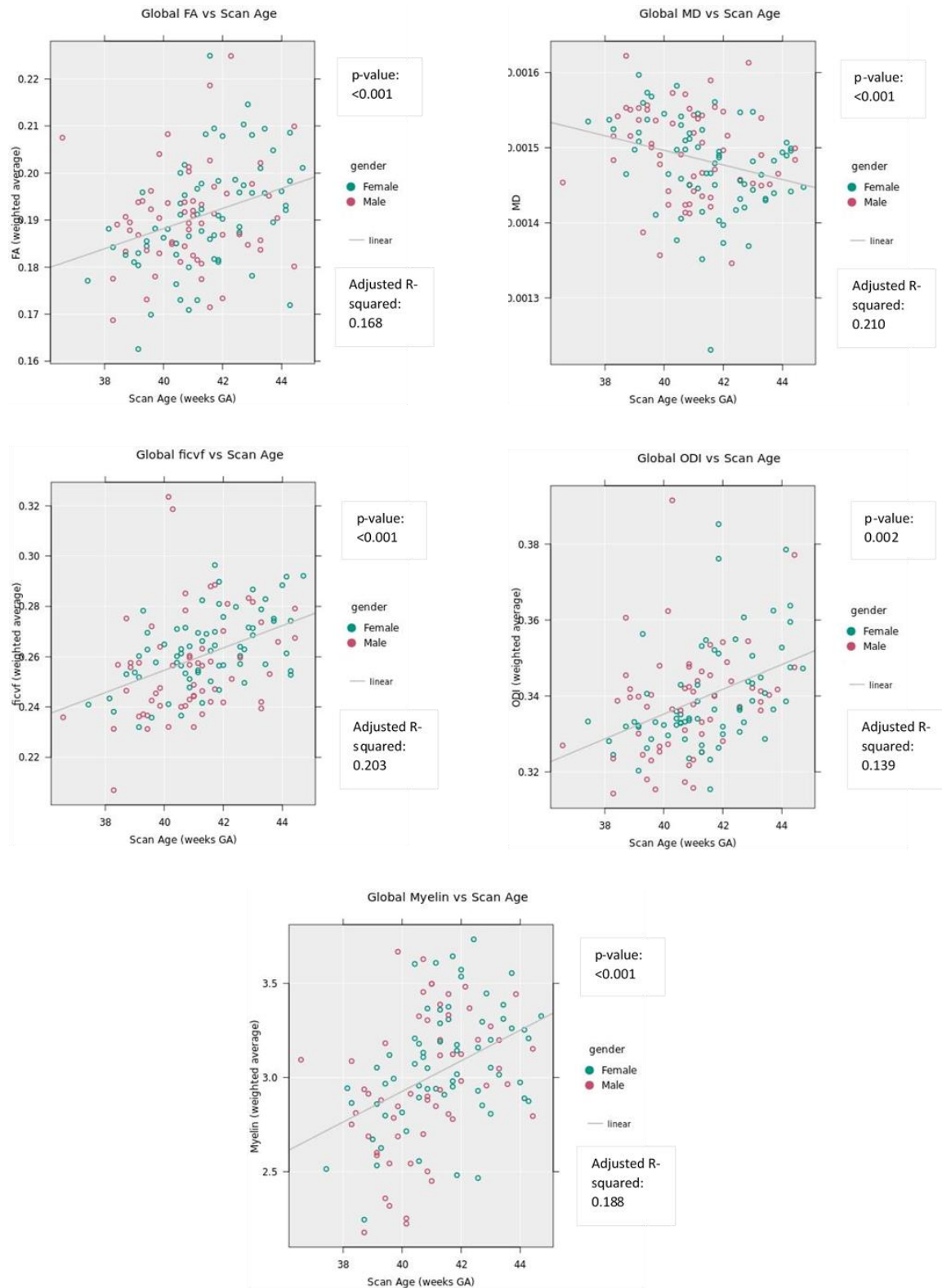


Figure 12. Global DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and global brain volume).

6.3.1 White Matter

WM was grouped into the following ROIs: frontal WM, temporal WM, parietal WM, occipital WM, Insular WM, and the corpus callosum. In frontal, temporal, and occipital WM, scan age was associated with increasing FA ($p<0.001$), NDI ($p<0.001$), and myelin ($p<0.001$), and decreasing MD ($p<0.001$), but not ODI ($p=0.643$, $p=0.043$, $p=0.425$) (figures 13-15). In parietal WM, scan age was associated with increasing NDI ($p<0.001$), ODI ($p<0.001$), and myelin ($p<0.001$), and decreasing MD ($p<0.001$), however, FA did not survive correction for multiple comparisons ($p=0.033$) (figure 16). In insular WM, scan age was associated with increasing FA ($p<0.001$), NDI ($p<0.001$), ODI ($p<0.001$), and myelin ($p<0.001$), and decreasing MD ($p<0.001$) (figure 17). In the corpus callosum, scan age was associated with increasing NDI ($p<0.001$) and ODI ($p=0.002$), but not FA ($p=0.620$) or MD ($p=0.265$). Myelin did not survive correction for multiple comparisons ($p=0.005$) (figure 18).

6.3.2 Cortical Grey Matter

CGM was grouped into the following ROIs: frontal GM, temporal GM, parietal GM, occipital GM, and Insular GM. In frontal GM scan age was associated with increasing FA ($p<0.001$), NDI ($p<0.001$), and myelin ($p<0.001$), but not MD ($p=0.127$) or ODI ($p=0.781$) (figure 19). In temporal, occipital, and insular GM, scan age was associated with increasing NDI ($p=0.001$, $p<0.001$, $p<0.001$), and myelin ($p<0.001$), but not MD ($p=0.115$, $p=0.279$, $p=0.010$) or ODI ($p=0.010$, $p=0.423$, $p=0.055$), although trends were observed for MD in the insular GM and for ODI in both the temporal and insular GM. FA was tentatively positively associated with scan age in the occipital and insular GM; however, these did not survive correction for multiple comparisons ($p=0.008$, $p=0.009$). FA was not associated with scan age in the temporal GM ($p=0.066$) (figures 20-22). In parietal GM, scan age was associated with increasing myelin ($p<0.001$), but not MD ($p=0.072$) or ODI ($p=0.051$) (figure 23). Again, a positive trend was observed for both FA and NDI, however, these did not survive correction for multiple comparisons ($p=0.039$, $p=0.007$).

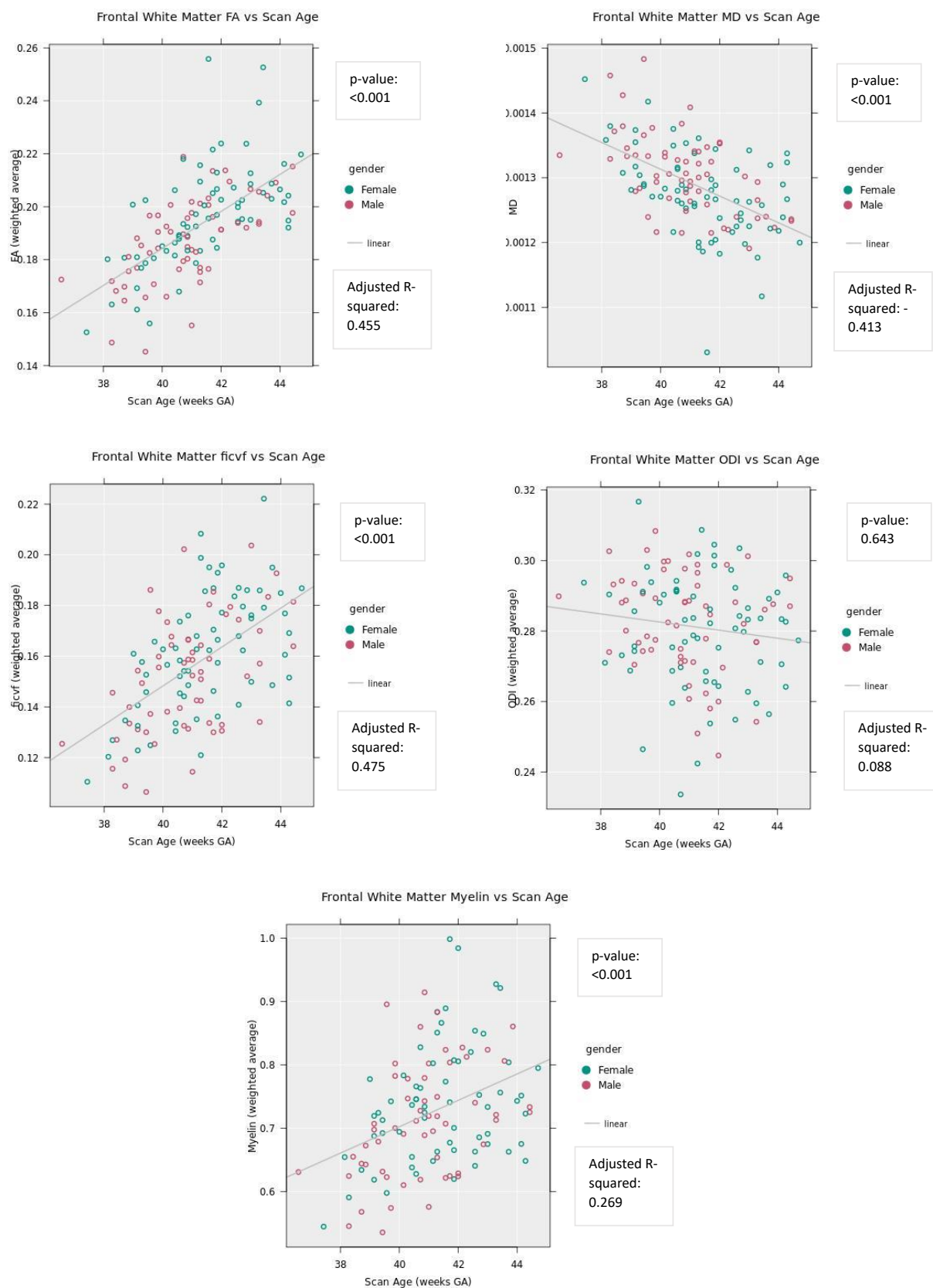


Figure 13. Frontal white matter DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and white matter volume).

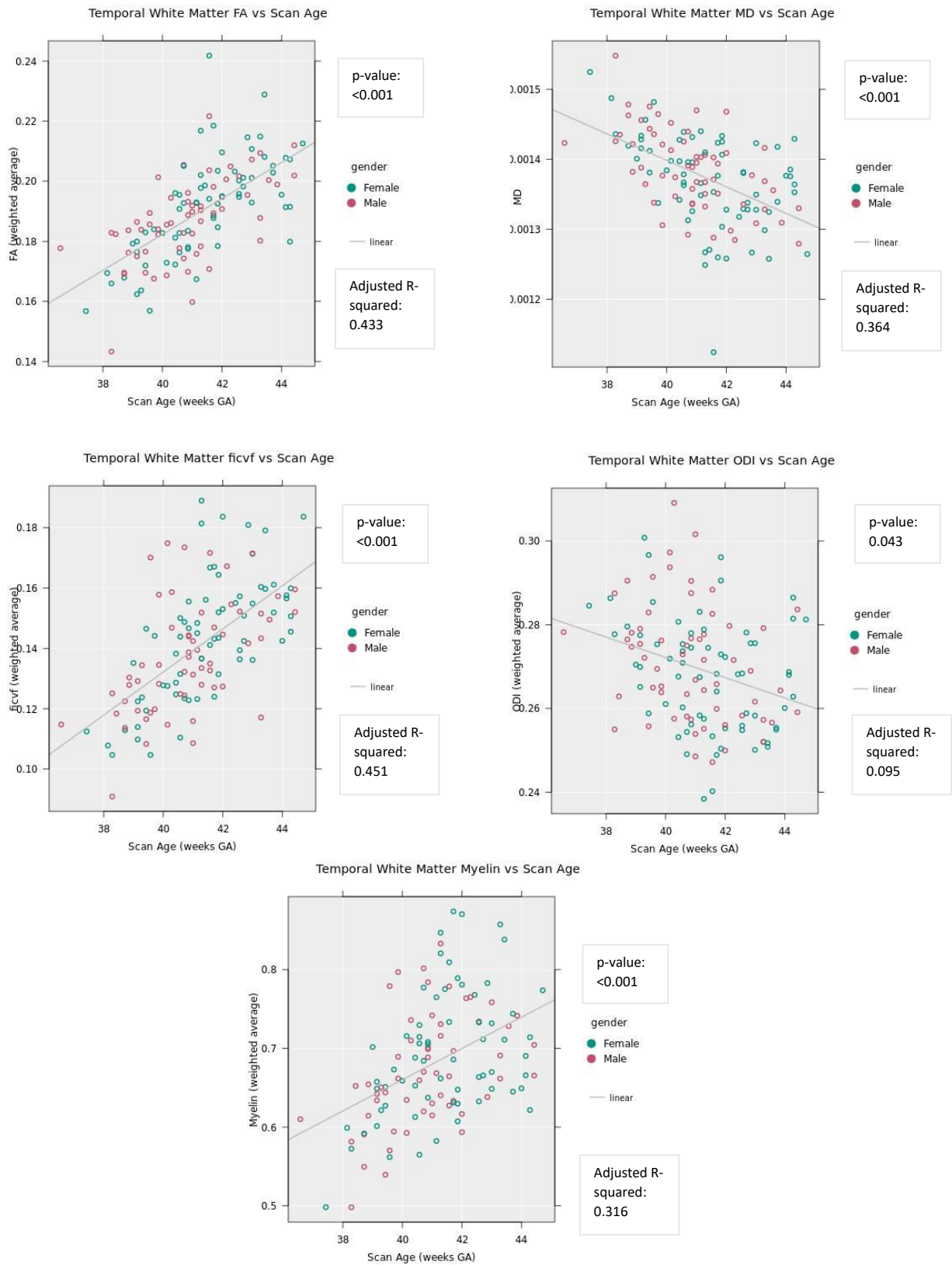


Figure 14. Temporal white matter DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and white matter volume).

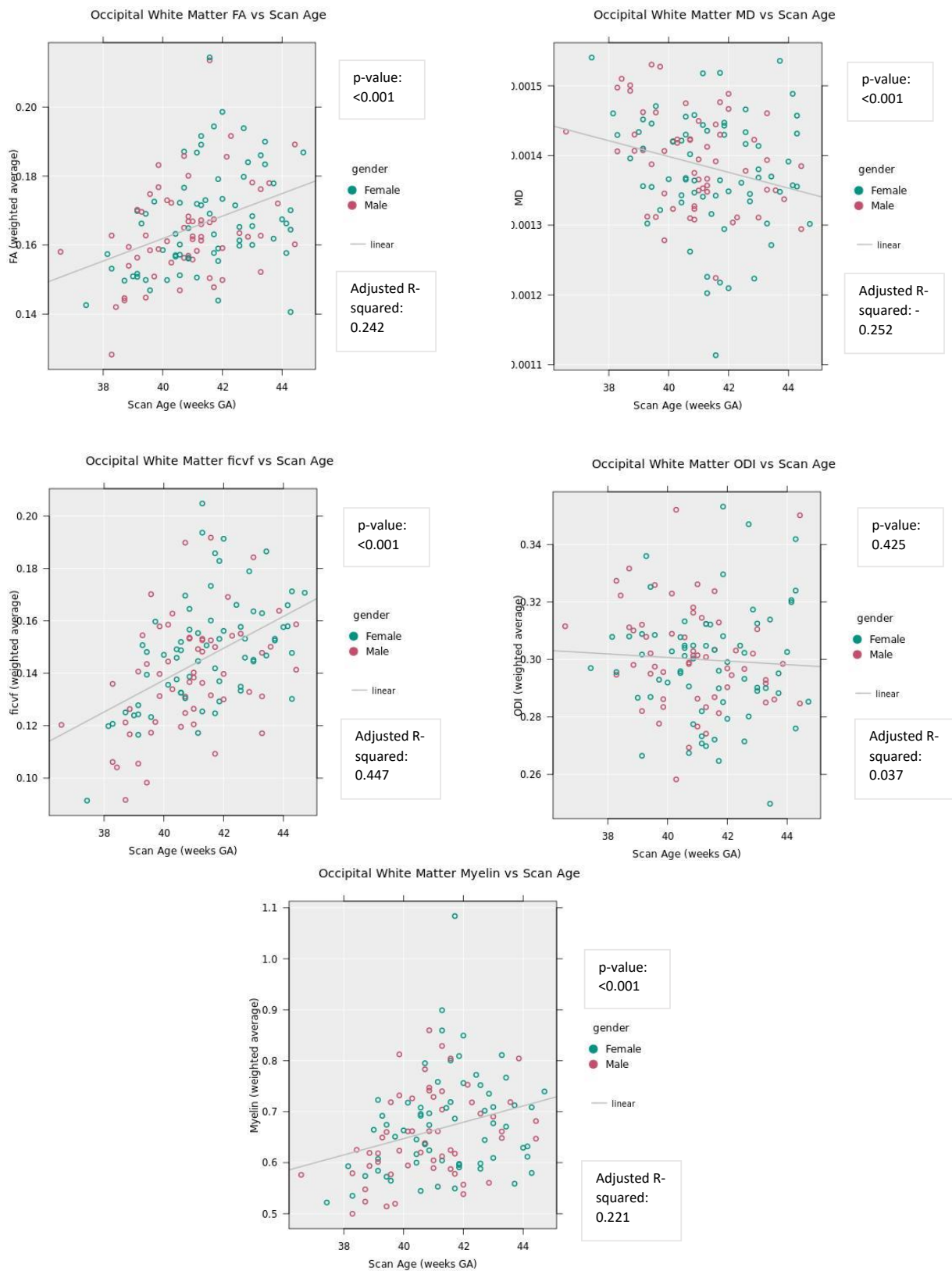


Figure 15. Occipital white matter DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and white matter volume).

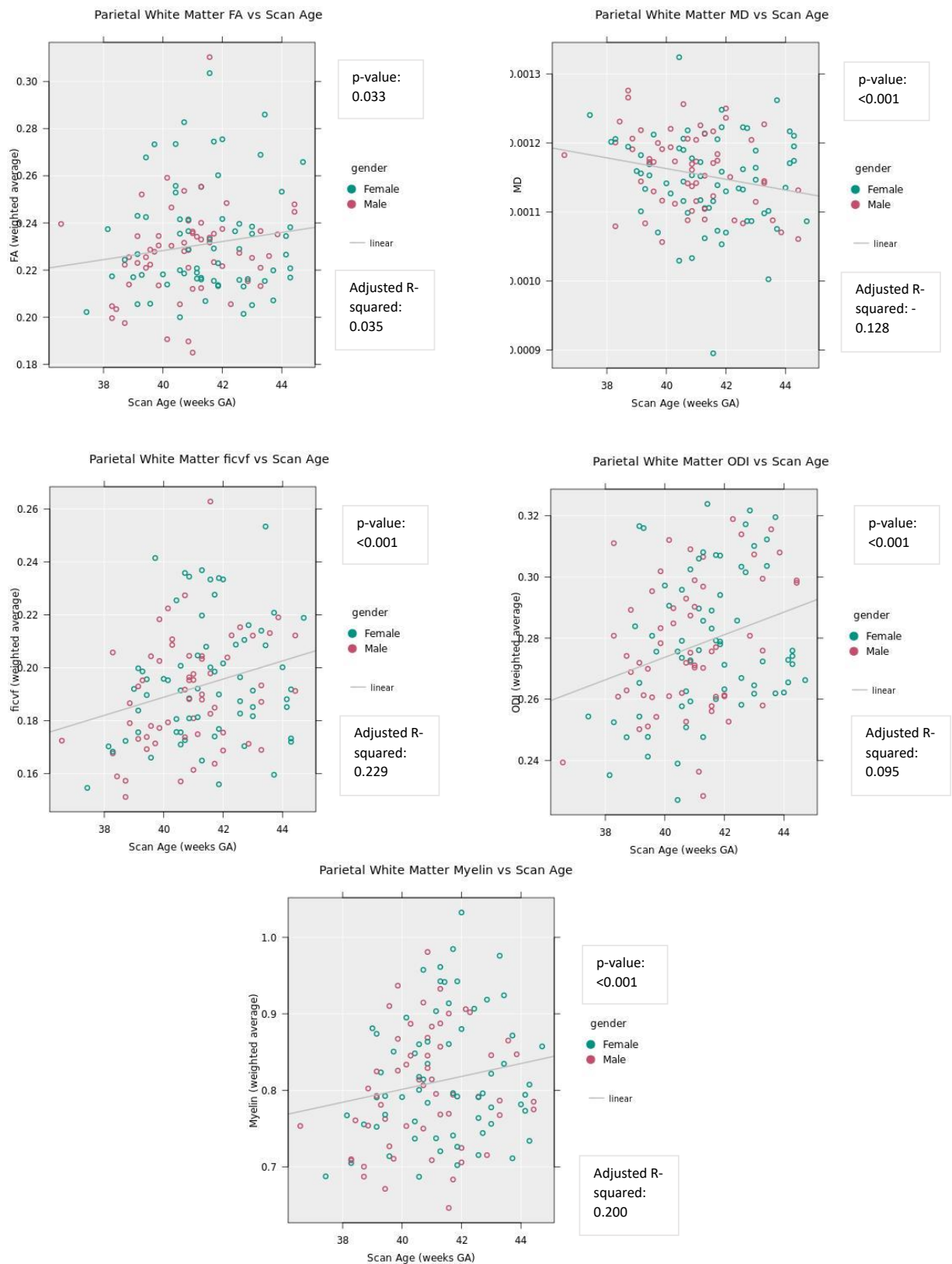


Figure 16. Parietal white matter DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and white matter volume).

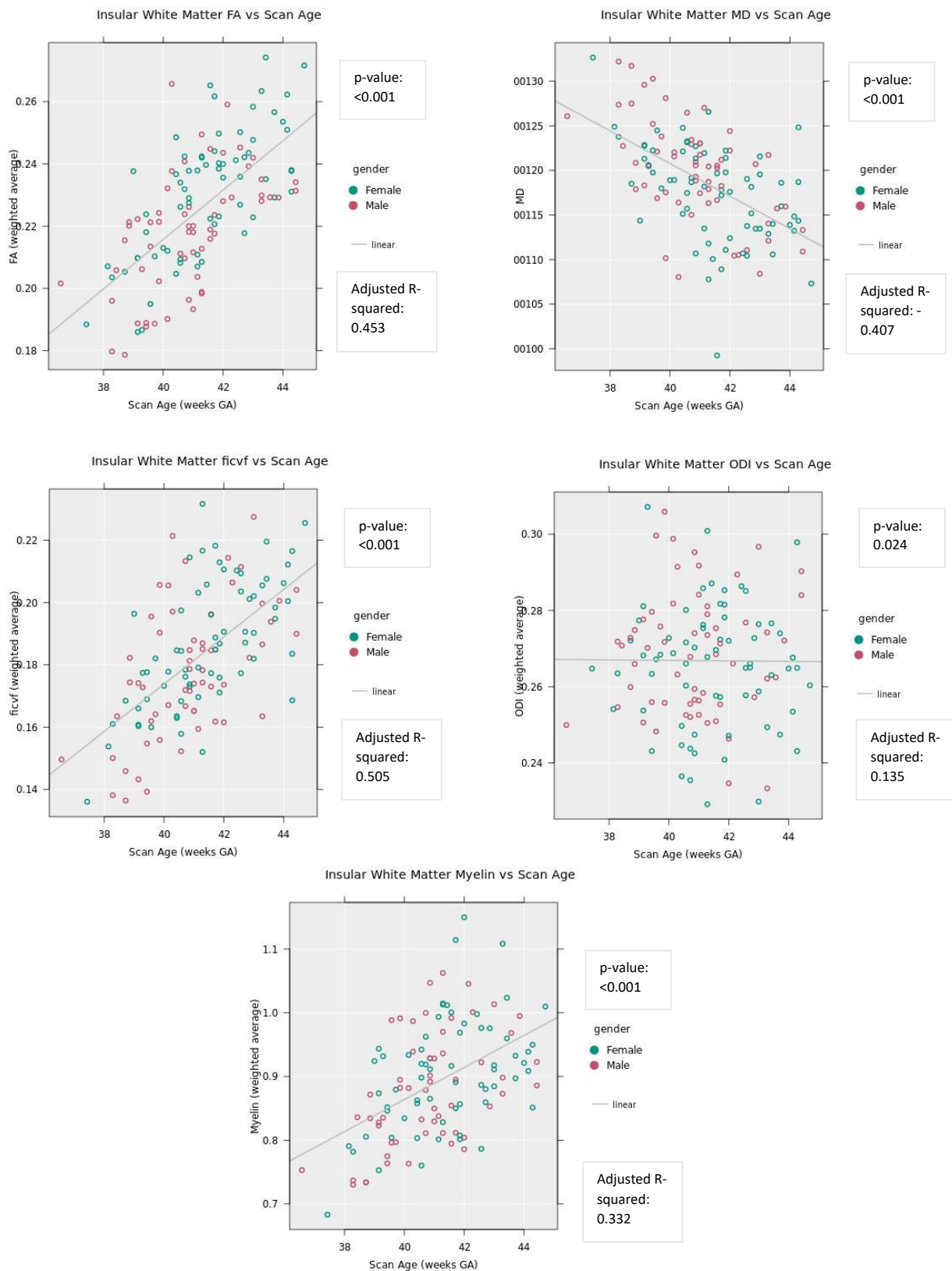


Figure 17. Insular white matter DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and white matter volume).

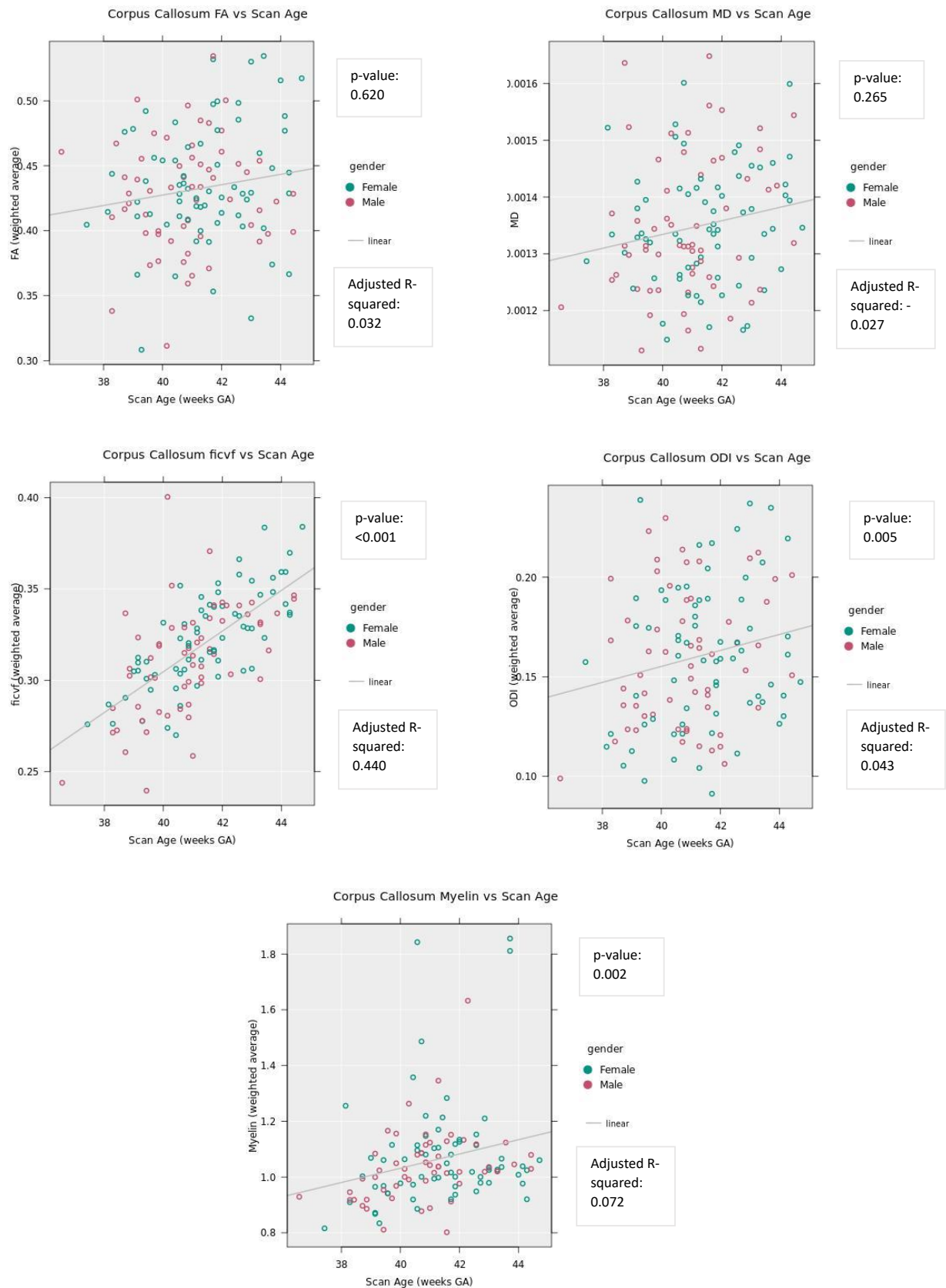


Figure 18. Corpus callosum DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and white matter volume).

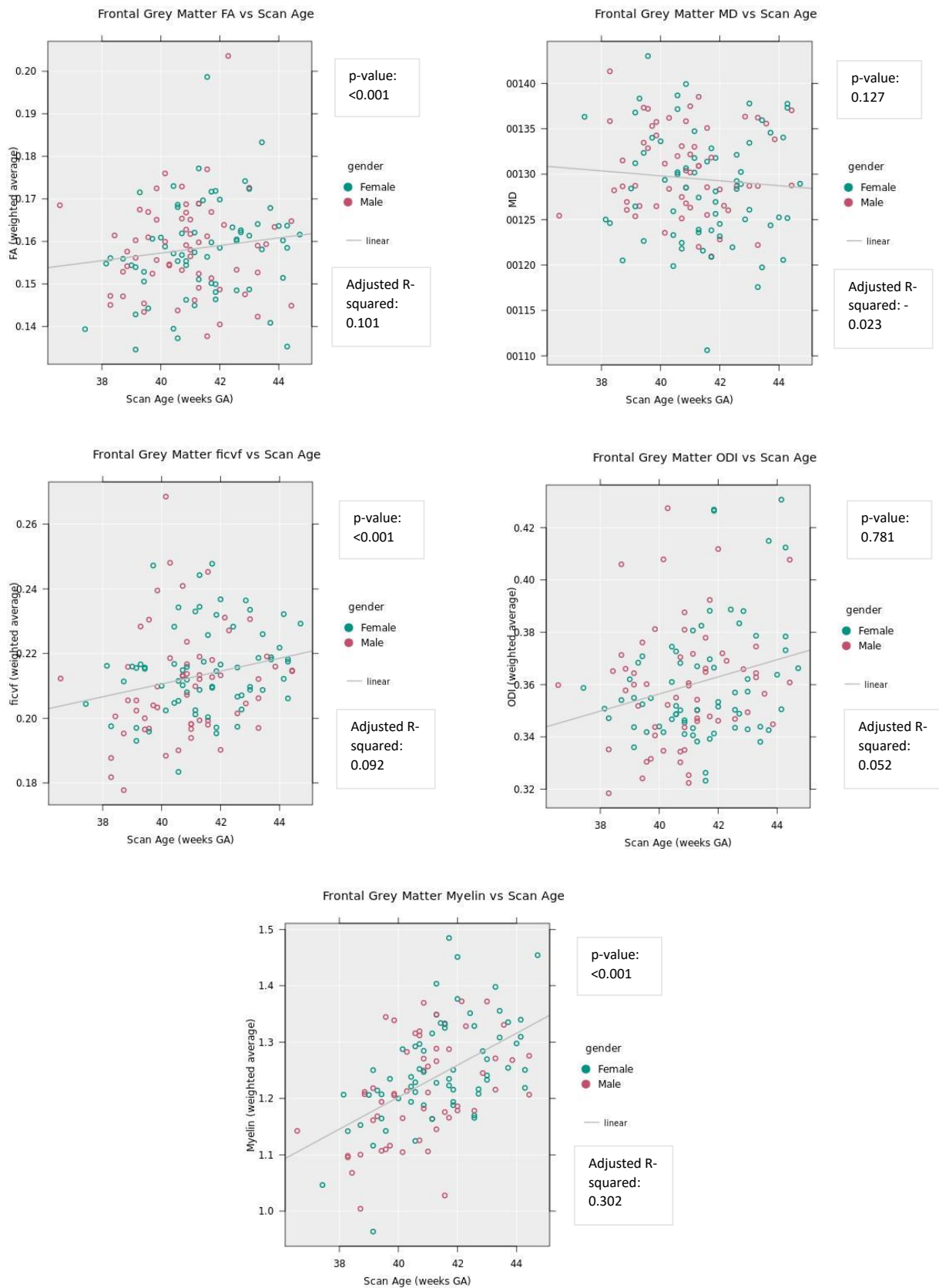


Figure 19. Frontal grey matter DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and cortical grey matter volume).

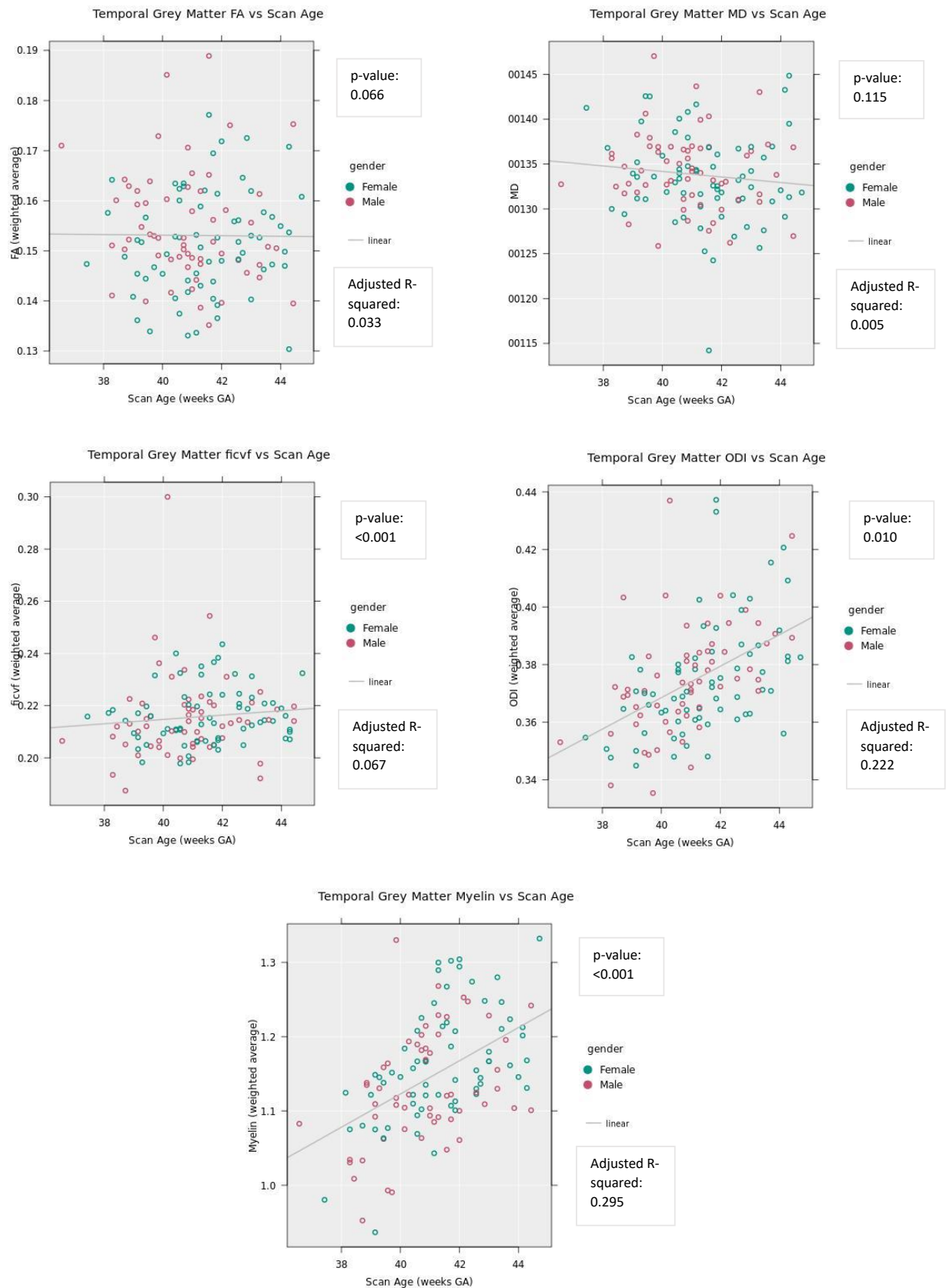


Figure 20. Temporal grey matter DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and cortical grey matter volume).

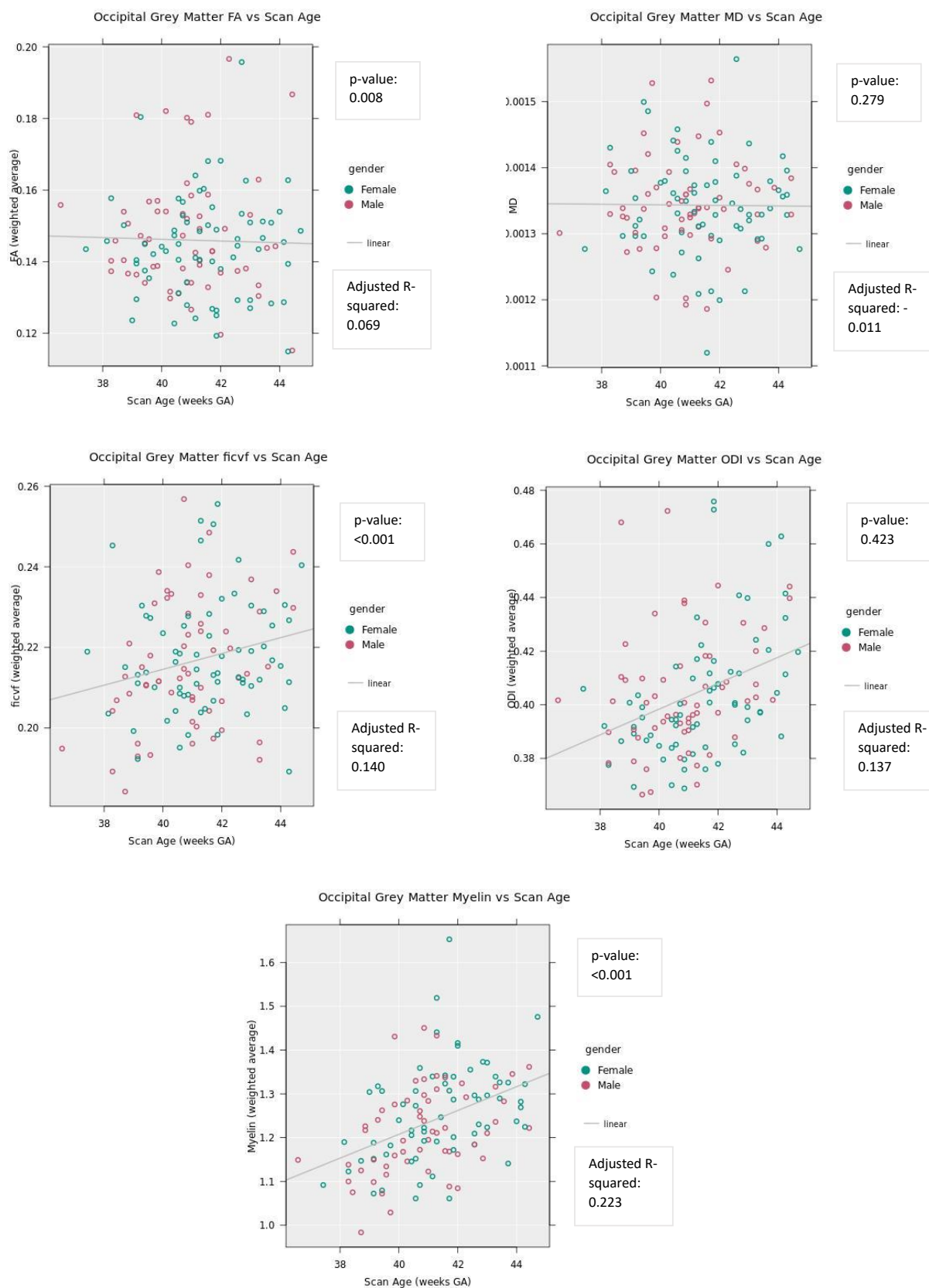


Figure 21. Occipital grey matter DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and cortical grey matter volume).

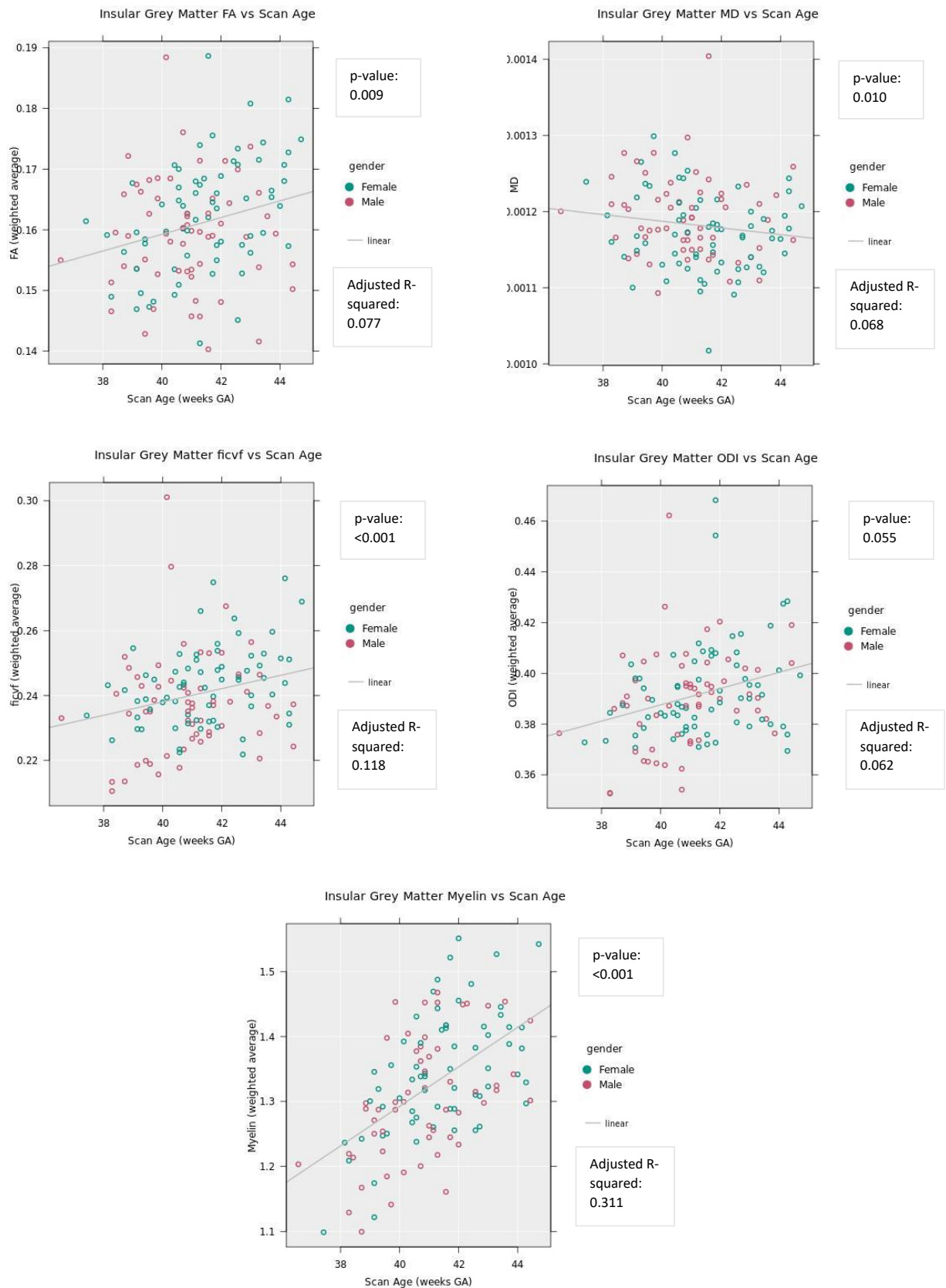


Figure 22. Insular grey matter DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and cortical grey matter volume).

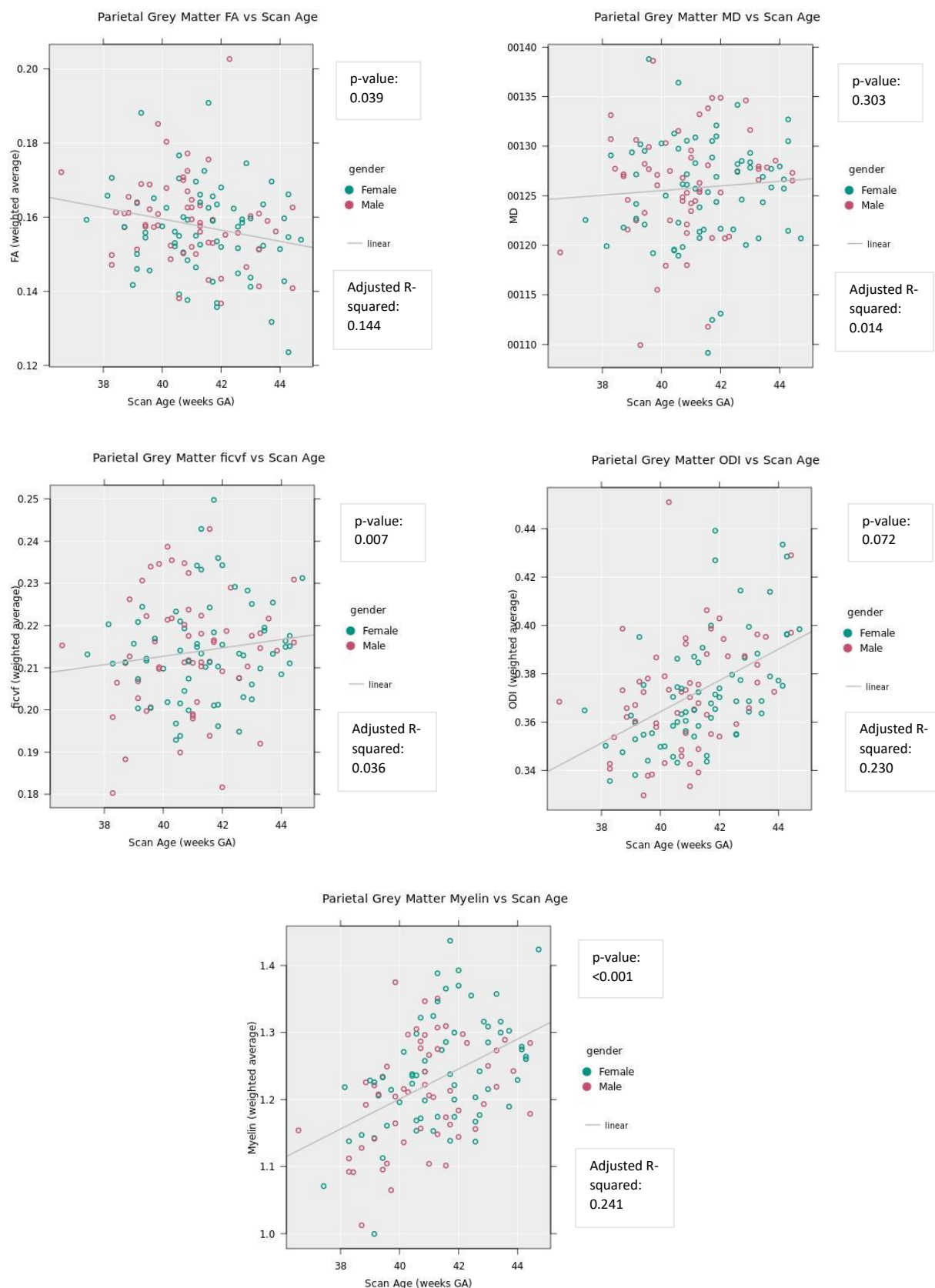


Figure 23. Parietal grey matter DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and cortical grey matter volume).

6.3.3 Deep Grey Matter

DGM was grouped into the following ROIs: the caudate nucleus, hippocampus and amygdala, lentiform nucleus, thalamus, subthalamic nucleus, and thalamus with low T2 intensity. In the caudate nucleus and the hippocampus and amygdala, scan age was associated with increasing FA ($p<0.001$), NDI ($p<0.001$), and myelin ($p<0.001$), but not MD ($p=0.190$, $p=0.150$) or ODI ($p=0.311$, $p=0.900$) (figures 24 and 25). In the lentiform nucleus, the thalamus, the subthalamic nucleus, and the thalamus with low T2 intensity, scan age was associated with increasing FA ($p<0.001$, $p<0.001$, $p<0.001$, $p=0.002$), NDI ($p<0.001$), and myelin ($p<0.001$, $p<0.001$, $p=0.002$, $p<0.001$), and decreasing MD ($p<0.001$), but not ODI ($p=0.040$, $p=0.059$, $p=0.456$, $p=0.071$) (figures 26-29). A negative trend was observed for ODI in the lentiform nucleus, and a positive trend for ODI in the thalamus, but these did not survive correction for multiple comparisons ($p=0.040$, $p=0.059$).

6.3.4 Cerebellum and Brainstem

In the cerebellum, scan age was associated with increasing FA ($p<0.001$), NDI ($p<0.001$), ODI ($p<0.001$), and myelin ($p<0.001$) and decreasing MD ($p<0.001$) (figure 30). In the brainstem, scan age was associated with increasing FA ($p<0.001$), NDI ($p<0.001$), and myelin ($p<0.001$), but not MD ($p=0.725$) or ODI ($p=0.803$) (figure 31).

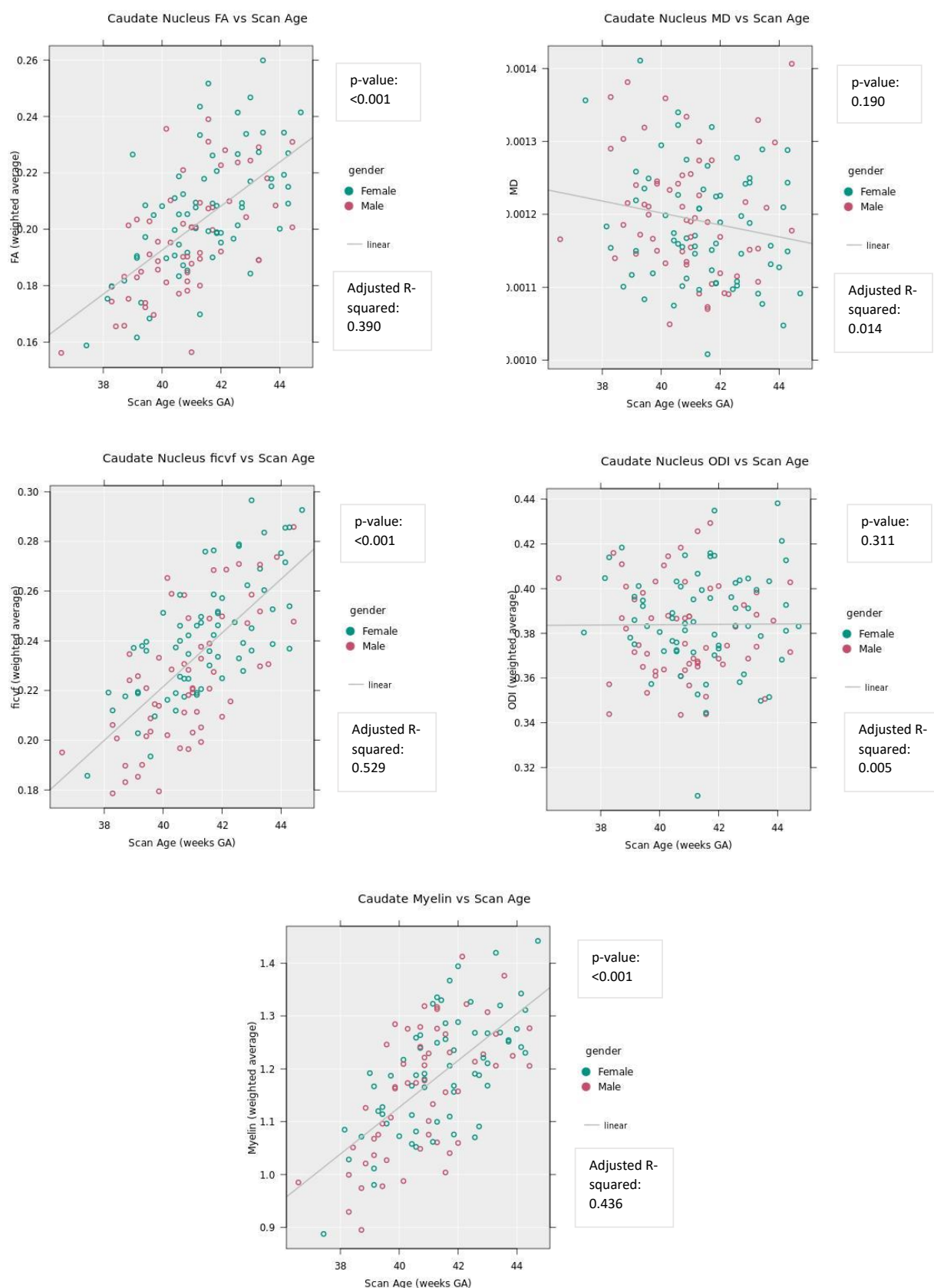


Figure 24. Caudate nucleus DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and deep grey matter volume).

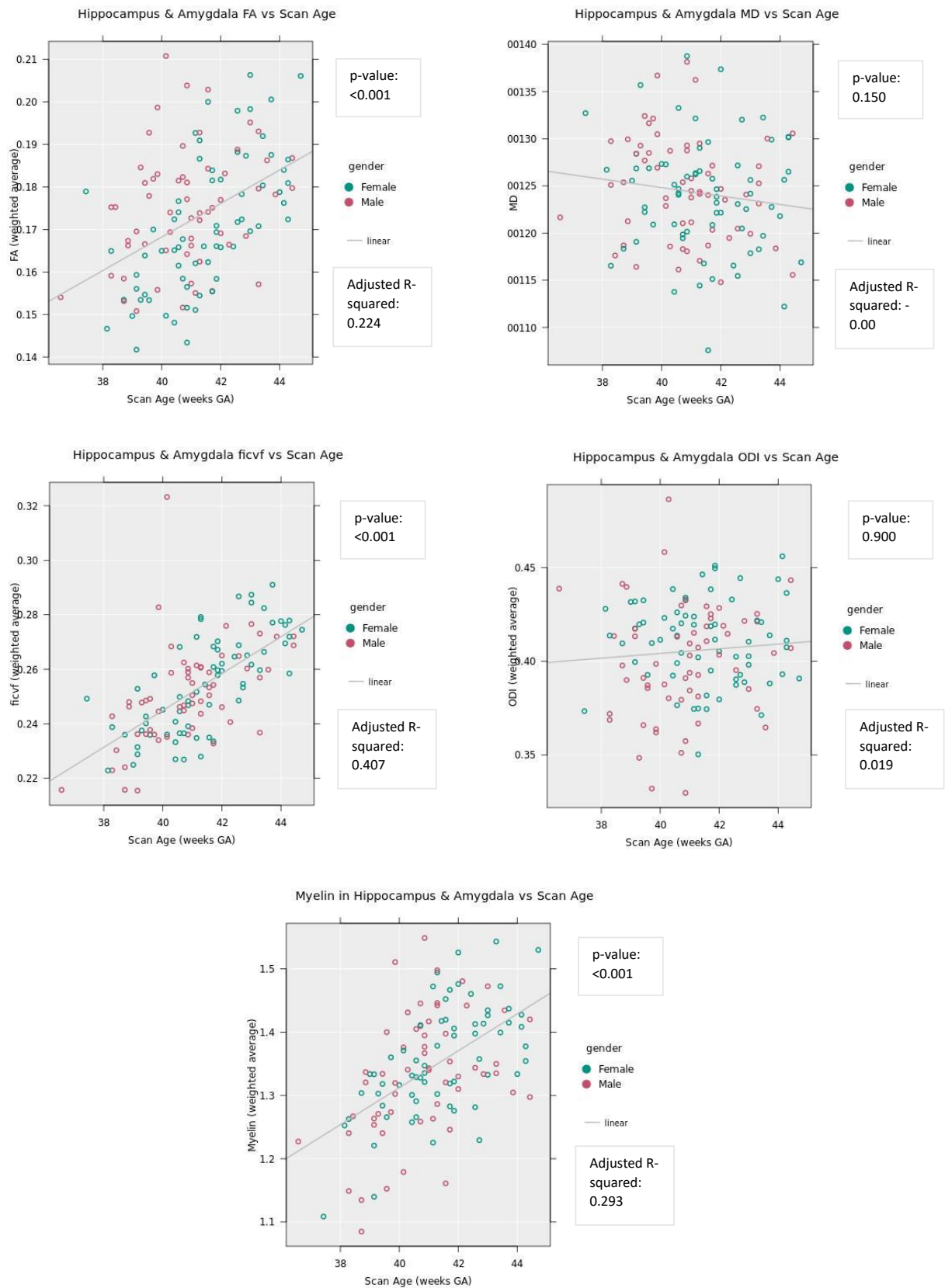


Figure 25. Hippocampus and amygdala DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and deep grey matter volume).

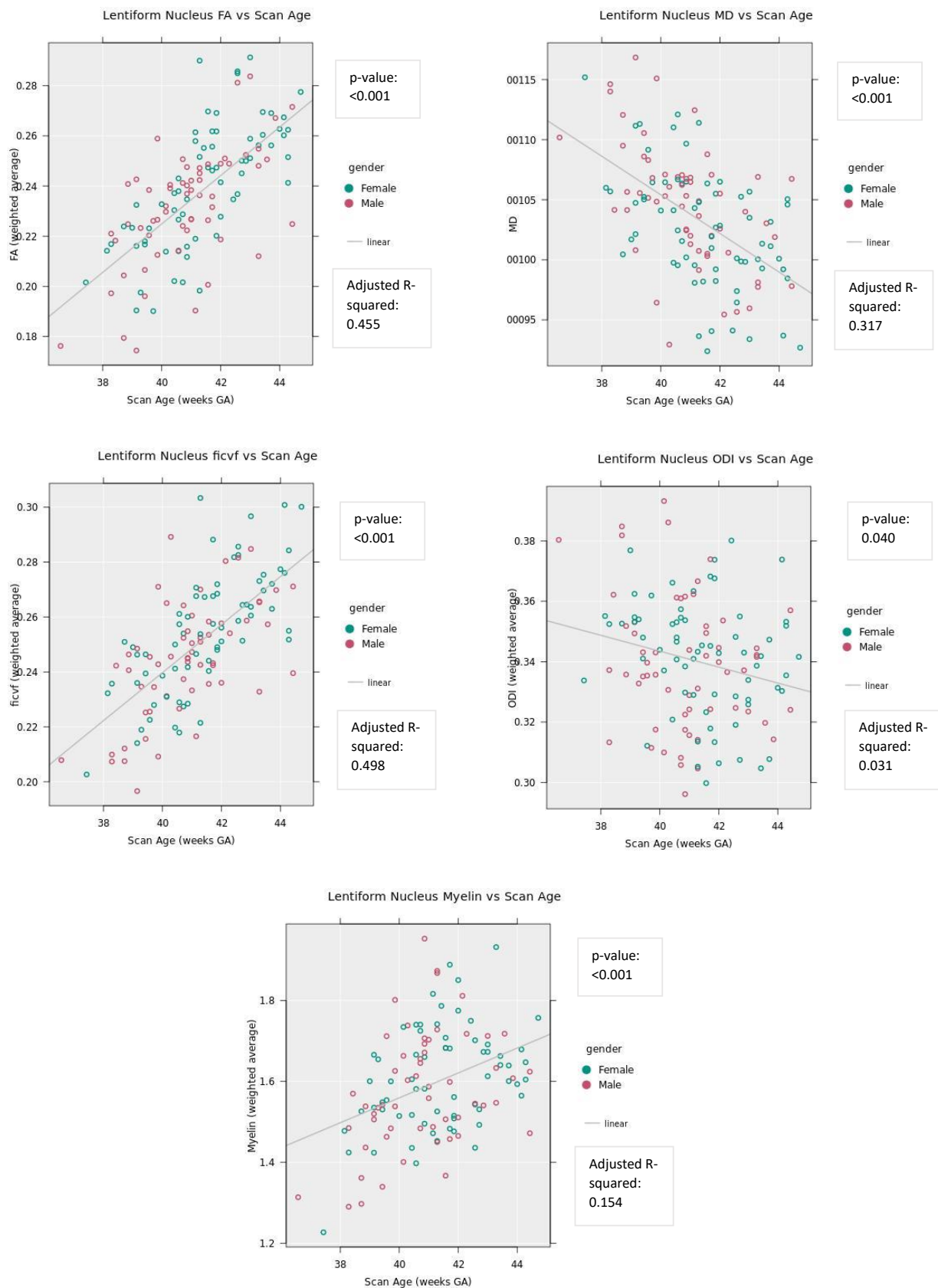


Figure 26. *Lentiform nucleus DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and deep grey matter volume).*

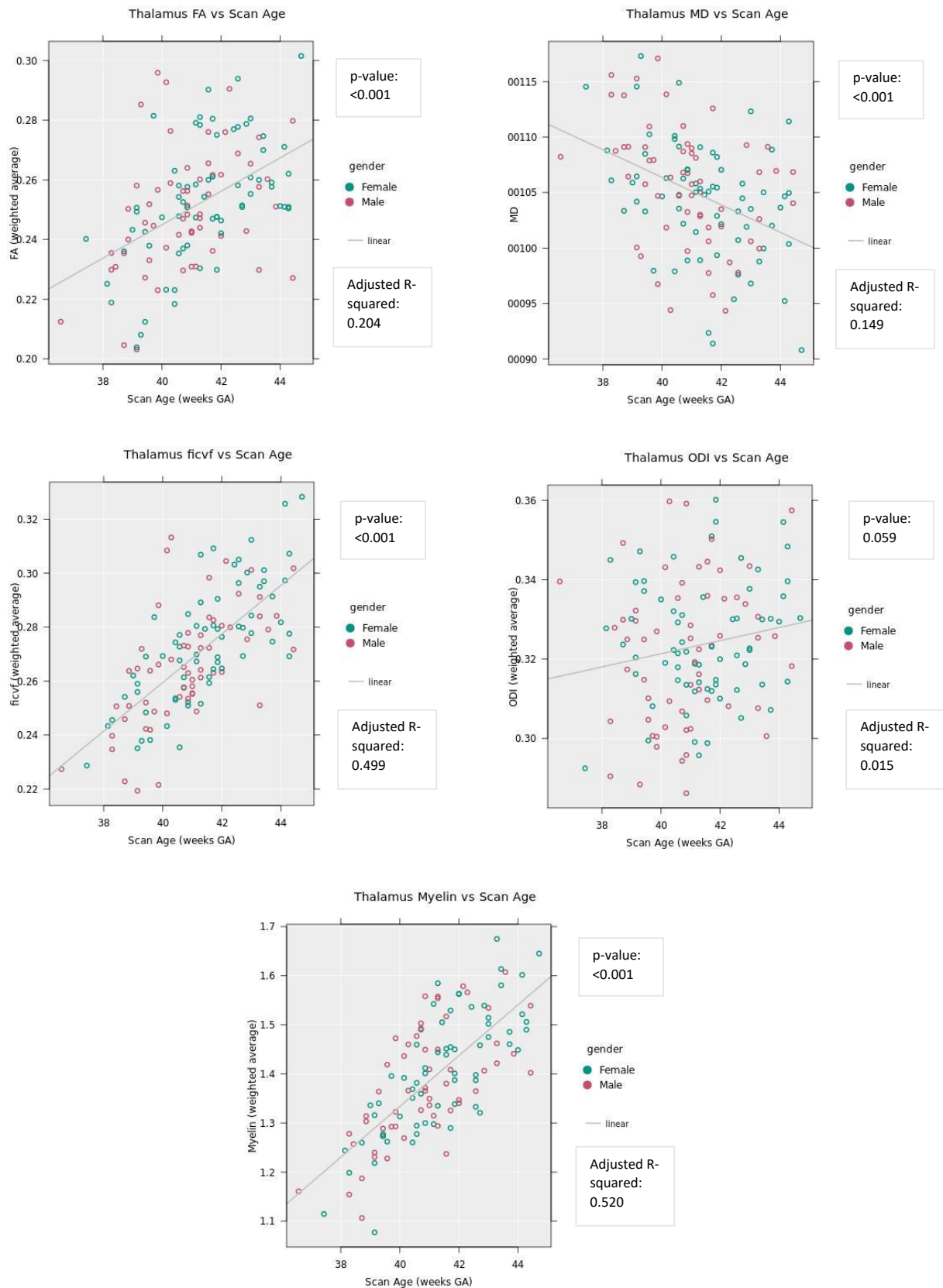


Figure 27. Thalamus DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and deep grey matter volume).

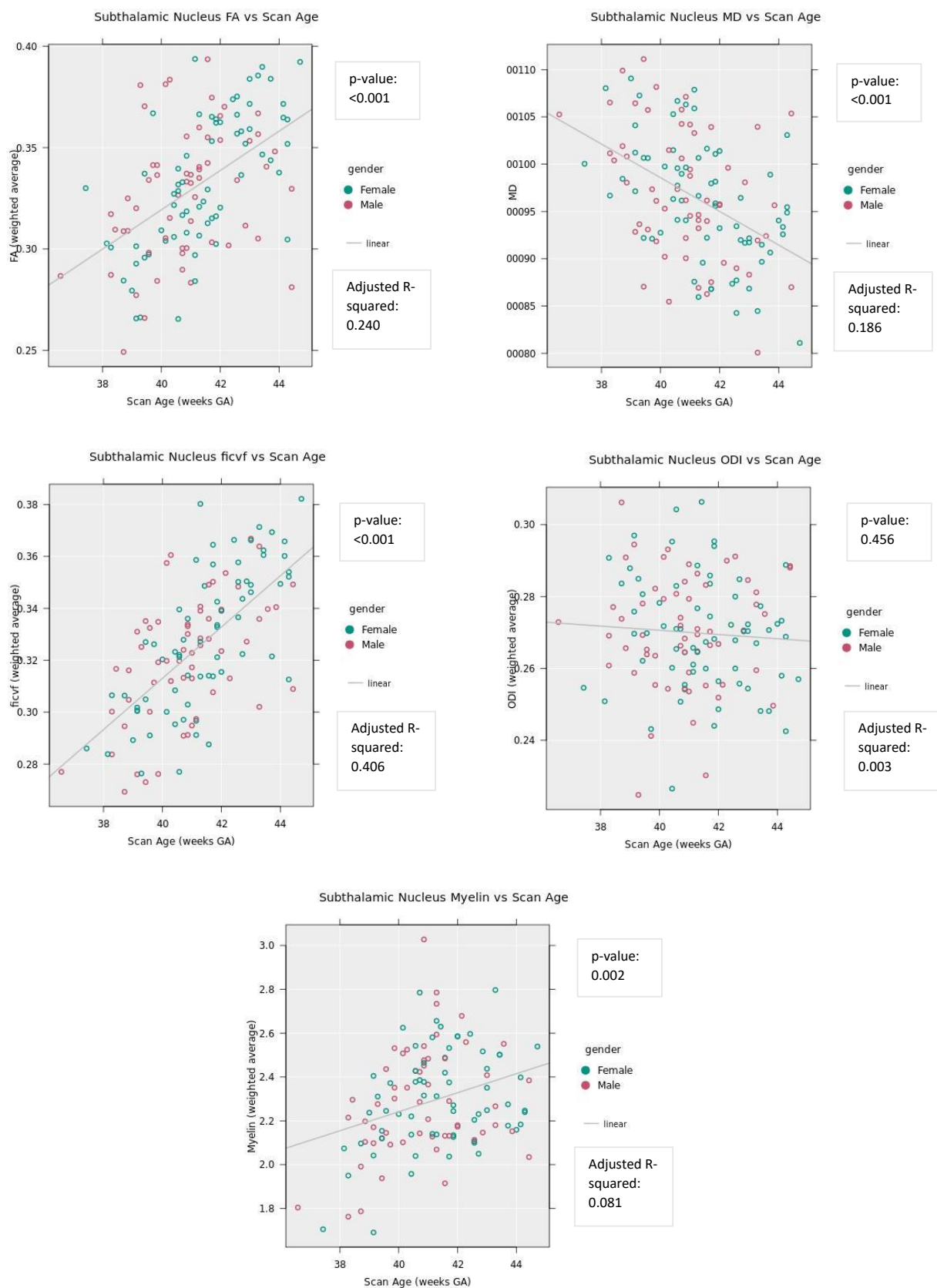


Figure 28. Subthalamic nucleus DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and deep grey matter volume).

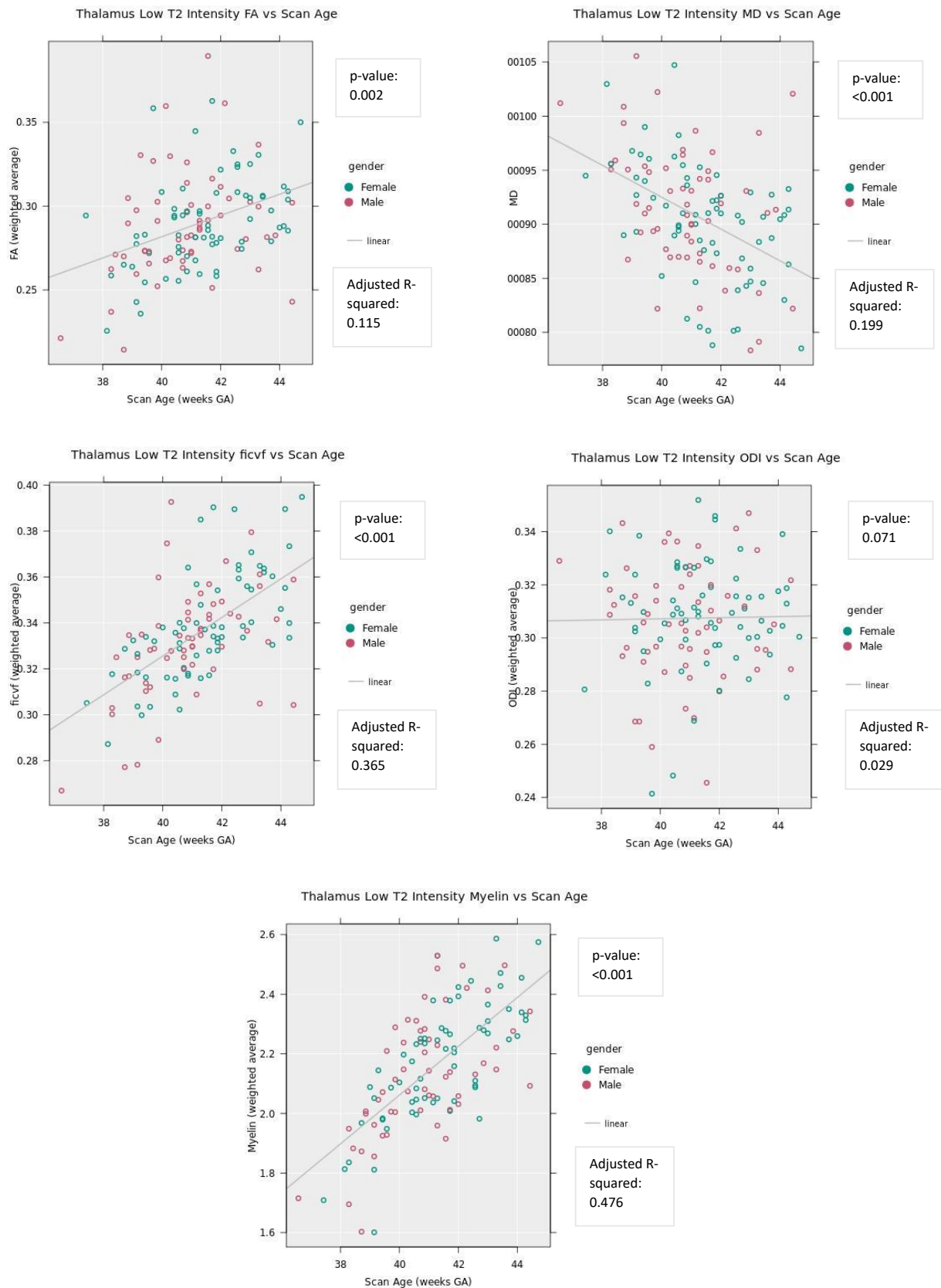


Figure 29. Thalamus with low T2 intensity DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and deep grey matter volume).

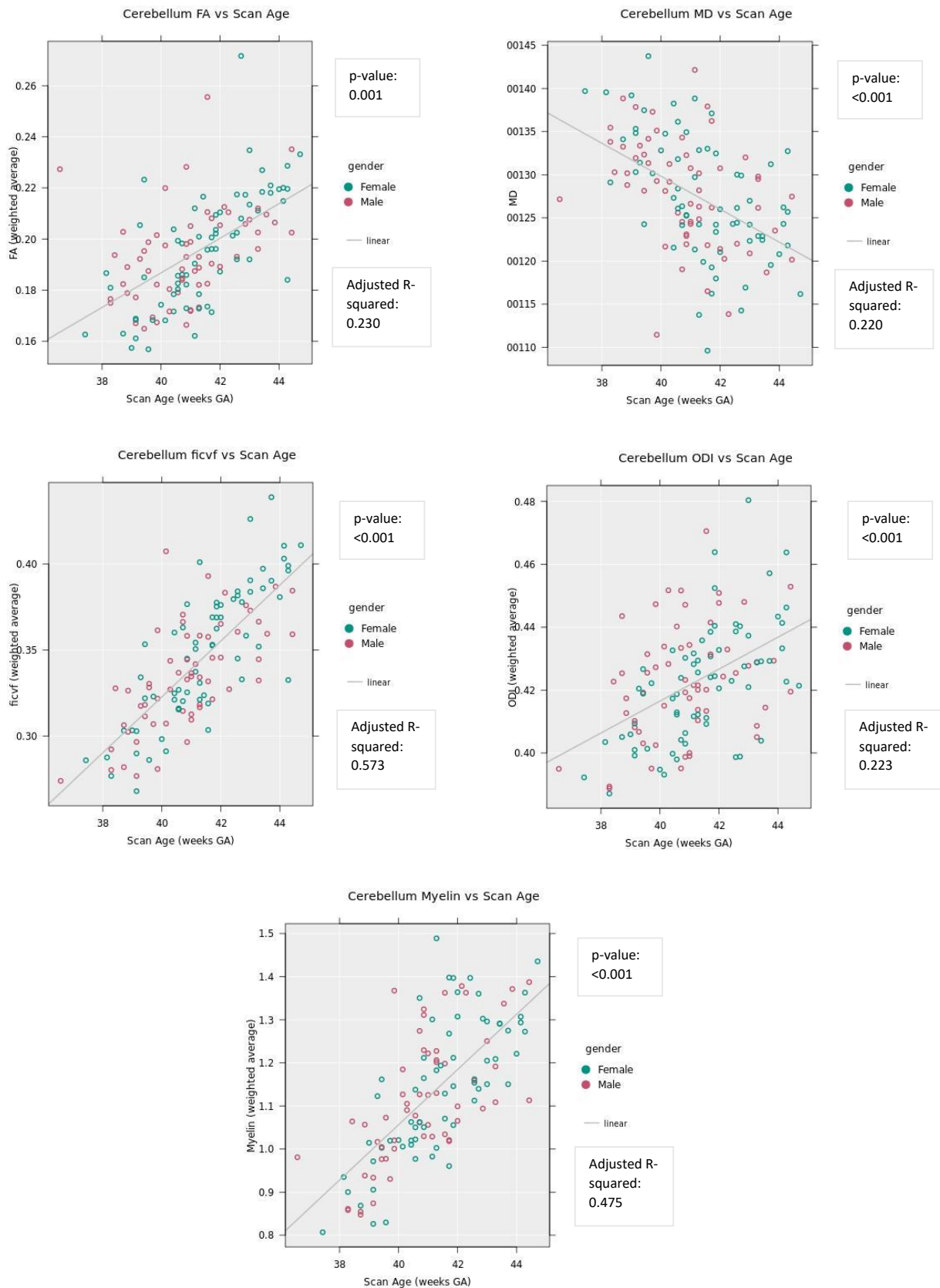


Figure 30. Cerebellum DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and cerebellar volume).

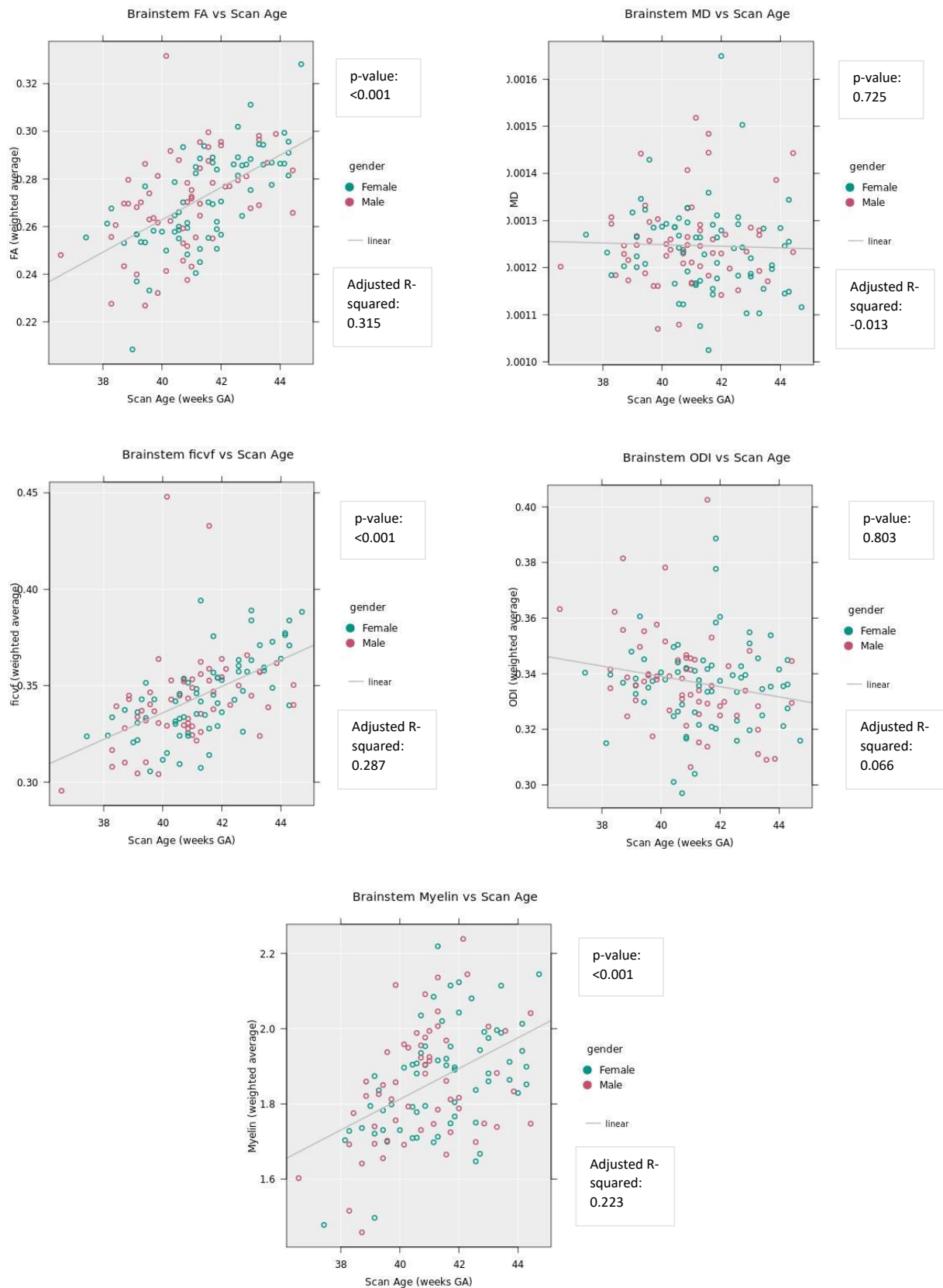


Figure 31. Brainstem DTI, NODDI, and myelin measures plotted against gestational age at scan (controlled for sex and brainstem volume).

6.4 Results - fMRI Measures vs Gestational Age

fMRI analyses were focused to regions of grey matter only. Globally (all CGM ROIs added together), linear regression revealed that fALFF was tentatively associated with increasing gestational age at scan, though this did not survive correction for multiple comparisons ($p=0.005$). No association was found between ALFF or mALFF and gestational age at scan ($p=0.787$, $p=0.603$) on a global level (figure 32). fALFF was also positively associated with gestational age at scan in several ROIs across the brain (results below), but no associations were found in any of the ROIs for ALFF or mALFF.

6.4.1 Cortical Grey Matter

fALFF was associated with increasing gestational age at scan in the frontal ($p=0.004$), temporal ($p=0.002$), and insular GM ($p<0.001$), but did not survive correction for multiple comparisons in the parietal ($p=0.022$) or occipital GM ($p=0.028$) (figure 33). In none of the CGM ROIs was gestational age at scan associated with ALFF ($p=0.731$, $p=0.828$, $p=0.376$, $p=0.707$, $p=0.699$) or mALFF ($p=0.556$, $p=0.348$, $p=0.096$, $p=0.461$, $p=0.659$).

6.4.2 Deep Grey Matter

fALFF was associated with increasing gestational age at scan in the caudate nucleus ($p=0.002$), and - although clear positive trends were seen - did not survive correction for multiple comparisons in the lentiform nucleus ($p=0.040$), hippocampus and amygdala ($p=0.051$), and thalamus ($p<0.031$). No association was seen between fALFF and scan age in the subthalamic nucleus ($p=0.590$) or the thalamus with low T2 intensity ($p=0.358$) (figure 34). In none of the DGM ROIs was gestational age at scan associated with ALFF ($p=0.912$, $p=0.907$, $p=0.970$, $p=0.454$, $p=0.521$, $p=0.991$) or mALFF ($p=0.644$, $p=0.548$, $p=0.856$, $p=0.264$, $p=0.213$, $p=0.615$).

The full DTI, NODDI, myelin and fMRI results for all regions can be seen in table 7.

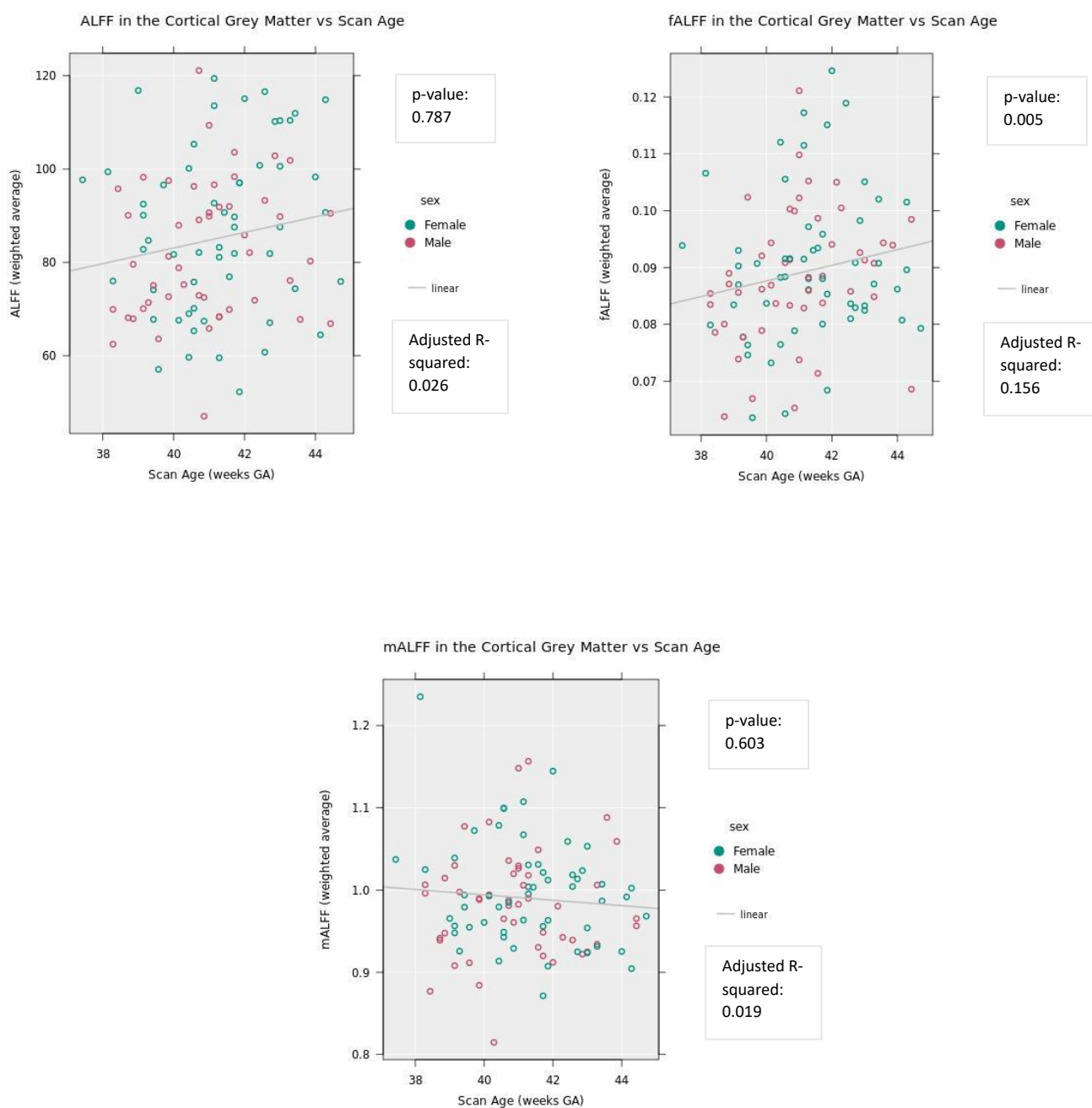


Figure 32. Global (grey matter) fMRI measures plotted against gestational age at scan (controlled for sex, cortical grey matter volume, and total framewise displacement).

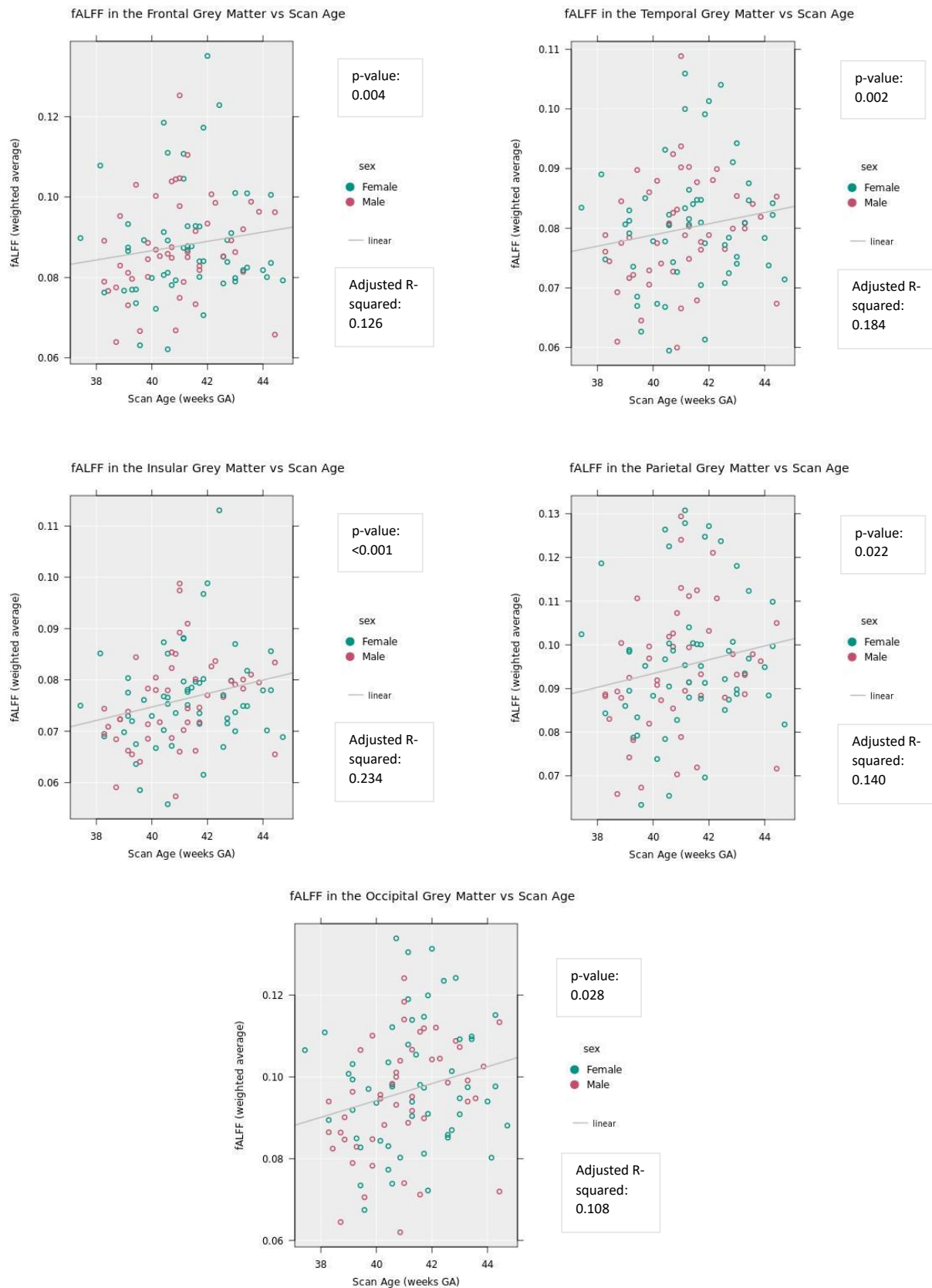


Figure 33. *fALFF in cortical grey matter regions of interest plotted against gestational age at scan (controlled for sex, cortical grey matter volume, and total framewise displacement).*

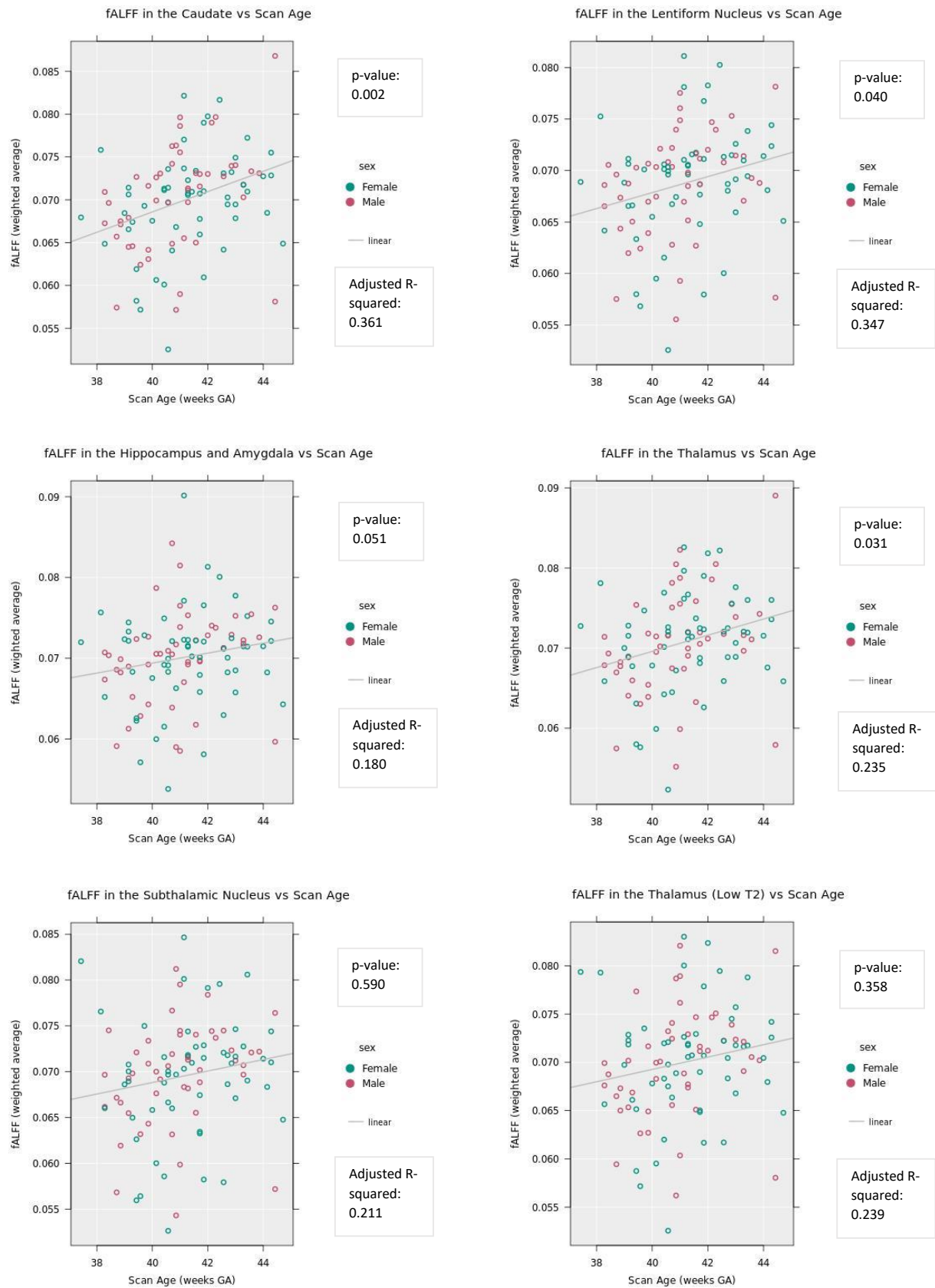


Figure 34. *fALFF in deep grey matter regions of interest plotted against gestational age at scan (controlled for sex, deep grey matter volume, and total framewise displacement).*

(GA) p-values	FA	MD	NDI	ODI	Myelin	ALFF	fALFF	mALFF
Global	<0.001+	<0.001-	<0.001+	0.002+	<0.001+	0.787	0.005	0.603
Frontal GM	<0.001+	0.127	0.001+	0.781	<0.001+	0.731	0.004+	0.556
Parietal GM	0.039	0.303	0.007	0.072	<0.001+	0.553	0.022	0.461
Temporal GM	0.066	0.115	0.001+	0.010	<0.001+	0.828	0.002+	0.348
Occipital GM	0.008	0.279	<0.001+	0.423	<0.001+	0.707	0.028	0.659
Insular GM	0.009	0.010	<0.001+	0.055	<0.001+	0.376	<0.001+	0.096
Thalamus	<0.001+	<0.001-	<0.001+	0.059	<0.001+	0.454	0.031	0.264
Subthalamic Nucleus	<0.001+	<0.001-	<0.001+	0.456	0.002+	0.521	0.590	0.213
Lentiform Nucleus	<0.001+	<0.001-	<0.001+	0.040	<0.001+	0.907	0.040	0.541
Caudate Nucleus	<0.001+	0.190	<0.001+	0.311	<0.001+	0.912	0.002+	0.644
Hippocampus & Amygdala	<0.001+	0.150	<0.001+	0.900	<0.001+	0.970	0.051	0.856
Thalamus (Low T2)	0.002+	<0.001-	<0.001+	0.071	<0.001+	0.991	0.358	0.615
Cerebellum	0.001+	<0.001-	<0.001+	<0.001+	<0.001+	-	-	-
Brainstem	<0.001+	0.725	<0.001+	0.803	<0.001+	-	-	-
Frontal WM	<0.001+	<0.001-	<0.001+	0.643	<0.001+	-	-	-
Parietal WM	0.033	<0.001-	<0.001+	<0.001+	<0.001+	-	-	-
Temporal WM	<0.001+	<0.001-	<0.001+	0.043	<0.001+	-	-	-
Occipital WM	<0.001+	<0.001-	<0.001+	0.425	<0.001+	-	-	-
Insular WM	<0.001+	<0.001-	<0.001+	0.024	<0.001+	-	-	-
Corpus Callosum	0.620	0.265	<0.001+	0.005	0.002+	-	-	-

Table 7. Summary of associations between MRI measures and gestational age (controlled for sex, brain volume by region, and motion (fMRI)). *p*-values in bold are those deemed statistically significant after Bonferroni correction for multiple comparisons, with a + or – denoting the direction of the association. Abbreviations: ALFF, amplitude of low-frequency fluctuation; FA, fractional anisotropy, fALFF, fractional amplitude of low-frequency fluctuation; GA, gestational age; GM, grey matter; mALFF, mean amplitude of low-frequency fluctuation; MD, mean diffusivity; NDI, neurite density index; ODI, orientation dispersion index; WM, white matter.

6.5 Results – DTI, NODDI and Myelin vs fMRI Measures

The relationship between DTI (FA, MD), NODDI (NDI, ODI) and myelin measures, and fMRI (ALFF, fALFF, mALFF) measures was looked at in regions of CGM and DGM, controlling for sex, brain volume by region, gestational age at scan, and motion. Table 8 shows the results in full, categorised by each fMRI measure. Linear regression found no statistically significant associations in any of the individual ROIs for ALFF, nor were there any global associations. Only one regional association was found for mALFF, which was positively associated with ODI in the subthalamic nucleus ($p=0.004$), whilst mALFF was negatively associated with ODI on a global level ($p=0.003$). A few regional trends were observed, but these did not survive correction for multiple comparisons. fALFF was found to be positively associated with myelin in the parietal GM ($p=0.001$). fALFF was also positively associated with myelin on a global (GM) level, though this just missed statistical significance after correction for multiple comparisons ($p=0.005$). Positive trends were observed for fALFF and myelin in several CGM and DGM ROIs but did not survive correction for multiple comparisons. A negative relationship was also found between fALFF and MD in the occipital GM ($p=0.004$). Figure 35 visualises these statistically significant associations.

<i>ALFF</i>					
p-values	FA	MD	NDI	ODI	Myelin
Global	0.463	0.958	0.366	0.833	0.630
Frontal GM	0.533	0.833	0.271	0.670	0.463
Parietal GM	0.728	0.344	0.274	0.185	0.231
Temporal GM	0.587	0.862	0.420	0.359	0.117
Occipital GM	0.092	0.087	0.773	0.963	0.573
Insular GM	0.037	0.273	0.027	0.978	0.637
Thalamus	0.732	0.581	0.769	0.200	0.542
Subthalamic Nucleus	0.752	0.343	0.802	0.223	0.972
Lentiform Nucleus	0.844	0.803	0.671	0.094	0.515
Caudate Nucleus	0.094	0.696	0.066	0.166	0.183

Hippocampus & Amygdala	0.499	0.632	0.354	0.052	0.595
Thalamus (Low T2)	0.848	0.641	0.528	0.681	0.481
<i>fALFF</i>					
p-values	FA	MD	NDI	ODI	Myelin
Global	0.799	0.074	0.038	0.749	0.005+
Frontal GM	0.665	0.790	0.077	0.552	0.027
Parietal GM	0.989	0.022	0.118	0.951	0.001+
Temporal GM	0.894	0.483	0.066	0.850	0.016
Occipital GM	0.369	0.004-	0.762	0.552	0.115
Insular GM	0.695	0.544	0.279	0.933	0.063
Thalamus	0.108	0.954	0.298	0.006	0.016
Subthalamic Nucleus	0.055	0.933	0.316	0.027	0.096
Lentiform Nucleus	0.277	0.194	0.030	0.671	0.061
Caudate Nucleus	0.575	0.622	0.217	0.946	0.094
Hippocampus & Amygdala	0.744	0.411	0.219	0.292	0.015
Thalamus (Low T2)	0.246	0.560	0.489	0.326	0.118
<i>mALFF</i>					
p-values	FA	MD	NDI	ODI	Myelin
Global	0.865	0.529	0.400	0.003-	0.290
Frontal GM	0.340	0.989	0.401	0.020	0.769
Parietal GM	0.438	0.099	0.512	0.159	0.031
Temporal GM	0.553	0.024	0.350	0.567	0.075
Occipital GM	0.917	0.167	0.625	0.515	0.095
Insular GM	0.900	0.499	0.489	0.013	0.653
Thalamus	0.273	0.143	0.199	0.980	0.186
Subthalamic Nucleus	0.626	0.751	0.427	0.004+	0.374
Lentiform Nucleus	0.319	0.093	0.103	0.469	0.063

Caudate Nucleus	0.136	0.251	0.392	0.603	0.027
Hippocampus & Amygdala	0.788	0.088	0.756	0.192	0.636
Thalamus (Low T2)	0.152	0.327	0.089	0.407	0.278

Table 8. *Summary of associations between DTI, NODDI and myelin measures, and fMRI measures (controlled for sex, brain volume by region, and motion). p-values in bold are those deemed statistically significant after Bonferroni correction for multiple comparisons, with a + or – denoting the direction of the association. Abbreviations: ALFF, amplitude of low-frequency fluctuation; FA, fractional anisotropy, fALFF, fractional amplitude of low-frequency fluctuation; GA, gestational age; GM, grey matter; mALFF, mean amplitude of low-frequency fluctuation; MD, mean diffusivity; NDI, neurite density index; ODI, orientation dispersion index; WM, white matter.*

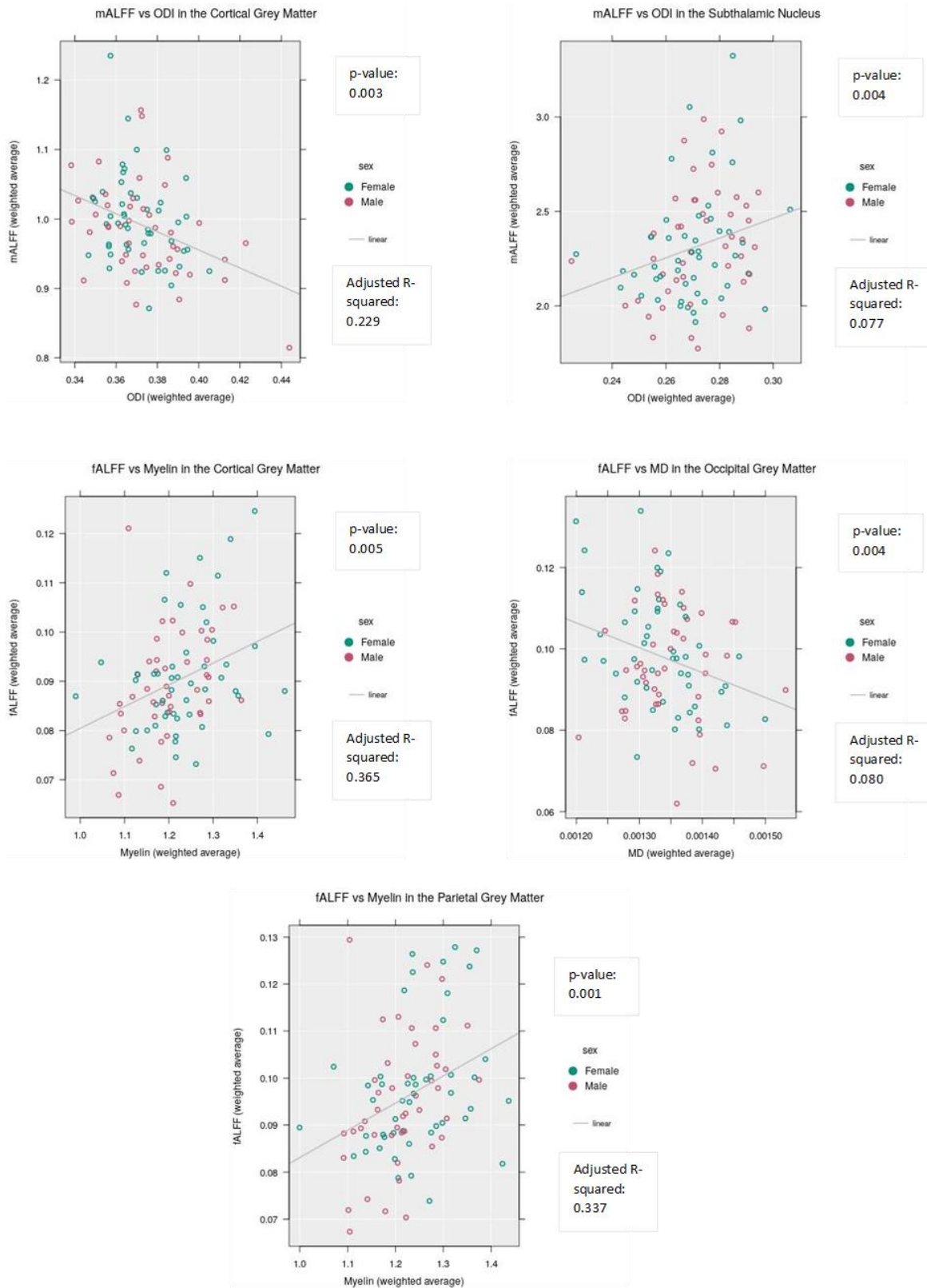


Figure 35. Statistically significant associations between mALFF/fALFF and structural MRI measures (controlled for sex, brain volume by region, and total framewise displacement). We

also include global (GM) fALFF vs myelin, which just missed significance after Bonferroni correction.

6.6 Results – Myelin (orthogonalized) vs all MRI Measures

Globally, we found no statistically significant associations between myelin and any of the DTI (FA, MD) or NODDI (NDI, ODI) measures. Regionally, however, we found numerous associations between different measures across the brain, which can be seen in full in table 9. Most notably, FA and NDI were positively associated with myelin in most WM regions and in some CGM and DGM regions, whilst MD showed a negative association with myelin in most regions. The exception was the corpus callosum, in which no associations were found. On the other hand, ODI was not associated with myelin in any region – a positive trend was observed in parietal, temporal, and insular WM, though these did not survive correction for multiple comparisons ($p=0.011$, $p=0.036$, $p=0.011$). Conversely, we found only one statistically significant regional association between fMRI measures and myelin – for fALFF in the parietal GM ($p=0.001$). We did also find a positive association between fALFF and myelin on a global (GM) level that just missed statistical significance after Bonferroni correction ($p=0.005$). It should be noted, however, that quite a few regional positive trends for fALFF were observed that didn't survive correction for multiple comparisons, in the frontal ($p=0.027$) and temporal ($p=0.016$) GM, the thalamus ($p=0.016$), and the hippocampus and amygdala ($p=0.015$). No statistically significant associations were found between ALFF/mALFF and myelin.

(Myelin) p-values	FA	MD	NDI	ODI	ALFF	fALFF	mALFF
Global	0.900	0.020	0.663	0.092	0.630	0.005+	0.290
Frontal GM	<0.001+	0.007	<0.001+	0.993	0.463	0.027	0.769
Parietal GM	0.005	<0.001-	<0.001+	0.380	0.231	0.001+	0.031
Temporal GM	0.148	0.002-	0.024	0.521	0.117	0.016	0.075
Occipital GM	0.677	<0.001-	<0.001+	0.214	0.573	0.115	0.095
Insular GM	0.002+	<0.001-	0.012	0.528	0.637	0.063	0.653
Thalamus	<0.001+	0.007	<0.001+	0.091	0.542	0.016	0.186

Subthalamic Nucleus	0.067	0.021	0.010	0.528	0.972	0.096	0.374
Lentiform Nucleus	0.102	0.002-	0.002+	0.427	0.515	0.061	0.063
Caudate Nucleus	0.153	0.098	0.184	0.130	0.183	0.094	0.027
Hippocampus & Amygdala	0.268	0.200	0.597	0.069	0.595	0.015	0.636
Thalamus (Low T2)	0.003	<0.001-	<0.001+	0.356	0.481	0.118	0.278
Cerebellum	0.004	<0.001-	<0.001+	0.279	-	-	-
Brainstem	0.123	0.003	0.119	0.330	-	-	-
Frontal WM	<0.001+	<0.001-	<0.001+	0.548	-	-	-
Parietal WM	0.004	<0.001-	<0.001+	0.011	-	-	-
Temporal WM	<0.001+	<0.001-	<0.001+	0.036	-	-	-
Occipital WM	<0.001+	<0.001-	<0.001+	0.487	-	-	-
Insular WM	0.004	<0.001-	<0.001+	0.011	-	-	-
Corpus Callosum	0.512	0.454	0.997	0.557	-	-	-

Table 9. Summary of associations between myelin (orthogonalized in relation to GA) and MRI measures (controlled for sex, brain volume by region, gestational age at scan and motion (fMRI)). *p*-values in bold are those deemed statistically significant after Bonferroni correction for multiple comparisons, with a + or – denoting the direction of the association. Abbreviations: ALFF, amplitude of low-frequency fluctuation; FA, fractional anisotropy, fALFF, fractional amplitude of low-frequency fluctuation; GM, grey matter; mALFF, mean amplitude of low-frequency fluctuation; MD, mean diffusivity; NDI, neurite density index; ODI, orientation dispersion index; WM, white matter.

6.7 Discussion

In this study, we investigated microstructural grey and white matter differences across the neonatal brain using structural and functional imaging. We first found that DTI, NODDI, and myelin measures were highly associated with the gestational age of the infant at scan, both at

the global and regional levels of the brain. Globally, increasing FA, NDI, ODI, and myelin, and decreasing MD values were strongly associated with increasing gestational age, which seems to reflect widespread patterns of microstructural development, such as decreases in total brain water content, enhanced organisation and complexity of fibre architecture, increases in membrane density, and myelination²⁰⁴. Regionally, however, we found more variability in the nature of these associations.

Both NDI and myelin similarly continued to show a strong association with gestational age in all regions of the brain (both GM and WM). This is consistent with previous studies that have found that the cortical distribution of NDI is highly similar to that of cortical myelin maps, indicating that neuron density co-varies with myelin content^{183,205}. Indeed, NDI has been found to increase significantly in the neonatal brain between 38-47 weeks in both the grey and white matter, consistent with increasing neuronal and organelle density^{206,207}. On the other hand, we found far fewer regional associations between ODI and gestational age (only two individual ROIs showed statistically significant relationships with scan age: the parietal WM and the cerebellum). It has been found that the developmental trajectory of ODI is slightly different to that of NDI/myelin in the foetal/neonatal brain, with studies showing that whilst ODI increases significantly between 25-38 weeks, it then plateaus and shows little change between 38-47 weeks^{206,207}. At 38 weeks gestation, increased dendritic arborisation replaces the columnar organisation that is seen in the foetal cortex, meaning there is little effect on ODI at a voxel level²⁰⁶, which is consistent with our regional ODI findings. Interestingly, the two ROIs (parietal WM and the cerebellum) in which we did find an association were positively associated with gestational age, suggesting that this plateau in ODI at around 38 weeks may occur slightly earlier in these particular regions.

Interestingly, in the corpus callosum, gestational age at scan was associated with NDI and myelin, but not DTI measures or ODI (although there was a trend for the latter). The corpus callosum - a central WM tract connecting the left and right hemispheres - is already a highly organised and aligned structure at birth, with high values of FA and low values of ODI^{74,204}. In fact, the key components of the corpus callosum are present by 20 weeks gestation³⁵, which may explain why we found no statistically significant associations between DTI metrics and ODI to gestational age in the full-term neonate. Despite this, between 20 weeks gestation and term, the corpus callosum shows robust growth in terms of length and thickness²⁰⁸. NDI is a

reflection of the density of neurons, and our results suggest that even slight differences in gestational age at birth will significantly affect NDI levels in the corpus callosum until it reaches its peak number of axons shortly after birth (after which the pruning process begins)⁷⁴. On the other hand, the corpus callosum is largely unmyelinated at birth and thus must go through extensive postnatal myelination⁷⁴, which may explain our finding of a strong association between myelin and gestational age. It should also be noted, however, that DTI and NODDI measures can significantly vary throughout the different regions of the corpus callosum (genu, body and splenium) in the neonate⁷⁴, therefore we may have overlooked various nuances within this region by only analysing it as a whole.

FA and MD were the two measures that showed the most variations between regions. Whilst it has previously been found that gestational age in the healthy term neonate is associated with increasing FA and decreasing MD (primarily in WM)^{204,206,209,210}, it has also been shown that this microstructural cortical maturation does not follow a consistent trajectory across the whole of the cortex^{206,210}. In our study, we similarly found that in cortical WM, most regions showed this positive association between FA and gestational age and a negative association between MD and gestational age, measures which are thought to be indicative of changes in WM microstructure such as premyelination, axon packing or density, internal axonal structure, and myelination²⁰⁹. The exception was the parietal WM, in which FA was not associated with gestational age, although there was a trend towards this relationship that didn't survive correction for multiple comparisons. However, we also found this pattern in the cerebellum and most deep GM structures. In fact, we found that gestational age was associated with increasing FA in every ROI in the deep GM and decreasing MD in all except the caudate, hippocampus and amygdala. These patterns in anisotropy and diffusivity have previously been found in the basal ganglia and thalamus in the early neonatal period (although to a lesser extent than WM)^{211,212} and may indicate that myelination and membrane proliferation are robust in the deep GM of the developing neonatal brain²¹³. We add to this by demonstrating these patterns in the subthalamic nucleus and lentiform nucleus, as well as increasing anisotropy in the caudate, hippocampus and amygdala.

In cortical GM, we were able to demonstrate a slightly different microstructural developmental trajectory in terms of FA and MD. Notably, MD was not associated with gestational age in any region of the cortical GM, whilst increasing FA was associated with

gestational age only in the frontal GM, however once again trends were seen for FA in the other cortical GM ROIs which did not survive correction for multiple comparisons. Anisotropy changes in the GM of the developing human cortex are thought to reflect the dendritic architectural changes of pyramidal cells, neuronal arborisation, and the presence or absence of radial glial cells²⁰⁹. Studies of the GM of preterm infants have found that diffusion is maximally anisotropic at approximately 26 weeks gestation, which is followed by a significant decrease in anisotropy through to 36-38 weeks gestation (term-equivalent), and then another subsequent increase from 38-47 weeks^{206,214,215}. Our results suggest that these changes in anisotropy may vary between cortical regions, and given that the oldest infant in this study was 44.71 weeks gestation at scan, it may be that the occipital, parietal, temporal and insular GM regions reach this final increase in anisotropy at the lower end of the 38-47 week range, whilst it occurs later for the frontal GM. Indeed, it is well documented that the frontal lobe is one of the latest maturing regions, particularly in terms of myelination and synapse production in the neonatal brain^{2,9}.

Our fMRI analyses found fALFF to be the only measure to show any statistically significant association with gestational age. Most regions of the cortex (GM) showed fALFF to increase with gestational age (although the parietal and occipital GM did not quite reach statistical significance after Bonferroni correction). This was also evident in some regions of the sub-cortical deep GM – most notably the caudate nucleus – however trends were also observed in the lentiform nucleus, thalamus, hippocampus and amygdala. No associations were found in the subthalamic nucleus or the thalamus with low T2 intensity. In rs-fMRI, fALFF has been proposed as an improved approach to detecting the amplitude of low-frequency fluctuations (ALFF), in which the ratio of the power spectrum of low-frequency (0.01–0.08Hz) to the entire frequency range is calculated¹⁸⁶. It is suggested to be a more sensitive and specific measure of regional spontaneous brain activity¹⁸⁶. Whilst task-fMRI is a widely used tool in researching functional connectivity in children, adolescents, and adults, research into the functional brain development of the neonate is still in its infancy, and rs-fMRI is the key sequence for this population as infants are unable to comply to tasks. A recent report on rs-fMRI in the infant brain²¹⁶ identifies that many large-scale RSNs can be identified at birth, although with slightly different topologies and weaker connection strengths than in adults, and that different functional systems follow different developmental trajectories. It has also been

found that preterm birth has a significant effect on functional connectivity metrics²¹⁷⁻²¹⁹. However, to our knowledge, there are few studies that examine ALFF, fALFF and mALFF in the typically developing neonatal brain. In our study, we were able to identify fALFF as a measure that is sensitive to gestational age in the term neonatal brain, across the grey matter of the cortex, and some sub-cortical structures, which may encourage further research using these metrics in the neonatal population.

We also examined the associations between DTI, NODDI, and myelin measures to fMRI measures. Whilst very few regional associations survived multiple comparisons, we did find a tentative positive relationship between myelin and fALFF on a global level, as well as in the parietal GM. As our previous findings show that both DTI, NODDI, myelin and fMRI (fALFF) measures change with the gestational age of the infant across most regions of the brain, it is surprising that these measures do not in fact seem to be related to each other in the term neonatal brain. Myelin and fALFF being related on a global but not a regional level perhaps suggests that whilst the global functional development of the neonate goes hand in hand with myelination and maturation, the more local and specialised functional networks may follow a different developmental trajectory to their respective microstructural development. Trends were observed between fALFF and myelin in a few GM regions which do suggest a tentative relationship between functional architecture and myelin throughout the GM of the neonatal brain, however, we hesitate to attribute too much significance to this until a larger sample size can be analysed. We also found mALFF to be negatively associated with ODI on a global level, whilst it was positively associated with ODI in the subthalamic nucleus, and while we are again cautious to attribute too much significance to this given that mALFF was not associated with gestational age, it may also support the notion that global and local functional development may follow different trajectories.

In order to further explore the relationship between myelin and the structural and functional maturation of the neonate, we reconducted the analysis with myelin independent of gestational age, through orthogonal regression. We looked at how myelin is related to all MRI measures (DTI, NODDI, and fMRI), again both globally and regionally. Interestingly we found inverse patterns between structural and functional measures. Myelin was associated with DTI and NODDI measures (all except ODI) on a regional level but not globally, whilst it was tentatively associated with fALFF on a global but not a regional level (with the exception of the

parietal GM). Myelin was positively associated with FA and NDI, and negatively with MD, in many regions throughout the brain, but most predominately in the cortical WM. We know that DTI and NODDI measures are sensitive to the maturational processes that alter the microstructure of the neonatal brain, such as changes in water content, axon packing density, dendritic architecture, and of course, myelination²⁰⁹. We were able to quantitatively demonstrate the link between these DTI and NODDI measures and myelination on a regional level, but not on a global level, which is supportive of the fact that myelination does not occur homogeneously throughout the brain⁹. On the other hand, we failed to find many significant associations between functional measures and myelination in individual regions, despite a positive trend being shown for a number of CGM and DGM regions. However, as fALFF was tentatively associated with myelin on a global level, this may suggest that although the development of local functional connections/networks may be protracted compared to the myelination process, there is a change in overall connectivity as myelination occurs. In this study, the T_1/T_2 ratio was used as a representation of myelin for both WM and GM, although it is typically used to assess WM where myelin is most abundant. However, it is possible that the T_1/T_2 ratio, when applied to GM, may include other aspects of GM development that could alter the myelin measurement. Although this method has been widely used across the dHCP, a 2017 histological study (albeit in a small group of patients with multiple sclerosis) found that the T_1/T_2 ratio correlated strongly with dendrite density but not with myelin²²⁰. It is, therefore, possible that the T_1/T_2 ratio may not reflect actual myelin in the GM of the brain, but rather dendritic architecture. Further research in the use of this technique to quantify myelin in the GM of the neonatal brain is required.

There are several other limitations to this study. Firstly, we must consider the parcellation of the brain into ROIs using a neonatal atlas²⁰¹. The brain was divided into regions of white matter, cortical grey matter, deep grey matter, as well as the cerebellum and brainstem, comprising a total of 87 ROIs. It should be noted that whilst the deep grey matter and the cortical white and grey matter of the temporal lobe were extensively divided into smaller more specific ROIs, the frontal, occipital, and parietal lobes were not parcellated as comprehensively, and in fact, each was composed of one large ROI, only separated by its left/right parts. Therefore, our analysis may have failed to discern more nuanced, regionally specific patterns of development within the lobes of the brain. Secondly, whilst DTI, NODDI

and fMRI metrics reflect changes to the structural and functional microstructure of the brain, they are inherently nonspecific²⁰⁴ and could reflect any one of the neurobiological substrates previously detailed in this chapter. Whilst it is possible to infer the developmental changes occurring in the brain using these metrics, we cannot be certain which they truly reflect. Finally, whilst our DTI, NODDI, and myelin analyses examined the whole brain (both grey and white matter) our fMRI analyses were limited to regions of cortical GM only. Although fMRI is typically used to study only grey matter, future research could potentially examine how fMRI metrics may vary across the white matter of the neonatal brain.

6.8 Conclusions

The first weeks of life are a critical period in brain development, and by understanding the microstructure of normal development, it is possible to then define the abnormal. In this study, we redemonstrate the ability of multi-sequence MRI techniques to examine the maturational trajectories of the white matter, cortical grey matter, and deep grey matter of the typically developing neonate, and how quantitative DTI, NODDI, and fMRI measures relate to the process of myelination. Our analysis found strong associations between DTI, NODDI, and myelin measures and gestational age on a global level. NDI and myelin showed this association throughout most regions of the brain, whilst FA and MD showed more regional variation, with the strongest associations in the WM and DGM. ODI showed the weakest associations with gestational age. We were also able to identify fALFF as an fMRI metric that is sensitive to the effects of gestational age, and this effect was widespread throughout the brain. On the other hand, we found that these DTI, NODDI, and myelin measures were not directly associated with fMRI measures on a regional level, however, fALFF and myelin were tentatively associated on a global level. Finally, by removing the effects of gestational age, we were able to demonstrate that FA, MD, and NDI were strongly associated with myelin on a regional but not global level, whilst fMRI measures were largely not associated with myelin - although fALFF was tentatively associated with myelin on a global but not a regional level. These results seem to reflect widespread patterns of microstructural development, such as decreases in total brain water content, enhanced organisation and complexity of fibre architecture, increases in membrane density, and myelination, as well as functional development. They also suggest that whilst

structural and functional development may be intertwined on a global level, they may follow slightly different developmental trajectories on an individual regional level. We, therefore, redemonstrate the ability of both DTI and NODDI to detect developmental changes in the white and grey matter of the typically developing neonate, as well as fMRI measures (though to a lesser extent). Importantly, despite these changes with gestational age, we found no clear direct link between DTI and NODDI measures to fMRI measures, which encourages further research to determine the exact nature of the interplay between structural and functional development in the neonatal brain.

Chapter 7: Empirical Study 2 - Is there a Linear Association Between Inflammatory Markers and Placental Histology with MRI Brain Scores in Neonatal Encephalopathy?

“Cytokines may represent an important link between perinatal infection and brain injury”.

- Dr Lina F Shalak²²¹

7.1 Introduction

Neonatal encephalopathy (NE) describes perinatal brain injury in the neonate, and although it is primarily characterised by neurological dysfunction and neurodevelopmental deficits, it can also commonly involve multiorgan injury that is associated with the release of organ-specific biomarkers^{222,223}. Inflammatory cytokines are small, secreted proteins that are modulators of the immune response to inflammation and are elevated in infants with NE. Research has shown that cytokines aid in assessing the severity of the injury, identifying infants in need of therapeutic hypothermia (TH), and predicting neurodevelopmental outcome^{221,223-225}.

MRI is an effective biomarker in NE, however, to our knowledge, there are only a few studies directly linking both inflammatory cytokine alterations and patterns of injury on MRI in infants with NE²²⁴⁻²²⁶. O'Hare et al²²⁴ examined ten pro- and anti-inflammatory cytokines in infants with NE with normal/abnormal MRI. Infants with NE and abnormal neuroimaging had significantly elevated granulocyte macrophage-colony stimulating factor (GM-CSF) at 0-24 hours. At 24-48 hours these infants had significantly elevated interleukins (IL) -8, -6, -10, and tumour necrosis factor (TNF)- α , and at 72-96 hours they had significantly lower TNF- α and Vascular Endothelial Growth Factor (VEGF). Additionally, Orrock et al²²⁶ found interleukins to be significantly elevated in infants with NE with moderate/severe MRI injury compared to those with mild/no MRI injury at 24 hours. However, Dietrick et al²²⁵ found that although interleukins were elevated in infants with NE compared to controls, they did not show any significant relationships with MRI abnormalities measured by the Barkovich score.

The first aim of this study was to examine whether there is a linear association between inflammatory markers and MRI brain scores. We include four inflammatory cytokines as biomarkers in this study. The first is IL-8, an interleukin that “promotes neutrophil chemotaxis, migration to the site of injury and production of bioactive lipids and oxygen radicals during the respiratory burst”²²⁴. The second is VEGF, a neurotrophic factor activated by tissue hypoxia²²². Finally, we include two hematopoietic cytokines – GM-CSF and EPO (erythropoietin). Both these cytokines are thought to be neuroprotective in the central nervous system as they cross the blood-brain barrier and counteract programmed cell death²²⁷. We also include three MRI scoring systems as assessment of brain injury in NE – Barkovich¹³², NICHD NRN¹³⁴, and Weeke¹³⁵ scoring systems. Because the appearance of brain injury in infants with NE is a consequence of impaired cerebral autoregulation, we aimed to look at which inflammatory cytokines, measured on days 1-2 of life, may be linearly associated with MRI brain scores in infants with NE, in order to establish a potential combined biomarker for severity of injury in NE.

NE is a condition with a multi-factorial aetiology, and placental pathology has been established as one of these aetiologies, including abnormalities such as umbilical cord lesion, chorioamnionitis, foetal vasculitis, chorionic plate meconium, and foetal thrombotic vasculopathy⁸³. Studies have found a high incidence of placental pathology in NE, have associated placental pathology with the severity of NE and found a synergistic effect between these placental pathologies and adverse neurodevelopmental outcomes²²⁸⁻²³¹. It has also been suggested that TH may be less effective in infants with chorioamnionitis and/or foetal vasculitis and chorionic plate meconium²³⁰. However, the evidence linking placental pathology to MRI brain scores is variable. Mir et al²²⁸ found that 38% of infants with chorioamnionitis and 62% with chronic villitis had abnormal brain MRI with evidence of WM injury and/or hemispheric devastation, however, they found no differences in severity or type of MRI abnormalities between these infants with placental inflammatory lesions and the rest of their cohort. Harteman et al²³² found that chorioamnionitis was common across all groups of MRI brain injury, with the highest incidence in the no injury and WM/watershed injury with BGT involvement groups. They also found that infants with a predominant BGT injury pattern were less like to show placental signs associated with chronic hypoxic ischaemia, whilst infants in the WM/watershed injury with BGT involvement group had the highest placental severity scores.

Other studies, on the other hand, have failed to find an association between placental pathology and MRI brain scores²²⁹.

It has been posited that cytokines may represent the final common pathway in the pathogenesis of perinatal brain injury that bridges both NE and placental pathology²²¹. By further understanding placental pathology and the inflammatory response in infants with NE in relation to the pattern of brain injury seen on MRI, it may also be possible to develop new preventative measures and neuroprotective therapies. Our second aim of this study, therefore, was to assess whether there is a linear association between placental pathology and MRI brain scores in infants with NE.

7.2 Methods

7.2.1 Ethical Approval

This study was part of three programmes: MODE (Multi-Organ Dysfunction and EEG Study)²³³, NEBULA (Neonatal Brain Injury: Understanding Systemic Inflammation & Immunomodulation)²³⁴, and NEPTuNE (Neonatal Encephalopathy PhD Training Network)²³⁵. MODE and NEBULA are prospective cohort studies that ran from 2011-2013, and 2013-2015, respectively. Data obtained from infants in these cohorts were used for retrospective analysis by the present study, which is part of the NEPTuNE programme. Ethical approval for all three programmes was granted by the Ethics Committee at National Maternity Hospital, Holles Street, Dublin. Recruitment was conducted and written informed parental consent was obtained for all voluntary participation by Dr Deirdre Sweetman for MODE and Dr Saima Aslam for NEBULA.

7.2.2 Patient Population

This prospective cohort study enrolled infants admitted to the NICU at the National Maternity Hospital (NMH), Holles Street, Dublin from February 2011 to June 2015. The NICU at NMH is a tertiary neonatal centre and is one of four referral centres for TH in Ireland. Infants with NE were included according to the criteria of Huang et al¹⁷⁹; abnormal neurological signs,

such as hypotonia or seizures in the immediate postnatal period, and/or other organ dysfunction (kidneys, liver, lung, heart, haematological) and at least two of the following three criteria:

- Evidence or suspicion of hypoxic-ischemic injury based on a history of foetal distress i.e., type II dips, loss of beat-to-beat variability on CTG and/or abnormal scalp pH.
- Need for resuscitation after birth i.e., bag and mask ventilation.
- Base deficit of $> 15\text{mmol/l}$ or $\text{pH} < 7.2$ in cord blood or admission arterial sample.

Infants were neurologically examined on postnatal day 1, 2, 3 and 7 using the Sarnat^{87,88} classification, which graded the severity of NE into normal (no neurological signs of encephalopathy), mild, moderate, or severe (see pages 56-57 for full classification), however the scores reported in this study are those given on day 1 only. Infants with congenital abnormalities, evidence of maternal substance abuse, or culture-positive sepsis were excluded. Infants with MRI performed after 14 days of life were also excluded. TH was administered according to the TOBY (Total Body Hypothermia for Neonatal Encephalopathy) criteria⁹⁷, with infants requiring TH treated for a duration of 72 hours.

7.2.3 MR Image Acquisition and Scoring

Imaging was carried out on a 1.5T GE Signa HDx MR scanner located at Children's Health Ireland at Temple Street, Dublin. MRI was obtained on or before 14 days of life. Harmonized MR protocols included T₁- and T₂-weighted sequences, ADC mapping, DWI and single-voxel ¹H-MRS. MR images were then scored according to the Barkovich¹³², NICHD NRN¹³⁴, and Weeke¹³⁵ scoring systems. All MRI scoring was completed by Megan Ní Bhroin – a PhD student working with the same data and trained in MRI scoring by a paediatric radiologist – who was blinded to infants' clinical course²³⁶. Inter-rater reliability was assessed using MRI scores given to the same cohort of infants by a previous study²⁴⁰. The inter-rater agreement was good for the Barkovich score and excellent for NICHD NRN and Weeke scores. T₁- and T₂-weighted images were used for the Barkovich scoring¹³². Infants were given a combined BG/W score grading the severity of injury from 0-4. Zero was given for a normal MRI, 1 for abnormal signal in the basal ganglia or thalamus, 2 for abnormal signal in the cortex, 3 for abnormal signal both in the

cortex and the basal ganglia/thalamus, and 4 for abnormal signal in the entire cortex and basal nuclei. The NICHD NRN¹³⁴ score also utilised the T₁- and T₂-weighted images, with scores described as follows: 0=normal; 1A=minimal cerebral lesions only; 1B=more extensive cerebral lesions without other involvement; 2A=basal ganglia, thalamic, internal capsule lesions only; 2B=basal ganglia, thalamic, internal capsule, and cerebral lesions; 3=cerebral hemispheric devastation. A numerical score from 0-5 was then assigned to each of these six domains (again ranging from normal to severe), to be used for analysis. The Weeke score¹³⁵ utilised the T₁- and T₂-weighted images, DWI (including ADC) and the ¹H-MRS to evaluate and score injury from 0-2 in 3 areas: grey matter, white matter/cortex, and the cerebellum. Scores were based on whether the injury was focal or extensive, and unilateral or bilateral. A total score was then given for the Weeke system by combining all four sub-scores for a possible maximum total of 57. Finally, we categorised each infant as having normal or abnormal MRI using the Barkovich score (0=normal, 1-4=abnormal).

7.2.4 Placental Histology, Blood Sampling, and Cytokine Analysis

Placentae of inborn infants (infants born at NMH) were processed and examined both grossly and microscopically in the Pathology Department of NMH²³³. Placental histology reports were reviewed and verified by two consultant perinatal histopathologists experienced in examining abnormalities of the placenta associated with NE (Dr E Mooney and Dr P Downey). Placental reports of outborn infants (infants born at other hospitals or at home) were sent to the Pathology Department of NMH for review, in order to ensure standardisation. All placentae were then graded according to the Redline classification systems for chorioamnionitis and maternal and foetal inflammatory responses²³⁷ (table 10) and disorders of placental circulation²³⁸ (table 11).

Diagnostic categories	Suggested diagnostic terminology	
Maternal inflammatory response		
Stage	1 = Early	Acute subchorionitis or chorionitis
	2 = Intermediate	Acute chorioamnionitis

	3 = Advanced	Necrotizing chorioamnionitis
Grade	1 = Mild-moderate	No special terminology required
	2 = Severe	Severe acute chorioamnionitis or with subchorionic microabscesses
Other		Chronic (or subacute) chorioamnionitis
Foetal inflammatory response		
Stage	1 = Early	With chorionic vasculitis or umbilical phlebitis
	2 = Intermediate	With umbilical vasculitis (one or two arteries ± vein) or umbilical panvasculitis (all vessels)
	3 = Advanced	With (subacute) necrotizing funisitis or with concentric umbilical perivasculitis
Grade	1 = Mild-moderate	No special terminology required
	2 = Severe	With a severe foetal inflammatory response or with intense chorionic (umbilical) vasculitis
Other		With associated foetal vessel thrombi
Other specific features		Peripheral funisitis Acute villitis Acute intervillitis with intervillous abscesses Decidual plasma cells

Table 10. Placenta reaction patterns related to amniotic fluid infection: nomenclature and definitions (adapted from Redline et al, 2003²³⁷)

Placental Regions		Timing
	<i>Maternal Circulation</i>	
	Reduced flow, large vessels	
Maternal hypotension		Acute
Findings consistent with underperfusion		Chronic
Villous infarcts		Chronic
	Decreased conductance, intervillous space	
Massive perivillous fibrinoid deposition		Chronic
Villitis of unknown aetiology with extensive perivillous fibrin		Chronic

Placental malaria		Chronic
Chronic histiocytic intervillitis		Chronic
Haemorrhages, retroplacental		
Abruptio placenta		Acute
Chronic abruption		Chronic
<i>Foetal circulation</i>		
Obstruction, umbilical cord		
Complete, total		Acute
Partial/intermittent	Cord entanglements	Subacute
	Pathologic umbilical cord lesions	Subacute
Thromboinflammatory lesions, large vessels		
Foetal thrombotic vasculopathy		Chronic
Villitis of unknown aetiology with obliterative foetal vasculopathy		Chronic
Meconium-associated vascular necrosis		Subacute
Chorioamnionitis with intense chorionic vasculitis		Subacute
Microcirculatory abnormalities		
Diffuse villous oedema		Subacute
Diffuse villitis of unknown aetiology		Chronic
Distal villous immaturity (Delayed villous maturation)		Chronic
Haemorrhages, foetal		
Massive foetomaternal haemorrhage (capillary)		Subacute
Foetal vessel rupture (large vessel)		Acute

Table 11. Categorization of placental circulatory abnormalities related to brain injury (adapted from Redline, 2009²³⁸).

Whole blood sampling was performed between days 1-7 of life²³³, however for the present study, samples on days 1-2 of life only were used. Sampling was performed at the time of routine patient phlebotomy, with 1.2ml of blood collected from either an arterial catheter sample or a peripheral venous sample. Samples were collected in serum blood bottles and immediately transported and processed at the laboratory in NMH. Blood was centrifuged at 3,000rpm for 10 minutes at a temperature of 4°C and stored at -80°C for later analysis. Samples were analysed according to the manufacturer's instructions (www.meso-scale.com). Cytokines IL-8, VEGF, GM-CSF and EPO – potential serum biomarkers of brain injury – were analysed using a 96 well singleplex plate. Cytokines were examined using a sandwich

immunoassay, where capture antibodies were coated in a patterned array on the bottom of the wells of the plate. Plates were then analysed on a SECTOR Imager and validated. The full blood sampling and cytokine analyses were conducted by Dr Deirdre Sweetman for MODE and Dr Saima Aslam for NEBULA, as well as postdoctoral researcher Lynne Kelly.

7.2.5 Statistical Analyses

Statistical analyses and visualization were carried out by myself using iNZight (v.4.2.0) (The University of Auckland, New Zealand). General linear regression was performed to assess the association between inflammatory cytokines (EPO, GM-CSF, IL-8, VEGF) and MRI brain scores (Barkovich, NICHD NRN, Weeke, normal/abnormal MRI). This was performed separately for each cytokine and each scoring system. Log values for each cytokine were used for this analysis, in order to transform the data from skewed to normally distributed. General linear regression was also performed to assess the association between placental histology (normal/abnormal) and MRI brain scores. A sub-group of those infants with abnormal placenta was then examined, in which the association between the presence/absence of chorioamnionitis and MRI brain scores was computed. All analyses were controlled for sex. A probability value of <0.05 was taken as statistically significant. Statistically significant associations were visualized as scatterplot graphs.

7.3 Results

7.3.1 Clinical Characteristics

136 infants with NE were prospectively recruited for the MODE and NEBULA studies between February 2011 and June 2015. MRI data was not available for 37 of these infants, and 6 had MRI performed after 14 days of life. Of the remaining 93 infants, 70 had full inflammatory cytokine data, and 68 had full placenta data available, whom we included in our final retrospective analyses (a total of 87 infants). 26 infants with abnormal placentae were included in a chorioamnionitis sub-group. The full clinical characteristics of each group can be seen in table 12.

Variable	Whole Sample (n=87)	Cytokines (n=70)	Placenta (n=68)
Gestational age (weeks)	40.33 (34.86-44.00)	40.28 (34.86-44.00)	40.46 (34.86-42.29)
Postmenstrual age (weeks)	41.30 (35.72-45.00)	41.27 (35.72-45.00)	41.43 (35.72-43.29)
Age at scan (days)	6.79 (0-14)	6.94 (2-12)	6.72 (0-14)
Birth weight (kg)	3.68 (2.30-5.86)	3.72 (2.30-5.86)	3.59 (2.30-5.04)
Male	60	48	45
Mode of delivery: LCSC	35	27	28
SVD	24	19	18
Inst.	28	23 (1 N/A)	22
Apgar: 1 minute	2.90 (0-9)	2.46 (0-9)	3.06 (0-9)
5 minutes	4.49 (0-9)	3.89 (0-9)	4.50 (0-9)
10 minutes	5.59 (1-10)	5.32 (1-10)	5.41 (1-10)
Therapeutic hypothermia	64	59	47
Seizures	55 (1 N/A)	43 (1 N/A)	44 (1 N/A)
Sarnat: 0=normal	3	2	2
1=mild	12	8	10
2=moderate	59	50	46
3=severe	10 (3 N/A)	10	7 (3 N/A)
Mortality	4 (5 N/A)	4 (4 N/A)	2 (5 N/A)
Cord pH 1	7.09 (6.79-7.37)	7.10 (6.79-7.37)	7.09 (6.79-7.37)
Cord pH 2	7.13 (6.80-7.34)	7.14 (6.81-7.34)	7.13 (6.80-7.34)
Cord BE 1 (mmol/l)	-10.85 (-21.30-5.90)	-10.81 (-21.30-5.90)	-10.77 (-20.60-5.90)
Cord BE 2 (mmol/l)	-9.73 (-19.00-5.60)	-9.99 (-19.00-5.30)	-9.50 (-17.00-5.60)

Table 12. Neonatal demographic characteristics (study 2). Data presented as means (with range) or n (number of infants). Abbreviations: BE, base excess; Inst., instrumental delivery; LSCS, lower segment Caesarean section; SVD, spontaneous vaginal delivery.

7.3.2 MRI Scoring Characteristics

All infants who underwent MRI were scored using all three MRI scoring systems²³⁶ – Barkovich¹³², NICHD NRN¹³⁴, and Weeke¹³⁵ (GM, WM, cerebellum, overall) – and were also

grouped into normal/abnormal MRI based upon the Barkovich score (0=normal, 1-4=abnormal). 59 of the 87 infants were scanned within 7 days, the remaining 28 were scanned within 14 days. The full MRI scoring characteristics for both analysis populations can be seen in table 13.

Variable	Whole Sample (n=87)	Cytokines (n=70)	Placenta (n=68)
Barkovich (BG/W):			
0	55	47	43
1	10	8	9
2	12	7	9
3	9	7	6
4	1	1	1
NICHD NRN:			
0	51	44	41
1A	4	3	2
1B	11	7	8
2A	9	7	8
2B	11	8	8
3	1	1	1
Weeks:			
Grey matter	2.66 (0-23) (n <11.50 = 81)	2.69 (0-23) (n <11.50 = 65)	2.62 (0-21) (n <11.50 = 63)
White matter	3.60 (0-17)	3.20 (0-17)	3.32 (0-13)
Cerebellum	1.84 (0-6)	1.76 (0-4)	1.93 (0-6)
Total overall score	8.53 (0-45)	8.06 (0-45)	8.29 (0-36)
Normal/abnormal MRI:			
Normal	55	47	43
Abnormal	32	23	25

Table 13. Neonatal MRI scoring characteristics (study 2). Data presented as means (with range) or n (number of infants). Abbreviations: BG/W, basal ganglia/watershed; MRI, magnetic resonance imaging; NICHD NRN, National Institute of Child Health and Human Development Neonatal Research Network.

7.3.3 Cytokines vs MRI Brain Scores

Analysis revealed a statistically significant positive association between GM-CSF and Weeke GM scores ($p=0.036$) (figure 36). No statistically significant associations were found amongst the other three cytokines (EPO, IL-8, VEGF) and MRI brain scores. Results can be seen in full in table 14.

p-values	Barkovich BG/W	NICHD NRN	Weeke GM	Weeke WM	Weeke Cerebellum	Weeke Overall	Normal/ Abnormal MRI
EPO	0.930	0.541	0.219	0.806	0.286	0.467	0.278
GM-CSF	0.398	0.199	0.036+	0.786	0.059	0.111	0.265
IL-8	0.339	0.936	0.750	0.376	0.343	0.483	0.234
VEGF	0.724	0.402	0.759	0.950	0.485	0.931	0.864

Table 14. Summary of linear regression analyses between cytokines and MRI brain scores. *p*-values in bold are those deemed statistically significant, with a + or – denoting the direction of the association. Abbreviations: BG/W, basal ganglia/watershed; EPO, erythropoietin; GM, grey matter; GM-CSF, Granulocyte Macrophage-Colony Stimulating Factor; IL, interleukin; MRI, magnetic resonance imaging; NICHD NRN, National Institute of Child Health and Human Development Neonatal Research Network; VEGF, Vascular Endothelial Growth Factor; WM, white matter.

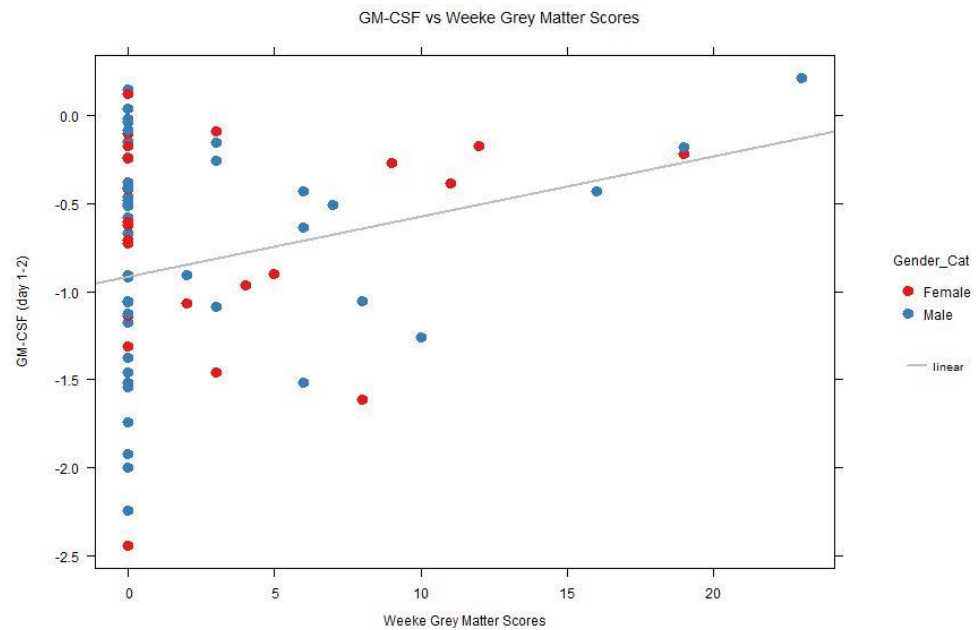


Figure 36. GM-CSF at day 1-2 plotted against Weeke grey matter scores (controlled for sex).

7.3.4 Placental Histology vs MRI Brain Scores

No statistically significant associations were found between placental histology and MRI brain scores, either for the normal/abnormal group or the chorioamnionitis/no chorioamnionitis group. Results can be seen in full in table 15.

p-values	Barkovich BG/W	NICHD NRN	Weeke GM	Weeke WM	Weeke Cerebellum	Weeke Overall	Normal/ Abnormal MRI
Normal/Abnormal Placenta	0.605	0.953	0.862	0.405	0.293	0.781	0.994
Chorio/No Chorio	0.606	0.932	0.151	0.267	0.360	0.127	0.714

Table 15. Summary of linear regression analyses between placental histology and MRI brain scores. Abbreviations: BG/W, basal ganglia/watershed; GM, grey matter; MRI, magnetic

resonance imaging; NICHD NRN, National Institute of Child Health and Human Development Neonatal Research Network; WM, white matter.

Given the small sample size for the chorioamnionitis/no chorioamnionitis sub-group, an a priori power analysis was then conducted using G*Power (v.3.1.9.7)²³⁹ to determine the minimum sample size required to test the study hypothesis for the Weeke GM and overall scores. Results showed that the required sample size in order to achieve .80 power for detecting a medium effect at a significance level of 0.05 was 83 and 65 participants, respectively.

7.4 Discussion

Higher levels of GM-CSF were associated with higher scores on the Weeke grey matter scoring system, indicating that GM-CSF levels increase along with increasing severity of grey matter injury in NE. However, none of the other scoring systems showed such associations, and whilst we are tentative in reporting this as a significant result, it does perhaps suggest that the Weeke grey matter scoring system may be the most sensitive to inflammatory markers in NE. On the other hand, we found that placental histology was not associated with MRI brain scores, either when looking at normal vs abnormal placenta, or the presence/absence of chorioamnionitis. These results lend support for the potential use of GM-CSF and the Weeke scoring system as a combined biomarker for NE, although further testing with a larger sample size should be conducted.

Although previous research has focused on inflammatory and placental biomarkers in NE, to our knowledge there are only a few studies directly linking both cytokine alterations and placental histology to patterns of injury seen on MRI in infants with NE^{224-226, 228,229,232}, and none of which use multiple scoring systems. The findings across these studies have been variable, with some reporting direct associations, and others finding no significant relationships. Our finding that GM-CSF can be directly linked to MRI brain scores is consistent with studies by O'Hare et al²²⁴ and O'Dea et al²⁴⁰, who found that GM-CSF was significantly elevated at 0-24 hours in infants with NE and abnormal neuroimaging. GM-CSF is a hematopoietic cytokine that is responsible for stimulating the proliferation, differentiation, and maturation of granulocytes

and monocytes²²⁷. GM-CSF is able to cross the blood-brain barrier, and it has been shown that the receptor for GM-CSF is induced in neurons across the brain by cerebral ischaemia, with GM-CSF counteracting apoptosis (or programmed cell death) in these neurons²²⁷. GM-CSF dysregulation (along with other cytokines including IL-8 and TNF- β) can persist through to school-age in children following NE²⁴¹. GM-CSF therefore may play an active role in the body's response to NE, with elevated levels indicating more severe injury as seen on MRI, and together may prove an effective biomarker for assessing the extent of injury in NE.

It is important to note that the O'Dea study was also a part of the NEPTuNE programme, and whilst the MR images from the O'Dea study were used to assess inter- and intra-rater reliability when scoring the MR images for the present study, the cohort of infants used for the present analysis is different from the O'Dea cohort, which also included the additional factor of lipopolysaccharide stimulation. It should also be noted that both the above studies (O'Hare, O'Dea) used the Barkovich scoring system to define normal/abnormal MRI, whilst our findings identified the Weeke grey matter scoring system as the only one to be associated with cytokine alterations. Several scoring systems have been developed for use in NE¹³²⁻¹³⁵, with the aim of guiding interpretation and reproducibility, reducing subjectivity and standardising the classification of brain injury²³⁶. The Barkovich and NICHD NRN scoring systems utilise T₁- and T₂-weighted images to group patterns of injury into watershed areas (mild-moderate injury) and to basal ganglia and thalamic injury (more severe injury) and are optimised for use beyond the first week of life. These scoring systems are both relatively quick and simple to use, and so are the most commonly used in clinical settings. The Weeke scoring system, on the other hand, is far more comprehensive, scoring injury in GM, WM, and the cerebellum, and taking into account whether the injury is focal or extensive, and unilateral or bilateral. It utilises not only T₁- and T₂-weighted imaging, but also DWI and MRS, and crucially for NE, is optimised for use within the first week of life – a critical time period where decisions regarding care are to be made. It is suggested that the Weeke scoring system is able to detect brain abnormalities with a higher frequency than the Barkovich and NICHD NRN scoring systems^{236,242}. The present study adds to this by suggesting that not only is the Weeke scoring system more sensitive to injury detection but may also be more sensitive to alterations in inflammatory cytokines that are indicative of brain injury. Specifically, the Weeke grey matter score can be directly linked to GM-CSF.

We did not find any significant associations between EPO, IL-8 and VEGF, and MRI brain scores. Whilst some studies have found EPO, IL-8, and VEGF to be elevated in infants with NE compared to controls, evidence linking these cytokines to specific MRI abnormalities again is inconsistent, with some finding a relationship and others not^{224-226,243}. O'Hare²²⁴ et al found that IL-8 was significantly elevated in infants with increased severity of NE and abnormal neuroimaging at 24-48 hours, whilst VEGF was significantly decreased at 72-96 hours of life in infants with NE with abnormal neuroimaging. Our study looked at cytokine levels at 0-24 hours due to a high level of missing data for days 3-5, and so it is possible that we may have missed the window in which the inflammatory response of these cytokines is most apparent. We found only one study linking EPO and MRI in NE, in which elevated serum EPO on days 2-3 of life was associated with severely abnormal MRI²⁴³, however, infants from this study came from the same cohort as the present study, and so this may be a potential area for future research.

Placental histology was not found to be associated with any MRI brain scores in the present study, either for infants with a normal/abnormal placenta, or for infants with/without chorioamnionitis. The presence of an abnormal placenta at birth has been associated with a doubling in risk of NE²⁴⁴, and indeed, placental pathology has been found to have a high incidence in infants with NE, with studies suggesting percentages as high as 87%²³⁰-95%²³³. Placental pathology has also been linked to short- and long-term outcomes in NE²²⁸⁻²³¹, however similarly to that of cytokines, evidence directly linking placental pathology to MRI brain scores is variable. Whilst Wintermark et al²³⁰ found that chorioamnionitis with foetal vasculitis and chorionic plate meconium were significantly associated with brain injury on MRI, a number of other studies by Orrock et al²²⁶, Hayes et al²²⁹, and Frank et al²⁴⁵ have also failed to find associations between placental and MRI brain abnormalities, which is consistent with our results. The former two studies^{226,229} cited small sample sizes as a possible reason for this. It is clear that placental pathologies play an important role in the aetiology of NE, however, there is not yet a consensus on exactly how they may or may not be associated with patterns and severity of injury on MRI, which presents an opportunity for future investigation.

Our study is not without limitations, which we must acknowledge. Firstly, despite our analysis groups of 70 and 68 infants, this is still a relatively small sample size and may lack the power to show significant associations between our variables under study, especially for the sub-group looking at infants with/without chorioamnionitis, which was only 26 infants. Secondly, a large portion of the infants included in our study did have a normal MRI – 55 of the

87 infants – which may have impacted our results, and it is possible that this may be due to pseudonormalisation given that some infants were imaged in the second week of life. Finally, whilst placental pathology reports of outborn infants were internally validated, the lack of in-house placental pathology review should also be considered a weakness. Strengths of our study include highly standardised protocols, and the use of published classification systems for scoring MRI injury.

7.5 Conclusions

Inflammation plays an important role in brain injury and altered cytokine levels have been found in infants with NE, although the evidence linking cytokine levels and MRI brain scores is quite varied, with some predating cooling therapies. The present study investigated whether there is an association between cytokines EPO, GM-CSF, IL-8, and VEGF, and MRI brain scores using the Barkovich, NICHD NRN, and Weeke scoring systems. We identify a positive linear relationship between GM-CSF levels on day 1 of life and Weeke grey matter scores. These results may have important therapeutic implications for NE, as a combined biomarker to assess the severity of injury and determine neuroprotective treatment options. We also investigated the association between placental pathology and MRI brain scores, however, found no direct link between these two variables, which encourages further research to tease apart potential interactions between placental abnormalities and patterns of brain injury in NE.

Chapter 8: Empirical Study 3 - Are MRI Brain Scores, Inflammatory Markers, and Placental Histology Associated with Neurodevelopmental Outcome in Neonatal Encephalopathy?

“We worried if she was going to be okay, if she was going to still keep living. And then after we got out of the woods with that and she was able to breathe, then it was time to think about you know, other things that could happen. Eventually, is she going to be able to do normal things that others can do?”

- *Parent of infant with NE (from Lemmon et al, 2016²⁴⁶).*

8.1 Introduction

Neonatal encephalopathy (NE) is a significant cause of enduring morbidity and mortality in the term neonate⁷⁶, and despite therapeutic hypothermia (TH), moderate-severe NE is associated with adverse outcomes at a rate of approximately 50%⁹⁸. Survivors are at risk of a multitude of neurodevelopmental impairments such as impaired cognition, language skills, or intellectual functioning, cerebral palsy, and behavioural problems¹⁴⁶. MRI has proven to be the single best predictor of outcome in infants with NE^{104,133,135-137}. Different patterns of injury are associated with different outcomes, with basal ganglia-thalamic (BGT) injury typically associated with more severe cognitive and motor deficits, and watershed (WS) typically with less severe cognitive impairment without motor deficits¹²⁶. Scoring systems to determine brain injury in NE have been shown to be associated with neurodevelopmental outcome at two years of age, with Barkovich¹³², NICHD NRN¹³⁴, and Weeke¹³⁵ scores associated with Bayley-III cognitive and motor composite scores, and the Weeke score associated with Bayley-III language composite scores²³⁶. Other biomarkers such as inflammatory cytokines and placental histology may also be predictive of outcomes, and it has been suggested that combining such biomarkers with other scoring system biomarkers may improve the sensitivity and specificity of outcome prediction²⁴⁷.

Studies have shown that altered levels of inflammatory cytokines are associated with outcome in NE^{225,248,249}. Dietrick et al²²⁵ identified a number of plasma and CSF markers that were associated with neurodevelopmental outcome at 15-30 months. When evaluating outcomes related to brain injury severity, they found that plasma tau, CSF GFAP, neurogranin, IL-6, IL-8, and IL-10 were inversely associated with Bayley-III scores. Specifically, IL-8 was associated with motor outcomes, tau, GFAP, IL-6, and IL-10 with cognitive and motor outcomes, and neurogranin with cognitive, motor, and language outcomes. Additionally, a study on the MODE cohort²⁴⁸ found that elevated GM-CSF but not IL-8 correlated negatively with composite language Bayley-III scores at 2 years of age, whilst Bartha et al²⁴⁹ found that IL-1 β , IL-6, and IL-8 were significantly higher, and IL-12 lower in infants with abnormal outcome at 30 months of age compared with those with normal neurodevelopment. However, to our knowledge, there are no studies directly investigating whether the addition of cytokine biomarkers can improve the prognostic ability of each of the different scoring systems used to assess brain injury in NE. The first aim of this study was to examine whether there is a linear association between MRI brain scores (measured by the Barkovich¹³², NICHD NRN¹³⁴ and Weeke¹³⁵ scoring systems) with the addition of four inflammatory cytokines as covariates (EPO, GM-CSF, IL-8, and VEGF) to predict neurodevelopmental outcome at two years of age (Bayley-III composite scores) in infants with NE.

Placental pathology has also been linked to adverse outcomes in NE. Hayes et al²²⁹ identified a number of placental lesions independently associated with an increased risk for NE, including haemorrhage, infection/inflammation, nucleated red blood cells, infarctions, thrombosis, meconium phagocytosis, accelerated villous maturation, and villous dysmaturity, and found that the presence of increased numbers of these placental lesions was associated with poorer scores on neurodevelopmental assessment. Moreover, one-third of infants with four or more placental lesions died or developed cerebral palsy. Mir et al²²⁸ found that although both chorioamnionitis and chronic villitis were independently associated with severity of NE, chronic villitis alone was able to predict abnormal neurodevelopmental outcome (Bayley-III scores) following TH. The connection between placental pathology, MRI brain injury, and outcomes is rather more unclear. We found only one study that aimed to link all three of these variables, and they found that in their population of infants receiving TH, no type of placental pathology was related to extensive brain injury or adverse

neurodevelopmental outcome²⁴⁵. Placental pathology has been described as the “oft-forgotten black box” of pregnancy²⁵⁰, and so the second aim of this study was to determine whether a linear relationship exists between MRI brain scores with the addition of placental pathology as a covariate to predict neurodevelopmental outcome at two years of age (Bayley-III composite scores) in infants with NE.

8.2 Methods

8.2.1 Ethical Approval

This study was part of three programmes: MODE (Multi-Organ Dysfunction and EEG Study)²³³, NEBULA (Neonatal Brain Injury: Understanding Systemic Inflammation & Immunomodulation)²³⁴, and NEPTuNE (Neonatal Encephalopathy PhD Training Network)²³⁵. MODE and NEBULA are prospective cohort studies that ran from 2011-2013, and 2013-2015, respectively. Data obtained from infants in these cohorts was used for retrospective analysis by the present study, which is part of the NEPTuNE programme. Ethical approval for all three programmes was granted by the Ethics Committee at National Maternity Hospital, Holles Street, Dublin. Recruitment was conducted and written informed parental consent was obtained for all voluntary participation by Dr Deirdre Sweetman for MODE and Dr Saima Aslam for NEBULA.

8.2.2 Patient Population

This study used the same NE patient population as the previous study, taken from the MODE²³³ and NEBULA²³⁴ cohorts (for full details, see page 143).

8.2.3 MR Image Acquisition and Scoring

MR image acquisition was the same as outlined in the previous chapter (see page 144 for the acquisition protocol). MR images were then scored according to the Barkovich¹³², NICHD NRN¹³⁴ and Weeke¹³⁵ scoring systems. All MRI scoring was completed by Megan Ní

Bhroin – a PhD student working with the same data and trained in MRI scoring by a paediatric radiologist – who was blinded to the infant’s clinical course, and inter-rater reliability was assessed for each scoring system²³⁶. For a full description of scoring, see page 144.

8.2.4 Placental Histology, Blood Sampling, and Cytokine Analysis

Collection and analyses of placentae, blood, and cytokines were the same as in the previous study and were conducted by Dr Deirdre Sweetman for MODE and Dr Saima Aslam for NEBULA (see pages 145-147 for details), as well as postdoctoral researcher Lynne Kelly.

8.2.5 Neurodevelopmental Outcome

The Bayley-III¹¹⁶ was used to assess neurodevelopmental outcome at two years of age, administered by a clinical developmental psychologist (Marie Slevin) blind to the MRI scores. Infants were scored in three categories; cognitive, motor, and language, and test norms were used to determine rates of developmental delay (mean=100, standard deviation [SD]=15). Scores of 70-84 (-1 SD) indicated mild impairment, and scores of <70 (-2 SD) indicated severe impairment.

8.2.6 Statistical Analyses

Statistical analyses and visualization were carried out by myself using iNZight (v.4.2.0) (The University of Auckland, New Zealand). General linear regression was performed to assess the association between MRI brain scores with cytokines/placenta as a covariate, and neurodevelopmental outcome (Bayley) scores. This was performed individually for each scoring system combined with each cytokine, as well as for normal/abnormal placenta. Log values for each cytokine were used for this analysis, in order to transform the data from skewed to normally distributed. All analyses were controlled for sex. A probability value of <0.05 was taken as statistically significant. Statistically significant associations were visualized as scatterplot graphs.

8.3 Results

8.3.1 Clinical Characteristics

136 infants with NE were recruited for the MODE and NEBULA studies between February 2011 and June 2015. Two infants died before MRI completion, MRI data was not available for a further 35 of these infants, and 6 had MRI performed after 14 days of life. Of the remaining 93 infants, 70 had full inflammatory cytokine data, and 68 had full placenta data available. 54 of the 70 infants for the cytokine analysis and 54 of the 68 infants for the placenta analysis had full outcome data available, whom we included in our final analyses (a total of 66 infants). The full clinical characteristics for each analysis group can be seen in table 16.

Variable	Whole Sample (n=66)	Cytokines (n=54)	Placenta (n=54)
Gestational age (weeks)	40.49 (34.86-42.29)	40.45 (34.86-42.29)	40.61 (34.86-42.49)
Postmenstrual age (weeks)	41.45 (35.72-43.29)	41.44 (35.72-43.29)	41.56 (35.72-43.29)
Age at scan (days)	6.74 (0-12)	6.94 (2-10)	6.63 (0-12)
Birth weight (kg)	3.71 (2.30-5.48)	3.72 (2.30-5.48)	3.67 (2.30-5.04)
Male	45	34	36
Mode of delivery:	27	21	24
LCSC	16	14	13
SVD	22	18	17
Inst.	(1 N/A)	(1 N/A)	
Apgar: 1 minute	2.97 (0-9)	2.78 (0-9)	2.98 (0-9)
5 minutes	4.38 (0-9)	4.02 (0-9)	4.33 (0-9)
10 minutes	5.58 (1-9)	5.35 (1-9)	5.45 (1-9)
Therapeutic hypothermia	49	44	38
Seizures	44	34	36
Sarnat: 0=normal	1	1	1
1=mild	9	7	8
2=moderate	50	42	40
3=severe	4	4	3
	(2 N/A)		(2 N/A)
Mortality	0	0	0
	(3 N/A)	(2 N/A)	(3 N/A)

Cord pH 1	7.10 (6.83-7.37)	7.11 (6.83-7.37)	7.11 (6.80-7.37)
Cord pH 2	7.13 (6.85-7.34)	7.14 (6.85-7.14)	7.14 (6.85-7.34)
Cord BE 1 (mmol/l)	-10.94 (-21.30-5.90)	-10.81 (-21.30-5.90)	-10.69 (-19.80-5.90)
Cord BE 2 (mmol/l)	-10.28 (-19.00-5.30)	-10.20 (-19.00-5.30)	-10.04 (-17.00-5.30)

Table 16. Neonatal demographic characteristics (study 3). Data presented as means (with range) or n (number of infants). Abbreviations: BE, base excess; Inst., instrumental delivery; LSCS, lower segment Caesarean section; SVD, spontaneous vaginal delivery.

8.3.2 MRI Scoring Characteristics

All infants who underwent MRI were scored using all three MRI scoring systems²³⁶ – Barkovich¹³², NICHD NRN¹³⁴, and Weeke¹³⁵ (GM, WM, cerebellum, overall) – and were also grouped into normal/abnormal MRI based upon the Barkovich score (0=normal, 1-4=abnormal). The full MRI scoring characteristics for both analysis populations can be seen in table 17.

Variable	Whole Sample (n=66)	Cytokines (n=54)	Placenta (n=54)
Barkovich (BG/W):			
0	41	35	34
1	10	8	9
2	10	7	7
3	5	4	4
4	0	0	0
NICHD NRN:			
0	38	33	32
1A	3	2	2
1B	9	7	6
2A	9	7	8
2B	7	5	6
3	0	0	0
Weeke:			
Grey matter	2.55 (0-21)	2.39 (0-19)	2.74 (0-21)

White matter	3.12 (0-13)	2.72 (0-13)	3.00 (0-13)
Cerebellum	1.98 (0-6)	1.94 (0-4)	2.07 (0-6)
Total overall score	8.12 (0-36)	7.50 (0-36)	8.30 (0-36)
Normal/abnormal MRI:			
Normal	41	35	34
Abnormal	25	19	20

Table 17. Neonatal MRI scoring characteristics (study 3). Data presented as means (with range) or n (number of infants). Abbreviations: BG/W, basal ganglia/watershed; MRI, magnetic resonance imaging; NICHD NRN, National Institute of Child Health and Human Development Neonatal Research Network.

8.3.3 Neurodevelopmental Characteristics

Neurodevelopmental outcome data for the 54 infants in each analysis group comprised of a set of three Bayley-III composite scores – cognitive, motor, and language. Means and ranges of scores can be seen in table 18, along with the number of infants in each category whose scores indicated mild (70-84) and severe (<70) impairment. Figure 37 shows the distribution of the three sets of composite scores.

Bayley-III Composite scores	Whole Sample (n=66)	Cytokines (n=54)	Placenta (n=54)
Cognitive	100.76 (55-140)	102.22 (55-140)	99.44 (55-140)
Mild (70-84)	5	3	5
Severe (<70)	5	3	5
Motor	101.53 (46-133)	103.57 (46-133)	100.96 (46-133)
Mild (70-84)	2	0	2
Severe (<70)	6	4	6
Language	100.77 (47-138)	101.30 (47-138)	99.96 (47-138)
Mild (70-84)	7	7	7
Severe (<70)	3	2	4

Table 18. Neonatal neurodevelopmental outcome characteristics (study 3). Data presented as means (with range) or n (number of infants).

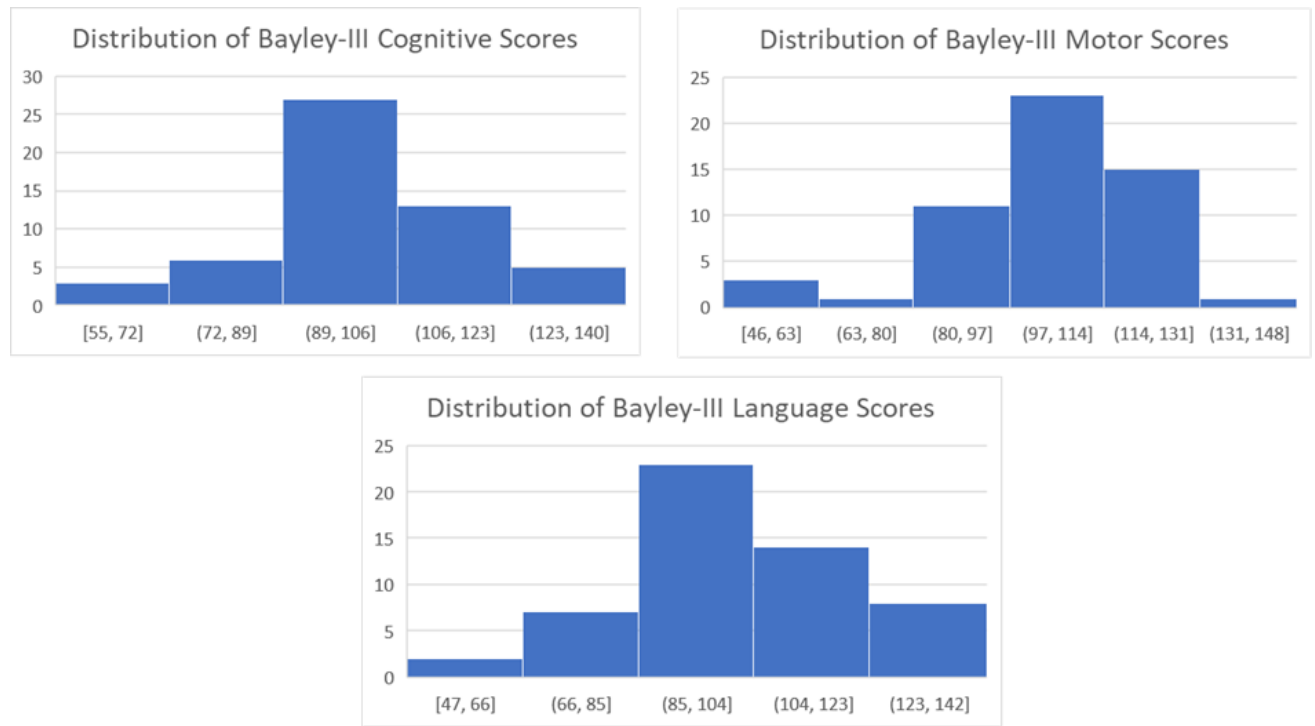


Figure 37. Distribution of cognitive, motor and language Bayley-III composite scores in our neonatal population.

8.3.4 Cytokines with MRI Brain Scores vs Neurodevelopmental Outcome

Full results can be seen in table 19. Analyses revealed that when GM-CSF was predictive of cognitive outcome when combined with the Barkovich ($p=0.022$), NICHD NRN ($p=0.033$), Weeke WM ($p=0.019$), Week cerebellum ($p=0.043$), and Weeke overall ($p=0.038$) scoring systems, as well as for the normal/abnormal MRI classification ($p=0.025$). Higher levels of GM-CSF were associated with lower cognitive composite scores. This trend was also observed for the Weeke GM scoring system but did not reach statistical significance ($p=0.067$). Because of the consistency across MRI scores for GM-CSF and cognitive outcome, we also report the FDR corrected p-values for these associations in table 20, in which it should be noted that all scores then just missed statistical significance. Figures 38 and 39 demonstrate the linear relationship

between GM-CSF and cognitive composite scores, with plot points coloured by the different MRI scoring systems. IL-8 was also found to be predictive of both cognitive ($p=0.031$) and motor ($p=0.048$) outcome when combined with the Weeke GM score (figures 40 and 41), with higher levels of IL-8 associated with lower cognitive and motor composite scores. No statistically significant associations were found between EPO or VEGF and outcome measures when combined with any MRI brain scores.

<i>Barkovich BG/W</i>				
p-values	EPO	GM-CSF	IL-8	VEGF
Cognitive	0.612	0.022-	0.407	0.784
Motor	0.434	0.363	0.676	0.453
Language	0.933	0.309	0.555	0.943
<i>NICHD NRN</i>				
p-values	EPO	GM-CSF	IL-8	VEGF
Cognitive	0.771	0.033-	0.281	0.643
Motor	0.606	0.512	0.466	0.306
Language	0.820	0.371	0.476	0.859
<i>Weeke Grey Matter</i>				
p-values	EPO	GM-CSF	IL-8	VEGF
Cognitive	0.477	0.067	0.031-	0.816
Motor	0.533	0.999	0.048-	0.388
Language	0.350	0.605	0.193	0.987
<i>Weeke White Matter</i>				
p-values	EPO	GM-CSF	IL-8	VEGF
Cognitive	0.567	0.019-	0.366	0.859
Motor	0.386	0.337	0.611	0.527
Language	0.958	0.299	0.536	0.995
<i>Weeke Cerebellum</i>				
p-values	EPO	GM-CSF	IL-8	VEGF
Cognitive	0.667	0.043-	0.457	0.774
Motor	0.461	0.466	0.690	0.460
Language	0.855	0.458	0.633	0.936
<i>Weeke Overall</i>				
p-values	EPO	GM-CSF	IL-8	VEGF
Cognitive	0.995	0.038-	0.139	0.851
Motor	0.790	0.651	0.268	0.461

Language	0.615	0.478	0.335	0.993
<i>Normal/Abnormal MRI (Barkovich)</i>				
p-values	EPO	GM-CSF	IL-8	VEGF
Cognitive	0.785	0.025-	0.459	0.829
Motor	0.667	0.406	0.791	0.500
Language	0.892	0.312	0.560	0.954

Table 19. Summary of linear regression analyses between cytokines combined with MRI brain scores, and neurodevelopmental outcome scores. *p*-values in bold are those deemed statistically significant, with a + or – denoting the direction of the association. Abbreviations: BG/W, basal ganglia/watershed; EPO, erythropoietin; GM-CSF, Granulocyte Macrophage-Colony Stimulating Factor; IL, interleukin; MRI, magnetic resonance imaging; NICHD NRN, National Institute of Child Health and Human Development Neonatal Research Network; VEGF, Vascular Endothelial Growth Factor.

Scoring System	FDR corrected p-value for GM-CSF and cognitive scores
Barkovich BG/W	0.077
NICHD NRN	0.058
Weeke Grey Matter	0.067
Weeke White Matter	0.133
Weeke Cerebellum	0.050
Weeke Overall	0.053
Normal/Abnormal MRI (Barkovich)	0.058

Table 20. FDR corrected *p*-values for GM-CSF combined with each MRI scoring system, and cognitive outcome scores. Abbreviations: BG/W, basal ganglia/watershed; FDR, false discovery rate; GM-CSF, Granulocyte Macrophage-Colony Stimulating Factor; MRI, magnetic resonance imaging; NICHD NRN, National Institute of Child Health and Human Development Neonatal Research Network.

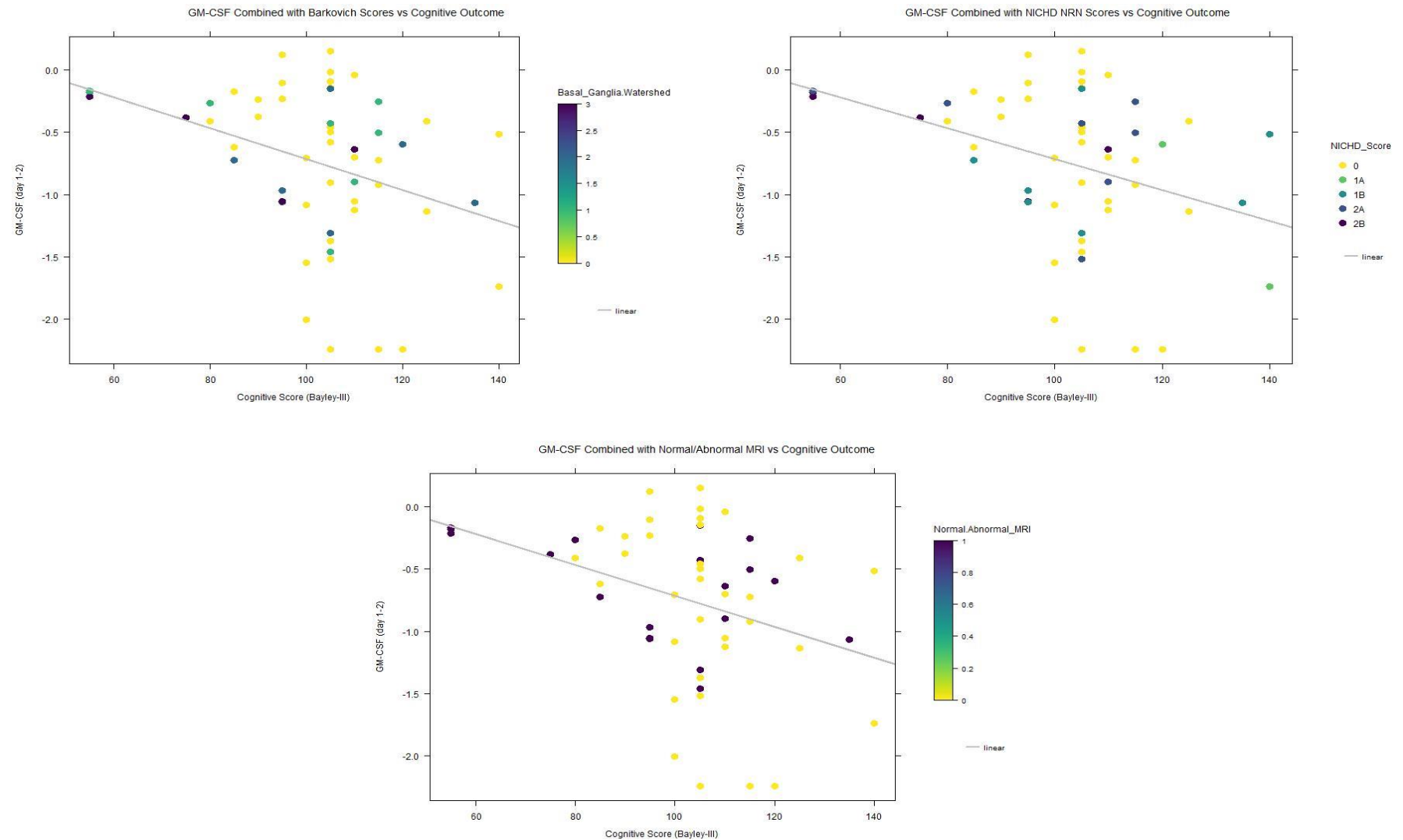


Figure 38. GM-CSF at day 1-2 plotted against cognitive outcome Bayley-III scores, coloured by MRI brain scores (Barkovich, NICHD NRN, normal/abnormal MRI) (controlled for sex).

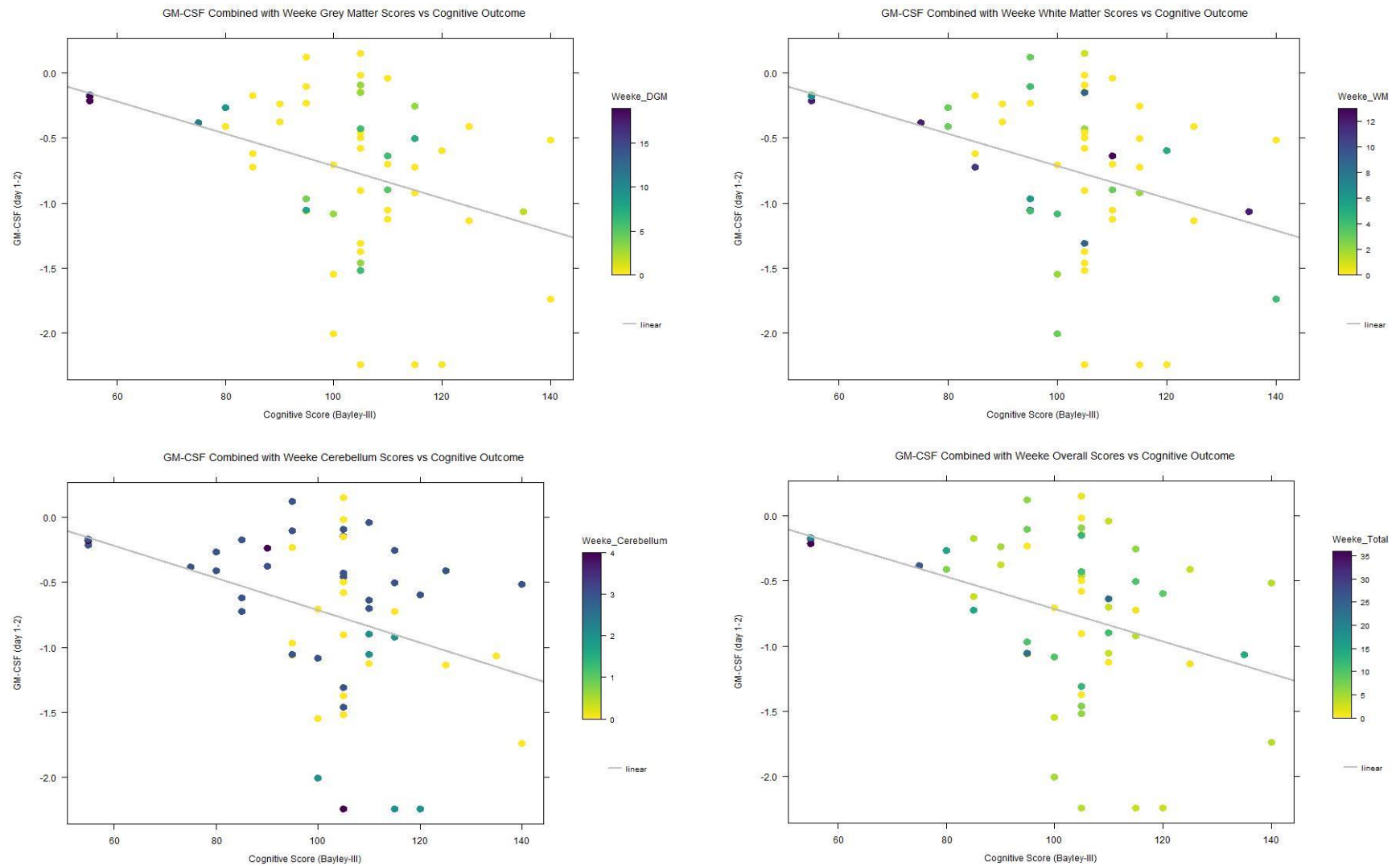


Figure 39. GM-CSF at day 1-2 plotted against cognitive outcome Bayley-III scores, coloured by MRI brain scores (Weeke GM, WM, cerebellum, and overall) (controlled for sex).

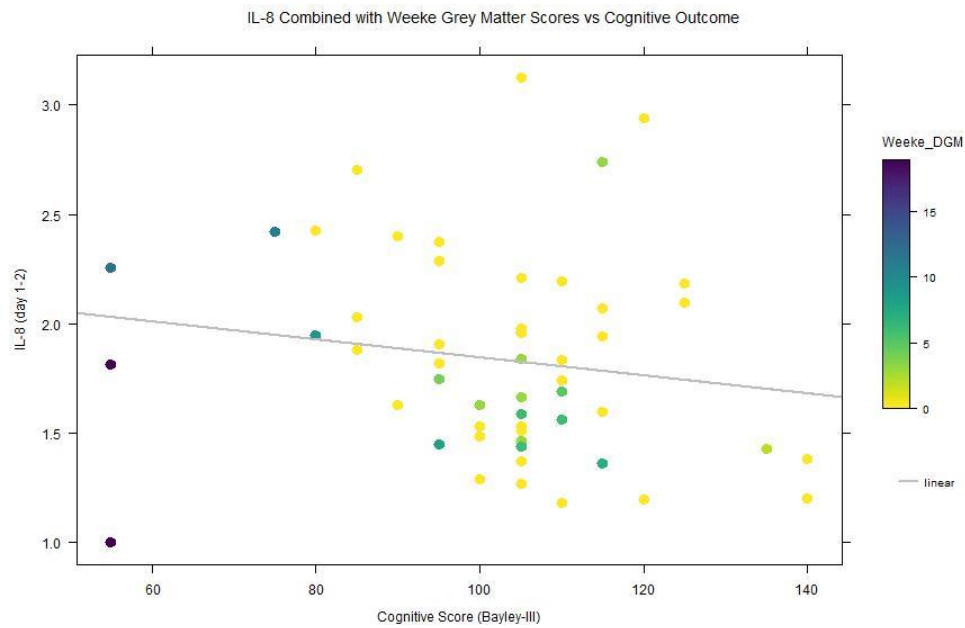


Figure 40. *IL-8 at day 1-2 combined with Weeke grey matter scores plotted against cognitive outcome Bayley-III scores (controlled for sex).*

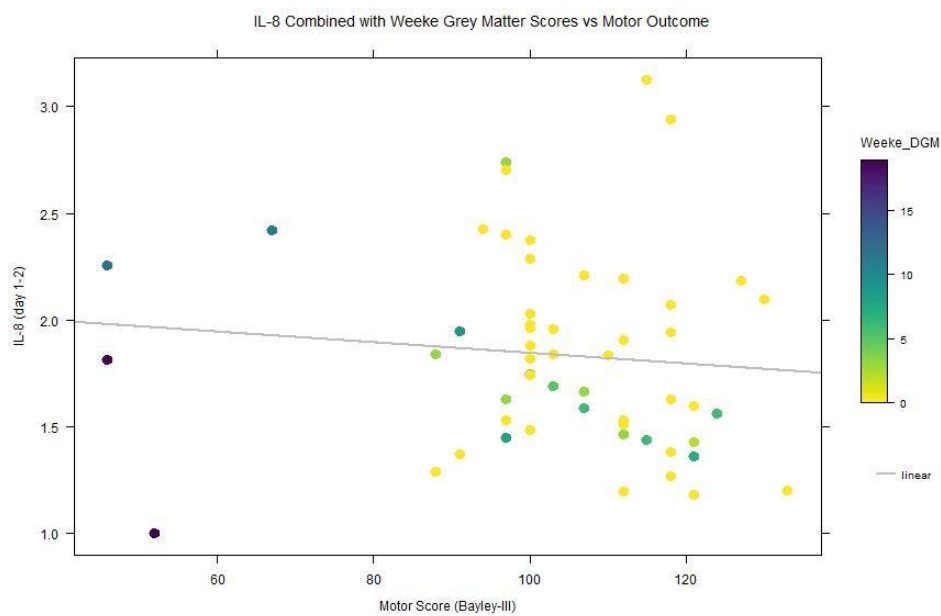


Figure 41. *IL-8 at day 1-2 combined with Weeke grey matter scores plotted against motor outcome Bayley-III scores (controlled for sex).*

8.3.5 Placental Histology with MRI Brain Scores vs Neurodevelopmental Outcome

No statistically significant associations were found between placental histology (normal vs abnormal placenta) and long-term neurodevelopmental outcome measures when combined with MRI brain scores. Results can be seen in full in table 21.

p-values	Barkovich BG/W	NICHD NRN	Weeke GM	Weeke WM	Weeke Cerebellum	Weeke Overall	Normal/ Abnormal MRI
Cognitive	0.612	0.786	0.603	0.592	0.685	0.612	0.755
Motor	0.556	0.723	0.491	0.533	0.699	0.518	0.683
Language	0.648	0.714	0.555	0.613	0.895	0.601	0.731

Table 21. Summary of linear regression analyses between placental histology combined with MRI brain scores, and neurodevelopmental outcome scores. Abbreviations: BG/W, basal ganglia/watershed; GM, grey matter; MRI, magnetic resonance imaging; NICHD NRN, National Institute of Child Health and Human Development Neonatal Research Network; WM, white matter.

8.4 Discussion

The present study investigated whether the addition of inflammatory markers and placental histology may improve the ability of MRI to predict long-term neurodevelopmental outcome in infants with NE. We identified GM-CSF as a biomarker to predict cognitive (but not motor or language) outcome when combined with MRI brain scores. For all MRI scoring systems – Barkovich, NICHD NRN, Weeke (WM, cerebellum, and overall), and normal/abnormal MRI – higher levels of GM-CSF on day 1-2 of life were associated with lower cognitive composite scores on the Bayley-III. It should be noted, however, that after FDR correction, these results did not reach statistical significance. We also identify IL-8 as a potential biomarker for predicting cognitive and motor outcome when combined with the Weeke GM score only. We

found no relationship between placental histology and neurodevelopmental outcome when combined with MRI brain scores. We can therefore report a definite trend for GM-CSF and IL-8, and outcome scores when combined with MRI brain scores.

Our previous study identified GM-CSF as the only cytokine associated with MRI brain scores in infants with NE, which reinforces our findings here that GM-CSF in addition to MRI brain scores may be used to predict cognitive outcomes. However, whilst our previous study only identified the Weeke scoring system as being associated with GM-CSF, here we found that the Barkovich, NICHD NRN and Weeke scoring systems, as well as a binary category of normal/abnormal MRI, were all able to predict outcome when combined with GM-CSF. It is interesting that GM-CSF was only predictive of cognitive outcome, a finding that was consistent across all scoring systems. Ní Bhroin et al²³⁶ found that all three of these scoring systems were able to predict cognitive outcome in NE (also measured by the Bayley-III) and we are able to add to this by suggesting that combining these MRI brain scores with GM-CSF levels at day 1-2 of life in the NE infant may be an even more powerful prognostic biomarker of cognitive outcome.

As mentioned in the previous chapter, the hematopoietic cytokine GM-CSF is thought to be neuroprotective, as it can cross the blood-brain barrier and seemingly counteract programmed cell death through stimulating the production of granulocytes and monocytes²²⁷. Altered GM-CSF levels have previously been reported in both the term and preterm population who are later diagnosed with cerebral palsy (CP), with lower levels of GM-CSF seen in the preterm infants²⁵¹ and higher GM-CSF in the term infants²⁵² compared to controls. Further research has also put forward the prognostic possibility of GM-CSF in NE, with O'Hare et al²²⁴ reporting that elevated GM-CSF was associated with mortality in NE. A previous study on the MODE cohort by Sweetman et al²⁴⁸ also found GM-CSF to be associated with mortality, as well as showing a negative correlation between GM-CSF and Bayley-III language scores. We, however, observed a negative correlation between GM-CSF and cognitive scores rather than language. Animal models have suggested the importance of GM-CSF in cognition, in particular learning and memory²⁵³, and have supported the neuroprotective ability of GM-CSF in a number of conditions that affect cognition, including stroke²⁵⁴, Down Syndrome²⁵⁵, traumatic brain injury²⁵⁶, and Alzheimer's Disease²⁵⁷. Short-term GM-CSF treatment has even been shown to improve cognition and memory in human participants with mild-moderate

Alzheimer's Disease²⁵⁸. In this study, we are able to demonstrate a direct link between GM-CSF, MRI brain scores and cognition in the infant population.

The Bayley-III cognitive scale includes 91 items that assess children's sensorimotor development, exploration and manipulation of objects, object relations, concept formation, and memory²⁵⁹. Infants at two years of age complete tasks such as finding hidden objects, stacking blocks, placing pegs in a pegboard, attending to a story, and relational play both with the self and others. Early infancy is a critical period in the development of structure and function in the brain, and the above cognitive functions that are measured by the Bayley-III have been linked to specific regions of the infant brain. In a review by Paterson et al²⁶⁰, they identify the key role of the frontal lobe in object permanence and joint attention in infants. They state the importance of months 8-12 of life in the development of the frontal lobe, which have far-reaching effects on cognition. The basal ganglia and thalamus – critical areas that are affected in NE – participate in a loop with frontal areas of the cortex (including the dorsolateral prefrontal cortex, the lateral orbitofrontal cortex, and the anterior cingulate/medial orbitofrontal cortices) that are known to be involved in cognitive functions such as planning, working memory, rule-based learning, and attention²⁶¹. It may be that brain injury in NE affects these loops, and in turn, affects the typical development of cognition.

Though to a lesser extent than GM-CSF, we were also able to demonstrate a link between IL-8 and cognitive and motor outcomes when combined with the Weekee GM scoring system. IL-8 belongs to the group of cytokines called interleukins, a large group of immunomodulatory proteins that are expressed and secreted by leukocytes and can have pro- and anti-inflammatory effects²⁶². IL-8 itself is a chemokine that plays a causative role in acute inflammation by recruiting and activating neutrophils, causing them to migrate to the site of injury/infection²⁶³. IL-8 has consistently been associated with NE, with higher levels seen in infants with NE during the first days of life compared to controls^{225,240,248,264,266,267}, higher levels in infants who underwent TH^{248,264}, and increasing levels of IL-8 with increasing severity of NE grade^{224,268}.

Research regarding IL-8 and outcomes in NE is mixed. Elevated IL-8 has been found to be associated with mortality in NE²²⁴, and IL-8 has been discovered to be elevated in term-born infants with CP compared to controls²⁵². IL-8 has also been found to be significantly higher in

school-age children with NE compared to controls²⁴¹. Bartha et al²⁴⁹ found that although not associated with MRI scores, IL-8 was higher in NE children with abnormal neurodevelopmental outcome, and Chalak et al²⁶⁴ also found IL-8 to be higher in infants with abnormal than normal outcome, as determined by the Bayley-III. On the other hand, in research from the MODE cohort, Sweetman et al²⁴⁸ found that although IL-8 was elevated in infants with NE compared to controls, it was not predictive of Bayley-III outcomes. There is support for the ability of IL-8 to predict motor outcomes. Foster-Barber et al²⁶⁵ found that IL-8 was associated with abnormal neuromotor outcome at one year of age in infants with NE, with higher levels of IL-8 seen in infants with more abnormal neuromotor scores, and in those who developed CP compared to those who did not. Akin to our findings, Dietrick et al²²⁵ found that IL-8 was inversely related to Bayley-III motor outcomes in NE, though IL-8 was not related to MRI brain scores. In this case, however, the Barkovich scoring system was used, and we identified IL-8 to be prognostic of outcome only when combined with the Weekee score – a scoring system that is arguably the most extensive and detailed in assessing MRI brain injury in NE.

In this study, we failed to find any association between placental histology (normal vs abnormal placenta) and outcome measures when combined with MRI brain scores. Whilst studies have independently linked placental pathology both with MRI brain scores^{228,232} and with neurodevelopmental outcome^{228,229}, to our knowledge, none have directly linked all three. Frank et al²⁴⁵ also assessed the relationship between these variables but found that most placental abnormalities were unrelated to patterns of brain injury in NE or to neurodevelopmental outcome after therapeutic hypothermia. In their study, only infants who had undergone TH were included, and they suggest that their negative results may be attributed to the neuroprotective effects of TH, with the effect of TH in preventing extensive brain injury and subsequent adverse neurodevelopmental outcome in NE not being adversely affected by placental pathology. This effect cannot be attributed in the present study, however, as we did not separate infants based on TH, and so included both infants with milder NE who did not reach the criteria for cooling, and the more severely affected infants who required TH. A factor that may have played a role in our negative results is that the number of infants with normal placenta was higher than those with abnormal placenta (n=35 vs n=19). A larger sample size of infants with placental pathologies would be beneficial in uncovering any potential interactions with MRI brain scores and outcomes.

The limitations of this study are similar to that of the previous study and include cytokine levels being taken only on day 1-2 of life due to missing data, and a relatively small sample size (54 in each analysis), which also meant we were unable to examine the sub-group of chorioamnionitis vs no chorioamnionitis within the placenta group due to small numbers. Whilst placental pathology reports of outborn infants were internally validated, the lack of in-house placental pathology review should also be considered a weakness. Additionally, only a small portion of infants was classified as having mild or severe impairment based on their Bayley-III composite scores, with many infants falling into the normal range. However, as our results still found an association between GM-CSF and cognitive scores across all MRI scoring systems, this suggests that these variables may be predictive of cognition within the normal range of the Bayley-III. Finally, as previously discussed, the gold-standard timeframe for scanning these neonates is between 3-7 days of life. Our study included infants that were scanned up until the second week of life, and therefore this is another definite weakness, as pseudonormalisation can occur after the first week. Strengths of our study again include highly standardised protocols, using published classification systems for scoring MRI injury, grading placentae, and for assessing neurodevelopmental outcome, as well as blind assessment of neurodevelopment in relation to MRI brain scores.

8.5 Conclusions

Whilst inflammatory cytokines have been independently linked to both MRI brain scores and to neurodevelopmental outcome in NE, there are very few studies attempting to link all three. Here we investigated whether the addition of inflammatory cytokines to MRI brain scores can improve prognostic ability. We identify GM-CSF to be associated with cognitive outcome measured by the Bayley-III when combined with MRI brain scores, and this was consistent across all scoring systems (Barkovich, NICHD NRN, Weeke, and normal/abnormal MRI). We also identify a relationship between IL-8 levels and both cognitive and motor outcomes when combined with Weeke GM scores. We also investigated whether the addition of placental pathology to MRI brain scores may improve outcome prediction, however, we found no relationship. Therefore, we advocate for the use of including cytokines (in particular GM-CSF) as a combined biomarker with MRI in predicting cognitive outcome in infants with NE,

however, we encourage further study with larger sample sizes to investigate whether there is any such relation with placental pathology.

Chapter 9: Discussion and Conclusions

“MRI provides invaluable information on the patterns and evolution of injury following HIE. This information will prove vital for monitoring the effects, both desired and not, of any future interventions”.

- *Prof Mary Rutherford*¹²³

The studies presented in this thesis provide important information about neonatal brain development and brain injury, through the lens of neuroimaging. We first examined the typically developing neonatal brain, and empirical study one used both structural and functional techniques to explore the changes in architecture with gestational age across the grey and white matter of the brain. We then focused on brain injury in neonatal encephalopathy (NE), presenting a systematic review and the remaining two empirical studies. The systematic review investigated the use of DTI to detect WM alterations in infants with NE. Empirical study two explored the linear relationship between the pattern of brain injury seen on MRI, and inflammatory markers and placental pathology in NE. Empirical study three elaborated on this by testing MRI brain scores and inflammatory markers/placental pathology as a combined biomarker to predict neurodevelopmental outcome. This final chapter will review the findings of each study, discuss the implications in a wider context and our contributions to the field, evaluate the strengths and limitations of the work, and finally, suggest directions for future research.

In chapter two, we outlined the incredibly complex and dynamic changes that the brain undergoes during development, highlighting the critical neonatal period in which synaptogenesis, pruning, and myelination are at their peak. Understanding these vast and rapid changes that occur in the postnatal period is an area of great interest, yet also great challenges in terms of data acquisition, processing, analysis, and interpretation²⁶⁹. The first empirical study of this thesis (chapter six) used an approach optimised for the neonatal population¹⁹⁰⁻¹⁹⁹ to quantify these microstructural changes in terms of DTI, NODDI, myelin and fMRI measures. Our study introduces a threefold novel approach. Firstly, whilst there is

existing literature applying DTI, NODDI and myelin measures to the white matter of the neonate, here we use these techniques to examine the structural developmental trajectories of white matter, cortical grey matter, sub-cortical grey matter, and the brain as a whole. Secondly, we combine these structural MRI measures with fMRI measures, in order to determine whether the developmental trajectories of both structural and functional architecture are consistent or homogeneous across the grey matter of the neonatal brain. We employ fMRI measures that have remained largely unexplored in the typically developing neonate – ALFF, fALFF and mALFF. These metrics employ a local voxel-level approach, which differs from the more commonly used resting-state networks to measure functional connectivity. To our knowledge, this is the first study that combines these specific multi-sequence MRI metrics to examine changes with gestational age and the relationship between structure and function in the typically developing neonate. Finally, by removing the effects of gestational age, we examine the relationship between MRI measures and myelination using the T_1/T_2 ratio. We not only conduct this analysis for DTI and NODDI metrics, but we also look at the relationship between fMRI metrics and myelin, which remains largely unexplored in the neonate.

We demonstrate that all DTI, NODDI, and myelin measures are strongly associated with gestational age on a global level, reflecting a widespread maturation of microarchitecture. On the other hand, there was more variation to be seen on a regional level. NDI and myelin, but not ODI, are consistently increasing with age across all regions of the brain, whilst FA and MD show strong associations in cortical white matter and deep grey matter, but less so in the cortical grey matter, and not at all in the corpus callosum. DTI measures (FA and MD) in the developing brain are a reflection of microstructural changes and decreasing water content. For white matter, increasing FA and decreasing MD with gestational age seemingly reflect the establishment, organisation, and maturation of fibre connections, as well as premyelination and myelination²⁰⁹. For deep grey matter, these anisotropy changes may reflect robust membrane proliferation and myelination²¹³. For grey matter, however, changes in DTI measures may be a reflection of synaptogenesis and arborisation that alters the radial organisation of the cortex²⁰⁹. This radial organisation disappears by approximately 36 weeks gestation, which leads to roughly equivalent hindrance of water motion both orthogonally and radially to the cortical surface, hence no significant changes in DTI measures with gestational

age in the term-born neonate. This lateral and radial fanning of dendrites also leads to a high ODI at term age, and subsequent increases in dendritic branching are unlikely to affect ODI on regional level²⁰⁶. On the other hand, increases in dendritic branching are likely to result in increases in NDI, a measure of neurite density, and indeed we found NDI to be consistently increasing with gestational age across all regions of the brain. Our findings demonstrate that rapid and widespread microstructural changes occur throughout the neonatal brain, even within merely a few weeks of postnatal life, and lend support to the idea that the maturation of structural architecture is heterogeneous across different tissue types and across the different lobes of the brain.

Our fMRI analyses also provide valuable information for neonatal fMRI researchers, which may encourage further research using voxel-based functional metrics in the neonatal population. Notably, we identify fALFF – the fractional amplitude of low-frequency fluctuations in the BOLD signal – as a measure that is sensitive to gestational age in the term neonatal brain, across the grey matter of the cortex, and some sub-cortical structures, whilst its similar counterparts ALFF and mALFF do not show the same sensitivity to changes in gestational age. Interestingly, however, is our finding that these fMRI metrics do not seem to be directly associated with DTI and NODDI measures in the neonatal brain. It is generally thought that the development of structure and function in the brain go hand in hand, however, this surprising negative finding suggests that this may not be the case in the first few postnatal days/weeks. It may be that, although structural and functional architecture develop with increasing gestational age, they may in fact follow distinct separate developmental trajectories very early in life, and only begin to merge with each other as the infant develops more complex neurodevelopmental skills. Our study certainly encourages future research to examine in more depth how the interplay between structure and function during early development may be delineated and quantified.

By removing the effects of gestational age, we demonstrate myelin to be strongly associated with DTI and NODDI measures (all except ODI) throughout many regions of the brain, but not on a global level. Therefore, we advocate for the use of these MRI techniques to identify local changes in myelination that may be sensitive to tissue type and to region, rather than overall global increases in myelin. Conversely, fMRI metrics were largely not associated with myelin on a regional level but fALFF was tentatively associated with myelin

globally. fALFF did also show several trends towards an increase with myelination in some regions the cortical and deep grey matter, as well as one significant association in the parietal grey matter. Here we again identify fALFF as a metric that is far more sensitive to developmental changes in the neonate than ALFF or mALFF. We suggest that whilst local functional maturation may follow a more protracted trajectory than myelination, there may be a change in overall functional development as myelination occurs. Whilst our DTI and NODDI analyses were conducted within all regions of the brain, we limited our fMRI analyses to regions of GM only. Our reasons were twofold: firstly, cerebral blood flow and volume – on which the BOLD signal depends – are lower in WM than GM; secondly, the fMRI signal is thought to arise primarily from post-synaptic potentials (which occur mainly in GM) rather than action potentials (neural activity in WM)²⁷⁰. However, we would encourage future research to examine whether there exists a relationship between functional activity (using fALFF) and myelin in the WM of the typically developing neonate, as WM is not merely a form of connecting regions of GM, but it also has its own functional significance. Indeed, our systematic review of DTI in the WM of infants with NE (chapter 5.3) identified two key WM regions – the CC and the PLIC – that were associated with poorer neurodevelopmental outcomes. WM fMRI therefore may open new research avenues not only to neonatal studies of neurological disorder, but also to the healthy developing neonate. Neurodevelopmental outcomes were not available for the cohort of infants at the time of analysis for this study, however, this data is to be made available by the dHCP in the near future, and so we would also invite further research to link our findings to long-term outcome measures in these infants.

In chapters four and five, we detail NE in-depth and how neuroimaging is used as a clinical tool to assess infants with this form of brain injury. NE is a heterogenous clinical syndrome described by disordered neurological functioning in the term-born neonate and is one of the leading causes of infant morbidity and mortality. The gold standard of care is therapeutic hypothermia (TH), a neuroprotective intervention started within six hours of birth that aims to slow down the progression of brain injury. MRI is widely deemed an effective biomarker in NE, and our systematic review in chapter five demonstrates the ability of DTI to detect specific patterns of injury in the WM of infants with NE that can even be related to adverse long-term neurodevelopmental outcome. We advocate for the addition of DWI and

DTI to the scanning protocol within the first week of life, with particular attention placed on obtaining FA measures in the PLIC, and the genu and splenium of the CC. This data may then be used to assess the impact of TH on the injury, and as a prognostic tool in infants with NE. Our goal in empirical studies two and three (chapters six and seven) was to then examine the potential of MRI as a combined biomarker along with inflammatory cytokines and placental histology to improve its diagnostic and prognostic efficacy.

Empirical study two investigated whether inflammatory cytokines and placental histology were linearly associated with MRI brain scores in NE, assessed by Barkovich¹³², NICHD NRN¹³⁴, and Weeke¹³⁵ scoring systems. Whilst each of these three variables has been independently investigated in NE, the evidence linking both cytokines and placental histology to MRI is scarce and variable, and thus warranted further investigation. Additionally, our study provides a novel perspective by utilizing multiple MRI scoring systems in order to identify more specific nuances in the relationship between these variables. Of the four cytokines under investigation, we identified higher levels of GM-CSF on day 1-2 of life to be associated with increasing severity of deep grey matter injury using the Weeke scoring system. GM-CSF is a hematopoietic cytokine that counteracts apoptosis in neurons whose receptors for GM-CSF are induced by cerebral ischaemia²²⁷. GM-CSF has previously been found to be significantly elevated in infants with NE and abnormal neuroimaging^{224, 240}, and GM-CSF dysregulation has even been found to persist through to childhood²⁴⁰. Additionally, injury to the deep grey matter structures of the basal ganglia and thalamus is most often seen in more acute and severe cases of NE¹²⁶. Our finding may therefore suggest that GM-CSF combined with the Weeke grey matter score may be an effective biomarker in identifying those infants with more severe NE who are most in need of TH intervention. Whilst we found no relationship between EPO, IL-8, and VEGF on day 1-2 of life, and MRI brain scores, we would suggest that future research examine these cytokines and MRI brain scores with a larger sample size, and with cytokine levels taken at multiple time points during the first week of life.

We also found no statistically significant associations between placental histology and MRI brain scores. Placental pathology is one of the many aetiologies of NE and includes abnormalities such as umbilical cord lesion, chorioamnionitis, foetal vasculitis, chorionic plate meconium, and foetal thrombotic vasculopathy⁸³. In the present study, we separated infants based on whether they had a normal or an abnormal placenta. We also sub-divided the group

with an abnormal placenta into those who had chorioamnionitis – an infection of the placenta and the amniotic fluid – and those who did not. Evidence regarding placental pathology, in particular chorioamnionitis, and MRI brain injury is variable and somewhat challenging in NE. Whilst there is a wide prevalence of this pathology in NE, studies have reported some ambiguous findings regarding its relationship with neuroimaging. Harteman et al²³² found chorioamnionitis to be most prevalent in neonates with NE both with no brain injury and with the most severe injury. Hayes et al²²⁹ on the other hand, have reported chorioamnionitis to be predictive of mild NE only. Further study using a much larger sample size is recommended. We would also encourage future research to examine the relationship between chorioamnionitis and inflammatory cytokines in different patterns of injury in NE. It is speculated that cytokines may represent the final common pathway leading to brain injury that bridges both chorioamnionitis and NE²²¹, and whilst we analysed these two variables independently in relation to MRI brain scores, key answers may lie in the interplay between all three.

Empirical study three examined whether the addition of inflammatory markers and placental histology would improve the ability of MRI to predict long-term neurodevelopmental outcome in infants with NE. This combination of biomarkers and neurodevelopmental outcome has scarcely been studied in the encephalopathic neonatal population; thus the present study provides new information for clinicians that may aid in the redirection of care for these infants. We identify GM-CSF as a biomarker to predict cognitive outcome (but not motor or language) when combined with almost all scoring systems: Barkovich, NICHD NRN, Weeke (white matter, cerebellum, and overall, but not grey matter), as well as for normal/abnormal MRI. Higher levels of GM-CSF were associated with lower composite scores on the Bayley-III. The consistency of these results for cognitive scores only across all scoring systems, in conjunction with the results of study two, reinforces our advocacy for GM-CSF as a combined biomarker alongside MRI brain scores to assess injury and predict cognitive outcome in infants with NE. Whilst both MRI scoring systems in NE and GM-CSF have independently been associated with cognitive outcome, we add to current knowledge by showing that they can be used hand in hand with each other as an even more powerful predictor of cognition at two years of age. However, we would again recommend reconducting the analysis with a larger sample size, as these associations did not survive FDR correction. We also identify IL-8 as a

predictor of both cognitive and motor outcome when combined with the Weeke GM score only. IL-8 has been linked to cerebral palsy in infants with NE^{252,265}, a movement disorder that is commonly associated with BGT injury in NE¹⁴¹. Given that in the present study IL-8 was associated only with the Weeke GM score, it may be that elevated levels of IL-8 may be predictive of more severe neurodevelopmental sequelae due to BGT injury in NE.

Consistent with empirical study two, the present study also failed to find any statistically significant associations between placental histology and neurodevelopmental outcome when combined with any MRI brain scores. Evidence regarding placental pathology and long-term outcomes in NE is again challenging, and whilst some studies have managed to link the two variables in terms of severity^{228,229}, Harteman²³² et al found no associations between placental pathology and neurodevelopmental outcomes. Interestingly, Frank et al²⁴⁵ found small placental infarcts to be common among infants with favourable neurodevelopmental outcome, and Jenster²⁷¹ et al even found that chorioamnionitis was associated with a lower risk of adverse neurodevelopmental outcomes. They posit that chorioamnionitis has a protective effect in infants with NE, suggesting that the prenatal timing may have a preconditioning effect that protects the infant against hypoxia–ischemic injury. In the present study, we did not have large enough numbers of infants to be able to conduct a separate analysis for those with/without chorioamnionitis, and we had a larger number of infants with normal placenta compared to abnormal. Therefore, further research should employ a larger sample size, with comparison groups of various different placental pathologies in order to tease out any possible associations with MRI brain scores and long-term outcomes.

In conclusion, multi-sequence MRI neuroimaging studies during early infancy offer an insight and understanding into the very beginnings of brain development, and the factors that may contribute to individual differences in cognitive functioning, behaviour, and risk for neurodevelopmental disorders, such as NE. NE can be a devastating diagnosis for parents and infants who otherwise experience healthy pregnancy, and early intervention and prognostication is crucial. MRI is an effective biomarker in NE, in particular DTI on day 3-7 of life. We suggest that combining MRI brain scores with other clinical biomarkers such as inflammatory cytokine GM-CSF may be an even more powerful tool to assess the severity of the injury, determine infants in need of TH, and predict long-term cognitive outcomes. We encourage further neuroimaging analyses of both typically developing infants and those with

NE, to correlate MRI measures with detailed long-term neurodevelopmental outcomes past two years of age, which was unfortunately beyond the scope of this thesis. Nevertheless, we provide important information both for MRI researchers and clinicians, on the details of early brain development and injury.

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Appendix

Diffusion Tensor Imaging in Very Preterm, Moderate-Late Preterm and Term-Born Neonates: A Systematic Review

The content of this section is part of a systematic review that was published in the *Journal of Pediatrics* (Dibble et al, 2021).

Abstract

Objective

To examine white matter abnormalities, measured by Diffusion Tensor Imaging (DTI), in very preterm (<32 weeks) and moderate-late preterm neonates (32-37 weeks) at term-equivalent age, compared to healthy full-term controls (≥37 weeks).

Study Design

A search of Medline (Pubmed) was conducted to identify studies with diffusion data collected on very preterm, moderate-late preterm and full-term neonates, using the guidelines from the Meta-analysis of Observational Studies in Epidemiology (MOOSE) and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statements.

Results

Eleven studies were included with DTI data from 554 very preterm, 575 moderate-late preterm, and 318 full-term neonates. Widespread statistically significant diffusion measures were found in all preterm sub-groups at term-equivalent age compared to full-term neonates, and this difference was more marked for the very preterm group. These abnormalities are suggestive of changes in white matter microstructure in the preterm groups. The corpus callosum was a region of interest in both early and moderate-late preterm groups, which showed statistically significant diffusion measures in all eleven studies.

Conclusions

Microstructural white matter changes may underpin the increased risk of neurodevelopmental disability seen in preterm infants in later life. DTI may therefore be a useful prognostic tool for neuro-disability in preterm neonates.

Introduction

Prenatally, there is accelerated brain development, involving the growth and elaboration of cortical and sub-cortical features¹. There is a relative slowing in development after birth. Cognitive development is coupled to the structural and functional maturation of the brain². Preterm birth perturbs this developmental trajectory of the brain and so poses a considerable risk to cognitive development³. Preterm birth is the leading cause of death in children under the age of five^{4,5}, and accounts for 3.1% of all Disability Adjusted Life Years (DALYs) in the Global Burden of Disease⁶. Preterm neonates who survive are at greater risk of a range neurodevelopmental sequelae, including cognitive dysfunction, cerebral palsy, impaired vision and hearing, and behavioural and emotional problems⁷.

The standard of care for preterm neonates at present is sequential cranial ultrasound (CUS), which is used to assess white matter injury - such as cystic periventricular leukomalacia, intraventricular haemorrhage and ventricular enlargement - in the brain at term-equivalent age^{8,9}. However, Magnetic Resonance Imaging (MRI) has been shown to be superior to CUS in its sensitivity and prognostic ability⁹⁻¹¹. Diffusion Weighted Imaging (DWI) is an advanced MRI technique to measure the diffusion of water in the brain, which differs depending on the type of tissue, and provides information from which one may infer the cellular structure of a region. In CSF, water is unrestricted and free to diffuse in any direction, and so is isotropic. However, in white matter, which mostly consists of axonal fibres pointing in a specific direction, diffusion is restricted and will occur along the same direction as the fibres, and so is anisotropic. In grey matter, which mostly consists of dendrites with a randomly oriented microstructure, diffusion may appear isotropic although it is in fact anisotropic – this appearance of isotropy is due to limitations in the DWI sequences. The three-dimensional shape of this diffusion is modelled through Diffusion Tensor Imaging (DTI). With the DTI model, it is possible to infer, in each

voxel, properties such as the molecular diffusion rate (mean diffusivity), the directional preference of diffusion (fractional anisotropy), the axial (diffusion rate along the main axis of diffusion), and radial (rate of diffusion in the transverse direction) diffusivity.

Preterm neonates are at significant risk of acute ischaemic and/or haemorrhagic brain injury, most notably periventricular leukomalacia, a distinctive variant of cerebral white matter injury^{12,13}. However, evidence suggests that when studied at term-equivalent age, preterm neonates show markedly different diffusion values compared to term neonates even in the absence of abnormalities on their clinical evaluation or CUS¹⁴. The purpose of this systematic review was to examine current DTI findings in very preterm (<32 weeks) and moderate-late preterm (32-37 weeks) neonates at term-equivalent age, as compared to healthy, full-term neonates (≥37 weeks). This would allow for the identification of white matter microstructural alterations present in preterm neonates at term-equivalent age which may contribute to later neurodevelopmental sequelae.

Methods

A systematic review of the literature was carried out using the guidelines from the Meta-analysis of Observational Studies in Epidemiology (MOOSE)¹⁵ and Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)¹⁶ statements. Medline (PubMed) was searched using combinations of the terms (preterm), (term), (white matter) and (diffusion tensor imaging). We selected the relevant papers using the PRISMA flowchart (Figure 1). This search of the literature was conducted over a period of ten weeks.

Studies were included based on the following inclusion criteria: (1) neonates were grouped by GA at birth, (2) at least one of these groups was very preterm or moderate-late preterm, (3) a control group of full-term neonates was present OR a comparison of the two preterm groups was made, (4) neonates were scanned at term-equivalent age and (5) diffusion weighted images were obtained and analysed. We also included studies with preterm neonates born with extremely low birth weight (ELBW) and very low birthweight (VLBW). Cross-sectional, cohort and case-control study designs were included in the search. Studies were excluded if there was insufficient data on the GA of the neonates, neonates were

not scanned at term-equivalent age, there was no comparison between groups based on GA, animal models were used, if grey matter alone was studied, or studies were not published in the English language. We selected papers in which neonates did not present with any illness/disease for any of the groups (very preterm, moderate-late preterm and full-term). However, we did not exclude the papers which also include infants with minor abnormalities if the above criteria were met. Studies were also assessed for quality and bias with the application of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement¹⁷ to selected articles.

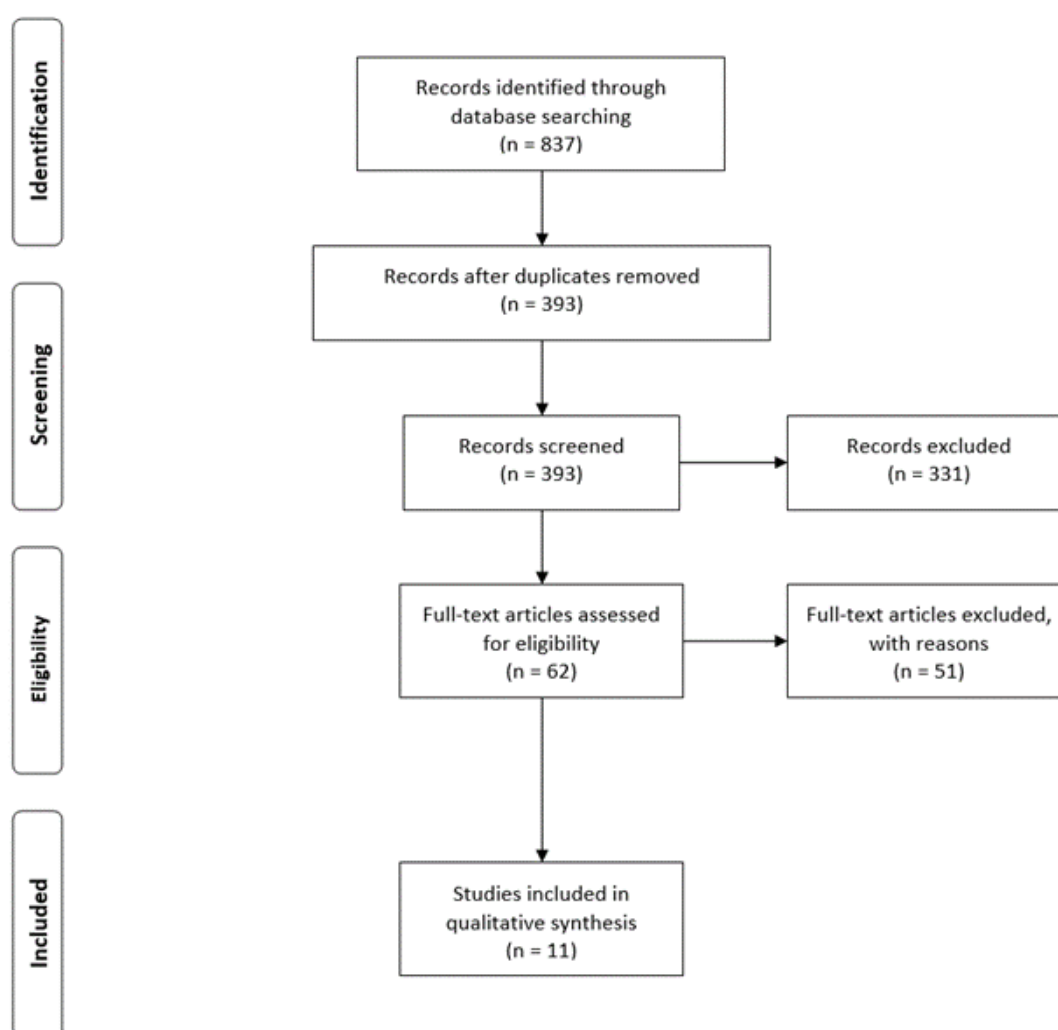


Figure 1. PRISMA flowchart of the search and selection process.

Data extracted from the studies included population characteristics, criteria for determining groups based on GA, diffusion metrics for white matter, and specific white matter tracts, or regions of interest, that showed statistically significant diffusion measures in preterm neonates compared to full-term neonates. These variables were then compiled into a summarising table (see Table 1).

We aimed to define and quantify the specific white matter tracts, or regions of interest, that seem to be implicated in preterm birth. By using the JHU-ICBM DTI-81 white matter labels¹⁸ as a starting point, we calculated the number of times each region of interest was mentioned in the results section of these studies as having statistically significant diffusion measures. We then visualised the results by creating a greyscale image using FSLeves (<http://www.fmrib.ox.ac.uk/fsl>). Using FSL's FMRIB58 fractional anisotropy image as a template, we placed the JHU-ICBM DTI-81 white matter labels atlas as an overlay, selecting the relevant tracts and removing the tracts that were not part of the results in the literature review. The relative weighting of the tracts was a function of the number of times it appeared in the literature review.

Study selection, data extraction and analysis, and quality assessment were conducted by three authors independently – one author for the early preterm group, one author for the moderate-late preterm group, and one author for the two groups combined.

Authors	Definition/Criteria	Neonates with DWI scan	Metrics	Whole brain / ROI	Findings
Brossard-Racine et al (2017)	VLBW : ≤32 weeks GA, ≤1500g. Full term: ≥37 weeks GA. Exclusion: multiple pregnancies, foetal CUS/neonatal MRI abnormalities, congenital infection, chromosomal abnormalities, and/or multiorgan dysmorphic conditions.	73 VLBW 73 term	FA, MD	ROI - 7 cerebellar regions (anterior vermis, right/left SCP, MCP, and dentate nuclei) and the genu and splenium of CC	- No significant difference between left and right side of bilateral ROIs measured, except MD in the MCP of FT infants where the left MCP shows significantly lower MD compared with the right MCP. - Preterm infants at TEA presented with higher FA in the dentate nuclei and middle cerebellar peduncle, and significant lower MD in the vermis compared with controls. - Conversely, preterm infants showed reduced FA and increased MD in both the genu and splenium of the CC.
Thompson et al (2011)	VLBW: <30 weeks GA, <1250g. Exclusion: congenital anomalies. Full term: ≥37 weeks GA. Exclusion: antenatal/perinatal/neonatal complications and congenital	106 VLBW 22 term	FA, MD, RD, AD	ROI - CC (genu, rostral body, anterior mid-body, posterior mid-body, isthmus and splenium)	- FA was lower and MD, RD were higher in CC of VPT, especially at the anterior and posterior ends. - Volume of tracts originating from the CC reduced in VPT, particularly for anterior mid-body and isthmus tracts. - Reductions in CC area in VPT at TEA. - Cross sectional area: absolute difference in CC size of 8.5% between FT and VPT. - Reductions in cross-sectional area of the VPT rostral body, anterior and posterior mid-body, isthmus, and splenium compared with FT. VPT CC more circular. - Mean FA within the CC lower in VPT, while mean MD and RD higher in VPT CC compared with FT. No difference in AD between VPT and FT. - FA and AD highest in the splenium, followed by the genu, and lowest in the mid-body, for both VPT and FT.

	or chromosomal abnormalities.				- Both MD and RD values were highest in the mid-body, and lowest in the genu and splenium of the CC. - Diffusion within CC subregions FA was lower in VPT compared with FT within the genu, rostral body, isthmus and splenium of the CC. - Significant increase in MD and RD values in the genu, posterior mid-body and isthmus of the VPT CC compared with FT, as well as significantly increased AD values in the genu. - No significant difference in gender between FT and VPT infants.
Pannek et al (2018)	Very preterm: <31 weeks GA. Exclusion: genetic or chromosomal abnormality, non-English speaking caregivers, lived >200 km from the hospital. Full term: 38-41 weeks GA. Exclusion: pregnancy/delivery complications, birth weight below 10th percentile, admission to NICU, abnormal neurological examination at the time of the MRI.	55 very preterm 20 term	Fibre density (FD), fibre cross-section (FC), fibre density and bundle cross-section (FDC)	Whole brain – fixel-based analysis	- Reduced FD, FC and FDC in VPT without brain abnormalities compared to FT. - FD reduced in the anterior commissure, genu, midbody, and splenium of the CC, and a small area of the right fornix bilaterally to occipital and temporal lobe. - FDC reduced within the same structures as FD except the anterior midbody of CC and anterior commissure. - FC was reduced in the: (1) CC (genu and splenium); (2) Optic radiations (left hemisphere - more extensive), cingulum bundle, cerebral and cerebellar peduncles bilaterally; (3) WM of the right postcentral gyrus, left SLF. - No significant differences between VPT with no and with minimal brain abnormality in FDC and FC when compared to term born babies. - Reduction of FD more spatially extensive in VPT with minimal brain abnormalities than VPT without brain abnormalities when compared with FT. - FD reduced in the anterior commissure extending to the temporal lobes and a small area within the splenium of the CC that showed statistically significant differences in FD between the two groups. - Correlation between FC and FDC, and ICV statistically significant throughout the white matter after correction for PMA, while correlation between FD and ICV significant only in the left PLIC, cerebral and cerebellar peduncles, and anterior commissure.

Pogribna et al (2013)	Very preterm: ≤ 29 weeks GA and/or ELBW: <1000g. Exclusion: Congenital anomalies, mechanically ventilated. Full term: 37-42 weeks GA. Exclusion: History of perinatal distress or complications.	75 very preterm 15 term	FA, MD	7 WM ROIs: ALIC, PLIC, FPVZ, OPVZ, centrum semiovale, subventricular zone, genu and splenium of CC	Compared to FT, VPT infants with significantly lower FA in 3 of 7 (PLIC, occipital periventricular zone, CC) and higher MD in 6 of 7 white matter study regions (PLIC, FPVZ, OPVZ, CC, centrum semiovale, subventricular zone).
Rose et al (2008)	Very preterm: 25-29 weeks GA. Preterm: 29-32 weeks GA. Full term: 37-42 weeks GA.	12 very preterm 11 preterm 10 term	FA	Whole brain & ROI – TBSS & sagittal striatum, frontal WM, CC genu, external capsule, cerebral peduncle, CC splenium, corona radiata, centrum semiovale	- Reduced FA within the frontal lobe, and a number of anterior and posterior commissural pathways in VPT compared to FT (indicating reduced WM maturity). - VPT show significant increased FA in corticospinal pathway within cerebellar peduncle compared to FT. - Increased FA within the sagittal striatum, frontal lobe white matter, genu of the CC, external capsule, and white matter at the level of the centrum semiovale within the preterm compared to the VPT group.
Kaur et al (2014)	ELBW: <1000 g. Exclusion: severe white matter injury, major congenital anomalies. Full term: 37-41 weeks GA	29 ELBW 15 term	FA, MD, RD, AD	ROI - CC, CST, ILF, IFO, UNC, CG, fornix, optic radiations, MCP, SCP	- Most tracts displayed a lower FA, higher MD, AD, and RD, and fewer fibre numbers and voxels in ELBW than FT. - FA significantly reduced in the CC, CG UNC and IFO tracts and significantly elevated MD in the CC, CG, CST, ILF and fornix in ELBW compared to FT.

	Exclusion: history of perinatal distress or complications.				
Kelly et al (2016)	Moderate-late preterm: 32-26 weeks GA. Exclusion: conditions known to affect development. Full term: ≥ 37 weeks GA, ≥ 2500g. Exclusion: resuscitation at birth, clinically unwell, admitted to the newborn nursery.	193 moderate-late preterm 83 term	FA, MD, RD, AD	Whole brain - TBSS	- Many voxels had lower FA in MLPT infants vs FT, encompassing $\approx 59\%$ of the white matter skeleton. - No regions in which FA was higher in MLPT infants than FT. - Many voxels with higher MD in MLPT infants, generally co-located on same tracts as FA differences, encompassing $\approx 64\%$ of the white matter skeleton. - Many voxels had higher RD in MLPT than FTs, overall encompassed $\approx 69\%$ of the white matter skeleton. - Voxels with higher AD in MLPT infants found in many of the same tracts as FA group differences, less continuous in length, encompassed $\approx 41\%$ of the white matter skeleton. - AD, RD, and MD all higher in the superior cerebellar region in MLPT vs. FT, no difference in FA between groups in this region. - Only a small region where GA at birth correlated positively with FA and negatively with RD in MLPT infants.
Knight et al (2018)	Very preterm: < 32 weeks GA (singletons). Late preterm: 33-36 weeks GA (twins).	11 very preterm 20 late preterm	FA, MD, RD, AD	Whole brain - TBSS	- FA lower, RD higher in VPT group compared to LPT group, with a bias toward early myelinating regions. - VPT group appears to show myelogenesis which is lacking behind the LPT group at TEA, with early myelinating regions suffering the greatest adverse effects. - Late-myelinating regions appear to follow a more similar myelogenesis pathway, being less affected by premature birth/
Thompson et al (2019a)	Very preterm: < 32 weeks GA. Moderate preterm: 32-33 weeks GA.	92 very preterm	FA, MD, AD, RD	Whole brain – TBSS	- GA group associated with FA, MD, AD, and RD in 45-67% of the mean FA skeleton. - The regions in which GA group was associated with FA, MD, AD, and RD overlapped with some of the regions in which GA group was associated with white matter volume (CC, fornix, occipital white matter, temporal WM). - Overall voxels that differed (lower FA, higher MD) between MPT and FT were ≈ 10-20% fewer than in the VPT-FT.

	Late preterm: 34-36 weeks GA. Full term: ≥37 weeks GA. Exclusion: congenital abnormalities likely to affect brain growth or development.	69 moderate preterm 120 late preterm 80 term			- Overall voxels that differed (lower FA, higher MD) between LPT and FT were ≈10-20% fewer than in the MPT-FT. - Small number of voxels with higher FA and lower MD in each preterm group vs. FT group. Mostly in approx. location of cerebellar & brainstem white matter, cerebral peduncle, internal capsule, corona radiata. - Relatively stable number of voxels with this opposite pattern was relatively stable between VPT-FT, MPT-FT, and LPT-FT. - Differences in FA and MD between each preterm group and FT group mostly driven by RD rather than AD.
Kelly et al (2019)	Very preterm: 24-29 weeks GA. Moderate-late preterm: 32-36 weeks GA.	90 very preterm 173 moderate to late preterm	FA, RD, MD, AD	Whole brain – TBSS	- Some evidence that alterations in white matter microstructure of various tracts at TEA were related to atypical performance on some neurological and behavioural domains of the NNNS and HNNE in preterm infants at TEA. - Relationships between brain structure and neurological and behavioural performances relatively similar between VPT & MLPT groups. - Association generally in the direction of suboptimal scores associated with lower FA and higher AD/RD/MD. - Except for suboptimal NNNS tonic, which was associated with lower AD and MD.
Thompson et al (2019b)	Very preterm: <32 weeks GA. Moderate preterm: 32-33 weeks GA.	92 very preterm 69 moderate preterm	FA, MD, AD, RD	Whole brain - TBSS	- Male sex associated with lower FA & higher AD, RD, MD in optic radiation, external & internal capsules, and corona radiata. - These associations were generally similar between GA groups. - Postnatal growth not associated with brain volumes or diffusion values. - Higher BWSDS associated with higher FA & lower RD, MD consistently in CC, sagittal striatum, posterior thalamic radiation, internal & external capsules, and corona radiata in MPT & LPT groups. - With higher BWSDS, FA increased in ≈33% of the white matter skeleton, covering many of the major WM tracts. Mainly driven by MPT group & also by LPT group to lesser extent.

	Late preterm: 34-36 weeks GA. Full term: ≥ 37 weeks GA. Exclusion: congenital abnormalities likely to affect brain growth or development.	120 late preterm 80 term			
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Table 1. Summarising table of data extracted from the literature for the main variables of interest.

Results

The search yielded 837 articles, which after the removal of duplicates, left 393 individual studies. Full texts of 62 studies were examined, and eleven studies were included in our final list for review. These eleven studies included DTI data from 554 very preterm, 575 moderate-late preterm, and 318 full-term neonates¹⁹⁻²⁹ (two studies used the same cohort of infants). These studies all met the guidelines outlined in the STROBE statement¹⁷ regarding quality and bias. Overall, all preterm sub-groups showed atypical brain microstructure at term-equivalent age when compared with a full-term group. Most white matter tracts in preterm sub-groups scanned at term-equivalent age presented with lower fractional anisotropy, higher mean diffusivity, radial diffusivity, axial diffusivity, and fewer fibre count compared to full-term controls. Furthermore, moderate-late preterm neonates were shown to have higher fractional anisotropy and lower axial diffusivity, radial diffusivity, and mean diffusivity when compared to very preterm infants, with one study demonstrating a ≈ 10 -20% difference in overall percentage of voxels between successive GA groups²⁷.

We also calculated the frequency of regions of interest implicated in both very and moderate-late preterm birth (Table 2). Whilst widespread diffusion abnormalities were observed, one particular region of interest was identified – the corpus callosum, which had statistically significant diffusion measures in all eleven studies reviewed. The frequencies of these regions of interest were then converted to a greyscale image, in which the tracts with the most mentions (such as the corpus callosum), are shown as the brightest (white), and the tracts with the least mentions are shown as the darkest (black), with various levels of grey in between (Figure 2). Three studies provided sufficient data to calculate the effect size of differences in diffusion measures in these regions of interest between groups (Table 4; online). Notably, Cohen's *d* and Hedges' *g* values for various regions of the corpus callosum were found to exceed the convention for a large effect size ($\geq .80$).

Region of interest (ROI)	n = ROI mentioned in results
CC	11
OR, CG, PLIC, Cerebellar WM	6
SS, EC, CR, IFO	5
Fornix, CP, SLF, ALIC	4
CST, UNC	3
CS, ILF	2
AC, OPVZ, FPVZ, SVZ, Forceps minor & major	1

Table 2. A summarising table of ROIs implicated in NE from the literature and how often they were mentioned (ROIs chosen based on the JHU-ICBM DTI-81 white matter atlas). Abbreviations: CC, corpus callosum; OR, optic radiations; CG, cingulum; PLIC, posterior limb of the internal capsule; WM, white matter; SS, sagittal striatum; EC, external capsule; CR, corona radiata; IFO, inferior fronto-occipital fasciculus; CP, cerebral peduncle; SLF, superior longitudinal fasciculus; ALIC, anterior limb of the internal capsule; CST, corticospinal tract; UNC,

uncinate fasciculus; CS, centrum semiovale; ILF, inferior longitudinal fasciculus; AC, anterior commissure; OPVZ, occipital periventricular zone; FPVZ, frontal periventricular zone; SVZ, subventricular zone.

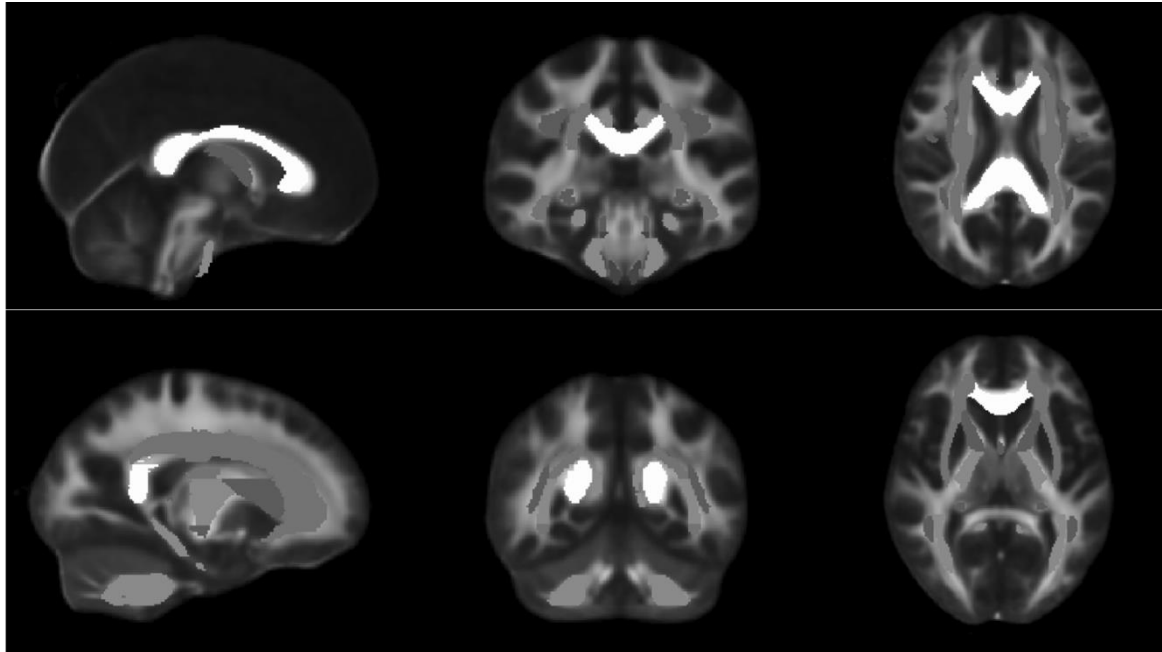


Figure 2. *Tract regions of interest as defined by JHU-ICBM DTI-81 white matter atlas, shown on the FMRIB58 fractional anisotropy template, in sagittal (left), coronal (centre) and axial (right) views. Tracts shown are those that arose as areas of interest in the literature search, with colour representing the relative amount of times that region of interest was mentioned; regions of interest in white being the most mentioned and regions of interest in black being the least.*

When considering very preterm neonates, data from 5 studies suggested that this group presents with a reduced fractional anisotropy and increased mean diffusivity in the genu and splenium of the corpus callosum when compared to full-term controls^{19,20,22-24}. This indicates a reduced white matter maturity in this region in the very preterm groups. On the other hand, very preterm infants present with higher fractional anisotropy and lower mean diffusivity in the corticospinal tract within the cerebellar peduncles compared to the

controls^{19,23}, however this finding may be due to the presence of crossing fibres in this region which can affect DTI parameters. A study measuring the fibre density, fibre cross-section and fibre density and bundle cross-section found that early preterm infants compared to term-born controls show a reduced fibre density, fibre cross-section and fibre density and bundle cross-section in many regions of the brain, including the corpus callosum, anterior commissure, cortico-spinal tract, optic radiations, and cingulum¹⁹. This is consistent with another study that found callosal area to be reduced by 8.5% in very preterm infants compared to full-term controls²⁰.

Each of the studies looking at moderate-late preterm neonates took a whole brain approach using tract-based spatial statistics (TBSS). Diffusion abnormalities encompassed up to 70% of the white matter skeleton, and the voxels were located bilaterally within several major fibre tracts; including the corpus callosum, cerebral peduncle, sagittal stratum, inferior fronto-occipital fasciculus, posterior thalamic (optic) radiation, uncinate fasciculus, forceps minor and major, cingulum, stria terminalis, external capsule, internal capsule (anterior limb, posterior limb and retrolenticular part), fornix, superior longitudinal fasciculus and corona radiata (anterior, superior and posterior sections)²⁵. The estimates of the percentage of the white matter skeleton with a lowered fractional anisotropy ranged between one third²⁹, $\approx 59\%$ ²⁵ and $\approx 45\text{--}67\%$ ²⁷.

The very preterm group appears to show myelogenesis which is lagging behind the moderate-late preterm group at term-equivalent age, with early myelinating regions suffering the greatest adverse effects. This is demonstrated by lower fractional anisotropy and higher radial diffusivity in the very preterm group compared to the moderate-late preterm group²⁶. Late-myelinating regions appear to follow a more similar myelogenesis pathway, being less affected by premature birth.

Discussion

All preterm sub-groups show widespread white matter abnormalities when scanned at term-equivalent age compared to full-term neonates. This is demonstrated by statistically significant diffusion measures in most white matter tracts throughout the brain. This

difference is more marked for the very preterm group compared to the moderate-late preterm group, with fractional anisotropy increasing and mean diffusivity, axial diffusivity and radial diffusivity decreasing as GA at birth increases.

DTI is increasingly being applied in studies of the developing brain, as these measures seem to be sensitive to maturational processes that alter the microstructure of the infant brain. Cellular substrates for diffusion changes seen during development may include decreases in axon packing density, number of oligodendroglia, degree of myelination or membrane integrity, and/or increases in tissue water content or extra-cellular space³⁰⁻³³. DTI measures have in fact been linked to myelination³⁰, the process in which the axons of neurons are surrounded in an insulating fatty sheath called myelin in order to facilitate neural connectivity and communication³⁴. Myelination is a major developmental process that begins during the second trimester and continues through to adulthood, with its most rapid development occurring during the first year of life³⁵. Myelination occurs in several predictable neuroanatomical sequences: from central to peripheral, from posterior to anterior, and from caudal to rostral³⁶. For this reason, premature birth may “interrupt” the normal scheme of myelination differently depending on gestational age.

Preterm infants often present with neurodevelopmental deficits later in life, such as lower performance IQ, learning difficulties and developmental coordination disorder³⁷. Our identification of the corpus callosum as a region of interest in preterm neonates is consistent with this - the corpus callosum is a large commissural fibre tract connecting the left and right hemispheres and is thought to play a key role in cognitive³⁸ and even motor³⁹ functioning. It has in fact been found that abnormal corpus callosum integrity is associated with poorer neurodevelopmental outcome in preterm infants, with callosal shape alterations associated with later cognitive impairment, and microstructural abnormalities within the tract associated with motor impairment at two years of age⁴⁰. Another study found that at term-equivalent age, extremely preterm infants (<26 weeks GA) had significantly lower fractional anisotropy in the splenium of the corpus callosum compared to preterm infants (>26 weeks GA). However, after a year of corrected age, there were no longer any significant between-group differences, suggesting that the white matter of younger GA infants may mature more quickly and eventually catch up with the older GA infants⁴¹.

It should be considered that, although this review focused solely on diffusion changes in the white matter tracts of the brain, DTI has also been used to demonstrate detectable changes in deep grey matter regions during the preterm period. It has been found that fractional anisotropy and mean diffusivity both decreases significantly in the thalamus during the preterm period, as well as an increase in the intra-neurite volume fraction. This measure represents how much of the remaining space is occupied by the neurites and suggests that the thalamus is also sensitive to myelination status in preterm infants⁴². Thalamic abnormalities are often a feature of brain injury in preterm neonates and have been associated with the neurodevelopmental sequelae seen in these infants later in life.

Several studies have found a relationship between diffusion measures at term-equivalent age and outcomes at 18-24 months corrected age; notably, associations between cognitive outcomes and fractional anisotropy values in the corpus callosum, as well as motor outcomes and fractional anisotropy values in the corpus callosum, fornix, cingulum, and internal and external capsules⁴³⁻⁴⁵.

Preterm neonates display alterations in white matter microstructure when compared to full-term controls, including a decrease in fractional anisotropy and an increase in axial diffusivity, radial diffusivity, and mean diffusivity. This difference is more marked for very preterm than moderate-late preterm neonates, potentially due to a disruption in the myelination process. Cellular substrates for these changes may include changes in water content and axon packing density and are suggestive of altered brain development. This may underpin the increased risk of neurodevelopmental disability seen in preterm infants in later life. DTI may be used, alongside other complimentary MRI parameters such as absolute T_1 and T_2 relaxation times, to assess white matter maturity in preterm neonates, and may therefore be a useful prognostic tool for neuro-disability in these infants.

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