Diffusion Weighted Magnetic Resonance Imaging of in utero and ex utero Human Brain Development

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Declaration of Originality

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For dad and mum
Abstract

The primary objective of this thesis was to establish quantitative measurements of normal fetal brain tissue across gestation using diffusion magnetic resonance imaging (MRI); the secondary aim was to compare diffusion metrics in fetuses with normal brain development to those with isolated ventriculomegaly (VM), congenital heart disease and in infants born preterm.

Fetal diffusion weighted imaging (DWI) was optimised and a motion-corrected diffusion tensor imaging (DTI) technique was utilized to produce apparent diffusion coefficient (ADC) and fractional anisotropy (FA) values across gestation in normal fetal cohorts. Tract-based spatial statistics (TBSS) was utilised to analyse DTI in neonates with isolated VM compared to controls. Diffusion and volumetric MR data in preterm infants were analysed using an objective segmentation approach to characterise ex utero neurodevelopment, and to establish the effects of perinatal clinical factors on brain development.

Normative ADC values were established in the fetal brain (n=52) across a large gestational age range. Increased ADC values were found in fetuses (n=24) and neonates (n=22) with isolated VM compared to controls; decreased FA was also demonstrated in neonates with VM. In preterm neonates (n=208), white and deep grey matter exhibited significantly increasing FA, and decreasing ADC, axial and radial diffusivity measures with increasing age at scan. DTI measures in the cortex significantly decreased with increasing age at scan; volume measures increased in all brain regions. Clinical factors including respiratory support and age at birth affected regional DTI and volumetric measures in preterm infants. FA values from a normal fetal cohort using motion-corrected DTI (n=26) were compared to preterm neonates (n=32) and significant differences were found.

This thesis produced normal fetal diffusion data comparable to that produced in neonates. Quantifiable MR techniques can be used to explore the relationship between in utero and ex utero brain development and study alterations of normal fetal maturation.
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List of Abbreviations

AD Axial diffusivity
ADC Apparent diffusion coefficient
ANOVA Analysis of variance
ASQ-3 Ages and Stages Questionnaires-III
AVSD Atrioventricular septal defects
B₀ External magnetic field
BMI Body mass index
BSID-III Bayley Scales of Infant Development-III
BPD Biparietal diameter
BW Birth weight
CHD Congenital heart disease
CLD Chronic lung disease
CI Confidence interval
CMV Cytomegalovirus
CNS Central nervous system
coef b coefficient
CONS Coagulase-negative staphylococcus
CPAP Continuous positive airway pressure
CSF Cerebrospinal fluid
CSO Centrum semiovale
CST Corticospinal tract
DEHSI Diffuse excessive high signal intensity
DT Diffusion tensor
DTI Diffusion tensor imaging
DWI Diffusion weighted imaging
EPI Echo planar imaging
FA Fractional anisotropy
FACT Fiber Assignment by Continuous Tracking
FDT FMRIB's Diffusion Toolbox
FoV Field of view
GA Gestational age
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>GBS</td>
<td>Group B streptococcal septicemia</td>
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<tr>
<td>HC</td>
<td>Head circumference</td>
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<td>LOT</td>
<td>Lateral occipito-temporal</td>
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<tr>
<td>ICNIRP</td>
<td>International Non-Ionizing Radiation Committee of the International Radiation Protection Association</td>
</tr>
<tr>
<td>MBP</td>
<td>Myelin basic protein</td>
</tr>
<tr>
<td>MCDA</td>
<td>Monochorionic diamniotic</td>
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<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
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<tr>
<td>MRS</td>
<td>Magnetic resonance spectroscopy</td>
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<tr>
<td>ms</td>
<td>Millisecond</td>
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<tr>
<td>NEC</td>
<td>Necrotising enterocolitis</td>
</tr>
<tr>
<td>NMI</td>
<td>Normalised mutual information</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic imaging</td>
</tr>
<tr>
<td>NRPB</td>
<td>National Radiological Protection Board</td>
</tr>
<tr>
<td>PDA</td>
<td>Patent ductus arteriosus</td>
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<tr>
<td>PLIC</td>
<td>Posterior limb of the internal capsule</td>
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<td>PMA</td>
<td>Postmenstrual age</td>
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<td>PROM</td>
<td>Premature rupture of membranes</td>
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<td>RD</td>
<td>Radial diffusivity</td>
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<tr>
<td>RF</td>
<td>Radiofrequency</td>
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<td>ROI</td>
<td>Region-of-interest</td>
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<tr>
<td>SAR</td>
<td>Specific absorption rate</td>
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<tr>
<td>sd</td>
<td>Standard deviation</td>
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<tr>
<td>SENSE</td>
<td>Sensitivity encoding</td>
</tr>
<tr>
<td>SGA</td>
<td>Small for gestational age</td>
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<tr>
<td>SNAPIR</td>
<td>T1-weighted snapshot inversion</td>
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<tr>
<td>SNR</td>
<td>Signal-to-noise ratio</td>
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<tr>
<td>SPIR</td>
<td>Spectral presaturation with inversion recovery</td>
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<tr>
<td>SVR</td>
<td>Snapshot MRI with Volume Reconstruction</td>
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<td>SVZ</td>
<td>Subventricular zone</td>
</tr>
<tr>
<td>TBSS</td>
<td>Tract-based spatial statistics</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>TEA</td>
<td>Term equivalent age</td>
</tr>
<tr>
<td>TGA</td>
<td>Transposition of the great arteries</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>TTTS</td>
<td>Twin-to-twin-transfusion syndrome</td>
</tr>
<tr>
<td>US</td>
<td>Ultrasound</td>
</tr>
<tr>
<td>VM</td>
<td>Ventriculomegaly</td>
</tr>
<tr>
<td>VZ</td>
<td>Ventricular zone</td>
</tr>
<tr>
<td>WM</td>
<td>White matter</td>
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Conference Abstracts


M. Kuklisova Murgasova, C. Malamateniou, G. Lockwood Estrin, ZQ. Wu, JV. Hajnal. Image driven distortion correction during slice to volume reconstruction of fetal MRI. European Society for Magnetic Resonance in Medicine and Biology, October 2013

1 Outline of thesis

1.1 Motivation

In utero magnetic resonance imaging (MRI) is becoming increasingly important for both clinical and research purposes. Diffusion weighted MRI exploits the molecular mobility of water within tissues to provide insight into normal and abnormal brain development. Two commonly used quantitative measures derived from diffusion data are the apparent diffusion coefficient (ADC) and fractional anisotropy (FA).

The majority of diffusion imaging studies investigating human brain development have focused on neonates born prematurely, yet ex utero development is not representative of normal intrauterine development. Normal brain development is better assessed by imaging the fetal brain in utero. However, fetal movement resulting in artefacts and poor signal-to-noise ratio (SNR) have posed major challenges to fetal diffusion imaging, and have limited its use in both the clinical and research setting. Fetal diffusion studies have rarely addressed these limitations, resulting in poor quality in utero data. Robust ADC and FA measures in the normal fetal brain across a wide range of gestational ages need to be established.

1.2 Aims and objectives

There were two main objectives of this thesis:

1) To establish quantitative measurements of normal development in the fetal brain across a range of gestational ages using diffusion MR imaging.

2) To compare diffusion metrics from data in normal fetuses to those with specific complications of pregnancy in order to investigate differences in brain development in these cases.

The first aim of this thesis was achieved using two methodologies: 1) optimisation of the diffusion weighted imaging (DWI) sequence and protocol to produce ADC maps of the fetal brain within a short time frame for use in clinical settings, and 2) using a motion-corrected diffusion tensor imaging (DTI) technique to produce FA and ADC measures.
To accomplish the second objective, the normal quantitative diffusion measures in the fetal brain established from the first aim, were used for comparison with diffusion data in fetuses diagnosed with isolated ventriculomegaly (VM). In addition, fetal diffusion measures were used to compare normal in utero development to ex utero development in infants born prematurely.

1.3 Thesis outline

The thesis is organised as follows:

- Chapter 2 outlines the background literature to this thesis. This chapter reviews early brain development, the basics of MRI, conventional MR imaging of the developing brain and the current roles of diffusion weighted and diffusion tensor imaging. The limitations of fetal MR to study normal brain development are also discussed.

- Chapter 3 seeks to obtain robust ADC measurements by optimising a fast DWI sequence that would be suitable for use within a clinical setting. To ensure that data are reliable and that there is minimal degrading of images due to motion, a strict criteria and protocol for data exclusion was designed. ADC values were obtained and analysed using this optimised protocol in a large cohort of normal fetal controls.

- Chapter 4 aims to assess white and grey matter development in fetuses with isolated VM and congenital heart disease (CHD) compared to normal controls. The large normal fetal cohort obtained in chapter 3 allowed for a powerful comparison with these patient cohorts. The optimised fetal DW sequence developed in chapter 3 was used to ensure good quality ADC data for comparison between groups.

- Chapter 5 aims to determine whether white matter structure in neonates with antenatally diagnosed isolated VM differed to healthy controls. Tract-based-spatial statistics (TBSS), an automated observer-independent technique which allows voxel-wise, whole brain analysis of DTI measures between groups, was used. It has the power to reveal DTI differences in white matter tracts between groups of neonates.

- Chapter 6 aims to characterise DTI and volume changes in white and grey matter with increasing age at scan in a large preterm population without focal lesions. A novel brain segmentation approach (Makropoulos et al, IN PRESS) was used. The effect of perinatal clinical factors on brain development in this population was also assessed.

- Chapter 7 aims to characterise fetal FA and ADC measures across a range of gestational ages in a large group of fetuses without brain abnormalities by using a motion correction
DTI technique. FA and ADC measures in the normal fetal brain were then used for comparison with diffusion data from premature neonates in order to detect developmental differences between in utero and ex utero brain maturation.

- Chapter 8 draws together the results from chapters 3 to 7 and provides a summary of the main findings and contributions. The chapter also explores the implications of this study for future research.
2 Background and literature review

2.1 The Normal Developing Brain

The development of the brain is carefully regulated by proliferation, migration, differentiation and apoptosis of neurons and glia. The high degree of organisation is achieved during development by specific timing and modulation of these processes, and their regulation is essential for establishing the functional organisation and connectivity of neurons (Chan et al. 2002) (Figure 2.1).

Whilst the first half of gestation is chiefly characterised by proliferation and migration, the second half of gestation and the neonatal period are the most important developmental periods for the formation of cerebral pathways (Kostovic and Jovanovmilosevic 2006) and neuronal connectivity is therefore particularly vulnerable to disruption during this developmental phase.
Figure 2.1 Timeline of events and processes in the developing brain.

Each line represents approximate timing of event; dashed lines represent the event occurring to a lesser degree. Timeline is based on information from the literature reviews of de Graaf-Peters and Hadders-Algra 2006, Kostovic and Jovanov-Milosevic 2006, Rados et al. 2006, Yuste and Bonhoeffer 2004 and Compston et al. 1997.
**Early cerebral development**

The first stage of intrauterine development is the period of blastocyst implantation during the first 3 weeks. Following this, from week 4 until day 57, is the embryonic period. During early embryonic development, a stage referred to as neural induction, the ectoderm becomes specified into either the neural plate, which serves the basis for the nervous system, or the epidermis (which develops into the skin). During this stage, ectodermal cells are exposed to signals which determine whether they become neural stem cells or precursor cells, or else they are diverted to alternative fates (Wilson and Edlund 2001). The ectodermal cells instructed to become neural plate receive signals produced by the notochord, which is a structure that not only induces the formation of the neural plate, but also synchronises the development of the neural tube.

At approximately 3-4 weeks gestation, during a process called neurulation, the neural plate begins to fold, forming neural folds and subsequently the neural tube (Monsoro-Burq et al. 1995, Volpe 2008). The initial fusion of neural folds to form the neural tube occurs in the region of the lower medulla at approximately 22 days. Neural tube closure occurs at different times along the cord, and generally progresses rostrally and caudally, though it is not a simple, zip-like process, and depends upon a variety of cellular and molecular mechanisms (Volpe 2008). The anterior end and the posterior end of the neural tube fuse at approximately 24 and 26 days respectively, and the neural tube goes through a series of folds, bends constrictions and rotations to shape the future brain. Subdivisions of the brain are established through these folds, and the superior section of the neural tube flexes at the level of the future midbrain, which becomes the mesencephalon. The prosencephalon is the region above the mesencephalon, which develops into the future forebrain; and below the mesencephalon is the rhombencephalon, which later develops into the hindbrain (O'Rahilly and Muller 2008).

Regional specification of neural tissue is determined and influenced by the pattern of gene expression of neurons in each region. Throughout the developing central nervous system (CNS), anterior-posterior and dorso-ventral gradients of transcription factors determine regional specification (Nichols et al. 2001); for example the ventral neural tube is patterned by Sonic Hedgehog from the notochord, whilst the dorsal neural tube is patterned by BMPs from the epidermal ectoderm flanking the neural plate.
Following neurulation, early gestation is characterised by proliferative processes, with systematic division of neuroepithelial cells in the germinal matrix, or ventricular zone (VZ) (Stephanova 2001); this process occurs from 5 weeks gestation (Iai et al. 1997) reaching its peak by 16 weeks (Paus et al. 1999). The VZ, which is formed from the innermost cellular layer of the closing neural tube, increases in size during this proliferative stage, with dividing cells capable of differentiating into both glial cells and neuroblasts (Blakemore 1995). Dividing cells extend radial processes towards the pial surface (Rakic 1988) and neurogenesis begins as post mitotic cells migrate along this radial scaffold (Hatten 1999). Neuronal migration is facilitated by the production of glycoproteins, such as reelin, which are essential for orderly neuronal migration and growth into the cerebral cortex (Ogawa et al. 1995). The initial migrating neurons form the preplate, which is later split into the subplate and subpial marginal zone by migrating neurons from both the VZ and the subventricular zone (SVZ). This allows for the development of the cortical plate that will eventually create the six layers of the cortex in an inside-out order. By 14 weeks gestation, a number of transient zones are present that are integral for axonal development and formation of neuronal pathways: the VZ; the SVZ; the intermediate zone; the subplate; and the cortical plate (Bystron et al. 2008). Figure 2.2 is a diagram presenting the development of these transient zones.
Figure 2.2 Representation of cortical cellular development.

A) The preplate is formed from the initial neurons migrating from the ventricular zone and along the radial glia towards the margin of the cerebral hemisphere. B) The preplate is split into the subplate and subpial marginal zone by an additional layer created by neurons migrating along radial glia into the cortical plate. The cortical plate begins to form from approximately 7th to 10th week as the first wave of postmitotic cells migrate towards the pial surface. From the 11th week, the cortical plate undergoes an exponential increase in cell numbers due to radially and tangentially migrating neurons. C) The subsequent layers of the cortex, initially the cortical plate, are formed ‘inside-out’ by additional migrating neurons from the germinal matrix; from which the sub ventricular zone becomes the dominant site for neuronal proliferation. The intermediate zone contains both radially and tangentially migrating cells. Image is adapted from (Sidman and Rakic 1973)
The SVZ emerges between the 8th and 10th week and takes over from the VZ as the main region of neuronal and glial proliferation during the second half of gestation (Rakic 1972). In the monkey cerebrum, the VZ gives rise to the majority of neurons, whilst the SVZ is the point of origin for later-developing neurons and the majority of glia (Rakic 1985, Rakic 1995, Zecevic et al. 2005). The intermediate zone appears between the 6th and 8th week, and it represents the prospective WM and contains both radially and tangentially migrating cells (Pearlman et al. 1998, Sidman and Rakic 1973). Radial migration, from the VZ and SVZ, is the primary mechanism for formation of the cortex and deep grey matter structures. Tangential migration occurs in a much smaller population of neurons, the majority of which form the GABAergic interneurons of the cerebral cortex (Volpe 2008, Noctor et al. 2007, Pearlman et al. 1998, Komuro and Rakic 1998). Radial neuronal migration to the cortex reaches its maximum between the third and fifth month of gestation (Rakic et al. 1994, Gressens 2000). Tangential migration appears to peak between 18 and 36 weeks gestation (Letinic and Kostovic 1997, Letinic and Rakic 2001).

The subplate contains migratory neurons as well as glial cells (Kostovic and Rakic 1990, Kostovic and Judas 2002). Normal development of the subplate is essential for normal cortical development and formation of synapses (Kostovic and Rakic 1990, Ghosh and Shatz 1993, Ghosh and Shatz 1992, Volpe 2008). It provides a site for synaptic contact for axons whilst accumulating afferent axons that form transient, functional circuits whilst they wait in this zone for a prolonged period (Kostovic and Jovanov-Milosevic 2006). This waiting phase is essential preparation for establishing normal thalamo-cortical and cortico-cortical connections. After waiting within the subplate, afferent fibres migrate to the cortical plate from approximately 26 weeks (Kostovic and Jovanov-Milosevic 2006, Kostovic and Judas 2002). The cortical plate then becomes the prominent site for dendritic differentiation, synaptic formation and glial proliferation, and the subplate begins to diminish (Kostovic and Jovanov-Milosevic 2006). This maturation of the cortical plate and the concurrent dissolution of the subplate signal the beginning of cortical connectivity.

**Cerebral organisation and connectivity**

As migratory neurons near their target, their axons arborize and branch with growth factors influencing their migration and retraction (Tau and Peterson 2010). This directed growth of axons is the first step towards neuronal connectivity. Branching axons and dendrites form important synaptic
connections that model neuronal architecture and remain largely the same throughout the life span. Glial cells and specifically astrocytes, play a major role by producing surface and extracellular matrix molecules, which modulate axon growth thereby influencing axonal path-finding during this initial period of development (Fitch and Silver 1997). Glial cells also play a crucial role in axonal organisation by providing a structural framework for axonal growth (Nedergaard et al. 2003). Some axonal bundles of major WM pathways appear as early as 8 weeks gestation (Vasung et al. 2010), although different tracts follow differential timelines for development; for example fibres of the internal capsule begin to develop at around 10 weeks gestation, with axons arising from the subplate; whilst axonal tracts forming the corpus callosum begin to develop in the 11th week, with axons arising from the cortical plate (Prayer et al. 2006).

Synaptogenesis, which is the formation of synapses, plays a key role in establishing connectivity and occurs in parallel with a high turnover of dendritic spines during the development of neuronal connectivity (Yuste and Bonhoeffer 2004). Synaptogenesis rapidly accelerates during the third trimester due to a surge in dendritic sprouting and arborisation (Bourgeois 1997, Huttenlocher and Dabholkar 1997), and continues after birth, in parallel with the sustained dendritic development and arborisation of both inhibitory and excitatory circuits (Andersen 2003, Tau and Peterson 2010). Synaptic activity is thought to stabilise dendritic structure in the later stages of development (Cline 2001). Synapses undergo continuous modification and pruning which is inherent to the fine tuning and maturation of neuronal circuits (Tau and Peterson 2010); as many as 50% of synapses and receptors are overproduced and eliminated, a process which may continue in certain brain regions until the 3rd decade of life (Petanjek et al. 2011), with two major stages of production occurring immediately before birth and during adolescence.

Apoptosis also plays an essential role in the development of connectivity. During early embryonic development, apoptosis is closely linked with proliferation and plays an important role in regulating the population of the progenitor cells needed for morphogenesis of neural stem cells (Chan et al. 2002). It is probably also involved in the establishment of neuronal activity later in development (Rakic and Zecevic 2000). Apoptosis is present from as early as 4.5 weeks in the VZ, but at a very low activity level which increases 5-fold by week 7, and is then seen in the preplate and cortical layers by week 11 (Zecevic and Rakic 2001). Later in gestation, when proliferation has declined, apoptosis occurs in neurons whose axons are unable to find their target (Chan et al. 2002). Neuronal number in
the human brain is at its peak by 28 weeks, and almost half this population undergo cell death by the end of adolescence (Lossi and Merighi 2003). From approximately 32 weeks gestation, apoptosis plays an integral role in the development of the cortical layers by reducing excess neurons to adjust the size of the cell population in order to match the input and the target population for synaptic connections (Kalinichenko and Matveeva 2008). This is crucial for regulation of the cortical size and shape as well as formation of functional connectivity. Delayed apoptosis has been suggested to result in atypical connectivity associated with cognitive dysfunction (Tail and Peterson 2010).

**White Matter Maturation**

In the fully developed brain, white matter (WM) consists of mainly glial cells and myelinated axons. The intermediate zone of the developing brain is the prospective mature WM, and contains both afferent and efferent fibres as well as migrating immature neurons (Prayer and Prayer 2003). It also contains a high proportion of oligodendrocyte progenitor cells, ready for the onset of myelination.

Myelination is a long process that results in fully myelinated axons important for the fast and effective transmission of neuronal signals (Jakovcevski and Zecevic 2005). Mature myelin itself consists of alternating layers of myelin sheath, which is a condensed lipid-rich membrane, wrapped around axons (Compston et al. 1997). Myelination describes the process where pre-myelin sheath, produced during the first ensheathment of axons by immature oligodendrocytes, compacts to form mature myelin (Prayer et al. 2006). A single oligodendrocyte provides myelin for many axons.

**Pre-myelination**

Pre-myelination takes place predominantly in utero and involves oligodendrocyte proliferation and differentiation before the synthesis of mature myelin (Kinney et al. 1994). The progression of oligodendrocyte maturation has a well-established lineage, as presented in Figure 2.3 (Compston et al. 1997).
Figure 2.3 Oligodendrocyte maturation.

The four stages of oligodendrocyte maturation are represented; each stage is marked by specific morphology. The accompanying major processes of neurodevelopment leading to myelination are also demonstrated. Myelin basic protein (MBP) is a myelin-specific protein stained during immunohistochemical analysis and is expressed in mature oligodendrocytes. Image is adapted from (Back 2006).

Oligodendrocytes originate from progenitors in the SVZ, as well as from radial glia progenitors (Volpe 2008). Oligodendrocyte progenitor cells differentiate to their preoligodendrocyte stage during migration from the SVZ into the intermediate zone or future cerebral WM. Preoligodendrocytes are present from 18 weeks and persist until near term (Back et al. 2001). Upon reaching their position in the WM, preoligodendrocytes differentiate into immature oligodendrocytes. A flat, membranous process containing a network of microtubules and microfilaments extends outwards from the immature oligodendrocyte cell body and wraps around neighbouring axons in a spiral fashion, forming the first encasing of the pre-myelin sheath (Prayer and Prayer 2003). At around 30 weeks gestation, immature oligodendrocytes increase in number and differentiate into mature oligodendrocytes, which give rise to myelination (Back et al. 2002b). Myelination describes the process of the initial loose wrappings of the axon to a progressively more compacted myelin sheath (Raine 1984).
Myelination

Myelination appears first in peripheral nervous system, with motor roots myelinating before sensory roots. Within the CNS, however, myelination in central sensory systems precedes that of central motor systems (Yakovlev 1967, Volpe 2008), and is generally known to progress in a posterior-to-anterior and medial-to-lateral direction (Yakovlev 1967, Benes 1989, Gilles et al. 1983), with proximal pathways myelinating before distal, and projection before associative pathways (Kinney and Armstrong 2002, Kinney et al. 1988). The first histological proof of myelination in the brain appears in the 21st gestational week at the medial longitudinal fasciculus (situated near the midline of the brainstem) and in the inferior cerebellar peduncles before 25 weeks gestation (Yakovlev 1967, Shiraishi et al. 2003, Jakovcevski and Zecevic 2005). Detection of myelination by magnetic resonance imaging (MRI) in the developing brain lags behind histological evidence by a few weeks; myelin can be observed on MRI in these regions, as well as in the inferior colliculi, posterior brain stem and ventro-lateral nuclei of thalamus, by 28 weeks (Counsell et al. 2002). Myelination can next be visualised on MRI in the corona radiata, posterior limb of the internal capsule (PLIC) and corticospinal tracts (CST) by 36 weeks (Counsell et al. 2002).

Myelination of the cerebral hemispheres is a predominantly post-term process and continues until the end of the second year (Kinney et al. 1994). In normal development, the optic radiation, for example, appears on MRI to be myelinated by three months and the frontal WM starts to myelinate at around 6 months after term (Barkovich et al. 1988).

2.2 Magnetic Resonance Imaging

MRI allows visualisation of human brain development in vivo and is therefore important for both clinical and research purposes. MRI is superior to other structural imaging techniques such as X-ray and positron emission tomography (Sperling et al. 1986) as it is non-invasive and non-ionising, meaning that serial scanning can safely be performed in vivo.

Nuclear magnetic resonance

Nuclear magnetic resonance (NMR) is the basis for MRI, and describes the quantum mechanical behaviour of nuclei within a magnetic field (Rabi et al. 1939). Nuclei containing an odd number of...
nucleons (protons or neutrons) have a non-zero spin and therefore possess an angular momentum, which combined with the charge of the nuclei can induce a magnetic moment along their rotational axis. Within the human body, the most abundant atom is hydrogen (\(^1\)H), with a nucleus of a single proton and a charge of +1. If an external magnetic field (\(B_0\)) is applied, the spins of the \(^1\)H nuclei align with the magnetic field in either a parallel (low energy) or anti-parallel (high energy) state.

At room temperature and with a \(B_0\) of approximately 1-Tesla, the number of spins aligned parallel to the \(B_0\) field will outnumber those aligned anti-parallel by approximately 3ppm, giving a net magnetisation parallel to \(B_0\). This is termed longitudinal magnetisation.

Due to their angular momentum, \(B_0\) induces spins to precess about the \(B_0\) axis. The frequency of the precession is proportional to the magnetic field strength and is defined by the Larmor equation (Equation 2.1). Under normal conditions, the spins precess around \(B_0\) out of phase from one another. The spins can be made to precess in phase, giving rise to a net magnetization in the XY-plane (perpendicular to \(B_0\)). This is termed transverse magnetisation.

\[
\omega = \gamma B_0
\]

*Equation 2.1 Larmor frequency. The precessional frequency, \(\omega\), also called the Larmor frequency, depends on the external magnetic field strength, \(B_0\), and the gyromagnetic ratio, \(\gamma = 42.57 \text{ MHz/T for } ^1\text{H} \).*

The overall magnetisation vector is the sum of the net longitudinal magnetisation vector and the net transverse magnetisation vector.

**MR signal and spatial encoding**

The transverse magnetisation created by spins precessing in phase rotates around \(B_0\) causing an induced current in a coil that can be measured as an MR signal.

Application of a radiofrequency (RF) pulse, with a frequency equal to the Larmor Frequency, causes nuclei to excite and enter the high energy anti-parallel state, thus reducing the longitudinal magnetisation. It also induces phase coherence in the precession of spins creating a transverse magnetisation.
Following application of the RF pulse, spins gradually revert back to their original state. This is characterised by two relaxation times: $T_1$ and $T_2$ (Figure 2.4). $T_1$ is also known as the spin-lattice relaxation, and is the time taken for longitudinal magnetisation to recover to equilibrium parallel to the $B_0$ field. $T_2$ relaxation, also known as spin-spin relaxation, is the time taken for the transverse magnetisation (and therefore MR signal) to decay due to loss of phase coherence. There are two main causes for spin dephasing; inhomogeneities in the $B_0$ field and inhomogeneities in the local magnetic fields of the studied tissue.

\[\text{Figure 2.4 Longitudinal and transverse relaxation.}\]

A) Increase in longitudinal component of magnetisation ($M_z$) corresponding to $T_1$ relaxation. B) $T_2$ relaxation occurs due to the loss of phase coherence and the resulting decay of transverse
magnetisation ($M_0$). C) Recovery of longitudinal magnetization and decay of net transverse magnetization follow an exponential curve characterised by the time constant $T_1$ and $T_2$ respectively. The time constant $T_1$ is defined as the time taken for 63% of longitudinal relaxation to occur; $T_2$ is time for 63% of transverse magnetisation to be lost. Image is adapted from Lmaios 2008.

Both $T_1$ and $T_2$ differ according to their tissue content and environment, which is important when imaging the brain, as it allows for contrast between tissues to be clearly visualised. Different MRI sequences can be designed to give different weightings to $T_1$ and $T_2$ within images, dependent on pulse sequence order and pulse timings. It is also possible to give weighting to other parameters such as diffusion.

A spin echo sequence, which is the most commonly used pulse sequence in MRI, begins by using a 90° excitation RF pulse to rotate the overall magnetisation vector into the XY-Plane. Spins then begin to dephase due to inhomogeneities in their local field and the transverse magnetisation decays. After a time ($TE/2$) a 180° RF pulse is applied, causing the direction of spin precession to reverse and spins to come back into phase at time $TE$, restoring the transverse magnetisation. The echo time ($TE$) is the time between the RF pulse and MR signal sampling, and the repetition time ($TR$), is the time between two excitation RF pulses.

Spatial localisation of each voxel of the MRI signal involves the use of slice selection and spatial encoding gradients that are superimposed onto the $B_0$ field, causing the intensity of the total magnetic field to vary linearly along the gradient axis.

A slice selection gradient ($G_{ss}$) is applied at the time of the initial excitation RF pulse, causing the Larmor Frequency of spins to vary with their position along the gradient axis. Use of a select group of frequencies in the initial RF pulse will then only excite spins in a slice perpendicular to the gradient axis. The thickness of this slice will be dependent on the bandwidth of RF frequencies used.

After slice selection, the position of each voxel within the slice can then be encoded by applying a phase encoding gradient ($G_{pe}$) along the second axis and frequency encoding gradient ($G_{fe}$) along the third. The phase encoding gradient is applied for a short period immediately after excitation, and encodes spins position along the gradient axis by altering their phase of precession. The frequency encoding gradient is applied during the measurement of the MR signal. In a similar way to the slice
selection gradient it encodes a spins position along its axis by causing a linear variation of the Larmor Frequency.

The MR signal can then be mapped into k-space and a Fourier transform used to reconstruct the MR Image. Echo Planar Imaging (EPI) is a fast MRI pulse sequence, where the entire MR signal is mapped into k-space in one TR, and is often used with DW sequences which require fast acquisition methods.

2.3 Diffusion Imaging

Diffusion MR imaging depends on the microscopic motion of water molecules (Le Bihan et al. 1986). The motion of diffusing water molecules results in dephasing of spins and a reduction in signal. This effect is small in conventional MRI, but, with the addition of two extra magnetic field gradients, MRI can be made sensitive to diffusion.

Quantitative measurements derived from diffusion weighted MR imaging describe this molecular motion of water in vivo (Le Bihan et al. 1986) and can be used to make inferences about the underlying tissue structure. Microstructural barriers in the brain hinder the diffusion of water, and so information about the overall direction and magnitude of water diffusion provides insights into tissue content (Beaulieu 2002). In a homogeneous medium, the diffusion of water molecules is equal in all directions, and called isotropic diffusion. However, structures such as WM tracts, cell membranes and macromolecules result in areas where diffusion is not uniform and some directions are more restricted than others; this is known as anisotropic diffusion.

**Diffusion weighted imaging**

The most common method to achieve diffusion-weighted sequences was introduced by Stejskal and Tanner, (1965) and is presented in Figure 2.5. After the application of the 90° RF pulse, the first diffusion gradient causes a position dependent phase shift of the proton spins. The second gradient is typically applied at the same amplitude and duration after the 180° refocusing pulse bringing spins into coherence to produce the MR signal. If spins remain stationary between the two diffusion gradients, there would be no net phase difference, no loss of phase coherence, and therefore no loss in MR signal amplitude. However, if the spins are randomly displaced, there would be a distribution of phase shifts, a loss of phase coherence, and a loss of signal. With greater diffusion, the spread of
displacements increases, resulting in a greater loss of phase coherence and signal. The extent to which phase dispersion occurs is also dependent upon the strength, duration and time between the two diffusion gradients, and together these factors characterise the degree of diffusion weighting \(b\) (Figure 2.5). DW sequences consist of diffusion gradients applied along three orthogonal directions together with a reference image (without diffusion weighting).

![Figure 2.5 Simplified diffusion sensitized spin-echo sequence (Stejskal and Tanner 1965).](image)

\(G\) - amplitude of diffusion pulsed gradient; \(\delta\) – duration of diffusion gradient; \(\Delta\) – time interval between diffusion gradient. Changing \(G\), \(\delta\) or \(\Delta\) has an effect on the diffusion sensitivity. All these factors combine to characterise the diffusion weighting, \(b\).

**Apparent diffusion coefficient**

Diffusion can be described by the diffusion coefficient. Diffusion MRI determines the coefficient from the observations of displacement over a certain time period. The diffusion coefficient at each voxel can be calculated by Equation 2.2. This measure is termed the apparent diffusion coefficient (ADC) and depends on the amount of diffusion weighting \(b\) as well as the underlying tissue structure (Le Bihan et al. 1986). Reductions in ADC values may result from a decrease in water content or an increase in the restriction to water motion (Le Bihan et al. 1986).
\[
\text{ADC} = -\frac{1}{b} \ln \left( \frac{D_{W}}{b_{0}} \right)
\]

Equation 2.2 Apparent diffusion coefficient.

\(D_{W}\) is the diffusion weighted image and \(b_{0}\) is the reference image with no diffusion weighting; \(b\) is the amount of diffusion weighting (unit = s/mm^2). ADC therefore represents the ratio between the DW image and the \(b_{0}\) image and is dependent upon the diffusion weighting (\(b\)).

In DWI, ADC measures from the 3 diffusion-sensitizing gradients are averaged to give the mean ADC value. This mean ADC is commonly used in a clinical and research setting, but is variably referred to mean diffusivity, \(D_{av}\), or simply ADC (Mukherjee et al. 2008). The term ADC will be used in this thesis to refer to this averaged value.

**Diffusion Tensor Imaging**

Diffusion tensor imaging (DTI) provides additional information to DWI as it probes water diffusion in the brain by applying at least six directions of diffusion gradients, which allows for quantification of anisotropic diffusion (Basser et al. 1994). Anisotropic diffusion provides insights into the underlying tissue structure (Pierpaoli et al. 1996, Beaulieu and Allen 1994) because microstructural barriers, such as macromolecules and cell membranes restrict water diffusion in certain directions; for example, within WM tracts water diffusion is relatively free parallel to axonal fibres but more restricted perpendicular to axonal fibres (Doran and Bydder 1990).

Diffusion properties can be described mathematically by a diffusion tensor (DT) (Equation 2.3).

\[
DT = \begin{pmatrix}
D_{xx} & D_{xy} & D_{xz} \\
D_{xy} & D_{yy} & D_{yz} \\
D_{xz} & D_{yz} & D_{zz}
\end{pmatrix}
\]

Equation 2.3 Diffusion tensor.

The DT can be conceptually represented as an ellipsoid, where the long axis represents the direction of the highest diffusivity (Figure 2.6). In vivo this long axis is oriented parallel to WM fibres (Moseley et al. 1990). The magnitude of its diffusivity is represented by the major eigenvalue (\(\lambda_1\)), and the median and minimus eigenvectors are perpendicular to this and their eigenvalues are \(\lambda_2\) and \(\lambda_3\), as is
demonstrated in Figure 2.6, the eigenvectors V₁, V₂, and V₃ represent their corresponding directional components (Denis Le Bihan et al. 1986, Mori and Zhang 2006). Diffusion along λ₁ is known as axial diffusivity (AD), whilst radial diffusivity (RD) is calculated as the average of λ₂ and λ₃. The average diffusivity across all three λs is known as the mean diffusivity (MD) (Equation 2.4). This MD is also often referred to as the ADC or D₀ in the literature; in this thesis the term ADC will be used.

\[
\text{Mean diffusivity} = \frac{\lambda_x + \lambda_y + \lambda_z}{3}
\]

*Equation 2.4 Mean Diffusivity.*

Fractional anisotropy (FA) is commonly used to describe the degree to which the movement of water molecules are restricted by their environment (Beaulieu 2002, Johnston 2008), and is the standard deviation of the eigenvalues of the DT divided by MD (Mori and Zhang 2006) (Equation 2.5).

\[
FA = \sqrt{\frac{1}{2} \left( \frac{(\lambda_1 - \lambda_2)^2 + (\lambda_2 - \lambda_3)^2 + (\lambda_3 - \lambda_1)^2}{\sqrt{\lambda_1^2 + \lambda_2^2 + \lambda_3^2}} \right)}
\]

*Equation 2.5 Fractional Anisotropy.*

As shown in Figure 2.6, the shape of the ellipsoid determines the FA value; isotropic diffusion can be modelled as a sphere where water displacement is equal in all directions, and FA values approach 0. Comparatively, anisotropic diffusion is modelled as an elongated ellipsoid, as there is greater hindrance of water motion in the intermediate and minimus eigenvectors compared to the primary eigenvector. As the degree of anisotropy increases within a tissue, FA values approach 1. As the degree of anisotropy is determined by the structure of its environment, FA values therefore provide useful information about tissue microarchitecture.
Figure 2.6 Ellipsoid representation of anisotropic and isotropic diffusion.

A) In brain regions such as the cerebral spinal fluid (CSF), water motion is unhindered and may move equally in all directions, which is termed isotropic diffusion. B) This can be represented by a spherically shaped ellipsoid with $\lambda_1 = \lambda_2 = \lambda_3$. C) Anisotropic diffusion, in brain regions such as the corpus callosum where diffusion occurs preferentially along axonal fibres can be represented by an elongated ellipsoid (D), with $\lambda_1 > \lambda_2$ and $\lambda_3$. In the case of C, the FA value would be close to 0, whereas in the case of D, FA would be approaching 1. Image adapted from (Dudink 2010).

Limitations of DTI: Signal-to-noise ratio and artefacts

Sequence parameters such as the TR, TE and the diffusion weighting (b value) affect the signal-to-noise ratio (SNR) of MR imaging and contrast between tissues. Noise appears as interferences on an image and presents as an irregular granular pattern that degrades the image. The SNR is the ratio of the average signal intensity against the standard deviation of the noise, and is dependent upon the
field intensity, pulse sequence design, tissue characteristics, RF coil and voxel size. Greater field strength and slice thickness increases the SNR.

Artefacts from motion are the most common form of artefact as diffusion imaging is very sensitive to motion. Motion corrupts spatial localisation which results in the presence of artefact. These artefacts occur mainly in the phase encoding direction resulting from movement occurring between phase-encoding steps. Comparatively, the time between signal sampling and spatial-encoding in the frequency-encode direction is very short, so that motion will only produce a small amount of spatial blurring in that direction. Repeated motion, such as breathing, creates the appearance of “ghost” images. Another type of artefact that degrades image quality is known as aliasing, or wrap, where objects that are outside of the field of view (FoV) appear on the opposite side of the image.

**DTI post-processing techniques in the immature brain**

Advanced post-processing DTI techniques have been employed to quantify normal WM development in the neonatal brain, as well as differences in DTI measures between patients and controls.

Region of interest (ROI) analyses are commonly used to define specific regions of the brain, extract measures for this specific region, and either examine changes over time or compare between two different cohorts. This method has the advantage of being a simple and relatively fast approach to detect differences, but may be prone to user variability and subjectivity because they rely on arbitrary definitions to manually delineate different brain regions. ROI analysis is a commonly used method, especially when automated approaches are unreliable due to limitations such as partial volume in small brain regions.

Diffusion tractography has been used to describe WM tracts in the neonatal brain, as it visualises and quantifies the trajectory of specific WM pathways from one region to another (Basser et al. 2000, Conturo et al. 1999, Mori et al. 1999). A WM fibre can be tracked using each voxel’s primary eigenvector of the DT, to trace an axonal tract from voxel to voxel from a seed region to the target region. Tractography has been used to quantify changes of the motor and somatosensory pathways over development in preterm infants (Berman et al. 2005). This method has also been used to show disrupted pathways, for example in the corpus callosum, PLIC and connections between the thalamus.
and cortex in children and adolescents with periventricular leukomalacia (PVL) and haemorrhagic parenchymal infarction (HPI) (Hoon et al. 2002, Nagae et al. 2007).

Tract-based spatial statistics (TBSS) is a powerful tool that improves the sensitivity, objectivity and interpretability of multi-subject DTI analysis (Smith et al. 2006). TBSS works by aligning FA images from multiple subjects to allow groupwise comparison of data and has the ability to reveal differences in the major WM pathways of the brain between different groups (Smith et al. 2006). TBSS has been modified to improve registration of FA images in neonates (Ball et al. 2010). It has previously been used to identify statistically significant DTI differences in WM tracts between groups of neonates (Anjari et al. 2007, Ball et al. 2010).

2.4 MRI of normal in utero brain development

In utero MRI is becoming increasingly important for both clinical and research purposes. T₂-weighted sequences are the most frequently used technique in fetal MR examinations, as they provide a good SNR and contrast between tissues due to the high water content and long T₂ relaxation time within the fetal brain (Garel 2004). This high contrast makes them particularly useful in the evaluation of brain structure such as the corpus callosum, ventricles, vermis and cerebellar hemispheres, which makes it ideal for anatomical scans. Comparatively, T₁-weighted scans may be used for visualising the degree of myelination.

To address problems caused by unpredictable movement of the fetus, current practice in fetal brain MRI is to use single-shot methods, rather than multishot imaging. This method freezes fetal motion at each slice and therefore acquires individual images that are relatively free of motion artefacts. For example, single-shot T₂-weighted sequences allow images to be acquired in less than 1 second, so that motion artefact is unlikely to occur. The T₁-weighted snapshot inversion recovery (SNAPIR) sequence optimised for the fetal brain (Malamatienou et al. 2011) also produces single-shot T₁-weighted images free of motion artefacts. Whilst individual slices through the brain are usually artefact free, using single-shot acquisition, slices may not be parallel and coverage of the brain is therefore incomplete. This limits the ability to objectively quantify the volume of the brain and its structures in utero.

Advances in fetal anatomical imaging have recently been made by offline reconstruction of T₂-weighted single-shot images for volume analysis, with a technique called Snapshot MRI with Volume
Reconstruction (SVR) (Jiang et al. 2007). This method first oversamples the fetal brain by repeatedly imaging it with multiple stacks of single-shot slices. These motion free images are then registered together and reconstructed into a volume image that offers the benefit of increased SNR and resolution, as well as a 3D image free of motion artefacts. These MR techniques have allowed for visualisation and quantification of brain maturation across gestation, with non-rotated and artefact free images that are comparable to those produced ex utero in the preterm infant.

The most obvious development seen on MRI from 23 to 40 weeks gestation are the overall increases in brain size and maturation of cortical gyration and sulcation (Rutherford 2002) (Figure 2.7). Transient as well as definitive anatomical layers described in the section above can also be visualised on fetal MRI with increasing gestational age, including the germinal matrix, subplate, developing WM and the cortex (Figure 2.8).

![Figure 2.7 Maturation of the fetal brain on conventional MR.](image)

The maturation of the brain can be seen in these sagittal T2-weighed images from fetal ages: A) 25.14 weeks, B) 27 weeks, C) 30 weeks and D) nearing term at 35.43 weeks. There is an increase in brain size as well as development of the sulci and gyri of the brain, which evolves in an ordered fashion.
Figure 2.8 Germinal matrix and subependymal zone on T2 fetal MRI

A) T2-weighted images in the transverse plane of the fetal brain at 24.57 weeks gestation at the high ventricular level. The germinal matrix and subependymal zone are characterised by low signal intensity on T2 images, with the germinal matrix lining the majority of the lateral ventricular wall and overlying the caudate nucleus (large arrow); whilst the thinner band of low signal seen around the remaining areas of the ventricle is consistent with the densely cellular area of the subependymal zone (small arrows). B) T2-weighted images in the transverse plane of the fetal brain at 29.29 weeks at the level of the centrum semiovale (CSO). The cortex is characterised by low signal intensity on T2 images, whereas, the intermediate zone of migrating cells representing developing WM, are characterised by higher signal.

Safety of fetal MRI

MRI is considered to be a safe imaging modality for assessing the developing fetus, however there are a number of safety concerns that must be considered. The safety of the mother and fetus are highest priority, and all sequences must comply with guidelines given by the National Radiological Protection Board (1991), the International Non-Ionizing Radiation Committee of the International Radiation Protection Association (ICNIRP) and the National Radiological Protection Board (NRPB), to ensure wellbeing and comfort of both.

Theoretical health risks come from static magnetic fields, the RF pulse and associated heating, and acoustic noise from time-varying magnetic gradients. There is currently no evidence in humans to suggest a harmful effect of MR imaging to the mother or the fetus (Myers et al. 1998, Baker et al.)
and current guidelines state that fetal MRI is safe at 3-Tesla or less during the second and third trimesters (Patenaude et al. 2014).

A literature review on the safety of strong static magnetic fields suggests that there is no established evidence for the adverse effect on human health (Schenck 2000), though some in vitro and animal studies have suggested altered early embryonic development (Narra et al. 1996, Levin and Ernst 1997) and increased risk of fetal loss (Levin and Ernst 1997, Mevissen et al. 1994). However, other studies on cell cultures exposed to static magnetic fields of up to 8T showed no significant effect on cell viability, growth or ability to differentiate (Ueno et al. 1994, Kula and Drozdz 1996). A study in rat pups exposed in utero to a 9.4T static magnetic field during the second and third trimester of pregnancy found no auditory, tactile or CNS effects (High et al. 2000). A further study in mice that had been repeatedly exposed to a 7T static magnetic field in utero did not observe any adverse emotional or cognitive behaviour alterations (Hoyer et al. 2012). In addition, a large study in humans also demonstrated no significant adverse effects on pregnant MRI workers (Kanal et al. 1993).

The risk associated with RF energy is thermal heating; which is of particular concern in fetuses as the CNS appears to be especially vulnerable to temperature rises (Edwards et al. 2003). MRI systems are developed according to an international standard (IEC 60601-2-33, 2002) and pulse sequences must conform to the guidelines on specific absorption rates (SAR) to ensure that body temperature rise does not exceed recommended levels. The ICNIRP guidelines (2004) state that ‘it seems reasonable to assume that adverse developmental effects will be avoided with a margin of safety if the body temperature of pregnant women does not rise by more than 0.5°C and the temperature of the fetus is less than 38°C; the temperature of the fetus is +0.5°C above the mothers’ (Schroder and Power 1997). Mathematical models of SAR during fetal scanning at 1.5-Tesla and 3-Tesla have demonstrated that maximum heat absorption was from the mother and levels in the fetus were safe and within the ICNIRP guidelines (Hand et al. 2006).

Acoustic noise produced from the rapid change of gradients within the gradient coils during pulse sequences are an additional safety limitation during MR imaging. The effect of MRI noise on the fetus is of concern because the mother’s hearing is protected by headphones or earplugs, but the same is not true for the fetus, and literature on the potential effects of noise is conflicting (American Academy of Pediatrics. Committee on Environmental Health, 1997, Brezinka et al. 1997). A simulation using a hydrophone in the stomach of a male participant showed that noise is attenuated by 30dB at the
frequencies generated by the scanner, and that this attenuation is sufficient to bring intrauterine sound levels down to within known safe limits (Glover et al. 1995); though this did not accurately model the noise attenuation to the fetus through amniotic fluid at all stages of the second and third trimester of pregnancy. However, studies on neonates and children who had been exposed to MR imaging during the second and third trimesters of pregnancy did not demonstrate an increased risk of hearing impairment (Kok et al. 2004, Reeves et al. 2010).

2.5 Diffusion MR in the Developing Brain

Diffusion imaging has been used to describe and quantify brain development with increasing age at scan in the term-born and preterm brain (Mukherjee et al. 2001, Mukherjee et al. 2002, Partridge et al. 2004, Miller et al. 2003, Dubois et al. 2006, Ball et al. 2013b). The infant born preterm cannot however be taken as demonstrating normal brain development as studies have shown that by term equivalent age (TEA) the typical preterm brain shows many differences from that of the term born control brain (Alexandrou et al. 2014, Anjari et al. 2007, Rose et al. 2008). In order to quantify normal development prior to term the fetal brain would need to be studied. However diffusion imaging is not commonly used in the fetal MRI protocol due to limitations discussed later in this chapter; although several studies have used the technique in a research setting.

Neurodevelopmental and neurobiological changes in ADC

ADC values in all regions of the preterm brain are consistently higher than those found in adults (Neil et al. 1998). The first papers using diffusion imaging to study human neurodevelopment focused on preterm neonates and term infants (Huppi et al. 1998a, Neil et al. 1998). These DTI studies in newborns over a range of gestational ages demonstrated that ADC decreased with age in both grey and white matter (Neil et al. 1998, Tanner et al. 2000, Huppi et al. 1998a). Further studies in older children demonstrated that ADC measures continued to decline throughout the first decade of postnatal life (Mukherjee et al. 2001), and through to young adulthood (Snook et al. 2005). More recently, regional decreases in ADC values with increasing gestation has also been observed in fetal studies, with a significant negative correlation with age in the pons, cerebellar hemispheres, thalamus (Schneider et al. 2007), periatrial WM, basal ganglia (Schneider et al. 2009), pyramidal tract and corpus callosum (Bui et al. 2006). Table 2.1 demonstrates the white and grey matter regions where
significant ADC changes have been observed with increasing gestation in preterm and fetal region of interest (ROI) studies.

A different pattern of ADC values between white and grey regions of the neonatal brain has also been established; regional differences between ADC values from diffusion papers can be seen in Table 2.1. The most rapidly maturing WM pathways, such as the PLIC demonstrate the lowest ADC values compared to later developing regions such as frontal WM, which have the highest ADC values (Neil et al. 1998, Mukherjee et al. 2001, Huppi et al. 1998a, Prayer and Prayer 2003, Miller et al. 2002). ADC have also been found to be greater in WM than grey matter in the preterm and fetal brain, for example (Viola et al. 2011) found ADC values in the frontal WM of the preterm brain to be $1.77 \times 10^{-3}$ mm$^2$/s, whereas in the grey matter of the pons ADC values were $0.98 \times 10^{-3}$ mm$^2$/s (as seen in Table 2.1). However, this white and grey matter ADC difference diminishes in the first 2 years of life, and by full maturation in adulthood ADC values are fairly uniform across the brain (Neil et al. 1998, Mukherjee and McKinstry 2006).
<table>
<thead>
<tr>
<th>Papers</th>
<th>age at scan</th>
<th>n</th>
<th>splenium</th>
<th>genu</th>
<th>PLIC</th>
<th>CSO</th>
<th>occipital WM</th>
<th>frontal WM</th>
<th>thalamus</th>
<th>cerebellum</th>
<th>pons</th>
</tr>
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<tr>
<td>Studies in Preterm neonates</td>
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<tr>
<td>Dudink et al, 2007</td>
<td>4 days after birth</td>
<td>28</td>
<td>1.27</td>
<td>1.24</td>
<td>1.09</td>
<td>*1.3</td>
<td></td>
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<tr>
<td>Partridge et al, 2004</td>
<td>28 - 43</td>
<td>14</td>
<td>1.33</td>
<td>1.40</td>
<td>*1.17</td>
<td>*1.57</td>
<td>*1.47</td>
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<td>Huppi et al, 1998a</td>
<td>28 - 30</td>
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<td>1.2</td>
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<tr>
<td>Tanner et al, 2000</td>
<td>18 days after birth</td>
<td>5</td>
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<td></td>
<td></td>
<td></td>
<td>1.43±0.14</td>
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<td>Viola et al, 2011</td>
<td>25 - 33</td>
<td>24</td>
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<td></td>
<td>1.44±0.03 1.56±0.02 1.77 ±0.03 1.10 ±0.01 1.19±0.02 0.98±0.02</td>
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<td>Studies in the fetal brain</td>
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<td>Viola et al, 2011</td>
<td>36 - 39</td>
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<td></td>
<td>1.44±0.03 1.50±0.03 1.75±0.02 1.14±0.02 1.20±0.02 1.07±0.02</td>
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<tr>
<td>Schneider et al, 2007</td>
<td>23 - 37</td>
<td>78</td>
<td></td>
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<td>1.85±0.04</td>
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<td>Richini et al, 2003</td>
<td>22 - 35</td>
<td>15</td>
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<td>1.95±0.1 1.96±0.1</td>
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<td>Cannie et al, 2007</td>
<td>17 - 37</td>
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<td><strong>1.89</strong> <strong>1.81</strong> 1.66 1.64</td>
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<tr>
<td>Philpott et al, 2013</td>
<td>21 - 31</td>
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<td><strong>1.37±0.07</strong></td>
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<td><strong>-2.1</strong> <strong>-1.45</strong> <strong>-1.75</strong> <strong>-1.4</strong></td>
</tr>
<tr>
<td>Bui et al, 2006</td>
<td>31 - 37.4</td>
<td>24</td>
<td>1.25±0.15</td>
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<td>*1.16±0.14</td>
<td>1.80±0.12</td>
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<td>Boyer et al, 2013</td>
<td>19.3 - 37.1</td>
<td>50</td>
<td>1.36±0.29</td>
<td>1.34±0.25</td>
<td>1.37±0.23</td>
<td>*1.06±0.19</td>
<td>*1.26±0.20</td>
<td>*1.05±0.17</td>
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<tr>
<td>Sartor et al, 2014</td>
<td>29 - 38</td>
<td>101</td>
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<td>1.76 1.89 1.91 1.47 1.09</td>
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</tbody>
</table>

Table 2.1 Results from previous studies of ADC values in the fetal and preterm brain.

* Significant decrease in ADC values with increasing gestation; ** significant increase in ADC values with increasing gestation; ~ estimated from graph in paper; ^ ADC values taken from a ROI in the optic radiation. Age at scan is reported in weeks. Abbreviations: n = cohort number.
Table 2.1 reports regional ADC values with increasing gestational age from previous studies. These studies have used ROI analyses in fetal and preterm brains with no structural abnormalities. In general, ADC values are highest in later developing regions such as frontal WM, and lower in earlier developing regions such as the PLIC. Significant ADC changes with increasing age at scan are seen in white and grey matter regions; although regions with significant relationships with age vary between studies.
It is likely that the decrease in ADC values with increasing gestation is associated with progressively reduced water content in the brain (Dobbing and Sands 1973). Increased binding of water to macromolecules such as myelin, and maturational processes leads to decreased extracellular space which reduces separation of structures such as cell membranes, and increasingly restricts water motion (Le Bihan 1995, Beaulieu 2002, Neil et al. 1998), and this may also contribute to the decline in ADC values with increasing age. These maturational processes include myelination, dendritic arborisation, axonal ramification, synaptogenesis and glial proliferation, which create new structural barriers to water diffusion in white and grey matter (Mukherjee and McKinstry 2006).

**Neurodevelopmental and neurobiological changes in measures of anisotropy**

FA measures have been shown to increase in white and grey matter with increasing age in preterm infants (Miller et al. 2002, Neil et al. 1998, Partridge et al. 2004), and this increase continues into childhood and adolescence (Mukherjee et al. 2001, Mukherjee et al. 2002). These DTI changes occur in parallel with white matter maturational processes, specifically an increase in myelination, reduced total brain water content and a higher degree of cohesiveness and compactness of WM tracts resulting in a decrease in extra-cellular space (Beaulieu 2002).

Anisotropy is influenced by a number of microstructural barriers, including axonal membranes, degree of axonal packing and myelin (Beaulieu 2009). The influence of myelin has been extensively investigated. The association between FA and myelination can be partially demonstrated by the hierarchy of anisotropy in the preterm brain, where high FA values are found in early myelinating regions such as the PLIC, compared to lower FA values which are observed in the later maturing regions such as peripheral WM, or grey matter regions (Neil et al. 1998). However changes in diffusion anisotropy are not indicative of the onset of myelination. Indeed, it has been observed that between myelinated rats and myelin deficient rats, the resulting reduction in FA was only 20% (Gulani et al. 2001, Beaulieu 2002).

An early study compared diffusion anisotropy in myelinated compared to unmyelinated nerves of the garfish and observed a similar degree of anisotropy (Beaulieu and Allen 1994). Importantly, this paper found that the number of cell membranes were influential to anisotropic diffusion. It has also been reported that diffusion anisotropy in WM tracts of rat pups precedes the onset of myelination, suggesting a sensitivity to microstructural development in the pre-myelination period (Wimberger et
al. 1995, Prayer et al. 1997). Consistent with this hypothesis, another study in the rabbit also demonstrated that FA increases with developmental age were greatest prior to myelination and corresponded with the appearance of immature oligodendrocytes (Drobyshevsky et al. 2005). This premyelination anisotropy may also be due to an increase in axonal diameter (Hildebrand and Waxman 1984), axonal membrane changes (Fields and Waxman 1988) or early oligodendrocyte wrapping around axons (Watson 1991).

A key influence on anisotropic diffusion appears to be increased parallel organisation and packing of axons as they mature (Beaulieu 2009, Budde et al. 2008, Takahashi et al. 2002). It has been observed that more orderly axonal packing leads to higher anisotropy measures and that anisotropy is largely dependent on axonal membranes (Song et al. 2003, Takahashi et al. 2002). For example, studies in human neonates have demonstrated that the developing unmyelinated corpus callosum, a region of highly ordered parallel fibres, has higher or similar anisotropy to the myelinating regions of the corticospinal tract in neonates (Partridge et al. 2004, Huppi et al. 1998a, Dudink et al. 2008, Gilmore et al. 2007).

It has been proposed that AD and RD provide more specific physiologic underpinnings of the structural changes in WM than those available through FA (Song et al. 2002, Song et al. 2003). DTI and histological studies in mouse models have demonstrated that AD and RD are specific markers of axonal and myelin injury respectively (Song et al. 2002, Song et al. 2003, Takahashi et al. 2002); AD reflects axonal alignment and coherent orientation organization, whilst RD indicates restriction perpendicular to the axon orientation and may be indicative of myelination or axonal membranes (Song et al. 2002, Song et al. 2003, Takahashi et al. 2002). Specifically, it has been shown that the observed reduction in RD with increasing gestation is the driving factor behind FA changes over development (Partridge et al. 2004, Suzuki et al. 2003).

Whilst DTI measures provide unique insight into WM maturation, only three papers have demonstrated FA changes with increasing gestation in the normal fetal brain (Bui et al. 2006, Kasprian et al. 2008, Zarin et al. 2011); this is due to the limitations of in utero DTI.
Limitations of fetal diffusion imaging

SNR and motion artefacts are the main limitations of diffusion imaging when studying the immature brain. Imaging of this population suffers from inherent poor SNR due to small brain size and fetal head distance from the coil around the maternal abdomen. Movement from both the fetus and maternal respiration is the cause of motion artefacts, meaning that a high proportion of images cannot be used to accurately study the brain. Figure 2.9 shows a fetal DW image that has been corrupted by motion; this is compared with a single-shot $T_2$-weighted image, which is relatively free of motion.

![Fetal DW image corrupted by motion artefacts compared to $T_2$-weighted single-shot image.](image)

_Fetal brain in transverse plane surrounded by maternal anatomy in A) $T_2$-weighted single-shot image, which is acquired in less than 1 second and relatively free of motion. B) The same fetus imaged with DWI; movement has degraded the quality of this image, leading to artefacts and apparent blurring of the fetal brain and regions of high signal (arrow)._  

Studies have used diffusion imaging with EPI, a technique that allows images to be produced with only a single RF excitation, which means that data can be acquired within a short timeframe. This therefore reduces the possibility of fetal movement during scanning. However, the use of EPI itself results in image artefacts such as distortion, which causes further difficulties when studying the small sized brain of the fetus. Additionally, even with the use of faster imaging techniques, fetal movement
remains the major limitation of fetal diffusion imaging; it is not uncommon for as many as 50% of diffusion images to be excluded from studies due to excessive artefact from motion (Bui et al. 2006).

**Fetal DWI: review of the literature**

A number of fetal diffusion imaging studies have been reported in the literature, although few have addressed the limitations described above. Table 2.2 outlines studies that have appeared to date which have used diffusion measures to describe in vivo fetal brain maturation. These studies can broadly be divided into 5 categories: 1) DWI studies aiming to establish ADC values with increasing gestational age in the fetal brain, 2) DWI studies aiming to evaluate the reproducibility of ADC values in the fetal brain, 3) DTI studies aiming to establish FA and ADC values with increasing gestational age in the fetal brain, 4) DWI papers aiming to compare normal to abnormal fetal brain maturation, 5) clinical case reports using fetal diffusion imaging. There are currently no studies that have used FA measures derived from in utero DTI to compare normal to abnormal development. Further studies, which are not detailed in Table 2.2, focus on methodologies to correct for fetal motion in DTI sequences and are discussed in the fetal DTI section below.
<table>
<thead>
<tr>
<th>Fetal Diffusion papers</th>
<th>Type of study</th>
<th>Age at scan weeks</th>
<th>Fetal cohort number</th>
<th>Fetal Cohort</th>
<th>Details of cohort</th>
<th>Motion Correction</th>
<th># Diffusion directions</th>
<th>b value sec/mm²</th>
<th>TR ms</th>
<th>TE ms</th>
<th>Scan time min:sec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Righini et al, 2003</td>
<td>DWI providing normal ADC across gestation</td>
<td>22 - 35</td>
<td>15</td>
<td>Normal</td>
<td>No structural abnormalities on US or MRI; normal neonatal examination. Included enlarged cisterna magna, TTTS, and other organ abnormalities</td>
<td>Maternal breath hold</td>
<td>3</td>
<td>0.600</td>
<td>5000</td>
<td>81</td>
<td>00:20</td>
</tr>
<tr>
<td>Cannie et al, 2007</td>
<td>DWI providing normal ADC across gestation</td>
<td>17 - 37</td>
<td>46</td>
<td>Normal</td>
<td>No structural abnormalities on US or MRI. Included fetuses with non-CNS abnormalities</td>
<td>Maternal Sedation</td>
<td>3</td>
<td>0, 100, 250, 500, 750, 1000</td>
<td>1500</td>
<td>84</td>
<td>01:42</td>
</tr>
<tr>
<td>Manganaro et al, 2007</td>
<td>DWI providing normal ADC across gestation</td>
<td>19 - 37</td>
<td>56</td>
<td>Normal</td>
<td>No structural abnormalities on US or MRI. Included fetuses with non-CNS abnormalities</td>
<td>-</td>
<td>3</td>
<td>0, 400, 700</td>
<td>3600</td>
<td>92</td>
<td>00:45</td>
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<tr>
<td>Schneider et al, 2007</td>
<td>DWI providing normal ADC across gestation</td>
<td>23 - 37</td>
<td>78</td>
<td>Normal</td>
<td>No structural abnormalities on US or MRI. Normal clinical and neurological evaluation at 6 months</td>
<td>Maternal Sedation</td>
<td>3</td>
<td>0, 500, 1000</td>
<td>3200</td>
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<td>Age (weeks)</td>
<td>Gender</td>
<td>Pregnancy Weight (kg)</td>
<td>Maternal Preparation</td>
<td>Duration (min)</td>
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<td>3</td>
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<td>No structural abnormalities on MRI.</td>
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<td>DWI providing ADC reproducibility</td>
<td>29.4 - 38.4</td>
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<td>Normal</td>
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<td>3</td>
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<td>Imaging Technique</td>
<td>Maternal Position</td>
<td>Maternal Breath Hold</td>
<td>Duration</td>
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<tr>
<td>Sanz-Cortés et al, 2010</td>
<td>DWI providing ADC comparison between groups</td>
<td>37 ± 1</td>
<td>8 vs 5</td>
<td>SGA vs Normal</td>
<td>&lt;10th centile for birth weight Vs. &gt;10th centile for birth weight</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>0,1000</td>
<td>3400</td>
<td>94</td>
</tr>
<tr>
<td>Erdem et al, 2007</td>
<td>DWI providing ADC comparison between groups</td>
<td>18 - 37</td>
<td>12</td>
<td>hydrocephalus cases</td>
<td>-</td>
<td>Maternal breath hold</td>
<td>-</td>
<td>3</td>
<td>0,1000</td>
<td>4393</td>
<td>81</td>
</tr>
<tr>
<td>Berman et al, 2011</td>
<td>DWI providing ADC comparison between groups</td>
<td>32 - 34</td>
<td>3</td>
<td>CHD</td>
<td>-</td>
<td>Maternal breath hold</td>
<td>-</td>
<td>3</td>
<td>0,600</td>
<td>4500</td>
<td>min</td>
</tr>
<tr>
<td>Viola et al, 2011</td>
<td>DWI providing ADC comparison between groups</td>
<td>36 - 39</td>
<td>24</td>
<td>Normal</td>
<td>No structural abnormalities on MRI</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>0,500, 1000</td>
<td>3200</td>
<td>102</td>
</tr>
<tr>
<td>Hoffmann et al, 2013</td>
<td>DWI providing ADC comparison between groups</td>
<td>within 6 days of twin death (17 - 32)</td>
<td>34</td>
<td>Monochorionic twin survivors</td>
<td>-</td>
<td>No eating or drinking for 4 hours prior to scan</td>
<td>-</td>
<td>3</td>
<td>0,700 or 1000</td>
<td>^</td>
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</tr>
<tr>
<td>Philpott et al, 2013</td>
<td>DWI providing ADC comparison between groups</td>
<td>20 - 29 vs 21 - 31</td>
<td>8 vs 23</td>
<td>Chiari II malformation vs Normal</td>
<td>Chiari II malformation Vs. no structural abnormalities on MRI</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>0,500, 1000</td>
<td>2800</td>
<td>721</td>
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<td>33</td>
<td>1</td>
<td>Hypoxic-ischemic brain lesions</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>0,1000</td>
<td>^</td>
<td>^</td>
</tr>
<tr>
<td>Study</td>
<td>Imaging Modality</td>
<td>Case Number</td>
<td>Diagnosis</td>
<td>Time</td>
<td>Duration</td>
<td>GA (Wks)</td>
<td>AGA (%)</td>
<td>GA (mm)</td>
<td>AGA (%)</td>
<td>Duration</td>
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<tr>
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<td>DWI Case report</td>
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<td>Hemimegalencephaly</td>
<td>-</td>
<td>-</td>
<td>3</td>
<td>0.1000</td>
<td>10000</td>
<td>123</td>
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<td>DTI Case report</td>
<td>34.9</td>
<td>Agenesis of the corpuscallosum</td>
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<td>-</td>
<td>6</td>
<td>0.800</td>
<td>3500</td>
<td>95</td>
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<td></td>
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<tr>
<td>Koob et al, 2012</td>
<td>DTI Case report</td>
<td>31</td>
<td>HPE</td>
<td>-</td>
<td>Fetal sedation</td>
<td>30</td>
<td>0.700</td>
<td>6800</td>
<td>99</td>
<td>07:10</td>
<td></td>
</tr>
</tbody>
</table>

Table 22 Existing studies using fetal diffusion imaging to describe brain maturation.

^ Information was not transparent in the study. Abbreviations: HPE: fetal septopreoptic holoprosencephaly associated with a thick corpus callosum; SGA = small for gestational age; TTTS = twin-to-twin-transfusion syndrome; VM = ventriculomegaly. The type of study broadly defines the 5 categories of fetal diffusion studies, as outlined above.
As outlined in Table 2.2, most DWI studies have aimed to reduce the probability of fetal movement by acquiring images within a short amount of time (Schneider et al. 2007, Schneider et al. 2009, Righini et al. 2003). To minimise motion from maternal breathing, some studies have acquired DW images within a maternal breath hold; for example Righini et al. (2003) acquired the DW image within a maternal breath hold of 20 seconds, as can be seen in Table 2.2. This short acquisition time also minimises the unpredictable motion from the fetus. Other studies have attempted to minimise the fetal movement with use of sedation. The majority of the fetal DWI studies also employed relatively thick slices to maintain SNR (Schneider et al. 2007, Schneider et al. 2009, Righini et al. 2003). However, the resulting ADC maps from fetal DWI papers remain markedly inferior when compared with datasets acquired postnatally, or with studies that have used robust motion correction techniques on fetal DTI data (Figure 2.10).

![Figure 2.10 Comparison of ADC maps from in utero and ex utero diffusion studies.](image)

*Published ADC maps from fetal DWI studies without correction for motion are demonstrated in A to D. E shows a fetal ADC map from a motion-correction DTI protocol, and F shows an ADC map of*
a preterm neonate produced from DTI acquired post-natally. ADC maps from fetal brains published in A) (Kim et al. 2008), B) (Schneider et al. 2007) at 28 weeks, C) (Righini et al. 2003) and D) (Manganaro et al. 2007) at 32 weeks (circular ROI demonstrated in the frontal WM (top), basal ganglia (middle), occipital WM (bottom) and cerebrospinal fluid (CSF)). On the bottom row, results from (Jiang et al. 2007) (E) illustrates an offline motion correction technique producing high quality ADC maps in a young fetus (26.7 weeks) with agenesis of the corpus callosum, which is comparable to ADC maps produced post-natally on 3-Tesla scanner in sedated neonates (F) at 32.6 weeks. Motion correction techniques are needed for high quality diffusion data of the fetal brain.

As can be seen in Table 2.2, a small number of studies have aimed to provide normal ADC values across a range of gestational ages, but study limitations have resulted in a wide range of ADC values reported between studies. For example in the frontal WM, mean ADC values have varied across studies from $2 \times 10^{-3}\text{mm}^2/\text{s}$ (Schneider et al. 2009) to $1.37 \times 10^{-3}\text{mm}^2/\text{s}$ (Boyer et al. 2013) (see Table 2.1). These variations between studies may be due to poor quality and unreliable in utero data. Differing range of gestations or imaging sequence parameters may have also led to variable ADC values. Some studies have also suffered from small cohort numbers; for example Righini et al. (2003) aimed to establish ADC values with increasing gestation, but the cohort consisted of only 15 cases distributed between 22 and 35 weeks gestation. Furthermore, some studies seeking to illustrate normal ADC values in the fetal brain have included cases of mild ventriculomegaly (VM) (Bui et al. 2006), and other organ abnormalities (Righini et al. 2003), which have been associated with abnormal neurological outcome (Lyall et al. 2012, Sadan et al. 2007, Partridge et al. 2006). The majority of studies also do not provide follow up information on their cohorts to ensure normal development.

As reported in Table 2.2, studies have sought to determine the feasibility of ADC to detect WM abnormalities in utero, by comparing a normal fetal cohort with cases of abnormalities, such as fetuses with severe congenital heart defects (Berman et al. 2011) or hydrocephalus (Erdem et al. 2007). There have also been a handful of case reports illustrating potential diagnostic use for DWI (Baldoli et al. 2002, Agid et al. 2006, Meoded et al. 2011), with 1 case study using DTI tractography to investigate differential tract development of a fetal case with septopreoptic holoprosencephaly (HPE) (Koob et al. 2012). These studies have been limited by poor quality datasets, as can be seen in Figure 2.11, which probably result from little or no correction of motion.
Figure 2.11 ADC maps of the abnormal fetal brain.

ADC maps without motion correction of a fetus with A) Hydrocephalus at 38 weeks; circles demonstrate ROIs in the frontal WM, basal ganglia, CSF and occipital WM (anterior to posterior) (Erdem et al. 2007). B) A fetus with acute hypoxic-ischemic brain lesion (arrow) at 33 weeks (Baldoli et al. 2002); C) A fetus with hemimegalencephaly at 32 weeks (Agid et al. 2006), D) A fetus with Chiari II malformation at the level of the cerebellum and pons (Mignone Philpott et al. 2013), E) The surviving twin after laser ablation with germinal matrix bleed (arrows) (Hoffmann et al. 2013).

These studies comparing normal to abnormal brain development were also limited by small cohort numbers (Sanz-Cortes et al. 2010, Berman et al. 2011, Mignone Philpott et al. 2013); for example Sanz-Cortés et al. (2010) sought to assess ADC differences between a normal control cohort of 5 fetuses and 8 fetuses that were small for gestational age (SGA). A final limitation was the use of different gestational age ranges and scanning parameters between control and clinical cohorts. For example, Erdem et al. (2007) compared mean ADC values from a group of 12 fetal hydrocephalus cases scanned between 18 and 37 weeks, to a control cohort from of 15 fetuses from Righini et al. (2003) scanned between 22 and 35 weeks. These two cohorts differed with respect to diffusion parameters, with b values ranging from 600 to 1000 s/mm². Consistent b values are particularly important when comparing cohorts, as ADC values have been shown to decrease with increasing b values in the neonatal brain (Dudink et al. 2008).

Only one study has investigated the differences between in utero and ex utero brain development using diffusion imaging (Viola et al. 2011). This study compared 24 in utero fetal cases to 24 preterm neonates of an equivalent age, and demonstrated significant ADC differences between groups in the pons and parietal WM. However, this study did not compensate for fetal motion and imaging was only performed at near term equivalent age (mean gestational age of 37.14 weeks),
hence any earlier differences or differential changes across gestation between fetal and preterm development have not been investigated.

**Fetal DTI: Review of the literature**

As shown in Table 2.2, some fetal DTI studies have also aimed to produce normative FA values across a range of gestational ages (Kasprian et al. 2008, Bui et al. 2004, Zanin et al. 2011); two of these studies used tractography (Kasprian et al. 2008, Zanin et al. 2011). However this was done without true compensation for limitations of fetal motion. Fetal DTI is even more sensitive to disruption from fetal motion than DWI, because it requires at least 6 non-collinear diffusion images for each slice studied. Motion disrupts the spatial correspondence between component images that are required to calculate diffusion tensor properties, causing images to be mis-aligned to one another, as well as producing artefacts in the individual diffusion weighted images. Therefore, a number of studies have developed techniques to correct for fetal motion in DTI to improve estimation of the DTI and resulting FA measures in the fetal brain (Jiang et al. 2009, Oubel et al. 2012, Fogtmann et al. 2014).

Motion correction techniques have involved alignment of diffusion weighted slices to a conventional anatomical MRI scan to provide a geometrically correct reference image (Kim et al. 2010) or b0 reconstruction methods (Oubel et al. 2012, Jiang et al. 2009). Jiang et al. (2009) extended their anatomical slice-to-volume registration technique, Snapshot Images with Volume Reconstruction (SVR) (as described above), to DTI of the fetal brain; thus presenting a methodology to achieve diffusion tensor image reconstruction of the brain in moving subjects. This method used oversampling and image registration to realign diffusion images to correct for subject motion, which produced reliable ADC reconstruction in moving subjects, which were validated in the adult brain using data with and without movement. The motion-corrected DTI technique also produced promising, but less robust, FA measures (Jiang et al. 2009). Figure 2.12 demonstrates an FA map in the fetal brain produced by using this motion-corrected technique (Jiang et al. 2009) compared to an FA map produced from DTI data acquired post-natally; FA maps produced in utero are of poorer quality, with SNR being a major limitation compared to those produced from ex-utero data. Improved registrations and methodologies have since enhanced the quality of the final FA image, enabling WM tracts to be obtained using standard tracking techniques (Bertelsen et al. 2009, Wu et al. 2012).
However, limitations of these techniques, including long acquisition times and lengthy post-acquisition processes to reconstruct and calculate the diffusion tensor, has meant that fetal DTI studies with adequate motion correction have had small subject numbers. For example Jiang et al, (2009) conducted motion-corrected DTI on 8 fetuses with a mixture of CNS abnormalities; whilst Bertelsen et al, (2009) tested their motion correction algorithm on 4 fetuses that were all referred for clinical scans. No study to date has used a motion correction technique to investigate FA values in the fetal brain across gestation in a large normal cohort.

![Image](image.png)

**Figure 2.12 FA maps produced in the fetal compared to preterm infant brain.**

*FA maps of the brain in a A) fetus with agenesis of the corpus callosum scanned at 26.7 weeks (Jiang et al. 2009); B) preterm infant with no brain structural abnormalities at 28.6 weeks.*

### 2.6 Abnormal brain development

Abnormal brain development may be subtle, as in the infant born preterm without overt pathology, or visually obvious and resulting from a variety of factors including chromosomal abnormalities, trauma or infection. There have been many studies over recent years describing the MR imaging findings in term and preterm infants with acquired brain injury (Rutherford et al. 1995, Byrne et al. 1990, Wilson and Steiner 1986, Garel et al. 2004, Huppi et al. 2001, Counsell et al. 2003); these have used both conventional or diffusion sequences. The literature on the role of MRI in antenataly diagnosed abnormal brain development is now increasing.

Fetal MRI has become an accepted clinical technique within many antenatal clinics, due to its ability to detect and confirm subtle brain anomalies that may not be fully visualised on ultrasound
(Whitby et al. 2001, Whitby et al. 2004, Dietrich and Cohen 2006, Rutherford et al. 2008). Fetal MRI is proving particularly useful in high-risk pregnancies such as twin-to-twin transfusion syndrome where there is a high fetal mortality and neurological morbidity or in cases in which there has been a significant event or maternal illness or infection (Rutherford 2009). Fetuses with CNS abnormalities often illustrate delayed maturation of the brain that can be identified on MRI (Levine and Barnes 1999). The most common reason for fetal MRI referral is cerebral ventriculomegaly (VM) (Rutherford 2009). In these cases, MRI is important to identify any potential underlying abnormalities associated with the enlarged ventricles (Ouahba et al. 2006), a cause for the dilation or in order to confirm a diagnosis of isolated VM (Sévély and Manelfe, 2002).

In this thesis, the effect of congenital heart disease (CHD) and antenatal isolated VM on normal in utero brain development, as well as the effects of preterm birth and the ex utero environment on cerebral development, specifically in cases without focal lesions, have been studied.

**Congenital heart disease**

CHD refers to a variety of malformations of the heart present at birth, and is a common cause of childhood morbidity. CHD occurs in 6-8 infants per 1000 live births and approximately 50% of these cases require open heart surgery to correct the defect (Hoffman and Kaplan 2002, Samanek 2000).

Neurodevelopmental deficits are identified in up to half of children with CHD, in a wide range of developmental domains including fine motor skills, visuospatial skills, and cognition such as memory, attention, and higher-order language skills (Bellinger et al. 1995, McGrath et al. 2004, Bellinger et al. 2003, Limperopoulos et al. 2007). The neurological basis for the high incidence of these deficits has been studied and is beginning to be understood with insight from neuroimaging. Over one third of infants with CHD have brain abnormalities seen on MRI prior to cardiac surgery, and an additional third of infants acquire injuries during or shortly after cardiac surgery (Miller and McQuillen 2007). Imaging studies have shown that infants with CHD specifically have a high incidence of WM injury and structural brain abnormalities (Galli et al. 2004, Mahle et al. 2002, McQuillen et al. 2007); brain abnormalities observed on MRI in fetuses and infants with CHD also include delayed cortical growth, smaller brain volumes compared to controls, focal lesions, diffuse WM abnormalities and increased ADC and decreased FA values in WM (Mahle et al. 2002, Miller et al. 2006, Miller and Ferriero 2009, Limperopoulos et al. 2010, Berman et al. 2011).
**Antenatal isolated ventriculomegaly**

VM is the most common detectable CNS abnormality in utero, affecting approximately 1% of fetuses and refers to the enlargement of the cerebral ventricles. It is defined as a lateral ventricle atrial measurement of >10mm on a transverse image at the level of the glomus of the choroid plexus as can be seen in Figure 2.13. VM can be described as borderline (10mm), mild (10.1-12mm), moderate (12.1-15mm) or severe (>15mm), although definitions may vary. VM is associated with abnormal development in childhood; with poorer neurodevelopmental outcome being dependent on greater degree of ventricular dilation, and presence of associated abnormalities (Kazan-Tannus et al. 2007). Antenatally diagnosed VM may occasionally result from obstruction to the flow or absorption of cerebrospinal fluid (CSF) (Sévely and Manelle, 2002); but usually there are no signs of obstruction and the VM may be associated with chromosomal abnormalities, such as trisomy 21, neuronal tube defects, congenital infection, such as cytomegalovirus (CMV), vascular insults, or aberrations of cortical development including neuronal proliferation or migration.

There is no obvious cause or additional brain abnormalities detected in approximately 50% of antenatally diagnosed VM cases; this is termed isolated VM, and the aetiology of the dilation of these ventricles remains unknown (Kelly et al. 2001). Isolated VM has been hypothesised to be a structural marker of altered brain development manifesting in increased total brain volume in fetuses (Kyriakopoulou et al. 2013), neonates (Gilmore et al. 2008) and children (Lyall et al. 2012), as discussed in the following section.

There is evidence that long-term neurodevelopmental outcome is affected by isolated VM (Melchiorre et al. 2009). Previous studies have indicated that the risk of neurological abnormality is 12% in cases where ventricular dilation is between 10.1-15mm (Ouahba et al. 2006, Devaseelan et al. 2010). However, poor outcome has been associated in >50% of cases with greater atrial diameter (> 15 mm). Asymmetrical VM and progression of the ventricular dilation prenatally (Ouahba et al. 2006, Arora et al. 1998, Gaglioti et al. 2005) have also been associated with a poorer outcome. In cases of mild isolated VM (10-12mm), the overall risk of abnormality decreases to 4% (Devaseelan et al. 2010). Studies have demonstrated that abnormal neurodevelopmental outcome in association with isolated VM is variable with deficits in cognitive, language and behavioural domains, whilst motor development was less affected or included mainly minor deviations (Sadana et al. 2007, Lyall et al. 2012, Gomez-Arriaga et al. 2012).

In addition to the increased risk of developmental problems in cases of isolated VM (Senat et al. 1999), an association has been found between mild enlargement of the ventricles and
neuropsychiatric disorders such as autism (Palmen et al. 2005), attention deficit hyperactivity disorder (Lyoo et al. 1996) and schizophrenia (Wright et al. 2000).

![Image](image.jpg)

**Figure 2.13 Diagnosis of antenatal ventriculomegaly on fetal MRI.**

*T₂*-weighted scans of two fetuses at 32 weeks gestation: (A) a fetus with normal brain appearance; (B) a fetus with isolated VM (atrial diameter 15.7mm).

**Brain development in infants with antenatally diagnosed VM**

MRI volumetric studies have identified increased total brain volume in cases with isolated VM compared to controls (Kyriakopoulou et al. 2013, Gilmore et al. 2008, Lyall et al. 2012). Studies in neonates have demonstrated that total brain volume increases were specifically due to significantly greater cortical grey matter volumes in infants with antenatally diagnosed VM compared to controls (Gilmore et al. 2008). Increased white and grey matter volumes have also been observed in older children with antenatally diagnosed VM at 1 and 2 years compared to controls (Lyall et al. 2012).

Fetal MRI analyses have been advanced with the use of SVR. SVR, as described above (Jiang et al. 2007), has furthered MRI’s contribution to the clinical analysis of the fetal brain in vivo by allowing for accurate volumetric analysis to compare between clinical and control cohorts (Kyriakopoulou et al. 2013, Vatansever et al. 2013, Damodaram et al. 2012). Using this technique, increased total brain volume was found in fetal isolated VM cases compared to controls; this increase was restricted to cortical enlargement (Kyriakopoulou et al. 2013). However previous fetal studies, which did not employ SVR or any MR reconstruction methods and had other limitations including...
use of non-homogeneous VM populations, failed to identify any significant volume changes between groups (Grossman et al. 2006, Kazan-Tannus et al. 2007, Pier et al. 2011, Scott et al. 2013). Cortical enlargement in fetal cases with isolated VM is consistent with findings in a fetal rat model where VM was associated with cortical overgrowth (Eyles et al. 2003). This finding, along with delays in gyrification of fetuses with isolated VM (Scott et al. 2013), indicate abnormal cortical development in this cohort.

Relative brain overgrowth in isolated VM cases has been suggested to result from a lack of normal developmental apoptosis, which is prominent in the cortex from approximately 32 weeks gestation (Kuan et al. 2000). Increased volumes have also been suggested to be associated with an increase in proliferating cells (Eyles et al. 2003), as studies have found a correlation between ventricle size and the amount of neuronal cell proliferation within the corresponding periventricular region (Sawamoto et al. 2006).

The observed alterations in WM (Gilmore et al. 2008, Goodlett et al. 2009, Lyall et al. 2012) and grey matter (Lyall et al. 2012, Kyriakopoulou et al. 2013) in isolated VM cases may be associated with neurodevelopmental outcome (Lyall et al. 2012).

**Premature birth**

Prematurity is defined as birth before 37 weeks gestation, and 7-8% of babies born in the UK are preterm (Petrou et al. 2011). Premature birth can be described as either extreme prematurity (<28 weeks), very preterm (28 to <32 weeks), or moderate to late preterm (32 to <37 weeks). 93% of preterm births occur after 28 weeks of gestation, but with the threshold of viability between 23 to 25 weeks of gestation, extremely preterm infants are at the greatest risk for a poor outcome (Eichenwald and Stark 2008).

Almost a third of all early neonatal deaths in the US are attributed to prematurity (Callaghan et al. 2006), and the incidence of preterm birth has not declined in the past few decades. This is mainly due to lack of understanding of the aetiology of spontaneous preterm birth. Between 65-70% of preterm births occur following spontaneous labour or preterm premature rupture of membranes (PROM) (Goldenberg et al. 2008). Epidemiological studies have illustrated an association of preterm birth with poverty, limited maternal education, young maternal age, inadequate prenatal care, maternal history of preterm birth and ethnic origin (Muglia and Katz 2010). Other strong risk factors of spontaneous preterm birth include multiple pregnancies (Gardner et al. 1995, Blondel and Kaminski 2002) and maternal infection during pregnancy (Romero et al. 2001).
WM injury is common following preterm birth, but advances in perinatal care have led to a decrease in the incidence of cystic WM lesions, and non-cystic lesions have become the predominant form of injury (Volpe et al, 2003). Advances in care have also led to an increase in the survival rate for preterm infants (Horbar et al. 2002, Slattery and Morrison 2002, Wilson-Costello et al. 2007). However, preterm infants have an increased risk of neurodevelopmental impairment (Bhatta et al. 2002, Johnson 2007, Moster et al. 2008, Saigal and Doyle 2008, Kerr-Wilson et al. 2012), even when no overt pathology is present (Saigal and Doyle 2008). Evidence from large population based studies demonstrate that over half of all infants born at less than 26 weeks gestation suffer some form of developmental impairment by 30 months of age (Wood et al. 2000) with adverse functional and behavioural consequences that may persist into adolescence and early adulthood (Rushe et al. 2001, Hille et al. 2007, Aarnoudse-Moens et al. 2009). Whilst the incidence of severe neurological disabilities such as cerebral palsy has decreased (van Haastert et al. 2011), a wide array of more subtle neurodevelopmental complications including visual and auditory impairment, cognitive deficiencies and behavioural disorders have become increasingly common (Latal 2009, Delobel-Ayoub et al. 2009, Moore et al. 2012). These impairments are more severe with prolonged exposure to the extra-uterine environment (Bhatta et al. 2002), and there is a significant increase in the risk of disability in male compared to female infants (Marlow et al. 2005).

**Premature birth and ex utero development compared to controls**

This thesis studies preterm infants without evidence of focal lesions in the brain. Preterm infants without focal lesions have subtle structural alterations in their cerebral development compared to term-born controls, and this along with an increased risk of neurodevelopmental deficits has led to research investigating the differences of in utero and ex-utero development in cases of no overt injury.

Structural alterations in preterm brains on MRI at TEA compared to controls include ventricular dilatation (Maalouf et al. 1999), abnormal cortical development (Inder et al. 2003, Ajayi-Obe et al. 2000) and diffuse excessive high signal intensity (DEHSI) in WM seen on T₂-weighted MR images (Maalouf et al. 1999, Inder et al. 2003) and manifesting in increased ADC values on DWI (Counsell et al. 2003).

MRI studies have been instrumental in showing volumetric alterations in preterm infants compared to controls. Some studies have demonstrated a reduction in total brain volume in
preterm infants at TEA compared to controls (Thompson et al. 2007, Inder et al. 2005). However, a further study suggested that attenuated global brain growth at TEA was not related to prematurity per se, but was associated with prolonged supplementary oxygen requirements in preterm infants without focal lesions (Boardman et al. 2007). MR segmentation approaches have been used to quantify volume changes within different regions of the neonatal brain. A significant reduction in WM (Mewes et al. 2006, Inder et al. 2005) and cortical grey matter (Inder et al. 2005) volumes have been found in preterm infants at TEA compared to controls. Volume reduction has also been observed in the sub-cortical grey matter regions of the thalamus, lentiform and hippocampus in preterm infants compared to term-controls; this volume reduction appears to be more marked with increasing prematurity (Boardman et al. 2006, Ball et al. 2012). These volume reductions in preterm brain compared to controls remain evident through to childhood and adolescence, and have been associated with decreased general cognitive functioning (Peterson et al. 2000, de Kieviet et al. 2012).

DTI has proved extremely useful in demonstrating white and grey matter differences in the preterm brain compared to term-born controls. ADC increases and FA reductions have been established in the WM of preterm neonates compared to controls (Huppi et al. 1998, Shim et al. 2012, Rose et al. 2008, Anjari et al. 2007, Alexandrou et al. 2014). ADC values in the central WM were significantly increased in preterm neonates without evidence of injury at TEA compared to infants born at term (Huppi et al. 1998a). TBSS studies have demonstrated significant FA reductions in the corpus callosum, frontal WM and CSO of preterm neonates at TEA without focal brain lesions compared to term-born controls (Anjari et al. 2007, Alexandrou et al. 2014). These results have suggested delayed or altered maturation of WM tracts of preterm infants in the absence of major focal lesions (Huppi et al. 1998a, Alexandrou et al. 2014, Gimenez et al. 2008, Ling et al. 2013). These DTI measures in the WM may be associated with neurodevelopmental outcome in preterm children (Krishnan et al. 2007, Counsell et al. 2008, van Kooij et al. 2012).

Few studies have investigated DTI differences in the grey matter of preterm neonates compared to controls. However reduction in thalamic volume in preterm infants has been associated with increased diffusivity (Ball et al. 2012). This was advanced by a recent study which demonstrated significantly diminished thalamo-cortical connectivity between preterm infants at TEA compared to term-born controls (Ball et al. 2013a). These results suggest that the thalamo-cortical system is vulnerable following preterm birth.

A number of studies have suggested that structural brain alterations observed in preterm infants compared to term-born controls are associated with common perinatal events (Boardman et al. 2000, de Kieviet et al. 2012).
Perinatal events such as chronic lung disease (CLD), defined as dependence on oxygen at 36 weeks, have been associated with reductions in FA and increased RD values (Anjari et al. 2009, Ball et al. 2010). Post-natal sepsis has also been found to correlate negatively with ADC values in central WM (Hart et al. 2010); although a recent study of preterm neonates without overt injury found no association between any DTI measure and post-natal sepsis (Hemels et al. 2012). These perinatal risk factors have been correlated to poor outcomes (Kiechl-Kohlendorfer et al. 2009, Short et al. 2003).

The neuropathology underlying the observed decreased volume, FA reductions and ADC and RD increases in preterm infants without focal lesions compared to controls remains unresolved. The majority of studies exploring the cellular basis of brain alteration in preterm infants have focused on cases of cystic WM injury such as PVL (Back et al. 2001, Damska et al. 1989, De Vries et al. 1988, Back and Volpe 1999). It is widely believed that cerebral WM abnormalities detected on MRI reflect a disturbance or alteration in myelination; and the neuropathology of diffuse WM alterations represents a milder form of PVL (Back 2006). Diffuse WM injury may be initiated through targeted injury to oligodendrocytes, perhaps due to their susceptibility to oxidative stress (Back and Volpe 1997). Previous work suggested that the early stages of diffuse WM injury are characterised by a depletion of premyelinating oligodendroglia (i.e. late oligodendrocyte progenitors and immature oligodendrocytes) within the diffuse lesion (Riddle et al. 2006), along with the presence of numerous reactive microglia (Kinney and Back 1998). However, recent work has suggested that WM injury is linked to an arrest in the maturation of pre-myelinating oligodendrocytes to their mature form, rather than depletion of their numbers, and that this is a potentially reversible process (Billiards et al. 2008, Back et al. 2005, Preston et al. 2013, Sloane et al. 2010, Back and Rosenberg 2014). Comparatively, astrocytes and axons appear to be more resistant to injury than oligodendrocytes (Back 2006). Chronic diffuse WM lesions demonstrate extensive myelin pallor and a reduction in MBP, indicating reduced myelin in these regions (Back 2006). This arrest in oligodendrocyte maturation along with inadequate repair and an inability of mature oligodendrocytes to produce sufficient myelin is likely to lead to the myelin deficits seen in WM injury of preterm infants (Billiards et al. 2008).

The susceptibility of oligodendrocytes depends on their lineage at the time of insult: mature oligodendrocytes are more resistant to injury than pre-oligodendrocytes (Back et al. 2002a). Therefore, preterm neonates may be particularly vulnerable to WM damage between 28 and 36 weeks' gestation, because at this time most oligodendrocytes in the WM are immature (Back et al. 1998).
Aims of this thesis

The primary objective of this thesis was to establish quantitative ADC and FA measures of normal development in the fetal brain using high quality diffusion MR data.

Diffusion imaging is also invaluable to detect white and grey matter alterations in cases of abnormal development. Differences in DTI measures in neonates with isolated VM and infants born prematurely have previously been found when compared to normal term-born controls. The secondary aim of this thesis was to compare diffusion metrics in normal fetuses to those with isolated VM and to preterm infants.
3 Diffusion weighted imaging of normal development in the fetal brain

3.1 Introduction

Diffusion weighted imaging (DWI) allows objective quantification of the molecular motion of water in brain tissue, which gives invaluable insight into underlying structure. Apparent diffusion coefficient (ADC) values derived from DWI have been used to characterise cerebral development in premature infants without evidence of focal lesions (Partridge et al. 2004), but preterm infants cannot be considered representative of normal intrauterine development (Dyet et al. 2006). Normal brain development can only be assessed by imaging the fetal brain in utero. This is a challenge for fetal MRI due to fetal motion, artefacts and low signal-to-noise ratio (SNR). A robust fetal DWI sequence, with established normal ADC values for the fetal brain, would be a valuable clinical tool.

Unpredictable fetal movement and maternal respiratory motion present serious challenges to fetal diffusion imaging due to its inherent sensitivity to motion artefacts. Data are scarce on fetal movement patterns during scanning, but it has been shown that the frequency of fetal movements decrease across gestation (Hayat et al. 2011). The findings in the literature on other factors affecting fetal movement, such as fetal lie, maternal state of relaxation and maternal food or drink intake, is conflicting (Hijazi and East 2009).

Artefacts arising from fetal motion are not the only limitation of fetal DWI. Further artefacts result from signal displacement of maternal anatomy, which may present on the fetal brain. Low SNR due to the small size of the fetal brain, and its position within maternal anatomy, also limits the quality of fetal DWI. Therefore, diffusion parameters must be adjusted for optimal SNR and reduction of artefacts. Short sequence acquisition time also minimises the likelihood of fetal motion to reduce motion artefacts, thereby improving image quality.

Despite the associated difficulties with fetal DWI, a number of papers have produced normative ADC values of the fetal brain across gestation (Righini et al. 2003, Manganaro et al. 2007, Schneider et al. 2007, Schneider et al. 2009) and two papers have illustrated the reproducibility of these ADC measures (Boyer et al. 2013, Sartor et al. 2014). However there is large variability of ADC values between fetal papers (Righini et al. 2003, Manganaro et al. 2007, Schneider et al. 2009).
2007, Schneider et al. 2009, Boyer et al. 2013). Some studies have attempted to reduce motion artefacts using maternal sedation (Schneider et al. 2007, Cannie et al. 2007) or short sequences acquired within a maternal breath hold to limit artefacts from maternal breathing (Righini et al. 2003, Schneider et al. 2009). Whilst these methods have improved the quality of images in some cases, no fetal DWI studies have yet identified criteria for exclusion of data with motion artefacts or provided the proportion of data excluded.

The main objective of this study was to establish ADC measurements of normal white and grey matter development in the fetal brain using an optimised DWI protocol. This involved 1) assessment of factors potentially influencing fetal head motion, in order to implement procedures that could minimise fetal movement and improve image quality; 2) optimisation of DW sequence parameters for a fast clinical application of fetal DWI; 3) image quality assessment and definition of exclusion criteria. The optimised fetal DWI was then utilised to establish normative ADC values with increasing gestational age (GA) at scan in a large cohort of fetal cases.

### 3.2 Methods

#### Normal Fetal Cohort

To establish normative ADC values in utero, all normal fetal cases between July 2011 and end of May 2013 were scanned with the optimised fetal DWI protocol. The normal control fetal population was selected from healthy volunteer participants, those who had a child with previous abnormalities, or those with a suspected abnormality on ultrasound but not detected on MRI. Fetal cases were included if fetal MRI confirmed a normal brain appearance as established by an experienced neuroradiologist, but were excluded in cases of delivery complications, low Apgar score (<7 at 1 minute of age), abnormal neurological signs, low birth weight (<3<sup>rd</sup> centile), congenital malformations, infection, chromosome abnormality, multiple pregnancy, premature delivery (<36 weeks gestation) or abnormal clinical neonatal examination.

#### Fetal MR imaging procedure

The fetal imaging programme allowed scanning over a range of GAs (Ethics 07-H0707-105). Healthy volunteers for the study were recruited from the antenatal clinic in Queen Charlotte’s and Chelsea Hospital and through voluntary enrolment. Clinical fetal cases were referred from antenatal departments across London for MRI assessment of brain development. Pregnant women
were provided with an information sheet that included details on the study and scanning procedure. Informed written consent was obtained from all participants prior to scanning, and a detailed metal check questionnaire was completed before entering the scanner to ensure safety guidelines were strictly met.

All scanning was conducted on a Philips 1.5-Tesla scanner using a 32-channel phase array cardiac coil placed around the mother's abdomen and as close to the fetal head as possible. Participants were not sedated, and were placed in a lateral tilt position using supportive cushioning to avoid inferior vena cava compression from the pregnant uterus. Total examination length was less than one hour, as long as the mother remained comfortable in the scanner, and the protocol included clinical anatomical scans that were acquired prior to research protocols. All mothers were provided with headphones to protect hearing during the scan, as well as allowing communication with the radiographer.

**Assessment of factors influencing fetal head motion**

A pre-scan motion-assessment was conducted on all women undergoing fetal MRI during an 8-month period between November 2010 and July 2011. Questions were designed to gather information concerning: recent maternal exercise, food and caffeine intake that morning, and maternal anxiety. Anxiety was assessed using a visual analogue scale (VAS). The VAS has frequently been used in both a research and clinical setting and has proved to be a useful and valid measure of pre-operative anxiety (Kindler et al. 2000). The VAS was designed as a 100mm scale, with the far left side indicating zero anxiety, and the far right side indicating maximum anxiety (VAS is shown in Appendix A). Mothers were asked to mark along the 100mm scale their level of anxiety at that moment. The VAS score was the length (in mm) along the line that the mark was made. The mothers were asked to rate their anxiety just before the scan and immediately afterwards, but before they received the clinical results from the scan.

Additional variables were documented at the time of the scan including: GA at scan, maternal body mass index (BMI), maternal temperature, gender and lie of the fetus, twins or singletons, reason for scan and fetal motion at different times during scanning. Variables such as recent intake of food, caffeine and exercise were graded on a 2-point scale, with 0 indicating no food/caffeine/exercise. Fetal head motion was graded on a 3-point scale relating to its shift away from a predefined axis in each slice of T2-weighted dynamic scans in three planes (transverse, coronal and sagittal); the sum of movements comprised the motion score of that fetus (Figure 3.1).
STATA 11/C was used for statistical analysis including multiple regressions to establish correlation of variables with fetal motion, and Wilcoxon signed-rank test for maternal anxiety (non-normally distributed data).

Figure 3.1 Fetal head motion.

Fetal head motion in a transverse $T_2$-weighted sequence of a fetus scanned at 26.14 week, scanned for ventriculomagaly. The axis (red line) was defined on the $T_2$-weighted slice on the left, which was taken immediately prior to the slice seen on the right - illustrating the degree of movement away from this axis.

Motion assessment scale:

A - B: minimal movement, defined as movement $0 \leq 15^\circ$ away from the axis (1 point)

C - D: medium movement, defined as movement $15 \leq 50^\circ$ away from the axis (2 points)
E - F: severe movement, defined as movement >50° away from the axis (3 points), or severe motion artefact present as can be seen in F.

This score was completed for every acquired slice (slice number 80) on T2-weighted images, in transverse, coronal and sagittal planes. The sum of movements comprised the motion score of that fetus.

**Optimisation of fetal DWI sequence parameters**

To obtain an improved fetal DWI sequence for clinical use, the preset echo planar imaging (EPI) DWI sequence was optimised in order to: 1) maintain optimal SNR; 2) minimise artefacts; 3) decrease scan time.

For sequence optimisation, 40 pregnant women (GA at scan ranged from 22 to 37 weeks; median 30.43 weeks) were scanned with different variations of the preset DWI protocol. A systematic investigation into the effect of different imaging parameters on fetal DW images was performed. Each parameter was modified separately and parameter testing was completed on 3-7 patients before deciding on optimised values, depending on the quality of the image, fetal movement and ambiguity of image differences.

**Signal-to-noise ratio**

Figure 3.2 demonstrates reasons for low SNR on fetal MRI. These include the position of the fetal head within the surrounding maternal anatomy and the position of the coil with respect to the fetal brain. The coil position with the fetal head at its centre was important for optimal SNR. Maternal factors such as a high BMI may also affect the SNR.
Figure 3.2 Factors influencing SNR.

*T₂*-weighted images of the fetal brain surrounded by maternal anatomy in a case of: (A) good SNR, and poor SNR (B and C), as can be seen with the increased graininess of the image. Poor SNR may be due to fetal brain positioning deep in the mother’s pelvis and not in the centre of the coil (arrow) as shown in (B). C demonstrates poor SNR in an image where there was a high maternal BMI (maternal booking BMI was >40).

SNR is influenced by DWI parameters such as the voxel size, repetition time (TR), echo time (TE) and diffusion weighting (b value). They were systematically assessed to reduce SNR for the optimised fetal DWI sequence.

Smaller voxel size reduces SNR (Figure 3.3), by reducing the hydrogen nuclei content and hence the signal. TR describes the time between 2 excitation pulses and TE is the time between the excitation pulse and signal sampling, as described in chapter 2. Decreases in TE and increases in TR lead to improved SNR due to higher signal intensity. TE was therefore set to ‘shortest’. Increasing TR values also increases scan time, which raises the probability of fetal movement. Therefore an optimal balance between lengthening TR to increase SNR and the time of scan needed to be found (Figure 3.4). The b value determines the diffusion weighting (Gillard et al. 2005); an increase in b value results in increased sensitivity to water diffusion, but a reduction in SNR, so this also needed to be optimised (Figure 3.5). Figure 3.3 to Figure 3.5 demonstrates the optimisation of parameters and explains reasons for chosen values.
Figure 3.3 Voxel size and SNR.

DW images of a fetal brain (GA at scan 36.86 weeks), with a voxel size of 2x2mm and a slice thickness of A) 3mm, B) 5mm, C) 6mm. SNR increases with increasing slice thickness, as can be seen with the reduced graininess of the image; however thicker slices result in fewer anatomical details and greater partial volume. A slice thickness of 5mm was chosen.

Figure 3.4 TR, SNR and time of acquisition.

DW images of a fetal brain in the transverse plane (GA at scan 36.86 weeks) surrounded by maternal anatomy: with a TR of A) 1000ms, B) 2000ms, C) 4000ms and D) 6000ms. SNR increases with increasing TR; however as SNR increases, so does scan time (doubling TR doubles scan time). TR 4000ms gave an optimised SNR within 24 seconds, allowing for a maternal breath hold.

Figure 3.5 Diffusion weighting and SNR.
DW images of fetal brain (GA 24.29 weeks), with b values of A) 500 s/mm², B) 600 s/mm², C) 700 s/mm². High b values lead to greater diffusion weighting, but also result in a reduction in SNR. A b value of 500s/mm² was chosen. Previous fetal and neonatal papers have also used a b value of 500s/mm² (Schneider et al, 2007; Jiang et al, 2009).

Artefacts

Artefacts appearing on the fetal brain reduce the quality of DW images. These artefacts were reduced by the fat saturation technique SPIR (spectral presaturation with inversion recovery) suggesting their source was maternal fat surrounding the fetal brain (Figure 3.6). Rest slabs, or regional saturation bands, were useful to further reduce artefacts (Figure 3.7); they saturate signal by immediately reading the MR signal following the excitation pulse. Their use was especially important to avoid wrap artefacts when utilising a small field of view (FoV) where it was not possible to cover the entire maternal anatomy. A small FoV was used to reduce distortion caused by EPI sequences. There is, however, a decrease in SNR as the FoV decreases; FoV of 180mm was chosen to balance between SNR and distortion of the fetal brain, which covered the region of the fetal brain, but did not extend over the whole of the maternal anatomy.

Figure 3.6 Fat saturation techniques reduce artefacts.

A) The T2-weighted image of the fetal brain surrounded by maternal anatomy; B) the corresponding DW image without the use of SPIR; and C) the DW image when using SPIR. Maternal fat (arrows) is displaced onto the DW images causing artefacts. Use of the SPIR is effective in reducing the appearance of artefacts from maternal fat.
Figure 3.7 Rest slabs reduce artefacts.

$T_2$-weighted image (1$^{st}$ row) and DW image (2$^{nd}$ row) of two fetal brains, without (column A) and with (column B) signal suppression rest slabs. Rest slabs saturate the majority of signal from maternal anatomy. This decreases artefacts from signal displacement in DW image (arrows). Rest slabs need to be positioned on maternal tissue surrounding the fetal brain in the phase encoding direction.

**Image quality assessment**

Following DW acquisition, ADC maps were produced on the scanner using in-built Philips software, and DW images and ADC maps were then assessed for quality before conducting the region of interest (ROI) analysis.

In order to ensure that ROI analyses were conducted on good quality images, each DW scan and its corresponding ADC map was coded according to the presence of motion and artefacts. DW scans with motion on more than 40% of slices were excluded from the analysis. In scans that were
not excluded, each slice was coded to assess if image quality was adequate for a regional analysis. Slices with any evidence of motion were excluded. Slices were coded according to presence of artefact: no artefact; artefact present but not on brain; or artefact present on brain. Slices were excluded if the artefact was present on the brain in or near the ROI.

**ADC ROI analysis on normal fetal cohort**

A ROI analysis was conducted on the good quality ADC maps. Regions in white matter (WM) included the centrum semiovale (CSO) (32 voxels), genu and splenium of the corpus callosum (6 voxels), frontal (50 voxels) and occipital WM (20 voxels); regions in grey matter included the thalamus (32 voxels), cerebellum (18 voxels), pons (10 voxels) and cortex (average 203 voxels, ranging from 130 - 268). Bilateral ROIs were taken in the CSO, frontal WM, occipital WM, thalamus and cerebellum Figure 4.1. A repeatability ROI analysis of ADC values was conducted: ROI measurements were repeated in the frontal WM, splenium and genu, with a gap of a week. In order to ensure appropriate sized ROIs were used for analysis, ROI sizes and shapes were compared using intra-class correlations and Bland Altman plots.

To achieve the main objective of this study, regression analysis of ADC values with GA at scan was performed. A quadratic term was included to test for non-linearities in the relationship between ADC and GA at scan. Analysis of variance (ANOVA) was used for regional comparison of mean ADC values. Bonferroni correction for multiple comparisons was applied with a level of significance set at p≤0.006.

**3.3 Results**

**Assessment of factors influencing fetal head motion**

120 pre-scan motion assessments were conducted on participants coming for a fetal MRI scan; seven were healthy controls, and the remainders were scanned for clinical reasons. However, it was not always possible to collect data on every variable for each participant. Table 3.1 and 3.2 report information on each variable and fetal motion scores.

A significant negative correlation between fetal head movement and GA at scan was found (p=0.012; R² =0.052; b coefficient -1.80), and significant differences in the anxiety measures are
illustrated in Figure 3.8 and Figure 3.9. No other factor was significantly associated with fetal head motion when taking into account fetal GA.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Participant number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caffeine No caffeine</td>
<td>49</td>
</tr>
<tr>
<td>Caffeine</td>
<td>55</td>
</tr>
<tr>
<td>Food No food</td>
<td>16</td>
</tr>
<tr>
<td>Food</td>
<td>88</td>
</tr>
<tr>
<td>Exercise No exercise</td>
<td>56</td>
</tr>
<tr>
<td>Exercise</td>
<td>51</td>
</tr>
<tr>
<td>Multiple pregnancies</td>
<td></td>
</tr>
<tr>
<td>Singleton</td>
<td>104</td>
</tr>
<tr>
<td>Twins</td>
<td>16</td>
</tr>
<tr>
<td>Fetal Lie Breech</td>
<td>32</td>
</tr>
<tr>
<td>Cephalic</td>
<td>88</td>
</tr>
<tr>
<td>Fetal gender Male</td>
<td>50</td>
</tr>
<tr>
<td>Fetal gender Female</td>
<td>42</td>
</tr>
</tbody>
</table>

Table 3.1 Variables noted for motion-assessment during fetal scan.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA at scan (weeks)</td>
<td>29.14</td>
<td>20.43 - 38.71</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>30.50</td>
<td>18 - 49</td>
</tr>
<tr>
<td>Maternal BMI</td>
<td>27.08</td>
<td>17.85 - 43.75</td>
</tr>
<tr>
<td>* Anxiety scores (mm)</td>
<td>0.20</td>
<td>-6.3 - 6.7</td>
</tr>
<tr>
<td>Fetal head movement score</td>
<td>26</td>
<td>3 - 197</td>
</tr>
</tbody>
</table>

Table 3.2 Fetal and maternal variables and fetal motion score.

* Difference between maternal anxiety scores before and after scanning
Figure 3.8 Fetal head motion and GA at scan.

A significant negative linear correlation was found between fetal head movement and GA at scan ($R^2=0.052; b$ coef $-1.80$, $p=0.012$) ($n=120$).

Figure 3.9 Maternal anxiety.

A significant decrease in maternal anxiety (assessed before scanning) of mothers coming for a repeat scan was found compared to their initial scan ($n=16$) ($p=0.008; z=2.637$). A reduction in maternal anxiety before scanning compared to after the scan, but prior to results being given, was also found ($n=102$) ($p=0.08; z=1.741$), however this did not reach significance.
**Optimised protocol for fetal DW imaging**

The fetal DWI sequence was optimised to be acquired within a maternal breath hold of 24 seconds, in order to minimise motion from maternal breathing. Mothers were instructed to breathe out slowly if they were unable to hold their breath for the whole period. Parameters for optimised fetal DWI sequence included: TR 4000ms, TE ‘smallest’ (~90ms), b value 500 s/mm², FoV 180x150x110mm³. SPIR, for fat saturation, and signal suppression rest slabs were utilised to decrease artefacts occurring over the fetal brain.

Full suppression of fat signal was not always possible, even with the use of SPIR. The optimised protocol included an initial acquisition of the DW sequence with SPIR off in order to clearly visualise the position of any artefact present. The following sequence, with SPIR on, was acquired with rest slabs positioned over the relevant maternal anatomy, and the phase encoding direction could be changed to move the artefact away from the fetal brain (Figure 3.10). This reduced the presence of artefacts on the fetal brain; thereby improving image quality and aiding clinical interpretation. The total time to acquire an initial DWI sequence without SPIR, correctly place the rest slabs, and then re-scan was less than 1 minute.

DW images from the optimised fetal DW sequence compared to the preset protocol can be seen in Figure 3.11.

![Figure 3.10 DW protocol for reduction of artefacts over the fetal brain.](image)

A) T₂-weighted image of the fetal brain surrounded by maternal anatomy (GA at scan 33.86 weeks); B) DW image with SPIR off and a phase encoding direction of anterior-posterior (AP), in the posterior direction; the artefact from maternal anterior fat appears on the fetal brain (arrow). C) the DW image, with phase encoding changed to be in the anterior direction, shows the fat artefact shifted off the fetal brain (arrow).
Figure 3.11 Comparison of the optimised fetal DWI sequence to the preset DWI protocol.

A) The preset DWI sequence (magnified), compared to B) the optimised fetal DWI protocol on the same fetus. A) Illustrates distortion of the fetal brain from a large FoV, and an artefact from signal displacement of maternal fat (arrow). B) Illustrates reduced distortion and reduction in artefacts over the fetal brain.

Scan time of preset DWI protocol was 1m53s, compared to the 24s optimised scan. This reduced scan time was completed within a maternal breath hold thus limiting artefacts from maternal respiration and fetal movement. All fetal DW sequences were acquired in the transverse plane.

**Image quality assessment**

Results of fetal DWI coding can be seen in Table 3.3. Entire scans were excluded in cases of excessive motion; an example can be seen in Figure 3.12. ROIs were excluded if there was an artefact on the fetal brain in or near to that region (Figure 3.13) or if image slices were off plane to the extent that the ROI could not be fully visualised. Examples of good quality DW images and their corresponding ADC maps used for ROI analysis are shown in Figure 3.14.
Table 3.3 Fetal DW image coding: motion and artefacts.

* Entire dataset was excluded from analysis; ** Individual image slice with movement or artefact was excluded from ROI analysis.

Figure 3.12 Exclusion criteria from ROI: movement.

A) An example of excessive movement seen in successive slices of a DW image in a fetus at 2229 weeks, leading to exclusion of entire fetal DWI dataset. Fetal motion has led to a loss of signal in the brain region; the image appears noisy, and there is no delineation of brain structure or the surrounding maternal anatomy. B) An example of movement seen in successive slices of a DW image in a fetus at 2686 weeks: the DW image (i) has little evidence of motion (but is slightly off the transverse plane) and would be eligible for ROI analysis. However (ii) has evidence of small movement compared to the previous slice, and is off-plane, so would be excluded from ROI analysis. The subsequent 3 slices show evidence of excessive movement (iii and iv), or movement between slices (v), and so would be excluded from ROI analysis. Movement leads to a noisy and blurred image, where the anatomy cannot be visualised.
Figure 3.13 Exclusion criteria from ROI: artefact.

*DW image of fetus scanned at 29.71 weeks; an artefact, from signal displacement of maternal fat, is present on the fetal brain (arrow).*
Figure 3.14 DW image and ADC maps used for ROI analysis.

Scans at the level of the CSO (first row), thalamus (second row), peduncle (third row) and cerebellum (final row) in a 26.86 week fetus (columns 1 and 2) and a 32.71 week fetus (columns 3 and 4). Columns 1 and 3 show the DW image, and columns 2 and 4 are the corresponding ADC maps. The images of the older fetus (columns 3 and 4) are slightly off plane, especially seen in the region of the cerebellum. ROIs are demonstrated in the next chapter in Figure 4.1.

**ROI analysis: repeatability and comparison of size and shape**

Repeatability ROI analyses of ADC values were undertaken in the frontal WM (n=16), genu (n=15) and splenium (n=17) of the corpus callosum. Significant agreement between the two measures was found in all regions (Figure 3.15).
Figure 3.15 Bland Altman plots for repeatability measures in frontal WM and corpus callosum.

The absolute mean difference between measures in the A) splenium, B) genu, and C) frontal WM was -32.2, -22.5, and -32.2 respectively. There was a very small percentage mean difference in the splenium (2.2%), genu (-1.5%) and frontal WM (-1.9%). The 95% confidence interval (CI) limits of agreement were between 303.61 and -368.09 (spleunium), 300.43 and -345.45 (genu), 28.82 and -93.13 (frontal WM). The repeatability index (RI) was 0.23, 0.21 and 0.03, and the intra-class correlation coefficient was 0.442 (p<0.0011), 0.631 (p<0.01) and 0.991 (p<0.0011) in the splenium, genu and frontal WM respectively.

Comparison of ROI size was conducted in the frontal WM (n=12). In order to account for increased brain volume with GA, an increasing ROI size with GA at scan was compared to a standard sized ROI; size was adjusted by 10% per week (the approximate brain volume increase (Kyriakopoulou et al, 2013). The standard ROI size was chosen to be the median sized ROI due to its applicability to all ages. There was significant agreement between ADC measures of both ROI sizes (Figure 3.16).

Figure 3.16 ROI size comparison in the frontal WM.

The absolute mean difference between measures in the frontal WM was -25.1. There was very small percentage mean difference in the frontal WM (1.5%). The 95% CI limits of agreement were
between 46.13 and -96.21. The RI index was 0.041, and the intra-class correlation coefficient was 0.879 (p<0.001).

Comparison of ROI shape was conducted in the corpus callosum. Anatomy in this region was variable and therefore ROI shape may need to be tailored to individuals, which could introduce an unwanted variable. However, differences between ADC values of different ROI shapes were not statistically significant (p<0.001) (Bland Altman plots can be seen in Appendix B). Therefore, whilst ROI size and shape were standardised for consistency, it was found to be appropriate to further adjust the ROI shape in some cases to ensure it fitted with the anatomy of the individual, especially in cases of younger gestational ages.

**ADC ROI analysis on normal fetal cohort**

For the normal control fetal cohort, a total of 58 fetal scans were performed with the optimised fetal DWI protocol between July 2011 and end of May 2013. This comprised of 47 participants, 11 of whom returned for a second scan. 6 DWI scans had to be excluded due to excessive movement (Table 3.3). Therefore 52 fetal DWI scans were used for the ROI analysis; although some ROIs were excluded due to motion or artefact on the image slice, as detailed in Table 3.3. The GA at scan of this fetal control cohort ranged from 20.43 to 37.71 (median: 30.43) weeks. Clinical reasons for referral from fetal ultrasound can be found in Table 3.4; all had normal MRI scans.

<table>
<thead>
<tr>
<th>Reason for fetal MRI</th>
<th>Number of participants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biparietal diameter (BPD) below 3rd centile</td>
<td>2</td>
</tr>
<tr>
<td>Query ventriculomegaly</td>
<td>2</td>
</tr>
<tr>
<td>Previous child with neurodevelopmental problems</td>
<td>5</td>
</tr>
<tr>
<td>Query Hypoplastic cerebellum</td>
<td>1</td>
</tr>
<tr>
<td>Query cyst</td>
<td>1</td>
</tr>
<tr>
<td>Query enlarged cisterna magna</td>
<td>1</td>
</tr>
<tr>
<td>Healthy volunteer</td>
<td>40</td>
</tr>
</tbody>
</table>

*Table 3.4 Clinical reasons for referral to fetal MRI.*
Table 3.5 illustrates normative fetal ADC values in each ROI. There was regional variation of ADC values ($p<0.001$), with significantly greater values in the frontal WM and CSO compared to the corpus callosum. Significantly lower values were found in grey matter compared to WM regions (Figure 3.17).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ADC ($\pm$sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>splenium</td>
<td>1.36 ($\pm$0.13)</td>
</tr>
<tr>
<td>genu</td>
<td>1.44 ($\pm$0.15)</td>
</tr>
<tr>
<td>frontal WM</td>
<td>1.77 ($\pm$0.20)</td>
</tr>
<tr>
<td>CSO</td>
<td>1.69 ($\pm$0.17)</td>
</tr>
<tr>
<td>occipital WM</td>
<td>1.53 ($\pm$0.14)</td>
</tr>
<tr>
<td>thalamus</td>
<td>1.28 ($\pm$0.14)</td>
</tr>
<tr>
<td>cerebellum</td>
<td>1.37 ($\pm$0.14)</td>
</tr>
<tr>
<td>pons</td>
<td>1.22 ($\pm$0.13)</td>
</tr>
<tr>
<td>cortex</td>
<td>1.35 ($\pm$0.09)</td>
</tr>
</tbody>
</table>

Table 3.5 Normal ADC values and ranges between 20.43 and 37.71 weeks gestation.

ADC units: $x10^{-3}$ mm$^2$/s. Abbreviations: Obs = number of observations in each region used for the ROI analysis; some ROIs were excluded due to motion or artefact on the individual slice.
Figure 3.17 Regional ADC variations.

A box plot illustrating ADC values in each region. ADC values were highest in the frontal WM and CSO, followed by the occipital WM. The corpus callosum had significantly lower ADC values than the proximal WM regions. All WM regions had significantly greater ADC values than the grey matter regions. The pons had significantly lower ADC values than all other regions except the thalamus.
Table 3.6 shows the results of linear regression between ADC measures and GA at scan from the normal fetal cohort. ADC values significantly decreased with increasing GA at scan in the thalamus and cortex. ADC values also decreased with increasing age at scan in the CSO and pons, although this did not reach significance after Bonferroni correction. In the frontal WM ADC values increased with increasing GA at scan; this occurred at a decelerating rate towards term, as can be seen in Figure 3.18 (quadratic relationship was significant \( p=0.005, R^2=0.730 \)).

<table>
<thead>
<tr>
<th>Region</th>
<th>( R^2 )</th>
<th>coef.</th>
<th>95% CI</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>splenium</td>
<td>0.041</td>
<td>-0.007</td>
<td>-0.019, 0.006</td>
<td>0.291</td>
</tr>
<tr>
<td>genu</td>
<td>0.006</td>
<td>-0.003</td>
<td>-0.015, 0.010</td>
<td>0.660</td>
</tr>
<tr>
<td>frontal WM</td>
<td>0.657</td>
<td>0.037</td>
<td>0.028, 0.046</td>
<td>*~ 0.000</td>
</tr>
<tr>
<td>CSO</td>
<td>0.142</td>
<td>-0.017</td>
<td>-0.032, -0.002</td>
<td>0.023</td>
</tr>
<tr>
<td>occipital WM</td>
<td>0.044</td>
<td>0.007</td>
<td>-0.003, 0.016</td>
<td>0.170</td>
</tr>
<tr>
<td>thalamus</td>
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<td>-0.022</td>
<td>-0.031, -0.013</td>
<td>* 0.000</td>
</tr>
<tr>
<td>cerebellum</td>
<td>0.065</td>
<td>-0.010</td>
<td>-0.023, 0.003</td>
<td>0.127</td>
</tr>
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<td>pons</td>
<td>0.131</td>
<td>-0.013</td>
<td>-0.024, -0.002</td>
<td>0.022</td>
</tr>
<tr>
<td>cortex</td>
<td>0.262</td>
<td>-0.010</td>
<td>-0.016, -0.004</td>
<td>* 0.002</td>
</tr>
</tbody>
</table>

*Table 3.6 Regression results of ADC with increasing gestational age at scan in fetal control cohort*

* indicates that linear relationships were significant after Bonferroni correction \( p \leq 0.006 \). ~Data were best explained using a quadratic model. Abbreviations: coef = b coefficients; \( p \) = \( p \) value.
Figure 3.18 ADC with increasing gestational age at scan in all regions.

Significant correlations are indicated by a line of best fit. In the thalamus and cortex, a significant negative linear correlation between ADC and GA at scan was found. The ADC values in frontal WM increased with age at scan.
3.4 Discussion

The main objective of this study was to use an optimised fast acquisition DWI sequence to establish ADC measurements in the normal fetal brain. This chapter provides normal ADC values in white and grey matter regions in a large fetal cohort across a wide range of GAs.

**Fetal motion assessment**

This is the first study to investigate the frequency of fetal head movements during scanning and results demonstrated a significant association with GA at scan. The observed reduction in the frequency of fetal head motion with increasing GA is consistent with studies illustrating significant decreases in whole body movement in the second half of pregnancy (Hayat et al. 2011). A longitudinal study illustrated that fetuses were active 17% of the time at 24 weeks, but only 7% nearer term (Ten Hof 2002). Hayat et al. (2011) also observed that the frequency of movements decreased with increasing GA, specifically they found a decrease in lower limb movements, and hypothesised that this was due to a decrease in intrauterine space. However, the proportion of variation in fetal head motion explained by GA at scan was very small ($R^2=0.052$); this indicates that other unidentified factors also contributed to fetal head movement.

The majority of studies looking at fetal behaviour have focused on general body and isolated limb movements, or breathing, heart rate and hiccups, rather than head movement (Hijazi and East 2009). For fetal brain MRI, it is important to understand whether any factors specifically affect the head motion of the fetus, as this is ultimately what decreases the quality of brain images. However, results from the pre-scan fetal motion assessment suggest that head motion cannot be reduced by the variables studied in this chapter such as monitoring maternal diet or exercise prior to scanning; although results were limited by a relatively small cohort for each variable.

Results suggested that patients were burdened with anxiety. This anxiety may be related to a lack of understanding of the examination procedure: anxiety was reduced in patients coming for a repeat scan compared to their initial scan. Anxiety was also reduced after compared with prior to the scan, but before clinical results were given to the patient; however this difference was not significant. In order to decrease maternal anxiety, a short film was produced - [http://vimeo.com/37368763](http://vimeo.com/37368763) - that explained the examination procedure to familiarise participants before the scan.
**Fetal DWI protocol**

The final fetal brain DWI sequence demonstrated a reduction of artefacts occurring over the fetal brain, an optimised SNR and a scan time of only 24 seconds. The short scan time could be achieved within a maternal breath hold and minimised motion artefacts. Three previous fetal DWI studies also used maternal breath holds or quiet breathing to minimise artefacts from motion (Righini et al. 2003, Schneider et al. 2009, Bui et al. 2006), although scan length was as long as 53 seconds in one study (Bui et al. 2006). A further two fetal DWI studies used maternal sedation, with longer scan times of approximately 2 minutes (Schneider et al. 2007, Cannie et al. 2007). Three articles did not report any methodology to minimise motion or adjust for other fetal imaging limitations (Manganaro et al. 2007, Boyer et al. 2013, Sartor et al. 2014).

Fetal DWI papers aiming to establish normative ADC values have not previously addressed the limitations of artefacts over the fetal brain. In addition, only two previous studies mentioned that they excluded diffusion images with evidence of motion artefacts (Schneider et al. 2009, Bui et al. 2006), and no details of criteria for exclusion were given. Therefore, an advantage of the study in this chapter was the detailed exclusion criteria for ADC maps based on image quality, leading to high quality data sets with which to establish normal fetal ADC values. An ADC map derived from the optimised fetal DWI protocol can be seen in comparison to ADC maps in the literature in Figure 3.19. ADC maps and ADC values produced from the optimized DWI protocol were similar to those from motion-corrected fetal DTI data (a scatter plot of fetal ADC values derived from the two methods can be seen in Appendix C).

![Figure 3.19 ADC map from the optimised DWI protocol compared to ADC maps in previous studies.](image_url)

**ADC maps in the fetal brain from previous fetal DWI papers are seen in:** A) Schneider et al, (2007) (28 weeks); B) Schneider et al (2009) (23 weeks); C) Boyer et al, (23 weeks) (Circles in B and C illustrate position of ROIs). D shows an ADC map of improved quality compared to the maps in A, B and C. There is improved visualisation of anatomy such as the cortex compared to previous ADC.
maps, and less blurring of the image due to motion artefacts. The ADC map from the optimised DWI protocol (D) is comparable to ADC maps from a motion-corrected technique of the fetal brain seen in (E) of a fetal brain at 28 weeks (Jiang et al. 2009).

**Normal fetal ADC values with increasing gestational age at scan**

This study established normal ADC values within the fetal brain using the optimised fetal DWI sequence. Fetal ADC values reported in previous papers have varied; for example, in the frontal WM, values have ranged from 1.37 x 10^{-3} \text{ mm}^2/\text{s} (Boyer et al. 2013) to approximately 2 x 10^{-3} \text{ mm}^2/\text{s} (Schneider et al. 2009, Righini et al. 2003). These values may differ due to poor quality diffusion data, as well as use of different sequence parameters, such as diffusion weighting (b value of 600s/mm² in Righini et al. (2003) compared to 1000s/mm² in Boyer et al. (2013)). As ADC values have been shown to significantly decrease with increasing b values in the neonatal brain (Dudink et al. 2008), this is likely to be an important explanation for variation of fetal ADC values. Other factors leading to variability include different sized ROI, different GA ranges, small cohort numbers (Righini et al. 2003, Berman et al. 2011) and relaxed inclusion criteria for normal controls (Boyer et al. 2013, Bui et al. 2006). Some studies reported normal ADC values, but included cases of mild abnormalities such as ventriculomegaly (Bui et al. 2006) or non-CNS abnormalities (Righini et al. 2003); these have been associated with increased risk of neurodevelopmental abnormalities and altered diffusion measures (Miller et al. 2007, Limperopoulos et al. 2001, Senat et al. 1999, Gilmore et al. 2008). This chapter demonstrated ADC values over a wide range of GAs in a large cohort of normal fetuses, with strict inclusion criteria for normal controls.

The normal fetal ADC values in this study were comparable to measures in preterm neonates. For example the mean ADC value observed in the splenium was 1.36 x 10^{-3} \text{ mm}^2/\text{s}, which was similar to 1.33 x 10^{-3} \text{ mm}^2/\text{s} in the splenium of preterm infants scanned between 28 and 43 weeks (Partridge et al. 2004). Preterm studies have also demonstrated that rapidly maturing WM pathways, such as the PLIC, and grey matter regions demonstrate low ADC values when compared to later developing regions such as frontal WM, which have high ADC values (Neil et al. 1998, Mukherjee et al. 2001, Huppi et al. 1998a, Miller et al. 2002). Similar ADC patterns have been found in the fetal brain (Schneider et al. 2007, Schneider et al. 2009), and results from this chapter showed that ADC measures were also higher in later maturing WM regions, such as frontal WM, compared to earlier developing WM regions, such as the corpus callosum.
ADC values were found to significantly decrease between 20 and 38 weeks gestation in the thalamus and cortex; ADC decreases with age were also observed in the pons and CSO, although this did not reach significance after Bonferroni correction. It is likely that the decrease in ADC values with increasing gestation are associated with progressively reduced water content (Dobbing and Sands 1973), increased binding of water to macromolecules such as myelin, and maturational processes leading to decreased extracellular space and increased restriction of water motion (Le Bihan 1995, Beaulieu 2002). These maturational processes include myelination, dendritic arborisation, axonal ramification, synaptogenesis and glial proliferation, which create new structural barriers to water diffusion in white and grey matter (Mukherjee and McKinstry 2006). This reduction in ADC values over development has also been found in previous fetal and neonatal studies, although regions that demonstrate significant changes vary between papers (Partridge et al. 2004, Schneider et al. 2007, Schneider et al. 2009, Boyer et al. 2013). Most papers have found a reduction in ADC values with increasing GA at scan in the sub-cortical grey matter (Boyer et al. 2013, Mignone Philpott et al. 2013, Schneider et al. 2007, Schneider et al. 2009). This is the first fetal study to demonstrate ADC reduction with increasing GA at scan in the cortex. This may be due to improved quality DWI data which allow ROI placement within the cortex; a region specifically vulnerable to partial volume due to its close proximity to cerebral spinal fluid (CSF). Reduction in ADC in the cortex with increasing GA at scan has however been previously demonstrated in preterm infants (Ball et al. 2013b).

A significant increase in frontal WM ADC values with increasing GA at scan was also demonstrated. An ADC increase between 17 and 37 weeks has previously been observed in the frontal and occipital WM of the fetal brain (Cannie et al. 2007), and was attributed to the decreased cellularity in these regions (Dobbing and Sands 1973). Another study (Zanin et al. 2011) hypothesised that ADC values would increase during the early WM developmental stage of axonal organisation with water becoming less restricted as WM fibres progress from a tortuous axonal state to more coherent bundles (Volpe 2008). As frontal WM is a late developing WM region, which only begins myelination at approximately 6 months (Barkovich et al. 1988), it is possible that over the GA range studied, the increase in ADC values reflect the initial WM developmental stage of axonal organisation. Following this, ADC values decrease in WM as pre-myelination and myelination processes cause water diffusion to be hindered (Zanin et al. 2011, Dubois et al. 2008). Figure 3.18 demonstrated that frontal WM ADC values plateau after approximately 35 weeks, which may represent the beginning of pre-myelination processes in this region. Further investigation into this ADC increase is needed, using a wider age range to allow better understanding of the maturational processes underlying ADC changes through development.
**Limitations**

This study has a number of limitations since despite sequence optimisation and strict criteria for exclusion of poor quality data, some images still suffer from artefacts and small movements which could affect ADC values. In order to ensure that motion has not affected ADC values, post-acquisition techniques to correct for movement must be used; this method has previously been validated in the adult brain with and without motion (Jiang et al. 2009). However, the technique requires lengthy acquisition times as well as manual intensive and long post-processing times. This limits its use in a clinical setting. The DW sequence optimised in this chapter has the advantage of producing ADC values within a short time during a clinical examination.

Voxel size, as well as motion and artefacts, led to some ROIs not being included in the analysis, thereby reducing the number of observations for certain regions such as the splenium. The relatively large size of the DWI voxel may also have caused partial volume, which limits the fetal ADC data. Due to the relatively larger voxel size in smaller brains, partial volume was especially a problem at younger GAs. However, a decrease in the size of the voxel would have meant a decrease in SNR, which was already a limitation of fetal DWI. The splenium was a particularly difficult region to analyse due to its small size and partial volume effects; however repeatability analyses, as well as comparisons of size and shape, demonstrated that the corpus callosum ADC measurements were robust in this region.

It is also possible that the large voxel size limited the ADC results within the frontal WM. The significant increase in frontal WM ADC values with increasing GA at scan was an unexpected result, as other white and grey matter regions show a decrease in ADC values over this time period. It might be argued that the frontal WM ROI captured tissue with lower ADC values such as the cortex at younger gestational ages due to a greater chance of partial volume. This could have led to the observed ADC increase, as partial volume error decreased with increasing GA at scan due to the relative voxel size in the fetal brain. However, a ROI size test in the frontal WM demonstrated that when ROI size was increased proportionally with the increase in total brain volume across gestation, frontal WM ADC values did not significantly change when compared to using a standard ROI size (Figure 3.16). This suggested that the increase in frontal WM ADC values with increasing GA at scan was not due to the ROI capturing tissue other than WM, but instead associated with the underlying structural changes over this time period. Further investigation into this relationship is needed.
Conclusions

This optimised DWI sequence can be used in utero to produce good quality ADC images of the fetal brain in a short time. The large fetal control cohort provides normal ADC values across a wide GA range, which can be used for comparison to clinical cases and to investigate disease states where water content is increased in the developing brain.
4 Diffusion weighted imaging of the brain in fetuses with isolated ventriculomegaly and congenital heart disease

4.1 Introduction

Diffusion weighted imaging (DWI) could be used to detect developmental abnormalities in the fetal brain in the presence of a complication of pregnancy or delivery. Previous studies in preterm neonates have demonstrated an increase in apparent diffusion coefficient (ADC) values in the white matter (WM) of preterm infants with overt cerebral pathology at term equivalent age (TEA) compared to those without injury (Cheong et al. 2009, Counsell et al. 2003, Miller et al. 2002). ADC values have also been shown to be increased in preterm infants with subtle white matter abnormalities such as diffuse excessive high signal intensity (DEHSI) compared to those with normal brain appearance (Counsell et al. 2003). DWI could therefore be an important clinical addition for fetal MR imaging, allowing an objective assessment of the effect of congenital abnormalities, infection or injury on the developing brain.

Previous in utero DWI studies have sought to determine the feasibility of detecting WM abnormalities in utero. These studies have compared normal fetal cohorts to fetuses with specific congenital abnormalities, such as severe congenital heart disease (CHD) (Berman et al. 2011) or hydrocephalus (Erdem et al. 2007). Only two previous fetal DWI papers have compared abnormal to normal brain development using their own control cohorts (Sanz-Cortés et al. 2010, Mignone Philpott et al. 2013), and both with very small numbers. Fetal DWI papers have also been limited by the practical and technical difficulties outlined in chapters 2 and 3, including poor signal-to-noise ratio (SNR), and artefacts arising from fetal motion or displacement of maternal anatomy, which have resulted in poor quality ADC maps. This chapter seeks to address these shortcomings by using the optimised protocol for improved quality fetal ADC maps to compare a large normal fetal control cohort (from chapter 3) to two groups of fetuses with congenital abnormalities: fetuses with isolated ventriculomegaly (VM) and fetuses with CHD.

Fetuses with VM are commonly referred for antenatal magnetic resonance imaging (MRI), and isolated VM is associated with a high risk for later neurodevelopmental impairment (Sadan et al. 2007). Elevated ADC values have been found postnatally in neonates with mild isolated VM, along
with decreased fractional anisotropy (FA) values and increased radial diffusivity (RD) values (Gilmore et al. 2008, Goodlett et al. 2009). These results suggested a developmental WM delay in neonates with VM, and this is likely to originate in utero. A more recent study using fetal MRI showed increased brain parenchymal volume, specifically in the cortex, in isolated VM cases compared to controls (Kyriakopoulou et al. 2013). These results suggest that WM alterations in isolated VM may be evident antenatally, and that these might be detected and quantified with the use of optimised fetal DWI sequences.

With improvement in neonatal and infant surgery and intensive care, neonates with previously life threatening CHD are now surviving into adult life. Childhood survivors are now known to be at high risk of long-term neurodevelopmental difficulties, including motor, sensory and cognitive deficits (Partridge et al. 2006). Imaging studies have shown that infants with CHD have a high incidence of WM injury and structural brain abnormalities (Galli et al. 2004, Mahle et al. 2002, McQuillen et al. 2007) and that antenatally fetuses with CHD have smaller brain volumes and altered brain metabolism compared with controls (Limperopoulou et al. 2010). Both acquired focal lesions and more diffuse WM abnormalities compared to controls have been identified in neonates with CHD (Mahle et al. 2002, Miller et al. 2006, Miller and Ferriero 2009). This abnormal WM has been associated with ADC increases and FA decreases in neonates with CHD (Miller et al. 2007) which may reflect abnormal brain development in utero.

This study aims to assess white and grey matter development in fetuses with isolated VM and CHD by comparison to a large cohort of normal fetal controls. The optimised clinical fetal DW sequence and stringent exclusion criteria developed in chapter 3 were used to ensure good quality ADC data for comparison between groups.

### 4.2 Methods

**Fetal MR imaging protocol**

The fetal imaging programme allowed scanning over a range of gestational ages (GA) for both control and clinical fetal cases (Ethics O7-H0707-105). Clinical fetal cases were referred from antenatal departments across London for MRI assessment of brain development; the most common reason for referral for fetal MRI was VM. Other reasons for referral included cases of central nervous system (CNS) abnormalities detected on ultrasound, non-CNS abnormalities and
specifically cases of CHD, potential maternal infection, and complications due to multiple pregnancies.

The fetal imaging examination protocol was described in chapter 3. Briefly, participants were provided with information on the study and details of the procedure prior to the scan. A short film that detailed the fetal MRI procedure provided additional information for parents to minimise anxiety leading up to the examination. Informed written consent was obtained from all participants, which established consent for clinical assessment of images, use and storage of research images, and permission to contact parents after the scan for follow up post-delivery.

Images were taken on a 1.5-Tesla Philips scanner, with a 32-channel cardiac coil. The clinical fetal MRI protocol included $T_2$-weighted single-shot images and $T_1$-weighted single-shot (SNAPIR) images for anatomical images of brain maturation; $T_2^*$ gradient echo for detection of haemorrhage, and DWI.

**Fetal DW imaging**

Fetal DW imaging was undertaken using the procedure and optimised protocol outlined in chapter 3. The DW sequence was acquired in the transverse plane and included: b value 0 and 500s/mm$^2$, TR 4000ms, TE 'shortest'; use of SPIR and rest slabs for signal saturation of surrounding maternal anatomy; and the sequence was acquired within a maternal breath hold of 24 seconds. Fetal DWI was part of the clinical MR protocol and was conducted on all clinical patients. Fetal DWI and their corresponding ADC maps were checked for quality and data was excluded if corrupted by motion or artefacts, using the procedure described in chapter 3. The DWI protocol for controls and clinical cases were identical.

**Clinical examination**

Following sequence acquisition, an experienced neuroradiologist visually assessed all clinical MR scans, producing a detailed report on all fetuses, including normal controls. This clinical examination included assessment of signal intensity, appearance and size of WM, basal ganglia, thalami, orbits, cerebellum, vermis, brainstem, cortex and biparietal diameter; as well as the size and shape of the lateral ventricles, $3^{rd}$ and $4^{th}$ ventricles, cavum septum pellucidum and vergae, and extra-cerebral space; and finally presence of the corpus callosum and age specific myelination of the internal capsule or presence of haemorrhage in the germinal matrix.
Participants

All clinical cases were scanned with the fetal DW sequence, beginning in July 2011 and ending in May 2013, except in cases where mothers were unable to stay in the scanner for the duration of the examination.

The population of normal controls consisted of the 52 fetuses with good quality DWI scans that were used and described in chapter 3. This control fetal population had a confirmed normal brain appearance on fetal MRI as established by an experienced neuroradiologist; control cases were excluded in cases of delivery complications, low Apgar score (<7 at 1 minute of age), abnormal neurological signs, low birth weight (<3rd centile), congenital malformations, infection, chromosome abnormality, multiple pregnancy, premature delivery (<36 weeks gestation) or abnormal clinical neonatal examination.

Isolated VM cases were diagnosed with VM initially on antenatal ultrasound (US) and were then confirmed to have isolated VM with an antenatal MRI brain scan. Exclusion criteria for isolated VM were: additional brain abnormalities on MRI or US, positive infection screen or chromosomal abnormality screening, maternal drug use, multiple pregnancy, intra-utero growth restriction, birth weight below 3rd centile. CHD patients were included if they had a congenital heart defect diagnosed on US by a fetal cardiologist. CHD cases with congenital brain abnormalities were excluded.

ADC region of interest analysis

ADC maps were produced using Philips software on the scanner, and region of interest (ROI) analyses were performed on each ADC map. WM regions of the centrum semiovale (CSO) (32 voxels), genu and splenium of the corpus callosum (6 voxels), frontal (50 voxels) and occipital WM (20 voxels) were analysed. Grey matter regions of the thalamus (32 voxels), cerebellum (18 voxels), pons (10 voxels) and cortex (average 203 voxels, ranging from 130 - 268) were also analysed. Regions of the CSO, frontal and occipital WM, thalamus and cerebellum were assessed with bilateral ROIs (Figure 4.1).

STATA 11/C was used for statistical analysis; to compare between the ADC trajectories – i.e. the relationship between ADC and GA at scan – of the clinical and control groups, a multiple regression was estimated with a dummy variable taking the value 1 if the observation was from the clinical group and 0 if the observation was from the control group. Differences in trajectories
were indicated by the significance of the dummy variable. In a further test, aged-matched control groups were selected from the larger fetal control cohort. Independent t-tests or Mann-Whitney (for non-normally distributed data) were used to compare aged-matched control data to clinical groups. Bonferroni correction for multiple comparisons indicated a level of significance at $p \leq 0.01$.

![Figure 4.1 ADC maps and regions of interest in white and grey matter.](image)

**Figure 4.1 ADC maps and regions of interest in white and grey matter.**

An ADC map in transverse plane from a control fetus at 26.86 weeks. ROIs in the A) CSO, B) genu, C) frontal WM, D) splenium, E) thalamus, F) cortex, G) occipital WM H) pons, and I) cerebellum. The cortical ROI, at the level of the thalamus, does not include voxels where partial volume makes the cortex difficult to visualise. The occipital WM ROI parallels the border of the lateral ventricles and is likely to capture the optic radiation.

### 4.3 Results

Good quality ADC maps were produced in 24 fetuses with VM, 11 with CHD and 52 normal control fetuses. Mean ADC values in the isolated VM cohort, CHD cohort, and normal control fetal cohort
can be seen in Table 4.1. The percentage of regions analysed is also stated, as not all ROIs were able to be analysed in every fetal case due to artefact, slight movement or the plane of the image not allowing full visualisation of the region.

<table>
<thead>
<tr>
<th></th>
<th>Isolated VM n=24</th>
<th>CHD n=11</th>
<th>Normal controls n=52</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ADC</td>
<td>% ROI analysed</td>
<td>ADC</td>
</tr>
<tr>
<td>splenium</td>
<td>1.38 ±0.18</td>
<td>75</td>
<td>1.31 ±0.12</td>
</tr>
<tr>
<td>genu</td>
<td>1.45 ±0.17</td>
<td>92</td>
<td>1.41 ±0.12</td>
</tr>
<tr>
<td>frontal WM</td>
<td>1.84 ±0.16</td>
<td>100</td>
<td>1.82 ±0.16</td>
</tr>
<tr>
<td>CSO</td>
<td>1.78 ±0.15</td>
<td>88</td>
<td>1.76 ±0.14</td>
</tr>
<tr>
<td>occipital WM</td>
<td>1.64 ±0.15</td>
<td>92</td>
<td>1.67 ±0.19</td>
</tr>
<tr>
<td>thalamus</td>
<td>1.29 ±0.11</td>
<td>88</td>
<td>1.29 ±0.15</td>
</tr>
<tr>
<td>cerebellum</td>
<td>1.35 ±0.12</td>
<td>83</td>
<td>1.36 ±0.13</td>
</tr>
<tr>
<td>pons</td>
<td>1.26 ±0.14</td>
<td>92</td>
<td>1.22 ±0.11</td>
</tr>
<tr>
<td>cortex</td>
<td>1.39 ±0.10</td>
<td>88</td>
<td>1.39 ±0.08</td>
</tr>
</tbody>
</table>

Table 4.1 ADC values in isolated VM and CHD groups compared to control fetal data.

Mean ADC values (units: x10⁻³mm²/s) and standard deviation (sd) for each group are demonstrated; percentage of ROIs analysed (% ROI) are also stated.

**Isolated VM**

25 cases of isolated VM were scanned; two participants had a repeat scan during pregnancy. Three DW scans had to be excluded due to excessive movement, therefore a total of 24 isolated VM DWI scans were available for ADC ROI analysis and comparison with fetal controls. GA at scan from the isolated VM cohort ranged from 22.86 to 35.43 (median 32) weeks. Of this group, 16 had mild unilateral isolated VM (posterior horn diameter ≥10-12mm), of which 2 cases normalised during pregnancy. A further 3 cases had mild bilateral isolated VM, and 3 cases had moderate bilateral isolated VM (posterior horn diameter ≥12-15mm).

Figure 4.2 demonstrates ADC values with increasing GA at scan in the control and isolated VM fetal cohorts.
Figure 4.2 Change in ADC values with increasing gestational age at scan in isolated VM cases compared to fetal controls.

ADC values (units: $\times 10^{-3 \text{mm}^2/\text{s}}$) plotted with increasing GA at scan in cases of isolated VM and fetal controls.
Differences in ADC values between the control cohort and the isolated VM cohort were found; multiple regression results for each ROI can be seen in Table 4.2. The occipital WM and cortex demonstrated significantly different ADC trajectories between groups; ADC values were greater in the isolated VM group compared to controls in these two regions.

<table>
<thead>
<tr>
<th></th>
<th>R²</th>
<th>coef</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>splenium</td>
<td>0.042</td>
<td>0.016</td>
<td>-0.077, 0.110</td>
<td>0.727</td>
</tr>
<tr>
<td>genu</td>
<td>0.004</td>
<td>0.014</td>
<td>-0.076, 0.103</td>
<td>0.763</td>
</tr>
<tr>
<td>frontal WM</td>
<td>0.487</td>
<td>0.059</td>
<td>-0.011, 0.129</td>
<td>0.097</td>
</tr>
<tr>
<td>CSO</td>
<td>0.073</td>
<td>0.077</td>
<td>-0.012, 0.166</td>
<td>0.087</td>
</tr>
<tr>
<td>occipital WM</td>
<td>0.150</td>
<td>0.102</td>
<td>0.028, 0.176</td>
<td>* 0.008</td>
</tr>
<tr>
<td>thalamus</td>
<td>0.348</td>
<td>0.006</td>
<td>-0.052, 0.064</td>
<td>0.841</td>
</tr>
<tr>
<td>cerebellum</td>
<td>0.133</td>
<td>-0.016</td>
<td>-0.083, 0.051</td>
<td>0.637</td>
</tr>
<tr>
<td>pons</td>
<td>0.153</td>
<td>0.030</td>
<td>-0.036, 0.097</td>
<td>0.364</td>
</tr>
<tr>
<td>cortex</td>
<td>0.297</td>
<td>0.058</td>
<td>0.015, 0.101</td>
<td>* 0.009</td>
</tr>
</tbody>
</table>

Table 4.2 Multiple regression results of ADC trajectories between isolated VM and control cohorts.

* Significantly different ADC trajectories between isolated VM and control fetal groups. Abbreviations: R² = Adjusted R²; coef = b coefficient for dummy variable; p = p value

To test the robustness of the ADC results between cohorts, an aged-matched comparison of means between isolated VM and controls was conducted. The aged-matched group of fetal controls was extracted from the larger fetal control group; both cohorts had 24 observations. The isolated VM cohort had a mean GA at scan of 30.45 (±4.07) weeks, and the aged-matched control cohort had a mean of 30.59 (±4.06) weeks.

The aged-matched analyses found a significant increase in ADC values in the isolated VM cohort compared to controls in the occipital WM (p=0.006, t-stat= -2.60, df= 40); the mean occipital WM ADC in the isolated VM group was 1.64 x10⁻³ mm²/s compared to 1.52 x10⁻³ mm²/s in the aged-matched control group. No other regions, including the cortex, demonstrated significant ADC differences between groups.

**CHD**

16 cases of CHD were scanned; two of these participants had repeat scans during pregnancy. Six CHD scans had to be excluded due to poor quality data from movement and artefacts; therefore 11 CHD scans were available for analysis, with a GA at scan ranging from 24.57 to 35.29 (median 29.14) weeks. Six fetuses were diagnosed with transposition of the great arteries (TGA); one case had pulmonary stenosis, and one hypoplasia of the aortic arch; the final cases had atrioventricular
septal defects (AVSD). One case demonstrated a prominent fourth ventricle and slight rotation of the cerebellar vermis, which were no longer present at the second MRI scan, but otherwise there were no reported CNS abnormalities. Figure 4.3 demonstrates ADC values with increasing gestation in the fetal control cohort (n=52) and CHD fetal cohorts. ADC values of the CHD cohort were reported in Table 4.1. Multiple regression analyses between fetal CHD and the control fetal cohort found no significant differences in ADC trajectories between groups.

An age-matched comparison between the fetal CHD group and a smaller control cohort was also conducted; both cohorts had 11 observations. The CHD cohort had a mean GA at scan of 29.36 (±3.68) weeks, and the aged-matched control cohort had a mean of 29.44 (±3.72) weeks. T-tests found no significant differences between ADC values in the CHD compared to the aged-matched control cohort.
Figure 4.3 ADC values with increasing gestational age at scan in fetal CHD cases and fetal controls.

ADC values (units: $10^{-3}$mm$^2$/s) plotted with increasing GA at scan in cases of CHD and fetal controls.
4.4 Discussion

Significantly higher ADC values were found in isolated VM cases compared to a large control cohort in the occipital WM and the cortex. The study demonstrates the ability of the optimised in utero DWI sequence to detect ADC alterations in the fetal brain between isolated VM cases and control cohorts; however no significant ADC alterations were found in the white or grey matter of fetal CHD cases compared to normal controls.

This study addresses many of the limitations of previous in utero DWI papers, as an optimised protocol for improved quality fetal ADC maps was utilised to compare a normal fetal cohort to cases of complications of pregnancy. Only two previous fetal DWI papers used their own control data to compare abnormal to normal fetal brain development (Sanz-Cortes et al. 2010, Mignone Philpott et al. 2013). Both of these papers suffered from small clinical and control cohort numbers; for example Sanz-Cortés et al. (2010) compared 5 normal controls to 8 fetal cases that were small for gestational age (SGA). Shortcomings of previous fetal DWI papers also arose due to differences in scanning parameters used between the control and patient cohorts; for example different b values (Erdem et al. 2007). Comparatively, the data presented in this study benefits from comparison to a large control group (n=52); and an identical DWI protocol was used for comparison between cohorts. Data in this chapter were also the first to use fetal DWI to compare cohorts of isolated VM and CHD to normal controls in utero.

Fetal ADC alterations with isolated VM

Regression results demonstrated a significantly altered ADC trajectory, with increased ADC values across gestation in the occipital WM and cortex of fetuses with isolated VM compared to controls. Regression results were confirmed in the occipital WM in an aged-matched analysis, which showed increased ADC values of isolated VM cases compared to controls; but this was not found in the aged-matched analysis in the cortex.

The results from the regression analysis are more powerful due to the large fetal control cohort. The aged-matched analysis is based on a smaller sample, but controls more effectively for differences in GA at scan between cohorts. Therefore, together these results provide evidence for increased ADC values in the occipital WM and significant support for increased ADC values across gestation in the cortex of isolated VM fetuses compared to controls.
Significant increases in ADC values have previously been found in neonates with isolated VM compared to controls (Gilmore et al. 2008); this study demonstrated WM alterations in the corpus callosum and corticospinal tracts of 34 neonates with isolated VM and compared to 34 controls. The same paper demonstrated significantly decreased FA values in multiple WM regions compared to control infants, suggesting that this was due to delayed or altered WM maturation in these regions (Gilmore et al. 2008). Results from this chapter extend this data to show that altered ADC values can be observed in utero, although significant WM ADC differences were found in the occipital WM and not in the corpus callosum or corticospinal tracts across the gestational age range 22.86 to 35.43 weeks.

Increased ADC values may be caused by an increase in water content and a decrease in restriction to water motion. ADC values decrease with increasing age in the WM of the preterm and fetal brain (Mukherjee and McKinstry 2006, Bui et al. 2006, Cannie et al. 2007, Partridge et al. 2004), and this is associated with progressively reduced water content in the brain (Dobbing and Sands 1973) and a decreased extracellular space, which reduces separation of structures such as cell membranes and so hinders water diffusion (Neil et al. 1998). Increased ADC values in fetuses with isolated VM may therefore represent delayed or altered WM maturation.

The observed ADC increase in occipital WM in isolated VM cases compared to controls is consistent with a whole brain tract based spatial statistics (TBSS) analysis of WM tracts in neonates with antenatally diagnosed isolated VM compared to controls (chapter 5). TBSS demonstrated an increase in ADC and decrease in FA values in multiple WM tracts, including the sagittal striatum and posterior thalamic radiation that contains the optic radiations; these WM tracts are likely to be captured in the occipital WM ROI on the fetal brain in this chapter (Figure 4.1G).

Injury or DTI alterations in WM tracts of the sagittal striatum and posterior thalamic radiation have previously been associated with deficits in language, motor, cognitive and attention skills (Dramsdahl et al. 2012, Catani 2007, Doricchi and Tomaiuolo 2003, Duffau et al. 2002, Gazzaniga 2000, Hynd et al. 1995, Leclercq et al. 2010, Nosarti et al. 2004, Qiu et al. 2011, Tanabe et al. 1987). As antenatally diagnosed isolated VM has been associated with the neurodevelopmental delays including cognitive, behavioural and communication deficits (Bloom et al. 1997, Gomez-Arriaga et al. 2012, Lyall et al. 2012, Sadan et al. 2007), it is possible that ADC alterations in these WM tracts in antenatally diagnosed isolated VM relate to the neurodevelopmental delays found in this population; this will be further discussed in chapter 5. Fetal DWI may therefore be used to aid in early clinical prediction of outcomes. However further investigation into the progression of ADC
alteration over a larger age range combined with neurodevelopmental follow up is needed to establish any relationship between these ADC alterations and cognitive delay.

The cortex also displayed significantly elevated ADC values across gestation in fetal isolated VM cases compared to controls. ADC values in the cortex of preterm neonates have been observed to decrease with increasing age at scan (Ball et al. 2013b). This ADC reduction in the cortex with increasing GA at scan was also found in normal fetuses in chapter 3, and may be due to the increased dendritic density, neurite number, cellular complexity, and synapse formation over this time period (de Graaf-Peters and Hadders-Algra 2006). It is therefore possible that the observed increased ADC values over development in isolated VM compared to controls may be due to differences in these cortical maturational processes. The results are consistent with literature that has described significant differences in cortical growth in isolated VM cases compared to controls. Increased cortical volumes have been found in fetuses (Kyriakopoulou et al. 2013) and neonates (Gilmore et al. 2008) with antenatally diagnosed isolated VM. In addition, delays in gyrification (Scott et al. 2013) indicate abnormal cortical development in this cohort. Previous authors have suggested that the increased surface area along the ventricular wall in VM may result in a larger number of progenitor cells migrating towards to cortex (Lyall et al. 2012) or that reduced apoptosis in the developing brain leads to overgrowth of the cortex (Kuan et al. 2000). Apoptosis begins early, at approximately 4.5 weeks gestation, but is only present in all layers of the cortex by 11 weeks, and increases rapidly between 12 and 22 weeks (Zecevic and Rakic 2001). Apoptosis plays an integral role in cortical development, as it is essential for the regulation of size and shape of the cortex (Chenn and Walsh 2002, Depaepe et al. 2005). These theories of abnormal cortical development in VM are supported by cortical overgrowth in a rat model of VM which was associated with both an increase in proliferating cells, and a decrease in apoptotic cells (Eyles et al. 2003). Results from this chapter suggest that ADC values are capable of detecting progressively altered cortical structure in the fetus across gestation in isolated VM compared to controls, perhaps due to the differences in cellular content between groups in this region.

**Fetal ADC values in congenital heart disease**

No significant differences were found between ADC values in fetal cases with CHD compared to controls. However, these results were limited by a relatively small size of CHD cohort (n=11), and inclusion of a mixture of CHD cases. Small cohort numbers prevented division into smaller groups of specific CHD cases in this study. The small cohort number was mainly due to movement in a large proportion of these cases leading to data exclusion from ROI analysis. This cohort had a
greater proportion of DWI artefacts from fetal movement than any other group, with almost 40% of scans having to be excluded due to excessive motion. This resulted in very small numbers of observations in certain brain regions being available for analysis, for example the cortex contained only 5 ADC values for comparison to control data.

Delayed cortical growth has previously been found in fetuses with hypoplastic left heart syndrome, which is one of the most severe forms of CHD (Clouchoux et al. 2013). Clouchoux et al. (2013) compared 18 fetuses with CHD to 30 controls and demonstrated cortical folding delays from as early as 25 weeks, which preceded progressive volumetric brain growth disturbances later in the third trimester; volume reduction was observed in the cortex as well as subcortical white and grey matter. They suggested that the delayed cortical gyrification might be an important early marker to detect increased risk of brain growth failure. However, ADC values in the cortex of CHD cases were not statistically different to controls in the study of this chapter; although numbers were limited.

Increased risk of WM injury has been observed in neonates with CHD (Mahle et al. 2002, Miller et al. 2006, Miller and Ferriero 2009). One study of 41 neonates with CHD (scanned before cardiac surgery) demonstrated significantly altered brain magnetic resonance spectroscopy (MRS) measures and ADC values compared to controls (Miller et al. 2007). They found a total brain ADC increase of 4% in neonates with CHD compared to controls, and showed that the thalamus, perirolandic WM, posterior WM, and optic radiations were the most affected regions (Miller et al. 2007). They suggested that these results reflected abnormal brain development that originated in utero; however cases of WM lesions were included in the analysis which may have been the driving factor behind ADC changes in the cohort. Similar ADC abnormalities have been found in the thalamus and posterior WM of fetuses with CHD (Berman et al. 2011); however, only 3 fetuses with CHD were compared to controls in this study, and these were severe cases. The larger, though still small, CHD cohort in this chapter (n=11) found no significant differences between ADC values in fetuses with CHD compared to controls, suggesting that altered tissue structure in cases without CNS abnormalities may not originate in utero. However, due to the limitations of this group discussed below, further investigation is needed before conclusions can be drawn.

**Limitations**

Fetal CHD results are limited by small numbers in the CHD cohort (n=11); this may be a reason for the lack of significant results compared to controls. It is also possible that the mixed group of CHD
cases affected the results; the cohort included mainly TGA and VSD cases, but also AVSD, pulmonary stenosis and hypoplasia of the aortic arch. Previous fetal CHD studies have focused mainly on hypoplastic left heart syndrome or TGA cases and found smaller brain volumes, altered brain metabolism and delayed cortical development in these cases compared with controls (Limperopoulos et al. 2010, Clouchoux et al. 2013). It is therefore possible that a larger and more homogeneous group of fetal CHD cases may uncover subtle ADC alterations compared to controls. These CHD cases were from a larger cohort which is currently being studied for brain volume comparisons with normal fetal controls.

This study demonstrates the feasibility of using the optimised fetal DWI sequence to assess abnormalities in white and grey matter development in utero. However, investigation into the clinical use of fetal DWI would be advanced by repeated DWI scans across gestation as well as the collection of follow up neurodevelopmental information through to childhood in order to elucidate in utero ADC's predictive value on neurodevelopment.

In addition, the fetal DWI sequence suffers from similar limitations outlined in chapter 3; specifically fetal DWI suffers from subtle motion artefacts and partial volume which may lead to less robust ADC values. Partial volume may have particularly affected regions such as the occipital WM and cortex due to their close proximity to CSF. These were the regions associated with ADC increases in the isolated VM cohort compared to controls found in this chapter. A further limitation was that the fetal isolated VM scans were clustered towards later GAs, which may have driven the ADC trajectory differences in the occipital WM and cortex between this cohort and controls. However, the aged-matched comparison controlled more effectively for differences in gestational age between cohorts, providing stronger evidence for increased ADC values in the occipital WM of VM cases. The following chapter further explores this WM ADC increase in isolated VM compared to controls by investigating DTI measures in the neonatal population using TBSS.

**Conclusions**

The primary finding of this study was that ADC values were increased in the occipital WM and the cortex of fetuses with isolated VM cases compared to controls. No significant difference in ADC values of fetuses with CHD were found compared to controls, although this may be due to small numbers and mixed cohort group, rather than a lack of white or grey matter alterations in these cases. Overall this study demonstrates the feasibility of the optimised fetal DWI sequence to
detect ADC alterations in white and grey matter in fetuses with abnormal development. Further investigation into ADC diagnostic use would be beneficial for the fetal DWI sequence to reach its full potential in the clinical setting; this could offer the opportunity for early diagnosis and future intervention strategies to prevent neurodevelopmental delay.
5  Assessment of white matter structure in neonates with isolated ventriculomegaly compared to controls

5.1 Introduction

Ventriculomegaly (VM) is the most common fetal central nervous system (CNS) abnormality diagnosed antenatally, affecting approximately 1% of fetuses and refers to the enlargement of the cerebral ventricles. It is defined as an atrial diameter measurement of the lateral ventricle equal to or greater than 10mm. Approximately half of all antenatal VM cases are isolated, where there are no associated CNS abnormalities present. The outcome of these isolated cases is variable; some studies have illustrated a more favourable outcome in mild (10.1-12mm) compared to moderate isolated VM (Falip et al. 2007), and a poorer outcome in assymetrical or cases where there was prenatal progression of ventricular dilatation (an increase of more than 3mm) (Ouahba et al. 2006). Neurodevelopmental outcomes in isolated VM cases have included difficulties in language (Falip et al. 2007, Sadan et al. 2007), cognitive (Bloom et al. 1997, Gomez-Arriaga et al. 2012, Leitner et al. 2009, Sadan et al. 2007), gross motor (Gomez-Arriaga et al. 2012, Leitner et al. 2009) and behavioural functions (Falip et al. 2007, Gomez-Arriaga et al. 2012, Leitner et al. 2009, Sadan et al. 2007). Despite the prevalence of isolated VM and its association with neurodevelopmental deficits and disorders such as autism (Palmen et al. 2005) very little is known about its aetiology.

The majority of magnetic resonance imaging (MRI) studies investigating brain development in isolated VM have focused on volumetric measures compared to normal infants. Enlarged ventricular size has been associated with significantly increased total brain tissue in isolated VM compared to controls, which appears to be restricted to increased cortical grey matter volume in fetuses (Kyriakopoulou et al. 2013) and neonates (Gilmore et al. 2008). Gilmore et al. (2008) also demonstrated a significant decrease in white matter (WM) volume when controlling for intracranial volume. Brain volume alterations of antenatally diagnosed isolated VM appear to persist into childhood, with enlargement of the lateral ventricles being associated with increased white and grey matter volumes at 1 and 2 years (Lyall et al. 2012). These results suggest that isolated VM may be a marker for altered brain development evident in both white and grey
matter. However, few studies have used diffusion tensor imaging (DTI) to assess WM structure in isolated VM cases compared to controls.

DTI has previously been used to demonstrate significant regional WM changes in neonates born with isolated VM, suggesting delayed or altered WM development (Gilmore et al. 2008, Goodlett et al. 2009). However to date there is no comprehensive study assessing global WM development in this cohort. Tract-based-spatial statistics (TBSS) is an automated observer-independent technique and allows voxel-wise, whole brain analysis of DTI measures between groups (Smith et al. 2006). TBSS has the power to reveal DTI differences in WM tracts between groups of neonates (Anjari et al. 2007, Ball et al. 2010). The aim of this study was to use an optimised TBSS protocol for neonatal data (Ball et al. 2010) to determine whether white matter structure in neonates with antenatally diagnosed isolated VM differed to healthy controls.

### 5.2 Methods

#### Subjects

Isolated VM and control neonatal infants were enrolled into the fetal MR research programme, which includes fetal and postnatal MRI and later neurodevelopmental assessments at 1 and 2 years of age (Ethics: 07/H0707/105). 15 healthy term born controls were enrolled post-natally as term-born controls in MR research studies of the preterm brain (Ethics: 07/H0707/101; 04/Q0406/125). Control neonates were scanned between October 2006 and January 2012, whilst neonates for the isolated VM cohort were scanned between August 2008 and June 2012.

#### Normal Controls

The normal control cohort consisted of 25 term-born infants, who had a normal brain appearance on MRI. Ten of the control cases had enrolled on the fetal research programme, and also had normal antenatal brain appearances on MRI. The controls were all from singleton pregnancies, had a normal uncomplicated delivery and were of normal birth weight. None of the control group had signs of infection, congenital anomalies or dysmorphic features, and all had normal findings on neonatal brain MRI and a normal neurodevelopmental outcome.
Isolated Ventriculomegaly

Twenty-two cases were included in the study and all subjects were diagnosed with VM initially on antenatal ultrasound (US), and then had the diagnosis of isolated VM confirmed on an antenatal MRI brain scan. Exclusion criteria for isolated VM were: additional brain abnormalities on MRI or US, positive infection screen or chromosomal abnormality screening, maternal drug use, multiple pregnancies, intra-uterine growth restriction, birth weight below 3rd centile. Not all fetuses underwent amniocentesis and therefore delivery summaries were also reviewed to exclude any dysmorphic facial features or additional undiagnosed congenital malformations that could indicate an underlying genetic syndrome.

Clinical information and systematically structured neurodevelopmental follow up for VM cases was collated and reviewed for analysis by colleagues within the department (VK and SD).

Neurodevelopmental assessment

Parents were invited for a detailed neurodevelopmental assessment of their child at 2 years. Assessments were performed by a clinical psychologist or paediatric neurologist. The Bayley Scales of Infant Development-III (BSID-III) assessment was chosen due to its wide use within research studies. The BSID-III assesses the developmental function of infants between 1 month and 42 months (Bayley 2006). It assesses 3 domains: cognition, language and motor.

In cases where parents were unable to attend a formal assessment, questionnaires were provided. Ages and Stages Questionnaires-III (ASQ-3) and PedQ are parent-completed developmental questionnaires that assess the child's development in the areas of communication, motor, problem solving and personal-social. The reliability and validity of both questionnaires have been demonstrated (Woodward et al. 2011, Gollenberg et al. 2010, Varni et al. 2003). ASQ-3 serves as a first-level screening system and can identify infants or young children (between 1 month and 5.5 years) who are delayed in their development. Comparatively, PedQ was designed to assess a paediatric population (5 to 7 years) for healthy outcome (Varni et al. 2003). PedQ was only used in control cases if the BSID-III or ASQ-3 had not been completed before 5.5 years.

Neonatal scanning procedure

MR imaging was performed on a 3-Tesla Philips Achieva system, using an eight-channel phased array head coil. Single-shot echo planar DTI was acquired in 32 non-collinear directions with the
following parameters: TR 8000ms, TE 49ms, slice thickness 2mm, field of view 224mm, voxel size 2x2x2mm³, b value 750 s/mm², SENSE factor 2.

All neonates were clinically assessed as stable prior to scanning by an experienced paediatrician, and scans of VM cases were performed under sedation (oral chloral-hydrate, 30-50 mg/kg). Neonatal heart rate, oxygen saturation and temperature were monitored throughout the scan. Ear protection during scanning comprised of neonatal earmuffs (Natus MiniMuffs; Natus Medical Inc., San Carlos, CA) as well as individually moulded earplugs using silicone-based dental putty (President Putty, Coltene/Whaledent, Mahwah, NJ), which were placed into the external ear. A neonatologist experienced in MRI procedures supervised all examinations. A perinatal radiologist carried out a clinical visual analysis of the images.

**DTI and Tract based spatial statistics**

DTI data were processed offline using FMRIB's Diffusion Tool Box (FDTv2.0), part of FSL (Smith et al. 2006, Smith et al. 2004). Initially, DTI data were affine registered to the non-diffusion weighted (b0) image to minimise distortions due to eddy currents. Non-brain tissue was then removed using the FSL Brain Extraction Tool, and fractional anisotropy (FA) and apparent diffusion coefficient (ADC) images were produced by fitting a tensor model to the raw diffusion data using FDT (FMRIB's Diffusion Toolbox).

TBSS (Smith et al. 2006, Smith et al. 2004) was used to register FA data for whole-brain comparison between groups. Image registration was carried out using an optimised protocol for neonatal DTI analysis (Ball et al. 2010), where two linear registration steps (6 and 12 degrees of freedom) were performed prior to nonlinear registration to improve global alignment between neonatal FA maps and therefore to increase reliability. A target FA map was chosen (the target had a median age at scan of 40.86 weeks), and each infant’s FA map was aligned in the target space. Data was up-sampled to 1x1x1 mm³ voxel size for better display and equal resolution, and a mean FA map was created. A second set of registrations was then performed to register every individual FA map to the mean FA map. The aligned images were then used to create the final mean FA map and a mean FA skeleton, which represents the centres of all tracts common to the group. An FA threshold of ≥0.15 was applied to the skeleton, to include the major WM pathways but exclude peripheral tracts where there was significant variability between subjects and partial volume effects with grey matter or cerebral spinal fluid (CSF). Each subject’s aligned FA data were then projected onto the skeleton. This process is demonstrated in Figure 5.1. To apply TBSS to
ADC data, the individual warps created during the second registration of FA maps to the final mean FA and the skeleton projections were then applied to the ADC images.

Voxel-wise cross-subjects linear regression was then performed to assess FA and ADC values between groups, correcting for post-menstrual age (PMA) at scan and gestational age (GA) at birth. The results were corrected for multiple comparisons by controlling family-wise error rate following threshold-free cluster enhancement, and $p<0.05$ was considered significant. The FSL Atlas (Mori et al. 2005, Wakana et al. 2007, Hua et al. 2008) was used to establish which WM tracts had significant differences between groups.

Figure 5.1 TBSS process.

Un-registered DW images (A) were registered to the non-diffusion weighted image to minimise distortions due to eddy currents. FA images (B) were then produced using FSL. FA maps were initially registered to a target producing a mean FA image. The mean FA map was then used as a target for a second set of registrations, creating a final mean FA map (C). A mean FA skeleton (green) was then produced from the aligned FA images, which represents the centre of WM tracts in the neonatal brain, and is seen overlayed on the final mean FA map (D). The FA skeleton was thresholded to only include the centre of the major WM tracts (E).

5.3 Results

Cohorts

The cohort characteristics for both groups can be seen in Table 5.1. There was no significant difference between GA at birth between cohorts. However, the PMA at scan in the isolated VM group was significantly greater than the control cohort ($p<0.001$). Delivery details such as birth weight and Apgar scores are also detailed in Table 5.1, and were within the normal range.
Table 5.1 Cohort characteristics.

GA at birth, PMA at scan and Apgar scores are presented as median (range). * Apgar scores were not available in 4 VM neonates.

**TBSS results**

TBSS analysis demonstrated significantly lower FA values and increased ADC values within WM tracts of isolated VM neonates compared to control neonates (Figure 5.2 and Figure 5.3). Results were corrected for multiple comparisons, and GA at birth and PMA at scan were included in the analysis. The percentage mean differences in global FA and MD between the two cohorts were 7.42% and 3.9% respectively.

FA reduction was found in the regions of the splenium, posterior thalamic radiation and sagittal stratum (Figure 5.2); and ADC increases were found in the splenium and body of the corpus callosum, and the posterior thalamic radiation (Figure 5.3).
Figure 5.2 Differences in FA values between the 2 groups of infants.

The mean FA skeleton (green) is overlaid on the mean FA map. Areas in red show regions where FA values were significantly lower in the VM group compared to controls (p<0.05), after correcting for multiple comparisons using threshold-free cluster enhancement. Arrows demonstrate regions of FA reduction in the splenium (A), posterior thalamic radiation (B) and sagittal stratum (C) in the transverse, coronal and sagittal plane.
Figure 5.3 Differences in ADC values between the 2 groups of infants.

ADC results are overlaid on the mean skeleton (green) and the mean FA map. Areas on the skeleton in red-yellow show regions where ADC values were significantly increased in the VM group compared to controls. Arrows demonstrate regions of ADC increase in the (A) splenium and (B) body of the corpus callosum, and (C) posterior thalamic radiation in the transverse, coronal and sagittal plane.
**Neurodevelopmental outcome**

Neurodevelopmental assessments are currently ongoing, and therefore this data have not yet been analysed. Neurodevelopmental follow up has so far been conducted on 19/25 control subjects, with normal outcome in all cases. Neurodevelopmental assessments at 2 years were conducted on 9 controls and ASQ-3 questionnaires were completed in 6 control participants, at a mean age of 4.5 years (range 3.9 – 5 years). PedQ questionnaires were conducted on 4 control cases at a mean age of 6.42 years (range 6.08 – 6.83 years). 2 control cases were lost to follow up, and the remaining cases are currently too young to have a neurodevelopmental assessment.

Comparatively, 8 VM subjects have had neurodevelopment assessments at 2 years, and the remaining 22 cases are currently too young to have a neurodevelopmental assessment. Normal outcome was found in 2 cases, but 6 VM cases demonstrated a neurodevelopmental delay. Two of these cases displayed delays in motor and language development, and the remaining 4 VM cases displayed delay in only the language domain.

**5.4 Discussion**

This TBSS study demonstrated a significant regional reduction in FA and increase in ADC values in the WM of neonates with isolated VM compared to controls. Observed DTI differences may be indicative of altered WM structure in neonates with isolated VM at term. This was the first study to use an objective whole-brain approach to determine DTI measures in WM tracts between infants with isolated VM and controls.

TBSS is an observer-independent method of analysing whole-brain DTI data on a voxel-wise basis (Smith et al. 2006). This approach overcomes many limitations of region of interest (ROI) techniques, which are prone to user variability and subjectivity because they rely on arbitrary definitions to manually delineate different brain regions. TBSS has previously been used to demonstrate FA alterations in the WM of neonates born prematurely compared to term born controls (Anjari et al. 2007), as well as to report reduced FA in preterm infants associated with chronic lung disease (Ball et al. 2010, Anjari et al. 2009). TBSS was therefore ideal to objectively study global DTI changes between cohorts, giving insight into underlying WM structure of isolated VM neonates compared to controls.

A previous DTI paper used a ROI approach to assess 34 neonates with isolated VM and compare data to 34 aged-matched and sex-matched term controls (Gilmore et al. 2008). This study
demonstrated that neonates with isolated VM had a significant decrease in FA and increase in ADC in the splenium and cortico-spinal tracts, as well as an increase in ADC in the genu compared to controls (Gilmore et al. 2008). This group furthered their work by developing a population-based registration method and compared FA values in predetermined regions based on their previous study. Using this approach, reduced FA values were observed in the splenium in neonates with isolated VM compared to controls, but not in the genu or corticospinal tracts (Goodlett et al. 2009). Comparatively, TBSS results from this chapter also demonstrated reductions in FA and increases in ADC values in the splenium of the corpus callosum in VM neonates compared to controls. TBSS found additional FA decreases and ADC increases in the posterior thalamic radiation in the VM group compared to controls; further FA reductions were also seen in the sagittal stratum and ADC increases in the body of the corpus callosum.

Decreased anisotropy values and elevated ADC values in WM have been previously reported in preterm infants at term equivalent age compared to term-born controls, and this has been attributed to delayed or altered WM maturation (Anjari et al. 2007, Jo et al. 2012). At term, normal myelination has only progressed from the brainstem to the PLIC (Yakovlev 1967), and so the observed FA alterations are likely illustrative of delayed processes that occur leading up to myelination, such as increased axonal thickness, alteration in axonal permeability and pre-myelination wrapping of oligodendrocytes around axons (Wimberger et al. 1995). Increased ADC values in infants with VM may be caused by an increase in water content and a decrease in restriction to water motion (as discussed in chapter 4), and may reflect delayed or altered WM maturation in these regions.

The corpus callosum, posterior thalamic radiation and sagittal stratum contain a number of WM tracts (Oishi et al. 2010, Wakana et al. 2004), and it is therefore not possible to extrapolate exactly which pathways contribute to the DTI changes observed in these regions between cohorts. Fibres passing through the splenium of the corpus callosum form the forceps major; both the posterior thalamic radiation and sagittal stratum contain the inferior and superior longitudinal fasciculus as well as the inferior fronto-occipital fasciculus. In addition, the posterior thalamic radiation contains the optic radiations. Injury or DTI alterations to these WM tracts, specifically the superior and inferior longitudinal fasciculi, inferior fronto-occipital fasciculus, posterior thalamic radiations and the corpus callosum, have previously been associated with deficits in language, motor, cognitive and attention skills (Dramsdahl et al. 2012, Catani 2007, Doricchi and Tomaiuolo 2003, Duffau et al. 2002, Gazzaniga 2000, Hynd et al. 1995, Leclercq et al. 2010, Nosarti et al. 2004, Giu et al. 2011, Tanabe et al. 1987). In particular, altered size and shape of the corpus callosum has been
associated with developmental language disorders in children (Preis et al. 2000). Altered diffusion measures in the splenium of the corpus callosum have also been associated with gross motor and cognitive scores in a TBSS study of 63 preterm infants at term equivalent age (van Kooij et al. 2012). Neurodevelopmental deficits in these domains of language, motor, cognitive and attention skills have also been associated with children with isolated VM (Gomez-Arriaga et al. 2012, Lyall et al. 2012, Sadan et al. 2007).

Neurodevelopmental outcome was not included in the TBSS analysis, and follow up data are not currently complete. Despite this, a high proportion of VM cases that so far completed a neurodevelopmental assessment at 2 years demonstrated a delay in neurodevelopment. Language and to a lesser extent motor delays were specifically identified in this cohort, which is consistent with previous follow up studies. Children with isolated VM diagnosed antenatally have been found to be at increased risk of abnormal communication, behavioural, motor and cognitive development (Beeghly et al. 2010, Bloom et al. 1997, Leitner et al. 2009, Lyall et al. 2012, Sadan et al. 2007). Sadan et al. (2007) assessed 20 children with antenatally diagnosed VM (10-15mm) at a mean age of 32.3 months using the BSID-II, and demonstrated that developmental delays were mostly of a cognitive (including language) and behavioural nature. A further study found reduced developmental scores in expressive language and fine motor scores, but no difference in gross motor, visual perception and receptive language (Lyall et al. 2012).

A previous paper hypothesised that fetal isolated VM was a structural marker of altered brain development, that may be associated with high risk for neuropsychiatric and neurodevelopmental disorders associated with enlargement of the lateral ventricles (Gilmore et al. 2008). Future TBSS studies including neurodevelopmental follow up are necessary to extrapolate whether the observed regional WM changes in isolated VM neonates compared to controls are associated with outcome and whether DTI measures may therefore be used as an early marker of delayed neurodevelopment.

**Limitations**

There has been limited neurodevelopmental follow up data to date, and assessments need to be completed for both cohorts and then analysed to be able to make any real inferences about WM tract DTI alterations and their association with neurodevelopmental delays.

There was a significant difference in PMA at scan between the two cohorts, with VM neonates having an average age at scan 3.4 weeks greater than that of controls. However, this difference
was taken into consideration, as PMA at scan as well as GA at birth was included in the voxel-wise cross subject linear regression. The reason for this difference was due to the recruitment protocol for control cases compared to clinical cases. Term-controls were mainly recruited shortly after birth in Queen Charlotte’s and Chelsea Hospital, which was adjacent to where neonatal scanning was performed. Clinical cases of isolated VM, however, were often born in other hospitals around London thereby delaying the time of scan.

The VM cohort also contained a greater number of male participants, which was not corrected for in the TBSS analysis. However, previous papers (Anjari et al. 2007, Ball et al. 2010, Aeby et al. 2012, Alexandrou et al. 2014), and results in the next chapter (chapter 6), found no significant DTI differences between male and female neonates. Another cohort limitation was the lack of genetic investigation in all cases. Genetic investigation was only completed when parents agreed antenatally or when clinical signs in neonates suggested a potential genetic abnormality. Therefore, it is possible that genetic abnormalities might be present in some of the apparently isolated VM cohort. However, delivery summaries were reviewed to exclude any obvious features that could indicate an underlying genetic syndrome.

An optimised TBSS approach for the neonatal brain was used (Ball et al. 2010). However, whilst voxel-wise approaches have an advantage over ROI approaches; TBSS is limited to analyse only the centre of WM tracts, meaning that any alterations in peripheral WM tracts would not be recognised.

Post-mortem studies in cases with isolated VM would advance understanding of the biological underpinnings of the altered diffusion measures in this cohort; however due to their predominantly good outcome and high survival rate, there is currently no post-mortem data from this group.

Conclusions

TBSS results demonstrated significantly reduced FA and increased ADC values in WM tracts of neonates with antenatally diagnosed VM compared to controls. Antenatally diagnosed isolated VM cases appeared to have an increased risk of neurodevelopmental deficits, specifically in the language and motor domain, although this data are currently not complete. The observed DTI WM alterations in isolated VM cases may be consistent with a delay in maturation or abnormal development of specific WM tracts that are involved in language, motor skills, cognition and attention. FA and ADC alterations may therefore represent neural correlates for later
neurodevelopmental deficits. However, future work is needed to establish neurodevelopmental outcome and its association with neonatal DTI measures.
6 DTI and volume measures in the developing preterm brain

6.1 Introduction

In the last trimester of pregnancy rapid and important developmental changes occur in the brain. Processes including neuronal migration, synaptic reorganisation, myelination and programmed cell death contribute to brain maturation during this perinatal period (Chan et al. 2002, Tau and Peterson 2010, de Graaf-Peters and Hadders-Algra 2006). Quantitative measures derived from diffusion tensor imaging (DTI) and T2-weighted anatomical scans allow assessment of the underlying tissue structure and brain volume, thereby providing information about brain development. Infants born prematurely and without brain lesions offer the opportunity to study neurodevelopment during this critical maturational period, with fewer of the limiting factors that affect in utero magnetic resonance imaging (MRI).

DTI has been used previously in the preterm brain to provide quantitative measures of water diffusion with increasing age at scan. Quantitative measures have included the apparent diffusion coefficient (ADC), fractional anisotropy (FA), axial diffusivity (AD) and radial diffusivity (RD). A number of studies have demonstrated a region specific decrease in ADC and increasing FA in the preterm brain with increasing age at scan (Neil et al. 1998, Huppi et al. 1998a, Partridge et al. 2004, Dudink et al. 2007, Berman et al. 2005); fewer papers have described AD and RD changes with increasing age at scan in preterm infants (Berman et al. 2005, Partridge et al. 2004). These previous studies have focused mainly on WM maturation, although some studies have investigated cortical, thalamic and cerebellar development (Ball et al. 2013b, delpolly et al. 2005, McKinstry et al. 2002, Tam et al. 2009).

Segmentation of T2-weighted anatomical images has been used previously to assess regional and total brain volume changes with increasing age at scan in the preterm brain (Huppi et al. 1998b, Tzarouchi et al. 2009). Preterm neonates at term equivalent age (TEA) exhibit significantly reduced volume in the white matter (Mewes et al. 2006), deep grey matter of the thalamus, lentiform (Boardman et al. 2006, Srinivasan et al. 2007), as well as the cortical grey matter (Inder et al. 2005) compared to term-born controls. In this chapter, the relationship between brain volume and DTI changes with increasing postmenstrual age (PMA) at scan in the preterm brain
was explored using a novel automated segmentation method specifically developed and adapted for the neonatal brain (Makropoulos et al. IN PRESS).

Altered regional brain development has previously been associated with the presence of perinatal risk factors in preterm infants (Bonifacio et al. 2010, Keunen et al. 2012, Boardman et al. 2007, Thompson et al. 2008). The degree of prematurity at birth is associated with decreased FA (Anjari et al. 2007), and decreased regional brain volumes (Boardman et al. 2006, Ball et al. 2012). Other clinical factors following preterm birth, which are independent of gestational age (GA) at birth, have also been associated with altered brain development. For example, chronic lung disease (CLD) (defined as dependence on oxygen at 36 weeks) is associated with decreased anisotropy in preterm infants without focal lesions (Ball et al. 2010, Anjari et al. 2009). CLD has also been identified as a risk factor for attenuated global brain growth (Boardman et al. 2007). It has been proposed that alterations in white matter (WM) DTI measures in preterm infants result from comorbid conditions including CLD or necrotizing enterocolitis (NEC) rather than extreme prematurity per se (Bonifacio et al. 2010).

The primary aim of this chapter was to characterise DTI and volume changes in white and grey matter over development in a large preterm population without focal lesions on MRI. The secondary aim was to determine the effect of perinatal clinical factors on brain development in this population. Characterisation of quantitative MR measures of preterm neonates without focal lesions will enable assessment of ex utero brain development and evaluation of the impact of perinatal clinical factors may give an insight into early predictors of long-term neurodevelopmental consequences.

### 6.2 Methods

**Participants**

This retrospective study included a cohort of prematurely born neonates scanned between June 2006 and March 2011 that had been recruited from the Neonatal Intensive Care Unit at Queen Charlotte's and Hammersmith Hospital. All preterm neonates scanned within these dates and had good quality T$_2$-weighted and DTI scans were included in the study. Neonates with evidence of focal lesions on conventional T$_1$ and T$_2$-weighted MRI scans, detected by an experienced perinatal radiologist, were excluded from the study. Clinical data were collected from hospital notes and discharge summaries from the neonatal unit for each infant. Ethical approval was obtained from
the Hammersmith Hospital Research Ethics Committee (Ethics no. 07/H0707/101), and all parents gave written consent prior to scanning.

**MR Imaging**

MR imaging was performed on a 3-Tesla Philips Achieva system, using an eight-channel phased array head coil, and was performed as previously described in chapter 5. Single-shot echo planar DTI was acquired in 32 non-collinear directions with the following parameters: TR 8000ms, TE 49ms, slice thickness 2mm, field of view 224mm, voxel size 2x2x2mm³, b value 750 s/mm², SENSE factor 2. T₂-weighted fast spin echo images were acquired using: TR: 8670ms; TE: 160ms; flip angle: 90 degrees; slice thickness: 2 mm with 1-mm overlap; field of view 220mm; voxel size: 0.86x0.86x1mm².

All infants were clinically assessed as stable prior to scanning by an experienced paediatrician, and scans of older neonates (>36 weeks) were usually performed under sedation (oral chloral-hydrate, 30-50 mg/kg). Neonatal heart rate, oxygen saturation and temperature were monitored throughout the scan. Ear protection during scanning comprised of neonatal earmuffs (Natus MiniMuffs; Natus Medical Inc., San Carlos, CA) as well as individually moulded earplugs using silicone-based dental putty (President Putty, Coltene/Whaledent, Mahwah, NJ), which were placed into the external ear. A neurological examination of the neonate was performed at the time of the scan by an experienced neonatologist, who also supervised all MR examinations. A perinatal radiologist carried out a visual analysis of the images.

**DTI processing and analysis**

DTI data were processed offline using FMRIB’s Diffusion Tool Box (FDTv2.0), part of FSL (Smith et al. 2006, Smith et al. 2004), as described in chapter 5. DTI data were initially affine registered to the non-diffusion weighted (b₀) image to minimise distortions due to eddy currents. Non-brain tissue was then removed using the FSL Brain Extraction Tool and FA images were produced by fitting a tensor model to the raw diffusion data using FDT (FMRIB's Diffusion Toolbox). ADC and λ₁ (AD), λ₂, λ₃ maps were also produced during this step. RD maps were produced by averaging the intermediate and minor eigenvalues [(λ₂+λ₃)/2].

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Work done by others

The segmentation approach used for analysis had been developed and adapted for multi-label segmentation of the neonatal brain (Makropoulos et al. IN PRESS). T2 segmentation, transformation to DTI space and extraction of volumetric and DTI measures were performed by the colleague who developed this segmentation technique (AM).

T2 segmentation

The neonatal brain was segmented by initially registering a number of segmented atlases to each individual’s T2-image. A total of 20 atlases were used, which were manually segmented (Gousias et al. 2012), and had an age at scan ranging from 36 to 44 weeks gestation. After registering these atlases to individual T2-images, a spatial prior was generated for each region. This spatial prior was combined with information on the intensity of the image in order to obtain the segmentation of the brain.

Using this technique, 21 regions of the neonatal brain were segmented in each individual. WM regions included the corpus callosum as well as the cingulum, lateral occipito-temporal (LOT), frontal, parietal, occipital, and temporal lobe WM. Cortical grey matter regions included the cingulate gyrus, LOT, frontal, parietal, occipital, and temporal lobe. Further regions included the subcortical grey matter of the hippocampus, amygdala, caudate nucleus, thalamus, subththalamic nuclei and lentiform nucleus, as well as the cerebellum and brainstem.

These segmentations were utilised to extract volumetric measures of each region, as well as the whole-brain volume. The segmentations were then transformed onto DTI space. This was achieved using a non-linear registration (Rueckert et al. 1999) to register T2 images to RD images. The estimated transformations from these registrations were then used to propagate the segmentation label from the T2 space to the DTI space. The DTI measures of FA, ADC, AD and RD were extracted for each segmented region.

Using this technique, quantitative volumetric and DTI measures were established in 21 regions of the neonatal brain (Figure 6.1).
Figure 6.1 Segmented regions in the preterm brain.

Neonates scanned at 26 weeks (A), 30.43 weeks (B) and 34 weeks (C); each neonate is illustrated with the $T_2$ weighted image in the top row and the corresponding $T_2$ image with segmented
regions on the row beneath. Column (i) and (ii) demonstrates the cerebellum (yellow) and brainstem (blue). Column (ii), (iii) and (iv) demonstrates the temporal (blue) and occipital lobe (purple) white and grey matter. The grey matter is presented by the darker colour compared to white matter. Columns (iii) to (v) show the frontal lobe white and grey matter (yellow); columns (iii) and (iv) show the lentiform (mustard yellow), sub-thalamic nuclei (dark purple), caudate nucleus (pink) and thalamus (red). The frontal (yellow) and parietal lobe white (green) and grey (blue) matter can be seen in (iv), (v) and (vi). The corpus callosum (green) and the cingulate white and grey matter (red) can be seen in (v) and (vi).

**Key:**

![Color Key Image]

**Clinical characteristics**

Perinatal clinical data was collected for each infant including: PMA at scan, GA at birth, birth weight (BW), head circumference (HC) at birth, weight change per week from birth until day of scan (referred to as weight increase in this chapter), gender, multiple pregnancy, prolonged premature rupture of membranes (>24 hours; PPROM), presence of culture-positive sepsis, presence of patent ductus arteriosus (PDA), antenatal and postnatal steroid treatment, necrotising enterocolitis (NEC), small for gestational age (SGA), and number of days on respiratory support at scan. Respiratory support information included the sum of days on mechanical ventilation, days on CPAP (continuous positive airway pressure) and days on supplementary oxygen at the time of scan. SGA was defined as a birth weight less than 10th centile. Associations between these perinatal clinical characteristics and DTI and volumetric measures were explored.

Statistical analysis was performed using Stata/IC 11. In every region of the brain, multiple linear regressions were performed for each quantitative DTI and volume measure. The independent variables in each regression comprised the 14 clinical variables; dummy variables were included in
cases of sepsis, PDA, gender, NEC, antenatal steroids, PPROM and multiple pregnancies. The test for the impact of each clinical condition on quantitative measures of brain development, controlling for PMA at scan, depended on the significance of the coefficient of each clinical variable, the direction of the effect was indicated by whether the coefficient was positive or negative. Analysis of relative volume measures included the total brain volume in the regression model. For results to withstand the Bonferroni test for multiple comparisons, significance was only accepted if \( p \leq 0.002 \). Quadratic and exponential terms were explored to test for non-linearities in the relationship between quantitative measures and PMA at scan. These are reported when the quadratic or exponential term was statistically significant and best fit the data in the sense of having a higher adjusted \( R^2 \).

6.3 Results

Participants

DTI data from 208 scans of neonates without focal lesions were retrospectively collected and analysed. This dataset comprised of 157 preterm neonates, 45 infants had 2 scans, and 3 infants had 3 scans. The GA at birth of these infants ranged from 23.3 to 36.1 weeks (median, 29.71 weeks) and the PMA at scan ranged from 26 to 45.1 weeks (median, 38.14 weeks).

Table 6.1 and Table 6.2 illustrate neonatal group demographic data and clinical characteristics. Mean DTI and volumetric measures for each structure are shown in Table 6.3.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>PMA at scan (weeks)</td>
<td>38.14</td>
<td>26 - 45.14</td>
</tr>
<tr>
<td>GA at birth (weeks)</td>
<td>29.71</td>
<td>23.29 - 36.14</td>
</tr>
<tr>
<td>HC at birth (cm)</td>
<td>27.00</td>
<td>20.5 - 35</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>1.18</td>
<td>0.56 - 3.71</td>
</tr>
<tr>
<td>Days Respiratory Support at scan</td>
<td>11</td>
<td>0 - 167</td>
</tr>
<tr>
<td>Weight increase (kg/week)</td>
<td>0.11</td>
<td>-0.21 - 0.31</td>
</tr>
</tbody>
</table>

Table 6.1 Neonatal group demographic data.
<table>
<thead>
<tr>
<th>Clinical detail</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>107</td>
</tr>
<tr>
<td>Female</td>
<td>101</td>
</tr>
<tr>
<td><strong>Multiple pregnancySingleton</strong></td>
<td></td>
</tr>
<tr>
<td>Singleton</td>
<td>86</td>
</tr>
<tr>
<td>Multiple pregnancy</td>
<td>116</td>
</tr>
<tr>
<td><strong>SGA</strong></td>
<td></td>
</tr>
<tr>
<td>No SGA</td>
<td>162</td>
</tr>
<tr>
<td>SGA</td>
<td>46</td>
</tr>
<tr>
<td><strong>PPROM</strong></td>
<td></td>
</tr>
<tr>
<td>No PPROM</td>
<td>163</td>
</tr>
<tr>
<td>PPROM</td>
<td>45</td>
</tr>
<tr>
<td><strong>Antenatal Steroids</strong></td>
<td></td>
</tr>
<tr>
<td>No Steroids</td>
<td>48</td>
</tr>
<tr>
<td>Steroids</td>
<td>160</td>
</tr>
<tr>
<td><strong>PDA</strong></td>
<td></td>
</tr>
<tr>
<td>No PDA</td>
<td>179</td>
</tr>
<tr>
<td>PDA</td>
<td>29</td>
</tr>
<tr>
<td><strong>NEC</strong></td>
<td></td>
</tr>
<tr>
<td>No NEC</td>
<td>204</td>
</tr>
<tr>
<td>NEC</td>
<td>4</td>
</tr>
<tr>
<td><strong>Sepsis</strong></td>
<td></td>
</tr>
<tr>
<td>No sepsis</td>
<td>195</td>
</tr>
<tr>
<td>sepsis</td>
<td>13</td>
</tr>
</tbody>
</table>

Table 6.2 Clinical characteristics.

**DTI and volume measures with increasing PMA at scan**

Figure 6.2 to Figure 6.19 shows scatter plots demonstrating the relationship between PMA at scan and DTI or volume measures in white matter, cortical grey matter and subcortical grey matter regions, and can be found at the end of the chapter.

Multiple regression results between PMA at scan and quantitative DTI and volumetric measures are detailed in Table 6.4; perinatal clinical data were included as co-variables within the regression model. Significant relationships in the majority of regions between PMA at scan and FA, ADC, RD, AD or volume were identified; further details of these relationships in white matter, cortical grey matter and sub-cortical grey matter are detailed in the following sections. The sections below report these relationships based on the statistically best fitting regressions, namely linear or quadratic, which are also presented in Table 6.4. A significant exponential increase in volume with PMA at scan was found in all regions (p<<0.001).
<table>
<thead>
<tr>
<th>Variable</th>
<th>FA</th>
<th>ADC</th>
<th>Axial diffusivity</th>
<th>Radial diffusivity</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippocampus</td>
<td>0.12 ± 0.02</td>
<td>1.24 ± 0.08</td>
<td>1.60 ± 0.09</td>
<td>1.16 ± 0.08</td>
<td>1.25 ± 0.48</td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.16 ± 0.02</td>
<td>1.12 ± 0.09</td>
<td>1.31 ± 0.09</td>
<td>1.03 ± 0.09</td>
<td>0.798 ± 0.31</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.17 ± 0.03</td>
<td>1.26 ± 0.17</td>
<td>1.46 ± 0.18</td>
<td>1.15 ± 0.16</td>
<td>20.04 ± 10.61</td>
</tr>
<tr>
<td>Brainstem</td>
<td>0.20 ± 0.03</td>
<td>1.05 ± 0.08</td>
<td>1.26 ± 0.07</td>
<td>0.94 ± 0.09</td>
<td>5.25 ± 1.79</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>0.15 ± 0.02</td>
<td>1.26 ± 0.11</td>
<td>1.46 ± 0.12</td>
<td>1.17 ± 0.11</td>
<td>3.15 ± 1.31</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.19 ± 0.02</td>
<td>1.13 ± 0.09</td>
<td>1.35 ± 0.10</td>
<td>1.02 ± 0.09</td>
<td>7.82 ± 3.01</td>
</tr>
<tr>
<td>Sub-thalamic nucleus</td>
<td>0.24 ± 0.03</td>
<td>1.02 ± 0.08</td>
<td>1.27 ± 0.08</td>
<td>0.89 ± 0.09</td>
<td>0.46 ± 0.16</td>
</tr>
<tr>
<td>Lentiform Nucleus</td>
<td>0.17 ± 0.02</td>
<td>1.16 ± 0.10</td>
<td>1.37 ± 0.10</td>
<td>1.05 ± 0.10</td>
<td>5.58 ± 2.17</td>
</tr>
<tr>
<td>Corpus Callosum</td>
<td>0.32 ± 0.04</td>
<td>1.50 ± 0.11</td>
<td>2.03 ± 0.11</td>
<td>1.23 ± 0.12</td>
<td>2.22 ± 0.99</td>
</tr>
<tr>
<td>Frontal lobe WM</td>
<td>0.13 ± 0.02</td>
<td>1.43 ± 0.10</td>
<td>1.62 ± 0.10</td>
<td>1.34 ± 0.11</td>
<td>56.84 ± 20.64</td>
</tr>
<tr>
<td>Parietal lobe WM</td>
<td>0.14 ± 0.02</td>
<td>1.45 ± 0.14</td>
<td>1.65 ± 0.13</td>
<td>1.36 ± 0.14</td>
<td>34.47 ± 13.31</td>
</tr>
<tr>
<td>Occipital lobe WM</td>
<td>0.14 ± 0.02</td>
<td>1.40 ± 0.14</td>
<td>1.59 ± 0.15</td>
<td>1.30 ± 0.14</td>
<td>16.62 ± 6.51</td>
</tr>
<tr>
<td>Temporal lobe WM</td>
<td>0.14 ± 0.02</td>
<td>1.41 ± 0.10</td>
<td>1.62 ± 0.09</td>
<td>1.31 ± 0.10</td>
<td>24.01 ± 9.02</td>
</tr>
<tr>
<td>Lateral occipito-temporal WM</td>
<td>0.14 ± 0.01</td>
<td>1.38 ± 0.09</td>
<td>1.57 ± 0.09</td>
<td>1.28 ± 0.09</td>
<td>5.91 ± 2.66</td>
</tr>
<tr>
<td>Cingulate gyrus WM</td>
<td>0.18 ± 0.02</td>
<td>1.35 ± 0.08</td>
<td>1.60 ± 0.08</td>
<td>1.23 ± 0.08</td>
<td>4.98 ± 2.25</td>
</tr>
<tr>
<td>Frontal lobe GM</td>
<td>0.14 ± 0.03</td>
<td>1.24 ± 0.07</td>
<td>1.42 ± 0.01</td>
<td>1.15 ± 0.06</td>
<td>32.31 ± 16.99</td>
</tr>
<tr>
<td>Parietal lobe GM</td>
<td>0.15 ± 0.03</td>
<td>1.21 ± 0.08</td>
<td>1.40 ± 0.12</td>
<td>1.12 ± 0.07</td>
<td>22.36 ± 11.54</td>
</tr>
<tr>
<td>Occipital lobe GM</td>
<td>0.15 ± 0.04</td>
<td>1.20 ± 0.09</td>
<td>1.38 ± 0.13</td>
<td>1.10 ± 0.08</td>
<td>13.93 ± 7.18</td>
</tr>
<tr>
<td>Temporal lobe GM</td>
<td>0.15 ± 0.03</td>
<td>1.23 ± 0.07</td>
<td>1.43 ± 0.11</td>
<td>1.14 ± 0.06</td>
<td>14.66 ± 7.65</td>
</tr>
<tr>
<td>Lateral occipito-temporal GM</td>
<td>0.14 ± 0.03</td>
<td>1.21 ± 0.08</td>
<td>1.39 ± 0.11</td>
<td>1.12 ± 0.07</td>
<td>4.49 ± 2.19</td>
</tr>
<tr>
<td>Cingulate GM</td>
<td>0.17 ± 0.03</td>
<td>1.21 ± 0.06</td>
<td>1.43 ± 0.07</td>
<td>1.10 ± 0.06</td>
<td>4.12 ± 1.90</td>
</tr>
</tbody>
</table>

Table 6.3 Mean and standard deviation of DTI and volume measures in each region.

Units: volume cm³, ADC, AD and RD x10⁻³ mm²/s. Mean (standard deviation) for each quantitative measure are presented in each region. Abbreviations: GM = grey matter; WM = white matter
<table>
<thead>
<tr>
<th>Region</th>
<th>FA R²</th>
<th>coef</th>
<th>p</th>
<th>ADC R²</th>
<th>coef</th>
<th>p</th>
<th>Axial diffusivity R²</th>
<th>coef</th>
<th>p</th>
<th>Radial diffusivity R²</th>
<th>coef</th>
<th>p</th>
<th>Relative volume R²</th>
<th>coef</th>
<th>p</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.031</td>
<td>&lt;0.001</td>
<td>0.639</td>
<td>0.444</td>
<td>&lt;0.001</td>
<td>0.688</td>
<td>&lt;0.001</td>
<td>0.411</td>
<td>&lt;0.001</td>
<td>0.941</td>
<td>&lt;0.001</td>
<td>7.482</td>
<td>0.088</td>
<td>0.754</td>
<td>72.731</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.157</td>
<td>0.001</td>
<td>0.035</td>
<td>0.583</td>
<td>&lt;0.001</td>
<td>0.519</td>
<td>&lt;0.001</td>
<td>0.578</td>
<td>&lt;0.001</td>
<td>0.964</td>
<td>&lt;0.001</td>
<td>-3.534</td>
<td>*&lt;0.109</td>
<td>0.761</td>
<td>50.156</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.122</td>
<td>0.002</td>
<td>*&lt;0.001</td>
<td>0.793</td>
<td>&lt;0.001</td>
<td>0.767</td>
<td>&lt;0.001</td>
<td>0.781</td>
<td>&lt;0.001</td>
<td>0.949</td>
<td>&lt;0.001</td>
<td>627.313</td>
<td>*&lt;0.001</td>
<td>0.857</td>
<td>1872.855</td>
<td>*&lt;0.001</td>
</tr>
<tr>
<td>Brainstem</td>
<td>0.639</td>
<td>0.009</td>
<td>*&lt;0.001</td>
<td>0.426</td>
<td>&lt;0.001</td>
<td>0.241</td>
<td>&lt;0.001</td>
<td>0.497</td>
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<tr>
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<td>0.771</td>
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Table 6.4 Relationship between PMA at scan and DTI and volume measures
Results are reported for PMA at scan from multiple regression analysis; all clinical variables were included in the regression model. Significant relationships with PMA at scan are highlighted in bold. * Linear relationships were significant after Bonferroni correction (p<0.002). "Data were best explained using a quadratic model; ^ Data were best explained using an exponential model. P values and coefficients for quadratic and exponential terms are summarised in Appendix D. Abbreviations: $R^2$ = Adjusted $R^2$; coef = PMA at scan b coefficients; p = p value.
White Matter

In the WM, FA measures increased significantly with PMA at scan in the corpus callosum, frontal, parietal, occipital, temporal and cingulate WM (Table 6.4). FA increased at an accelerating rate towards TEA in all these regions with the exception of the corpus callosum, where FA increased at a uniform rate (Figure 6.2). No significant changes with FA and PMA at scan were found in the LOT WM after Bonferroni correction.

ADC measures significantly decreased with increasing PMA at scan in all WM regions. In the frontal, occipital, temporal, LOT and cingulate WM, this ADC decline occurred at an accelerating rate towards TEA (Figure 6.5).

AD measures also significantly decreased with increasing PMA at scan in all WM regions, with the exception of the corpus callosum which did not demonstrate any significant change with PMA at scan. In the occipital, temporal and LOT WM, the AD decline occurred at an accelerating rate towards TEA (Figure 6.8).

RD measures significantly decreased with increasing PMA at scan in all WM regions. In the frontal, occipital, temporal, LOT and cingulate WM, the RD decline occurred at an accelerating rate towards TEA (Figure 6.11).

A significant increase in volume with PMA at scan was found in all WM regions (Figure 6.17). Relative volume significantly decreased with increasing PMA at scan in the frontal, occipital and temporal lobe WM; this decrease decelerated towards TEA (Figure 6.14). In comparison, the cingulate WM significantly increased with increasing PMA at scan, although this increase also declined towards TEA. The parietal lobe WM significantly decreased at a uniform rate, whilst the corpus callosum and LOT WM demonstrated no significant change with increasing PMA at scan.

Cortical Grey Matter

In the cortex, FA measures were found to decrease significantly with PMA at scan in all regions (Table 6.4). This decline decelerated towards TEA in all regions (Figure 6.3).

ADC measures significantly decreased with increasing PMA at scan in all cortical regions. In the occipital and cingulate cortical grey matter, this ADC decline occurred at a slightly accelerating rate towards TEA (Figure 6.6).
AD measures decreased significantly with increasing PMA at scan at a uniform rate in all cortical regions (Figure 6.9).

RD measures significantly decreased with increasing PMA at scan in all cortical regions. This RD decline occurred at a uniform rate in the temporal lobe, but at an accelerating rate towards TEA in all other cortical regions (Figure 6.12).

Relative volume measures significantly increased with increasing PMA at scan in the frontal, parietal, occipital, temporal and LOT cortical grey matter. This relative volume increase was uniform in the LOT, but increased towards TEA at a slightly accelerating rate in the frontal, parietal, occipital, temporal lobe cortical grey matter (Figure 6.15). There was no significant relationship between relative volume and PMA at scan in the cingulate grey matter.

**Sub-cortical grey matter**

As reported in Table 6.4, in the sub-cortical grey matter FA measures were found to increase significantly with PMA at scan in the cerebellum, brain stem, caudate, thalamus and sub-thalamic nuclei. In the brain stem, thalamus and sub-thalamic nuclei, FA increased at a uniform rate with PMA at scan; however in the cerebellum, lentiform and caudate nucleus, the FA increase accelerated towards TEA (Figure 6.4). FA measures were not significantly affected by PMA at scan in hippocampus and amygdala after Bonferroni correction.

ADC measures were found to decrease significantly with increasing PMA at scan in all sub-cortical grey matter regions. In the caudate nucleus, this ADC decline was at a slightly accelerating rate towards TEA (Figure 6.7)

AD measures significantly decreased with increasing PMA at scan at a uniform rate in all sub-cortical grey matter regions (Figure 6.10).

RD measures decreased significantly with increasing PMA at scan in all sub-cortical grey matter regions. The rate of decrease was uniform in the hippocampus, amygdala, cerebellum, brainstem, thalamus and sub-thalamic nuclei. As can be seen in Figure 6.13, in the caudate and lentiform nucleus the rate of decrease accelerated towards TEA.

Relative volume measures significantly decreased with increasing PMA at scan in the amygdala, sub-thalamic and lentiform nuclei. This relative volume decrease was at a uniform rate in the sub-thalamic nuclei, and at an accelerated rate towards TEA in the amygdala and lentiform nuclei.
Relative volume measures significantly increased with increasing PMA at scan in the cerebellum and caudate nuclei; the former at an accelerated rate, and the latter at a decelerating rate towards TEA. No significant changes of relative volume with increasing PMA at scan were found in the hippocampus, brainstem or thalamus.

In summary of the results above, FA values increased with increasing PMA at scan in the WM (except LOT WM) and sub-cortical grey matter (except in the hippocampus and amygdala). All regions demonstrated significant ADC, AD and RD decreases with PMA at scan; with the exception of AD in the corpus callosum. Relative volumes were significantly increased in the cerebellum, caudate and cortical grey matter (except the cingulate grey matter); whereas relative volume decreased in the WM (except LOT) and amygdala, lentiform and sub-thalamic nuclei with increasing PMA at scan.

**Relationship with Clinical variables**

Perinatal data are outlined in Table 6.1 and Table 6.2. No neonates in this cohort had postnatal steroid treatment, and so this factor was not included in the analysis. Only a small number of neonates had NEC (n=3) and sepsis (n=13). Culture positive sepsis included six cases of Coagulase-negative staphylococcus (CONS), and cases each of: Staphylococcal aureus, Group B streptococcal septicemia (GBS), Escherichia coli and Klebsiella pneumoniae, Candida and Enterobacter, and Acinetobacter infections.

Results outlined below are extracted from the multiple regressions seen in Table 6.5 to Table 6.12, and demonstrate which clinical variables were significantly associated with quantitative measures of brain development.

Multiple regression analysis demonstrated that GA at birth was significantly associated with relative volume measures: a significant negative impact of GA at birth on relative volume was found in the frontal and parietal lobe cortical grey matter (Table 6.9). GA at birth also was significantly positively associated with relative volume in the LOT WM, hippocampus and amygdala.

Days of respiratory support at scan was significantly associated with relative volume as well as FA and AD measures. A significant negative relationship was found between days of respiratory support and relative volume in the cerebellum and frontal lobe grey matter; and a significant positive relationship in the frontal lobe WM (Table 6.9). A significant positive relationship was
found between days of respiratory support and FA values in the cingulate WM (Table 6.5). Finally, respiratory support was positively associated with AD measures in the corpus callosum (Table 6.7).

HC at birth had a significant effect on volume and AD measures. A significant positive relationship was found between HC at birth and AD in the cingulate WM (Table 6.7). Absolute volume measures were positively affected by HC at birth in the corpus callosum and frontal lobe WM (Table 6.11).

Weight increase was significantly associated with DTI and volume measures: a significant negative relationship was found between weight increase and relative volume in the caudate, frontal and temporal lobe WM (Table 6.9). Comparatively, a significant positive relationship was found between weight increase and relative volume in the frontal and occipital cortical grey matter. A significant positive relationship was also found between weight increase and AD in the LOT and parietal lobe grey matter (Table 6.7).

In summary, the clinical variables associated with altered structural brain development in preterm neonates were GA at birth, number of days on respiratory support, HC at birth and weight increase. These clinical variables were significantly associated with measures of absolute and relative volume, as well as AD and FA; but they were not associated with ADC or RD.
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<th>Region</th>
<th>R²</th>
<th>GA at birth</th>
<th>BW</th>
<th>Respiratory Support</th>
<th>HC at birth</th>
<th>Weight increase</th>
<th>Multiple pregnancy</th>
<th>Gender</th>
<th>SGA</th>
<th>PPROM</th>
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Table 6.5 FA multiple regression results
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Table 6.6 ADC multiple regression results

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Table 6.10 Relative volume multiple regression results continued
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Table 6.11 Volume multiple regression results
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<td>0.90</td>
<td></td>
<td>-1096.94</td>
<td>0.18</td>
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<td>Temporal lobe GM</td>
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<td>678.15</td>
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<td>LOT GM</td>
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<td>-281.76</td>
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Table 6.12 Volume multiple regression results continued.

* Relationships are significant after Bonferroni correction (p<0.002). Abbreviations: $R^2$ = Adjusted $R^2$; coef = b coefficients; p = p value.
6.4 Discussion

These results are the first to demonstrate quantitative volumetric and DTI measures in a large cohort of preterm neonates, using an automated objective segmentation approach to establish white and grey matter brain maturation between 26 and 45 weeks. This period of neurodevelopment is critical as the majority of brain maturational processes including neuronal migration, synaptic re-organisation, myelination and programmed cell death, all take place during this perinatal time (Chan et al. 2002, Tau and Peterson 2010, de Graaf-Peters and Hadders-Algra 2006).

Cerebral WM, colloidal WM, cortical grey matter and sub-cortical grey matter regions displayed differences in their patterns of maturation expressed by volumetric and DTI measures. These regional differences likely reflect variances between timing of maturational processes as well as extra-cellular content and micro-architecture in each region. In all regions there was an overall increase in volume with increasing PMA at scan, which was consistent with the finding that brain volume increases ten-fold between 20 weeks gestation to term (Guilhard-Costa and Larroche 1990), as well as the increase in brain size and cortical folding from 23 to 40 weeks observed on fetal MRI (Battin and Rutherford 2002).

The following sections discuss relative volume and DTI changes with increasing PMA at scan in the white matter, cortex and sub-cortical grey matter. Together changes in DTI and volumetric measures over development highlight the dynamic maturation of complex neuronal and dendritic architecture, including synaptic processes, apoptosis and myelination (Ramakers 2005).

White matter

Changes in DTI measures with increasing PMA at scan in WM corresponded with previous studies that have established regional ADC reduction and FA increase over development, both in preterm neonates (Partridge et al. 2004, Neil et al. 1998, Huppi et al. 1998a, Berman et al. 2005) and in older infants and children (Gao et al. 2009). The majority of these studies have focused on DTI changes in small regions of interest (ROIs), such as the posterior limb of the internal capsule (PLIC) or centrum semiovale (CSO); results from this chapter have the advantage of segmenting large regions of WM for analysis.
All WM regions demonstrated significant FA increases with increasing PMA at scan, except in the LOT WM; a significant decrease in ADC, AD and RD in all regions except AD in the corpus callosum was also demonstrated. Measures of anisotropy are influenced by a number of microstructural barriers, including axonal membranes, degree of axonal packing and myelin (Beaulieu 2009). It has been demonstrated that the influence of myelin on FA values is approximately 20% (Gulani et al. 2001, Beaulieu 2002). However, the large WM regions studied in this chapter are not myelinating between 26 and 45 weeks. As mentioned in chapter 5, at TEA normal myelination has only progressed from the brainstem to the PLIC (Yakovlev 1967), and in normal development, the occipital lobe WM, for example, does not show evidence of myelination until 3 months and the frontal WM until around 6 months after term age (Barkovich et al. 1988). Over the third trimester, these WM regions are likely undergoing axonal organisation and pre-myelination processes, which includes changes in axonal integrity such as increases in axonal thickness and alteration of axonal permeability, as well as pre-myelination wrapping of oligodendrocytes around axons (Wimberger et al. 1995). These axonal changes, and number of cell membranes, appear to be the driving force behind changes in the two minor λs, which corresponds to RD measures (Song et al. 2002, Song et al. 2003, Takahashi et al. 2002). Previous studies have suggested that reduction in RD values with increasing age lead to the observed reductions in ADC and increases in FA values in WM (Song et al. 2002). Previous preterm studies have not observed significant decreases in AD with increasing age (Partridge et al. 2004); however, perhaps this was due to a limited number of subjects in previous studies (n=14). Reductions in AD in WM have been observed over the first 2 years of life, and has been hypothesized to be due to the intermingling of axonal branches, the elimination of overabundant axons and the reduction of the length of axons during the refinement processes (Gao et al. 2009). Whilst this process is predominant after birth, apoptosis of axons is evident from approximately 19 weeks gestation (Rakic and Zecovic 2000, de Graaf-Peters and Hadders-Algra 2006), and therefore might contribute to the observed decrease in AD seen in the study in this chapter.

Previous studies have hypothesised that the phases of WM development, namely three phases beginning with axonal organisation, pre-myelination and myelination, leads to a developmental model in which the rate of maturation assessed by DTI measures is not uniform (Zanin et al. 2011, Dubois et al. 2008, Aeby et al. 2009, Aeby et al. 2012). Non-uniform FA increases with PMA at scan were found in the corticospinal tract (CST) and CSO (Zanin et al. 2011); it was suggested that the accelerated FA increase was due to myelination in these regions. Results from this chapter provide the first evidence that FA values increase at an accelerating rate (with the exception of the corpus callosum and LOT WM), and that ADC and RD values decrease at an accelerating rate towards
TEA in all WM regions. AD values also decreased at an accelerated rate towards TEA in the occipital, temporal and LOT WM. As myelination has not yet begun in the WM regions studied in this chapter (Yakovlev 1967), these accelerated DTI changes may indicate the beginning of pre-myelination processes (Aeby et al. 2012). In addition to axonal integrity changes outlined above, the premyelination stage is characterised by a significant increase in the abundance of immature oligodendrocytes (Back et al. 2002b), and pre-myelination has been associated with changes in diffusion anisotropy measures (Prayer et al. 2001, Wimberger et al. 1995). However, further evaluation of these DTI values are needed, along with corresponding histological analysis to fully understand the processes behind the DTI changes with increasing maturation.

The corpus callosum was an exception, and did not demonstrate accelerated changes towards TEA in FA or RD, or any significant change in AD values with increasing PMA at scan. These results may be due to maturational differences within the region of the corpus callosum: myelination begins within its body at the second postnatal month, within the splenium at the third month, and within the rostrum at the fourth month (Damska and Wisniewsk 1999).

Cortical grey matter

Cortical growth is achieved predominantly by an increase in surface area during the third trimester. The rapid increase in brain size occurs with exuberant development of cortical surface area relative to cerebral volume, manifested in the development and progression of cortical folding, which increases rapidly between 25 and 30 weeks in the fetal brain, but slows when approaching term (Wright et al. 2014, Battin and Rutherford 2002). Relative volume results reflect this, with an increase in cortical grey matter compared to the decrease in relative volume of WM. This is a critical period of growth in the cortex, and the age-related relative volume increase likely reflects the dynamic changes of a complex architecture in this time period (Ramakers 2005). Neuronal proliferation and radial migration to the cerebral cortex are mainly completed by the 24th week of gestation (Rakic 1988), and so cortical volume increase after this time may be mainly related to the axonal and dendritic growth which accelerates during the third trimester (de Graaf-Peters and Hadders-Algra 2006), and development of new synapses which accelerates from approximately 28 weeks (Huttenlocher and Dabholkar 1997, de Graaf-Peters and Hadders-Algra 2006).

During this period, a decline in FA, ADC, AD and RD in the cortex was observed. The decline in cortical FA between 26 and 45 weeks was consistent with previous reports (delpolyi et al. 2005,
McKinstry et al. 2002, Ball et al. 2013b). Aeby et al, (2012) observed an initial cortical decrease in FA between 34 and 39 weeks, but then noted an FA increase between 40 and 43 weeks gestation. Comparatively, FA values in this chapter appear to plateau from approximately this age, as can be seen in Figure 6.3. These results are consistent with a recent study that extended the voxel-wise observer-independent whole brain approach to study DTI measures of the cortex in a cohort of 65 neonates between 27 and 46 weeks gestation (Ball, 2013 #103). Ball et al, (2013) demonstrated a fast rate of FA decline until 38 weeks, after which no significant FA change was apparent. This initial FA reduction before approximately 38 weeks is likely to reflect neurite outgrowth and maturing dendritic cytoarchitecture, including elaboration of dendrites, ingrowths of thalamocortical afferents, formation of synapse (Sidman and Rakic 1973), which transform the cortex from a predominantly radial formation into a denser, more complex structure (Bystron et al. 2008). These processes restrict water motion both orthogonally and radially to the cortical surface which may explain the reduction in anisotropy.

**Sub-cortical grey matter**

In sub-cortical grey matter, FA and volume measures increased with increasing PMA at scan, whilst ADC, AD and RD measures decreased; with the exception of FA measures in the hippocampus and amygdala, which were not significantly altered. The hippocampus and amygdala are difficult regions to image and segment in the young preterm brain, due to their small size and location; therefore few papers have studied their maturational growth in vivo (Thompson et al. 2014, Thompson et al. 2013) and these are the first results to show their DTI maturational pattern.

Sub-cortical grey matter ADC reduction with increasing age at scan has previously been found in the fetal brain (Righini et al. 2003, Manganaro et al. 2007, Schneider et al. 2007, Schneider et al. 2009, Cannie et al. 2007). Results from chapter 3 demonstrated a significant reduction in ADC values between 20 and 38 weeks in the pons and thalamus of the fetal brain, and ADC reduction with age in deep grey matter regions have also been observed in neonates through to late childhood (Mukherjee et al. 2001, Huppi et al. 1998).

FA increases and AD and RD decreases in sub-cortical grey matter with increasing age at scan have also previously been demonstrated, specifically in the thalamus and basal ganglia, in preterm neonates (delpolyi et al. 2006) and in infants and children from day 1 to 11 years (Mukherjee et al. 2001, Mukherjee et al. 2002). It has been suggested that this FA increase results from maturation of the internal WM tracts contained within these grey matter structures, rather than an FA
increase in grey matter itself (Mukherjee and McKinstry 2006). For example, myelination in the brainstem, cerebellum and the sub-thalamic nuclei can first be observed on T1- and T2-weighted images before 28 weeks in the preterm brain (Counsell et al. 2002), and WM tract maturation is likely to be detected earlier by anisotropy measures. This is reflected in the increased FA values with increasing PMA at scan in the brainstem, cerebellum, thalamus, lentiform, caudate and subthalamic nuclei seen in Figure 6.4.

Regions within the basal ganglia (caudate and lentiform nucleus) demonstrated increased FA and decreased ADC and RD with increasing PMA at scan, occurring at an accelerating rate towards TEA. Histological examination of the globus pallidus of the basal ganglia (included in region of the lentiform nucleus in this study) show myelin at 37 weeks (Dąmbska and Wisniewsk 1999), this may be associated with the accelerated FA increase observed in this region at this approximate age (Figure 6.4 in the lentiform nucleus); although myelin basic protein (MBP) examinations indicate that pre-myelination processes begin as early as 25 weeks (Dąmbska and Wisniewsk 1999).

FA values did not increase with increasing PMA at scan in the hippocampus or amygdala. This may be because myelin formation, and pre-myelination processes, start later in these regions, for example myelination occurs in the hippocampus from approximately TEA and may continue into adulthood (Dąmbska and Wisniewsk 1999, Abraham et al. 2010).

Significant absolute volume increases were found in all regions, including the sub-cortical grey matter, with increasing PMA at scan. This is consistent with previous studies demonstrating an age-related increase in sub-cortical grey matter volume through early childhood (Matsuzawa et al. 2001, Pfefferbaum et al. 1994). Tzarouchi et al. (2009) investigated grey matter volume changes across gestation in preterm infants and showed an age-related increase in volume of all grey matter regions. This study also demonstrated that the regions of the thalamus and lentiform nucleus reached their final volume earlier than cortical grey matter areas. Results from this chapter also show that the relative volume of these regions decreased with PMA at scan.

Comparatively, relative volume was found to increase with PMA at scan in the cerebellum. The cerebellum undergoes a rapid period of growth between 28 weeks to term, and this rate of cerebellar growth far exceeds that of the cerebral hemispheres during this time (Limmeropoulos et al. 2005). The mature cerebellum consists of several molecular layers (Purkinje cell layer, molecular layer, external and internal granular layer), which develop at different time periods: the formation and migration of Purkinje cells layer takes place during early gestation, but granule cells migrate into the internal granular layer during postnatal life (Millen and Gleeson 2008, Triulzi et
al. 2005). The rapid increase in relative cerebellar volume over this time period may therefore reflect either an increase in cell numbers (external and internal granular layers), or increases in neuropil (Purkinje cell layer) (Millen and Gleeson 2008, Triulzi et al. 2005); during this period the processes of proliferation and migration of the cerebellar granule cells are particularly prominent (Berry et al. 1995).

**Association between DTI and volume measures and clinical variables**

A number of perinatal clinical variables were associated with brain development between 26 and 45 weeks. Requirement for respiratory support was significantly associated with relative volume, FA and AD measures in cortical grey and white matter. GA at birth was significantly associated with relative volume measures in cortical and sub-cortical grey matter, but DTI measures were not associated with the degree of prematurity. HC at birth was significantly associated with AD and volume measures in WM; whereas weight increase was associated with AD and relative volume measures in white and grey matter.

No other perinatal clinical factors were significantly associated with DTI or volume measures in preterm infants, although there were only a small number of neonates with NEC and sepsis. Previous studies have also demonstrated no significant effect of sepsis on DTI (Hemels et al. 2012, Alexandrou et al. 2014) or volumetric measures (Boardman et al. 2007) in preterm neonates, despite it being a risk factor for widespread abnormalities in brain development (Chau et al. 2012, Hart et al. 2010). There was also no significant effect of gender or between singletons and neonates from multiple pregnancies. The literature on brain development between genders has not been consistent. Male preterm neonates are known to have poorer neurological outcomes than females (Marlow et al. 2005), and lower FA values when scanned at adolescence (Constable et al. 2008). However many studies of preterm infants have found no significant difference between genders (Anjari et al. 2009, Aeby et al. 2012, Thompson et al. 2007), which is consistent with data from this chapter. Studies investigating brain development in multiple pregnancies are limited; abnormalities compared to singletons have been suggested because of an increased likelihood that twins would experience adverse prenatal and perinatal events (Rao et al. 2004). However the few studies that have investigated the difference between twin or triplet and singleton brain development have found no significant differences in ADC values (Hart et al. 2010) or WM volumes in childhood (Ordaz et al. 2010) or neonates (Knickmeyer et al. 2011); this is consistent with the data from this chapter.
Prematurity

The degree of prematurity at birth was significantly associated with relative volume, principally in the grey matter. GA at birth was positively related to relative volume in the hippocampus, amygdala and LOT WM, but negatively related to relative volume in the frontal and parietal cortex. As relative volume significantly increased with increasing PMA at scan in the cortex, and remained constant or increased in the WM and sub-cortical grey matter, it appears that increasing prematurity was associated with an altered cortical, hippocampal and amygdala volume. Consistent with these results are previous papers where preterm birth has been shown to perturb the trajectory of development in both the grey matter and WM in childhood and early adolescence (Ment et al. 2009). In particular, increasing prematurity has been related to cortical and sub-cortical grey matter volume reduction in infants at TEA (Ball et al. 2012, Keunen et al. 2012), and in children born prematurely compared to controls (de Kieviet et al. 2012). Moreover, sulcation in preterm neonates at TEA appears to be reduced when compared to term-born controls (Ajayi-Obe et al. 2000). One study associated increased prematurity with a reduced cortical surface area compared to cerebral volume in 274 preterm neonates scanned between 23 and 48 weeks (Kapellou et al. 2006). These results are consistent with the negative association of prematurity with cortical relative volume between 26 and 45 weeks gestation found in this chapter. Little is known about why the cortex is particularly vulnerable in preterm infants, but it has been suggested that the ex utero environment disrupts cortical development (Kapellou et al. 2006), thus affecting synapse formation, which occurs at a fast rate before TEA, neural number (Leuba and Garey 1987) and the integrity of the subplate zone (Kanold et al. 2003, McQuillen et al. 2003) leading to reduced cortical surface area growth.

The hippocampus and amygdala have previously been associated with reduced volume in preterm children compared to term born controls (Peterson et al. 2000). The hippocampus has been found to be reduced by 12% in preterm children compared to control data (Brunnemann et al. 2013, Peterson et al. 2000) and this reduction is evident through childhood and adolescence (de Kieviet et al. 2012). Reduced hippocampal volume has also been found in adults who were born prematurely, with a greater effect depending on the degree of prematurity at birth (Lawrence et al. 2010). These results are consistent with data from this chapter, where increased prematurity was associated with decreased hippocampal and amygdala volume.

It has been suggested that clinical risk factors have a greater effect on DTI measures of brain development than extreme prematurity itself (Bonifacio et al. 2010). Previous studies have
demonstrated that GA at birth was not associated with DTI measures in preterm neonates (Aeby et al. 2012), or found only a small effect of GA at birth for FA prediction, and no effect for prediction on ADC in WM of preterm infants (Alexandrou et al. 2014). These studies are consistent with results of this chapter that show that DTI measures were not associated with GA at birth when other clinical risk factors were included in the regression model.

**Respiratory support**

This chapter found significant brain alterations in preterm infants with increased requirement for respiratory support; this is consistent with previous literature (Boardman et al. 2007, Thompson et al. 2007, Anjari et al. 2009, Ball et al. 2010). In addition, preterm children who required prolonged respiratory support have an increased risk of neurological impairment compared to those without (Meisels et al. 1986, Short et al. 2003). Specifically, this chapter showed that requirement for respiratory support in preterm neonates was associated with alterations in relative volume. CLD has previously been associated with reductions in whole brain volume in preterm infants at TEA (Boardman et al. 2007, Thompson et al. 2007). However, this chapter demonstrated regional volume alterations; respiratory support was positively associated with relative volume in the frontal WM, and negatively associated in the frontal grey matter and cerebellum.

In accordance with results in the frontal cortex, reduced cortical surface area has previously been predicted by development of CLD (Kaukola et al. 2009); this study suggested that adverse perinatal events such as CLD disrupt cortical development, specifically affecting cortical synapse formation and the integrity of the subplate zone during the preterm period. The paper studied the cortex as a whole, whilst results from this chapter suggest that the frontal cortex is the most vulnerable cortical region affected by respiratory support.

The finding that frontal WM relative volume increased with increased requirement for respiratory support was an interesting observation. As frontal WM relative volume was found to decrease with increasing PMA at scan, which may reflect axonal pruning occurring during the preterm period (de Graaf-Peters and Hadders-Algra 2006), requirement for respiratory support may delay these developmental processes over this time period, thereby leading to the observed relative volume increase. The frontal lobe may be especially affected by environmental influences such as respiratory support, due to its late maturation, making it particularly vulnerable compared to other brain regions during this period (Stuss and Knight 2002). An alternative explanation may be provided by a study on a baboon model of preterm birth, which demonstrated that infants on
CPAP (including both delayed or early intervention) displayed a persistence of radial glia in WM compared to controls; in gestational controls, radial glia had predominantly differentiated into astrocytes (Loeliger et al. 2006). This study also showed that the presence of radial glia was more pronounced when there was a greater fluctuation of oxygen requirements. Preterm infants receiving respiratory support commonly have fluctuating oxygen levels (Cunningham et al. 1995). Therefore, it is possible that the persisting radial glia divide and give rise to increasing numbers of neurons (Ganat et al. 2002, Malatesta et al. 2000), which may be associated with the increased relative volume in the frontal WM.

The cerebellum has been increasingly recognised to be vulnerable to injury and altered development (Volpe 2009, Tam et al. 2009); which is in accordance with results from this chapter demonstrating that increased requirement for respiratory support was significantly associated with lower relative volume in this region. A previous study found similar results; cerebellar growth was negatively affected by respiratory support, specifically mechanical ventilation, in a cohort of 169 preterm infants between 28 weeks and TEA (Limperopoulos et al. 2005). Reduced cerebellar volume has also been found in association with CLD in preterm infants at TEA (Keunen et al. 2012). It is possible that reduced cerebellar volume in preterm infants resulted from this region's particular vulnerability during the third trimester. As mentioned above, the third trimester of pregnancy is a phase of rapid cerebellar development (Berry et al. 1995). It is therefore probable that during this period, the cerebellum is vulnerable to environmental influences such as alteration of oxygen levels that can be affected by the need for respiratory support.

Requirement for respiratory support was also positively associated with FA and AD values in the cingulate WM and corpus callosum respectively. In the corpus callosum, it is possible that the increased AD associated with increased respiratory support may reflect a delay in developmental processes such as axonal pruning; axonal pruning has been hypothesised to lead to a decrease in AD values with increasing age in normal development (Gao et al. 2009). The effect of respiratory support on DTI measures in preterm infants has previously been demonstrated using TBSS (Ball et al. 2010, Alexandrou et al. 2014, Anjari et al. 2009). However, these papers found reduced FA in preterm infants with CLD in the centrum semiovale, corpus callosum and inferior longitudinal fasciculus. The reasons for differences between results is unclear, however, these previous papers used TBSS to compare between groups. TBSS captures only the centre of WM tracts; comparatively this study assesses larger segmentations of the WM and corpus callosum. In addition, these previous papers assessed infants at TEA with CLD, whereas this chapter assessed infants across the preterm period and analysed the number of days required for respiratory
support; further studies are needed to examine more fully the relationship between respiratory support and brain development over this developmental period.

**Head circumference at birth and weight increase**

Both HC at birth and weight increase per week was associated with altered absolute volume or relative volume measures. HC at birth may be clinically useful as a surrogate for looking at volume changes in the neonatal brain and it has previously been shown to be a powerful predictor of total brain volume in the neonate (Lindley et al. 1999). It has also previously been correlated positively with grey and white matter volume in late preterm infants (Munakata et al. 2013).

Impaired neonatal growth, assessed in terms of weight, HC and increase in length, has previously been associated with delayed cortical DTI maturation, but not WM volume between 25 and 40 weeks (Vinall et al. 2013). This is consistent with results from this chapter, which demonstrated that increases in weight was significantly associated with increased AD measures in the cortical grey matter of parietal and LOT.

In conclusion, respiratory support, HC at birth, weight increase and GA at birth are significantly associated with preterm brain development. This demonstrates the regional vulnerability of the developing preterm brain, even in cases without focal lesions.

**Limitations**

The automated segmentation approach allows for an objective whole brain analysis of a large preterm cohort; however a number of limitations of this technique exist. Whilst partial volume correction was performed in the boundary between cortical grey matter and cerebral spinal fluid, partial volume remained a limitation; the relatively large voxel size of the younger neonates led to some partial volume effects that were evident specifically in cingulate grey matter. In order to further compensate for this limitation, an additional step was added to the segmentation approach, which corrected for over-estimation of the cortex in regions where sulci were not fully delineated due to partial volume effects. However, despite these compensational methods, partial volume influenced results within the cingulate grey matter and may have led to it being an anomaly compared to other cortical regions; this was apparent in the regression analysis as no significant change in relative volume was observed with increasing PMA at scan.
An additional methodological limitation was that the segmentation of small regions of the brain was particularly difficult in younger neonates, as the atlases used to produce priors for segmentation were derived from neonates of TEA (36 to 44 weeks). It is possible that this influenced measures in small regions and may be a reason for the finding of no significant changes in relative volume and FA with increasing gestation in the LOT and cingulate gyrus WM. However, there are currently no atlases that delineate regional structures for preterm brains younger than TEA; the only atlases that exist in the early period are for large tissues and subcortical structures (Kuklisova-Murgasova et al. 2011, Serag et al. 2012).

The data were also partially limited due to the absence of preterm scans between 37 and 39 weeks; this was likely due to the retrospective nature of data collection and the scanning protocol: preterm neonates would either have been scanned within a week of birth or at TEA. A prospective study scanning preterm neonates at consistent time points over the third trimester of pregnancy may give greater detail about change in quantitative measures over this time point.

A final limitation was related to the relatively small numbers of neonates with clinical risk factors such as NEC (n=4) and sepsis (n=13); the potential effect of these clinical variables may therefore be masked in this analysis. It is also possible that there are other important influences on brain development at this vulnerable maturational age, which have not been addressed in this study, such as nutritional or genetic effects. Further work should aim at incorporating these factors and establishing their contribution to preterm brain development.

**Conclusion**

These results are the first to provide quantitative volumetric and DTI measures in the preterm brain between 26 and 45 week, using an objective segmentation approach to establish brain maturation. A significant reduction in ADC, AD and RD measures was found in white and grey matter with increasing age at scan, whilst FA measures increased in WM and most sub-cortical grey matter regions, but decreased in the cortex over this time period. DTI and volume changes occurred at a non-uniform rate with increasing PMA at scan. Volumetric measures exponentially increased in all regions, although relative volume decreased in white matter and the majority of sub-cortical grey matter regions, whilst increasing in cortical grey matter and the cerebellum with increasing gestation. GA at birth, respiratory support, HC at birth and weight increase all significantly affected quantitative measures across gestation in this large preterm cohort. Within a clinical setting, these results may allow identification of neonates at the highest risk of alterations...
in brain structure. By providing detailed characterisation of DTI and volumetric measures in the majority of brain regions, these results increase our understanding of maturation in the neonatal brain, and may assist in developing future strategies for early detection of abnormal maturation and design of protective approaches for the management of infants born prematurely.
Figure 6.2 FA values in white matter with increasing PMA at scan
Figure 6.3 FA values in the cortex with increasing PMA at scan
Figure 6.4 FA values in the sub-cortical grey matter with increasing PMA at scan
Figure 6.5 ADC values in white matter with increasing PMA at scan
Figure 6.6 ADC values in the cortex with increasing PMA at scan
Figure 6.7 ADC values in the sub-cortical grey matter with increasing PMA at scan
Figure 6.8 AD values in white matter with increasing PMA at scan
Figure 6.9 AD values in the cortex with increasing PMA at scan
Figure 6.10 AD values in the sub-cortical grey matter with increasing PMA at scan
Figure 6.11 RD values in white matter with increasing PMA at scan
Figure 6.12 RD values in the cortex with increasing PMA at scan
Figure 6.13 RD values in the sub-cortical grey matter with increasing PMA at scan
Figure 6.14 Relative volume measures in white matter with increasing PMA at scan
Figure 6.15 Relative volume measures in the cortex with increasing PMA at scan
Figure 6.16 Relative volume measures in the sub-cortical grey matter with increasing PMA at scan
Figure 6.17 Volume measures in white matter with increasing PMA at scan
Figure 6.18 Volume measures in the cortex with increasing PMA at scan
Figure 6.19 Volume measures in the sub-cortical grey matter with increasing PMA at scan.

Figure 6.2 to 6.19: units - volume \( \text{mm}^3 \); ADC, AD and RD: \( \times 10^3 \text{mm}^2/\text{s} \).
7 Motion-corrected diffusion tensor imaging in the fetal brain

7.1 Introduction

Diffusion tensor imaging (DTI) of the fetal brain provides more quantitative information on underlying brain structure than can be obtained from diffusion weighted imaging (DWI). However, previous studies using fetal DTI have been limited by small sample sizes or lack of motion correction.

Unpredictable motion from the fetus, as well as from maternal breathing, provides an on-going challenge that is exacerbated in DTI compared to DWI due to the greater number of diffusion-sensitising gradients and longer acquisition times. Despite these limitations, a small number of fetal studies have used DTI to produce anisotropy measures across gestation in fetuses with minimal head movement (Zanin et al. 2011, Kasprian et al. 2008). However, lack of motion correction methods has led to data exclusion rates as high as 72%, and low fractional anisotropy (FA) values (Zanin et al. 2011) that may be due to poor signal-to-noise ratio (SNR). For improved analysis of the fetal brain it is therefore important to develop a methodology to produce reliable estimation of anisotropic water diffusion in the fetal brain.

A number of studies have presented registration techniques that correct for fetal motion, thereby producing improved rendition of brain anatomy and delineation of white matter (WM) tracts (Oubel et al. 2012, Fogtmann et al. 2014, Jiang et al. 2009). The Snapshot to Volume Reconstruction (SVR) technique for anatomical T2-weighted images was extended to DTI (Jiang et al. 2009) thus presenting a methodology to achieve DTI reconstruction of the brain in moving subjects. This method used oversampling and image registration to realign diffusion images to correct for subject motion and successfully produced FA maps in the fetal brain, but was limited by poor SNR. Using more robust model-driven registrations (Bertelsen et al. 2009) and addressing limitations from distortion caused by echo planar imaging (EPI) (Wu et al. 2012) have further improved the results of final reconstructed images.

These studies have produced FA maps that are capable of delineating WM tracts in the fetal brain. However, to date no study has performed motion-corrected fetal DTI on a large cohort of normal
cases to establish normative FA and apparent diffusion coefficient (ADC) values over a range of gestational ages. This technique may also allow comparison of DTI measures across gestation between in utero and ex utero development, which has previously been restricted to post-natal DTI acquisition (Counsell et al. 2006, Anjari et al. 2007, Gimenez et al. 2008). In this study, an optimised motion correction DTI technique was used to quantify fetal FA and ADC measures across gestation in fetuses without brain abnormalities. Results obtained were compared with data from premature neonates to determine whether DTI could be used to detect developmental differences between in utero and ex utero brain maturation.

7.2 Methods

Work done by others

Optimisation and implementation of the fetal DTI protocol and algorithm for motion correction was conducted by a colleague (ZOW), and was based on work previously done within the department (Jiang et al. 2007, Jiang et al. 2009, Bertenssen et al. 2009, Wu et al. 2012). The methods were specifically designed to improve SNR and the accuracy of registration, as well as to overcome distortions from EPI sequences.

Calculation of the diffusion tensor in the fetal brain

Below the pipeline for calculating the diffusion tensor in the fetal brain is detailed. The procedure included: 1) scanning protocol and sequence acquisition 2) data preparation, including distortion correction of all images (Wu et al. 2012); 3) reconstruction of a volumetric $b_0$ image using the SVR technique (Jiang et al. 2007); 4) registration of DW images to the volumetric $b_0$ image (Bertenlsen et al. 2009); 5) final registration of DW images 6) calculation of the diffusion tensor (DT) in each voxel.

Scanning protocol and acquisition

Fetal scanning

Fetal DTI was conducted on a Philips 1.5-Tesla scanner using a 32-channel phase array cardiac coil. The sequence parameters consisted of: DTI obtained in 15 non-collinear directions, three $b_0$ spin-echo EPI images in both axial and coronal orientation for accurate reconstruction of $b_0$.
images; and B₀ field maps for distortion correction. The overall DTI scan time was approximately 11 minutes. DTI sequence parameters were as described previously (Wu et al. 2012): b value 0 and 500s/mm², 15 non-collinear directions, TE 121ms, TR 8500ms, field of view (FoV) 290×290×128mm³, voxel size 2.3×2.3×3.5mm³, slice gap -1.75mm (5 minutes 6 seconds). The b₀ sequence parameters were identical to the DTI sequence, except b value=0 (1 minute 42 seconds). B₀ field maps (29.5 seconds, TE1 4.6ms, TE2 9.2ms; TR 10ms, Flip Angle 10 degree, voxel size 2.27×2.27×10mm³; FoV 400×400×150mm³) were acquired just prior to the start and at end of each full DTI acquisition. Total scan acquisition time was approximately 12 minutes.

**Neonatal scanning**

The neonatal images used in this study were separately acquired on a 3-Tesla Philips Achieva system, using an eight-channel phased array head coil, as described in chapters 5 and 6. Briefly, single-shot echo planar DTI was acquired in 32 non-collinear directions with the following parameters: b-value 750 s/mm², TE 49ms, TR 8000ms, FoV 224×224×98mm³, voxel size 2x2x2mm³, no slice gap, and a SENSE factor 2 (scan acquisition time: 5 minutes 30 seconds). All infants were clinically assessed as stable prior to scanning by an experienced paediatrician, and scans in neonates older than 36 weeks were performed under sedation (oral chloral-hydrate, 30-50 mg/kg). For both fetal and preterm MRI, all parents gave written consent prior to scanning (Ethics 07/H0707/105, 07/H0707/101).

**Fetal DTI data preparation**

DW and b₀ images were initially assessed visually on a slice-by-slice basis. Any slice that was damaged due to movement or other artefacts were excluded from the data set (Figure 7.1). For later evaluation purposes, image data sets were coded according to the presence of fetal motion and other artefacts, percentage of damaged slices and the resulting difficulty for successful FA map production.
Figure 7.1 Fetal DW data with and without motion artifacts.

DW images from a fetus at A) 34.1 weeks and B) at 29.5 weeks. A) Demonstrates a DW image of good quality and was included in reconstruction for FA map production. B) Demonstrates a slice corrupted by motion artefacts, as can be observed from the blurred appearance of the image and patches of high and low signal as well as distortions to the appearance of the brain. This slice was therefore excluded from further processing.

DW data were registered to the $b_0$ image to minimise distortions due to eddy currents using FSL (Smith et al. 2004). Non-brain tissue was removed from each image to exclude the surrounding maternal and fetal anatomy.

Geometric distortion resulting from magnetic field inhomogeneities is a major limitation of EPI sequences, which also results in signal loss. By implementation of the FSL-FUGUE method (Smith et al. 2004) - which used the calculated difference in phase between the two undistorted $b_0$ images acquired at the beginning and end of the fetal DTI sequence - distortion was corrected on each $b_0$ and DW image, as previously reported (Wu et al. 2012).

**Reconstruction of a $b_0$ volume image**

Generating an accurate $b_0$ volume image is vital for calculation of the diffusion tensor, as it is used as the target for all DW images during registration. The SVR approach (Jiang et al. 2007), was adopted to generate the $b_0$ volume. The fetal brain was first oversampled by repeatedly imaging it with multiple stacks of single-shot $b_0$ slices. The $b_0$ image stack that was least corrupted by motion was chosen as a target and all the remaining images were registered to this target in order to align the slices.
**DTI registration**

DW images were registered first to the \( b_0 \) volume, and then to each other to create the final DT image. During the initial stage, DW stacks of images were initially aligned to the \( b_0 \) volume using normalized mutual information (NMI), as it has proved effective in accommodating contrast differences between images being aligned. Following this, registration was completed using an alternative model-based strategy, which was more effective in refining the registrations and the resulting DT calculations in each voxel. The model-based strategy used a cross-correlation technique to refine the registration result by aligning acquired DW slices to simulated DW volumes which were obtained from the latest DT model and direction of gradient for each slice.

Once the final stage of registration was completed, eigenvalues (\( \lambda_1, \lambda_2, \) and \( \lambda_3 \)), were derived from the final estimation of the DT and used to calculate FA and ADC maps for analysis and comparison with preterm neonatal DTI data.

**Subjects**

**Fetal Cohort**

DTI was conducted on a total of 36 fetal cases with no detected central nervous system (CNS) abnormalities (assessed on conventional MRI by an experienced neuroradiologist), and without CNS abnormalities following birth. The gestational age (GA) at scan of the fetuses ranged from 21.29 to 37.57 weeks (median 31.86 weeks).

**Premature neonatal cohort**

For comparison of fetal DTI with preterm data, 32 preterm neonate DTI maps were produced using FSL4 (Smith et al. 2004), as described in chapters 5 and 6. These preterm neonates were chosen to be of similar ages compared to the fetal group. Preterm neonates were scanned between 27.43 to 37.14 weeks post-menstrual age (PMA) (median 33.07 weeks), and had a GA at birth ranging from 24.57 to 34.71 weeks (median 29.86 weeks). Preterm neonates were only included for comparison if there was no evidence of focal lesions on conventional T1- and T2-weighted MRI scans.
Post-processing and analysis of DTI data

Following successful reconstruction, FA and ADC maps were generated and then tractography and region of interest (ROI) analysis performed. DTI measurements from ROIs obtained in the fetal brain were then compared to values from the preterm cohort.

Deterministic Tractography

DW images, $b_0$ image and the gradient table were inputted into Diffusion toolkit (Wang et al. 2007) to generate fibers. The fibre bundles were reconstructed using Fiber Assignment by Continuous Tracking (FACT) (Mori et al. 1999), which follows the orientation of the primary eigenvector on a voxel-by-voxel basis passing through a “seed” positioned on the fibre tract. An FA threshold of 0.1 and an angulation threshold of 35° were used, consistent with previous fetal and neonatal tractography papers (Berman et al. 2005, Dubois et al. 2006, Kasprian et al. 2008). Using Trackvis (Wang et al. 2007), tractography seeds with a radius of 5mm (4mm at an age at scan <26 weeks), were positioned on the cerebral peduncle to generate fibres in the cortico-spinal tract (CST) for each hemisphere. CST tracts were constrained by ROIs positioned in the posterior limb of the internal capsule (PLIC). Only the CST tracts that progressed from the peduncle and beyond the PLIC in both hemispheres were used for analysis. Tracts were also generated in the forceps minor and the forceps major, and constrained by ROI placed on coronal slices anterior to the frontal horns of the lateral ventricles, and posterior to lateral ventricles at the level of the 4th ventricle respectively. As described in the adult brain, use of a “NOT” ROIs was sometimes necessary to remove a subset of unwanted tracts (Wakana et al. 2007).

Region of interest analysis

FA and ADC measurements were taken from multiple ROIs, using FSL4 (Smith et al. 2004). ROIs were analysed in both white and grey matter, and were chosen due to their visibility across all gestational ages, and for comparison with previous fetal and preterm papers. WM regions were analysed in the splenium and genu of the corpus callosum, PLIC, frontal WM, occipital WM, and centrum semiovale (CSO). Grey matter regions were analysed in the pons, cerebellum and thalamus (Figure 7.2). ROIs were excluded if difficult to visualise due to mis-registration or artefacts.

Statistical analyses were performed using Stata/IC 11. In the normal fetal cohort, Analysis of variance (ANOVA) was performed to assess DTI measures between different regions. Regression
was performed between DTI measures and age at scan. For preterm data, multiple regression was performed between DTI measures and PMA at scan, including GA at birth as a co-variable in the regression model. In addition, in order to establish the effect on DTI measures of ex utero compared to in utero development, multiple regression analyses were utilised to compare between trajectories with increasing age at scan; GA at birth was also included as a co-variable. Bonferroni correction for multiple comparisons, due to analysis in 9 ROIs, indicated a level of significance at $p=0.006$.

*Figure 7.2 Regions of interest in white and grey matter.*

Regions of interest on the transverse plane of an ADC map of a 29.57 week old fetus in the CSO, frontal WM, genu, PLIC, thalamus, occipital WM, splenium, pons and cerebellum (left to right).
7.3 Results

Fetal DTI motion-corrected data and FA map production

26 DTI fetal scans were successfully reconstructed to produce FA maps (Figure 7.3) from a total of 36 DTI scans; this gave a 72% success rate of fetal DTI reconstruction. Images were coded according to the presence of motion and damaged slices, which corresponded to the success and quality of FA map production seen in Table 7.1. In 5 scans, there was excessive fetal movement that was not consistent with successful reconstruction, a further 5 cases were not successfully registered due to artefacts and movements in the DWI and $b_0$ images.

Figure 7.3 FA map produced from DTI reconstruction and motion correction algorithm.

A fetus scanned at 29.5 weeks. The first row demonstrates an FA map created with motion-corrected technique, in the transverse, coronal and sagittal plane (left to right). The corpus callosum and CST are clearly visible. The corresponding ADC map is seen in the bottom row.
<table>
<thead>
<tr>
<th>Category</th>
<th>Code 1</th>
<th>Code 2</th>
<th>Code 3</th>
<th>Code 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of slices to be discarded due to movement and artefact</td>
<td>&lt;10%</td>
<td>Approx. 10%</td>
<td>20% - 35%</td>
<td>More than 35%</td>
</tr>
<tr>
<td>Details of brain structure visualised in the FA map</td>
<td>Structure could be identified</td>
<td>Some structure could be identified</td>
<td>Almost no structure could be identified</td>
<td>No structure could be identified</td>
</tr>
<tr>
<td>Number of successful reconstructions/number of scans acquired with this code</td>
<td>18/18</td>
<td>5/6</td>
<td>3/7</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Table 7.1 DW images and categories of difficulty to calculate the diffusion tensor

**Fetal cohort**

The distribution of GA at scan of the 26 fetal scans can be seen in Figure 7.4; two participants had a repeat DTI scan during pregnancy. Fetuses were scanned for a variety of reasons, but all cases had normal brain appearance on MRI: eight were healthy volunteers, two were the surviving fetus from a monochorionic diamniotic (MCDA) twin pregnancy, two had atrioventricular septal defects (AVSD), and the remaining cases were scanned for query abnormalities suspected from an ultrasound scan. In addition to normal brain appearance on MRI, all infants had normal deliveries: no infants required resuscitation at birth and all had 1 and 5 minute Apgar scores >8 (data not available for two cases). Their GA at birth ranged from 36.57 - 42.57 (median 39.57) weeks. Thirteen cases were male, and nine female (data not available for one case).

![Figure 7.4 Histogram of fetal GA at scan](image)
Fetal Tractography

CST tractography was successful in both hemispheres in twelve fetal cases. DTI measurements for the CST tract were averaged from left and right hemispheres. The forceps minor was successfully tracked in 24 cases, and the forceps major in 20. There was only 1 case where there was no successful tracking in the forceps major or minor or CST, and this case corresponded to the lowest quality FA map and registration. There were 10 fetal cases where tractography could be performed in all 3 tracts. The GA at scan of these 10 fetal cases ranged from 22.14 to 37.5 weeks (median 31.85 weeks). The mean DTI values extracted from each tract are shown in Table 7.2.

![Image of fetal tractography](image)

*Figure 7.5 Fetal tractography in the CST.*

The CST in both hemispheres of a fetus at (A) an early GA (25.2 weeks), and (B) a later GA (34.1 weeks). The tractography seed was placed at the peduncle, and the tract was constrained by a waypoint ROI placed at the level of the PLIC.
The forceps major and minor of a fetus at (A) an early GA (25.2 weeks), and (B) a later GA (34.1 weeks). ROIs were placed anteriorly to the anterior horns of the lateral ventricle, and posterior to the posterior horn of the lateral ventricles for the forceps minor and major respectively.

DTI measurements were extracted from the tracts. A significant increase in FA measures of the forceps major was found with increasing GA at scan ($R^2=0.220$; b coef. 0.007; $p=0.037$). An increase in FA was also observed in the CST ($R^2=0.311$; b coef. 0.004; $p=0.059$), but this did not reach significance. FA measures were not associated with GA at scan in the forceps minor; ADC measures were not associated with GA at scan in any tracts. There was a significant increase in length of tract with increasing GA at scan ($p<0.05$).

<table>
<thead>
<tr>
<th></th>
<th>FA mean (sd)</th>
<th>ADC mean (sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(units: x10^{-3}mm^2/s)</td>
<td></td>
</tr>
<tr>
<td>Forceps Major</td>
<td>0.34 ($\pm0.06$)</td>
<td>1.62 ($\pm0.10$)</td>
</tr>
<tr>
<td>Forceps Minor</td>
<td>0.27 ($\pm0.04$)</td>
<td>1.62 ($\pm0.15$)</td>
</tr>
<tr>
<td>CST</td>
<td>0.27 ($\pm0.03$)</td>
<td>1.42 ($\pm0.11$)</td>
</tr>
</tbody>
</table>

*Table 7.2 FA and ADC values from tractography*
Region of Interest Analysis

ROI analysis was performed on all 26 successfully produced FA maps from the fetal cohort. Table 7.3 reports the mean FA and ADC values in each ROI.

<table>
<thead>
<tr>
<th>ROI (Obs)</th>
<th>Mean FA (±sd)</th>
<th>Mean ADC (±sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLIC (21)</td>
<td>0.37 (±0.07)</td>
<td>1.31 (±0.12)</td>
</tr>
<tr>
<td>Genu (25)</td>
<td>0.46 (±0.08)</td>
<td>1.44 (±0.14)</td>
</tr>
<tr>
<td>Splenium (21)</td>
<td>0.49 (±0.09)</td>
<td>1.48 (±0.16)</td>
</tr>
<tr>
<td>Frontal WM (23)</td>
<td>0.14 (±0.04)</td>
<td>1.89 (±0.20)</td>
</tr>
<tr>
<td>CSO (24)</td>
<td>0.14 (±0.03)</td>
<td>1.86 (±0.19)</td>
</tr>
<tr>
<td>Occipital WM (24)</td>
<td>0.20 (±0.06)</td>
<td>1.62 (±0.19)</td>
</tr>
<tr>
<td>Thalamus (24)</td>
<td>0.17 (±0.03)</td>
<td>1.29 (±0.15)</td>
</tr>
<tr>
<td>Cerebellum (23)</td>
<td>0.15 (±0.04)</td>
<td>1.45 (±0.13)</td>
</tr>
<tr>
<td>Pons (24)</td>
<td>0.18 (±0.06)</td>
<td>1.28 (±0.14)</td>
</tr>
</tbody>
</table>

Table 7.3 Fetal DTI Descriptive statistics.

Abbreviations: Obs = number of observations for the ROI analysis; units: ADC x10⁻³mm²/s

Regional variance in FA and ADC measures in the fetal brain

Significant FA and ADC differences were found between regions (p<0.001); box plots represent the FA (Figure 7.7) and ADC (Figure 7.8) values in each region. The corpus callosum exhibited the highest FA values, which were significantly greater than in the PLIC; other WM regions had significantly lower FA values. The CSO and frontal WM had lower FA values and were not significantly different from grey matter ROIs.

ADC values were significantly higher in the frontal and occipital WM, and lowest in the PLIC, compared to other regions. ADC values were significantly lower in grey matter than WM, except in the cerebellum, where ADC values were equivalent to those found in the corpus callosum and PLIC.
Figure 7.7 FA values in each region

Figure 7.8 ADC values in each region.

Box plots in Figure 7.7 and Figure 7.8 demonstrate FA and ADC values in each region; the median is represented by the line within the box. The box represents the interquartile range. ADC units: $10^{-3} \text{mm}^2/\text{s}$
Fetal FA and ADC values with increasing PMA at scan

<table>
<thead>
<tr>
<th></th>
<th>PLIC</th>
<th>genu</th>
<th>splenium</th>
<th>frontal WM</th>
<th>CSO</th>
<th>occipital WM</th>
<th>thalamus</th>
<th>cerebellum</th>
<th>pons</th>
</tr>
</thead>
<tbody>
<tr>
<td>FA</td>
<td></td>
<td>*</td>
<td>&lt;0.001</td>
<td>0.081</td>
<td>0.556</td>
<td>0.074</td>
<td>0.039</td>
<td>0.081</td>
<td>0.654</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.469</td>
<td>0.145</td>
<td>0.597</td>
<td>0.138</td>
<td>0.016</td>
<td>0.138</td>
<td>0.180</td>
<td>0.138</td>
</tr>
<tr>
<td></td>
<td>coef.</td>
<td>0.011</td>
<td>0.007</td>
<td>0.019</td>
<td>-0.003</td>
<td>0.001</td>
<td>0.005</td>
<td>0.004</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>0.005, 0.016</td>
<td>0.0003, 0.014</td>
<td>0.012, 0.027</td>
<td>-0.007, 0.0004</td>
<td>-0.002, 0.004</td>
<td>-0.001, 0.011</td>
<td>0.0001, 0.007</td>
<td>-0.0004, 0.007</td>
</tr>
<tr>
<td>ADC</td>
<td></td>
<td>0.018</td>
<td>0.056</td>
<td>0.749</td>
<td>0.021</td>
<td>0.840</td>
<td>* 0.001</td>
<td>* 0.007</td>
<td>0.456</td>
</tr>
<tr>
<td></td>
<td>R²</td>
<td>0.260</td>
<td>0.150</td>
<td>0.006</td>
<td>0.220</td>
<td>0.002</td>
<td>0.004</td>
<td>0.420</td>
<td>0.299</td>
</tr>
<tr>
<td></td>
<td>coef.</td>
<td>-0.013</td>
<td>0.013</td>
<td>-0.003</td>
<td>0.021</td>
<td>0.002</td>
<td>-0.003</td>
<td>-0.023</td>
<td>-0.015</td>
</tr>
<tr>
<td></td>
<td>95% CI</td>
<td>-0.024, -0.003</td>
<td>-0.0003, 0.026</td>
<td>-0.024, 0.018</td>
<td>0.004, 0.039</td>
<td>-0.017, 0.020</td>
<td>-0.021, 0.016</td>
<td>-0.035, -0.004</td>
<td>-0.025, -0.004</td>
</tr>
</tbody>
</table>

Table 7.4 reports regression results for FA or ADC values with GA at scan in the normal fetal cohort. FA values significantly increased with increasing GA at scan in the PLIC and splenium. FA values also increased with increasing GA at scan in the thalamus, but this was not significant after Bonferroni correction (p<0.006).

ADC values significantly decreased with increasing PMA at scan in the thalamus and cerebellum, though the cerebellum was not significant after Bonferroni correction. ADC values also decreased in the PLIC with increasing age at scan, but this was not significant after Bonferroni correction. Conversely, ADC values increased with increasing PMA at scan in the frontal WM, although this was not significant after Bonferroni correction. Figure 7.9 and Figure 7.10 are scatterplots of FA and ADC values with increasing PMA at scan in each region; the line of best fit is demonstrated in regions where there were significant relationships with PMA at scan.
<table>
<thead>
<tr>
<th></th>
<th>PLIC</th>
<th>genu</th>
<th>splenium</th>
<th>frontal WM</th>
<th>CSO</th>
<th>occipital WM</th>
<th>thalamus</th>
<th>cerebellum</th>
<th>pons</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>p</em></td>
<td><em>0.001</em></td>
<td>0.060</td>
<td><em>&lt;0.001</em></td>
<td>0.081</td>
<td>0.556</td>
<td>0.074</td>
<td>0.039</td>
<td>0.081</td>
<td>0.654</td>
</tr>
<tr>
<td><em>R^2</em></td>
<td>0.469</td>
<td>0.145</td>
<td>0.597</td>
<td>0.138</td>
<td>0.016</td>
<td>0.138</td>
<td>0.180</td>
<td>0.138</td>
<td>0.009</td>
</tr>
<tr>
<td>coeff.</td>
<td>0.011</td>
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<td>0.019</td>
<td>-0.003</td>
<td>0.001</td>
<td>0.005</td>
<td>0.004</td>
<td>0.003</td>
<td>0.001</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.005, 0.016</td>
<td>0.0003, 0.014</td>
<td>0.012, 0.027</td>
<td>-0.007, 0.0004</td>
<td>-0.002, 0.004</td>
<td>-0.001, 0.011</td>
<td>0.0001, 0.007</td>
<td>-0.0004, 0.007</td>
<td>-0.004, 0.007</td>
</tr>
<tr>
<td><strong>ADC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>p</em></td>
<td>0.018</td>
<td>0.056</td>
<td>0.749</td>
<td>0.021</td>
<td>0.840</td>
<td>0.761</td>
<td><em>0.001</em></td>
<td><em>0.007</em></td>
<td></td>
</tr>
<tr>
<td><em>R^2</em></td>
<td>0.260</td>
<td>0.150</td>
<td>0.006</td>
<td>0.220</td>
<td>0.002</td>
<td>0.004</td>
<td>0.420</td>
<td>0.299</td>
<td>0.026</td>
</tr>
<tr>
<td>coeff.</td>
<td>-0.013</td>
<td>0.013</td>
<td>-0.003</td>
<td>0.021</td>
<td>0.002</td>
<td>-0.003</td>
<td>-0.023</td>
<td>-0.015</td>
<td>-0.005</td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.024, -0.003</td>
<td>-0.0003, 0.026</td>
<td>-0.024, 0.018</td>
<td>0.004, 0.039</td>
<td>-0.017, 0.020</td>
<td>-0.021, 0.016</td>
<td>-0.035, -0.011</td>
<td>-0.025, -0.004</td>
<td>-0.017, 0.008</td>
</tr>
</tbody>
</table>

Table 7.4 Linear regression analysis of FA and ADC values and PMA at scan.

* indicates that relationships with age at scan were significant after Bonferroni correction p≤0.006; Abbreviations: coef = age at scan b coefficient; p = p value
Figure 7.9 FA values with age at scan in a normal fetal cohort.

Lines of best fit are fitted in cases of significant changes over gestation: FA significantly increased with increasing GA at scan in the PLIC and splenium.
Figure 7.10 ADC values with age at scan in a normal fetal cohort.

Lines of best fit are fitted in cases of significant changes with increasing age at scan: ADC significantly decreased with increasing age at scan in the thalamus and cerebellum. ADC units: $10^{-3}\text{mm}^2/\text{s}$. 
Comparison of fetal and ex-utero preterm FA and ADC values

Figure 7.11 demonstrates FA maps of a 30 week fetus (on the left) compared to preterm neonate scanned at an equivalent age. Both FA maps clearly depict the genu and splenium of the corpus callosum and the PLIC; however FA maps produced with DTI data obtained ex utero in the preterm brain exhibit improved SNR compared to in utero FA maps.

![FA maps in a fetus at 30 weeks compared to an age-matched preterm neonate.](image)

FA maps in the transverse plane of a fetal brain at 30.5 weeks (left) compared to a preterm infant at 30.7 weeks (right); the genu and splenium of the corpus callosum and the PLIC are clearly visible in both FA images.

Fetal DTI measures were compared to those from preterm neonates. Scatter plots of FA and ADC values of the fetus compared to preterm neonates can be seen in Figure 7.12 and Figure 7.13 respectively. Comparison of number of ROIs visualised and used for analysis in fetal compared to preterm DTI data are presented in Table 7.5.
<table>
<thead>
<tr>
<th></th>
<th>% Visible tracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fetus</td>
</tr>
<tr>
<td>PLIC</td>
<td>81</td>
</tr>
<tr>
<td>Genu</td>
<td>96</td>
</tr>
<tr>
<td>Splenium</td>
<td>81</td>
</tr>
<tr>
<td>Frontal WM</td>
<td>88</td>
</tr>
<tr>
<td>Occipital WM</td>
<td>92</td>
</tr>
<tr>
<td>CSO</td>
<td>92</td>
</tr>
<tr>
<td>Thalamus</td>
<td>92</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>88</td>
</tr>
<tr>
<td>Pons</td>
<td>92</td>
</tr>
</tbody>
</table>

Table 7.5 Regions of interest visualised and analysed in fetal and preterm data.

A higher proportion of regions can be visualised in preterm FA and ADC maps compared to fetal data. Specifically, the PLIC and splenium were difficult regions to visualise in the fetus.
Figure 7.12 FA values with age at scan in the fetal brain compared to preterm neonates.

Fetal FA values significantly increased with increasing age at scan in the PLIC and splenium; a similar increase in FA values is demonstrated in the preterm brain (PLIC: Adj. $R^2 = 0.697$; $b$ coef 0.010, p<0.001; splenium: Adj. $R^2 = 0.164$, $b$ coef 0.014, p=0.025).
Figure 7.13 ADC values with age at scan in the fetal brain compared to preterm neonates.

ADC (units: $10^{-3} \text{mm}^2/\text{s}$) values in the fetal and preterm brain with increasing age at scan. ADC values in the fetal brain decreased with increasing age at scan in the PLIC, thalamus and
cerebellum; a similar reduction in ADC with increasing age at scan was seen in the preterm brain (PLIC: Adj. $R^2=0.323$, $b$ coef. -0.013, $p=0.011$; cerebellum: Adj. $R^2=0.277$, $b$ coef. 0.020, $p=0.026$; splenium: Adj. $R^2=0.240$, $b$ coef. -0.033, $p=0.004$).

Table 7.6 reports the multiple regression results on DTI differences with increasing age at scan in the fetal and preterm brains. FA measures were significantly lower in preterm neonates compared to fetuses in the CSO and thalamus; although only the former was significant after Bonferroni correction. ADC measures were also lower in preterm neonates compared to fetuses in the PLIC and genu; although this was not significant after Bonferroni correction. GA at birth was included as a co-variable in these multiple regression analyses.

<table>
<thead>
<tr>
<th>Region</th>
<th>FA</th>
<th>ADC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>coef.</td>
</tr>
<tr>
<td>PLIC</td>
<td>0.559</td>
<td>0.043</td>
</tr>
<tr>
<td>genu</td>
<td>0.314</td>
<td>-0.001</td>
</tr>
<tr>
<td>splenium</td>
<td>0.490</td>
<td>-0.014</td>
</tr>
<tr>
<td>frontal WM</td>
<td>0.357</td>
<td>-0.030</td>
</tr>
<tr>
<td>CSO</td>
<td>0.368</td>
<td>-0.056</td>
</tr>
<tr>
<td>occipital WM</td>
<td>0.217</td>
<td>-0.020</td>
</tr>
<tr>
<td>thalamus</td>
<td>0.288</td>
<td>-0.046</td>
</tr>
<tr>
<td>cerebellum</td>
<td>0.043</td>
<td>-0.050</td>
</tr>
<tr>
<td>pons</td>
<td>-0.022</td>
<td>-0.032</td>
</tr>
</tbody>
</table>

Table 7.6 Multiple regression results of fetal compared to preterm cohorts of DTI measures over PMA at scan, including GA as a covariate.

* indicates significant results to $p=0.006$ (Bonferroni correction for multiple comparisons).

Abbreviations: coef. = $b$ coefficient for dummy variable on preterm neonates; $p$ = $p$ value

### 7.4 Discussion

To the best of our knowledge, this is the first study of FA and ADC changes with increasing GA at scan in a large normal fetal cohort using an optimised DTI protocol and algorithm for motion correction. It is also the first to compare FA values in the normal fetal brain to premature neonates over a large gestational age range.
The optimised motion-corrected protocol for DTI provides improved quality fetal diffusion imaging data that are comparable to those of preterm neonates; for example our mean FA value from ROI analysis in the splenium was 0.49, which was consistent with a mean FA value of 0.47 reported in a ROI analysis of preterm infants imaged between 33 and 37.5 weeks (Partridge et al. 2004). Previously, pilot data based on a smaller fetal cohort provided motion-corrected DTI results in a comparison with an age-matched group of neonates, demonstrating similar FA values in each group (Lockwood Estrin et al. 2013). These pilot results and the results from this chapter in a larger fetal cohort illustrate the potential of fetal DTI to explore normal brain development in utero.

Three previous studies have provided normal fetal FA values across gestation (Bui et al. 2006, Zanin et al. 2011, Kasprian et al. 2008). One study used a ROI approach to establish FA and ADC values in the CSO, splenium and pyramidal tract of 24 fetuses scanned between 31 and 37.43 weeks (Bui et al. 2006). The two other studies used tractography to extract FA measures in the CST and corpus callosum (Kasprian et al. 2008, Zanin et al. 2011). However, these studies used protocols that had little or no motion correction; they also showed limited evidence of addressing the low SNR, artefacts and distortions which limit fetal imaging. In addition, no FA images were presented in these studies and it is therefore difficult to ascertain the quality of the diffusion imaging acquired. It has been shown that even in fetal cases with minimal motion, FA maps were clearly enhanced using motion correction techniques, with greater depiction of anatomical detail and more consistent FA values (Oubel et al. 2010, Oubel et al. 2012, Jiang et al. 2009, Bertelsen et al. 2009). This study therefore adds to the literature by providing FA results from fetal DTI images, which are optimised with respect to SNR, artefacts and distortion and are not corrupted by motion. In addition, this technique allows for a higher rate of data inclusion compared to previous papers: this chapter has demonstrated that in 28% (10/36) of fetal cases FA maps could not be produced due to extreme fetal motion and artefacts. In comparison, previous fetal DTI papers without motion correction have quoted data exclusion rates of up to 72% (Zanin et al. 2011). The motion correction technique used here therefore leads to less wasted data from a time consuming and difficult cohort from which to acquire data.

**Fetal tractography**

Table 7.7 illustrates the comparison between the tractography results and values from two previous fetal tractography papers and those from a preterm paper. Zanin et al. (2011) conducted tractography on 17 fetuses imaged between 23 and 38 weeks gestation; however they found very
low FA values compared to preterm as well as other fetal DTI studies. This may be due to lack of compensation for fetal motion or from a low FA threshold (FA threshold: 0.08). In this chapter, using the motion-corrected approach, FA values were more consistent with those of preterm neonates, suggesting that this optimised technique generates improved results.

Kasprian et al, (2008) assessed the potential of in utero tractography on fetuses imaged from 18 to 37 weeks, and visualised the main projection and commissural pathways in 40 cases. This study did not correct for fetal motion, but the authors did pay close attention to scans corrupted by motion artefacts; whilst it is unclear whether they excluded corrupted data from analysis, this may have led to inclusion of only high quality data resulting in FA values that are also similar to those found in preterm neonates.

<table>
<thead>
<tr>
<th></th>
<th>Fetal Tractography</th>
<th>Preterm Tractography</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>fetal DTI data</td>
<td>0.27 ± 0.08</td>
</tr>
<tr>
<td>CST mean FA</td>
<td></td>
<td>0.28 ± 0.04</td>
</tr>
<tr>
<td>Forceps minor/genu</td>
<td>0.28</td>
<td>0.17 ± 0.01</td>
</tr>
<tr>
<td>mean FA</td>
<td></td>
<td>~0.3</td>
</tr>
<tr>
<td>Forceps major/splenium</td>
<td>0.34</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>mean FA</td>
<td></td>
<td>~0.33</td>
</tr>
<tr>
<td>Age at scan range</td>
<td>22 - 37</td>
<td>23 - 38</td>
</tr>
<tr>
<td>FA threshold</td>
<td>0.1</td>
<td>0.08</td>
</tr>
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<td></td>
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</table>

Table 7.7 Tractography result comparison between papers.

^ no data available

Tractography has the advantage over 2D ROI analysis of being less susceptible to variation from scan plane orientations, as well as allowing 3D visualisation of entire WM tracts. However, only in 40% of the fetal data were all three tracts visualised; this is in comparison to ROIs, where an average of 90% of regions were suitable for analysis. DTI measures obtained from ROIs were therefore used in this chapter for comparison with preterm data.

Region of Interest Analyses

Regional variance in FA and ADC measures in the fetal brain

In the WM, FA was highest in the corpus callosum, followed by the projection tracts of the PLIC, then the CSO with the lowest FA values found in the frontal and occipital WM. This same regional pattern of FA values has also been found in fully matured WM tracts of adults (Shimony et al. 1999), as well as in previous studies in prematurely born infants (Partridge et al. 2004, Dudink et
Myelination in the corpus callosum does not begin until approximately 6 weeks post-term (Barkovich et al. 1988) and starts posteriorly in the splenium before moving forward to include the genu. Therefore the main contributor to the high FA values observed in the corpus callosum in the fetal brain is likely to be the high degree of coherent parallel organization of the WM bundles (Kinney et al. 1988, Barkovich 2000). Comparatively, the PLIC is an early myelinating WM tract, a process that is likely to contribute to the high FA values in this region. Previous studies have demonstrated that myelin contributes about 20% towards the measure of FA (Gulani et al. 2001, Beaulieu 2002). Other studies have found that measures of anisotropy are also influenced by factors such as improved parallel organisation and packing of axons as they mature (Beaulieu 2009, Budde et al. 2008, Takahashi et al. 2002).

The frontal and occipital WM contain the lowest FA and highest ADC values; these are regions of less mature WM tracts than the commissure fibres and the internal capsule, with less coherence of WM fibres and later myelination (Barkovich et al. 1988).

The grey matter regions of the thalamus, cerebellum and pons demonstrated relatively low ADC and FA values, which is in agreement with neonatal data showing ADC values of grey matter are lower than those of the WM (Huppi and Dubois 2006, Neil et al. 1998). This is also similar to the fetal ADC results from chapter 3.

Fetal FA and ADC values with increasing PMA at scan

The observed changes in fetal DTI measures with increasing age at scan were consistent with established regional FA increases and ADC reduction found in neonates born prematurely (Huppi et al. 1998a, Neil et al. 1998, Partridge et al. 2004, Berman et al. 2005).

In WM, there was a significant increase of FA values with age at scan in the splenium of the corpus callosum and PLIC. Previous fetal DTI studies without motion correction have found similar maturational changes in these regions. Bui et al, (2006) found a significant FA increase and ADC decrease in the splenium of the corpus callosum and pyramidal tract in 24 fetuses, but they imaged over a limited GA range between 31 and 37 weeks. The same increase in FA measures with increasing age at scan has previously been found in the PLIC and splenium of preterm neonates (Partridge et al. 2004); results from chapter 6 also demonstrated FA increases with age at scan in the corpus callosum between 26 and 45 weeks in the preterm brain.
Results from this chapter also showed significant reduction in ADC values with increasing PMA at scan in the PLIC, although this was not significant after Bonferroni correction. A significant reduction in the PLIC with increasing GA at scan has been reported previously in both fetal (Bui et al. 2006) and preterm DTI studies (Huppi et al. 1998a, Neil et al. 1998, Partridge et al. 2004).

In the thalamus and cerebellum, ADC decreased with increasing age at scan; though this was not significant to Bonferroni level in the cerebellum. Previous fetal DW papers have also shown ADC reduction in these regions with increasing age. Schneider et al, (2007) demonstrated significant ADC reduction in the thalamus, cerebellum and pons in a large cohort of 78 fetuses without CNS abnormalities imaged between 23 and 37 weeks. The same research group published similar grey matter ADC decreases with increasing age in a smaller cohort of 28 fetuses, which had normal developmental follow up at 15 months (Schneider et al. 2009). This is also consistent with results from chapter 3, which demonstrated decreasing ADC values with increasing age at scan in the thalamus in 52 normal fetal controls between 20.43 and 37.71 weeks.

An FA increase with increasing age at scan was also found in the thalamus, but this was not significant after Bonferroni correction. No previous papers have investigated grey matter FA values in the fetus; however an ADC decrease and FA increase with age is consistent with data in preterm neonates (Delpolyi et al. 2005, delpolyi et al. 2006, Mukherjee et al. 2001, Mukherjee et al. 2002). Delpolyi et al, (2006) found a significant increase in FA and decrease in ADC values in the thalamus of 66 preterm neonates imaged between 27 and 43 weeks. This same pattern of development was found in deep grey matter of 153 infants and children scanned from day 1 to 12 years (Mukherjee et al. 2001, Mukherjee et al. 2002).

Fetal and preterm DTI data comparison

This is the first known study to compare FA values in the fetus with preterm neonates across gestation. FA measures were significantly lower in preterm neonates compared to fetuses in the CSO; whilst ADC measures were lower in preterms compared to fetuses in the PLIC and genu, though this was not significant to the Bonferroni level. Previous studies have demonstrated that degree of prematurity significantly affects DTI measures in preterm infants (Anjari et al. 2007), and GA at birth was therefore included in the analysis in this study. Only one previous in vivo study has compared ADC measures of preterm infants to fetuses (Viola et al. 2011), but they focused on near term equivalent age (TEA) and were therefore unable to discern any differences in development over a large gestational age range.
The FA reduction found in the CSO of preterm neonates compared to fetuses is comparable to studies comparing preterm infants at term equivalent age (TEA) to term-born controls (Anjari et al. 2007, Huppi et al. 1998). A study using tract based spatial statistics (TBSS) demonstrated a significant reduction of FA in the CSO, as well as in frontal WM and the genu of preterm neonates at TEA compared to controls (Anjari et al. 2007). They suggested that this was a result of delayed or altered maturation of WM tracts in preterm neonates in these regions. Huppi et al, (1998) also found reduced anisotropy measures of preterm neonates compared to controls, but using a ROI approach, and found a significant reduction in the PLIC. FA reductions are also evident later in life in children born prematurely compared to term born controls; and these observed WM alterations extend to adolescence and adulthood (Vangberg et al. 2006, Constable et al. 2008).

The lower (but not significant) FA values observed in the thalamus of preterm infants compared to fetuses are consistent with evidence of disruption of thalamic development in neonates born prematurely (Ball et al. 2012, Ball et al. 2013a). Histological evidence has shown that thalamocortical connections have been established by TEA in humans and other primates (Kostovic and Rakic 1984, LaMantia and Rakic 1990, Kostovic and Jovanov-Milosevic 2006), and so it is possible that premature birth leads to disruptions in these pathways (Ball et al, 2012). Premature birth has been associated with significantly reduced thalamic volume (Boardman et al. 2006), and reduction in thalamocortical projections in preterm infants compared to controls, which may reflect reduced cell and axon numbers (Ball et al. 2012). Reduction in volume of deep grey matter in preterm infants has also been correlated with neurodevelopmental disability at 1 year (Inder et al. 2005).

ADC results comparing preterm and fetal cohorts were different from DTI data acquired postnatally. ADC measures were lower in preterm infants compared to fetuses in the PLIC and the genu; but this was not significant after Bonferroni correction. Previous studies have found an increase in ADC values of preterm neonates at TEA compared to controls. Huppi et al, (1998a) found significantly increased ADC values in the central WM of 17 preterm infants imaged at TEA compared to 7 term-control infants. A larger study of 60 preterm infants demonstrated significantly increased ADC values compared to 21 term controls in the frontal and occipital WM (Ling et al. 2013). In contrast, a significant reduction in ADC values of preterm neonates compared to fetal controls was demonstrated in this chapter. The reason for this may be due to the comparison of in utero versus ex utero data; as body water mass rapidly decreases shortly after birth (Zelenina et al. 2005) and ADC values reflect overall brain water content (Neil et al. 1998,
Neil et al. 2002). It is therefore probable that the increased ADC values in the fetal brain represent the increased molecular motion due to an increased water content compared to neonates.

Only one previous paper has used diffusion ADC measures to compare fetal and preterm brain development (Viola et al. 2011). This study scanned 24 fetal and preterm neonates at 37 weeks, but made no correction for fetal motion. They found a significant ADC reduction in preterm neonates in the region of the pons and an increase in ADC in the parietal WM. This study concluded that their finding reflected greater water restriction in preterm neonates from modification to the organization of the pontine WM tracts, such as myelination, and microstructural WM anomalies and possible gliosis (Hagen et al. 2007, Neil et al. 2002). However, these conclusions may be superseded by the difference in overall water content between pre- and post-birth in the fetal and preterm brains.

These FA and ADC results between preterm neonates and fetuses, demonstrated the potential of the motion-corrected fetal DTI technique to detect alterations in DTI measures between these two groups. However, methodological reasons for the differences between groups must also be taken into account and are discussed below.

**Limitations**

The fetal DTI motion correction protocol provides FA and ADC measures which are similar to values produced in preterm infants. However, a greater proportion of ROIs from the fetal cohort compared to the preterm cohort could not be analysed due to artefacts or difficulty visualising the region, as is demonstrated in Table 7.5. This together with the low success of tracking fibres compared to neonatal studies (tractography in neonates can be achieved in 100% of studied WM tracts (Berman et al. 2005), compared to 40% found in these fetal data) suggests that motion-corrected fetal DTI data are not as robust as data obtained ex utero. In addition, in cases of excessive motion, FA maps could not be produced in the fetus. Despite this, we achieved a fetal FA production success rate of 72%, which is greater than previously reported for fetal DTI (Bui et al. 2006, Zanin et al. 2011, Kasprian et al. 2008).

The normal fetal cohort was limited for two reasons. First, it included cases of MCDA twin survivors and CHD, which are populations at high risk of adverse development (Partridge et al. 2006, Rao et al. 2004, Martinez-Biarge et al. 2013). However, none of these cases demonstrated CNS abnormalities on MRI and they all had normal deliveries; therefore it was felt to be justified to use this data in the fetal cohort. Neurodevelopmental follow up in these cases will also be
assessed and is currently ongoing. The second cohort limitation was the lack of fetal FA maps at certain GAs. No FA maps were successfully produced in fetuses at the gestational age of 23 weeks. Although one DTI scan was acquired on a fetus of this age, FA map production was not successful due to excessive movement. No DTI scans were acquired in fetuses of 28 or 36 weeks gestation (Figure 7.4). This was due to the lengthy acquisition scan times of the DTI sequence; for example, it was not always possible to perform a lengthy DTI scan on a particular fetal case if the mother became uncomfortable in the scanner; or where excessive fetal movement meant that conventional MRI sequences had to be repeated for clinical purposes.

The comparison between fetal and preterm DTI data suffers from some limitations, for example SNR and motion may have affected results. The field strength of the scanner, which was 1.5-Tesla for fetal scanning compared to 3-Tesla for preterm neonates would have contributed to differences in SNR of the DTI scans. Higher SNR in the fetus compared to the neonatal DTI could potentially lead to low FA values of the fetal cohort; however, significantly greater FA values in the fetal brain were still identified in the CSO compared to preterm infants. DTI sequence parameters may also have played a role in differential diffusion measures between cohorts; ADC values have been shown to decrease with increasing b values in the neonatal brain (Dudink et al. 2008). In this chapter the preterm DTI protocol contained a higher b value of 750 compared to a b value of 500 in the fetal protocol, and so this may have been influential on the ADC results. Additionally, the voxel size of the fetal DTI sequence was greater than for the preterm neonates, as it was set to increase SNR in fetal scans. However, increased voxel size may lead to increased partial volume, especially in regions immediately adjacent to the cerebral-spinal fluid (CSF), such as in the splenium, genu or the occipital WM. Further studies using identical DTI sequence parameters and protocols for fetal and neonatal data are therefore needed for a close comparison between white and grey matter development of these two cohorts.

The lengthy post-processing time for production of fetal FA maps were a further limitation. Post-processing time to produce FA maps took an average of 48 hours; but a longer time was required when there was a greater degree of fetal movement. Further work to reduce the time needed to produce reliable FA maps is necessary if this technique is to be useful in a clinical setting.
**Conclusion**

DTI data presented in this chapter produced diffusion maps that provided FA values across gestation which are comparable to data obtained ex utero. The fetal DTI motion correction technique also results in less exclusion of data compared to previous papers, allowing analysis on larger fetal cohorts. FA and ADC changes were quantified with increasing age at scan in a cohort of fetuses without CNS abnormalities. The potential of the technique to provide tractography data in this fetal cohort was also demonstrated, although its use is currently limited compared to ex utero data. This is the first study comparing FA values of the fetus compared to preterm neonates. Significant white and grey matter alterations in DTI measures were found between these groups, which is consistent with previous studies suggesting that development is affected by exposure to an ex utero environment. This optimised fetal DTI approach therefore has the potential to explore the relationship between in utero and ex utero brain development; and may be used in the future to study abnormalities of pregnancies in utero.
8 Main discussion

The primary objective of this thesis was to establish quantitative measurements of normal development in the fetal brain across a range of gestational ages using diffusion magnetic resonance imaging (MRI). The secondary aim was to compare diffusion metrics from these data in normal fetuses to those with isolated ventriculomegaly (VM) and to preterm infants.

This primary aim was achieved using two methodologies. In the first, a diffusion weighted imaging (DWI) sequence and protocol was optimised to produce ADC maps of the fetal brain with a short acquisition time for application in clinical settings (chapter 3). In the second a motion-corrected diffusion tensor imaging (DTI) technique was used to produce fractional anisotropy (FA) and apparent diffusion coefficient (ADC) measures in utero (chapter 7). Whilst motion-corrected DTI techniques have previously produced reliable ADC values (Jiang et al. 2009), the protocol required long acquisition times and the post-processing was manually intensive and lengthy. As a result, this technique was of limited value within a clinical setting, highlighting the importance of a fast fetal DWI acquisition to produce good quality ADC values within a short time frame.

The study described in chapter 3 provided improved quality ADC maps in the fetal brain derived from a short DWI sequence. Significant decreases in the thalamus and cortex with increasing gestational age were found. These results were consistent with established ADC data in the preterm neonatal brain (Partridge et al. 2004, Huppi et al. 1998, Neil et al. 1998). Reduced ADC measures over this developmental period may represent a reduction in total brain water content and an increased hindrance to water molecular motion due to maturation of microstructural barriers such as cells membranes (Dobbing and Sands 1973, Le Bihan 1995, Mukherjee and McKinstry 2006).

Previous fetal DWI studies have also observed similar ADC changes with increasing gestation, but they have been limited by poor quality data (Righini et al. 2003, Boyer et al. 2013, Schneider et al. 2007). The limitations include artefacts from fetal motion and maternal anatomy surrounding the fetal head, image distortion and low signal-to-noise ratio (SNR). Further shortcomings from previous fetal DWI studies result from inappropriate criteria for selection of normal controls. The study in chapter 3 was therefore designed to address these limitations. To ensure that data were reliable and that there was minimal degrading of images due to motion, strict criteria and protocol for data exclusion were designed. The resulting ADC values derived from the optimised fetal DWI
protocol were similar to results from the motion-corrected DTI technique used in chapter 7, that has previously been validated by comparison of data in the adult brain with and without head movement (Jiang et al. 2009).

The improved DWI technique produced images within 24 seconds, which is particularly valuable within a clinical setting. DWI is clinically useful for identifying acute ischaemia in neonates, and might also prove valuable in the fetus; such as in cases of sudden death of a monochorionic twin where the surviving twin is at immediate risk of widespread ischaemic brain injury. Fetal DWI could be used in this setting to detect abnormalities before they become apparent on conventional imaging.

Fetal DWI is also suitable for objective quantification of the fetal brain where there is a high risk of complications or abnormal development. In chapter 4, normal brain development in the large fetal control cohort was compared to cases of antenatal isolated VM and congenital heart disease (CHD). These two clinical cohorts were chosen due to their high referral rate for fetal MRI and because previous studies in VM and CHD neonates have found increased ADC values compared to controls (Gilmore et al. 2008, Miller et al. 2007). The study assessed whether these altered ADC values were also apparent in utero.

No previous studies have measured ADC values in fetuses with isolated VM. Only one study has compared ADC measures in fetal CHD cases to controls; but this paper included only 3 cases studies with severe CHD and did not address the limitations associated with fetal DWI mentioned above. Chapter 4 therefore provided the first in utero ADC results in cohorts of isolated VM and CHD. These results also had the advantage of comparison to ADC values in a large (n=52) normal fetal control cohort. Use of small cohort numbers, and specifically control cohort numbers, has been a major shortcoming of previous papers (Sanz-Cortes et al. 2010, Mignone Philpott et al. 2013).

No significant differences in ADC values were found in the white or grey matter of the fetal CHD cases compared to controls. This may be due to relatively small numbers (n=11) or the non-homogeneous group of CHD cases. Further research is therefore warranted in this cohort to establish whether these results were due to lack of underlying differences in structure between cohorts in utero or due to the limitations of the study. The optimised in utero DWI sequence did, however, detect increased ADC values in isolated VM cases compared to controls in the occipital WM and cortex. Differences in ADC values between cohorts were further tested with an aged-
matched comparison, which supported the evidence of elevated ADC values in occipital WM, but not in the cortex.

In chapter 5, tract-based spatial statistics (TBSS) was used to assess WM differences between neonates with isolated VM compared to controls. This analysis demonstrated significant ADC increases and FA decreases in the posterior thalamic radiation, sagittal stratum and corpus callosum. The posterior thalamic radiation contains the optic radiations, which are likely to be captured in the occipital WM region of interest (ROI) analysed in the fetal brain in chapter 4. Taken together, the results from chapters 4 and 5 provided consistent evidence of altered occipital lobe WM structure in isolated VM, which appears to originate in utero. These are the first results to demonstrate significant alterations of DTI values in this region in isolated VM compared to controls.

Chapter 5 also identified the splenium of the corpus callosum as a region of significantly altered FA and ADC values between isolated VM and control neonates. An ADC increase and FA reduction in isolated VM cases was found, which was consistent with previous papers (Gilmore et al. 2008, Goodlett et al. 2009). However, chapter 4 did not identify the splenium as a region of significant difference between cohorts in utero. The splenium is a particularly difficult region for ROI analysis in the fetal brain due to its small size. The effects of partial volume due to its close proximity to cerebral spinal fluid (CSF) and the large voxel size produce further difficulties in accurate quantification; this may have affected ADC values and concealed any underlying ADC difference between cohorts in utero. This highlights the ongoing limitations of in utero DWI, which are further discussed in the next section.

Results from chapters 3 and 7 demonstrated the frontal WM to be a particularly interesting region to study in the developing fetal brain. There was a significant increase in ADC values from 20^{\text{th}} to 37^{\text{th}} weeks gestation in the frontal WM in chapter 3 (this increase appeared to plateau at approximately 34 weeks), and from 21^{\text{st}} to 37^{\text{th}} weeks using the motion-corrected fetal DTI data in chapter 7 (although this increase was not significant after Bonferroni correction). Significant increases in ADC values with increasing age at scan in WM have previously been observed in the fetal brain (Cannie et al. 2007). They have been hypothesised to result from progression of WM fibres from a tortuous axonal state to more coherent bundles (Zanin et al. 2011). However, in the preterm brain (chapter 6), ADC values did not increase in any WM region with increasing age at scan in the frontal WM, ADC values significantly decreased after approximately 35 weeks. However the region encompassed the entire frontal lobe WM, rather than the smaller ROI employed to study the fetal brain in chapters 3 and 7, and so these results are not directly
comparable. The age at scan was greater in preterm neonates compared to fetuses, ranging from 26 to 45\textsuperscript{th} weeks, which may also have contributed to the different ADC trajectory found between chapters. The frontal WM is therefore discussed further with regards to limitations and further research in the following section.

Ex utero development is not representative of normal intrauterine development, even in the absence of focal lesions (Dyet et al. 2006); however advanced MRI techniques can be used to study neurodevelopment in the neonatal brain which are not yet possible in the fetal brain. Chapter 6 established volumetric and DTI measures with increasing age at scan in a large cohort of 208 preterm neonates without focal lesions using a novel brain segmentation approach (Makropoulos et al. IN PRESS). WM, cortical and sub-cortical grey matter demonstrated distinctive patterns of maturation. DTI and volumetric measures changed at a non-uniform rate with increasing age at scan between 26 and 45\textsuperscript{th} weeks. Whilst non-uniform FA changes with increasing age at scan have previously been observed in WM (Aebly et al. 2012) and the cortex of preterm infants (Ball et al. 2013b), this was the first study to demonstrate widespread WM, cortical and sub-cortical non-uniform changes obtained with FA, ADC, AD and RD with age at scan in preterm infants. These results highlighted the dynamic maturation of complex neuronal and dendritic architecture, including synaptic processes, apoptosis, pre-myelination and the onset of myelination. Histological examination alongside DTI analysis would be valuable to identify the exact maturational processes associated with changes in DTI measures across the third trimester. However the high survival rate of preterm neonates, especially in cases without overt injury or lesions, means that post-mortem data from this group are limited.

A decrease in ADC values with increasing age at scan was observed in all regions of the preterm brain in chapter 6. This was similar to results in the fetal brain from chapters 3 and 7 that showed significant ADC reductions across gestation in the thalamus, cortex and cerebellum using the optimised in utero DWI and motion-corrected DTI techniques. Axial diffusivity (AD) and radial diffusivity (RD) measures also significantly decreased with increasing age at scan in the preterm brain in all regions (except AD in the corpus callosum). A previous study in the preterm brain found that RD decreased with increasing age, but no significant change was found with AD (Partridge et al. 2004). This has led to the hypothesis that RD decrease is the driving force behind FA increase with increasing age (Partridge et al. 2004, Dubois et al. 2008). Results from chapter 6 demonstrated decreases in AD as well as RD values with increasing age at scan during early post-natal development; although the rate of AD reduction was lower than that of RD. This AD reduction may be attributed to the intermingling of axonal branches, the elimination of

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overabundant axons and the reduction of the length of axons during refinement processes (Gao et al. 2009).

FA values significantly increased in the majority of WM and sub-cortical grey matter regions with increasing age at scan, and significant FA reductions were observed in the cortex of the preterm brain in chapter 6. Significantly increased FA values with increasing gestational age were also found in the fetal brain (chapter 7) in the PLIC and splenium of the corpus callosum, and the thalamus, although the latter was not significant after Bonferroni correction. FA measures in the WM, cortex and thalamus have previously been the focus of research in preterm infants (Ball et al. 2013b, Partridge et al. 2004, Mukherjee and McKinstry 2006); however other sub-cortical grey matter regions have been less intensively investigated. The hippocampus, amygdala, sub-thalamus, lentiform and caudate nucleus are difficult regions to study due to their small size and position within the brain and have therefore not been the focus of previous DTI research in the preterm brain. Using the novel segmentation technique, results from chapter 6 therefore provides the first detailed characterisation of DTI and volumetric measures within these regions during the post-natal preterm period, giving insight into cell proliferation, migration, axonal growth, dendritic arborisation and pre-myelination and myelination within these regions.

The large cohort of preterm neonates in chapter 6 allowed detailed assessment of clinical perinatal factors that may be associated with changes in DTI or volumetric measures in the developing brain. The degree of prematurity significantly affected volumetric measures, but did not affect any DTI measures when other perinatal clinical factors were included in the regression model. This confirmed previous results in smaller cohorts of preterm infants (Bonifacio et al. 2010). The analysis found that head circumference (HC) at birth, weight gain per week and number of days on respiratory support were significantly associated with altered white and grey matter development of the preterm brain. Within a clinical setting, these results may allow identification of neonates at the highest risk of alterations in brain structure.

Chapter 7 was the first study demonstrating normal neurodevelopmental FA and ADC changes in the fetal brain across gestational age using a motion-corrected DTI protocol. Increased FA values in the PLIC and splenium, and decreased ADC values in the thalamus and cerebellum were observed between 21\textsuperscript{2} and 37\textsuperscript{15} weeks gestation in a cohort of 26 fetuses without CNS abnormalities. The study demonstrated that the advantages of this technique included the production of FA and ADC images that were comparable to images produced ex utero. In addition, the use of this technique resulted in less exclusion of data compared to previous papers, allowing analysis on larger fetal cohorts. The potential of the in utero DTI technique to provide
tractography data in this fetal cohort was also demonstrated, although the study showed that its use remains limited compared to ex utero data.

This study was also the first to compare FA values in the fetal brain to preterm neonates over a large age range. Differences in the trajectories of white and grey matter DTI measures were found between these groups. This was consistent with previous studies assessing term born infants and preterm infants at term equivalent age and provides further evidence that brain development is affected by early exposure to an ex utero environment.

8.1 Further research

Optimisation of the in utero DWI protocol has implications for use within the clinical setting and facilitates future research into the use of ADC values to aid diagnosis of abnormal brain development. Further studies investigating ADC differences between patient and normal control groups would particularly benefit from a study design which included large cohort numbers and aged-matched groups for comparison. Repeated scans during pregnancy and neurodevelopmental follow-up would allow for a more powerful longitudinal analysis. Neurodevelopmental follow up is currently ongoing in the fetal cohorts. Longitudinal studies would be useful to elucidate whether early alterations in fetal ADC values between clinical and control cohorts could be used as early predictors of long term neurodevelopmental outcomes.

Exploratory results using fetal DWI, reported in Appendix E, indicate the potential importance of fetal DWI in cases of abnormal or high risk pregnancies. Frontal WM ADC values appeared to be elevated in case studies compared to controls at gestational ages below 25 weeks. Whilst no conclusions can be drawn from this preliminary data, they suggest the importance of further research into the potential of frontal WM ADC values to provide structural biomarkers of abnormal development during early gestation.

The fetal DWI sequence suffers from a relatively large voxel size which may have led to partial volume of neighbouring tissue, especially in small regions such as the cerebellum or the genu and splenium of the corpus callosum. This was specifically a problem at younger gestational ages, due to the smaller brain size. However, a decrease in the size of the voxel meant a decrease in SNR, which was already a limitation of fetal DWI. Research is currently underway within the department to optimise and implement fetal DWI for use in a 3-Tesla MR scanner. 3-Tesla MR scanners have the advantage of increased SNR due to higher magnetic field strength. This may allow smaller
voxel sizes to be used in future research whilst maintaining the SNR achieved at 1.5-Tesla, which
would reduce current limitations of partial volume within small regions of the brain and may allow
more precise assessment of these regions. Plans to build an optimised dedicated coil for fetal
imaging will also improve SNR.

In chapter 4, significant ADC increases were found in the cortex of fetal isolated VM cases with
increasing gestation compared to controls. However this result was not confirmed when using an
aged-matched comparison between cohorts. Further investigation into cohort differences in
neonates would help establish whether ADC differences resulted from alterations in the cortical
structure of the isolated VM fetal brain, or whether limitations, such as partial volume described
above, drove these results. A novel technique that extends the TBSS methodology to the cortex
(Ball et al. 2013b), could be used to provide an observer-independent analysis on DTI differences
between cortical structure of groups with isolated VM compared to controls. Results from such
analysis in neonates could provide evidence to support cortical ADC increases in the fetal brain in
isolated VM cases compared to controls.

TBSS revealed significant ADC increases and FA decreases in neonates with isolated VM
compared to controls in WM tracts that have been associated with deficits in language, motor,
cognitive and attention skills (Dramsdahl et al. 2012, Catani 2007, Doricchi and Tomaiuolo 2003,
et al. 2011, Tanabe et al. 1987). Children with antenatal isolated VM have also demonstrated
deficits in these domains (Gomez-Arriaga et al. 2012, Lyall et al. 2012, Sadan et al. 2007).
Neurodevelopmental data in the VM cohort in chapter 5 is currently on-going. Future TBSS
studies that include neurodevelopmental follow up could determine whether the observed
regional ADC and FA changes in isolated VM neonates compared to controls are associated with
impaired neurodevelopmental outcome; thereby aiding antenatal counselling.

The potential of the fetal DTI motion correction technique to provide tractography data in a fetal
cohort was also demonstrated, but its use remains limited compared to ex utero data. The motion
correction algorithm and protocol could be further developed to address remaining limitations
including the long post-processing time required to produce FA maps, and SNR. These advances
may produce FA data that is capable of supporting post-acquisition techniques, such as TBSS, and
enable fetal tractography to be comparable with what can be achieved in the post-natal brain.
8.2 Conclusion

This thesis has shown that fetal DWI is feasible for use within a clinical setting. The optimised DWI sequence produced improved quality ADC images within 24 seconds. The feasibility of producing FA images of the fetal brain over a wide range of gestational ages was also demonstrated. However fetal DTI is still limited when compared to DTI in neonates.

These diffusion MR imaging approaches can provide valuable information on normal and abnormal brain development in vivo. Both of these techniques have the potential to explore the relationship between in utero and ex utero brain development. This enables investigation into ex utero preterm growth compared to normal development, thereby allowing assessment of the impact of environmental influences on the premature brain. Moreover, these techniques can be used to study aberrant brain development in utero and may assist in developing future strategies for early detection of abnormal maturation and design of protective approaches for the management of complicated pregnancies.
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Appendix

Appendix A

Maternal anxiety was assessed using a visual analogue scale (VAS) seen below. The VAS was designed as a 100mm scale, with the far left side indicating zero anxiety, and the far right side indicating maximum anxiety. Mothers were asked to mark along the 100mm scale their level of anxiety at that moment. The VAS score comprises the length (in mm) along the line that the mark was made.

**Before Scan**

How anxious do you feel today? Place a vertical mark on the line below to illustrate how anxious you feel at this moment

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all anxious</td>
<td>Extremely anxious</td>
</tr>
</tbody>
</table>

**After Scan**

How anxious do you feel? Place a vertical mark on the line below to illustrate how anxious you feel at this moment

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Not at all anxious</td>
<td>Extremely anxious</td>
</tr>
</tbody>
</table>

*Not to scale*
Appendix B

The figure below shows Bland Altman plots comparing ROI shapes within the corpus callosum reported in chapter 3. There was significant agreement between measures in each case.

The figure above shows different shaped ROI (6 voxels) in the genu of an ADC map of a 24.14 week fetus: box (A), line (B) and freehand (C) were compared. There was significant agreement between measures in each case ($p<0.0011$). Intra-class correlation coefficients in all regions were significant (genu: line to box=0.831, box to free=0.776, free to line=0.822; splenium: line to box=0.713, box to free=0.784, free to line=0.776).
Appendix C

Three groups of ADC values are plotted against age at scan in the graph below. ADC values are derived from 1) the optimised fetal DWI sequence (chapter 3); 2) the motion-corrected fetal DTI protocol (chapter 7) and 3) term-born control neonatal DTI scans (chapter 5). ADC measures (units: $\times 10^{-3}\text{mm}^2/\text{s}$) in the fetal brain are similar between those derived from DWI or DTI. In all regions ADC values decrease with age after birth.
## Appendix D

Non-linear relationships between PMA at scan and quantitative measures of segmented neonatal regions found in Chapter 6.

<table>
<thead>
<tr>
<th>Region</th>
<th>FA</th>
<th>ADC</th>
<th>Axial diffusivity</th>
<th>Radial diffusivity</th>
<th>Relative volume</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R²</td>
<td>Coef 1</td>
<td>Coef 2</td>
<td>R²</td>
<td>Coef 1</td>
<td>Coef 2</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>*0.03</td>
<td>-0.02</td>
<td>0.00</td>
<td>0.44</td>
<td>-0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>Amygdala</td>
<td>0.18</td>
<td>-0.01</td>
<td>0.00</td>
<td>0.58</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.12</td>
<td>0.00</td>
<td>0.00</td>
<td>0.80</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Brainstem</td>
<td>0.65</td>
<td>-0.01</td>
<td>0.00</td>
<td>0.43</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>*0.43</td>
<td>-0.02</td>
<td>0.00</td>
<td>*0.69</td>
<td>0.06</td>
<td>0.00</td>
</tr>
<tr>
<td>Thalamus</td>
<td>0.44</td>
<td>0.00</td>
<td>0.00</td>
<td>0.75</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Subthalamic nucleus</td>
<td>0.68</td>
<td>-0.01</td>
<td>0.00</td>
<td>0.59</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Lentiform nucleus</td>
<td>*0.61</td>
<td>-0.03</td>
<td>0.00</td>
<td>0.72</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>Corpus callosum</td>
<td>0.42</td>
<td>-0.01</td>
<td>0.00</td>
<td>0.36</td>
<td>-0.05</td>
<td>0.00</td>
</tr>
<tr>
<td>Frontal lobe WM</td>
<td>*0.58</td>
<td>-0.02</td>
<td>0.00</td>
<td>*0.52</td>
<td>0.06</td>
<td>0.00</td>
</tr>
<tr>
<td>Parietal lobe WM</td>
<td>*0.60</td>
<td>-0.01</td>
<td>0.00</td>
<td>0.73</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>Occipital lobe WM</td>
<td>*0.27</td>
<td>-0.02</td>
<td>0.00</td>
<td>*0.74</td>
<td>0.09</td>
<td>0.00</td>
</tr>
<tr>
<td>Temporal lobe WM</td>
<td>*0.30</td>
<td>-0.01</td>
<td>0.00</td>
<td>*0.58</td>
<td>0.06</td>
<td>0.00</td>
</tr>
<tr>
<td>LOT WM</td>
<td>0.06</td>
<td>-0.01</td>
<td>0.00</td>
<td>*0.55</td>
<td>0.08</td>
<td>0.00</td>
</tr>
<tr>
<td>Cingulate WM</td>
<td>*0.19</td>
<td>-0.03</td>
<td>0.00</td>
<td>*0.42</td>
<td>0.05</td>
<td>0.00</td>
</tr>
<tr>
<td>Frontal lobe GM</td>
<td>*0.65</td>
<td>-0.04</td>
<td>0.00</td>
<td>0.38</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Parietal lobe GM</td>
<td>*0.66</td>
<td>-0.06</td>
<td>0.00</td>
<td>0.50</td>
<td>0.04</td>
<td>0.00</td>
</tr>
<tr>
<td>Occipital lobe GM</td>
<td>*0.70</td>
<td>-0.06</td>
<td>0.00</td>
<td>*0.58</td>
<td>0.07</td>
<td>0.00</td>
</tr>
<tr>
<td>Temporal lobe GM</td>
<td>*0.71</td>
<td>-0.03</td>
<td>0.00</td>
<td>0.44</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>LOT GM</td>
<td>-0.03</td>
<td>0.00</td>
<td>0.56</td>
<td>0.03</td>
<td>0.00</td>
</tr>
<tr>
<td>----------</td>
<td>--------</td>
<td>-------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Cingulate GM</td>
<td>*0.54</td>
<td>-0.07</td>
<td>0.00</td>
<td>*0.19</td>
<td>0.05</td>
<td>0.00</td>
</tr>
</tbody>
</table>

* Significant quadratic relationships best fit the data. R² = Adjusted R²; coef = b coefficient; Coef 1 = PMA at scan b coefficient; Coef 2 = PMA square b coefficient; Exponential relationships are demonstrated for volume measures.
Appendix E

The graph below shows ADC values derived from the optimised fetal DWI sequence in the fetal normal control cohort from chapter 3, compared to clinical case studies in the frontal WM. ADC values did not appear to be altered in any other white or grey matter regions.

ADC values (units: x10^{-3}mm^2/s) in the frontal WM of clinical fetal cases appear higher than control values in early gestation. At GA<25 weeks, ADC values from the case studies in frontal WM values fall outside the 5th-95th percentile (dashed lines). This can be seen cases of later developing neonatal seizures, trisomy 21, microcephaly, one of the haemorrhage cases and the encephalocele case.
The table below gives the clinical details of these clinical cases.

<table>
<thead>
<tr>
<th>Case</th>
<th>GA at scan</th>
<th>Clinical details</th>
<th>Fetal MRI report details</th>
<th>GA at birth</th>
<th>Apgar scores at 1 and 5 mins</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>27.57 and 32.57</td>
<td>severe IUGR</td>
<td>bilateral mild VM, with delayed cortical folding</td>
<td>36</td>
<td>7/1, 8/5</td>
<td>F</td>
</tr>
<tr>
<td>2</td>
<td>22.29</td>
<td>Trisomy 21 and AVSD with small atrial compartment and small ventricular septal defect</td>
<td>normal MRI brain appearance</td>
<td>38</td>
<td>9/1, 10/5</td>
<td>M</td>
</tr>
<tr>
<td>3</td>
<td>29.29</td>
<td>CMV positive on Amniotic fluid</td>
<td>mild VM and cerebellar vermis rotation</td>
<td>37</td>
<td>9/1, 10/5</td>
<td>F</td>
</tr>
<tr>
<td>4</td>
<td>20.14 and 30.14</td>
<td>Neonatal seizures following birth</td>
<td>lemon shaped brain, but normal appearance to brain on MRI</td>
<td>41.3</td>
<td>9/1, 10/5</td>
<td>M</td>
</tr>
<tr>
<td>5</td>
<td>24.14</td>
<td>Abnormal fetal MRI report</td>
<td>Fetal MRI results showed haemorrhage in the cerebellum and small subependymal cyst in the germinal matrix</td>
<td>TOP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>33.43</td>
<td>Abnormal fetal MRI report</td>
<td>Fetal MRI results showed antenatal haemorrhagic venous infarct with possible calcification, and involvement of the corpus callosum and asymmetry and atrophy of the brainstem</td>
<td>39</td>
<td>9/1, 10/5</td>
<td>F</td>
</tr>
<tr>
<td>7</td>
<td>35.85</td>
<td>Abnormal fetal MRI report</td>
<td>Fetal MRI results showed severe VM and evidence of an old haemorrhage along the germinal matrix and in the ventricles</td>
<td>38</td>
<td>9/1, 10/5</td>
<td>M</td>
</tr>
<tr>
<td>8</td>
<td>22.57</td>
<td>Abnormal fetal MRI report</td>
<td>Fetal MRI results showed microcephaly, a rudimentary brain with fusion of structures across the midline and a single isolated ventricle.</td>
<td>TOP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>21.85 and 27.85</td>
<td>Abnormal fetal MRI report</td>
<td>Fetal MRI results showed occipital encephalocele and severe ventricular dilatation with dilated third ventricle</td>
<td>TOP</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: CMV = cytomegalovirus*