The neonatal brain in Down Syndrome : white matter alterations and the relationship between brain volumes and childhood cognitive abilities

The neonatal brain in Down Syndrome: White matter alterations and the relationship between brain volumes and childhood cognitive abilities

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Declaration of Originality

The research presented in this thesis is my own and was conducted at the Babylab, Birkbeck, University of London, and at the Centre for the Developing Brain, King's College London. All sources have been appropriately referenced and any work that was conducted by others, has been declared.

Publications

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Abstract

Down Syndrome (DS) is the most common genetic cause of intellectual disability. This project is the first attempt to (1) determine associations between early structural brain imaging with subsequent neurodevelopmental outcomes in DS; and (2) explore white matter (WM) alterations in neonates with DS. Magnetic resonance imaging (MRI) was performed on neonates with DS and age- and sex-matched controls from 32 to 45 weeks postmenstrual age. WM microstructure alterations were explored with Tract-Based Spatial Statistics (TBSS) and Fixel-based Analysis (FBA) in a subsample of 10 neonates with DS and 39 typical developing (TD) controls. Planned and exploratory correlations were performed to analyse the relationship between neonatal brain volumes and cognitive outcome in infants with DS in an independent sample of 12 individuals with DS, who had neuropsychological assessments performed, between 6 months and 5 years of age. The diffusion data showed that all TBSS metrics (including fractional anisotropy and mean diffusivity) were reduced in the neonates with DS, relative to TD, in anterior WM tracts, corpus callosum and cerebral peduncles. FBA results showed statistically significant differences between DS and TD groups in WM organisation, but image registration was compromised by underlying volumetric difference between the groups and therefore the results were deemed unreliable. Regarding the structural data, there were no significant correlations between any of the brain tissue volumes and the outcome measures, but there were medium to large effect sizes (e.g., correlations between cortical grey matter and Mullen Receptive Language scores). However, there was a significant strong negative correlation between lateral ventricle volumes and Mullen Receptive Language Developmental quotient (DQ) scores (i.e., larger ventricle volumes relate to lower DQ

scores, or bigger delay). Overall, the research demonstrated that WM alterations in DS are present from early on in life, consistent with previous findings. However, it is too early to determine whether neonatal brain volumes might help predict measures of cognitive abilities in DS. Further research is needed to support these findings, to investigate whether other brain measures might be better predictors of outcomes (e.g., functional measures), and to understand the developmental mechanisms underlying the DS genotype-phenotype relationship across multiple levels of description.

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This PhD has been possible because of the constant support of my partner and my family. Thank you, Stefan for believing in my dream and unconditionally supporting me through the journey. Aita, Ama eta Ibone, eskerrik asko zuen laguntza eta animoengatik. I would not have been able to get where I am without all of you by my side.

To S. Lewis, we did it!

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Abbreviations

AD	Axial diffusivity
AE	Age equivalent
B0	Main magnetic field
B1	Oscillating magnetic field
BSID	Bayley Scales of Infant Development
BVS	Brain ventricular system
CA	Chronological age
CC	Corpus callosum
CCS	Cognitive composite score in BSID-III
CFE	Connectivity-based fixel enhancement
cGM	Cortical grey matter
CHD	Congenital Heart Defect
CNS	Central nervous system
CP	Cortical plate
CSF	Cerebrospinal fluid
dHCP	Developing Human Connectome Project
dMRI	Diffusion weighted MRI
dODF	Diffusion orientation density function
DS	Down syndrome
DT	Diffusion tensor
DTBM	DTI driven tensor-based morphometry
DTI	Diffusion tensor imaging
DTI-TK	DTI-ToolKit
eCSF	Extracerebral cerebrospinal fluid
ED	Embryonic day
EEG	Electroencephalography
EGL	Extra granular layer
EL	Expressive language
EPI	Echo planar Imaging

FA	Fractional anisotropy
FBA	Fixel-based analysis
FC	Fibre cross-section
FD	Fibre density
FDC	Combination of Fibre density and cross-section
FEW	Family-wise error
FM	Fine motor
fMRI	Functional Magnetic resonance imaging
fNIRS	Functional near infrared spectroscopy
FOD	Fibre orientation distribution
fODF	Fibre orientation density function
FSL	FMRIB's Software Library
GA	Gestational age
GLM	General Linear Model
GM	Grey matter
G_{PE}	Phase encoding gradient
GrM	Gross motor
G_{SS}	Slice selection gradient
GW	Gestational Week
HARDI	High Angular Resolution Imaging
HC	Head circumference
ICV	Intracranial volume
IZ	Intermediate zone
LCS	Language Composite score in BSID-III
M0	net magnetisation
MA	Mental age
MCS	Motor Composite score in BSID-III
MD	mean diffusivity
MDI	Mental developmental Index score in BSID-II
Mdn	Median
MR	Magnetic resonance

MRI Magnetic resonance imaging

MSEL Mullen Scales of Early Learning

MZ Marginal zone

NDC Neurodevelopmental condition

NMR Nuclear Magnetic Resonance

NODDI Neurite orientation dispersion and density imaging

PCI Parent child interaction

PDI Psychomotor developmental Index score in BSID-II

PMA Post menstrual age

PP Preplate

RD Radial diffusivity

RF Radiofrequency

RL Receptive language

ROI Region of Interest

SD Standard deviation

SES Socioeconomic status

SNR Signal-to-noise ratio

SP Subplate

TBSS Tract-based spatial statistic

TBV Total brain volume

TD Typical development/developing

TE Echo time

TFCE Threshold free cluster enhancement

TR Repetition time

TTV Total tissue volume

VR Visual reception

VZ Ventricular zone

WM White Matter

Preface

My contributions

The data presented in this thesis were obtained in collaboration with others, and any work that was conducted by others, has been documented in this preface and declared in text. To be able to access all the MRI data presented in this thesis, I entered a formal collaboration with Guy's and St Thomas' NHS Foundation Trust and was appointed a position in the Perinatal Imaging and Health Department, King's College London.

All MRI data from typically developing controls presented in this thesis were obtained as part of the developing Human Connectome Project (http://www.developingconnectome.org). The dHCP is a multi-site cross-sectional observational study, led by King´s College London, Imperial College London and Oxford University, with the aim to map the development of functional and structural connections in the human brain using Magnetic Resonance Imaging. I was not involved in these data collection but was granted access to it.

All MRI data from neonates with DS was obtained at St. Thomas´ Hospital, London as part of the Early Brain Imaging in Down Syndrome (eBiDS) project at the Perinatal Imaging and Health Department, King´s College London. The eBiDS project aims to map and further investigate the developing brain in Down Syndrome. I entered a formal collaboration with eBiDS investigators and was involved in the data collection and liaison with the medical imaging team. I also contributed to data collection by way of attending scanning sessions and answering any queries and doubts the participating families might have had about the project.

The diffusion data presented in empirical Chapter 3 were automatically pre-processed as part of a pipeline set up for the dHCP project, so I was not involved in this step. The remainder of the data analysis was performed by me under the mentorship of researchers at the Perinatal Imaging and Health Department, King´s College London.

The extraction and calculation of the volumetric data presented in empirical Chapter 4 was performed by a fellow PhD student part of the eBiDS project. Part of the neurodevelopmental data presented in this chapter were obtained as part of the dHCP project (for all the typically developing controls) and the LonDownS project (for the non-imaged children with DS, and 5 of the 12 participants in the sample). I collected the neurodevelopmental follow-up data for the remainder 7 children with DS included in the study in Chapter 4, that had been scanned as part of the eBiDS project when they were neonates. I also conducted all the analysis presented in this chapter.

Use of abbreviations

To aid the reader with the large number of abbreviations used in this thesis, aside from the list of abbreviations provided above, each abbreviation will be redefined in each chapter, the first time the term is used.

Chapter 1: Introduction

1.1 Down Syndrome or Trisomy 21

1.1.1 Cause

Down Syndrome (DS) or Trisomy 21 is one of the most common forms of intellectual disability, affecting one in every thousand births (Down Syndrome Association, 2018; Diamandopoulos & Green, 2018) first described by British Physician John Langdon Down in 1866 (Shapiro, 2001). Down Syndrome is caused by either a full or partial additional copy of human chromosome 21, and based on the pattern of this triplication, three different forms can be found (Diamandopoulos & Green, 2018). Trisomy 21 or non-disjunction is the most common form of DS, accounting for 95% of all cases. This occurs due to a failure during sperm or ovum meiosis, resulting in an extra full copy of chromosome 21. Robertsonian translocation is a less common form of DS, accounting for 4% of all cases. In this case, the long arm of chromosome 21 is attached to another chromosome, which will lead to the individual having 46 chromosomes, but the genetic material of 47. Finally, mosaicism is the most uncommon form of DS, accounting

for 1% of all cases. This genetic alteration results in multi division after fertilisation, leading to a combination of tissues and organ cells with either 46 or 47 chromosomes (Diamandopoulos & Green, 2018).

1.1.2 Prenatal testing and diagnosis

In the UK, around 750 babies are born with DS each year, and there are approximately 40,000 people with DS living in the country. Current screening and diagnostic tests can be divided into two main groups, based on how invasive they are (see Table 1.1 for a summary). Screening tests are routine tests offered to all pregnant women, with the aim of identifying those patients that might be at a higher risk of an abnormality (Brindley, 2019). They can be performed as early as the first trimester, and do not confer any risk to the pregnancy as they normally consist of maternal blood or serum testing (Allyse et al., 2015; Anderson & Brown, 2009; Brindley, 2019). When a screening test is positive, the diagnosis can be confirmed or excluded by an invasive diagnostic test, such as chorionic villus sampling (CVS) or amniocentesis (Brindley, 2019). The different tests can be performed at different stages of pregnancy, and they all have different detections rates and miscarriage risks. Whilst there has been an increase in screening testing in the last decade (Ravitsky et al., 2021), termination rates after the detection of DS are unchanged or even decreased compared to historical termination rates (Hill et al., 2017; Jacobs et al., 2016).

Table 1.1 *Prenatal testing and diagnosis summary.*

Name	Timing	Technique description	Detection rate	Miscarriage risk
		Screening (non-invasive)		
Nucal Translucency (NT)	10-14 gw	Ultrasound assessment of sonolucency in the posterior fetal neck.	70-71% with a 3.5-5% false-positive rate.	None
Combined screening		NT Plus pregnancy-associated plasma protein A (PAPP-A) and free beta subunit of human chorionic gonadotropin (B-hCG) maternal serum testing.	78.7- 89% with a 5% false-positive rate.	None
NIPT/Harmony test	≥ 10 gw	Analysing the cell-free fetal DNA (cffDNA) present in a sample of maternal blood to determine the likelihood of a fetal aneuploidy.	99% with a 1%–3%false positive rate	None
Triple test	≥ 12gw	Maternal serum testing.	55-77%	None
Quadruple test	≥ 12gw	Maternal serum testing.	66-84%	None
Diagnosis (invasive)				
Amniocentesis	≥ 15 gw	Transabdominal amniotic fluid sampling.	99.4%	1%
Chorion Villus Sampling (CVS)	≥ 10 gw	Transabdominal or transplacental placental tissue sampling.	97.8%	1%-3%

Note. GW= gestation weeks (information taken from (Allyse et al., 2015; Anderson & Brown, 2009; Brindley, 2019; Reynolds, 2010).

1.1.3 Phenotype and Comorbidities

The phenotype of this genetic alteration is variable, including congenital heart defects (CHD), gastrointestinal abnormalities, craniofacial and skeletal abnormalities, abnormal brain development and functioning, cognitive delays, and increased risk of psychiatric comorbidities, such as, autism, ADHD, depression or dementia (Haydar & Reeves, 2012; Lagan et al., 2020; Startin et al., 2020). The prevalence of these conditions varies through the lifespan of individuals with DS (e.g., Alexander et al., 2016; Startin et al., 2020).

Startin et al. (2020) conducted a study that included 602 individuals with DS from England and Wales, recruited as part of the LonDownS Consortium between 2013 and

2016. The age of the participants ranged from 3 months to 73 years of age and were divided into four age groups: young children (3 months to 5.5 years) (*n*=115), older children (5.5 to 15 years) (*n*= 35), young adults (16 to 35 years) (*n*=170) and older adults (over 36 years) (*n*= 282). They reported cognitive impairment in all individuals. Comorbidities of epilepsy, sleep apnoea, CHD, hypothyroidism, and vision and hearing impairments were consistently found throughout all four age groups. The prevalence of comorbidities increased with age, except for CHD and vision and hearing impairments, which remained similar across the lifespan. More than 50% of the young children with DS had a CHD, with only 19% having corrective surgery, and nearly 40% of those in the older group had dementia (Startin et al., 2020). When examining how these comorbidities related to cognitive functioning the results showed that, in younger children (0-5.5 years) individual differences in raw Mullen Scale of Early Learning (MSEL)(Mullen, 1995) scores (a measure of cognitive functioning for infants and children from birth through 68 months) were mainly explained by age, with no variability explained by comorbidities. However, in younger adults (16-35 years), whilst age did not predict cognitive outcome, higher socioeconomic status (SES) was associated with higher cognitive scores, and autism and epilepsy reliably explained additional variance, with the presence of either comorbidity associated with poorer scores. Therefore, the variables that should be controlled for when conducting research with a DS population seem to be dependent on the age of the sample being studied.

1.2 Cognitive profile in Down Syndrome

The large majority of individuals with DS have IQ scores ranging from moderate to severe intellectual disability (e.g., IQ<70), and their mental age is commonly below

their chronological age (Edgin, 2013; Vicari, 2006). Nevertheless, attempting to describe the cognitive abilities of individuals with DS using a single measure like IQ is insufficient (Wang, 1996). In fact, there is extensive literature showing that individuals with DS have a specific cognitive profile characterised by relative weaknesses in language and memory related abilities, while visual processes are a relative strength (Grieco et al., 2015; Mundy et al., 1995; Vicari, 2006; Wang, 1996).

This uneven cognitive profile, present early on in life, was examined by Fidler et al. (2006), who compared the performance of 2–3-year-old children with DS on the MSEL and the Vineland Scale of adaptive behaviour (Sparrow et al., 2005), with the performance of MSEL mental age (MA) matched typically developing (TD) children, and MSEL MA-matched children with mixed neurodevelopmental conditions. The results showed that the only significant difference in the performance on the Mullen scales was in expressive language, which was significantly better in the TD group compared to the other two groups. More interestingly, the analysis within the DS group showed distinct areas of relative strength (visual perception and receptive language) and weaknesses (gross motor and expressive language). The adaptive behaviour results were similar, where the DS group showed strength in the socialisation area, relative to communication and motor skills. Furthermore, the socialisation skill was at the same level as the MA matched TD group (Fidler et al., 2006).

There is also extensive literature examining the performance of individuals with DS in specific cognitive abilities, and whether this is syndrome specific. For instance, Abbeduto et al. (2001) examined the functioning in receptive and expressive language in people with DS, aged 11 to 23 years, and compared it to a non-verbal IQ matched group

of people with Fragile X syndrome and a MA-matched group of typically developing children, aged 3 to 6 years old. Their preliminary data showed that the group with DS had relative impairments in some receptive language domains, and that this was syndrome specific. More interestingly, the receptive language impairments were more pronounced in grammar related tasks than in semantically oriented ones. The group with DS performed significantly worse in expressive language, compared to receptive language, which was consistent with the results from Fidler et al. (2006). Their expressive language performance was also significantly worse that the Fragile X syndrome and the TD group, showing, once more, syndrome specificity (Abbeduto et al., 2001).

Moreover, evidence from infants with DS shows impaired or abnormal performance relative to MA matched TD infants, in abilities that could be seen as precursors to language, or steppingstones in language development. For instance, Mundy et al. (1995) found that, when compared to MA-matched TD infants, 12–36-month-old infants with DS displayed consistent differences in nonverbal requesting and responding to joint attention, and that individual differences in nonverbal requesting within the group with DS were associated with their development of expressive language. Visual face scanning and integration of audio-visual perception are two other processes that have been related to language development and have been found to be delayed or atypical in infants with DS when compared to matched TD controls (D'Souza et al., 2015, 2016). Similarly, infants with DS have a more extensive use of communicative gestures compared with TD infants at the same stage of communicative-linguistic development, which has been interpreted as an indirect sign of inadequate development of a verbal ability (Vicari, 2006).

Joint engagement, normally assessed during parent-child interactions (PCI), another ability intertwined with cognitive development, is atypical in infants with DS (Adamson et al., 2008; Soukup-Ascençao et al., 2016). In a study examining infants with DS, autistic infants and TD infants, Adamson and colleagues (2008) found that both neurodevelopmental conditions affected the infants' joint engagement experiences during social interactions with a parent. However, this effect was different for each condition, where in the case of children with DS, the ability to infuse symbols into joint engagement was reduced relative to both control groups. Interestingly, the data also showed that, for the three groups, a context for early language learning was facilitated when symbol-infused supported joint engagement was used during parent-child interactions (Adamson et al., 2008). So, the reduced ability to infuse symbols in infants with DS might suggest a hindered context for early language learning. Similar results were found by Soukup-Ascençao et al. (2016), who studied parent-child interactions in toddlers with DS, William Syndrome (WS) and TD. Overall, TD infants were more attentive to the parent, and their interactions were higher in mutuality and intensity of engagement than the interactions of infants with DS and WS. Moreover, different patterns of correlations were found between the different aspects of PCI (e.g., parent sensitive responsiveness, parent directiveness, infant liveliness) in DS and WS, showing an additional syndrome specific impairment.

Attention, another neuropsychological aspect considered a steppingstone in cognitive development, is also impaired in children with DS beyond their expected mental age (Grieco et al., 2015). Brown et al. (2003) studied sustained attention in infants with WS, infants with DS and two control groups (chronological age and MA matched), and they found that the group with DS had significantly shorter total duration of periods of

sustained attention than all other groups. In a recent study, the authors also found that latency to shift attention in 9-month-old infants with DS was negatively correlated to their scores in the Bayley scales, a measure of cognitive and motor development in infants aged 1–42 months (Fidler et al., 2018).

There is evidence that early measures of cognitive abilities may predict later cognitive outcomes. Marchal et al. (2016) undertook a longitudinal study, assessing 196 infants with DS during their first two years of life, and relating this to their intelligence, adaptive functioning, and motor skills outcomes at 10.7 years old. Their study showed that developmental outcomes at the age of 24 months appeared to be predictive of later functioning. Subsequent motor skills were less well predicted by early development than later intelligence and adaptive functioning.

The majority of studies, to date, have focused on comparing the cognitive abilities of people with DS with other matched groups. However, to truly understand developmental conditions, such as DS, static single snapshot measures might not be appropriate, and developmental trajectories should be taken into account instead (D'Souza et al., 2021; Karmiloff-Smith, 2018). In fact, there are few cognitive differences between DS and TD infants during the first year of life, with most of them emerging subsequently (Karmiloff-Smith et al., 2016; Rodríguez-Barrera & Chaves-Castaño, 2017). However, cognitive delays may start to appear from 12 months in infants with DS, and they show fewer cognitive gains from 12 to 30 months when compared to MA-matched children with other developmental delays (Fidler et al., 2018).

Moreover, different development patterns emerge, both between individuals and between cognitive domains. For instance, general intelligence changes across the lifespan,

as IQ scores tend to drop with age in DS, from a mean of 70 standard score at 6 months to 50 standard scores by 4 years (Edgin, 2013), and it is later affected by early onset dementia risk (Grieco et al., 2015; Vicari, 2006). In spite of this, children with DS continue to acquire different skills, but at a slower rate (Grieco et al., 2015; Wang, 1996). For instance, Tsao and Kindelberger (2009) found in their cross-sectional sample of children with DS that both verbal and non-verbal abilities improved between 6 and 10 years of age, but stagnation or regression happened between 10 and 11 years of age.

When looking at the development of specific skills, different developmental patterns emerge. For instance, although infants with DS follow the same sequence of motor milestones as TD infants, the development of these skills is slower, with the later ones, such as crawling or walking, showing greatest delay (Vicari, 2006). On the other hand, visuospatial abilities seem to develop to a level consistent with their mental age (Grieco et al., 2015; Vicari, 2006). Moreover, as mentioned before, in children and adults with DS language comprehension seems to be less impaired than language production. However, this uneven profile only starts to be evident at around 18 months old (Vicari, 2006). Similarly, long- and short-term memory differences from the TD population are detected across the lifespan, but become more pronounced with age (Grieco et al., 2015). Finally, executive functions, like inhibition, planning or attention seem to be impaired from toddlerhood, and continue to be so through adulthood (Grieco et al., 2015).

Finally, aside from considering cognitive and brain development when studying the population with DS, individual differences should be taken into account, too. In fact, there is a wide variability in both the occurrence and the severity of the phenotypes across the population (Haydar & Reeves, 2012), which are hidden when only studying group

differences. There is extensive literature demonstrating this variability, such as infants' standard composite scores in the Mullen ranging from floor level to scores reaching 80s to 90s (50^{th} centile= 100) (Karmiloff-Smith et al., 2016). Moreover, Marchal et al. (2016) found in their DS cohort at 10.7 years old, that age equivalent intelligence ranged from 10 months to 6 years and 10 months, and the age equivalent adaptive functioning ranged from 1 year and 2 months to 7 ½ years. Similarly, Tsao and Kindelberger (2009) ran a cluster analysis with 88 children with DS whose age ranged from 6 to 11 years old, and the results revealed different patterns of cognitive profiles: the first profile was characterised by equivalent, average performance in verbal and non-verbal tests; the second one had difficulties in all tests, but more prominent in the verbal ones; the third one, however, was noteworthy for its relatively superior performances on the verbal subtests; and, finally, the last profile was characterized by its relatively strong performances on the nonverbal tests. Although the sample was relatively small, his study clearly supports the importance of studying Down syndrome with an acknowledgment of marked individual differences.

1.2.1 Mullen Scales of Early Learning (MSEL)

Several different tools have been used to assess the cognitive abilities of infants and children with DS. In the current thesis, the chosen tool was the Mullen Scales of Early Learning (Mullen, 1995) (methodological considerations included in section 2.2.4), designed to measure cognitive functioning for infants and children from birth through 68 months using 5 subscales: Gross Motor, Visual Reception, Fine Motor, Receptive Language, and Expressive Language. The MSEL were chosen so the collected data could be compared to a larger LonDownS cohort of children with DS, presented in Chapter 4.

1.3 Brain development

The brain is arguably the most complex organ in the human body, and yet much of its development has occurred prior to birth at term. Considering that the majority of the processes are genetically driven, understanding and detecting typical development during the prenatal period in a fetus may assist in the detection of abnormalities in fetuses with DS, and may in turn provide a window of opportunity for interventional strategies designed to improve neurodevelopmental outcomes.

1.3.1 Structural brain development in the TD population

Brain development begins in the third gestational week (embryonic day (ED) 20-27) with the formation of the neural tube, through a process called neurulation (Stiles, 2008a; Stiles & Jernigan, 2010). This process begins when neural folds appear in the previously formed trilaminar germ layer, which progressively fuse together to form a hollow tube, starting from the centre, continuing to the rostral end (ED 25) and finishing in the caudal end by ED27. Before the end of the neural tube closure, the anterior end of the tube starts expanding and changing shape, forming the three primary vesicles or subdivisions of the neural tube: Prosencephalon (forebrain), Mesencephalon (midbrain) and Rhombencephalon (hindbrain). These will further subdivide into the primary five subdivisions of the primitive nervous system by ED49 (~GW7), which will later develop into the major subdivisions of the adult brain (Table 1.2).

Table 1.2 *Primary and secondary neural tube divisions and their derivates in the adult brain.*

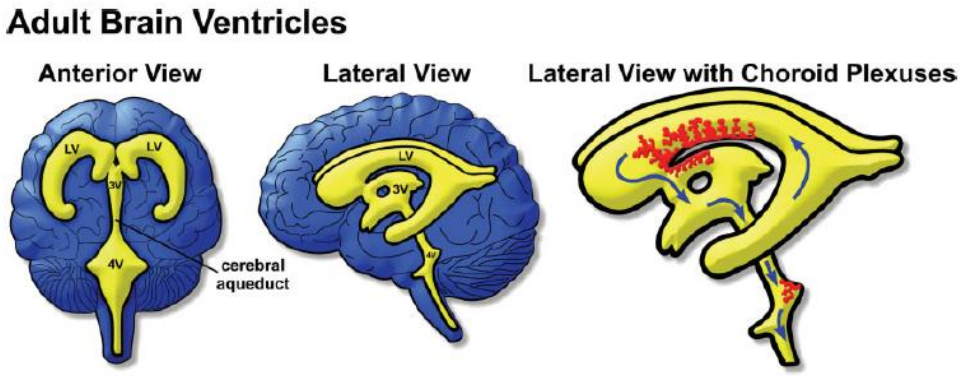
Primary subdivisions	Secondary subdivisions	Mature derivates	Central cavity
Prosencephalon	Telencephalon	Cerebral cortex	Lateral ventricle
		Basal ganglia	
		Basal forebrain	
	Diencephalon	Thalamus	3rd ventricle
		Hypothalamus	
Mesencephalon	Mesencephalon	Mid-brain-tectum	Cerebral aqueduct
Rhombencephalon	Metencephalon	Hindbrains, pons, Cerebellum	4th ventricle
	Myelencephalon	Medulla	
Spinal cord	Spinal cord	Spinal cord	Central canal

This rudimentary tube is the precursor to the central nervous system (CNS), containing a pool of neural progenitor cells which form a single layer of cells lining the centre of the neural tube. As this layer is adjacent to the hollow centre of the tube, the lumen, which will develop into the ventricular system of the brain, it is known as the ventricular zone (VZ). The fate of these progenitor neural cells is determined by their spatial location within the neural tube, with the cells in the most rostral regions giving rise to the brain, and the ones in the most caudal region will give rise to hindbrain and spinal column (Stiles, 2008a; Stiles & Jernigan, 2010).

With the closure of the neural tube, the brain ventricular system (BVS) also begins to develop (O'Rahilly & Müller, 1990). The BVS is a series of connected cavities lying deep within the brain and filled with cerebrospinal fluid (CSF). In mammals the BVS consists of two lateral ventricles (LV), that connect to the 3rd ventricle (3V) via the

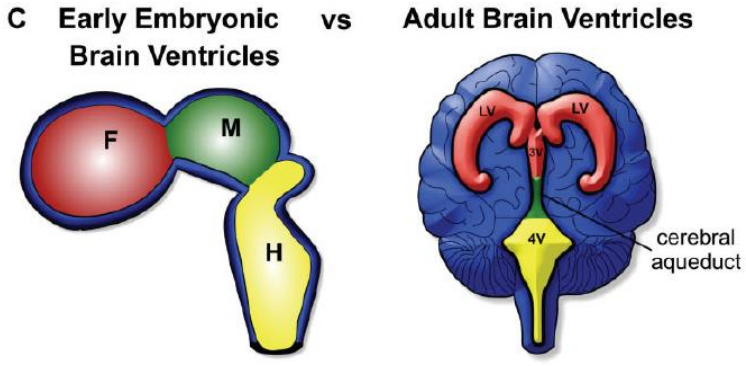
intraventricular foramina, which in turn connects to the 4th ventricle (4V) via the cerebral aqueduct, which is connected to the subarachnoid space (see Figure 1.1) (Fame et al., 2020; Korzh, 2018; Lowery & Sive, 2009).

Figure 1.1 *The adult ventricular system (in yellow). The choroid plexus are represented in red, and the blue arrows represent the flow of the CSF (from Lowery & Sive, 2009).*



During early BVS development, the primary brain vesicles are filled with CSF forming the embryonic forebrain, midbrain and hindbrain vesicles. The embryonic forebrain ventricle eventually splits into the two lateral ventricles and the third ventricle. The midbrain ventricle becomes the narrow cerebral aqueduct which connects the third and fourth ventricles, and the hindbrain ventricle becomes the fourth ventricle (Lowery & Sive, 2009) (see Figure 1.2). The precise positioning of the ventricles, as well as their characteristics and constrictions and bends within each brain region are regulated by patterning genes present since neurulation (Lowery & Sive 2009).

Figure 1.2 *Illustration of early embryonic brain vesicles (Forebrain in red, Midbrain in green and Hindbrain vesicle in yellow) and their adult equivalents (from Lowery & Sive, 2009).*

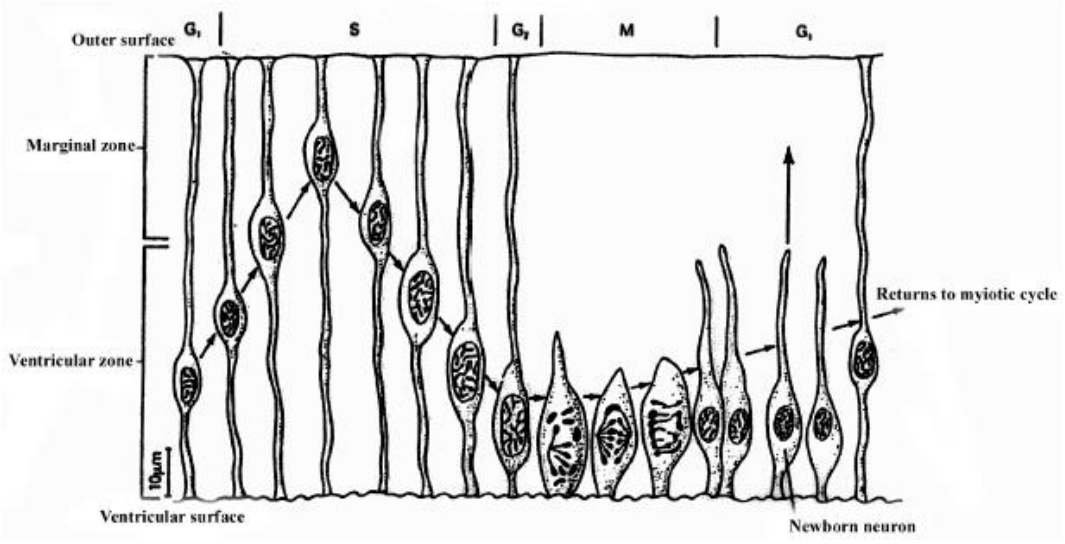


Following early brain ventricle shaping, the ventricles inflate through the secretion of embryonic CSF into the ventricular lumen (Lowery & Sive, 2009). Early in development CSF is produced by the neuroepithelium, while later, most is produced by specialized vascularized epithelial sheets called the choroid plexuses. In fact, the ventricles inflate with CSF ~3–4 weeks before choroid plexus maturation (Fame et al., 2020; Lowery & Sive, 2009). While the function of CSF in adults include protection, nutrient transport, and waste removal, the role of embryonic CSF has only recently been considered. Evidence suggests that embryonic CSF is important for brain development, as it is found to regulate neuronal proliferation and determination, and it contains a complex protein composition substantially different from adult CSF, which are crucial to in brain ventricle development and function (Lowery & Sive, 2009).

Aside from these macroscopic changes, the human brain also undergoes immense changes at a microscopic level during gestation. By the middle of the second month in gestation (~GW 6), the anterior regions of the embryo, especially in the telencephalon, have grown considerably more than the posterior regions, clearly defined regional boundaries have been defined, and interaction between gene products have begun to

specify cell fates (Stiles, 2008d). At a microscopic level, the production of neurons, or neurogenesis begins at this stage. The original pool of neural progenitor cells found in the VZ starts to multiply, in order to generate a sufficient numbers of progenitor cells to support brain development. This increase in the progenitor cell pool happens first through symmetrical cell division, a cycle consisting of 4 phases (Figure 1.3): Gap 1 (G1): the cell elongates and makes contact with the outer surface of the VZ; Synthesis (S): the cell nucleus moves towards the outer surface of the VZ and DNA synthesis begins; Gap 2 (G2): DNA synthesis continues; Mitosis (M): once the cell nucleus has returned to the ventricular surface, the cell divides into two identical daughter cells. During symmetric division of neural progenitor cells, both daughter cells will enter the mitotic cycle and cell division process begins again.

Figure 1.3 *Phases of cell division (from Stiles, 2008b).*



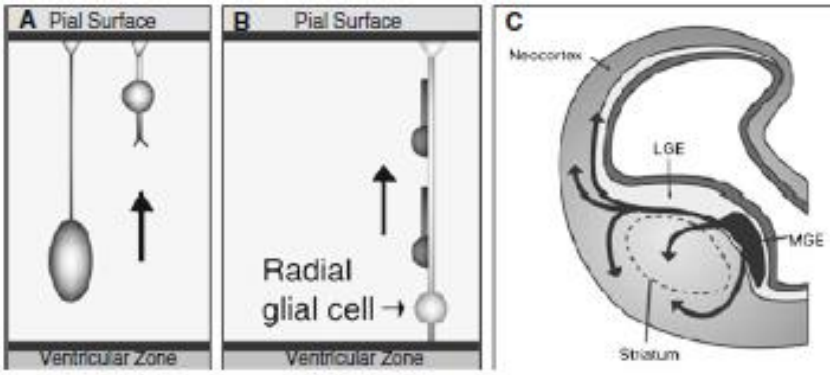
By GW7, the first cortical neurons start being produced when a small subset of progenitor cells begins dividing asymmetrically, meaning that the two daughter cells created from cell division differ in their role and their specified fate. One of them will re-

enter the mitotic cycle, while the other one will exit the proliferative cycle and migrate away from the VZ, an event known as *neuronal cell birthday* (Stiles, 2008b; Stiles & Jernigan, 2010). In humans, the period of neurogenesis lasts approximately 100 days (~GW21).

Once the newborn neuron leaves the proliferative zone, it moves toward the position it will assume in the developing cortex in a process known as neuronal migration (Stiles, 2008c). There are different types of migration depending on when and where the neuron was born (Figure 1.4). Early on in neocortical development, neurons migrate through *somal translocation* (Figure 1.4a), as the distance to be travelled is small. In this form of migration, the neuron extends part of its body (basal process) to the outer surface of the developing brain, the pial surface. Then the nucleus moves radially away from the VZ through the cytoplasm of the basal process (Nadarajah & Parnavelas, 2002). Later in development, as the distance the neurons have to travel increases, migration happens along *radial glia scaffolding* (Figure 1.4b). During this process, the neuron attaches itself to radial glial guides that traverse the intermediate zone (IZ) and move along the basal process that are attached to the outer pial surface of the developing brain. One glial cell can support the migration of many neurons (Nadarajah & Parnavelas, 2002; Stiles, 2008c). More recent evidence suggests that cortical development in humans is supported by two different types of glial cells, truncated and outer radial glia, each of them appearing at different stages of cortical development (Nowakowski et al., 2016). Finally, neurons born in the ventral telencephalon, that will later develop into the basal ganglia, reach their final location using a mode of migration labelled *tangential migration* (Figure 1.4c). This movement is tangential to the other two types of migration, and involves unique signalling

pathways (Nadarajah & Parnavelas, 2002; Stiles, 2008b). GABAergic interneurons, a specific type of neurons that originate in the ventral telencephalon, follow a more complex migration route. In fact, they leave their birthplace following a tangential migration, and eventually switch to radial migration through the intermediate zone to reach their final position in the cortex (Bartolini et al., 2013; Kolasinski et al., 2013). Neuronal migration peaks between the third and fifth month of gestation (Keunen et al., 2017).

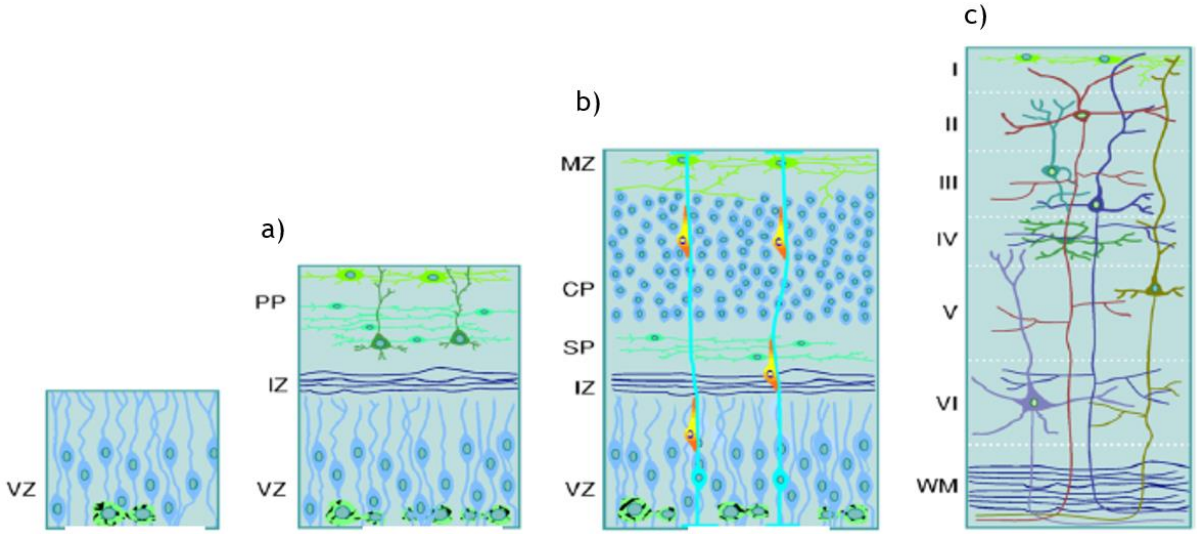
Figure 1.4 *Modes of neuronal migration: (a) somal translocation, (b) radial glia scaffolding, and (c) tangential migration (from Stiles & Jernigan, 2010).*



The migration of neurons into the developing neocortex results in the formation of an orderly 6-layered structure (Figure 1.5) (Stiles & Jernigan, 2010). This happens in an inside-out fashion, where earlier migrating neurons form the deepest cortical layer and later migrating neurons form subsequently more superficial layers. However, there is one exception to this process, where the earliest migrating neurons from a primitive structure called the preplate (PP) (Figure 5a). The next wave of migrating neurons (~GW15) creates the cortical plate (CP) and divide the PP into the outer marginal zone (MZ) and the inner subplate (SP) (Figure 1.5b). The first neurons arriving to the cortical plate, pass through the SP, then create the deepest layer, layer 6, and later migrating neurons from the more

superficial layers. The IZ will transform into the white matter layer (WM) in the mature brain (Keunen et al., 2017; Stiles & Jernigan, 2010).

Figure 1.5 *Cortical layering in the fetal brain.*



Although the MZ and the SP are transient layers that disappear by the time of birth, they play a critical role in brain development. For instance, the MZ contains Cajal-Retzius cells, which produce a molecular signal (Reelin) that helps control the positioning of neurons into cortical layers by signalling when to stop migrating and take up their positions (Stiles & Jernigan, 2010). Similarly, the SP neurons play a critical role in the development of early functional connectivity in the brain. More precisely, they help direct the formation of major sensory and motor pathways by helping establish thalamocortical connections to cortical layer 4 (Arber, 2004; Kanold & Luhmann, 2010; Stiles, 2008c). The SP also has been described as a waiting zone for afferent fibres travelling from the thalamus to the cortex (Kostović et al., 2014; Kostovic & Rakic, 1990). Axonal projections traveling from the thalamus enter the SP around GW18 and stay there until

approximately GW26, when they travel to CP (Stiles, 2008c). Finally, the SP also has an important role in the migration of late-generated neurons (Kostović et al., 2014).

During the initial period of rapid cortical expansion, a macroscopic transformation of the cortical surface happens, known as gyrification. This is the process by which the cortical surface starts folding within itself and sulci (grooves) and gyri (folds) start appearing. In fact, up until GW18 the brain is lissencephalic, has a smooth surface, with little gyri and sulci, and it is not until postnatal week 14 that the cortical folding and gyrification resembles an adult brain (Stiles, 2008b). Gyral and sulcal formation follows a temporospatial pattern with primary fissures appearing earlier and tertiary sulci appearing last (Fogliarini et al., 2005; Stiles, 2008b). Primary sulci and rudimentary secondary sulci emerge by GW26. The earliest fissure to emerge is the interhemispheric fissure, separating the two cerebral hemispheres, appearing first rostrally and progressing caudally, being clearly defined by ~GW22. By approximately GW26, the Sylvian fissure clearly divides the frontal and temporal lobes. Secondary sulci are well defined by GW34, and at birth, tertiary sulci have begun to emerge, their formation continuing well into the postnatal period (Stiles, 2008b).

Once neurons have reached their final destination in the cortical plate, processes to develop functional networks begin, that is, neurons start extending axons and dendrites (Webb et al., 2001). Early in development, dendrites appear as thick processes extending from the neuronal bodies and have few spines and then they thicken and increase in number providing greater area for synaptic contact. Dendritic arborisation follows a spatiotemporal pattern, with visual cortex and hippocampus presenting more numbers than the frontal cortex at birth, for example (Webb et al., 2001). With respect to neuronal

axons, pathways connecting different brain regions start to form by approximately GW8-9, and a series of sequential events lead to the establishment of major long trajectories by the neonatal period (Vasung et al., 2010).

Another crucial process for developing a functional brain is myelination. Myelin forms a fatty sheath that insulates axons allowing more rapid and energy efficient neuronal impulses (Webb et al., 2001), and evidence shows that the completion of myelination within a neural system signals the onset of full functional capacity (Flechsig 1920, cited in Stiles 2008b). Myelination is one of the last processes to happen during brain development and is largely a postnatal process, and although most of it is completed by the end of second year, research has shown that it is a lifelong process that might continue up until the 60s (Stiles, 2008b). It has been shown to follow a systematic progression within the brain, starting in caudal regions, following central regions, and finishing in anterior and posterior poles. Moreover, early histological work (Flechsig 1920; Yakovlev & Lecours, cited in Stiles 2008b), has shown that motor and sensory systems myelinate earlier than systems that serve higher functional cognitive processes, and that not only different areas start myelination at different times, but they also progress at different rates, taking variable times to complete.

Finally, the development of the cerebellum should also be considered, as the human cerebellum has almost 80% of the surface area of the neocortex (Sereno et al., 2020), and is one of the brain areas with reduced brain volumes in DS (Patkee et al., 2020). The cerebellum develops over a long period, extending from early embryonic stages until the first postnatal year (ten Donkelaar et al., 2014), and it arises close to the boundary (the ‘isthmus’) between the midbrain and the hindbrain (Butts et al., 2014). Although the

cerebellum is the most architecturally complex region of the CNS, it has a simple histological organisation: the cerebellar cortex is composed of a single layer of inhibitory Purkinje cells lying between a dense layer of excitatory granule cells, and layer of granule cell axons and Purkinje cell dendritic trees (Butts et al., 2014).

There are several steps in cerebellar development, starting with the specification of the cerebellar territory through molecular boundaries (Butts et al., 2014; ten Donkelaar et al., 2003). Once the boundaries of the cerebellar territory are defined, cerebellar histogenesis starts. At this point the cerebellar region is comprised of two separate symmetric bulges that will eventually grow and fuse together, giving rise to the cerebellar plate comprising the vermis and two hemispheres (Leto et al., 2016). During this process, two proliferative zones appear just above the opening of the fourth ventricle: the dorsally located rhombic lip and the ventrally located VZ (Hoshino, 2016; Leto et al., 2016). The cerebellum contains about ten different types of neurons, but they can be categorised into glutamatergic excitatory and GABAergic inhibitory neurons, which arise from the rhombic lip and the VZ, respectively (Hoshino, 2016). These two proliferative zones disappear by the time of birth. Dividing VZ precursors migrate into the cerebellar prospective white matter, whereas those of the rhombic lip move tangentially along the cerebellar surface, where they form the external granular layer (EGL) (Leto et al., 2016). Granule cells are created in the EGL, which form axons, and migrate along the processes of Bergmann glia cells to their deeper, definitive site, the internal granular layer, situated below the layer of Purkinje cells (ten Donkelaar et al., 2003). The final output structures of the cerebellum are the four cerebellar nuclei (the medial (fastigial), anterior and

posterior interposed, and lateral (dentate) nuclei), containing a mixture of inhibitory and excitatory neurons (Prekop & Wingate, 2016).

1.3.2 Brain development in DS

Due to the behavioural and cognitive phenotype present in people with DS, the study of the brain in this population has been of great interest, since DS was first described in 1866 (Shapiro, 2001). In fact, around 300 genes are triplicated in DS, several of which have been identified as being involved in brain structure and function (Haydar, 2020; Haydar & Reeves, 2012). More specifically, some of these genes, such as *DYRK1A*, are involved in key developmental processes like neuron proliferation and differentiation (Dierssen et al., 2001; Dierssen, 2012). However, the gene brain interaction in DS seems to go further than simply an overexpression of genes. In fact, evidence suggests that the impact of genes on brain development might also be due to deregulation of non-coding genetic element, misexpression of non-chromosome 21 genes and proteins relevant to brain development and function, and epigenetic influences (Dierssen, 2012; Engidawork & Lubec, 2003).

Using different approaches, such as human brain tissue studies, mouse models or cell cultures, decades of research have provided consistent evidence of alterations in early brain development in DS, both at a macroscopic and microscopic level. As early as the second trimester in pregnancy (~15GW onward), evidence from post-mortem studies shows smaller overall brain measures (e.g., 2D, 3D and brain weight) in DS when compared to age-matched controls (Guihard-Costa et al., 2006; Shapiro, 2001). Reduced neuronal numbers have also been found in the cortex (Larsen et al., 2008; Lu et al., 2011), hippocampus (Guidi et al., 2008), cerebellum (Contestabile et al., 2007; Guidi et al., 2011;

Haydar & Reeves, 2012) and temporal lobe (Guidi et al., 2018) of fetuses with DS. This reduction is believed to be a result of impaired progenitor proliferation or neurogenesis disruption (Contestabile et al., 2007; Guidi et al., 2008, 2011; Stagni et al., 2018), increased neurodegeneration or apoptosis (Busciglio & Yankner, 1995; Guidi et al., 2008) and a reduced level of neurotransmitter, such as, serotonin, taurine or dopamine, crucial for brain development (Whittle et al., 2007). More recent research has also suggested that there is a shift toward neural progenitor cells differentiating into glia (microglia, astrocytes, and oligodendrocyte) that might also contribute to reduced neuronal numbers (Reiche et al., 2019).

Aside from reduced neuronal numbers, there is also evidence of reduced radial glia progenitors in DS brain tissue, as early as the second trimester (Baburamani et al., 2020). Radial glia cells are essential in cortical development as they create a scaffolding structure for migrating neurons. So, a reduction in their number might be one of the underlying mechanisms leading to abnormal cortical development in DS, which involves delayed and disorganised cortical lamination (Golden & Hyman, 1994) and reduced dendrite density and proteins markers linked to synaptogenesis (Weitzdoerfer et al., 2001).

Finally, brain tissue and mouse model studies have also found prenatal alterations in myelination in DS. More precisely, post-mortem studies have found delays in myelination and decreased density of myelinated axons in the hippocampus (Ábrahám et al., 2012), as well as reduced complexity in myelinated pathways, and reduced number in nodes of Ranvier in the corpus callosum and external capsule (Olmos-Serrano et al., 2016). Moreover, results from mouse models have also shown slower action potentials in myelinated axons of DS mice when compared to euploid controls, indicating that WM

alterations in DS contribute to structural and functional defects in neurotransmission (Olmos-Serrano et al., 2016).

All these studies have been invaluable to get a deeper understanding on the neuropathology resulting from triplication of genes on chromosome 21. However, there is still a lack of understanding about how these underlying alterations relate to the impaired cognitive and functional phenotype found postnatally. Developments in antenatal Magnetic Resonance Imaging (MRI) techniques have provided imaging correlates for the atypical development of whole, and regional brain structures in DS. A recent neonatal MRI in vivo study found that babies with DS had reduced cerebral volumes when compared to age matched TD controls, as early as 21 GW. These volume reductions were more specifically found in the cortex and the cerebellum (Patkee et al., 2020). The use of fetal MRI has also allowed indirect comparisons of in vivo brain anatomy to postmortem histological findings (Baburamani et al., 2020).

1.4 Brain and cognition

The study of the relationship between the brain, especially brain volumes, and cognition or intelligence in humans has been greatly influenced by knowledge acquired from the study of brain evolution across species. In fact, investigating the size of brain parts to species-typical adaptive behaviours has been the goal of brain allometry —the growth of body parts at different rates, resulting in a change of brain proportions— studies for decades (Finlay et al., 2011; Finlay, 2019). Evidence from cross-species studies has shown that increases in relative brain size with respect to body size over evolutionary time is associated with behavioural complexity (Finlay et al., 2011; Finlay & Uchiyama, 2015). There have also been many MRI studies in human adults that have found significant

associations between individual variability in brain size and variability in intelligence (Finlay et al., 2011; Ritchie et al., 2015; Takeuchi et al., 2017).

Cross-species studies of brain evolution have also shown that, although it seems that the human cortex is proportionally larger than in other species, when considering the allomeric ratio within the brain, the cortex is exactly the size it should be (Finlay & Uchiyama, 2017), suggesting that the evolution of complex abilities in humans might not be solely a result of the cortical evolution. This notion is supported by Ritchie et al.'s (2015) study, which found that overall brain volume explained most of the variance in intelligence (~12%) when compared to other types of brain measures (e.g., cortical thickness, WM structure or fractional anisotropy). The literature in human cognitive abilities has also provided evidence of the association between regional brain volumes (e.g., putamen, caudate nucleus, frontal, temporal and parietal lobes) and different cognitive abilities (e.g., memory, attention, language, visuospatial and motor abilities) at different ages (Armstrong et al., 2020; Kennedy & Raz, 2005; Pangelinan et al., 2011).

1.4.1 Paediatric MRI and cognition

With recent developments in MRI techniques, *in vivo* quantification of both macro- and micro-structure and function within the neonatal brain can now be gained (Ferrazzi et al., 2014; Jiang et al., 2009; Malamateniou et al., 2013). These advances have consequently resulted in many studies exploring the relationship between MRI measures and cognitive abilities in the paediatric population. The relationship between neonatal brain volumes (e.g., overall, hippocampal, cerebellar and regional volumes) and cognitive outcome in toddlers has been investigated in the preterm population (e.g., Gui et al., 2018; Kooij et al., 2012; Peterson et al., 2003; Thompson et al., 2008) and the population of

babies born with CHD (Bonthrone et al., 2021; Meuwly et al., 2019), as well as the relationship between neonatal WM microstructure (measured with diffusion MRI) and cognitive outcome in toddlers in the term- and preterm-born population (e.g., Ball et al., 2015; Feng et al., 2019; Rose et al., 2009). It would also be feasible to extend these methods to the neonatal population with DS (Baburamani et al., 2019).

1.5 Doctorate Research

1.5.1 Aim

The main aim of this doctoral research was to examine whether there are any early structural biomarkers in the brain of neonates with DS that may help predict an individual's cognitive outcome. To answers this question, MRI neuroimaging techniques, as well as neuropsychological measures to assess cognitive outcome were used to: (1) investigate whether there are any early WM microstructure alterations in neonates with DS; and (2) examine whether neonatal brain volumes in DS may be used to predict cognitive outcome and its variability in toddlers with DS. The finding from this work would not only provide the stepping stone for future research but could also provide invaluable information for clinicians that could help better inform carers on how their child with DS might develop.

1.5.2 Thesis overview

Following this introductory chapter, Chapter 2 provides an overview of the key methods and theoretical approaches applied in this thesis. The first part of the chapter will cover key MRI concepts, image acquisition processes, data analysis approaches and neonatal image acquisition safety and considerations. The second part of the chapter presents contemporary approaches to studying neurodevelopmental conditions (NDCs),

including the theoretical framework of the thesis and methodological considerations. Chapter 3 presents a secondary data analysis study exploring neonatal diffusion MRI data investigating WM microstructural differences between neonates with DS and age-matched TD controls. A study examining the relationship between neonatal brain volumes and later cognitive outcomes in toddlers with DS is presented in Chapter 4, which also includes four case studies exploring the relationship between individual brain volume variability and cognitive developmental trajectories. The final chapter in this thesis, Chapter 5, is a general discussion of the results of this doctoral research. Key findings are highlighted exploring the potential associations between them, and theoretical and clinical implications are discussed. Avenues for future research are presented so that the results from this work may be extended to further the understanding of how the early neuro phenotype in the population with DS relates to their cognitive phenotype.

Chapter 2: Methods

2.1 Magnetic Resonance Imaging

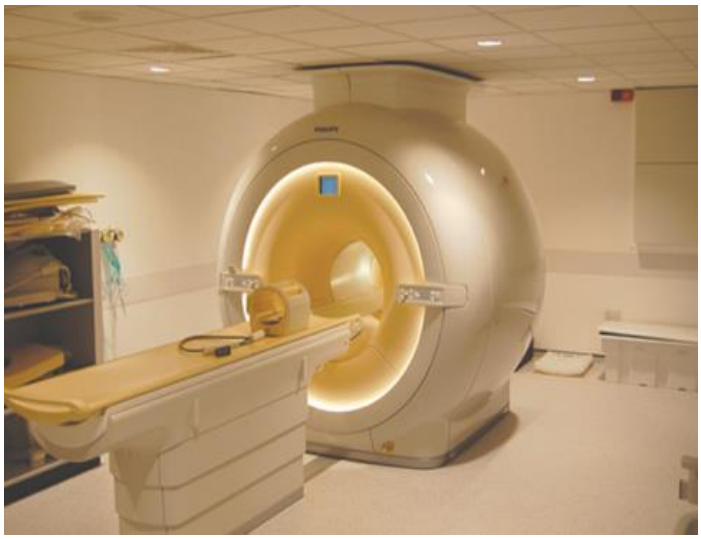
2.1.1 Introduction of MRI

The interest in studying the human brain to understand behaviour has a long history, but it was not until the early 20th century that efforts to visualise the brain in vivo were made, and not until the late 1970s that MRI was used outside dedicated academic research centres (Modo & Bulte, 2011). Although computerised tomography and positron emission tomography were the most commonly used methods to study the brain, they use ionising radiation to acquire images of the brain, which can potentially be harmful.

Since the beginning of the 1990s, the preferred method for in vivo visualisation of the brain in research and clinical settings has been MRI, as it is a non-invasive technique that allows to safely acquire three dimensional images of the brain.

2.1.2 The scanner

Figure 2.1 *Picture of the 3T scanner in the Neonatal Intensive Care Unit in St. Thomas' Hospital, London.*

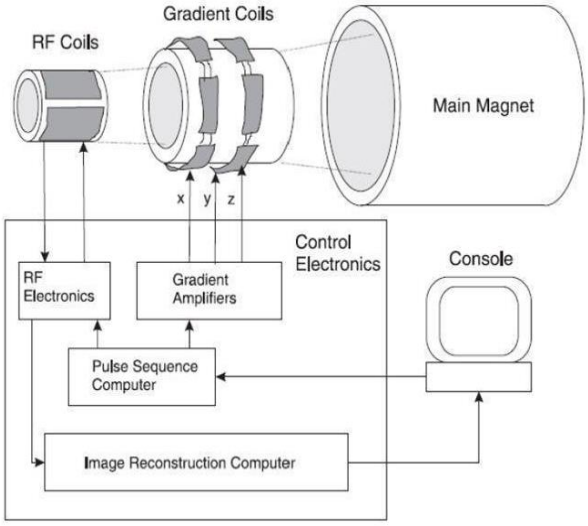


An MRI scanner consists of several components (see Figure 2.2): a Main magnet, gradient coils, and radio frequency coils, which contribute to the creation and acquisition of the MRI signal from an object. The *main magnet* is an array of coils cooled to

superconductive temperatures using liquid helium. This is what creates the main magnetic field (B0), measured in Tesla units (e.g., 1.5T, 3T, 7T, etc.). The *gradient coils* are loops of wire or thin conductive sheets and are the components of the scanner that allow the spatial encoding of the magnetic resonance (MR) signal. When an electric current is passed through these gradient coils, a secondary magnetic field is created, which slightly distorts the B0 in a predictable pattern. The radiofrequency (RF) coils can be either transmitter RF coils or receiver RF coils. Transmitter coils generate an oscillating magnetic field (B1), perpendicular to the B0 magnetic field, that is turned on only for brief periods of time known as RF pulses. Receiver RF coils are placed close to the body, and are responsible for detecting the MR signal, which is created through changes in magnetic flux, in accordance with Faraday's Law of induction.

To create and receive the MR signal in the body, and convert this to images, the system also contains a set of Control electronics. These include RF electronics — comprising an RF amplifier and an RF receiver— gradient amplifier, pulse sequence computer, and an image reconstruction computer. The control electronics are controlled by a radiographer via a console.

Figure 2.2 *Schematic representation of the MRI scanner system (from Gruber et al. 2018).*



2.1.3 Basics of MRI physics

Nuclear Magnetic Resonance (NMR)

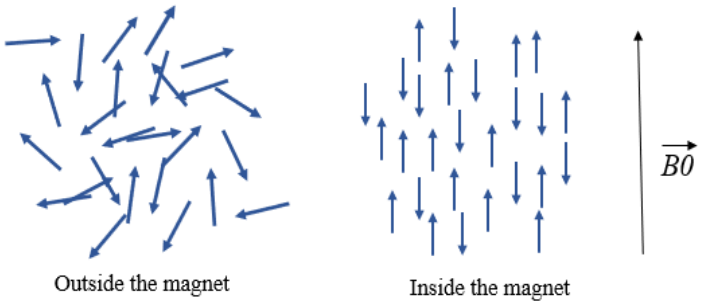
In conventional MRI, the signal used to create brain images derives from the hydrogen protons in the water that comprises a large part of the brain. More precisely, the signal comes from the spins of the hydrogen protons (Figure 2.3), a quantum magnetic property that results in the protons behaving as small magnets. These spins can be manipulated by a magnetic field or a radio frequency pulse, which is what MRI exploits.

Figure 2.3 *Schematic representation of a hydrogen proton and its spin.*

While outside the MRI scanner, all the spins in the brain are randomly oriented and have the same energy level (Figure 2.4 left). However, when the body is placed into the scanner, and the spins within the brain are inside the magnetic field created by the Main Magnet, the energy levels of the spins divide into a higher energy (anti-parallel to

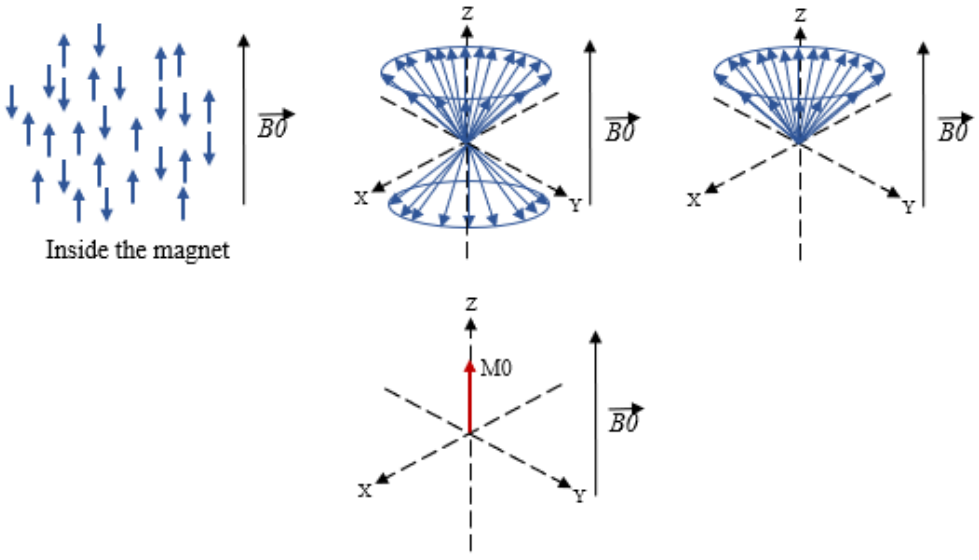
the main magnetic field) and a lower energy state (parallel to the main magnetic field), known as the Zeeman effect (Figure 2.4 right).

Figure 2.4 *The Zeeman effect. Representation of spin orientations outside and inside an MRI scanner.*



A slightly higher proportion of the spins are in a lower energy state, and this population difference causes an energy gap which generates a macroscopic magnetisation in the brain parallel to the external magnetic field, known as the net magnetisation (M0) (Figure 2.5).

Figure 2.5 *Schematic representation of the formation of the net magnetisation, where B0 is the base external magnetic field, x, y, and z are the three dimensions of space, and M0 is the net magnetisation of hydrogen protons.*



The placement of the protons in a strong magnetic field also causes them to experience a rotational turning force, known as a torque. This results in the hydrogen

nuclei rotating, or precessing, about the B0 axis, at an angular velocity of ω proportional to the strength of the magnetic field, called the Lamor frequency and defined by the Lamor equation (see Equation 1).

$$\omega = \gamma \cdot B0$$

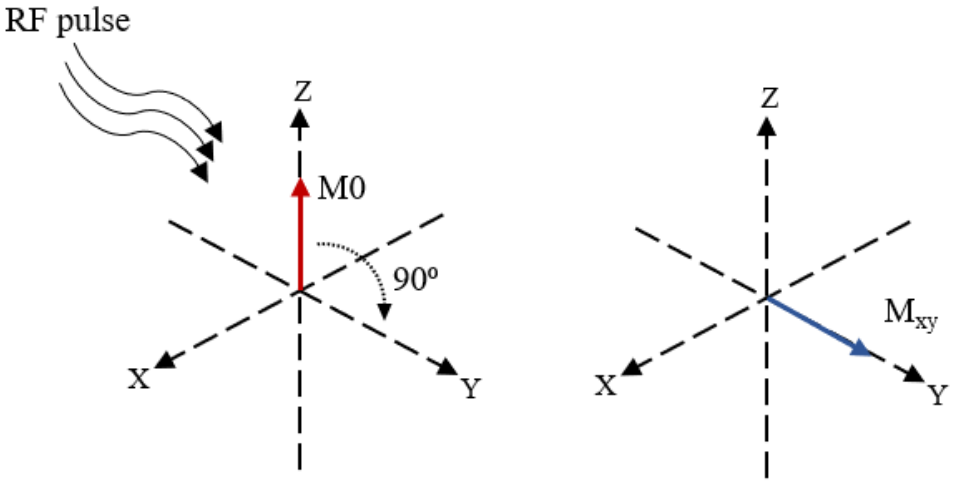
Equation 1. Lamor equation: where ω is the frequency of precession measured in Mega Hertz [MHz]; γ is the constant gyromagnetic ratio for specific nuclei in a magnetic field at 1T (for hydrogen γ= 42.58 MHz/T) and B0 is the strength of the magnetic (T) field in which the nuclei are precessing.

Image generation

Radio frequency pulse

While no scanning is taking place, the M0 is in equilibrium and longitudinal, that is, it is oriented in the z-direction. To obtain a MR signal from the body, M0 needs to be forced to precess, which is done by applying a 90º RF pulse that matches the Lamor frequency of the spins. This process is also known as excitation, and it causes the M0 to be flipped into the orthogonal plane (M_{xy}) (Figure 2.6). The precession of M0 in the orthogonal plane produces a varying magnetic field, which in turn creates an electric current that can be picked by the receiver coil (a loop of wires placed perpendicular to the transverse plane). This induced voltage, or the MR signal, is also known as free induction decay (Dale et al., 2015a).

Figure 2.6 *Schematic representation of the excitation, a process by which M0 is flipped into the orthogonal plane (M_{xy}).*



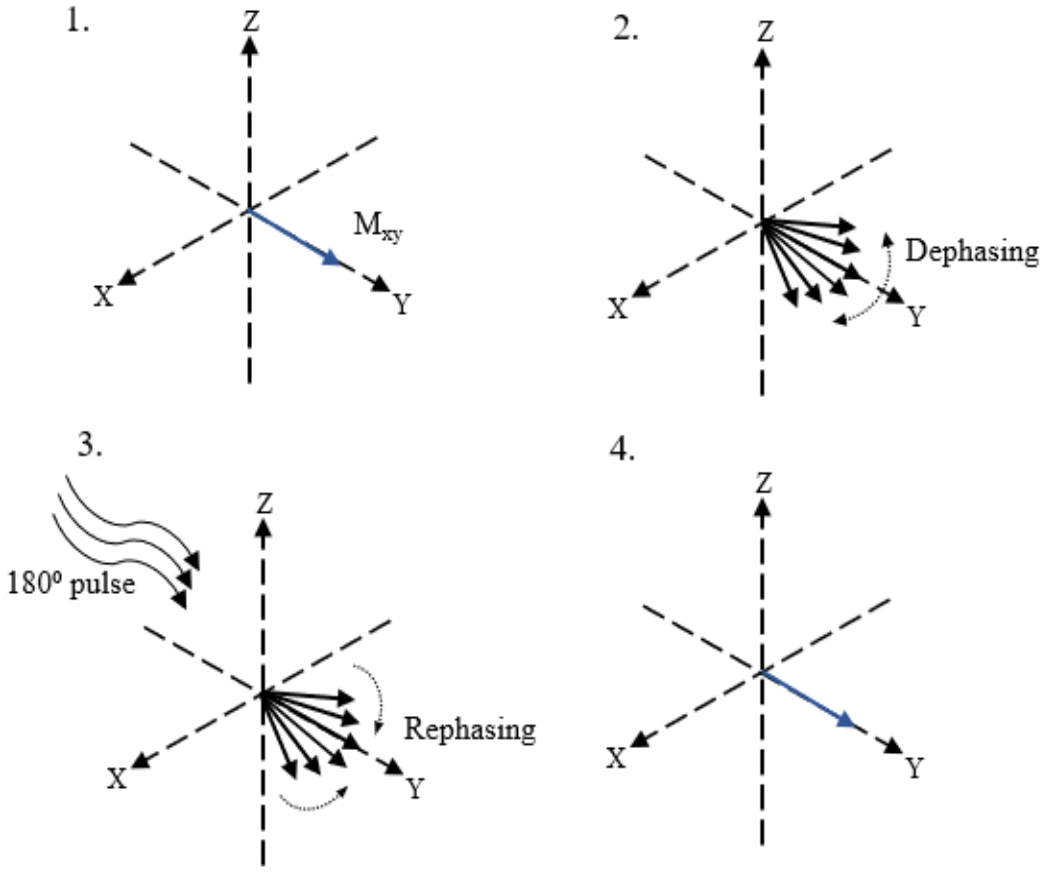
MR signal (relaxation)

When the transmitter that applies the RF pulse is turned off, the protons start to return to their state of equilibrium (oriented in the z direction), and they emit their added energy at a frequency ω_0 as they do so. This process is known as relaxation, and it is a time-dependent process, characterised by a rate constant known as relaxation time (Dale et al., 2015e).

There are two relaxation times: T1, or longitudinal relaxation, which measures the time required for the z component of M to return to 63% of its original value after an RF pulse; and T2, or transverse relaxation, which measures the time required for the transverse magnetisation to decay to 37% of its value (Dale et al., 2015e). This decay in transverse magnetisation is caused by microscopic and macroscopic inhomogeneities. The former one is irreversible, however, the loss caused by macroscopic field inhomogeneities, T2* relaxation, can be recovered. When the M0 is flipped into the transverse plane, after a period of time (t) the spins will start to dephase; they become asynchronous, reducing the transverse coherence. Applying an additional 180º RF pulse will cause the spins to

rephase, regaining some of their transverse coherence, which will induce another signal in the receiver coil, known as a spin echo (Dale et al., 2015e) (Figure 2.7).

Figure 2.7 *Spin echo. (1) After excitation, M0 is flipped into the transverse plane, (2) and due to inhomogeneities, the spins become asynchronous. (3) an additional 180º RF pulse causes the spins to rephase (4) inducing another spin echo signal.*



Signal read out and Image generation

To create a spatially accurate MR image, three different gradients are used in the image acquisition process for spatial encoding. The slice selection gradient (G_{SS}) is used to select the slice of the brain from which the signal will be induced. The process of detecting the MR signal from the body with the receiver coil is known as read out or frequency encoding. This step is always done in the presence of two gradients known as the readout gradient (G_{RO}) and phase encoding gradient, G_{PE} (perpendicular to both the

G_{SS} and the G_{RO}), which produce the other two spatial dimensions of the MR image (e.g., the location of a voxel within the brain)(Dale et al., 2015b).

Once the signal has been acquired, there are two types of data that can be used to analyse the measured MR data: the raw data and the image data. Although both types of data contain the same information, the raw data is a complex two-dimensional array of data points, also known as the k-space, where each data point (k_x, k_y) corresponds to different echo signal amplitudes influenced by the readout and phase encoding gradients (Dale et al., 2015c). The image data are the format used for image viewing and data interpretation, and both formats are related by the Fourier transformation.

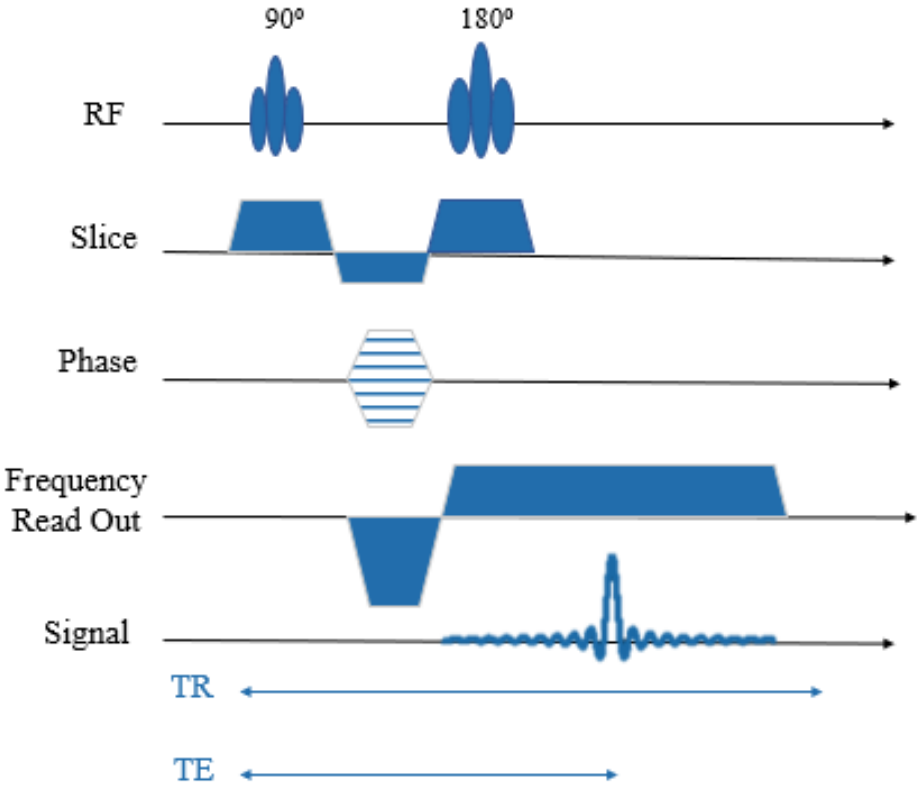
Pulse sequence

MRI allows the study of several aspects of the brain (e.g., anatomy, diffusivity, function, spectroscopy, etc.) by acquiring different types of data using different pulse sequences. A pulse sequence is the set of instructions given to the scanner used to obtain an MR image. It contains hardware instructions, such as RF pulses, gradient pulses, and timings, that can be modified to acquire the data in the desired manner. During the image acquisition, some parameters are directly selected by the radiographer (e.g., field of view) and other variables are defined in pre-existing template files (e.g., basic relationships between RF pulses and slice selection gradients)(Dale et al., 2015d). Multiple pulse sequences can be put together in an MRI protocol to acquire different types of images during a single scanning session.

A commonly used pulse sequence is a spin echo sequence, which includes at least two RF pulses, an excitation pulse and one or more 180° refocusing pulses that generate the spin echo(es) (Figure 2.8). This pulse can be combined with Echo Planar Imaging

(EPI) sequence, which allows to capture the whole brain images in under 100 milliseconds through the rapid switching of phase encoding gradients, G_{PE}.

Figure 2.8 *Time diagram for a single echo spin-echo sequence. The repetition time, TR, is the time between successive excitation pulses for a given slice. The echo time, TE, is the time from the excitation pulse to the echo maximum.*



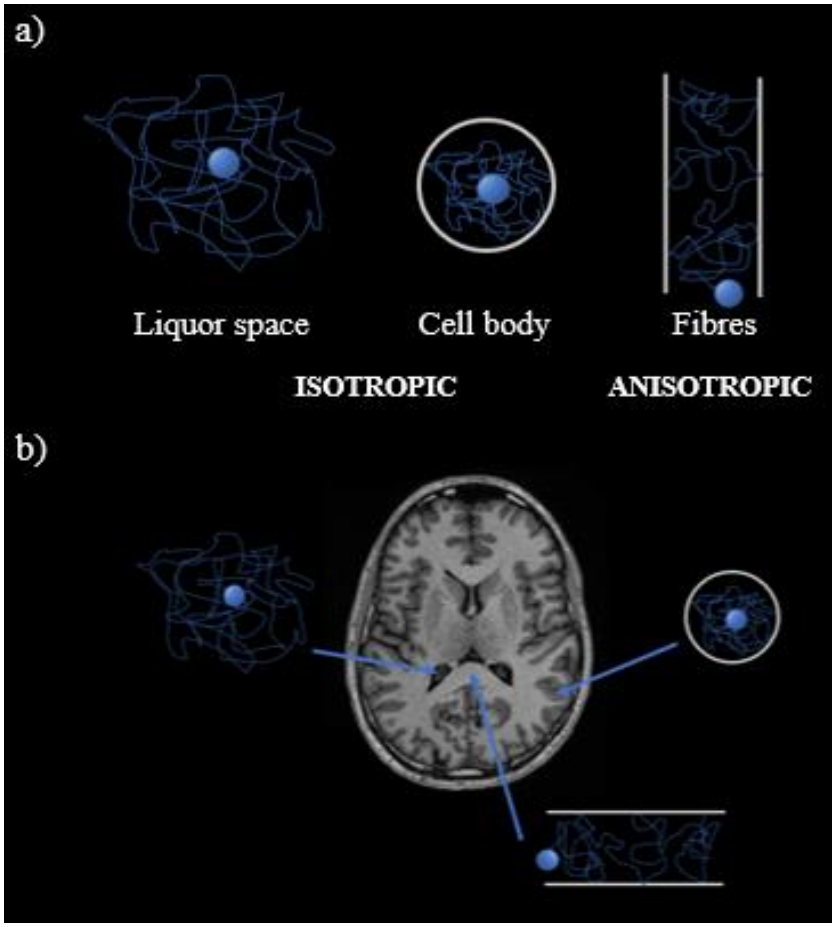
2.1.4 Diffusion weighted MRI (dMRI)

Basis of dMRI

The basis of diffusion weighted MRI is the random movement of the water molecules in the brain, also known as Brownian motion. Depending on the tissue type in which a molecule resides, its movement will be more or less restricted: in the cerebrospinal fluid, the movement will be unrestricted in all directions (isotropic); in grey matter (GM), the membrane of the neuronal body will somewhat restrict the movement of the water molecule (isotropic); in white matter, the movement of the water molecule

will be free in the direction of the myelinated or unmyelinated axon fibre bundles, but restricted in the perpendicular direction (anisotropic) (Figure 2.9).

Figure 2.9 *Representation of the random movement of a water molecule in different mediums (a) and in different brain tissues (b).*



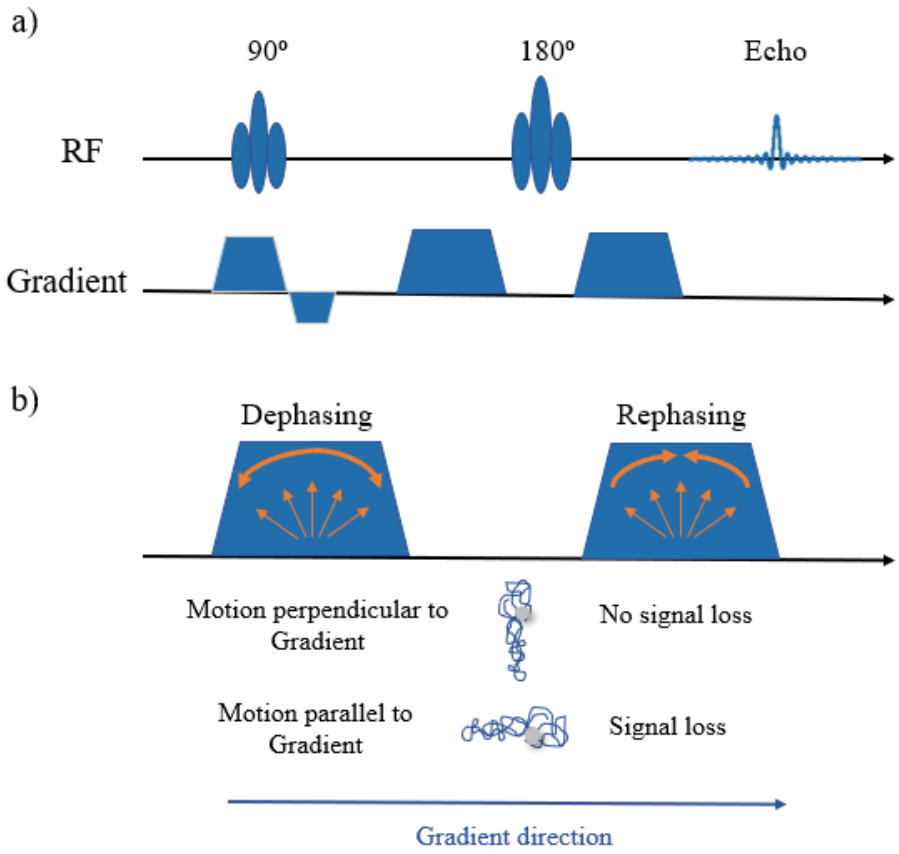
dMRI in the scanner

All MRI pulse sequences have built-in gradients for spatial encoding; however, they are too short and not strong enough to induce any significant diffusion effect. In dMRI, the directional movement of the water molecules in the brain is mapped by using additional diffusion gradients applied in several directions.

As previously mentioned, when an RF excitation pulse is applied and the M0 is flipped into the orthogonal plane (M_{xy}), due to inhomogeneities in the field, the spins

become asynchronous, they start to dephase. Some of the lost signal can be recovered, the spin-echo, by applying an additional rephasing 180° RF pulse. However, the echo formation depends on the spins remaining in the same local field over the whole process. Diffusion weighted MRI takes advantage of this phenomenon. In essence, dMRI measures the loss of signal between the excitation RF pulses and the rephasing RF pulse, caused by the motion of the spins in the direction of the applied gradient (Figure 2.10 b).

Figure 2.10 *Simplified Timeline diagram of relation between RF pulses and diffusion gradients (a). Relationship between spin-echo signal produced by the rephasing 180 RF pulse, diffusion gradient direction and water molecule motion direction (b).*



There are different types of diffusion acquisition strategies that can be chosen based on the aims of the experiment. A commonly used acquisition is the High Angular Resolution DW Imaging (HARDI) acquisition, which has been proven to be the optimal

approach to address the issue of crossing fibres within the brain (Tournier et al., 2011) (see next section).

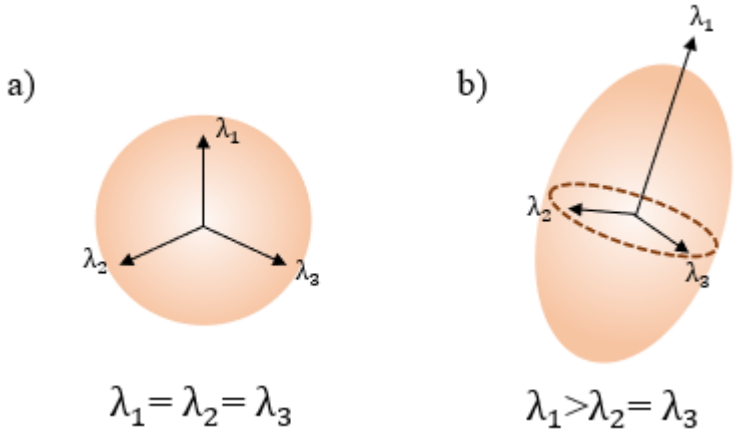
Diffusion data modelling

To acquire quantitative orientation information and anisotropy descriptions within the brain from dMRI, data modelling is required. There are two main ways to mathematically represent diffusion orientation and the subsequent underlying fibre orientation reflected by the dMRI signal in each voxel: to estimate the diffusion orientation density function (dODF) or the fibre orientation density function (fODF) (Jones et al., 2013). Whilst dODF is a spherical function that represents the relative number of particles that have diffused along an axis, fODF is a function that represents the relative number of fibres oriented along a given axis (Jones et al., 2013). The use of these two mathematical approaches leads to different ways to model the dMRI data, which leads to different metrics representing several aspects of the underlying tissue.

The diffusion tensor model

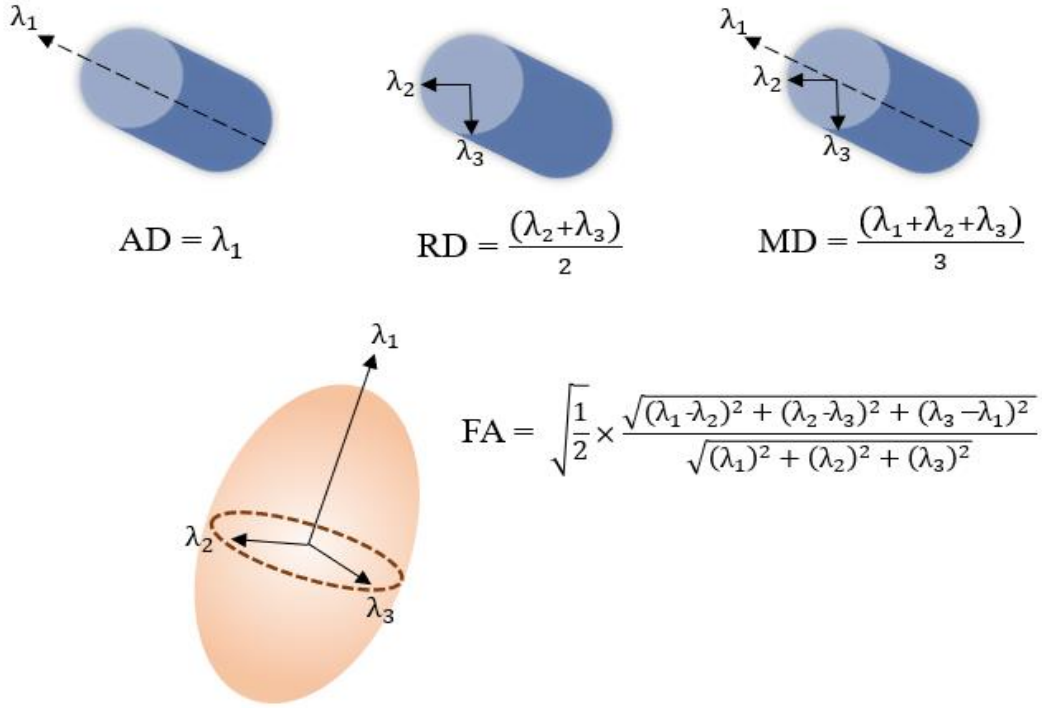
One of the most widely used models based on the dODF approach is the diffusion tensor model (DT), introduced in the beginning of the 1990s (Pierpaoli et al., 1996). The model involves depicting the magnitude and direction of diffusion within each voxel by computing a tensor, which is a mathematical description of a three-dimensional ellipsoid (Figure 2.11) (Chanraud et al., 2010). The ellipsoid is commonly described in terms of its major orientation axis. These are referred to as the tensor's orientation vectors or eigenvectors ($\varepsilon1$, $\varepsilon2$, $\varepsilon3$), and their length depends on the tensor eigenvalues ($\lambda1$, $\lambda2$, $\lambda3$) (Chanraud et al., 2010; Hüppi & Dubois, 2006).

Figure 2.11 *Diffusion tensor in an a) isotropic and b) anisotropic mean (modified from Basser & Ozarslan, 2010; Chanraud et al., 2010).*



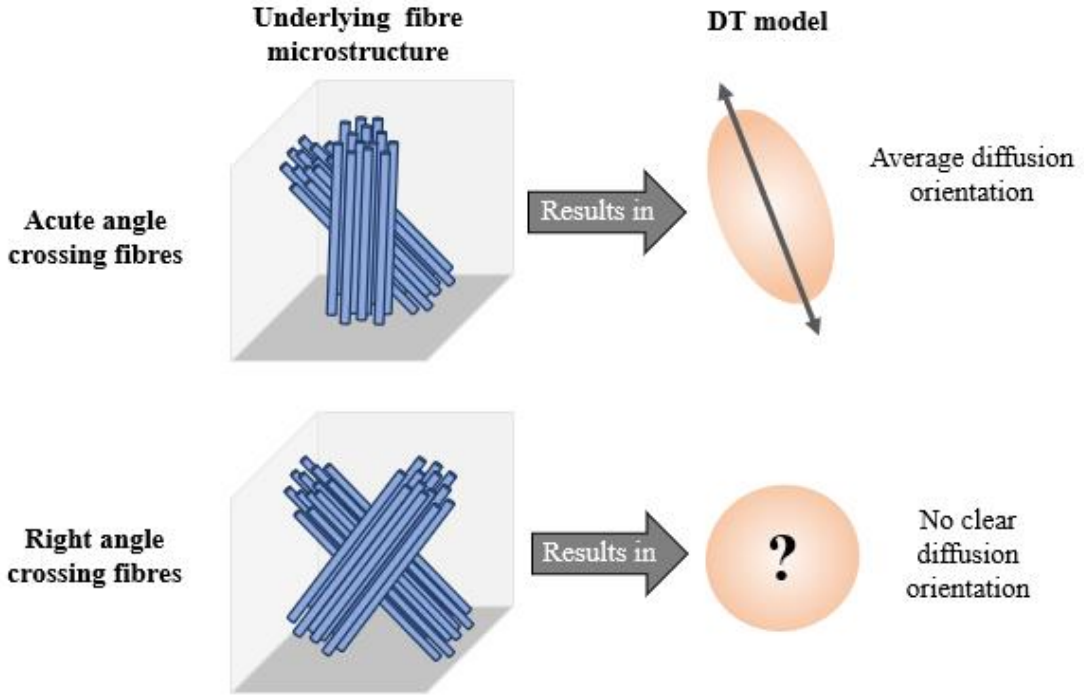
From the six components of the tensor, four different metrics can be extracted: fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD). FA measures the orientational preference within a voxel, that is, the extent to which the main eigenvalue (λ1) dominates the other two (λ2 and λ3). FA is the most commonly reported metric, with values ranging from 0 to 1, and its magnitude is tissue dependent. For instance, in a CSF filled space, such as the ventricles, FA values will be near zero, whereas in a tightly packed and organised WM structure, such as the corpus callosum, FA values will be closer to one. AD represents the largest eigenvalue (λ1) and RD quantifies the diffusivity in the transverse direction (λ2 and λ3). MD is simply the average of all the eigenvalues (Figure 2.12).

Figure 2.12 *Schematic representation of how the four different metrics derived from the tensor are calculated (modified from Moayedi & Hodaie, 2019).*



Although DT imaging (DTI) has been widely used in the literature, this approach has some inherent limitations and difficulties with the interpretation of its metrics. The main limitation of the tensor model is related to its inability to accurately represent the dMRI signal when it is reflecting underlying fibres oriented in different directions, such as crossing fibres (Figure 2.13) (Jones et al., 2013; Tournier et al., 2007). This is a considerable caveat as it has been estimated that one third of white matter voxels in the brain contain more than one fibre population (Behrens et al., 2007, as cited in Tournier et al., 2007), many of which might be part of the major WM tract bundles. Using the DT model in this situation might result in unreliable results when creating a tractogram.

Figure 2.13 *Representation of the diffusion tensor model in a voxel with crossing fibres (modified from Seunarine & Alexander, 2014).*



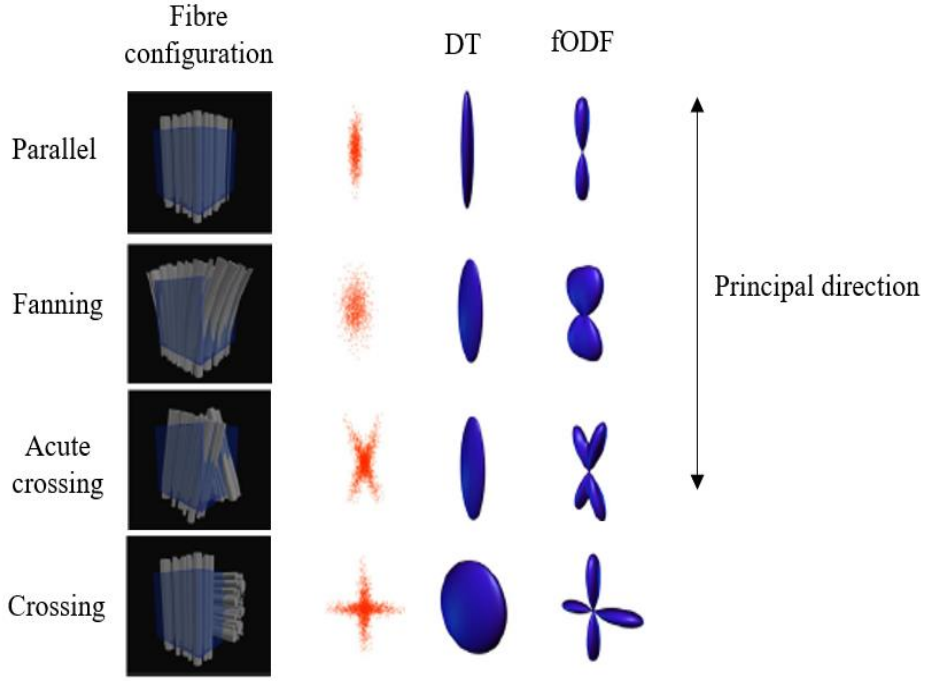
Aside from the difficulty of accurately representing differently oriented fibre populations within a voxel, the metrics extracted from the tensor model (FA, MD, AD and RD) are difficult to interpret. The reason for this is that the metrics are not sensitive to one single aspect of the underlying WM anatomy they are reflecting. More precisely, these measures are sensitive to partial volumes in the voxel (e.g., adjoining GM structures to the WM fibres), as well as to a number of tissue properties, such as, axonal ordering, axonal density or degree of myelination, without being specific to a single one of them (Chanraud et al., 2010; Jones et al., 2013). This results in difficulties in interpreting what aspects of the underlying anatomy exactly these measures are representing.

The spherical deconvolution model and Fixel-based analysis (FBA)

Different dMRI modelling techniques have been developed to overcome the DT model's multiple orientation fibre limitation (Figure 2.14). One of these techniques is the

spherical deconvolution model, which aims to reconstruct the fODF in each voxel (Tournier et al., 2004, 2007). This model can also be constrained by the underlying tissue properties (multi-tissue constrained spherical deconvolution), which addresses the issue of partial voluming in the voxel (Jeurissen et al., 2014a). Partial voluming occurs when tissue not belonging to white matter is also included within a voxel (e.g., CSF in voxels around the ventricles, or GM in voxels in the WM-GM boundary).

Figure 2.14 *Representation of how the dODF and the fODF based modelling approaches would reflect different underlying fibre configurations. The first column shows different possible fibre configurations existing in the brain, within a voxel. The second column shows the expected scatter pattern of water molecules for each of the fibre configurations. The third and fourth columns show the best-fit DT model and the fODF modelled with spherical deconvolution for each of the fibre configurations, respectively. Principal direction for the first 3 fibre configurations is known, but for the 90 degree crossing fibres it is not (modified from Seunarine & Alexander, 2014).*



The use of the spherical deconvolution model has led to more sophisticated dMRI analysis techniques, like the recently developed Fixel-based analysis (FBA) (Raffelt et al., 2017), which uses fibre population specific information to estimate the morphology of the underlying WM fibre bundles. This method offers advantages over existing single-fibre

models such as DT, as it allows measurement of voxel-wise alterations in multiple fibre orientations. For each voxel, a fibre orientation distribution (FOD) is calculated, which estimates the probability distribution of a fibre population running in a given direction. A fixel is then assigned to each voxel based on the magnitude and direction of the FOD, and subsequent fixel-wise statistics can be applied to compare fibre density and morphology (Raffelt et al., 2015).

Another advantage of the FBA is that it offers metrics that reflect several fibre properties, such as fibre density (FD), fibre cross-section (FC), and the combined effect of fibre density and cross-section (FDC). Whilst a fibre population's FD is proportional to the volume of the intra-axonal compartment, FC reflects the cross-sectional area of the white matter fibre bundle. These metrics are easier to interpret than the ones extracted from the tensor model (FA, MD, AD and RD). For instance, a decrease in FD within a fibre population can represent a decrease in axonal count, or less dense packing of axons, or even reduced myelination in a fibre area, whereas decrease in FC can suggest a reduced cross-sectional area of a fibre bundle.

2.1.5 Neonatal MRI and safety

Although MRI is a non-invasive, safe technique, there are certain safety measures that need to be taken when scanning neonates (Tocchio et al., 2015). These measures are associated with safety concerns related to (1) exposure to a static magnetic field, (2) heating and (3) acoustic noise.

The safety concerns related to the exposure to a static magnetic field refers to the hazard of ferromagnetic objects becoming projectiles near the MRI magnetic field (Tocchio et al., 2015), or becoming overheated if they are inside the body (e.g., implants,

surgical clips, etc.). To overcome this safety hazard, for studies within our department an MRI safety check questionnaire is used, administered by the person taking consent from the parents and double checked by our radiographers before placing the baby in the scanner.

The application of RF pulses during image acquisition deposits energy in the body, which dissipates as heat in the surrounding tissue (Tocchio et al., 2015). This is a considerable safety concern as it can cause serious local tissue burns and undesirable increases in body temperature in the baby. To prevent this from happening, our radiographers interchange low and high specific absorption rate sequences to reduce heat absorption by the baby. Moreover, the baby's oxygen levels, temperature and heart rates are monitored throughout the whole scanning session.

The switching of gradient fields during image acquisition produces a considerable amount of acoustic noise, which may be increased at higher magnetic field strengths (e.g., 1.5T, 3T) (Tocchio et al., 2015). Acoustic protection is provided by placing earplugs moulded from putty, neonatal earmuffs and an acoustic hood to further reduce noise (Hughes et al., 2017).

Aside from the general safety concerns involved in any MRI acquisition, when scanning neonates, there are also reduced signal-to-noise-ratio (SNR) and motion concerns. These concerns are reduced by acquiring the images during natural sleep after feeding and using a dedicated neonatal brain imaging system developed by Hughes et al. (2017) for the Developing Human Connectome Project (dHCP). A dedicated 32-channel neonatal head coil is used to maximise SNR. To reduce motion, the baby is swaddled, and

the body immobilised using an air-evacuated beanbag, and foam padding is then placed around the infant's head to prevent gross motion (Hughes et al., 2017).

2.2 Contemporary approaches to studying Neurodevelopmental Conditions (NDCs)

2.2.1 Studying development

The study of infant and child development and how humans learn and interact with their environment has been of interest for the last century, however, the approach taken to do so has changed with time. Since the 1950s, there has been a shift away from studying development through simple observation, to gathering information on cognitive abilities through experimental approaches (Johnson & de Haan, 2015). This experimental approach has presented several challenges, such as designing behavioural tasks that rely on simple verbal instructions or motor responses, or overcoming infants' and children's short attentional span, given that a certain level of engagement is often needed to cooperate with the experimenter. To overcome these challenges when studying cognitive development, several methods have been developed that build on infants' and toddlers' natural behaviours, such as their tendency to look at conspicuous and novel visual stimuli but to become bored of them relatively quickly These methods rely on the infant's preferential looking (i.e., infants will look at novel or preferred stimuli for longer), or habituation to stimuli (i.e., an infant's looking time will wane after the novelty of a stimulus has worn off), as a measure of attention or interest, and commonly use an eye-tracker to detect the exact pattern of looking (Aslin, 2012; Tafreshi et al., 2014).

Another useful method to study the relationship between brain development and behaviour, and how these change with age, is the *marker task* (Johnson & de Haan, 2015). This involves designing behavioural tasks (e.g., building a tower of blocks, or naming

pictures in a stimuli book) based on behaviours closely related to one or more brain regions or networks in human adults or non-human primate neuropsychological or imaging studies. The Mullen Scales of Early Learning (MSEL) (Mullen, 1995) or the Bayley Scales of Infant and Toddler Development (BSID) (Bayley, 1993, 2006) are examples of standardised test batteries based on the *marker task*. Studying an infant's performance in the marker tasks across different ages provides evidence that potentially reflects the relationship between known brain development patterns and the observed behavioural changes.

There are, however, several weaknesses to the *marker task*. For instance, different brain regions might be essential for a single task at different ages, complicating the interpretation of the results. Moreover, it is difficult to design tasks that are easy enough for the infants to perform but sufficiently demanding that higher cognitive capacities are tapped into (e.g., using preverbal cues, such as looking or orienting the head toward objects to assess receptive language in toddlers) (Johnson & de Haan, 2015). These challenges are applicable when studying development in a typically developing population (TD) or a population with any kind of neurodevelopmental condition (NDC), such as DS. However, there are additional challenges when this method is used with the latter population, especially when using standardised batteries. For example, these batteries often rely on verbal instructions given by the experimenter for each of the tasks the participant must perform. If the population with NDC being studied has difficulties with receptive language, a task might be incorrectly performed, or not performed at all due to a lack of understanding of the instructions, and not necessarily due to the inability of the participant to perform the task. Similarly, the scoring on these tests also relies on

the participants' motor responses (e.g., pointing at a picture in a book, or standing up from the desk and taking an object as a measure of receptive language), which means that slower developing motor skills may compromise the ability to assess cognitive abilities. In standardised tests, this might result in scores that misrepresent the child's true ability (the test is invalid) or in a floor effect of the standardised scores (the test is not sensitive enough).

The study of NDCs does not only have clinical and societal implications, but it can also inform about causal factors and basic processes and mechanisms important in typical development. In this thesis, NDCs will be classified as genetic syndromes, caused by well understood genetic abnormalities (e.g., DS, Williams syndrome, Fragile X syndrome), or behaviourally defined conditions, more usually associated with polygenic causes and an accumulation of common variants (e.g., developmental dyslexia, Developmental Language Disorder, autism) (Thomas & Karmiloff-Smith, 2002).

2.2.2 Theoretical approaches

When studying NDCs with or without their origin in a genetic syndrome, the theory chosen to explain the development and of their cognitive phenotype will not only inform the type of research questions asked, but also the interpretation of the results (D'Souza & Karmiloff-Smith, 2017). There are two main approaches that have been taken when conducting research with populations with NDC: the neuropsychological approach, often associated with nativist assumptions, and the neuroconstructivist approach, which places a greater emphasis on experience-dependent developmental processes.

The Neuropsychological approach

The neuropsychological or nativist approach views the brain as an ensemble of separate *functional modules*, each responsible for a specific cognitive function (Carruthers, 2006; Sperber, 2001). Functional modules are viewed as genetically defined brain areas or parts dedicated to specific cognitive functions, that can be selectively activated or impaired (Sperber, 2001). Examples from adult neuropsychological cases of acquired brain damage are often used as evidence to support this modularity view. The common assumption is that brain damage is the cause for poor cognitive performance, leading to the conclusion that the affected brain area in Patient 1 is necessary to perform cognitive task A (e.g., impaired expressive language) but not cognitive task B (e.g., intact receptive language). The existence of Patient 2 with a different damaged brain area that displays the reverse pattern of cognitive impairment (e.g., impaired receptive language and intact expressive language) leads to the use of cognitive double dissociations to relate brain and cognitive data directly (D'Souza & Karmiloff-Smith, 2017).

Many researchers have applied the logic of this approach when studying infants with NDC or genetic disorders, seeking evidence of modular impairment and cognitive dissociations. This leads to the conclusion that observed individual differences result from deficits or impairments, in one or more innately specified cognitive modules, and probably defined by genetic faults (D'Souza & Karmiloff-Smith, 2017; Fan et al., 2017; Wang & Bellugi, 1993). Nevertheless, NDCs do not display a pattern of behaviour interpretable in terms of a neat pattern of intact and impaired modules, that can be mapped onto specific genes (Karmiloff-Smith, 2006). Moreover, the neuropsychological approach views brain modules as innately specified (i.e., experience only provides the content for

the module but has no impact on what function it supports) (Sperber, 2001). It completely disregards the effects of the environment in the emergence of functional specialisation (widely supported by an extensive body of evidence) and how this might impact cognitive outcome (e.g., D'Souza et al., 2020). Empirical evidence from functional imaging demonstrating the emergence of functional specialisation (e.g., Blasi et al., 2011; Lloyd-Fox et al., 2012), supports the view that if any specific cognitive modules were to exist in the adult brain, these would be the result of ontogenesis over developmental time and not genetically predefined at the starting point. It is for this reason that adopting a developmental perspective when studying populations with NDCs is essential (Karmiloff-Smith, 2006).

The Neuroconstructivist approach

Unlike the static nativist approach, neuroconstructivism views development as a process of self-organisation that results from the interaction between multiple systems within a context, and is shaped by intrinsic (e.g., genetic, neural, psychological), as well as extrinsic factors (e.g., social context) (D'Souza & Karmiloff-Smith, 2017; Mareschal et al., 2007). In other words, proponents of this theory argue that humans are not born with fixed, function-specific brain modules, instead, we are born with *domain-relevant* functional modules that only become domain-specific with the process of development and environmental interactions (Karmiloff-Smith, 1998).

Regarding the approach to studying atypical phenotypes, instead of trying to identify impaired and intact cognitive modules in older children and adults, neuroconstructivism aims to identify more indirect low-level causes of atypicality earlier in development, ultimately tracing them back to infancy (Karmiloff-Smith, 1998). It is for

this reason that a multilevel approach (e.g., the genetic, the spatial and temporal brain dynamics, the cognitive, the behavioural and the environmental) is encouraged when investigating NDCs. Moreover, if the brain does indeed start as a highly interactive system, and its development is influenced by complex multilevel interactions, an initial impairment in one component (e.g., a basic-level deficit driven by genetic alterations, like reduced progenitor cells) is likely to have cascading effects on other parts of the developing system and at higher-level cognitive functions, and developmental plasticity may allow compensatory pathways (D'Souza & Karmiloff-Smith, 2017). Hence, research in neurodevelopmental conditions should start from early infancy.

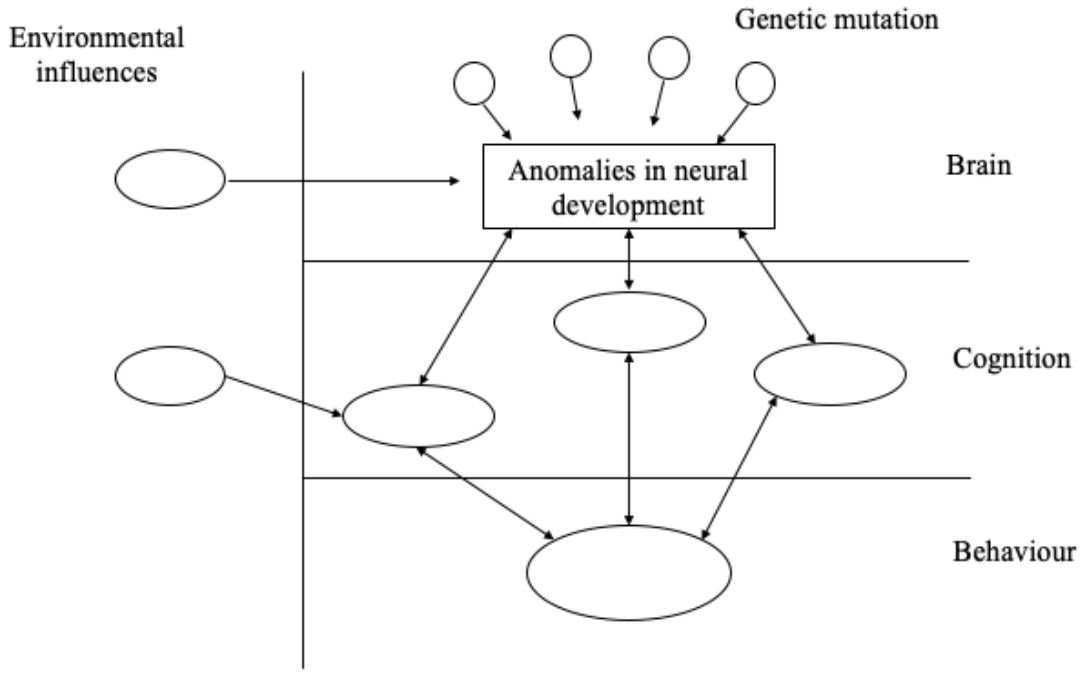
As a consequence of the cascading effect —resulting in higher level components being impacted by lower-level impairments— and multilevel interactions, such as when atypical environments lead to atypical functional systems that influence the operation of lower-level mechanisms', proponents of the neuroconstructivist approach argue that comparing the brains of children with NDCs to adults with acquired brain lesions is inappropriate. Instead, they believe that it is more likely that children with NDCs develop atypical neural and cognitive trajectories with potentially numerous widespread impairments, proposing that their brains are better described as an *"atypical system developing under different constraints*" (D'Souza & Karmiloff-Smith, 2017, p. 4). It is for this reason that research in NDCs should be carried out from early infant stages and longitudinally, keeping development (e.g., brain development, cognitive development) at the forefront of every explanation. The work presented in this thesis has been carried out within the neuroconstructivist framework, which has guided the methodological choices,

specifically a focus on infancy, on longitudinal designs, and a combination of brain and behavioural measures.

2.2.3 The multilevel approach: genes, the brain, cognition and behaviour, and the environment

According to Morton and Frith (1995, 2008) when investigating the causes of NDCs, there are several levels of description that should be taken into consideration: genes, biology or the brain, cognition, behaviour, and environment. Figure 2.15 shows a possible causal model of the cognitive and behavioural phenotype found in a genetic syndrome, like DS (Morton, 2008). A known genetic alteration, like the triplication of chromosome 21, causes anomalies in neural development (e.g., reduced neurons, delayed myelination, disorganised cortical layering). These neural anomalies cause atypical cognitive processes, which might in turn influence neural development (e.g., developmental plasticity and synaptic pruning), and result in the observed behavioural phenotype. The environment might have an overarching effect in the causal model, influencing one or many of the descriptive levels (e.g., prenatal or postnatal injuries affecting the brain, or a parent's interaction with their child influencing their cognitive development) (Morton, 2008).

Figure 2.15 *Possible causal model of the cognitive and behavioural phenotype in a genetic syndrome (modified from Morton, 2008).*



Genes

With over 86 billion neurons and trillions of synaptic connections, the brain is the most complex organ in the human body. Although its development is largely influenced by postnatal experiences and the environment in which it is formed, the control and coordination necessary to build a brain is largely predetermined by genetic programming, especially in the earlier embryonic stages (Osborne, 2012). Considering the influence of genes in brain formation and development, and consequently in cognitive abilities, it is crucial to investigate the genetic level of explanation when attempting to understand the phenotype in NDCs. This is especially true in NDCs caused by genetic alterations, such as DS because basic processes in brain development, like neuronal proliferation or migration may have been disrupted.

Depending on their role at any given time, the expression of mammalian genes changes throughout development, so to truly understand the impact of genes in any NDC's

phenotype, the precise spatial and temporal expression of the genes should be examined (Osborne, 2012). The role of epigenetics should also be taken into consideration, that is, how an individual's behaviour and environment might modify their gene expression. Finally, when describing the relationship between genotype and phenotype, careful consideration should be taken not to commit the fallacies of simplistically assuming that any combination of gene effects will alone determine a cognitive function, or that genes code for neural structures that solely relate to a single cognitive domain (Karmiloff-Smith et al., 2002). In truth, it still remains relatively unknown how genetic variation might result in uneven cognitive profiles via the influence of genes on brain development (e.g., Grasby et al., 2020). In fact, the genes involved in fundamental processes of brain development are highly conserved, that is they do not vary widely in the population, obscuring the main mechanisms underlying variation in brain development.

There are several methods that can be applied to investigate the genotype-phenotype relations, such as quantitative and molecular genetics, computational studies of NDCs, or the use of animal models (Johnson & de Haan, 2015; Karmiloff-Smith et al., 2002). Although homologies between species need to be carefully considered, these models can directly or indirectly inform how genes relate to brain organisation and development, and provide useful theoretical and empirical insights into cognitive development in humans (Johnson & de Haan, 2015). A commonly used animal model is the murine one. Selective breeding of natural traits, or the creation of mice strains with specific genes knocked out or altered (i.e., transgenic mice), allow the study of how

specific genetic changes affect brain development at different prenatal and postnatal stages (Karmiloff-Smith et al., 2002).

The use of animal models to understand cognitive development in humans is especially helpful in the case of genetic syndromes, where the genetic alterations related to the behavioural and cognitive phenotypes are better defined than in behaviourally defined NDCs. For instance, in DS, little is known about the molecular mechanisms (e.g., neurotransmitter functioning) underlying the associated developmental cognitive disabilities (Liu et al., 2011). However, it is well known that there is an imbalance in the expression of more than 300 genes present in the extra copy of chromosome 21, which has a clear homology to 3 chromosomes in mice. The existence of this conserved synteny allows the creation of several mouse models of DS, where different homologous genes have been knocked-out or altered, which can be used to explore the neuroanatomical and cognitive phenotype. The latter one, however, is limited by the extent to which human and animal behaviours can be matched. That is, although tasks measuring memory or sensorimotor abilities can be aligned between human and animal studies, it is not possible to do so with tasks measuring higher-level cognitive functions, like language (D'Souza et al., 2020; Karmiloff-Smith et al., 2002). Aside from valuable theoretical and empirical insights to understand the phenotype in humans with DS, these mouse models have also allowed the identification of brain mechanisms (e.g., brain volumes, myelination, cell proliferation) particularly affected by the trisomy, which have then informed the pharmacological approaches to correct underlying imbalances that disrupt cognitive functions in DS (Das & Reeves, 2011; Liu et al., 2011).

The brain

The next level of description to consider, is the biological one (Morton, 2008; Morton & Frith, 1995), more precisely, the brain. There are different levels in which the brain can be described at: (1) gross brain anatomy (e.g., regional brain volumes), (2) functional neural systems and pathways (e.g., default mode networks), and (3) neurochemistry and microcircuitry (e.g., neurotransmitter imbalance) (Johnson & de Haan, 2015). Thanks to new technological advances, many methods of measurement exist nowadays to explore these different levels of description, from early developmental stages (e.g., fetuses and new-born babies). These methods include: post-mortem studies of brain tissue to explore microanatomy and biochemistry; different modes of Magnetic Resonance Imaging (MRI) to explore in vivo brain anatomy (structural MRI), biochemistry (magnetic resonance spectroscopy), activity (functional MRI (fMRI)), or water diffusivity (diffusion MRI (dMRI)); and other methods to measure brain function, such as, magnetoencephalography, electroencephalography (EEG), and functional near-infrared spectroscopy (fNIRS) (Karmiloff-Smith, 2010, 2012). Each of these methods allows exploration of a single aspect of the brain, so a combination of these methods should be used to obtain complementary data about changes in the time course, spatial location, and connectivity of neural activity in the brain (Karmiloff-Smith, 2012).

As with any other methodology, neuroimaging studies with NDCs could also apply a neuroconstructivist approach to their interpretations. Brain imaging is not intrinsically developmental, but a developmental perspective can be deliberately taken by using neuroimaging techniques to measure developmental changes and mechanisms, rather than static snapshots of system states at different ages. The relationship between

brain imaging results and cognition could also be viewed as a bidirectional one. For instance, for the nativist perspective, a reduction in volume of a particular brain area (e.g., parietal cortex) would be the cause for poorer performance in a cognitive task (e.g., numerical tasks). The neuroconstructivist approach would acknowledge this relationship, but it would also argue that the reduced brain volume could be a consequence of atypical cognitive processing in that area (Karmiloff-Smith, 2012).

Moreover, the method used for analysing neuroimaging data should also be carefully considered. Rather than using a region of interest (ROI) approach, like in many adult studies, where a specific part of the brain is selected and isolated for analysis, a hypothesis-driven whole brain volume analysis approach might be favoured when studying NDCs (Karmiloff-Smith, 2010). That is, ROI analyses encourage a modular, and therefore non-developmental perspective. However, per neuroconstructivism, any region is developing in the context of the other regions to which it is connected, and therefore a whole brain perspective is more appropriate. This could be especially helpful when studying the atypical brain in cases were cerebral volumes or regional boundaries may differ substantially from their TD peers.

Cognition and behaviour

According to the neuroconstructivist point of view, it is possible for compensatory pathways to develop in children with NDCs. This would mean that children with NDCs could display behaviours that look equivalent to typically developing children, even though the underlying cognitive process may be different (D'Souza & Karmiloff-Smith, 2017). Therefore, cognition and behaviour should be considered as two separate descriptive levels. More precisely, it would be more suitable to consider cognition as a

necessary intermediary between brain and behaviour (Morton, 2008). In this case, cognition refers to functions of the brain, like perception, memory, or language, whilst behaviour refers to directly observable actions. According to Morton (2008; Morton & Frith, 1995) behaviour itself might not be reliable when studying NDCs, because (1) different causes can underlie the same atypical behaviour (e.g., atypical neural pathways, or peripheral motor control problems might be the cause of speech impairments), (2) the same cause can lead to different patterns of behaviour, and (3) even if there is no reason to believe that the underlying processes have changed, the atypical behaviour might vanish if circumstances change (e.g., for autism, learnt coping strategies might lead to better social skills, even though the cause of the original atypicality, a theory of mind impairment, is still present).

The environment

Proponents of the neuroconstructivist approach argue that the genetic makeup of an individual and their brain alone will not solely determine the resulting cognitive function. They propose that the emergence of cognitive functions is context dependent, and that the environment plays a causal role in generating the ultimate cognitive structures. In this context, the environment refers to everything from the biochemical environment affecting cell differentiation in the embryo and the prenatal nutritional and maternal environment affecting the development of the fetus, to the external world with which infants interact during the process of cognitive development (D'Souza & D'Souza, 2019; Karmiloff-Smith et al., 2002; Mareschal et al., 2007).

Regarding the external, social environment in which an infant develops, the researcher should be aware of the possibility that this may differ when a child has an NDC.

The parental knowledge of their infant's condition might change their expectations, the sensitivity of parenting, or their ability to scaffold the development of different cognitive skills (Karmiloff-Smith, 2006; Thomas et al., 2020). Having a child with a NDC might also have an impact on parents' mental health which might affect the way they interact with the child and their cognitive development (D'Souza, Lathan, et al., 2020). Outside the home environment, the family's socioeconomic status or neighbourhood might impact the time and resources available to support the child in the home or school, and in the therapeutic resources available to the family (Thomas et al., 2020).

2.2.4. Methodological considerations when studying NDCs

Standardised tests and scores

When conducting research with populations with NDCs *standardised tests* are commonly used to assess participants' cognitive, adaptive, or affective functioning. Standardised tests are psychometric measures designed to test a sample of behaviours relevant to cognitive or affective abilities, or both (Johnson & Marlow, 2006; Urbina, 2014). They are considered standardised because the administration, scoring and result interpretation is done in a specific way (Urbina, 2014). In fact, when these tests are created, they are administered following a specific procedure to a large group of people, meant to be representative of the population the test is focused on and referred to as *the norm* or *normative sample*, whose scores can then be used as a standard to compare participants' scores to (Johnson & Marlow, 2006; Purser & Van Herwegen, 2016; Urbina, 2014).

Different scores can be extracted from standardised tests. The most direct score that can be extracted is the *raw score*, which is the number of correct answers a child has given. Although easy to compute, raw scores cannot normally be compared to each other

as children from different ages will have different starting points, and the amount of items administered by differ between tasks (Purser & Van Herwegen, 2016). For this reason, standardised scores can also be extracted from these tests, which allow not only to compare scores to each other, but also allows to compare the individual's performance against the normative sample.

Standardised scores are age-adjusted scores, with a normal distribution and prescribed mean and standard deviation (SD) and include statistical metrics like *z*-scores (Mean 0, SD 1) and *t*-scores (Mean 50, SD 10) (Johnson & Marlow, 2006; Purser & Van Herwegen, 2016). When interpreting the results, SD becomes the unit of measurement, representing an individual's deviation from the normative sample's mean and used to classify the child's developmental level (e.g., a child considered to have a delay in development would have a large negative score). Aside from standardised scores, other norm referenced scores, like percentiles or Age Equivalents (AE), can also be extracted from standardised tests, and in those that are composed of subscales or domains, composite or overall scores are also commonly available. Whilst percentile ranks represent the percentage of scores in the normative sample that are lower than an obtained test score, AE scores represent the age at which the assessed individual's score is the mean or median observed in the normative sample.

Although widely used in research with populations with NDCs, there are some disadvantages to using AE scores. For instance, if a child obtains low raw scores in a scale, there might not be available AE scores to extrapolate (Maloney & Larrivee, 2007; Sullivan et al., 2014). It has also been suggested that AE scores extrapolated from a normative sample might not be valid to accurately represent the abilities of children with NDCs, as

they might use different underlying processes to TD children when approaching the same test stimuli items (Couzens et al., 2004). Finally, AE scores reflect a child's ability as absolutes, so as children grow, AE scores will increase too (Toffalini et al., 2019). If a sample has a wide age range (like the sample with DS presented in Chapter 4), variability in AE scores might simply be reflecting range of CA, rather than actual variability in AE scores.

Some of the most commonly used standardised tests to assess cognitive abilities in developmental research in infancy and early childhood are the MSEL (Mullen, 1995) and the Bayley Scales of Infant Development II (BSID-II) and III (BSID-III) (Bayley, 1993, 2006). They are both designed to assess a child's global developmental level by providing an inventory of key developmental milestones in different cognitive domains, such as language or fine and gross motor abilities. The MSEL provides separate measures of Gross Motor, Fine Motor, Visual Reception, Receptive Language and Expressive Language abilities, as well as a measure of global cognitive functioning (Early Learning Composite standardised score) (Mullen, 1995). However, the BSID-II and BSID-III differentiate between higher and lower cognitive abilities, as they provide scores that pull apart cognitive (BSID-II Mental developmental Index score (MDI), and BSID-III Cognitive composite score (CCS) and Language Composite score (LCS)) and motor abilities (BSID-II Psychomotor developmental Index (PDI) score, and BSID-III Motor composite score (MCS)) (Bayley, 1993, 2006). For the current thesis, the MSEL was chosen instead of the BSID-II/-III, as I had access to existing MSEL data from a larger sample with DS from the LonDownS cohort, and there was an interest in separately examining fine motor and gross motor skills. For the purposes of this thesis, I will refer

to all subtests of the Mullen as measuring cognitive abilities, albeit relying on higher or lower levels of cognition, since the relevant motor behaviours assessed are not reflexes, and therefore require cognitive initiation.

Aside from these two lab based standardised tests, parental questionnaires, like the Vineland Adaptive Behaviour Scales (VABS II) (Sparrow et al., 2005) are also commonly used in developmental research to assess adaptive functioning (e.g., Mattie et al., 2023). The Vineland II measures motors skills, and personal and social sufficiency across three domains: Communication, Daily Living Skills, and Socialisation. Unlike the MSEL or the BSID-III that are valid only for the first few years of life (up to five years of age), the VABS II can be used to assess adaptive functioning from birth to age 90, making it a powerful tool to study development.

When using standardised tests to assess populations with NDCs there are certain limitations and challenges that should be considered. For instance, some adaptations of the administration procedure might be necessary (Johnson & Marlow, 2006; Purser & Van Herwegen, 2016). A child with a NDC, like DS, might have visual, hearing or language impairments, so additional prompts might be necessary to get a response. They might also struggle to maintain their attention, so modification might be made to shorten the testing time (e.g., modify the procedure to select the starting point), or caregiver reports might have to be accepted instead of direct observation to score an item.

During the acquisition of the neurodevelopmental data collected for this project, and presented in Chapter 4, such adaptations were made. When administering the MSEL, rather than selecting the starting item based on the child's CA and finding the basal level (i.e., three consecutive items scoring at least 1), a ceiling level (i.e., scoring 0 in three

consecutive items) was found first. This adaptation was implemented due to the developmental delay present in the population with DS (D'Souza et al. 2021), and to reduce the amount of time needed to assess the full cognitive ability of the participant, overcoming the challenge that the short attention span common in infants and children with DS (Brown et al., 2003; Fidler et al., 2018; Grieco et al., 2015) presents. When completing the VABS II, parents were asked to complete each of the subscales starting from the first item, not based on the CA of the child for which they were completing the questionnaire. This adaptation was implemented because the developmental delay present in the population with DS (D'Souza et al. 2021) often meant that the standard stating point (i.e., based on their CA) depicted activities beyond the abilities of the children.

However, most tests do not allow such adaptations, which means the normative value of the test might be compromised (Johnson & Marlow, 2006). The appropriateness of each of the scores should also be considered. In populations that show an uneven cognitive profile, for example, overall or composite scores should be used cautiously, as they might underestimate a child's ability in a domain, whilst overestimating it in another one (Purser & Van Herwegen, 2016). Similarly, if a child's development is delayed (e.g., is lower than expected for their chronological age) it could be that normative scores are not available because there are simply no tabulated norm scores for their age group. This is the case, for example, for MSEL gross motor abilities scores for older children with DS. Finally, in cases where development is significantly delayed or impaired, floor effects might be encountered when using standardised scores, hindering the identification of subtle difference in abilities (Karmiloff-smith, 2009; Karmiloff-Smith et al., 2003).

Study approaches: matching and trajectories

For many years, the most common approach to studying NDCs has been to use group matching (Burack et al., 2004; Jarrold & Brock, 2004; Thomas et al., 2009). In this approach the group with an NDC is matched to one or more control groups and their performance compared, whilst the control group acts as a reference point for the performance of the group with NDCs (Knowland et al., 2015). The purpose of matching is to cancel out any effect of variables that are not under investigations and might explain group differences (e.g., understanding instructions, cognitive abilities related to the task under investigation, response behaviours, and so forth) (Jarrold & Brock, 2004).

The two most common variables to match for are chronological age (CA) and mental age (MA) (Caplan et al., 2015; Flanagan et al., 2008). CA-matching allows one to investigate whether the group with the condition is developing or performing on a task at the level expected for their actual age. However, this method is essentially comparing groups that by definition operate at different developmental levels, which will often lead to results where the group with the condition has a lower performance, simply suggesting that they have a deficit or impairment in a specific cognitive domain (Caplan et al., 2015; Flanagan et al., 2008). MA-matching, on the contrary, allows one to investigate whether the group with a NDC is performing at a level expected from peers matched on some relevant indicator of developmental level, providing a more significant indication of the cognitive delay (Caplan et al., 2015; Flanagan et al., 2008). Therefore, the selection of the matching approach will be based on the research question, and whether events of interest are more experience dependent (e.g., onset of expressive language, myelination of axons)

or part of biological maturation (e.g., whether the child has started to walk yet), that is, linked to CA.

MA matching is commonly carried out using standardised test, and can be based on global measures of development (e.g., IQ, global verbal/nonverbal ability) or based on more specific single measures that mirror the ability under investigation (e.g., attention, receptive language, expressive language) (Flanagan et al., 2008; Jarrold & Brock, 2004). This latter approach is theory dependent as it is based on the theoretical assumption that the task used for matching is the best one to tap into the cognitive ability of interest. Similarly, the global or composite MA measure is a commonly used alternative but might not be ideal in cases where an uneven cognitive profile exist. In fact, global MA measures rely on the idea that the abilities in all the subtests are closely correlated, which might be true in a TD group, but not in the NDC one (Karmiloff-smith, 2009; Thomas et al., 2009). This would compromise the interpretation of the results as it could lead to a selection of a control group that surpasses the ability of the condition group in some measures but falls short on others. Regardless of the measure chosen, matching between groups can be done either at the group level or individually. Whilst the later one entails selecting a control participant with the same CA or MA for each of the participants in the NDC group, group matching is done based on the group CA or MA means, which is less demanding on recruitment (Thomas et al., 2009).

A more recent theory-neutral approach that places development at its core is the developmental trajectory approach. This approach, rather than simply comparing static performance between groups, consists on creating functions of cognitive performance against age (either chronological or mental) to allow developmental trajectories to be

compared between groups (Thomas et al., 2009). Trajectories can either be cross-sectional or longitudinal. Cross-sectional trajectories are created by collecting data at single points in time from different age groups, which is easier in practice, but does not account for individual variability. This limitation can be overcome by creating the trajectory using longitudinal data. That is, data are collected from the same individuals at several points in times and developmental trajectories are created for each individual. This, however, is a very time-consuming method, so cross-sectional trajectories are more commonly used, which give an approximation of developmental trajectories, and can be validated by longitudinal research (Knowland et al., 2015).

The trajectory approach can also be combined to the matching approach for richer interpretation of results and better understanding of NDCs. For instance, if the NDC group shows a developmental trajectory different from a CA-matched group, but not from a MA-matched group, individuals with the NDC are considered to have a developmental delay. If, on the other hand, the NDC group has a development trajectory different from both CA- and MA-matched groups, then the development is considered to be atypical (Thomas et al., 2009). Different patterns of development can also be explored with this approach. For instance, D'Souza et al. (2021) conducted a cross-sectional developmental trajectory study in a sample of 104 children with DS to investigate the emergence of the cognitive profile in the first five years of life. They found not only that the divergence from the TD trajectory was gradual, but also that the developmental trajectory of different cognitive domains differed between each other. For example, whilst expressive language seemed to further diverge from the TD trajectory with time, receptive language seemed to catch up (i.e., it was initially low but rapidly increased) to the TD trajectory. Finally the trajectory

approach also allows to investigate developmental relations between different abilities (Thomas et al., 2009). That is, developmental trajectories can be created for each individual for different cognitive abilities, such as, gross motor, fine motor, receptive language, or expressive language. Then the pattern of these trajectories (i.e., whether their trajectory is similar or different) can be used to make inferences about how these different abilities relate to each other and whether they are developing equally (even cognitive profile) or unequally (uneven cognitive profile).

2.3 Summary

The current chapter has presented key concepts and considerations on the methodological and theoretical approaches underpinning the work presented in this thesis. The basics of MRI data acquisition have been presented, differences and limitations in diffusion data analysis approaches have been considered, and safety precautions for neonatal image acquisition have been explained. Finally, contemporary approaches to studying NDCs have been presented, establishing the theoretical framework of the thesis, and methodological considerations have been presented.

The following chapter presents the study where WM microstructure differences between neonates with DS and age-matched TD controls were investigated using two different dMRI analysis approaches.

Chapter 3: White matter microstructure in neonates with DS

3.1 Introduction

As presented in the introduction, the cerebral phenotype in Down Syndrome (DS) is complex, manifesting at both macrostructural (e.g., reduced brain volumes) and microstructural levels (e.g., reduced cell numbers). White matter features including myelination have been extensively studied ex vivo and in animals but have scarcely been investigated in vivo. Most studies focusing on the in vivo brain in the DS population have

focused on exploring volumetric (e.g., Carducci et al., 2013; Pinter et al., 2001) or functional (e.g., Anderson et al., 2013; Pujol et al., 2015) rather than microstructural alterations when compared to a typically developing (TD) or healthy population.

There are, however, a small number of studies exploring the alteration in MRI-measured white matter diffusion (dMRI) in the DS population (see Table 3.1 for summary). Most of these studies have focused on investigating the effect of aging or dementia on diffusion properties in white matter (Fenoll et al., 2017; Powell et al., 2014; Romano et al., 2018). In fact, by the age of 65, 68–80% of individuals with DS have developed dementia, with memory and attention deficits, as well as neuropathological changes consistent with Alzheimer's disease (e.g., development of amyloid plaques and neurofibrillary tangles) being present as early as 40 years-old (McCarron et al., 2014; Startin et al., 2019; Wiseman et al., 2015).

For instance, Powell et al. (2014) investigated the differences in white matter (WM) between 50-year-old adults with DS with (n= 10) and without Alzheimer's (n= 10) (ADDS and DS, respectively) and age-matched controls (n= 10). Tract-based spatial statistic (TBSS), a tensor-based diffusion data analysis approach, was used to extract fractional anisotropy (FA) values of the main white matter fibre bundles, and the results showed significant reductions of FA in the anterior parts of the brain (e.g., inferior fronto-occipital fasciculus, uncinate fasciculus, forceps minor, cingulum, thalamic radiations, inferior longitudinal fasciculus) in the DS groups when compared to controls, and further FA reductions in these same areas in the ADDS group compared to the DS group. Moreover, these FA reductions were also related to lower scores in a motor skills test

Table 3.1 *Summary of DS animal models and human MRI/post-mortem studies examining white matter alterations when compared to TD controls. Results are divided between post-mortem/mouse model and in vivo studies and are presented following the chronological age of the sample.*

Authors	Method	Sample size	Age	Brain area	Results
Post-mortem and mouse model					
Ábrahám et al. (2012)	Human tissue histology	*n*= 47 (23 DS; 24TD)	17 weeks GA - 65 years	Hippocampus	Same myelin developmental pattern but delayed. Decreased density of myelinated axons in hilus of dentate gyrus.
Olmos-Serrano et al. (2016)	Human tissue histology and Ts65Dn mouse model	*n*= 30 (15 DS; 15 TD)	14 weeks GA - 42 years	Whole brain	Co-dysregulation of genes associated with oligodendrocyte differentiation and myelination.
In vivo human studies					
Gunbey et al. (2017)	TBSS FA and MD	*n*= 18 (10 DS; 8 TD)	2.6 ± 0.69 years	Whole brain (TBSS mask)	Reduced FA in right CP, right UF, CC body, right ALIC and bilateral ILF and IFOF. Increased MD in clusters of the right CP, ILF and IFOF, left EC and PTR and bilateral ALIC, ATR, UF, IFO
Lee et al. (2020)	TFCE FA and MD	*n*= 30 (15 DS; 15 TD)	6-23 years	Whole brain	Reduced FA in inferior and middle cerebellar peduncles, CP, EC, anterior corona radiata, internal capsule, fornix, and medial lemniscus. No difference in MD

Romano et al. (2018)	TBSS FA, MD, AD, and RD	*n*= 46 (26 DS; 17 TD)	14-27 years	Whole brain (TBSS mask)	Reduced FA bilaterally in ATR, IFOF, the ILF, and the cortico-spinal tract. Increased MD, RD and AD bilaterally in ATR, IFOF, ILF, cortico-spinal tract, minor and major forceps, SLF, the cingulum, and UF.
Fenoll et al. (2017)	TBSS FA	*n*= 90 (45 DS; 45 TD)	18-52 years	Whole brain (TBSS mask)	Reduced FA in frontal lobes, semioval centres, CC, EC, internal capsule, putamen, thalamus, pyramidal tracts, and brainstem.
Powell et al. (2014)	TBSS FA	*n*= 30 (10 DS; 10 ADDS; 10 TD)	50+ years	Whole brain (TBSS mask)	Reduced FA in IFOF, SLF, forceps minor, thalamic radiations, frontal and temporal UF, ILF, and cingulum.

Note. ADDS= participants with Down syndrome and Alzheimer's disease; ALIC= Anterior limb of internal capsule; ATR= anterior thalamic radiation; CC= corpus; callosum; CP= cerebral peduncle; EC= External capsule; FA= Fractional anisotropy; GA= Gestational age; IFOF= inferior fronto-occipital fasciculus; ILF= inferior longitudinal fasciculus; MD= Mean diffusivity; PTR= posterior thalamic radiation; SLF= superior longitudinal fasciculus; TBSS= Tract-based spatial statistics; TFCE= threshold free cluster enhancement; UF= uncinate fasciculus.

A later study by Fenoll et al. (2017) conducted the same diffusion data analysis in a sample of non-demented adults with DS (*n*= 45) across a wider age range (18-52-years-old) aiming to investigate WM alterations in DS and to determine whether changes in WM are detectable before dementia becomes clinically evident. The results showed FA reductions in the DS group compared to the age-matched controls (*n*= 45) in the anterior parts of the brain, as in Powell et al. (2014), as well as the corpus callosum (CC), external capsule, internal capsule, putamen, thalamus, pyramidal tracts, and brainstem, indicating a more widespread alteration of the WM in DS compared to controls. In both groups, FA decreased with age at a similar rate, suggesting that WM changes with age were not significantly different between the DS and the control group. Moreover, none of the participant with DS were diagnosed with Alzheimer's diseases, so the authors were unable to conclude whether premature aging resulting in WM changes happened prior to dementia becoming clinically relevant.

Romano et al. (2018) also focused on studying the effects of aging in WM in the DS population (*n*= 26) before any signs of dementia but recruited a younger sample whose age ranged 14-27 years old. TBSS analysis was used to analyse the diffusion data, but in this case differences between the DS and control group (*n*= 17) in FA, mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD) metrics were explored. After controlling for age and sex, the result showed significant decreased FA and increased MD, AD and RD in the participants with DS in the anterior thalamic radiation, the inferior fronto-occipital fasciculus, the inferior longitudinal fasciculus, and the cortico-spinal tract. There were also increased MD, AD and RD in the forceps minor and major, superior

longitudinal fasciculus, the cingulum, and the uncinate fasciculus in the participants with DS.

These studies have used the same approach to analyse the diffusion data and have found WM alterations in people with DS throughout adulthood (from 14 to 52 years) and across the brain, showing some consistent altered brain areas (e.g., inferior fronto-occipital fasciculus, forceps minor, inferior longitudinal fasciculus, thalamo-cortical tracts, cingulum, etc.). This suggests not only that there are indeed widespread WM alterations in individuals with DS, but also that in vivo techniques, such as dMRI, are appropriate to capture them. Changes in WM microstructure are part of normal aging (Bendlin et al., 2010; Bennett & Madden, 2014; Madden et al., 2009), and its development is a lifelong process that widely varies between individuals (Lebel et al., 2012) and it is influenced by external factors. Studies with younger populations would allow to discern whether WM microstructure alterations in adults with DS are due to properties of the system that are an outcome of prior atypical development (e.g., less robust WM structures), or they are a proximal later-life pathology that is independently caused by the trisomy (e.g., the extra copy of the amyloid precursor protein gene in chromosome 21 leading to excessive amyloid deposition (Tcw & Goate, 2017)).

A recent study investigated WM alterations in a younger sample with both children and young adults with DS (n= 15) aged 6–23 years old (Lee et al., 2020). Whole-brain TBSS and threshold free cluster enhancement (TFCE) analyses, an approach that enhances areas of signal that exhibit some spatial contiguity, were performed to assess FA and MD differences between participants with DS and age-matched controls (n= 15). In this study, as opposed to previous literature, TBSS showed no significant differences

between the groups either in FA or in MD, but TFCE showed significantly reduced FA in the DS groups in the inferior and middle cerebellar peduncles, cerebral peduncles, external capsule, anterior corona radiata, internal capsule, fornix, and medial lemniscus. The authors also performed a DTI driven tensor-based morphometry (DTBM) analysis, which uses the level of deformation needed to spatially normalise each subject as a measure of a structure's size deviation from the study template (Lee et al., 2020). In this study a template was created with an independent set of 14 TD controls, so the results from the DTBM analysis represent the deviation from TD, negative results meaning smaller structures than the average volume. In this case, supratentorial brain[1], brainstem and cerebellum grey and white matter were parcellated into several regions of interests (ROI) for analysis. The results showed significant hypoplasia in the fornix, the cingulate and the cerebellum in the DS group. These latter results were found in the cerebellar hemispheres, pons (including transverse pontine fibres), inferior olivary nuclei, and inferior and middle cerebellar peduncles. The results from this study suggest that the presence or absence of differences in WM between DS and TD depend on the choice of analytical methods.

Although Lee et al.'s (2020) study included a paediatric population, the age range was very wide, during a period when myelination in the brain is maturing rapidly (Dean et al., 2014; Stiles, 2008b), and this was not reported as part of their analysis, not even as a covariate. This limitation was addressed in Gunbey et al.'s (2017) study, where they explored WM alterations in a paediatric sample of toddlers with DS with a narrower age

[1] *Supratentorial brain:* the brain area above the cerebellar tentorium, containing the cerebrum, and excluding the cerebellum.

range (mean age 2.6 ± 0.69 years). They conducted a whole brain voxel-wise TBSS analysis to compared FA and MD measures between toddler with DS (*n*= 10) and age-matched controls (*n*= 8). The results showed significantly reduced FA in children with DS in association tracts of the fronto-temporo-occipital regions as well as the CC and anterior limb of the internal capsule, areas which have previously found to be altered in the adult DS population.

Although myelination is a lifelong process, most of it is completed within the first two years of life (Stiles, 2008b), and increasing evidence suggests that it is an experience-dependent process (Kaller et al., 2017; Monje, 2018; Mount & Monje, 2017; Purger et al., 2016) introducing further variables to cohort comparison studies. In order to understand the effect of DS on developing white matter more specifically, the effects of variables such as age and experience need to be minimised. To that end, study of the newborn brain might allow a better understanding of the implications of the triplication of chromosome 21 in white matter development.

Established protocols used to image the adult brain cannot be used to image the neonatal brain because of its higher water content (Batalle et al., 2018; Rutherford et al., 2006). In fact, at term-equivalent age (40 weeks gestational age[2] (GA)), the average diffusion MRI signal from the grey matter and the white matter is nearly indistinguishable due to the low myelination rate (Pietsch et al., 2019), which poses some challenges and considerations for both practical and technical aspects of MRI when acquiring diffusion

[2] Gestational age (GA) is a measure of the age of a baby during pregnancy, which is based on the beginning of the woman's last menstrual period, or on the earliest antenatal ultrasound.

weighted images (Hüppi & Dubois, 2006; Pietsch et al., 2019). For neonatal dMRI acquisitions, field strength, image resolution, number of encoding directions and gradient strengths need to be considered to produce optimised imaging data for the immature brain (Pannek et al., 2012). For instance, a higher field strength of 3T, as opposed to 1.5T, provides an increase in signal-to-noise ratio (SNR), but also increases the severity of artefacts and the specific absorption rate. To counteract these artefacts, an increased number of encoding directions can be used. Finally, the optimal b values should be selected to address the high water content in the neonatal brain, without compromising angular resolution (Pannek et al., 2012).

In recent years, diffusion weighted acquisition protocols for neonatal imaging have been optimised, addressing all those parameters (e.g., Hutter et al., 2018). The optimisation of acquisition protocols during the last decade has led to an increase in the number of studies investigating diffusion properties of the neonatal brain to explore typical early WM development (e.g., Kunz et al., 2014; Telford et al., 2017), WM alterations (Dimitrova et al., 2020) and structural connectivity (Batalle et al., 2017) in babies born prematurely, and even cortical development and maturation from mid-gestation to 47 weeks post menstrual age[3] (PMA) (Batalle et al., 2019; Fenchel et al., 2020). WM maturation is associated with cognitive function development (Nagy et al., 2004), and the assessment of its microstructural characteristics through dMRI has provided clinically relevant associations with later neurodevelopment in TD (e.g., Feng

[3] Postmenstrual age (PMA) is a measure of the age of a baby in weeks, used once the baby is born, that takes GA into account. A preterm baby born at 35 weeks GA will be 36 weeks PMA one week after birth.

et al., 2019) and atypical populations (e.g., Jeong et al., 2021; Kelly et al., 2020; Kooij et al., 2012; Rose et al., 2009).

3.1.1 Aim and hypothesis of the current study

Studying WM microstructure in neonates with DS might produce early brain biomarkers that relate to later cognitive outcomes and provide *in vivo* surrogate outcome measures for future early interventions designed to reduce alterations in brain development. To the best of our knowledge, the current study is the first to use diffusion imaging to assess early white matter microstructure in neonates with DS. The primary aim of the study was to explore in vivo WM alterations in neonates with DS compared to age-matched controls. It was hypothesised that the alterations found in children and adults with DS would be present from the perinatal period in the anterior parts of the brain. More precisely, it was hypothesised that FA would be reduced in neonates with DS in the CC, internal capsule and inferior longitudinal fasciculus. Moreover, previous dMRI studies in the DS population have used tensor-based modelling to analyse their data, which has some limitations and difficulties in the interpretation of the extracted metrics (FA, MD, RD and AD) (Chanraud et al., 2010; Jones et al., 2013). So, the second aim of the study was to apply a novel diffusion data analysis technique (FBA) to examine different aspects of WM microstructure, including fibre density and fibre bundle cross-section, in neonates with DS and compare them to TD controls. Based on the findings from post-mortem studies showing delayed myelination and reduced density of myelinated axons in the hippocampus in the DS population (Ábrahám et al., 2012), it was hypothesised that these metrics would also significantly differ between groups, in different areas of the brain. More precisely, that fibre density would be reduced in the neonates with DS in tracts that

have been previously found to have altered WM microstructure in the DS population (e.g., in the CC, internal capsule and inferior longitudinal fasciculus).

3.2 Methods

3.2.1 Participants

Neonates with DS

Thirteen neonates with confirmed DS diagnosis were recruited from the neonatal unit or postnatal wards at St Thomas' Hospital (London, UK) by clinical research staff between 2016 and 2021. Data from three of the babies were removed from the sample, two due to an incomplete acquisition of the diffusion weighted image and one due to excessive motion artefacts that could not be corrected after pre-processing.

The final sample consisted of image data from 10 neonates with DS (5 females), born between 31^{+5} to 39^{+6} weeks GA[4] (31.71-39.86; *Mdn*= 37) and scanned between 32^{+3} to 44^{+4} weeks PMA (32.43-44.57; *Mdn*= 38.43). Participants with DS who had additional non-brain congenital abnormalities, such as CHDs or gastrointestinal malformations, were also included in this study. No participants with DS who had acquired neural pathology, like periventricular leukomalacia, were included in this study. Clinical details for neonates with DS can be found in Table 3.2.

[4] GA and PMA are a measure of age, and are hereafter written as weeks plus days (e.g., a baby born at 36 weeks and 4 days will have a GA of 36^{+4})

Table 3.2 *Summary of clinical information for the neonates with DS. Where the column* Total n with data *is less than 10, this represents instances of missing data.*

	Range	Total n with data	Frequency per category	% per category
Birth information				
Term (≥ 37 wGA)	-	10	6	60%
Preterm (32-37 wGA)	-	10	3	30%
Very preterm (28-32 wGA)	-	10	1	10%
Weight (kg)	1.66-3.55	10	-	-
HC (cm)	29-34.5	9	-	-
APGAR scores				
1 minute	6-9	9	-	-
5 minutes	6-10	9	-	-
Mode of delivery				
SVD	-	10	5	50%
EmCS		10	5	50%
Resuscitation	-	10	3	30%
Neonatal and Scan information				
Weight at Scan (kg)	1.8-3.83	10	-	-
HC at scan (cm)	29.5-36	8	-	-
Ventilation	-	10	5	50%
Hypotonia	-	9	5	56%
Sepsis/infection	-	10	6	60%
Jaundice	-	10	3	30%
Comorbidities				
CHD	-	10	6	60%
Gastrointestinal	-	10	3	30%
Surgery prior to scan	-	10	2	20%

Note. HC= head circumference, SVD= Spontaneous Vaginal Delivery, EmCS= Emergency C section, CHD= Congenital heart defect

TD controls

Typically developing controls were selected from the Developing Human Connectome Project (dHCP) (http://www.developingconnectome.org/project/) database. Thirty-nine age and sex matched controls (19 females) were selected. TD controls were included if they had been scanned at a PMA that was within the week range that matched each participant with DS (e.g., 40^{+0} to 40^{+6}), and were born between 30 to 40 weeks GA.

The final TD sample was born between 31^{+0} to 40^{+6} weeks GA (31.00-40.86; *Mdn*= 37.86) and scanned between 32^{+5} to 44^{+3} weeks PMA (32.71-44.43; *Mdn*= 38.29). Control neonates were included based on a normal neonatal brain appearance on MRI, classified as having no structural anomalies (e.g., no periventricular leukomalacia) and no clinically significant acquired findings, with no other congenital or chromosomal abnormalities, and a normal neurodevelopmental outcome at 18 months of age. Any participant whose image did not include the whole brain in the field of view (e.g., cropped cerebellum) were excluded from the sample.

Ethics

Informed consent was obtained from legal guardians, prior to imaging at the Evelina Newborn Imaging Centre, St. Thomas' Hospital, London, UK. Ethical approval for this study was obtained from the West London and GTAC Research Ethics Committee for DS participants (07/H0707/105 and 19/LO/0667); and from the National Research Ethics (14/LO/1169) for controls participants in the developing Human Connectome Project (dHCP).

3.2.2 Neonatal MRI data acquisition and pre-processing

Neonatal MR scanning was performed on a Philips Achieva 3-Tesla system (Best, The Netherlands) at the Evelina Newborn Imaging Centre (St. Thomas' Hospital, London) using a dedicated 32-channel neonatal head coil. Infants were placed and secured within the scanning shell and a neonatal positioning device was used to stabilise the head to reduce movement (Hughes et al., 2017). Auditory protection was comprised of earplugs moulded from silicone-based putty placed in the outer ear (President Putty, Coltene/Whaledent Inc., NJ, USA), neonatal earmuffs over the ear

(MiniMuffs, Natus Medical Inc., CA, USA) and an acoustic hood placed over the scanning shell. Sedation was not administered, and all babies were scanned during natural sleep after feeding. Scan time was restricted to 60 minutes. An experienced neonatologist was present during the examinations and pulse oximetry, heart rate, and temperature were monitored throughout the scan.

Data acquisition

Diffusion weighted images were acquired using a multi-shell High Angular Resolution (HARDI) single-shot spinecho echo-planar sequence with a maximum gradient amplitude of 70 mT/m. Three hundred volumes per image were sampled with four phase-encode directions on four shells with b-values of 0 (n= 20), 400 (n= 64), 1000 (n= 88) and 2600 (n=128) with TE= 90, TR= 3800 ms (Hutter et al., 2018; Tournier et al., 2015) and reconstructed to a resolution of 1.5 mm.

T2-weighted images were also obtained using a turbo spin echo sequence, acquired in sagittal and axial planes, using parameters: TR= 12 s, TE= 156 ms, and SENSE factor 2.11 (axial) and 2.58 (sagittal) with overlapping slices (resolution 0.8×0.8×1.6 mm). Anatomical images were visually inspected to ensure normal brain appearances and no acquired pathology (e.g., periventricular leukomalacia) and used for pre-processing.

Image pre-processing

Pre-processing of the data for this study was carried out as part of the dHCP processing pipeline (https://biomedia.github.io/dHCP-release-notes/dwi-shard.html#ref1). The pipeline consisted of image denoising, gibbs ringing correction, susceptibility-induced distortion correction and motion correction. Brain masks were also extracted

from this pipeline, and they were manually edited to remove any non-brain part of the image (e.g., eyeballs) included in the automated mask.

3.2.3 Diffusion data analysis

The diffusion data were analysed using two different approaches to examine diffusivity measures (FA, MD, RD and AD), as well as fibre density and cross-section: Tract-based spatial statistics (TBSS) analysis based on the diffusion tensor model, and Fixel-based analysis (FBA) (Raffelt et al., 2017). The rationale for using both FBA and TBSS was twofold: (1) previous diffusion studies with people with DS have solely used TBSS to analyse the data, so this would allow comparison of the early brain WM microstructure results from this study to previous findings and discern whether WM alterations found in adults with DS are solely due to aging, part of the phenotype, or both; (2) as outlined in section 2.1.4., the spherical deconvolution model and FBA analysis provide a more accurate representation of the underlying WM anatomy, and result in more intuitive metrics, which could result in new more accurate findings on WM microstructure differences in DS.

Tract-based spatial statistics (TBSS) pipeline

Previous literature has shown that the optimal b value for tensor-based analysis in adults is 1,000 s/mm^2 (Bisdas et al., 2008; Seo, 2013), and is commonly favoured in neonatal diffusion studies (e.g., Fenchel et al., 2020). Therefore, all b= 0 and b= 1000 shell volumes were first extracted from the pre-processed images. The extracted images were registered and analysed using FMRIB's Diffusion Toolbox (v6.0.2), DTI-ToolKit (v2.3.1 www.dti-tk.sourceforge.net) (DTI-TK) (Zhang et al., 2006) and TBSS (v6.0.2

http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/TBSS/UserGuide) as implemented in FMRIB's Software Library (FSL) (Smith et al., 2004, 2006).

Diffusion tensors were calculated on a per voxel basis for each of the participants. Image registration was performed using DTI-TK and integrated within the TBSS pipeline to produce a population-specific high resolution DTI template from a subset of 10 DS and 11 age-matched controls. From this template a mean FA map was derived and then thinned by perpendicular non-maximum suppression to create a mean FA skeleton (Figure 3.1). AD, RD, MD and FA maps were calculated for each subject (Figure 3.2). An FA threshold of ≥0.15 was used to limit the inclusion of voxels with high inter-subject variability and non-white matter voxels. FA, AD, RD and MD were projected onto this skeleton prior to statistical analysis.

Figure 3.1 *Mean FA map (top row) and the derived skeleton in blue (bottom row).*

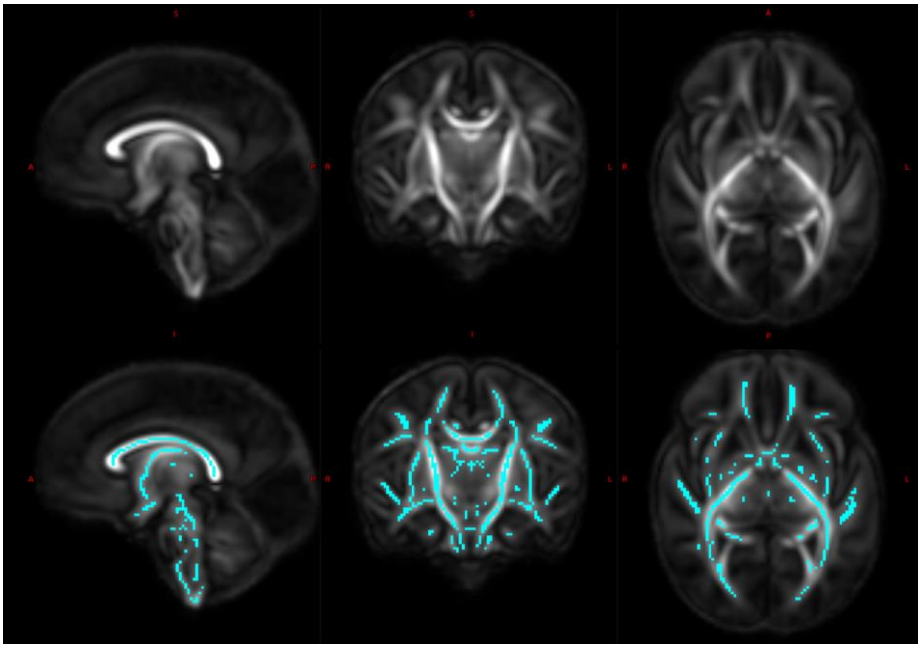
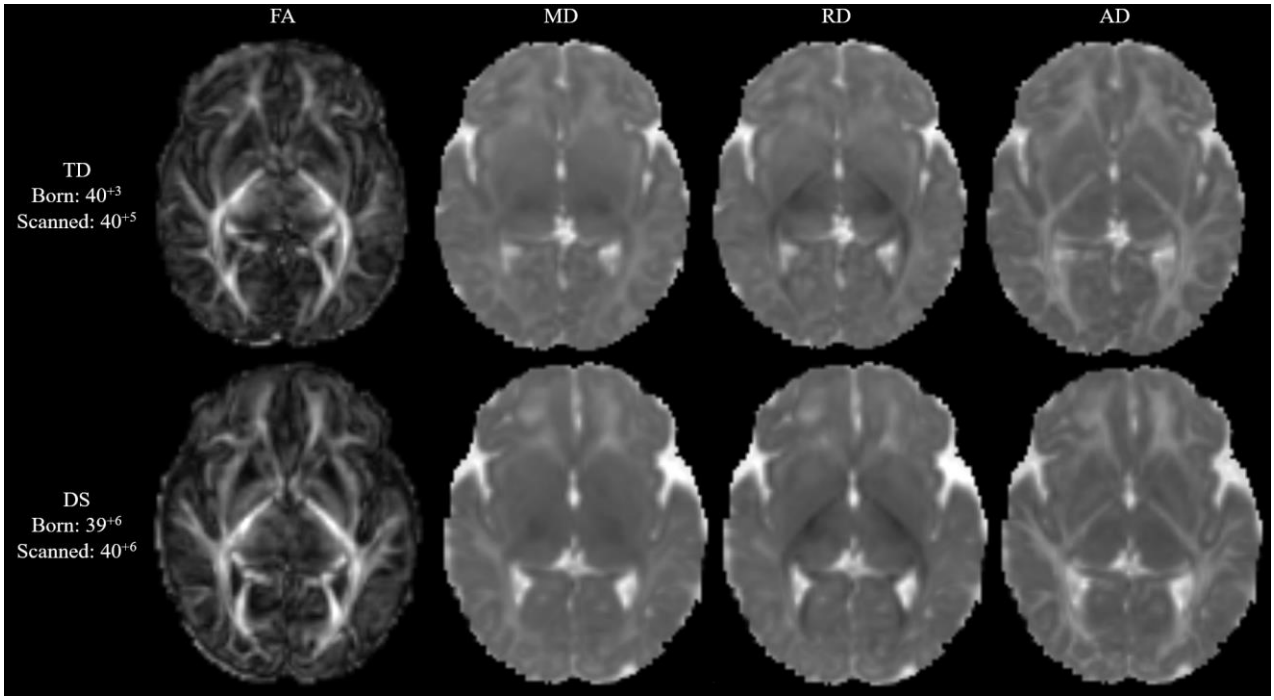


Figure 3.2 *Example of FA, MD, RD and AD metrics for a term-born control neonate (top row) and a neonate with DS (bottom row).*



Fixel-based analysis (FBA) pipeline

MRtrix3 Software (Tournier et al., 2019) was used to perform fixel-based analysis (Raffelt et al., 2017) in accordance with a recommended pipeline (https://mrtrix.readthedocs.io/en/latest/fixel_based_analysis/mt_fibre_density_crosssecton.html). Firstly, average tissue response functions for WM and cerebrospinal fluid (CSF) were computed (Pietsch et al., 2019), and fibre-orientation distributions (FODs) were estimated for each participant using multi-tissue spherical deconvolution (Jeurissen et al., 2014b). An unbiased study-specific FOD template was created using 21 participants (10 DS and 11 controls), matched as closely as possible for age at birth and scan (Table 3.3), and each subject's FOD image was registered to it. A template mask was created and manually edited to ensure that all regions of interest in the brain were included in the analysis.

Table 3.3 *Age and sex of the participants with DS and matched TD controls used to create the study-specific template.*

TD control neonates (*n*= 11)				Neonates with DS (*n*= 10)			
	GA at birth	PMA at scan	Sex		GA at birth	PMA at scan	Sex
TD1	31.86	32.71	F	*DS1*	32.00	32.43	F
TD2	31.57	34.14	M	*DS2*	31.71	34.14	F
TD3	36.86	37.14	F	*DS3*	37.00	37.57	M
TD4	36.86	37.57	F	*DS4*	37.00	37.86	F
TD5	37.14	37.71	M				
TD6	37.14	37.86	F	*DS5*	37.14	38.00	M
TD7	36.86	38.71	M	*DS6*	35.29	38.86	M
TD8	37.86	38.86	M	*DS7*	37.57	38.86	F
TD9	40.29	40.86	F	*DS8*	39.86	40.86	F
TD10	40.71	43.57	F	*DS9*	37.71	43.57	F
TD11	40.86	44.43	M	*DS10*	36.71	44.57	M
Mdn	37.14	37.86	54.55%F	*Mdn*	37.00	38.43	60% F

Note. There were no significant differences in GA and PMA between the two groups.

A fixel-mask was then created from the template FOD that defined the fixels to be included in the statistical analysis. Individual participant's fixels were reoriented and assigned to the template fixels. FBA output metrics fibre density (FD), fibre cross-section (FC) and fibre density and cross-section (FDC) were calculated for each participant. Whole brain tractography was performed on the FOD template where 20 million tracts were generated, and spherical-deconvolution informed filtering of tractograms was implemented to filter this down to 2 million tracts, reduce any bias created in the tractogram and increase biological plausibility (Smith et al., 2013). Due to working with neonatal diffusion data which has less myelinated tracts, a more conservative approach than the one suggested in the pipeline was taken in this step to reduce noise in the final whole brain tractogram. Finally, a fixel-fixel connectivity matrix was generated from the filtered tractogram, used to inform the Connectivity-based fixel enhancement (CFE) during the statistical analysis (Raffelt et al., 2015).

3.2.4 Statistical analysis

Group differences in global measures

Group differences in global measures were examined. Whole brain volume differences were investigated using the SPSS software package (version 25). Total brain volume (TBV) and total tissue volume (TTV) variables were tested for normality of distribution using the Shapiro-Wilk goodness-of-fit test, alongside Q-Q plots and histograms. Differences between groups were assessed using independent sample t-test for normally distributed data, and Mann-Whitney U test for non-normally distributed data.

Whole brain FA voxel-wise group comparison was performed using Randomise in FSL. A General Linear Model (GLM) with PMA at scan and sex (Rose et al., 2009; van

Kooij et al., 2011) as co-variates was used and two hypothesis were tested: (1) that the control group would have higher FA than the group with DS (main effect of group: Controls>DS), and (2) that the opposite would be true (main effect of group: Controls<DS). A multiple comparison correction of the cross-sectional analyses was performed using TFCE, with randomised (5,000 permutations) nonparametric permutation. The results reported below were generated using 5,000 permutations and reached family-wise error (FWE) corrected statistical significance at pFWE< .05.

TBSS

Cross subject voxel-wise statistical analysis was performed using Randomise in FSL. Separate GLM were used to assess the difference between DS and TD neonates in FA, MD, AD and RD, with PMA at scan and sex (Rose et al., 2009; van Kooij et al., 2011) as co-variates. Two hypotheses were tested for each of the GLMs: (1) that the control group would have higher metrics than the group with DS (main effect of group: Controls>DS), and (2) that the opposite would be true (main effect of group: Controls<DS). A multiple comparison correction of the cross-sectional analyses was performed using TFCE, with randomised (5,000 permutations) nonparametric permutation. The results reported below were generated using 5,000 permutations and reached family-wise error (FWE) corrected statistical significance at pFWE< .05.

FBA

GLMs were computed for permutation-based testing of FBA metrics, covarying for PMA at scan and sex. Two hypotheses were tested for each of the GLMs: (1) that the control group would have higher metrics than the group with DS (Controls>DS), and (2) that the opposite would be true (Controls<DS). FD, FC and FDC were compared between

neonates with DS and controls using CFE (Raffelt et al., 2015) in MRtrix3 (Tournier et al., 2019). CFE uses the created tractogram to identify fixels that are structurally connected and likely to share underlying anatomy and pathology, and then uses this connectivity information to enhance the statistical map. For the analysis of FC and FDC, total brain tissue volumes were included as an additional covariate, as recommended by Smith et al. (2019). The FBA results reported below were generated using 5,000 permutations and reached FWE corrected statistical significance at pFWE< .05.

3.3 Results

There were no significant differences in age at birth (t(47)= 1.17; *p*=.25) and age at scan (t(47)= -0.16; *p*=.87) between the DS group (*n*= 10) and the control group (*n*= 39).

Shapiro-Wilk test results showed that TBV and TTV were normally distributed for both the DS (W= .94, *p*=.55; W= .94, *p*=.57, respectively) and the TD groups (W= .98, *p*= .79; W= .98, *p*= .81, respectively). Two separate independent sample t-test were performed, and the results showed that both TBV (t(47)= -2.23; *p*<.025) and TTV (t(47)= -2.38; *p*<.025) were significantly reduced in the group with DS (*M*= 272.59, *SD*= 53.84; *M*= 265.65, *SD*= 54.86, respectively) when compared to the TD group (*M*= 316.76, *SD*= 56.52; *M*= 312.65, *SD*= 56.06, respectively).

Regarding the whole brain FA group comparison, Figure 3.3 shows the areas where FA was increased (in blue) and decreased (in red) in the group with DS when compared to the TD group (pFWE < .05).

Figure 3.3 *Axial (top) and sagittal (bottom) view of whole brain tissue voxels where FA was increased (in blue) and decreased (on red) in the group with DS.*

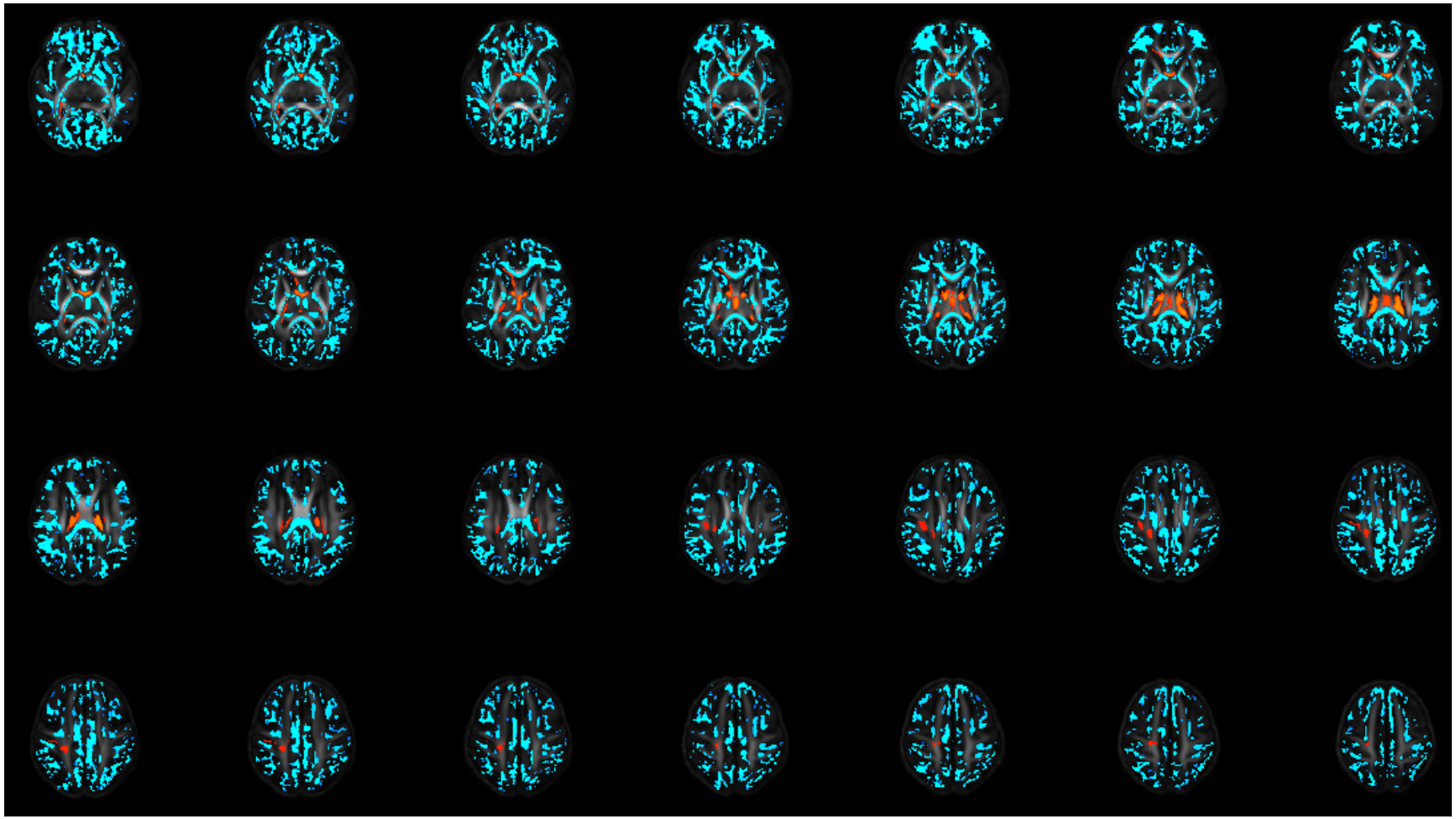


Figure 3.3 *(continued)*

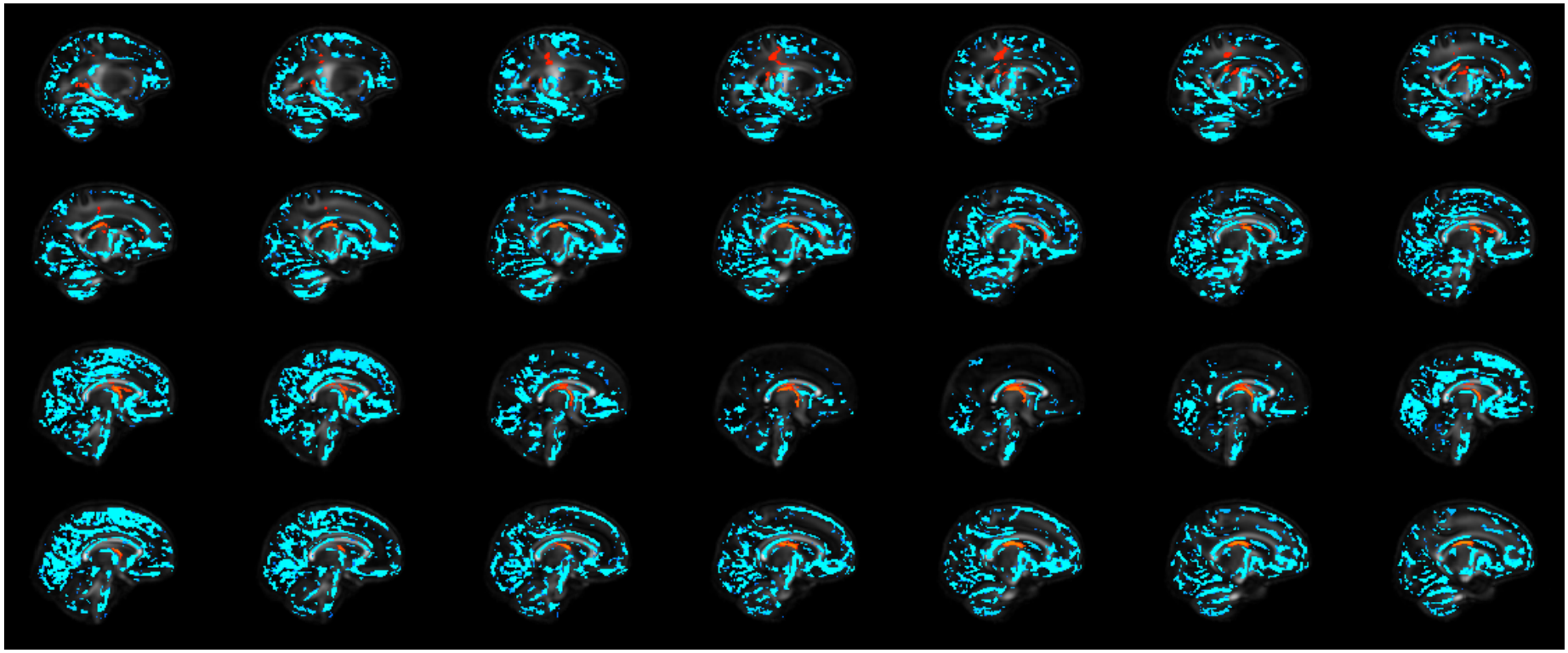
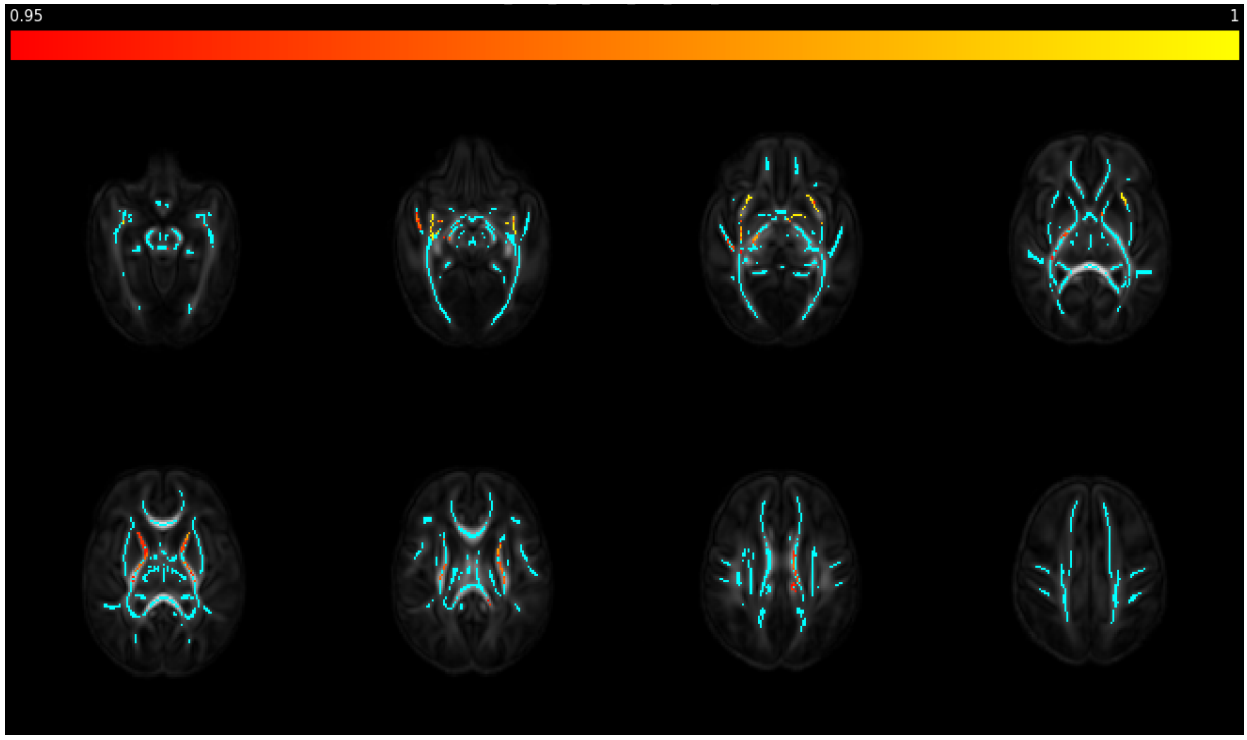
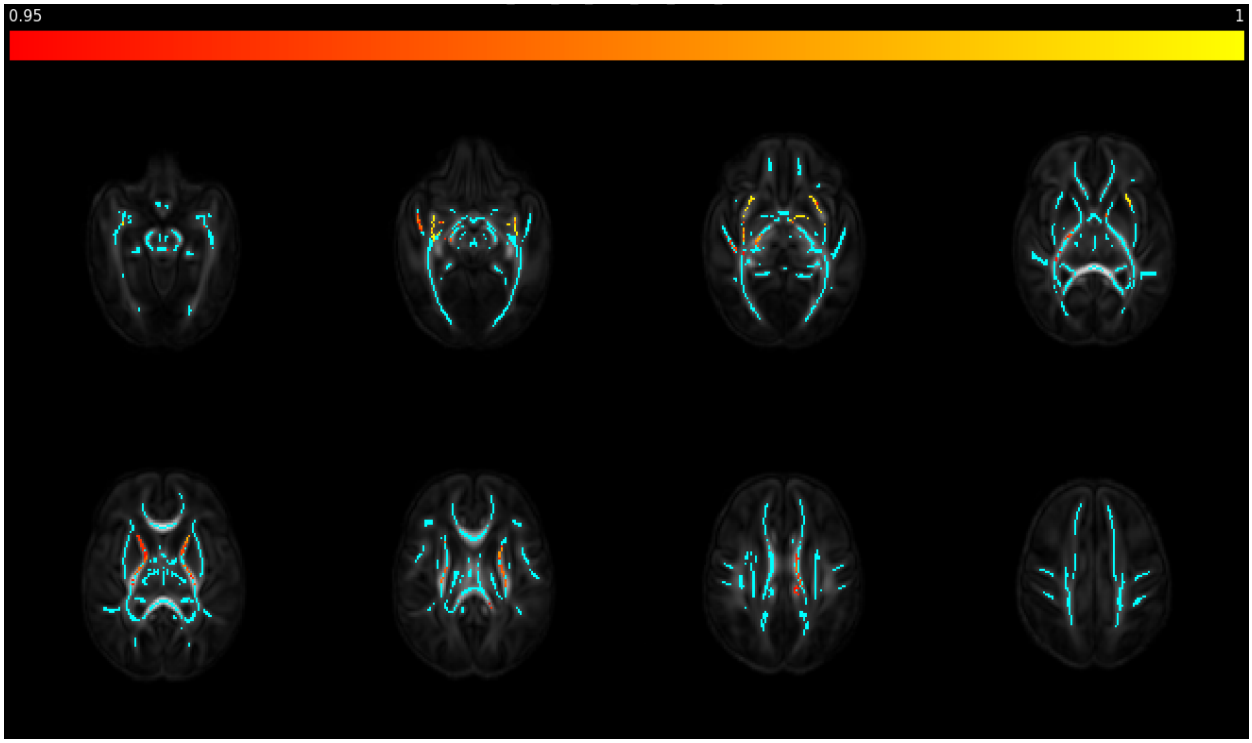


Figure 3. 4 *Axial view of white matter tract skeleton (in blue) where (a) FA, and (b) AD were reduced in neonates with DS compared to controls (voxels in yellow to red) (controlling for age at scan and sex). Colour bar represents 1 – p values.*

a)



b)

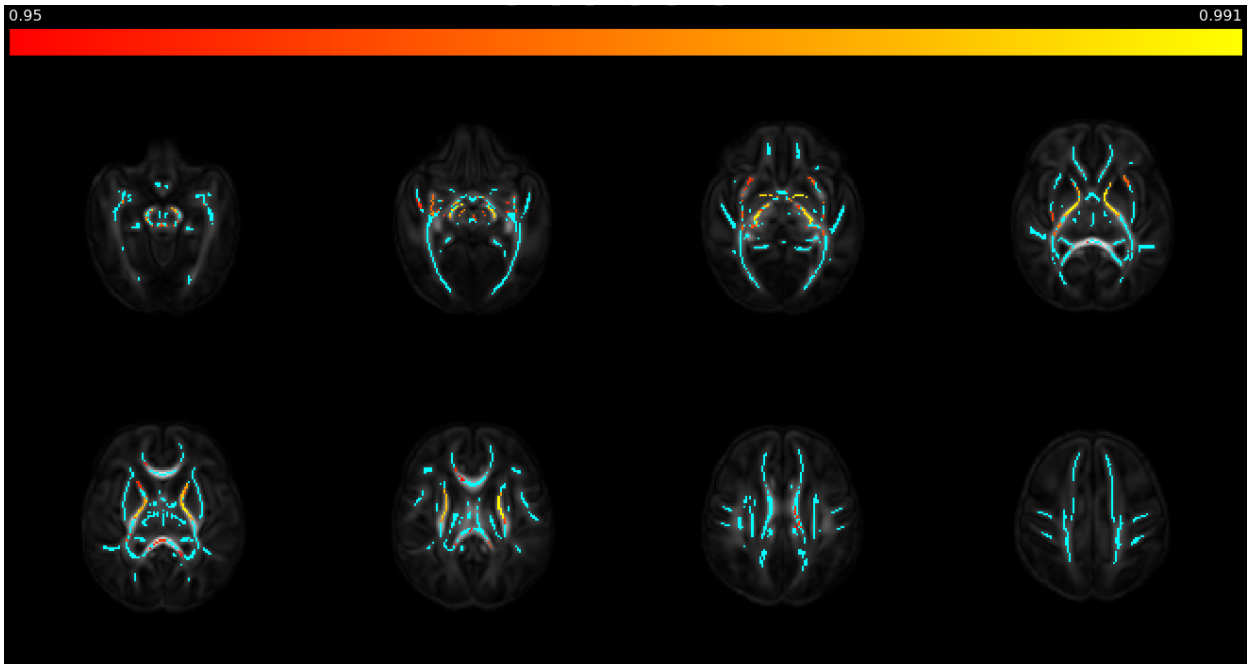


3.3.1 TBSS

The results from the TBSS analysis showed a significant reduction in all four metrics in the neonates with DS compared to controls ($p < .05$). FA and AD were reduced in 9.44% of the mask voxels, in the left cingulum, bilateral internal capsule, left splenium of the CC, bilateral anterior external capsule and left anterior commissure (Figure 3.4).

The reduction of MD was more extensive (15.87% of mask voxels), localised in the left cingulum, bilateral internal capsule, splenium and right genu of the CC, bilateral external capsule, anterior commissure, cerebral peduncle and superior cerebellar peduncle (Figure 3.5).

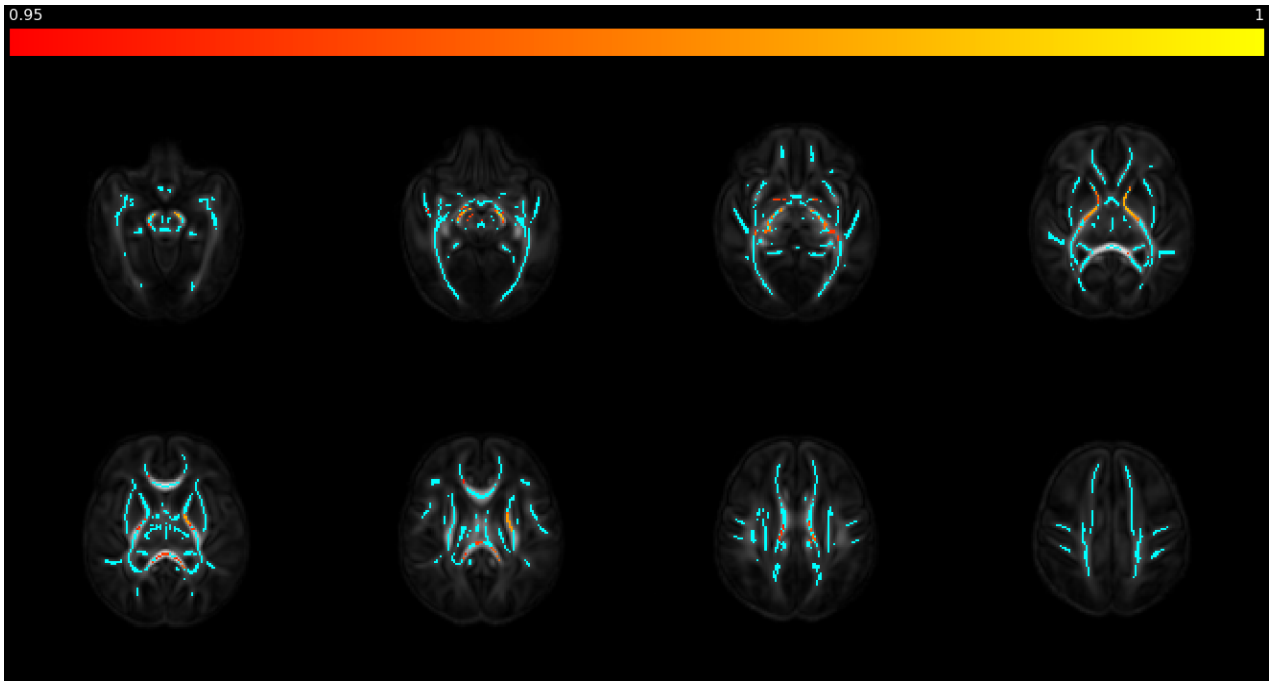
Figure 3.5 *Axial view of white matter tract skeleton (in blue) where MD was reduced in neonates with DS compared to controls (voxels in yellow to red) (controlling for age at scan and sex). Colour bar represents 1 – p values.*



Finally, RD was reduced in the posterior cingulum bilaterally, splenium and right genu of CC, internal capsule bilaterally, anterior commissure and cerebral peduncles (Figure 3.6). In this case 8.78% of the mask voxels were significant.

No TBSS metric was higher in the DS group than in TD controls at $p<0.05$ threshold.

Figure 3.6 *Axial view of white matter tract skeleton (in blue) where RD was reduced in neonates with DS compared to controls (voxels in yellow to red) (controlling for age at scan and sex). Colour bar represents 1 – p-values.*

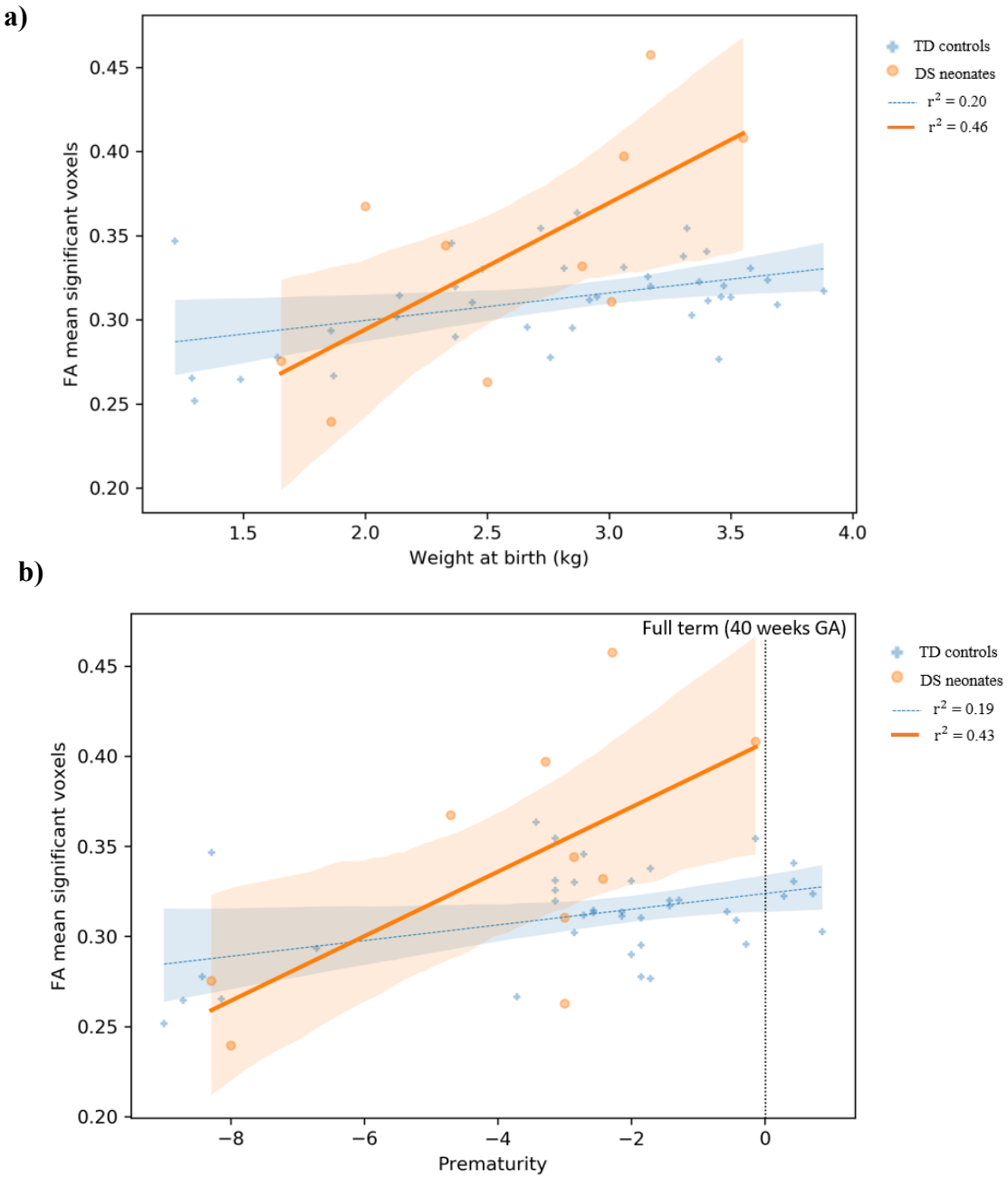


Effect of birth weight and prematurity

Previous literature has shown that low birth weight is also related to FA reductions in TD babies born prematurely (e.g., Dudink et al., 2007; Kim et al., 2023; Zhu et al., 2021). Figure 3.7 shows the effect of weight at birth (a) and of prematurity (b) on FA in those voxels that were significantly different between groups. Although the relationship between birth weight and FA is positive for both groups (i.e., the higher the weight at birth, the higher the FA, or the more mature WM microstructure), the effect seems to be four times stronger for the group with DS (β_1= 0.08) than for the TD group (β_1= 0.02) (Figure 3.7 a). A similar pattern is present when looking at the relationship between prematurity

and FA, but in this case, the effect seems to be five times stronger for the group with DS ($\beta1 = 0.02$) than for the TD group ($\beta1 = 0.004$) (Figure 3.7 b).

Figure 3.7 *Scatterplot showing the effect of weight at birth (a) and prematurity (b) on FA in neonates with DS (in orange) and TD neonates (in blue). FA was extracted from those voxels in the TBSS mask that were significantly different between groups. Prematurity reflects how many weeks before term (40 weeks, marked by the vertical dotted line) were the participants born.*



The above presented TBSS results have been controlled for age at scan, so the effect of prematurity has been accounted for in the analysis. However, weight at birth was not accounted for, which is a limitation of the current study, considering the different effect it seems to have in the two groups.

3.3.2 Whole brain FBA

The results from the fixel-based analysis, controlling for age and sex, showed a significant increase in fibre cross-section (FC) and fibre density and cross-section (FDC) in the DS group when compared to the TD control group in several areas, including the CC, cingulum and anterior limb of the internal capsule (Figure 3.8 and Figure 3.9)

Figure 3.8 *Areas where FC was higher in the DS group at p<0.05 (axial and sagittal view). Green represents fibre bundles running anterior-posterior, blue represents fibre bundles running inferior superior and red represents fibre bundles running left-right.*

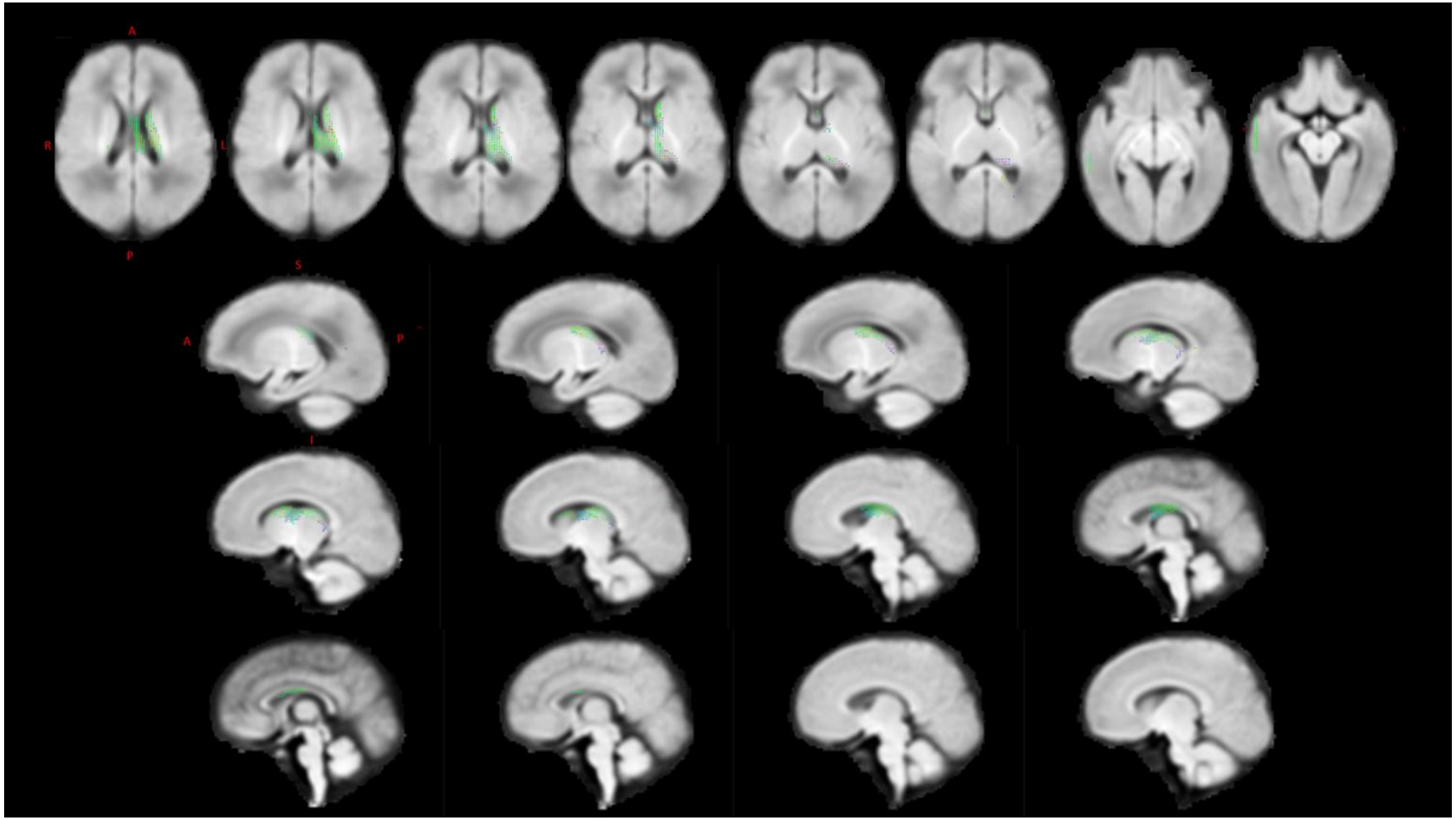
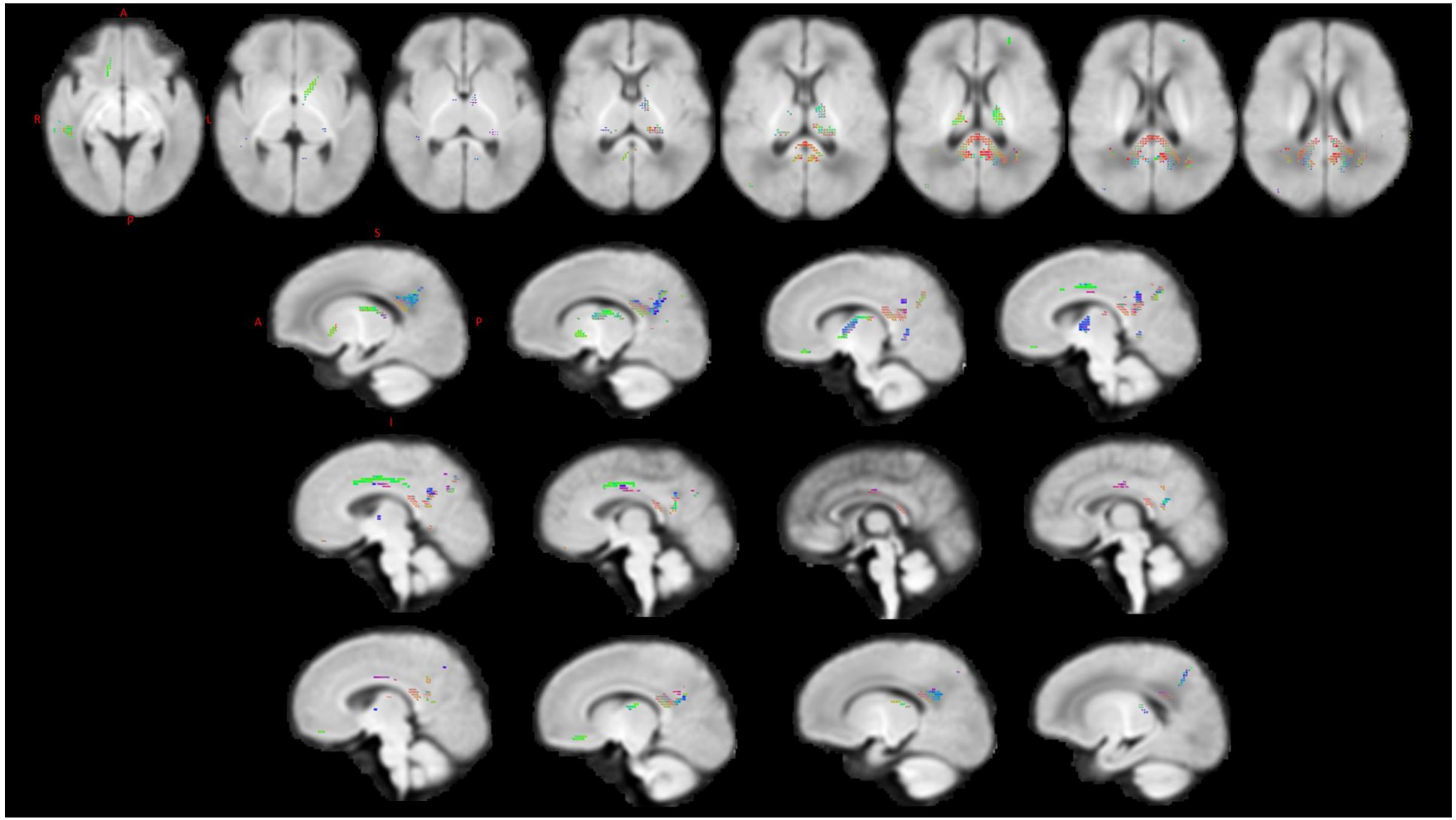


Figure 3.9 *Areas where FDC was higher in the DS group at p<0.05 (axial and sagittal view). Green represents fibre bundles running anterior-posterior, blue represents fibre bundles running inferior superior and red represents fibre bundles running left-right.*



The results also showed higher FD in controls than in DS at $p < 0.05$, within the pons and into the cerebellum, the body of the CC and the left cingulum (Figure 3.10), and higher FD in DS than in controls in the CC and the cingulum bilaterally (Figure 3.11). However, these findings seem implausible, as they suggest that fibre density is simultaneously lower and higher in DS in some of these brain areas, like the body of the CC, which is not anatomically possible. Upon further inspection of the images, a misalignment of the image registration was found (e.g., in the CC in Figure 3.11), rendering these results unreliable as they are mostly likely reflecting an effect of the misalignment rather than of the actual anatomical differences between groups (considered in further depth in the discussion section).

Figure 3.10 *Areas where FD was higher in the control group at p<0.05. Green represents fibre bundles running anterior-posterior, blue represents fibre bundles running inferior superior and red represents fibre bundles running left-right.*

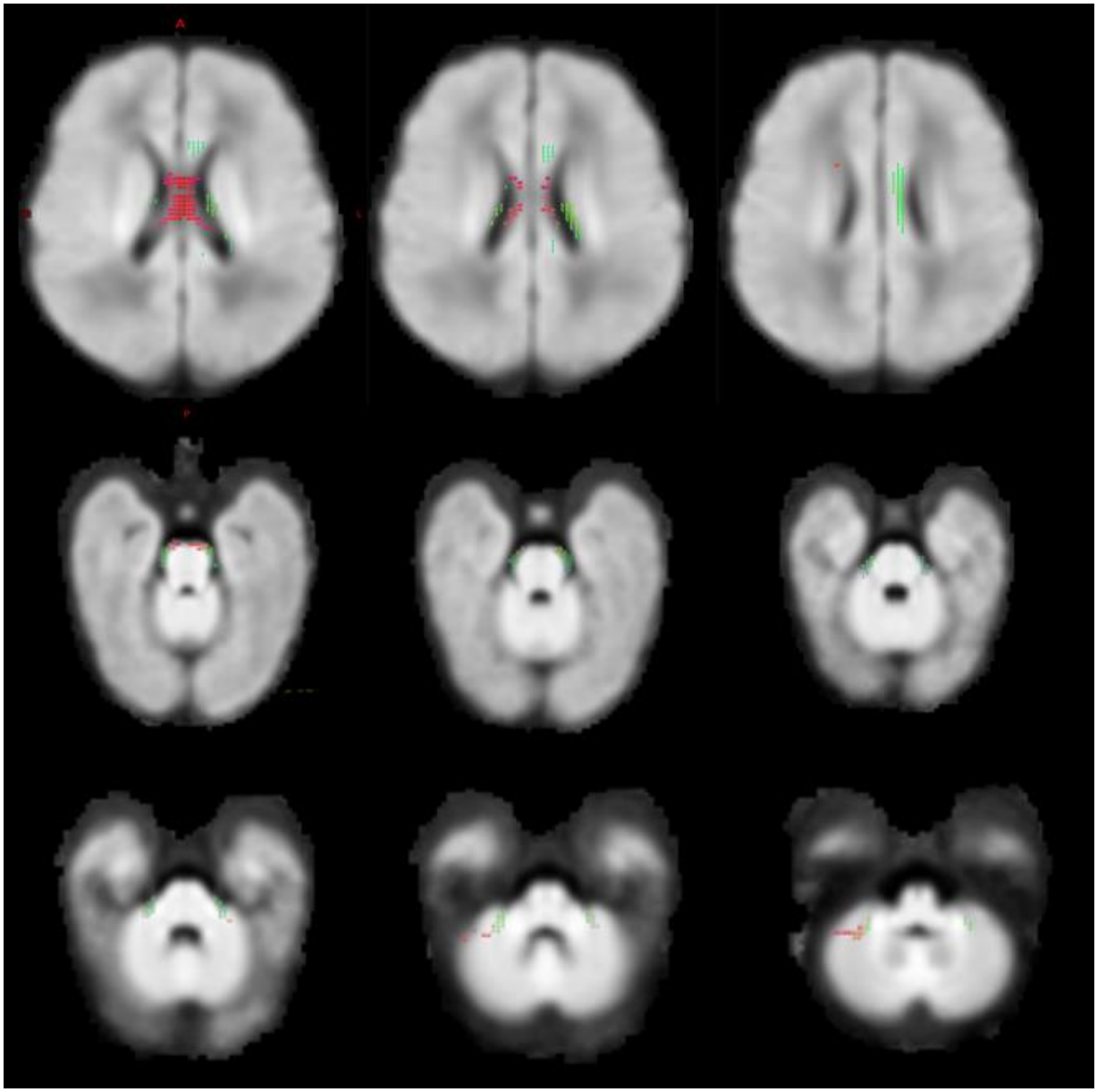
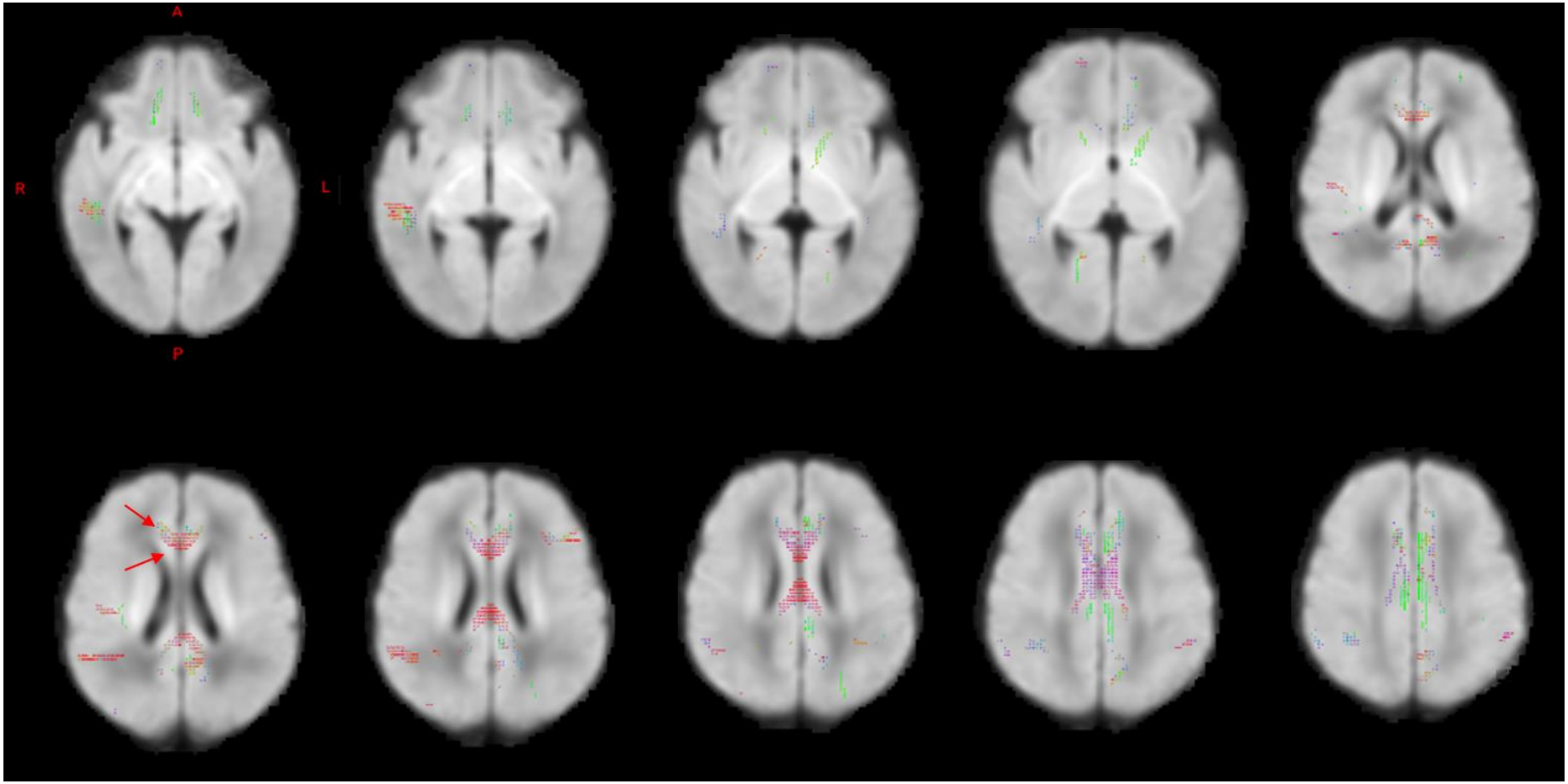


Figure 3.11 *Areas where FD was higher in the DS group at p<0.05. The red arrows show the misalignment in the corpus callosum between significantly different fixels and the underlying template anatomy. Green represents fibre bundles running anterior-posterior, blue represents fibre bundles running inferior superior and red represents fibre bundles running left-right.*



3.3.3 Case studies

Four of the 10 participants in the sample had a single neurodevelopmental assessment between 6 months and 4 years old, which allowed to visually explore any emergent pattern in the relationship between neonatal diffusion measures and later cognitive outcomes. Table 3.4 shows clinical and demographic information for the case studies. To contextualise the results for these children with DS and visualise individual variability in the sample, their diffusion data were plotted against the whole sample of neonates with DS and TD controls included in this study. Their AE MSEL scores were plotted against a larger sample of infants and children with DS (n= 98) (age 6-63 months) and TD children (n= 47) (age 4-35 months) tested as part of the LonDownS cohort. As previously done for a proof of concept with brain volumes in Tomas et al. (2020), the relationships between each child's MSLE scores' positions relative to the rest of the DS and TD sample and the relative position of each child's FA measures were explored.

Since the goal was to consider the position of each child with respect to other individuals with DS, standardised residuals against the DS trajectory were generated for the FA measures and all the behavioural MSEL measures (i.e., composite, Visual reception, Gross rotor, Fine rotor, Receptive language, and Expressive language AE scores) (see Table 3.5).

Table 3.4 *Demographic and clinical details for case studies who had a follow-up behavioural assessment.*

	DS7	**DS8**	**DS11**	**DS12**
Sex	Male	Male	Male	Male
GA at birth	37+1	31+5	36+5	35+2
GA at scan	38	34+1	44+4	38+6
Days between birth and scan	7	18	55	26
Age at visit (months)	8.7	8.87	36.77	45.93
Birth weight (kg)	2.33	1.66	3.06	2.00
Weight at scan (kg)	2.26	1.80	3.27	2.20
CHD	None	None	Resolved PDA	ASD
Other conditions	None	Duodenal atresia	None	Hirschsprung, IUGR
APGAR scores (1 minute/5 minute)	9/9	9/10	8/9	9/10
Ventilation after birth	Yes	Yes	Yes	Yes

Note. CHD= Congenital heart defect; ASD= Atrial Septal Defect; APGAR= Appearance, Pulse, Grimace, Activity, Respiration; IUGR= Intrauterine growth restriction.

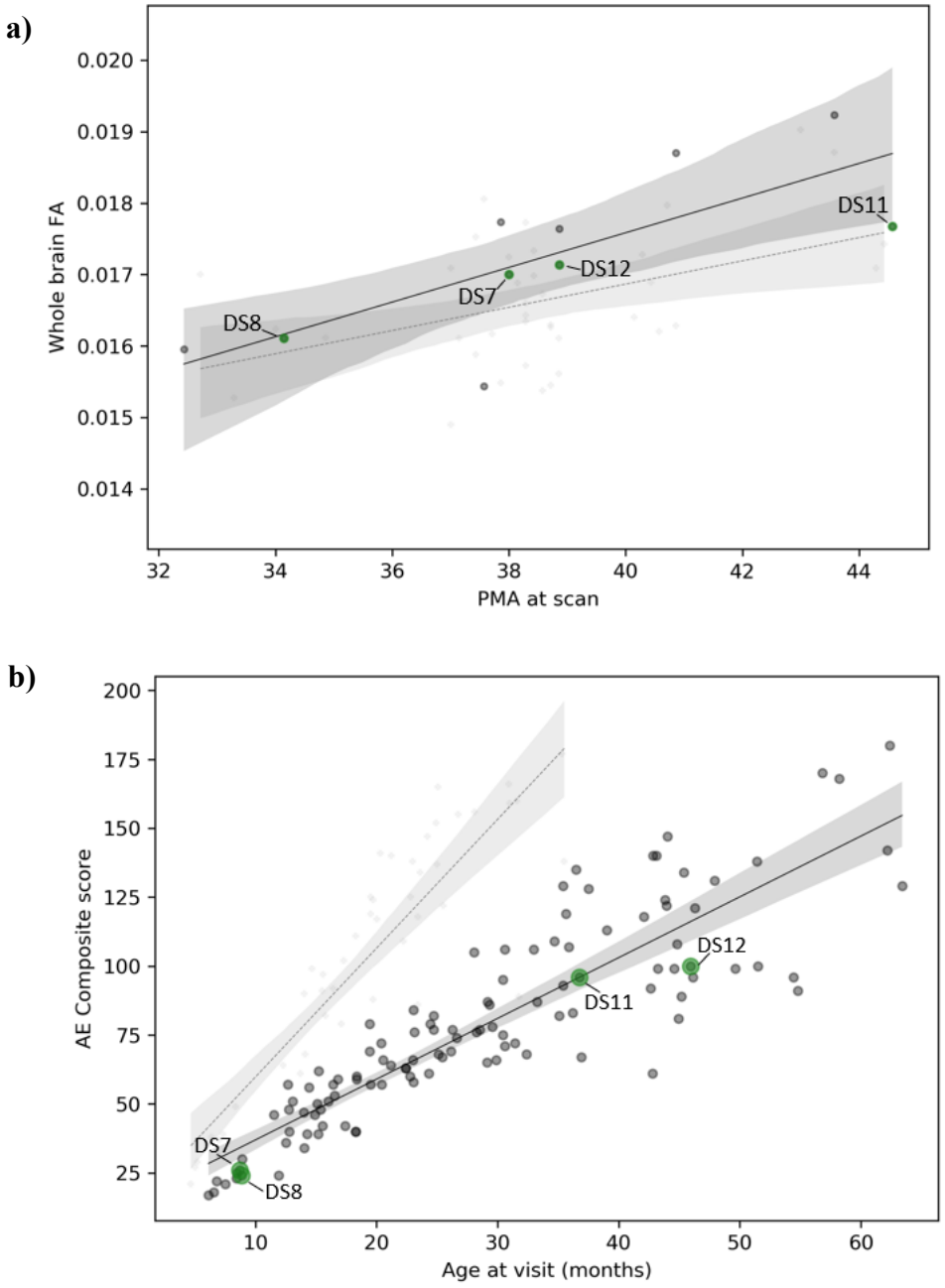
Table 3.5 *Standardised residuals for each of the 4 DS case studies for FA measures (whole brain and mean FA in voxels where there was a significant difference between groups) and MSEL AE composite scores.*

Case ID	**FA**		**MSEL**					
	Whole brain	*sig voxels*	*Composite*	*VR*	*GM*	*FM*	*RL*	*EL*
DS7	-.061	.470	-.485	-.336	-.831	-.574	-.206	-.128
DS8	-.119	.329	-.627	-.350	-.539	-1.194	-.027	-.515
DS11	-1.227	-1.139	.003	-.398	.077	.004	.539	-.280
DS12	-.206	.783	-.968	-1.019	-.536	-.576	-.564	-.880

Note. EL= Expressive language; FA= Fractional anisotropy; FM= Fine motor; GM= Gross motor; RL= Receptive language; VR= Visual reception.

Figure 3.12 shows (a) the position of each case's whole brain FA relative to the larger imaged DS and TD controls, and (b) the position of each case's MSEL AE composite score relative to the larger imaged DS and TD controls. Comparison of neonatal whole brain FA and MSEL AE composite score plots case by case suggests different patterns of relationship. Whilst being below the DS cohort average in the FA measure matched also being below the DS cohort average in the MSEL composite measure for cases DS7, DS8 and DS12, this was not the pattern for case DS11. This neonate had whole brain FA values well below the DS cohort average (zres= -1.227) and within the TD cohort range, and MSEL scores close to the DS cohort mean (zres= .003) (and above the other three case studies, Table 3.5), but still well below the TD cohort. This seems to suggest that having neonatal whole brain FA values closer to what it would be expected from the TD population might be related to slightly better cognitive outcomes during childhood. However, this might be the case only for FA measures from older neonates, as cases DS8's whole brain FA value also falls within the TD cohort range, but the MSLE composite score is below the DS cohort's average (zres= -.627).

Figure 3.12 *Whole brain FA (a) and MSEL composite score (b) scatterplots for the four case studies. FA data plotted against the larger cohort of neonates with DS (dark grey) and TD controls (light grey). MSEL data plotted against cross-sectional trajectories created from a larger sample of infants and children with DS and TD controls tested as part of the LonDownS cohort (D'Souza et al., 2021).*



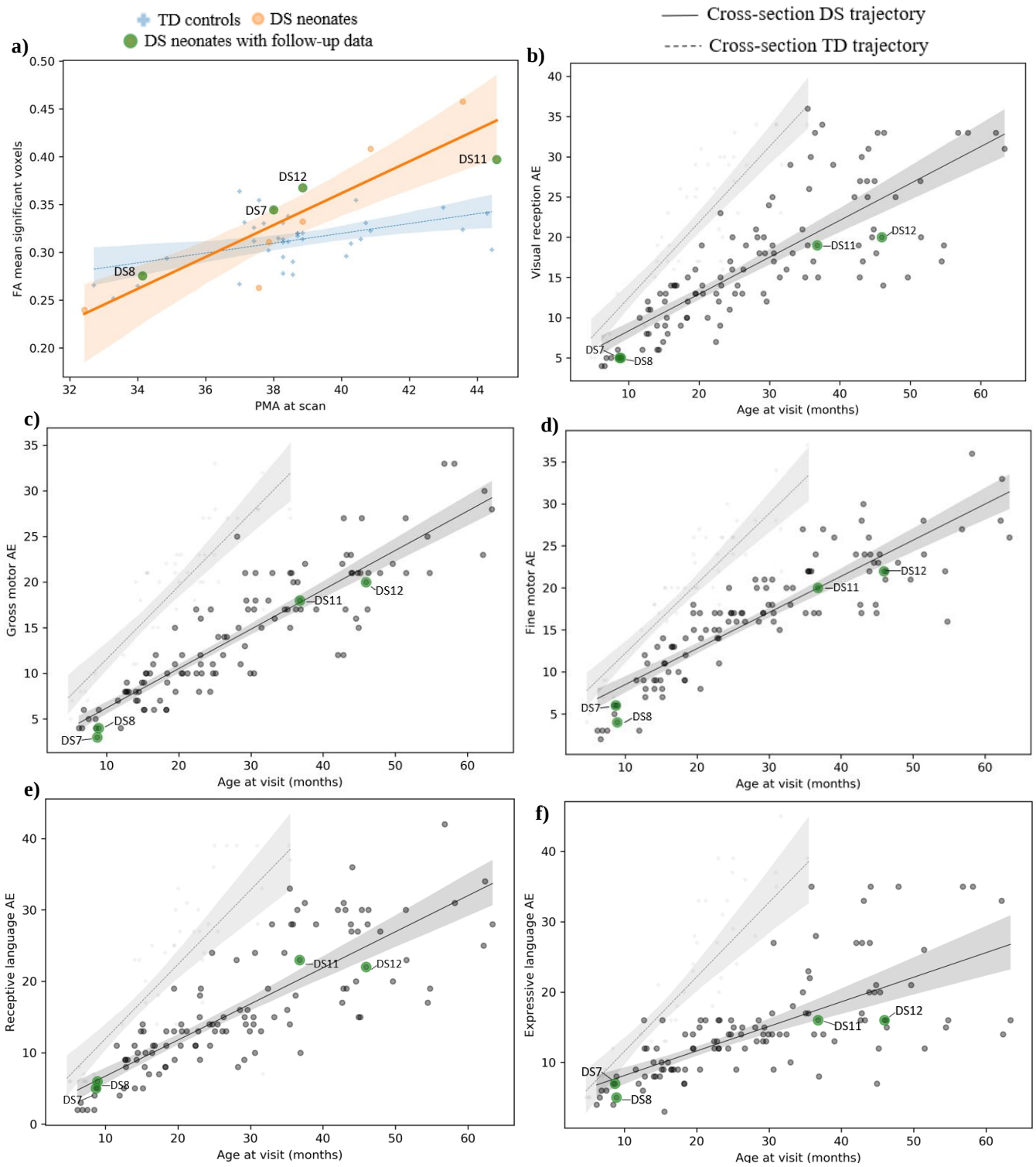
The mean FA of the TBSS mask voxels that were significantly different between the DS and TD neonates was extracted for each of the participants in the study, and cross-sectional DS and TD trajectories were created (Figure 3.13 a) to investigate the individual variability in the sample and the relative position of the four case studies. This was compared to their relative position in the MSEL subscales (Figure 3.13 b-f).

Having FA values above the DS cohort relates to having MSEL subscale score below DS cohort for cases DS7, DS8 and DS12. For case DS8, who was born at 31+5 weeks GA and scanned at 34+1 weeks GA, having FA values slightly above the DS cohort (zres= .329) relates to having Receptive language scores around the mean (zres= -.027), but below the mean for the rest of the subscales (Visual reception, zres= -.350; Expressive language, zres= -.515; Gross motor, zres= -.539) and well below the mean Fine motor scores (zres= -1.194). Cases DS7 and DS12 were born prematurely (37+1 and 35+2 weeks GA, respectively) but scanned around term equivalent age (38 and 38+6 weeks GA, respectively). Whilst they both have FA values above the DS cohort (DS7, zres= .470; DS12, zres= .783) the relationship to MSEL subscale scores seems to be slightly different. For case DS7, this relates to larger deviations from the DS cohort mean in Gross motor (zres= -.831) and Fine motor scores (zres= -.574) and scores slightly below the cohort mean in the rest of subscales (Visual reception zres=-.336; Receptive language, zres= -.206; Expressive language, zres= -.128). For case DS12, however, having FA scores above the DS cohort relates to having MSEL score below the cohort for all subscales (Gross motor, zres= -.536; Fine motor, zres= -.576; Receptive language, zres= -.564), and especially Visual reception (zres= -1.019) and Expressive language (zres= -.880).

Case DS11 follows a different pattern of relationship between FA and MSEL subscales. In fact, whilst this neonate has an FA value well below the cohort average (zres= -1.139), only Visual reception (zres= -.398) and Expressive language (zres= -.280) AE scores are below the larger DS cohort average. In fact, Gross motor (zres= .077) and Fine motor (zres= .004) scores are around the average, and Receptive language score is (zres= .539) above the cohort average, and has the largest deviation from the "DS norm" relative to the other subscales.

Overall, the mixed pattern of relationships between neonatal FA values and later MSEL scores shown in these case studies has highlighted the wide individual variability found in the population with DS. Nevertheless, the relationship between neonatal FA and outcomes seems to be affected by prematurity and/or weight at birth, and FA values in the splenium of the CC and cingulum closer to what it is expected in the TD population seem to be related to higher Receptive language scores.

Figure 3.13 *Mean FA in the voxels that were significantly different (a) and MSEL subscales (b-f) scatterplots with the four case studies highlighted. FA data plotted against the larger cohort of neonates with DS (dark grey) and TD controls (light grey). MSEL data plotted against cross-sectional trajectories created from a larger sample of infants and children with DS and TD controls tested as part of the LonDownS cohort (D'Souza et al., 2021).*



3.4 Discussion

The focus of this study was to investigate WM integrity in a sample of neonates with DS compared to TD age-matched controls, using two different dMRI analyses.

3.4.1 TBSS

As hypothesised, TBSS results showed significant differences between the DS and the TD group in the anterior parts of the brain (e.g., genu of the CC, anterior internal capsule, anterior commissure, anterior external capsule), with DS having reduced FA, MD, RD and AD. Additionally, the differences between groups were not limited to the anterior part of the brain. In fact, DS group also had reduced TBSS metrics across the cingulum and the splenium of the CC, as well as in the cerebral and superior cerebellar peduncles.

Reductions in FA when compared to TD neonates have been previously found in the same WM tracts as the current study in several studies examining WM microstructure alterations in population with different conditions, like CHD (e.g., Karmacharya et al., 2018; Mulkey et al., 2014), ventriculomegaly (e.g., Lockwood Estrin et al., 2016), and perinatal asphyxia (e.g., Gao et al., 2012; Porter et al., 2010), or in neonates born prematurely (e.g., Alexandrou et al., 2014; Glass et al., 2017; Shim et al., 2012). One of the WM tracts consistently found to have reduced FA in atypical neonates, is the CC, especially the splenium (Alexandrou et al., 2014; Gao et al., 2012; Karmacharya et al., 2018; Lockwood Estrin et al., 2016; Mulkey et al., 2014; Paquette et al., 2013), as well as the external capsule (Alexandrou et al., 2014; Glass et al., 2017; Porter et al., 2010). The anterior limb of the internal capsule has also been found to have reduced FA in neonates born prematurely (Shim et al., 2012), and after perinatal asphyxia in term born neonates

(Porter et al., 2010). WM alterations in the cingulum have been reported less consistently, but reduced FA in this tract was reported by Porter et al. (2010) in their sample of term born neonates with perinatal asphyxia. Nearly half of the DS neonates in the sample were born before 37 weeks GA and half of them had to be ventilated after birth for several reasons, but neither prematurity nor ventilation were included as confounding variables in the current study. So, it cannot be concluded that the WM alterations in these tracts are solely due to the trisomy, and not affected by the clinical comorbidities present in the sample.

The alteration in diffusion in the peduncles were also reported by Lee et al. (2020). More precisely, they found reduced FA in the DS group in the inferior and middle cerebellar peduncles. However, they did not control for age, even though the sample included primary school children and young adults with DS, and no other diffusion study with older adults with DS has previously reported any alteration in the cerebellar peduncles. This suggests that alterations in these tracts might be present in the younger population with DS, and be influenced by the different rates at which myelin develops in different areas of the brain (Dubois et al., 2008). In fact, the cerebellum develops over a long period of time, extending from early embryonic stages until the first postnatal year (ten Donkelaar et al., 2014), and this protracted period of development might also apply to the cerebellum's fibre connections. So, the alteration in myelination in neonates with DS in the cerebellar peduncles might be due to a delay in development.

The reduction of FA in the anterior part of the brain, CC and cingulum is in line with previous studies conducted in the aging DS population (e.g., Fenoll et al., 2017; Powell et al., 2014) who found reduced FA in the CC, external capsule, internal capsule

and cingulum in adults with DS with and without Alzheimer's. Reductions in FA in anterior association tracts (e.g., the fronto-temporo-occipital regions, the CC, and anterior limb of the internal capsule) were also reported in Gunbey et al.'s (2017) study conducted in toddlers with DS. However, the reduction of the other TBSS in the DS groups (i.e., MD, RD and AD) is inconsistent with Romano et al.'s (2018) findings, which showed increased MD, AD and RD in the anterior thalamic radiation, the inferior fronto-occipital fasciculus, the inferior longitudinal fasciculus, and the cortico-spinal tract, the forceps minor and major, superior longitudinal fasciculus, the cingulum, and the uncinate fasciculus in the participants with DS. In fact, in the current study, no WM tract bundle had increased TBSS metrics in the group with DS.

The reason for this discrepancy could be due to the two samples' differences in age. In fact, whilst the current study has been conducted in a sample of neonates, when most of the WM tract are not yet myelinated, Romano et al.'s (2018) study was conducted in a sample ranging from 14 to 27 years old. Lebel et al. (2012) used DTI tractography in a sample of 403 healthy subjects aged 5–83 year and found that FA increased during childhood, reached a peak between 20 and 42 years of age, and then decreases, whilst MD decreased first, reaching a minimum at 18–41 years, and then increased later in life. As these results are based on snapshot measures of TBSS metrics, it could be that the discrepancies between the studies are due to simply assessing WM microstructure at different point in development. Similarly, it was suggested that investigating WM early in life in DS would allow to discern what alterations are due to simply atypical lifelong development versus additional pathology in later life caused by the genetic dysregulation. Perhaps these inconsistencies in findings are a reflection of both these aspects: some

differences in WM are the products of atypical early development and some are part of later life pathology (e.g., where Romano et al.'s (2018) results diverge from the current finding).

It should also be noted that the current study did not consider weight or gestational age at birth as confounding variables, but they both seem to have a different effect on FA on the neonates with DS and the TD neonates included in this study. Lower birth weight has been consistently been found to be relate to reduced FA values in neonates (e.g., Dudink et al., 2007; Kim et al., 2023; Zhu et al., 2021) and in a recent study, del Hoyo Soriano et al. (2020) found that GA at birth strongly relates to symptoms of attention-deficit/hyperactivity disorder in children and adolescents with DS, where an earlier GA at birth relates to more symptoms.

Regardless of some of the discrepancies between the current findings and previous in vivo literature, the results from the current study suggest that some of the alterations in WM found later on in life in the DS population are actually present from the neonatal period. Nevertheless, due to the limitations related to the TBSS metrics, it is not possible to determine exactly what aspect of the WM microstructure is contributing to those difference (Chanraud et al., 2010; Jones et al., 2013): that is, if the neonates with DS have reduced myelin, thinner white matter bundles, or more disorganised WM bundles.

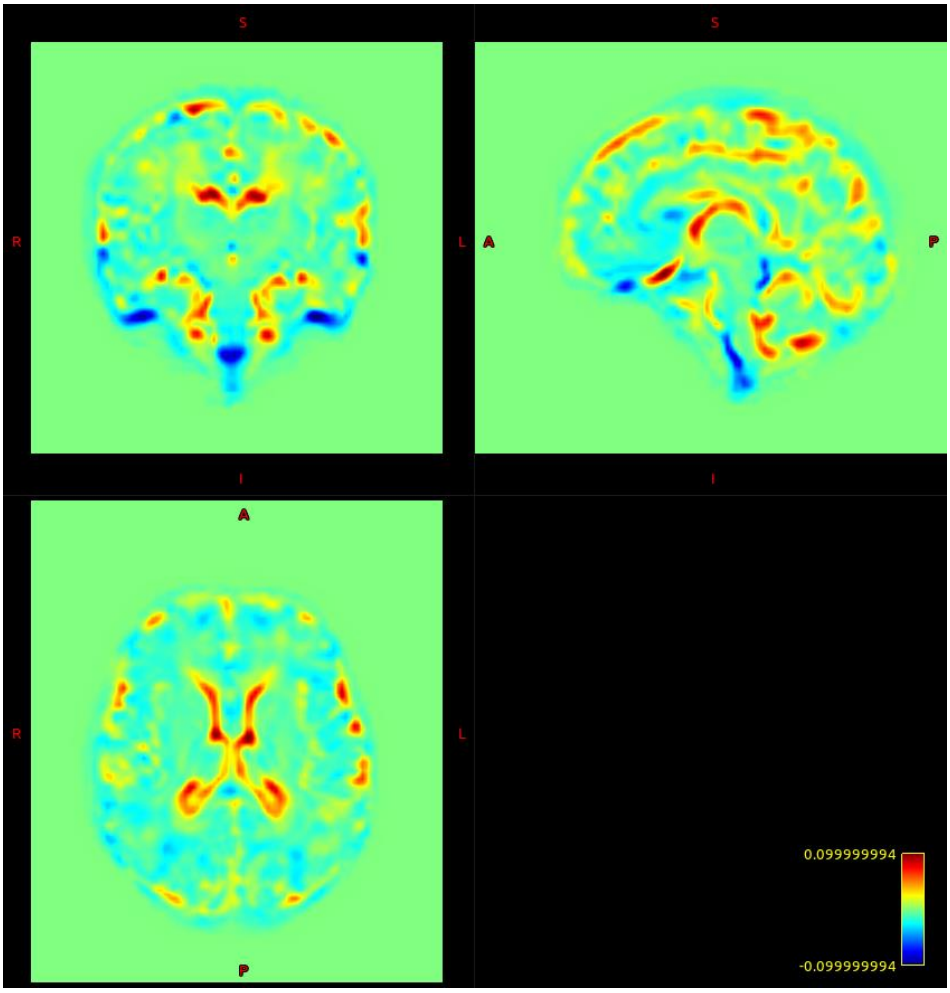
3.4.2 FBA

The second dMRI analysis was performed to explore different components of WM microstructures, such as, fibre density (FD), fibre cross-section (FC) and fibre density and cross-section (FDC). As originally hypothesised, the results showed significant differences between the DS and the TD groups, for all of the metrics. More precisely, FD

and FDC were higher in the DS group in the CC, cingulum and anterior limb or the internal capsule. However, the analysis also showed some implausible results for FD, that is, FD being increased and decreased in the CC for the DS group.

Upon further investigation, it was found that the FOD registration step, necessary to ensure that the voxels for all the participants align (i.e., voxels in the CC in participant A align with CC voxels in participant B) had not worked properly. To prove this, separate mean FOD images were created for the TD and DS groups and subtracted from one another. The resulting heatmap image is shown in Figure 3.14. The warm (red and yellow) and cool (dark blue) areas represents the parts of the brain where the registration has failed more considerably. If the registration had worked correctly, this heatmap should have no anatomical detail in it (e.g., ventricles would not be discernible) and warm and cool areas would only be present in the brain areas where there was a real group difference in fibre density. In this case, the map shows considerable anatomical details, especially in the areas where there is an edge between different densities (e.g., the cortex, the ventricles, around the pons and the cerebellum). These areas also coincide with the ones that the results showed as being significantly different between groups for FD, FC and FDC. Hence, the results of the current study are not reliable and should be interpreted with caution.

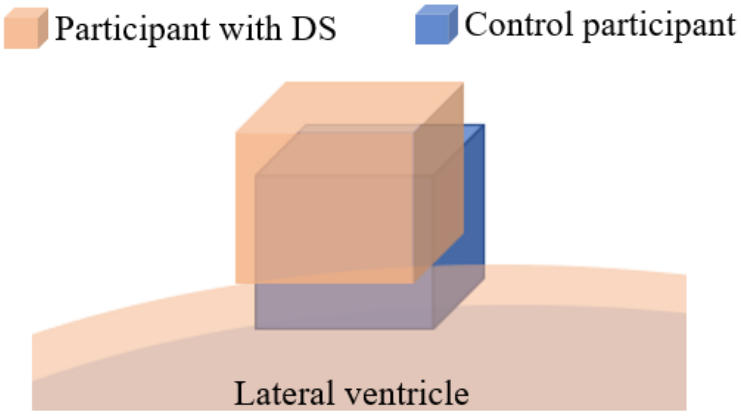
Figure 3.14 *Image showing differences in mean fibre-orientation distributions (FODs) between TD control group and DS groups caused by a misalignment during registration. The more intense the colour, the bigger the difference.*



The reason behind this misalignment might have been driven by two factors affecting the quality and validity of the study template: the inherent volumetric differences between the DS and the TD groups, and the age range of the sample with respect to the rate of anatomical developmental change. In has been consistently reported that brain volumes of individuals with DS significantly differ from those of the TD population, as early as the second trimester of pregnancy (Patkee et al., 2020). More precisely, lateral ventricles volumes are significantly larger than in the TD population, and the cortical and

the cerebellar volumes are significantly smaller. The boundaries around these altered brain regions, coincide with the areas in Figure 3.14 that show a higher difference between groups in mean FODs. Figure 3.15 shows a schematic representation of what might have happened during registration in the voxels surrounding the CSF filled cavities. Volumetric differences in the lateral ventricle volumes would cause the surrounding voxels to be misaligned (i.e., the voxels in the DS group being pushed upwards), which could have led to the implausible results acquired in this study. That is, as the voxels do not fully overlap for both groups, there is a part of the voxel where the fibre density is being compared to non-WM voxels in the statistical analysis step.

Figure 3.15 *Schematic representation of what the effect of larger lateral ventricles in the DS group might have had in the adjacent voxels.*



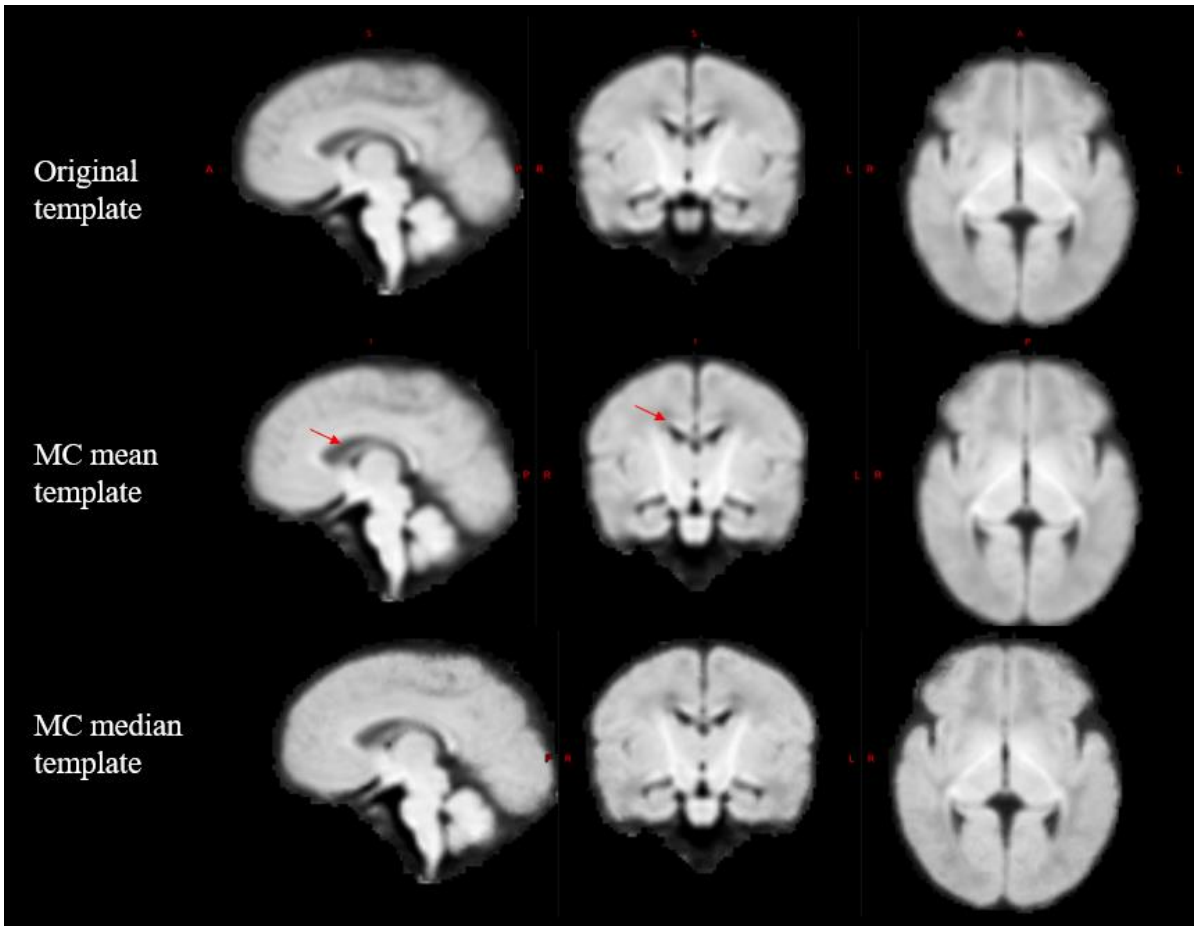
The second factor that might have affected the quality of the study template, and hence, the reliability of the current results, is the age range at which the current sample was scanned with respect to the rate of anatomical developmental change. In fact, during the neonatal period, not only does brain size rapidly change, but also the water content in the brain, which has an impact on the acquired diffusion signal (Pietsch et al., 2019). Although there is no optimal technique for generating study-templates, the common

approach is to average the WM response functions, which was the approach followed in the current study. Alas, the wide age range in the sample and the variability on diffusion signal in the brain, has resulted in a study-template that was not representative of the individual subjects. To overcome this issue in a sample of 113 neonates scanned between 32 to 44 weeks PMA, Pietsch et al. (2019) created age-specific study templates by dividing the sample into weekly cohorts and creating a group average template for each week group. This approach was not feasible in the current study, due to the small sample size.

There are different approaches and solutions that could be implemented to the study-template building step to overcome the mentioned issue. To overcome the issue of working with a small sample scanned during wide age range, the template could be created using the median rather than the average of the WM FODs. In addition, following the recommendations, the current study-template was created using only the WM FODs extracted from the WM response functions. This means that only the WM contrast was used to align the images from all the subjects in the template creation. To overcome the issue of the inherent volumetric difference between the neonates with DS and TD neonates, a multi-contrast template could be created instead, that is, a template using the FODs for white matter and CSF. So far, a group mean multi contrast template and a multi contrast median template have been created and compared to the original mean single contrast template (Figure 3.16). After visual inspection, it appears that the mean multi contrast template is the best one, as it is the one with the sharpest edges in the tissue-CSF intersections, like the lateral ventricles. The next steps will be to run the registration again and create a new group difference heatmap and compare to the original one (Figure 3.14)

to check if the anatomical details have been reduced. This alternative data processing is currently being undertaken. If this alternative approach does not result in an improved registration, it could be an indication that the FBA approach might not yet be optimal to analyse dMRI data in the neonatal DS population, or any two groups with significant macroanatomical differences.

Figure 3.16 *Comparison between the originally created single tissue contrast template and the two new multi-contrast templates. All templates were created using the same subsample of DS and control participants. The red arrows show the sharper tissue-CSF edge in the lateral ventricles.*



3.4.3 Future directions

This study is, to the best of our knowledge, the first to show in vivo that the WM alterations found in mouse model studies and post-mortem studies (see section 1.3.2 Brain

Development in DS) are also present in the perinatal period in human neonates with DS, which might be a contributing underlying factor to the cognitive phenotype in the DS population. Historically, when studying the neuroanatomical correlates of cognitive abilities, most of the focus was set on the cortical grey matter. However, in the last two decades the importance of WM in development, aging, and many neurologic and psychiatric conditions across the life span have been highlighted (Filley, 2005). Individual variability in WM microstructure, measured with diffusion weighted imaging, has been consistently shown to relate to cognition and behaviour in human adults (Johansen-Berg, 2010).

For instance, measures of WM microstructure in temporal lobe connections (e.g., superior longitudinal fasciculus, the arcuate fasciculus, the uncinate fasciculus, the inferior longitudinal fasciculus) have been associated with measures of behaviours associated with cognitive functions, such as executive function, information processing speed and memory (Sasson et al., 2013), as well as language abilities (Mandelli et al., 2014; Warren et al., 2009). Similarly, DTI metrics (e.g., FA and MD) in the cingulum have been related to sustained attention (Takahashi et al., 2010), executive control, emotion, and episodic memory (Bubb et al., 2018); and the anterior limb of the internal capsule has been related to attention, inhibition and processing speed (Mithani et al., 2020).

The CC is the largest WM bundle in the human brain, connecting the left and the right hemispheres and conventionally divided into the genu (anterior portion), body, and splenium (posterior part) (Catani & Thiebaut de Schotten, 2008). As a whole, the CC has been found to be responsible for bilateral representation of language, functional interhemispheric inhibition, and the maintenance of hemispheric differences in arousal

(Clarke & Zaidel, 1994). When investigating the parts of the CC separately, the genu has been related to overall IQ (Kontis et al., 2009), and the body and the splenium with visuospatial abilities, language, processing speed and motor abilities (Fryer et al., 2008).

The role of white matter bundles in cognition is not only limited to tracts within the supratentorial brain. Aside from basic functions like equilibrium or motor function (e.g., Caeyenberghs et al., 2010; Shany et al., 2017; Thomas et al., 2017), cerebro-cerebellar tracts and WM tracts within the pons, like the cerebellar peduncles, have also been found to be involved in higher cognitive functions, such as, general IQ and language (Shany et al., 2017), working memory (Takahashi et al. 2010), visuospatial skills and memory (Thomas et al., 2017) and even reading (Bruckert et al., 2020; Travis et al., 2015).

The existence of these relationships between WM bundle microstructure and cognitive abilities highlights the potential for identification of neonatal imaging biomarkers, that could help predict cognitive outcomes later in the DS population. It was not possible to statistically test this in the current study, but the presented case studies seem to suggest that FA values in the CC and cingulum and anterior IC closer to what would be expected in a TD neonate might relate to better language outcomes in neonates with DS. However, this should be further investigated with a larger sample, and taking into account other factors that might impact neonatal FA values, such as weight at birth (Dudink et al., 2007; Kim et al., 2023; Zhu et al., 2021).

The relationship between neonatal WM microstructure and toddlerhood or childhood outcomes has been widely investigated in the typical and some atypical populations. Feng et al. (2019) investigated the relationship between neonatal variations of white matter microstructures measured with TBSS and Bayley Scales of Infant

Development (BSID) outcomes at 2 years-old in a sample of term-born babies and found a positive correlation between FA and BSID language, cognitive, and motor subscales.

Neonatal TBSS measures have also been related to BSID outcomes in the preterm-born population. For instance, Rose et al. (2009) found that neonatal FA in the splenium and posterior limb of the internal capsule (PLIC) related to neurodevelopmental outcomes at 18-months-old in a sample of very-low-birthweight preterm children. Kooij et al. (2012) also found evidence that FA and RD in the CC and PLIC, as well as the fornix, were related to cognitive and gross motor BSID scores, and that FA throughout the brain was related to fine motor scores. The associations between neonatal WM microstructure have also been found to be different for males and females (van Kooij et al., 2011). A more recent study by Kelly et al. (2020) also found supporting evidence for the relationship between preterm-born neonatal TBSS metrics in CC and internal capsule, as well as the bilateral cerebellar peduncles and the BSID cognitive and language scores at 2-years-old, but they found no significance relationship to motor outcomes.

Despite some of the inconsistencies, this evidence suggests that early CC and internal capsule microstructure could indeed be helpful in predicting outcomes in toddlerhood. These two WM bundles have been found to have atypical WM microstructure measures in the DS sample of neonates in the current study, so future research should investigate if they can help predict outcome variability in the DS population too. In the current study, a whole-brain analysis approach was taken, as suggested in Karmiloff-Smith (2010). However, with this approach it can be hard to identify specific WM bundles, so future studies could follow a tractography based analysis, where single WM tracts could be identified and extracted.

3.4.4 Conclusion

The current study has provided in vivo evidence of quantifiable alterations in WM microstructure in the brain of neonates with DS, previously found in older populations with DS. Some of these DS-TD differences were consistent with what was found in the older individuals with DS, but some other DS-TD differences were inconsistent with previous findings in older samples. Further research is needed to discern what WM alterations present in adults are part of early diverging trajectories, and what are experience-dependent or later developmental pathology independently caused by the trisomy. Nevertheless, further efforts are needed to optimise the latest diffusion data analysis approaches to accommodate populations where gross anatomical alterations are present, and be able to investigate WM microstructure properties, like density or fibre cross-section.

Finally, the early WM alterations found in the current study provide a novel in vivo biomarker that could be used to predict the cognitive individual variability found in the population with DS. This would be extremely helpful not only for research and clinical purposes, but also to provide more detailed information to parents on how their child with DS will develop.

Chapter 4: Neonatal brain volumes and childhood cognitive outcomes

CHAPTER 4: BRAIN VOLUMES AND OUTCOMES

4.1 Introduction

One of the consistently found neuropathological phenotypes in DS is that of hypocellularity throughout the brain (see Table 4.1 for summary). Evidence from mouse models and histological studies has shown reduced cell numbers in different parts of the trisomic brain in embryonic and fetal stages, as a consequence of different mechanisms (e.g., increased cell death, decreased neurogenesis and cell proliferation) (Contestabile et al., 2007; Guidi et al., 2008, 2011; Larsen et al., 2008). The combination of these alterations is associated with reduced 2D and volumetric measures of the brain (Contestabile et al., 2010), found in several MRI in vivo human studies as early as the 1990s. For instance, Weis et al. (1991) explored brain volume alterations in a sample of seven adults with DS and matched controls, aged 30 to 45 years old. The images were acquired using a 0.5 Tesla magnetic resonance scanner and segmented into main brain tissue types. The results showed absolute smaller volumes of the whole brain, cerebral cortex, white matter, and cerebellum in the cohort with DS. Similar brain volume reductions have been consistently found in the literature when compared to healthy participants, showing greater than 20% brain volume reductions (Contestabile et al., 2010), and even further reductions in adults with both DS and Alzheimer's Disease (Neale et al., 2018).

Table 4.1 *Summary of research examining hypocellularity and reduced brain volumes in mouse models and human studies when compared to TD controls. Results are divided between post-mortem/mouse model and in vivo studies and are presented following the chronological age of the sample.*

Authors	Methods	Sample size	Age	Key results
Post-mortem and mouse model studies				
Contestabile et al. 2007	Fetal Histology and Ts65Dn mice	*n*= 10 (5 DS; 5 TD)	17–21 weeks GA	Reduced number of proliferative cells in hippocampal dentate gyrus (DG) and germinal matrix of the latera ventricles.
Guidi et al. 2008	Fetal Histology	*n*= 13 (6 DS; 7 TD)	17–21 weeks GA	Reduced volume and cell number in hippocampal dentate gyrus (DG) and parahippocampal gyrus. Higher percentage of cells with astrocyte phenotype and smaller percentage with neuronal phenotype
Guidi et al. 2011	Fetal Histology	*n*= 13 (7 DS; 6TD)	17–21 weeks GA	Reduced volume and cell numbers in all cerebellar layers.
Larsen et al. 2008	Fetal Histology	*n*= 12 (4 DS; 8 TD)	19 weeks GA	34% reduction of cell numbers in the neocortex
In vivo human studies				
Patkee et al. 2020	Fetal and neonatal volumetric MRI growth trajectories	*n*= 117 (44 DS; 73 TD)	21weeks GA–46 weeks PMA	Reduced whole brain and cerebellar volumes from 21 weeks GA. Reduced cortical volumes from 28 weeks GA onwards. Increased lateral ventricular volumes from 28 weeks GA onwards.
Fukami-Gatner 2021	Neonatal volumetric MRI deviation from the normative mean	*n*=525 (25 DS; 500 TD)	32–46 weeks PMA	*Absolute volumes*: Reduced whole brain volume and all regional volumes except lentiform nuclei (n.s.). Increased lateral ventricle volumes. *Relative volumes:* Reduced cerebellum, cingulate, frontal, insular and occipital WM. Increased thalami, lentiform nuclei, lateral ventricle and eCSF volumes.

Gunbey et al. 2017	Regional volumetric MRI	*n*= 18 (10 DS; 8 TD)	2.6 ± 0.69 years	Reduced total cortical GM, cerebellar GM and WM volumes, basal ganglia, thalamus, brainstem, and CC
Śmigielska-Kuzia et al. 2011	Volumetric MRI	*n*= 49 (23 DS; 26 TD)	6.7 years	Reduced whole brain, frontal and temporal lobes, hippocampi amygdala volumes.
Carducci et al. 2013	Volumetric MRI	*n*= 48 (21 DS; 27 TD)	7-17 years	Reduced whole brain, cerebellar, frontal lobes, frontal region of the limbic lobe, parahippocampal gyri and hippocampi GM volume and cerebellar, frontal, and parietal lobes, sub-lobar regions and brainstem WM volumes.
Pinter et al. 2001	Volumetric MRI	*n*= 31 (16 DS; 15 TD)	5-23 years	Reduced whole brain volumes, and relative cerebellar volumes. Increased relative subcortical GM volumes.
Weis et al. 1991	Volumetric MRI	*n*= 14 (7 DS; 7 TD)	30-45 years	Reduced whole brain, and relative cortical, total WM, and cerebellar volumes.

Note. CC= Corpus callosum; eCSF= Extra-cerebral corticospinal fluid; GA = Gestational age; GM= Grey matter; n.s.= no significant difference; PMA= Post menstrual age; WM= White matter.

An interpretation of these findings would be that the volume reduction in DS is due to atrophy caused by the early onset of Alzheimer's or dementia associated with the trisomy, and it would not be possible to determine additional prior effects of hypocellularity found in fetal and neonatal ex vivo and non-human studies. However, these volume reductions have been found in individuals with DS at all ages. Pinter et al. (2001) found reduced whole brain volumes and significantly smaller cerebellums but proportionately larger subcortical grey matter volumes in a sample of individuals with DS aged 5–23 years. Carducci et al. (2013) found similar whole brain volume reduction in a sample of children and adolescents with DS, aged 7-17-years-old. In a more detailed analysis of brain regions, the results also showed reductions of the grey matter (GM) in the cerebellum, frontal lobes, parahippocampal gyri and hippocampi and of the white matter (WM) in the cerebellum, frontal and parietal lobes, and brainstem. Reduced frontal lobe and hippocampal volumes were also found in a sample of primary school aged children (mean age 6.7 years) with DS (Śmigielska-Kuzia et al., 2011), and reduced deep GM (i.e., left putamen, bilateral thalamus, caudate nucleus), cerebellar cortex brain stem and corpus callosum in a sample of toddlers with DS (mean age 2.6 years) (Gunbey et al., 2017). These neuroanatomical abnormalities are believed to be a consequence of earlier neurodevelopmental disruptions, and the neural correlates to the intellectual disability (Contestabile et al., 2010).

When studying the relationship between brain size and cognition, two approaches could be taken: (1) exploring how global brain measures (e.g., intracranial volume[5] (ICV),

[5] *Intracranial volume* includes all brain structures and fluid inside the skull, excluding extracranial background (i.e., the skull and the background of the MRI image)

total brain volume[6] (TBV), total tissue volume[7] (TTV)) relate to overall cognitive and motor abilities (i.e., using composite scores), or (2) exploring variability in specific brain regions (e.g., smaller cerebellum or larger cortex) and comparing this to variability in the cognitive profile. The large individual variability found in the phenotype of the population with DS (Haydar & Reeves, 2012; Karmiloff-Smith et al., 2016) suggests that the latter approach is more appropriate. Considering the overwhelming evidence on not only allometry between the body and the brain, but also between different parts of the brain (i.e., there is a constant proportionality maintained between different brain areas, both within and between species) (Finlay, 2019; Finlay et al., 2011), it becomes clear that when studying regional brain volumes, these should be corrected for some measure of whole brain size (e.g., head circumference, ICV, TBV) if the goal is to explore true individual regional differences.

There is evidence showing significant correlations between several brain area volumes (e.g., cerebellum, temporal lobe, occipital lobe, orbitofrontal cortex, cingulum) and cognitive abilities (e.g., language, short-term memory and long-term memory) in a small sample of adolescents with DS. Menghini et al. (2011) investigated the association between regional GM volumes and cognitive scores in a sample of 12 adolescents (ages 12-20 years) with DS. Participants were recruited from a pool of individuals undergoing clinical and rehabilitative follow-up at the Children's Hospital Bambino Gesu` in Rome. The reason for the follow-up or the existence of any medical comorbidities were not

[6] *Total brain volume* includes all brain structures and lateral ventricles and excludes extracranial background and extracerebral cerebrospinal fluid.
[7] *Total tissue volume* includes all brain structures and excludes extracranial background, extracerebral cerebrospinal fluid and lateral ventricles.

reported by the authors. A large battery of measures were used to assess participants' cognitive abilities: intelligence (Stanford-Binet Intelligence Scale, Italian adaptation, Bozzo & Zecca, 1993), language abilities, like lexical production (Boston Naming test, Kaplan et al., 1983) and comprehension (The Peabody Picture Vocabulary Test, Italian adaptation, Stella et al., 2000) and morphosyntactic comprehension (The Grammar Comprehension Test, Rustioni, 1994) and production (The Phrase Repetition Test, Vender et al., 1981); short- term verbal (The Verbal Span Task; PROMEA, Vicari, 2007), visual (The Object Span Task; PROMEA, Vicari, 2007) and spatial (The Spatial Span Task; PROMEA, Vicari, 2007) memory; long-term verbal (The Verbal Long-term Memory Task; PROMEA, Vicari, 2007) and spatial memory (The Spatial Long-term Memory Task ; PROMEA, Vicari, 2007); and visual motor (The Visual Motor Integration Test, Preda, 2000) and perception (Visual Perception Test, Hammill et al., 1994) abilities. All measures' raw scores were converted to z scores, using mental age normative data. The Statistical Parametric Mapping (SPM2) software was used to pre-process and segment MRI images into GM, WM, and cerebrospinal fluid (CSF) maps, and GM was further divided into more regions, but no details are provided for this procedure.

Associations between regional GM density and cognitive assessments in adolescents with DS were investigated using several multiple regression models (one for each cognitive ability), with overall GM density as a confounding variable. Results showed significant positive correlations between cerebellar GM densities, and language comprehension and spatial short-term memory. Temporal GM density was also positively associated with language production, visual short-term memory and spatial long-term memory. Moreover, parietal, occipital and insular GM density was positively associated

with the three measured short-term memory scores (visual, verbal and spatial). Finally orbitofrontal cortical GM and frontal GM densities were positively associated with verbal long-term memory and visual perception abilities, respectively (Menghini et al., 2011).

Nevertheless, reported studies have focused on adulthood, adolescence, and toddlers, by which time the individual's external environment and experience may have contributed to the possible differences in brain and cognitive development. From a neuroconstructivist point of view, it would be more appropriate to assess the brain earlier in development. This could provide neural markers that could help target earlier interventions, potentially reducing the impact of the cascading effect that leads to the emergence of the cognitive phenotype in DS (D'Souza & D'Souza, 2019; D'Souza & Karmiloff-Smith, 2017). At the least, the early study of the brain in the DS population would provide an understanding of the size of the differences, and the extent to which they are exaggerated or attenuated by the developmental process. Historically, ultrasonography has been the only method to assess the brain during gestation, and has been used to show reductions in the brain size of fetuses with DS as early as the second trimester (16-20 weeks gestational age (GA)) (Contestabile et al., 2010). However, the development of MRI techniques now allows better quality images of the fetal and neonatal brain enabling the visualisation and quantification of smaller brain regions. This has been the aim of the Early Brain Imaging in Down Syndrome (eBiDS) project carried out by our team.

An initial volumetric study was carried out with 30 fetuses (scanned between 22-36 weeks gestation) and 21 neonates (scanned 36- 46 weeks postmenstrual age (PMA)) (six of whom also had fetal imaging) with DS. Healthy pregnant volunteers whose fetus

had no congenital or chromosomal abnormalities and no apparent anomalies in MRI scans were recruited as controls from antenatal clinics at Queen Charlotte's and Chelsea Hospital and St. Thomas' Hospital, London at approximately 20 weeks GA. Age-matched neonatal controls with no brain structural anomalies and normal neurodevelopmental outcome at 2 years of age were recruited from South London and Southeast of England antenatal centres as part of the Brain Imaging in Babies Study (BIBS). Anatomical images were acquired and pre-processed using optimised acquisition protocols and processing pipelines (see Chapter 2) (Patkee et al., 2020). Fetal brain images were segmented semi-automatically for some regions (e.g., supratentorial brain tissue, lateral ventricles, extra cerebral CSF (eCSF)) and manually for others (cerebellum and cortex) with appropriate additional manual fine editing. Neonatal images were segmented using a fully automated algorithm optimised for the neonatal brain into supratentorial brain tissue, cortical, cerebellar, eCSF and lateral ventricular volumes. The results from group comparison showed significantly smaller whole brain (Cohen's d= 1.65) and cerebellar (d= 1.06) volumes in the group with DS as early as the second trimester, and smaller cortical volumes (d= 0.93) as early as 28 weeks gestation. On the contrary, lateral ventricle volumes were significantly larger (d= 1.15) in the babies with DS, as early as the second trimester (Patkee et al., 2020).

More recent ongoing work from our team has focused on looking at more detailed brain volume alterations in neonates with DS by parcellating the brain into structures (e.g., the four cerebral lobes, insula, cingulate gyrus, hippocampus, amygdala) (Fukami-Gartner et al., 2021). In this case, rather than looking at absolute brain volumes, the methodology used in Bonthrone et al. (2021) was followed, and Gaussian Process Regression used, to

extract multiple regional brain volume z-scores (e.g., GM and WM for the four brain lobes, white matter, amygdala, hippocampus, and so forth), that represent deviation from a normative typically developing (TD) population of 493 neonates (32-46 weeks PMA) scanned as part of the Developing Human Connectome Project (dHCP). Scores controlled for age and sex were extracted for 20 neonates with DS (scanned 32-44 weeks PMA). Results from group-level absolute brain volume differences showed that all whole brain volume measures (ICV, TBV and TTV) (Cliff's *d*= .5-.76) and nearly all segmented regions (e.g., thalamus, amygdala, putamen, brainstem, cerebellum) were significantly smaller than the controls (Cliff's *d*= .35-.95). The lateral ventricles were the only volumes significantly larger in the group with DS (Cliff's *d*= .52). When the regional volumes were controlled for total tissue volume, the areas that appeared to be significantly negatively deviated from the norm (i.e., significantly smaller) were the cerebellum and several of the WM segmentations (cingulate, frontal lobes, insula, and the occipital lobes). So, when considering relative regional volumes (i.e., taking into account overall brain size) only these areas were significantly smaller, suggesting that the reduction found in the other regions was driven by the overall brain volumes being smaller.

Although cross sectional, these studies have been the first to show brain volume development alterations, in vivo, so early in brain development in a sample with DS. The question now remains whether these brain measures have any predictive value when it comes to the cognitive phenotype related to trisomy 21. This would be of great interest considering the wide individual variability found in both the occurrence and the severity of the neuroanatomical (Haydar & Reeves, 2012) and cognitive (Karmiloff-Smith et al., 2016; Marchal et al., 2016; Tsao & Kindelberger, 2009) phenotypes. This study is the first

step towards answering this question, as it aims to explore the relationship between neonatal brain volumes and subsequent cognitive abilities in infants and children with DS.

Although such an endeavour has never been undertaken before with this population, there have been several studies seeking to achieve the same goal in other atypical populations, such as, the preterm population (see Table 4.2 for summary). For instance, Peterson et al.'s (2003) study explored the relationship between brain volumes in preterm babies scanned at term-equivalent age and their cognitive outcomes at 18-20 months old. Neurodevelopmental outcomes were assessed using the Bayley Scales of Infant Development (BSID II) (Bayley, 1993) and motor and mental domain scores were extracted. Brain tissue segmentation was based on manually selected sample voxel intensities for CSF, cortical grey matter and white matter. Subcortical GM, ventricles, brainstem, and cerebellum were extracted, and cortical grey and white matter tissues were parcellated into 8 subregions using a combination of 3 coronal planes. When no controlling variables were included in the analysis, both the mental (d= 2.08-6.08) and motor (d= 2.41-2.87) subscale scores were significantly correlated with several brain regions (e.g., WM in the bilateral premotor region, bilateral sensorimotor region, etc.). However, when GA at birth and head circumference were controlled for, only the WM in the right sensorimotor and right mid-temporal regions significantly correlated with the mental subscales.

Table 4.2 *Summary of studies exploring relationships between neonatal brain volumes and later cognitive outcomes. All reported associations are positive (i.e., smaller volumes are associated to lower scores), unless otherwise stated. Results are organised based on the use of absolute brain volumes or relative brain volumes (measure used to correct for overall brain stated between brackets) for analysis. Brain areas that were significantly associated with outcome measures are listed for each of the outcome composite scores (or subscale scores) and the range of effect sizes (Cohen's d) is provided for the studies that provided enough information*.

Authors	**Sample population (*n*)**	**Brain volumes**	**ND assessment**	**Controlled variables**	**Results**
Bonthrone et al. 2021	CHD (*46*)	cGM, WM, cerebellum, brainstem, hippocampus, amygdala, ventricles, eCSF, and left/right lentiform, caudate nucleus and thalamus.	Age: 18-24 mo Measure: Bayley III CCS and MCS	sex, scan PMA, SES	**Absolute volume z scores** *CCS:* bilateral thalamus and caudate and left lentiform nucleus, TTV[c] and cGM[c] (*d*= .77-1.00) *LCS:* not reported *MCS:* n.s.
Kelly et al. 2020	Preterm *(201)*	cGM and WM regions (segmented using MANTiS)	Age: 24 mo Measure: Bayley III CCS, LCS, MCS	sex, scan PMA, ICV	**Relative volumes (corrected for ICV)** *CCS*: right temporal grey matter, left frontal white matter *LCS*: GM (Bilateral lingual gyrus; right precentral gyrus, postcentral gyrus, cingular gyrus, occipital gyrus and cuneus) Bilateral frontal WM *MCS:* n.s.
Meuwly et al. 2019	CHD postop (*62*)	TBV, cGM, Total WM, frontal, parietal, temporal and occipital WM, cerebellum.	Age: 12 mo Measure: Bayley III CCS, LCS, MCS	sex, scan PMA, SES	**Absolute volumes**

					CCS: TBV, cGM, total white matter, frontal lobe WM, temporal lobe WM and cerebellar (*d*= .25-.35) *LCS:* TBV, cGM, frontal lobe WM, temporal lobe WM (*d*= .35-.41) *MCS:* nothing reported **Relative volume (corrected for TTV)** No significant associations
Gui et al. 2018	Preterm (*74*)	ICV, cGM, uWM, dGM, cerebellum, and CSF	Age: 18-24 mo Bayley II MDI and PDI	Sex, GA birth, SES	**Absolute volumes** *MDI:* n.s *PDI*: ICV, cGM, uWM, dGM, cerebellum, and CSF (*d*= .56)
Keunen et al. 2016	Preterm (*112*)	cGM, ventricles, eCSF, uWM, and cerebellum basal ganglia and thalami	Age: 24 mo Measure [a]: Bayley III cognitive and fine and gross motor subtests	Maternal education	**Absolute volumes** *Cognition:* cGM [b], ventricles [b], cerebellum *Fine Motor:* ventricles [b] and UWM *Gross motor:* cGM [b], ventricles and UWM **Relative volumes (corrected for ICV)** *Cognition:* ventricles [b] *Fine Motor:* cGM [b], ventricles [b] *Gross motor:* cGM [b], ventricles [b] and UWM

Hansen-Pupp et al. 2013	Very preterm (*49*)	TBV, GM, uWM and cerebellum.	Age: 24 mo Measures: Bayley II MDI and PDI	-	**Absolute volumes** [b] *MDI:* TBV, Cerebellum and uWM (*d*= .64-.91) *PDI:* Cerebellum and uWM (*d*= .66-.78)
Peterson et al. 2003	Preterm (*9*)	cGM and WM parcellated into 8 subregions	Age:18-20 mo Measure: Bayley II MDI and PDI	GA at birth	**Absolute volumes (no covariates)** *MDI*: WM in the bilateral premotor region, bilateral sensorimotor region, the right subgenual region and the bilateral midtemporal regions, GM in the left sensorimotor and left midtemporal cortices (*d*= 2.08-6.08) *PDI*: WM volumes in bilateral subgenual regions (Cingulate cortex) (*d*= 2.41-2.87) **Relative volumes (corrected for HC):** MDI: WM in the right sensorimotor and right midtemporal regions PDI: n.s.
Kooij et al. 2012	Preterm (112)	Cerebellum	Age:24 mo Measure[a]: Bayley cognitive and fine and gross motor subscales	Scan PMA, sex, maternal education	**Absolute volumes** *Cognition:* Cerebellum (*d*= 1.09) *Gross motor:* n.s *Fine motor:* n.s.

Thompson et al. 2008	Preterm (184)	Hippocampus	Age: 24 mo Measure: Bayley II MDI and PDI	Sex	**Relative volume (corrected for head size):** *MDI:* right and left hippocampus *PDI:* right and left hippocampus

Note. Bayley II (Bayley, 1993) mental and psychomotor developmental indices (MDI and PDI, respectively). Bayley III (Bayley, 2006) cognitive composite score (CCS), the language composite score (LCS) and the motor composite score (MCS). cGM= cortical grey matter; CHD= congenital heart defect; CSF= cerebrospinal fluid; dGM= deep grey matter; eCSF= extra cerebral cerebrospinal fluid; GM= grey matter; HC= Head circumference; ICV= intracranial volume; MANTiS= morphologically adaptive neonatal tissue segmentation (Beare et al., 2016); mo= months old; PMA= post menstrual age; SES= Socioeconomic status; TBV= Total brain volume; TTV= total tissue volume; uWM= unmyelinated white matter; WM= white matter;

[a] Subscales rather than composite scores were used.

[b] Associations were negative

[c] Significant association found only when the analysis was performed with the participants born ≥37 weeks GA (*n*= 42)

n.s. no significant associations were found.

Throughout the years, there have been other studies with the same goal as Peterson et al. (2003), which have also used the BSID II (Bayley, 1993) to assess cognitive abilities around the same age in preterm-born toddlers, showing mixed results. The reason for this might be due the different methods used for brain segmentation, potentially resulting in different target brain areas. As an example, Gui et al. (2018) only segmented the brain into cortical GM (cGM), unmyelinated white matter (uWM), deep GM (dGM), cerebellum, brainstem, and CSF. Although myelination is mostly a postnatal process (Stiles, 2008b)(in the neonatal brain most of the axons have not yet been myelinated) no explanation was provided as to why the authors decided to, not only differentiate myelinated and uWM and the criteria to do so, but also not to include myelinated WM in the segmentation and subsequent analysis. The results from several general linear models showed that neonatal brain volumes (either at birth, or at term equivalent age (40 weeks GA), or both) had no predictive power over the Bayley mental development index (MDI) scores, but the combination of brain volumes at birth and at term equivalent age did predict Bayley psychomotor development index (PDI) scores at 18–24 months (d= 0.56). In this case, no analyses were run to explore the predictive value for each of the separate regional volumes, and only absolute volumes were considered (i.e., they were not controlled for individual overall brain volume differences).

Hansen-Pupp et al. (2013) applied a similar brain segmentation (TBV, GM volume, WM and uWM volume, and cerebellar volume) in their study, and assessed neurodevelopmental outcomes in preterm-born toddlers with the BSID at two years old. Their analysis showed that lower cerebellar and uWM volumes were associated with MDI (cerebellar volume d= 0.78; uWM volume d= 0.91) and PDI (cerebellar volume d= 0.66;

uWM volume *d*= 0.78) scores of 1 standard deviations below the mean. Significant associations were found also for TBVs, but only with the MDI scores (*d*= 0.64). Once again, only absolute brain volumes were considered, as in Gui et al. (2018).

Previous studies have also focused on the predictive value of specific brain areas in preterm-born babies, such as the hippocampus (Thompson et al., 2008) and the cerebellum (Kooij et al., 2012), and their Bayley scores later in childhood. Aside from different segmentation methods and the chosen region of interests, the mixed results in the predictive value of brain volumes in preterm babies might also be due to the decision to focus on different Bayley scale scores and subscales as outcome measures. In fact, the two different Bayley editions used (BSID II and III) allow to extract different composite scores, with the later edition being more specific allowing to differentiate between cognitive and language composite scores (Bos, 2013; Lowe et al., 2012). However, the association between the composite scores in the second and the third edition is rarely mentioned in the literature, which further complicates comparisons between studies (Bos, 2013).

More recently, the association between neonatal brain volumes and neurodevelopmental outcomes has also become of interest in other clinical populations, such as in babies born with congenital heart defects (CHD). In some forms of CHD there may be an alteration of both blood flow and /or oxygen to the fetal brain , which can have an impact in brain development (Bonthrone et al., 2021; Meuwly et al., 2019). A recent study explored the deviations from the norm (*n*= 219) in absolute brain volumes in 46 neonates with CHD, and their relationship to Bayley cognitive and motor composite scores at 18-month-old, controlling for socio economic status (calculated from postcode

at birth). When the analysis was run with the whole sample, the results showed a significant positive correlation between bilateral thalamus (*d*= .80), caudate (*d*= .80) and left lentiform (*d*= .77) nucleus volumes and the Bayley cognitive composite score, and no correlations were found with any other brain regions. Nevertheless, when the analysis was run excluding those participants born preterm (<37 weeks GA, *n*= 42), cGM (*d*= .87) and total tissue (*d*= .87) volumes were also positively significantly correlated to the Bayley cognitive composite score, and the effect sizes for the thalamus (*d*= 1.00), caudate (*d*= 1.00) and left lentiform nucleus (*d*= .93) and outcome correlations were larger. These results suggest that time of birth (i.e., being born preterm or at term) might have an effect on the associations between neonatal brain volumes and later cognitive outcomes. Finally, no correlations were found between brain volumes and motor abilities, whether preterm participants were included in the analysis or not (Bonthrone et al., 2021).

As shown in Table 4.2, the studies looking at the association between neonatal brain volumes and cognitive abilities in atypical populations have used a mix of analysis and volumes extraction methodologies. Nevertheless, cGM, WM, ventricles and cerebellum volumes have been consistently measured across the literature. However, little to no rationale is provided for the different parcellation criteria used (e.g., total WM vs. regional WM vs. unmyelinated grey matter) hindering the comparison between studies and the replication of the results in a different population. Regarding the associations between brain volumes and cognitive outcomes, overall, small to large effect sizes have been found when exploring the association between overall brain size and overall cognitive ability (Bonthrone et al., 2021; Hansen-Pupp et al., 2013; Meuwly et al., 2019). In those studies that used the same outcome assessment method (e.g., Bayley III, Bayley,

2006) cognitive scores have consistently been positively associated to cGM and cerebellar volumes. Moreover, cognitive scores have consistently been found to be associated to frontal and temporal lobe WM, despite the different segmentation method used. It should also be noted that none of these studies have included a TD control sample to compare the brain-outcome pattern of relationships to. Therefore, no conclusion can be drawn on whether these relationships are atypical and how they differ from TD.

The results regarding motor scores have been more inconsistent, with some studies finding no associations at all. There have also been inconsistencies regarding the use of absolute versus relative brain volumes, but most of them (5 out of the 9 studies presented in Table 4.2) have controlled for overall brain sizes although the measure used for correction is not consistent (HC, TBV, ICV, TTV). Although this literature has been invaluable in determining whether early brain volumes can be used to predict cognitive outcome, none of these studies have looked at the brain-cognition association in the TD control group, if included. So, it is still not possible to determine if and how the brain-cognition associations found in the atypical populations are different from those found in typical populations.

4.1.1 Aim and hypothesis of the current study

Despite the somewhat inconsistent methodologies and sometimes contradicting results, the literature at least illustrates persistent attempts to identify brain structure–behaviour associations in neonates and implicitly, therefore, the belief among researchers that these relationships can be established and will be informative. The aim of the current study was to explore the relationship between neonatal brain volumes and later cognitive and motor abilities in a cohort of babies with DS. It was hypothesised that overall brain

volumes would be related to composite scores of overall cognitive abilities, and that cerebellar volumes would be related to fine and gross motor scores. Moreover, as this is the first study of its kind within the DS population, some exploratory analyses were also conducted, to investigate the relationship between more detailed brain regional volumes (cGM, dGM, lateral ventricles and total white matter) and more specific cognitive abilities (e.g., gross and fine motor, expressive and receptive language, and so forth). Based on previous research, it was hypothesised that relative cGM would be related to language and visual reception outcomes, that cerebellar volumes would be related to fine and gross motor scores, and that lateral ventricles would be related to all subscales.

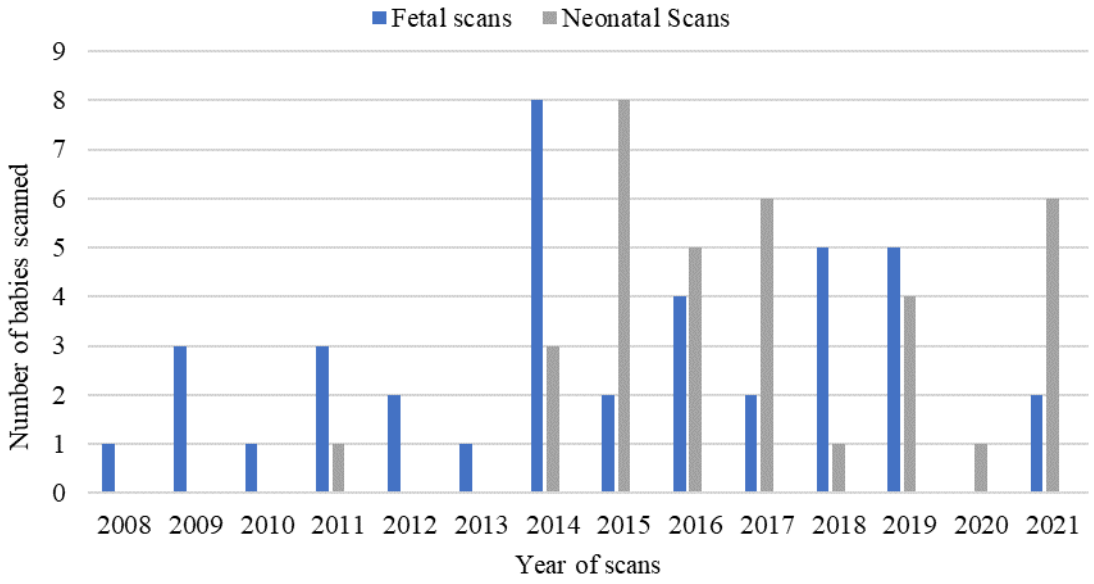
4.2 Methods

4.2.1 Participants

Over the last decade, fetuses and neonates with DS have been recruited with ethical approval at the Centre of the Developing Brain at St. Thomas' Hospital, London, UK. Figure 4.1 demonstrates the number of each type of scan per year since the beginning of the project in 2008. Volumetric data from this large sample are presented in Patkee et al. (2020) and Fukami-Gartner et al. (2021). To date, twelve of these infants have been followed up for cognitive testing at Birkbeck's Centre for Brain and Cognitive Development. The current study presents the neonatal and neuropsychological follow-up data from those twelve participants (7 males). Participants were born at 31^{+5} to 39^{+0} weeks GA (*Mdn*= 37.07 weeks GA) and scanned 34^{+1} to 44^{+4} weeks PMA (*Mdn*= 40.93 weeks PMA). At neurodevelopmental testing, these children were aged between 6 months and 4 years old (*Mdn*= 13.42 months). This wide age range poses a limitation as it introduces

variability in developmental stages and responses, which were not controlled for, due to the small sample size.

Figure 4.1 *Number of fetuses and neonates with DS scanned each year since the project started.*



To visually represent the deviation from normative data and the individual variability present in the DS population, data from the twelve cases were compared with data from larger DS groups, as well as TD cross-sectional growth trajectories (brain volume: Fukami-Gartner et al., 2021; behavioural data for Mullen Scales of Early Learning: D'Souza et al., 2021). For brain volume, the comparison groups comprised 30 scans from individuals with DS, aged 32^{+3} to 45^{+4} weeks PMA (*M*= 40.49 weeks; *SD*= 3.23), and 488 TD individuals, aged 31^{+1} to 45^{+1} weeks PMA (*M*= 40.50 weeks; *SD*= 2.89), part of the dHCP. For cognitive outcome, the comparison groups comprised 98 individuals with DS, age 6-63 months (*M*= 29.55; *SD*= 13.98) and 47 TD individuals from the LonDownS cohort, age 4-35 months (*M*= 23.60 months; *SD*= 11.33).

4.2.2 Neuropsychological assessment

Neurodevelopmental assessments were performed by the researchers using the Mullen Scales of Early Learning (MSEL) (Mullen, 1995). The MSEL are designed to measure cognitive functioning for infants and children from birth through 68 months. The test consists of 5 subscales: Gross Motor (GrM), Visual Reception (VR), Fine Motor (FM), Receptive Language (RL), and Expressive Language (EL). In a TD population, the concurrent validity of the MSEL VR, FM, RL and EL scales with BSID II MDI score were higher (r= .53-.59) than with the PDI score (r= .21-.52), and the reverse was true for the MSEL GrM scale (MDI, r= .30; PDI, r= .76) (Mullen, 1995), supporting the validity of each of the MSEL scales. All participants had data for MSEL.

Aside from the lab-based assessments, participants' parents were asked to complete the Vineland Adaptive behaviour Scales (VABS II) (Sparrow et al., 2005) Parent/Caregiver rating form at home, prior to their visit to the lab. The Vineland II is a parental questionnaire that measure motors skills, and personal and social sufficiency across three domains: Communication, including receptive, expressive and written skills; Daily Living Skills, or behaviours relating to personal grooming, domestic chores, and functioning in the community; and Socialisation, or behaviours involved in getting along with others, playing, and coping with everyday demands. Test-retest reliability analysis for the Survey interview form (administered by the researchers) and the Parent/caregiver rating form showed strong correlations (r= .75-.98) between forms for all the domains, suggesting that caregiver reported scores were as accurate as those reported by the researchers in the semi structured interview form (Sparrow et al., 2005). Only 10 participants had Vineland data available.

Following the previous literature (D'Souza et al., 2021; Dykens et al., 2006; Fidler et al., 2006; Marchal et al., 2016), age equivalent (AE) MSEL subscale and Vineland domain scores were used as opposed to standard scores in data analysis, as standard scores are often at floor in DS. AE scores indicate the age at which the child's score is the median observed in the typically developing standardisation sample for the test. MSEL and Vineland also allow calculation of composite scores. However, these are calculated with standard scores. As a surrogate measure of general cognitive abilities, MSEL and Vineland composite score were calculated using the AE scores for all the subscales and domains (Dykens et al., 2006).

There are some disadvantages to using AE scores, like not being able to extrapolate AE scores for a child with low raw scores (Maloney & Larrivee, 2007; Sullivan et al., 2014), the potential limited validity of using norm extrapolated AE scores to represent abilities of a population with NDCs (Couzens et al., 2004) or their limited reliability in samples with a wide age range (Toffalini et al., 2019) (see section 2.2.4 for a detailed discussion). For this reason, developmental quotients (DQ), previously used in the LonDownS sample (D'Souza et al., 2021), were also used as outcome measure scores. DQ scores were calculated as AE divided by chronological age, multiplied by 100 ([AE/chronological age]*100). Thus, DQ is a measure of how close the child is to the level expected of a TD child of the same chronological age.

4.2.3 Neonatal MRI data acquisition and volume extraction

Neonatal scanning was performed following the procedure described in Chapter 2 (see section 2.1.5. Neonatal MRI and safety).

T2-weighted anatomical images were acquired on a Philips Achieva 3T MRI scanner in the sagittal and transverse planes using a multi-slice turbo spin echo sequence. Two stacks of 2D slices were acquired using the scanning parameters: TR= 12 s; TE= 156 ms; slice thickness= 1.6 mm with a slice overlap= 0.8 mm; flip angle= 90 degrees and an in-plane resolution: 0.8x0.8 mm.

After motion correction and reconstruction, T2 images were segmented into multiple brain regions using *The Developing Brain Region Annotation With Expectation-Maximization (Draw-EM) MIRTK Package*, a fully automated tissue segmentation algorithm, optimised for the neonatal brain (Makropoulos et al., 2014, 2016, 2018)**.**

Normative curves of typical volumetric brain development were generated following Bonthrone et al.'s (2021) methodology, and z-scores [8] were derived for each neonate with DS by Fukami-Gartner et al. (2021), representing the degree of positive or negative deviation of a regional volume (in cm^3) from the normative mean for a given sex and age (in weeks PMA)(see Fukami-Gartner et al., 2021 for full details on how z-scores were calculated). Aside from absolute brain volumes and z-scores, relative volumes and relative z-scores were calculated as the proportion of each regional volume over total tissue volume (TTV) (i.e., regional volumes were divided by TTV). Relative volume for the lateral ventricles was calculated as a proportion of TBV.

4.2.4 Analysis

Statistical analysis was performed using the SPSS software package (version 25). Each variable of interest (i.e., brain volumes and outcome measures) was tested for

[8] Regional volumetric data and the derived z-score were obtained from a researcher in the eBiDS team in the Perinatal Imaging and Health Department, King's College London.

normality of distribution using the Kolmogorov-Smirnov goodness-of-fit test or the Shapiro-Wilk goodness-of-fit test, alongside Q-Q plots and histograms. Correlations between variables were assessed using the Pearson's correlation coefficient for normally distributed data, and the non-parametric Spearman's correlation coefficient for not normally distributed data. Bayes factors in favour of the alternative hypothesis were also computed from Bayesian Pearson's correlations, using the default prior included in JASP 0.10.0.0.

Based on previous literature, correlations between outcome overall composite scores and z-scores for the different gross brain volumes (ICV, TBV and TTV) were performed, as well as correlations between relative cerebellar volume z-scores and MSEL GrM and FM AE scores. As an exploratory analysis, correlations between the five MSEL subscales and four Vineland domains (Communication, Daily living skills, Socialisation and Motor), and relative brain regional volume z-scores (cGM, total WM, lateral ventricles, cerebellum, dGM) were also performed.

4.3 Results

4.3.1 Individual variability in DS

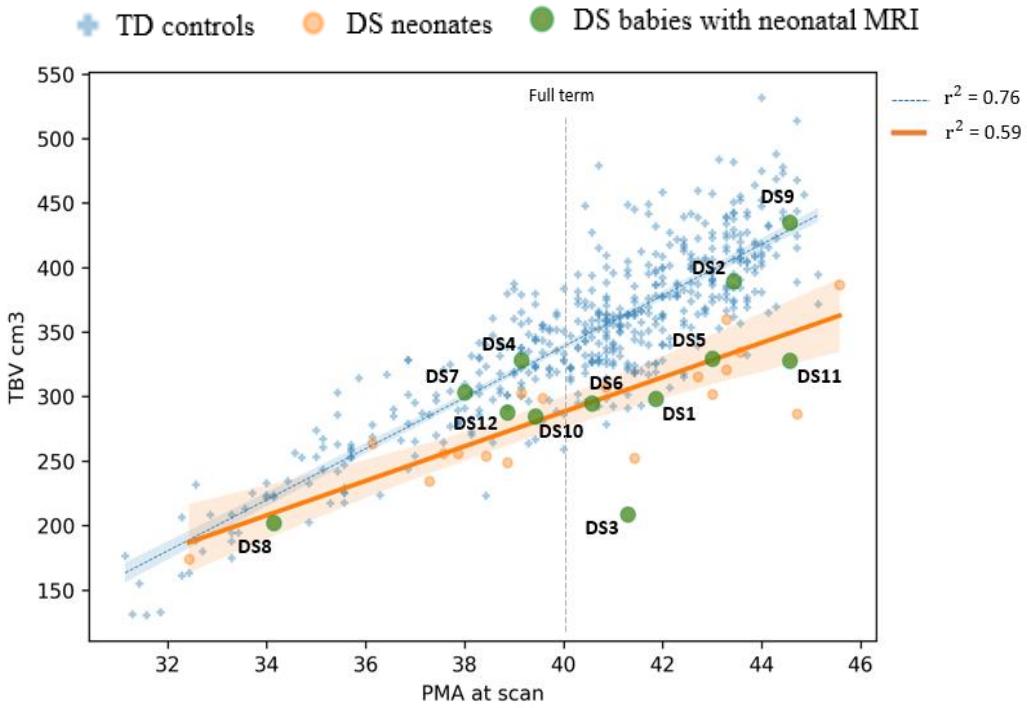
In order to contextualise the results for the 12 children with DS and visualise individual variability in the sample, their MRI data were plotted against the larger cohort (*n*= 18) of neonates with DS reported in Fukami-Gartner et al. (2021), and TD controls (*n*= 488). Their MSEL behavioural profiles were plotted against a larger sample of infants and children with DS (*n*= 98) (age 6-63 months) and TD children (*n*= 47) (age 4-35 months) tested as part of the LonDownS cohort. Since the goal was to consider the position of each child with respect to other individuals with DS, we generated

standardised residuals against the DS trajectory for total brain volumes measures and five behavioural MSEL measures, indicating how far above or below each child fell compared to the group trajectory for their age at testing. Finally, to test whether the cognitive abilities of the 12 children with DS were representative of the larger DS group, a subset of 25 age-matched children with DS were selected from the LonDownS cohort. (D'Souza et al., 2021).

Variability in brain volumes

Figure 4.2 shows the TBV (brain tissue and ventricles included) changes related to age. It revealed increased deviation of TBV in neonates with DS from the TD population with increasing age, represented by the reliably different gradients of the regression lines for TD (β_1= 19.81) and DS (β_1= 13.38) but the same intercept at the earliest point of overlap of trajectories (~32wks). Figure 4.2 also shows individual variability in the DS cohort and in the 12 babies with DS included in the current study sample, represented by the spread of the datapoints. For instance, whilst case DS9 had a TBV that could be found in a TD neonate, case DS3 had a TBV significantly smaller, not only when compared to the TD population, but also when compared to the DS cohort itself.

Figure 4.2 *Total brain volume (cm^3) changes with age in a sample of neonates with DS (aged 32 to 46 weeks PMA). Labels are attached to those cases who were later assessed with the MSEL. TD growth trajectory shown in blue (DS and TD group data taken from Fukami-Gartner et al., 2021). The dotted vertical line shows usual full term, though babies may be born prematurely (i.e., babies to the left of the line).*



Nevertheless, when the variance in TBV controlled for age at scan was compared between the DS and the TD group with an ANCOVA analysis, Levene's test of homogeneity showed no significant differences between the groups, $F(1,516)= 2.61$, $p= .107$. That is, the individual variability in TBV in the DS cohort is what it would be expected from an age-matched TD population.

Variability in cognition

Figure 4.3 shows the changes in the five MSEL subscales related to chronological age and as seen in TBV measures, the figure shows deviation from the TD population with increasing age. Linear regression equations predicting MSEL AE composite and subscales scores from chronological age (CA) were generated from the DS and the TD cohorts' cross-sectional developmental trajectories to explore this further. As shown in

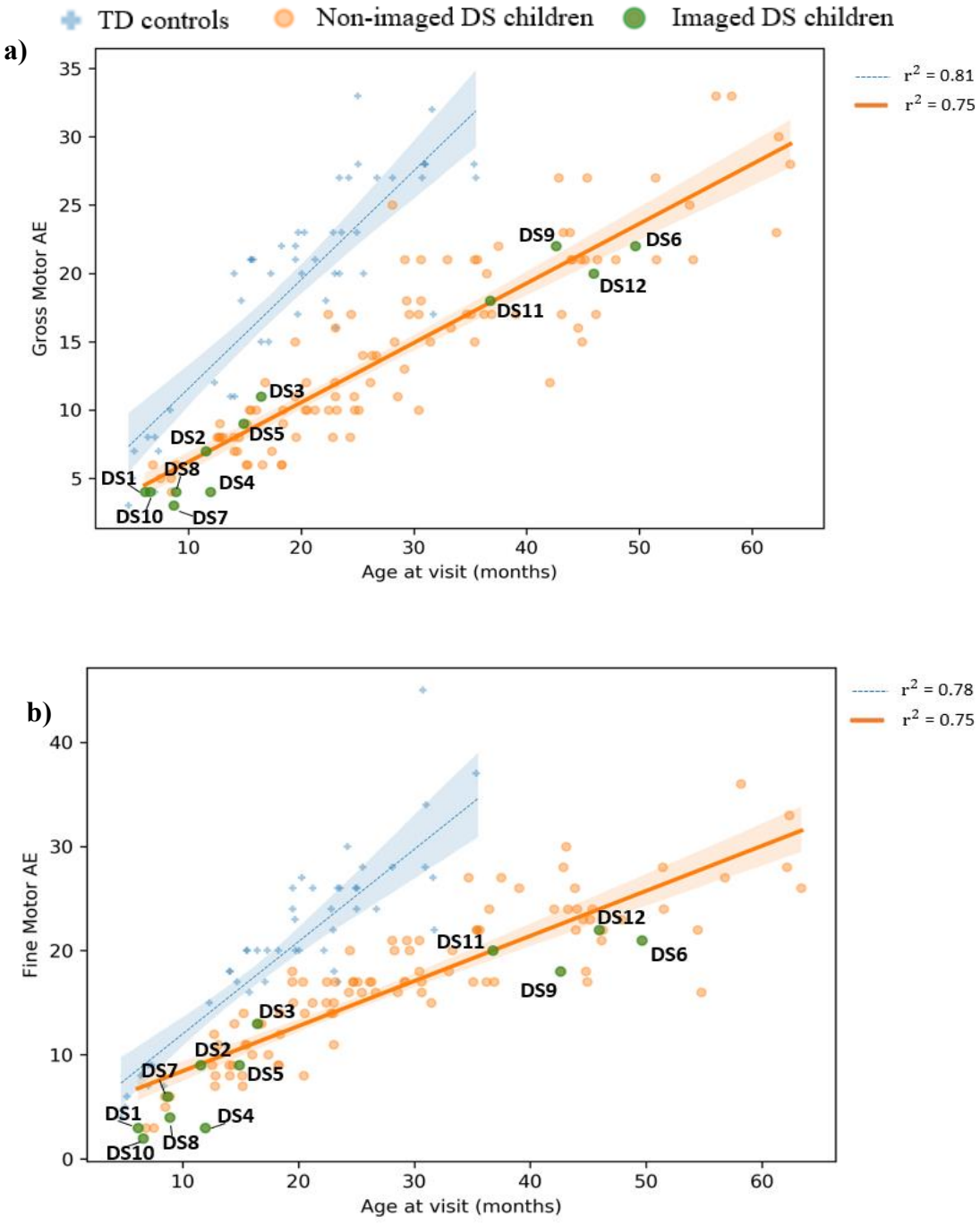
Table 4.3, the slopes for all the DS cross-sectional trajectories are shallower than the TD slopes. Specifically, they are approximately half the gradient of the TD trajectory slopes. This is the case for all scores, except EL (β_1= .35), which is three times shallower, suggesting that developmental delay in DS is especially pronounced in this cognitive ability.

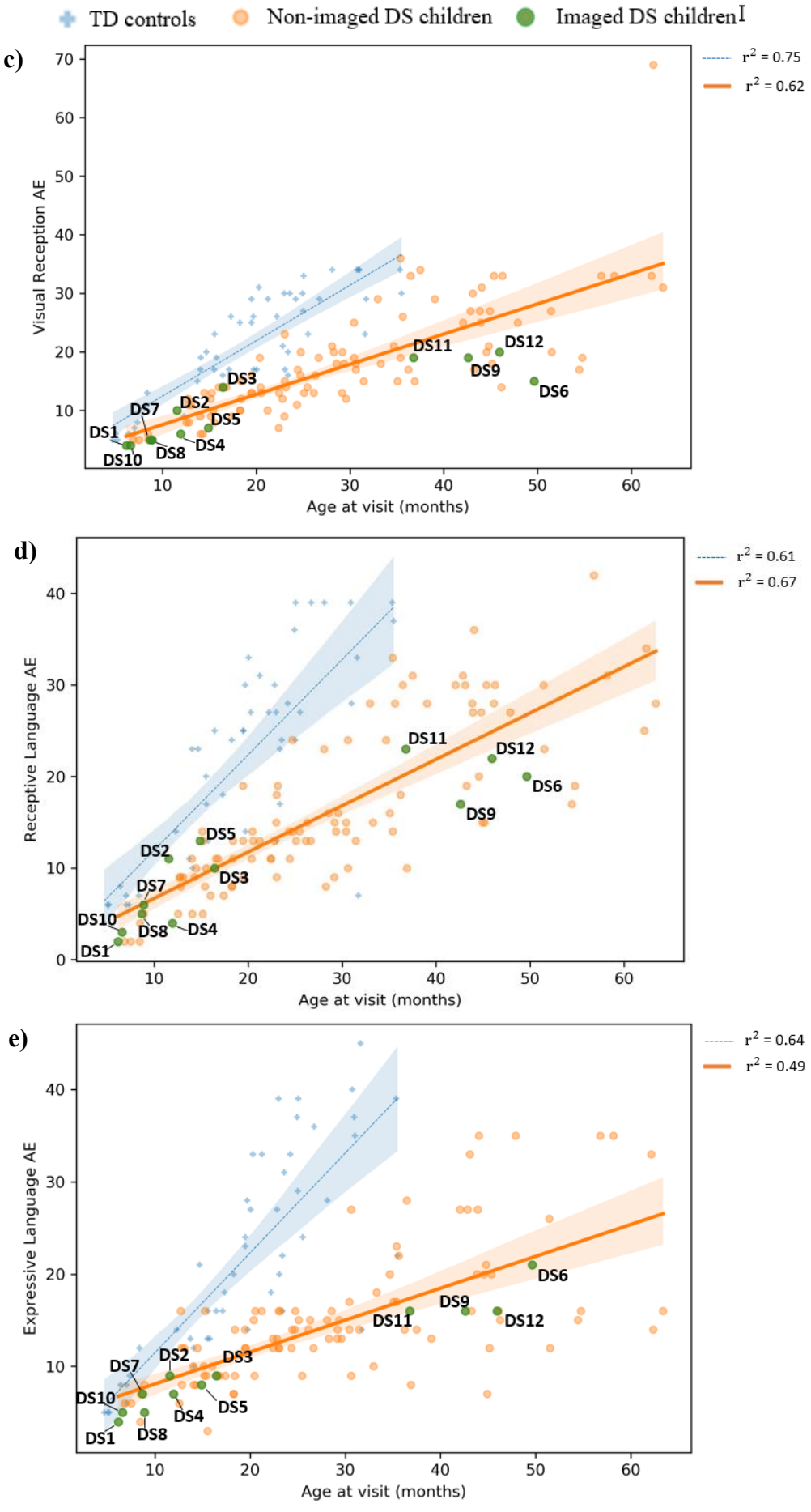
Table 4.3 *Statistics from cross-sectional MSEL AE composite and subscale AE trajectories. Extracted from linear regression equations predicting MSEL AE scores from CA for the DS (n= 110) and the TD (n= 47) cohorts (data taken from D'Souza et al., 2021).*

	DS cross-sectional trajectory			**TD cross-sectional trajectory**		
	Intercept (β_0)	Slope (β_1)	β_1 95% CI	Intercept (β_0)	Slope (β_1)	β_1 95% CI
Composite	14.67	2.23	2.02-2.45	13.02	4.67	3.94-5.41
GrM	1.84	.44	.39-.48	3.55	.80	.66-.94
FM	4.12	.43	.39-.48	3.88	.83	.69-.97
VR	3.66	.46	.39-.53	3.07	.94	.77-1.11
RL	1.64	.51	.44-.57	1.54	1.04	.79-1.29
EL	4.64	.35	.28-.41	.98	1.06	.81-1.30

Figure 4.3 also show individual variability within the DS cohort. Whilst some of the children in the DS cohort performed well below what would be expected from their CA, some performed more typically. Although present in all the subscale measures, individual variability was especially pronounced in the RL (σ^2= 79.04) and VR subscales (VR σ^2= 89.47), followed by EL (σ^2= 50.93), with the two motor scores having the least variability (GrM σ^2= 48.93; FM σ^2= 49.97).

Figure 4.3 *MSEL a) Gross Motor, b) Fine Motor, c) Visual Reception, d) Receptive Language and e) Expressive Language age equivalent score changes with age in a sample of infants and children with DS and TD controls. Labels are attached to those cases who had a neonatal MRI scan (DS and TD group data taken from D'Souza et al., 2021).*




TD controls
Non-imaged DS children
Imaged DS children
c)
Visual Reception AE
Age at visit (months)
r² = 0.75
r² = 0.62
d)
Receptive Language AE
Age at visit (months)
r² = 0.61
r² = 0.67
e)
Expressive Language AE
Age at visit (months)
r² = 0.64
r² = 0.49
DS1
DS2
DS3
DS4
DS5
DS6
DS7
DS8
DS9
DS10
DS11
DS12

For all the subscales, the current group of imaged babies with DS performed below the TD cohort average, and in some MSEL scales, even below the whole DS cohort average, especially the younger babies. A larger non-imaged but age-matched cohort of DS infants and children (*n*= 26, 53.8%/14 males) was selected, and independent sample t-tests were run to test whether there were significant differences between the 12 imaged DS cases and the larger age-matched non-imaged DS group in the 5 MSEL subscales. The two groups did not significantly differ on age, *U*= 116, *p*= .209, although the imaged cohort were slightly younger (*Mdn*= 13.42) than the larger non-imaged group (*Mdn*= 16.67). A Mann-Whitney U test was performed to explore whether there were any differences between the two groups in their MSEL subscales' AE scores. The results showed no significant differences between the two groups in any of the MSEL subscales (Table 4.4). That is, although having lower MSEL AE scores, the imaged sample appears to be representative of a larger age-matched UK DS cohort.

Table 4.4 *Mann Whitney U results for differences in MSEL scales between study sample and age-matched DS control subsample*.

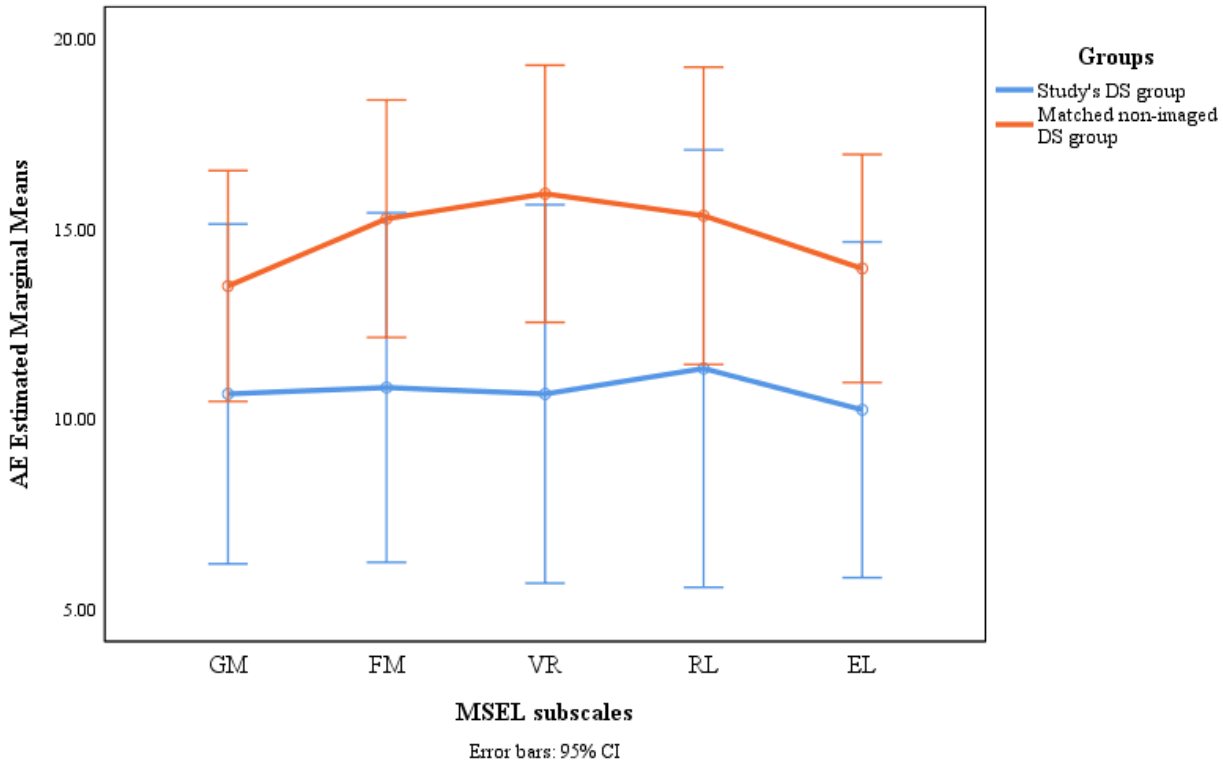
MSEL scale	Group (*n*)	*Mdn*	*U*	*z*	*p*
GrM	DS sample (12)	8.00	115.00	-1.29	.196
	Control DS (26)	11.00			
FM	DS sample (12)	9.00	99.50	-1.78	.075
	Control DS (26)	13.00			
VR	DS sample (12)	8.5	104.50	-1.62	.105
	Control DS (26)	14.00			
RL	DS sample (12)	10.50	124.50	-.99	.321
	Control DS (26)	10.50			
EL	DS sample (12)	8.50	114.00	-1.33	.185
	Control DS (26)	12.00			

Whilst some of the DS participants consistently had scores below the DS cohort average in all the subscales (e.g., DS12), the findings were heterogenous. For instance,

case DS9 had a score slightly above average in the GrM subscale, but below average in the rest, especially in the RL subscale. Similarly, case DS3 had scores above average for both motor subscales and VR subscale, but average scores for RL and below average scores for EL. These patterns reflect the uneven cognitive profile present in the population with DS, and the individual variability in the pattern (i.e., not all children with DS will have higher scores in one specific domain and lower in others).

To test whether the MSEL profile in this study's sample significantly differed to the profile of the UK recruited non-imaged DS cohort of equivalent age, a two-way 5x2 mixed ANOVA was performed, with MSEL subscales as the within-subject factor and group (DS cases and age-matched non-imaged DS infants) as the between-subject factor. Mauchly's test showed that sphericity was violated, χ^2 (9)= 26.60, p= .002, and since Greenhouse-Geisser ε= 0.79, Huynh-Feldt corrected results will be reported. The result showed that there was not a significant main effect of MSEL scales, F(4,144)= 1.35, p= .257, η_p^2 = .036 (Huynh-Feldt). This suggests the MSEL cognitive profile was not uneven. The main effect of groups was not significant either, F(1,36)= 2.20, p= .147, η_p^2= .058, which suggest that this study's sample's cognitive performance was similar to that of an age-matched UK DS sample. Finally, the cognitive profile did not differ between the two groups as indicated by the MSEL scales and group interaction, F(4,144)= .68, p= .591, η_p^2= .019 (Huynh-Feldt) (Figure 4.4).

Figure 4.4 *MSEL cross-sectional cognitive profile for the study sample and age-matched non-imaged DS group.*



4.3.2 Correlations between neonatal brain volumes and outcome in TD

The TD sample (*n*= 488) from dHCP against which the DS brain volumes were to be compared also had neurodevelopmental follow-up assessments carried out at 18-months of age, using the BSID III. To explore the type of effect sizes that could be expected when looking at neonatal brain volumes and neurodevelopmental outcomes in toddlers, I performed a correlation analysis with these data[9]. Based on previous evidence, it was expected that there would be medium to large (Cohen's d~ 0.60-1.0) effect sizes.

[9] The data used to conduct this analysis were acquired as part of the dHCP, to which I was given access. To the best of my knowledge, I have been the first person to run these correlations.

Table 4.5 *Partial correlation results showing relationship between neonatal brain volumes and BSID III composite scores in a TD sample, controlling for sex and age at scan.*

	ICV	**TBV**	**TTV**	**Cerebellum**
Coefficient	r_s	r_s	r_s	r_s
BSID Motor	-.038	-.028	-.022	-.089*
BSID Language	.072	.069	.072	.056
BSID Cognition	.048	.054	.057	.018

Note. ICV= Intracranial volume; TBV= Total brain volume; TTV= Total tissue volumes
n= 488; df= 484
** $p<0.05$*

Partial correlations were performed to explore the relationship between BSID III motor, language and cognitive composite scores, and neonatal brain volume, controlling for sex and PMA at scan. The following neonatal brain volumes were included in the analysis: intracranial volume (ICV), total brain volume (TBV), total tissue volume (TTV), and cerebellum. The results showed a small significant negative correlation between BSID motor composite score and cerebellar volumes ($r_s(484)= -.089$, $p= 0.049$), but this was no longer significant after Bonferroni correction for multiple comparisons (corrected for twelve multiple comparisons). As shown in Table 4.5, none of the other correlations were significant. Contrary to what was expected, the effect sizes were all small (Cohen's $d< 0.2$).

In a large TD sample, then, variations in gross brain structure volumes did not associate with variation in BSID composite scores. This was then considered in our DS cohort.

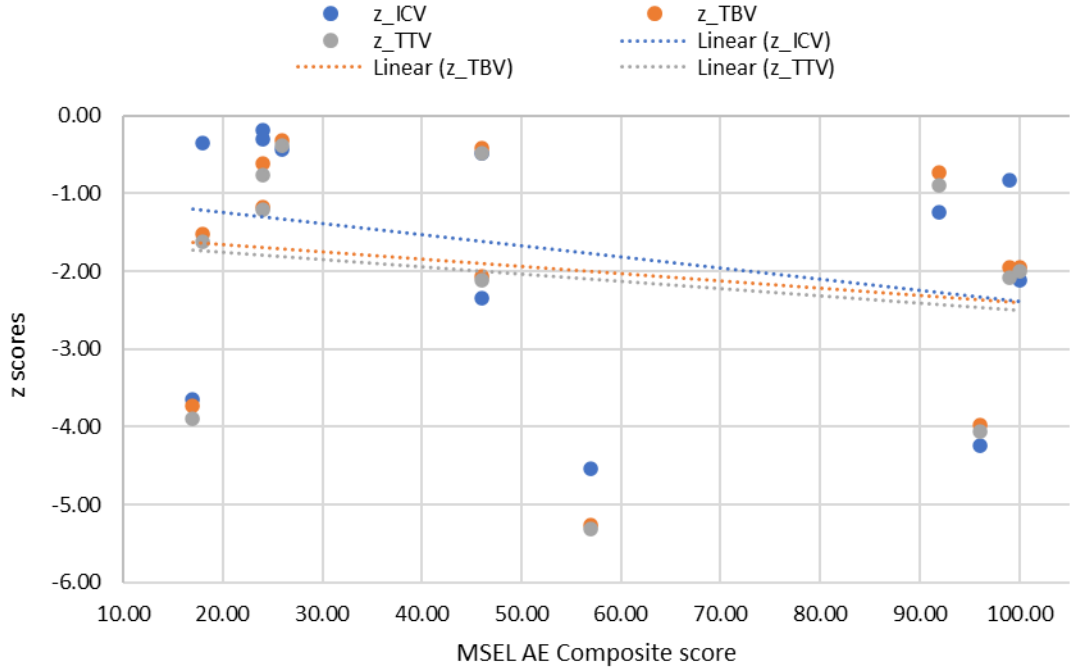
4.3.3 Correlations between brain volumes and outcome in the DS sample

Correlation between brain volumes and MSEL scores

Total brain volumes

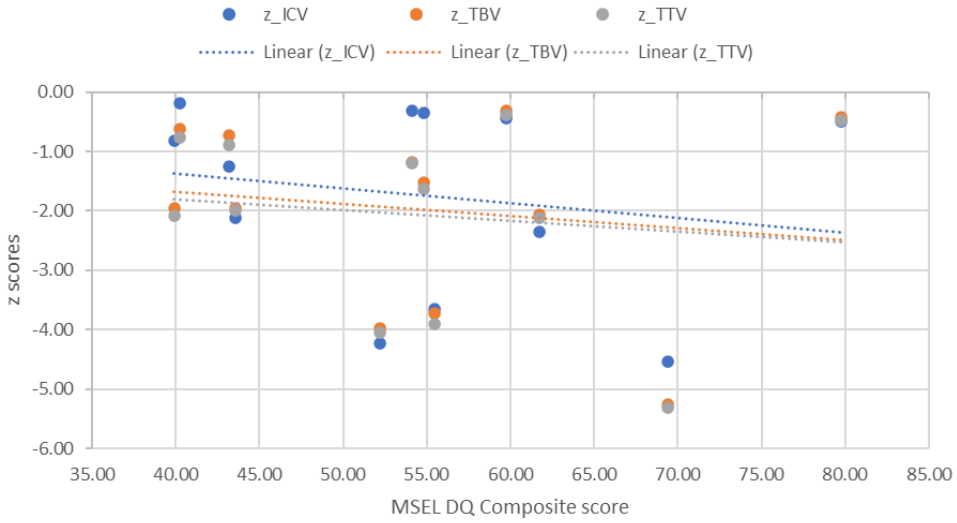
A Spearman correlation was performed to analyse the relationship between overall brain volume z scores (ICV, TBV and TTV) and the MSEL composite scores. The results showed no significant correlation between the composite score and any of the brain volumes (ICV r_s= -.432, p= 0.161; TBV, r_s= -.242, p= .448; TTV r_s= -.235, p= .462), but there was a large effect size between ICV (d= 0.96) and outcome composite MSEL scores. Moreover, the correlations were all negative, suggesting that the higher the deviation from the norm (i.e., the smaller the brain volume) the higher the composite score, which is not what was expected. Figure 4.5 shows the scatterplots for the correlations between the three brain volumes and MSEL AE composite scores. The large spread of the data suggests that the current results might be driven by noise, and not a true effect. Follow up Bayesian analysis showed weak evidence for the alternate hypothesis (ICV BF_{10} = 0.529; TBV, BF_{10} = 0.424; TTV BF_{10} = 0.424).

Figure 4.5 *Scatterplot showing relationship between overall brain volume (ICV, TBV and TTV) z scores and MSEL AE composite score.*



A Spearman correlation was also performed to analyse the relationship between overall brain volume z scores (ICV, TBV and TTV) and the MSEL DQ composite scores. The results showed no significant correlation between the composite score and any of the brain volumes (ICV r_s= -.280, p= 0.379; TBV, r_s= -.084, p= .795; TTV r_s= -.063, p= .846), and there was a moderate effect size between ICV (d=0.583) and MSEL DQ composite score. Like MSEL AE composite scores, and as shown in Figure 4.6, all correlations were negative, suggesting higher DQ composite scores when the overall brain volumes were further below the norm. The spread of the data shown in the figure might indicate the results are due to noise, rather than a true effect. Bayesian correlations also confirmed weak evidence in favour correlations between the MSEL DQ composite score and all three overall brain volume z scores (ICV BF_{10} = 0.415; TBV, BF_{10} = 0.395; TTV BF_{10} = 0.386).

Figure 4.6 *Scatterplot showing relationship between overall brain volume (ICV, TBV and TTV) z scores and MSEL DQ composite score.*



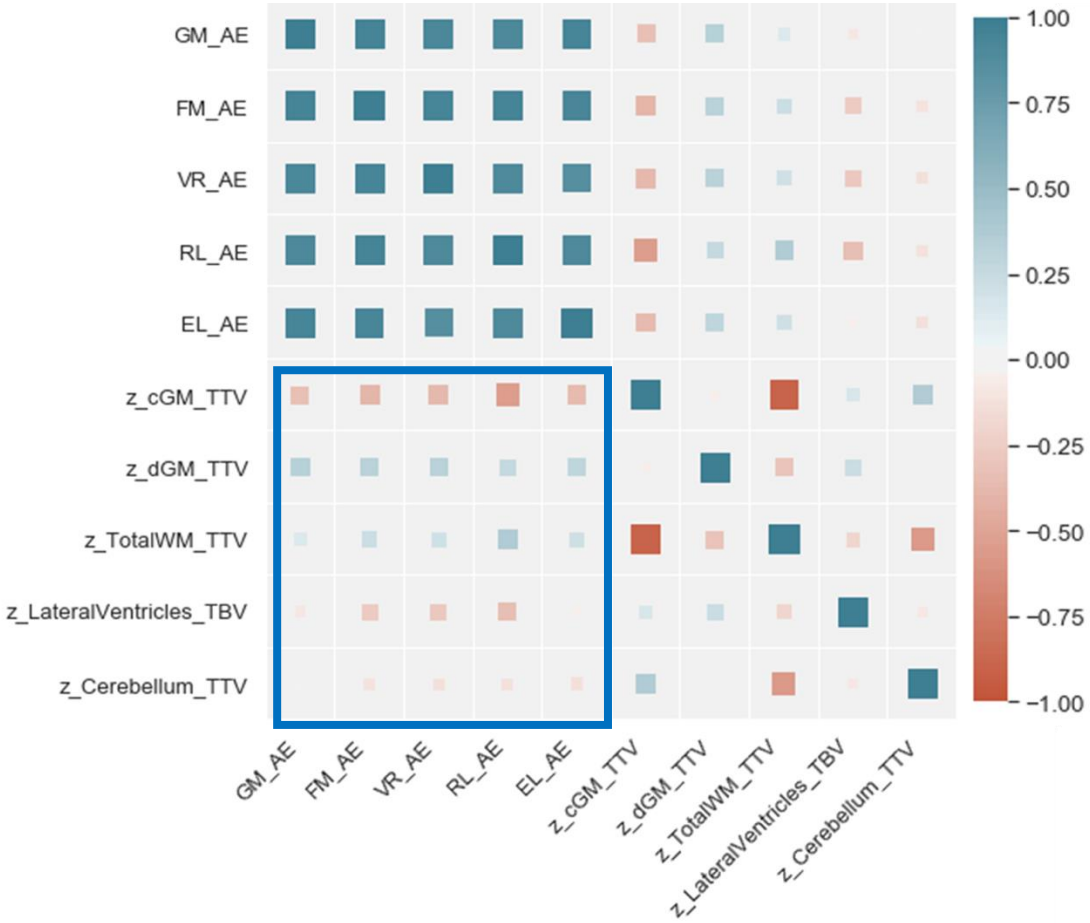
Relative regional brain volumes

A Spearman correlation was also performed to explore the relationship between regional brain volumes and MSEL subscale AE scores. No correlation was significant, but the effect sizes were between small and medium. Figure 4.7 shows a correlation matrix heatmap, where the size of squares represents the correlation size between variables (i.e., bigger square, stronger correlation) and the colour represents direction of correlation (i.e., green for positive correlations and red for negative ones). The correlations between relative cGM, dGM, Total WM, Lateral ventricles, and Cerebellar volumes, and MSEL subscale scores are highlighted by the blue square.

As shown in Figure 4.7, there were medium to large effect sizes between Receptive language and cGM (r_s= -.573), total WM (r_s= .343) and lateral ventricle (r_s= -.420) volumes. There were also medium effect sizes between Fine motor scores and cGM (r_s= -.425) and lateral ventricles (r_s= -.407), and between Visual Reception scores and cGM (r_s= -.376) and lateral ventricle (r_s= -.383) volumes. Expressive Language was only

mildly correlated with cGM (r_s= -.361) and Gross motor scores with dGM (r_s= .346). The correlation between cerebellar volume and MSEL subscales were the weakest, and most likely driven by noise. The directions of the correlations were as expected for dGM total WM (larger volumes relate to higher scores) and lateral ventricles (larger volumes related to lower scores), but not for cGM (larger volumes relate to lower scores).

Figure 4.7 *Correlation matrix heatmap showing the relationship between MSEL subscale AE scores and regional relative brain volume z-scores. Spearman's rho coefficients represented, where larger squares represent larger correlation coefficients.*



Bayesian correlations confirmed weak evidence for a relationship between any of the regional brain volume z scores, and MSEL subscale AE scores (see Table 4.6).

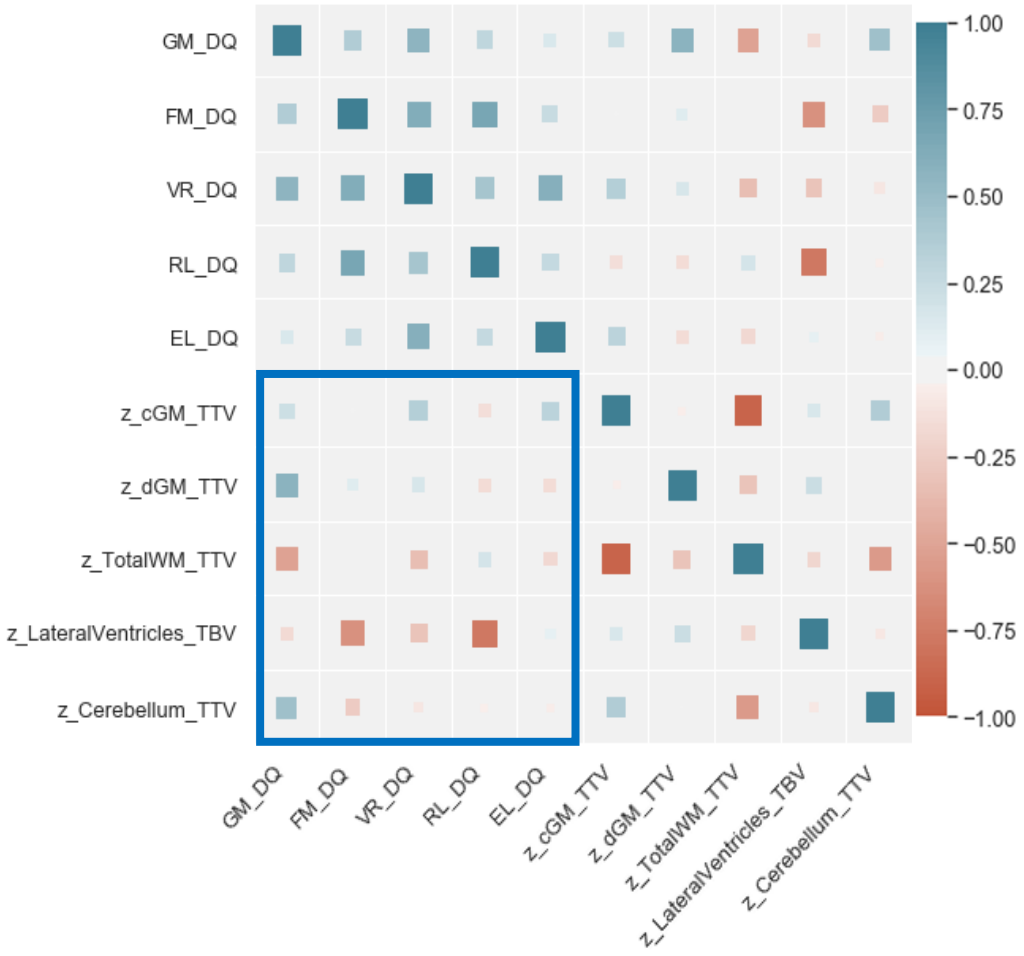
Table 4.6 *Bayes factors for correlations between MSEL subscale AE scores and relative regional brain volume z scores.*

	GrM	**FM**	**VR**	**RL**	**EL**
Bayes factor	BF_{10}	BF_{10}	BF_{10}	BF_{10}	BF_{10}
cGM	0.582	0.731	0.670	1.540	0.644
dGM	0.605	0.579	0.584	0.498	0.534
Total WM	0.385	0.451	0.434	0.684	0.438
Lateral Ventricles	0.367	0.477	0.495	0.604	0.356
Cerebellum	0.356	0.373	0.378	0.375	0.380

Note. cGM= Cortical grey matter; dGM= deep grey matter; EL= Expressive language; FM= Fine motor; GrM= Gross motor; RL= Receptive language; VR= Visual reception; WM= White matter.

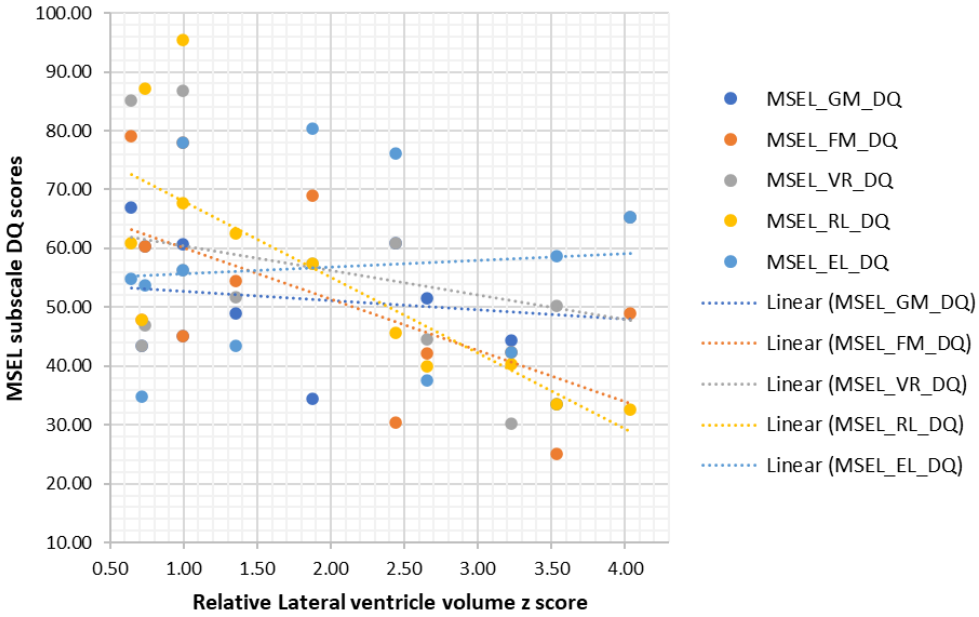
A Spearman correlation was also performed to explore the relationship between relative regional brain volumes and MSEL subscale DQ scores. The results showed several significant correlations between MSLE subscale DQ scores and several regional brain volume z scores before multiple comparison correction. Gross Motor DQ scores were positive correlated to dGM (r_s= .633, p= .027) and negatively correlated to Total WM (r_s= -.629., p=.028) relative volume z scores. Fine motor DQ scores were negatively correlated to relative lateral ventricle z scores (r_s= -.641., p= .025), and lateral ventricle z score were also negative correlated to Receptive Language DQ scores (r_s= -.788, p= .002). Finally, Visual Reception DQ scores were positively correlated to relative cGM z scores (r_s= .657., p= .020). As shown in Figure 4.8, there were also medium to large effect sizes between Gross Motor and Cerebellum (r_s= .348) and cGM (r_s= .566), Visual Reception and Total WM (r_s= -.343), and Expressive Language and cGM (r_s= .427).

Figure 4.8 *Correlation matrix heatmap showing the relationship between MSEL subscale DQ scores and regional relative brain volume z-scores. Spearman's rho coefficients represented, where larger squares represent larger correlation coefficients.*



After Bonferroni correction, only the correlation between Receptive Language DQ scores and relative lateral ventricle z score volume remained significant remain (r_s= -.788, p= .002). As shown in Figure 4.9, this was a negative correlation, meaning that the higher the volumetric z score (i.e., the further away from the norm, the larger the ventricles) the lower the Receptive Language DQ (i.e., the bigger the delay).

Figure 4.9 *Scatterplot showing relationship between lateral ventricle volume z scores and MSEL subscale DQ scores.*



These findings were further supported by the results of the Bayesian correlation (see Table 4.7), which showed weak for the alternative hypothesis for all the correlations, except for the relationship between Receptive Language and lateral ventricle volumes, for which there was strong evidence for the alternative hypothesis (BF_{10}= 18.28).

Table 4.7 *Bayes factors for correlations between MSEL subscale DQ scores and relative regional brain volume z scores.*

	GrM	**FM**	**VR**	**RL**	**EL**
Bayes factor	BF_{10}	BF_{10}	BF_{10}	BF_{10}	BF_{10}
cGM	0.448	0.356	0.635	0.387	0.556
dGM	2.328	0.377	0.411	0.393	0.391
Total WM	1.395	0.354	0.610	0.414	0.409
Lateral Ventricles	0.401	2.713	0.556	**18.280**	0.364
Cerebellum	1.077	0.479	0.367	0.356	0.359

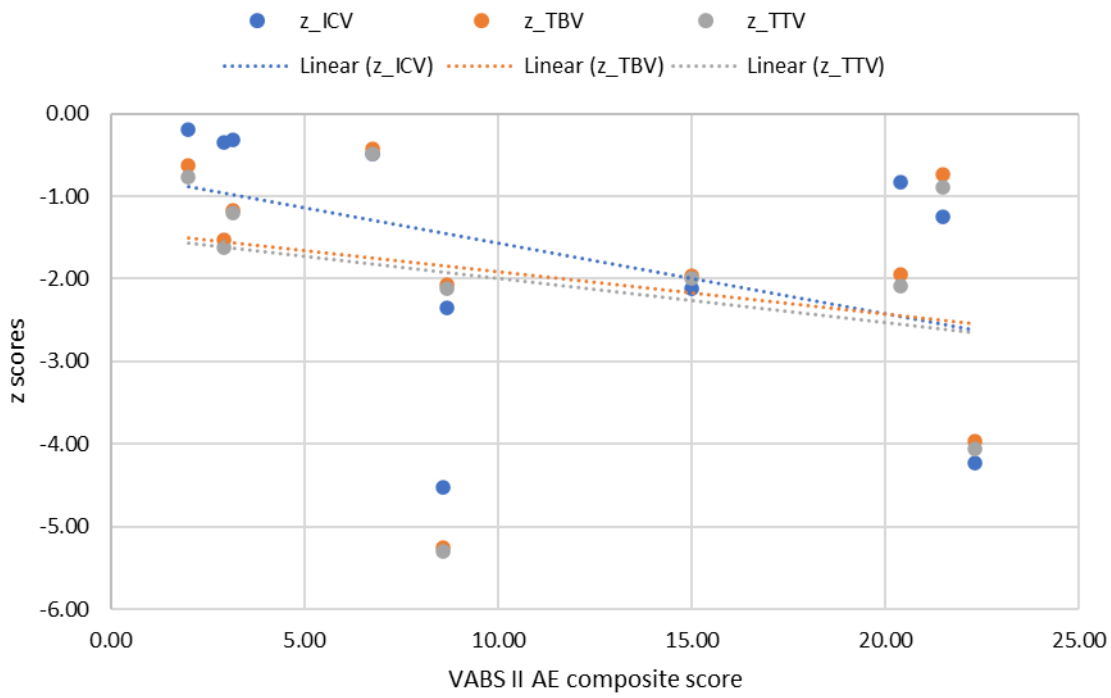
Note. cGM= Cortical grey matter; dGM= deep grey matter; EL= Expressive language; FM= Fine motor; GrM= Gross motor; RL= Receptive language; VR= Visual reception; WM= White matter.

Correlation between brain volumes and Vineland scores

Total brain volumes

A Spearman correlation was performed to analyse the relationship between overall brain volume z scores (ICV, TBV and TTV) and the Vineland composite score. In this case, ICV was significantly negatively correlated with Vineland overall score (r_s= -.697, p= .025). The correlation was no longer significant after Bonferroni correction for multiple comparisons (corrected for three multiple comparisons). There was no significant correlation between the Vineland composite score and the other overall brain volumes, but there were medium effect sizes (TBV r_s= -.455; TTV r_s= -.467). As with the MSEL, all correlations were negative, suggesting higher VABS II AE composite scores predicted by lower brain volumes (i.e., higher negative z scores, suggesting a larger deviation from the normative mean). Figure 4.10 shows the scatterplots for the correlations between the three brain volumes and VABS II AE composite scores. Once again, the large spread of the data points suggests that the current results might be driven by noise, and not a true effect. Follow up Bayesian analysis showed weak evidence for the correlations between overall brain volumes and VABS II AE composite score (ICV, BF_{10} = 0.754; TBV, BF_{10} = 0.497; TTV BF_{10} = 0.510).

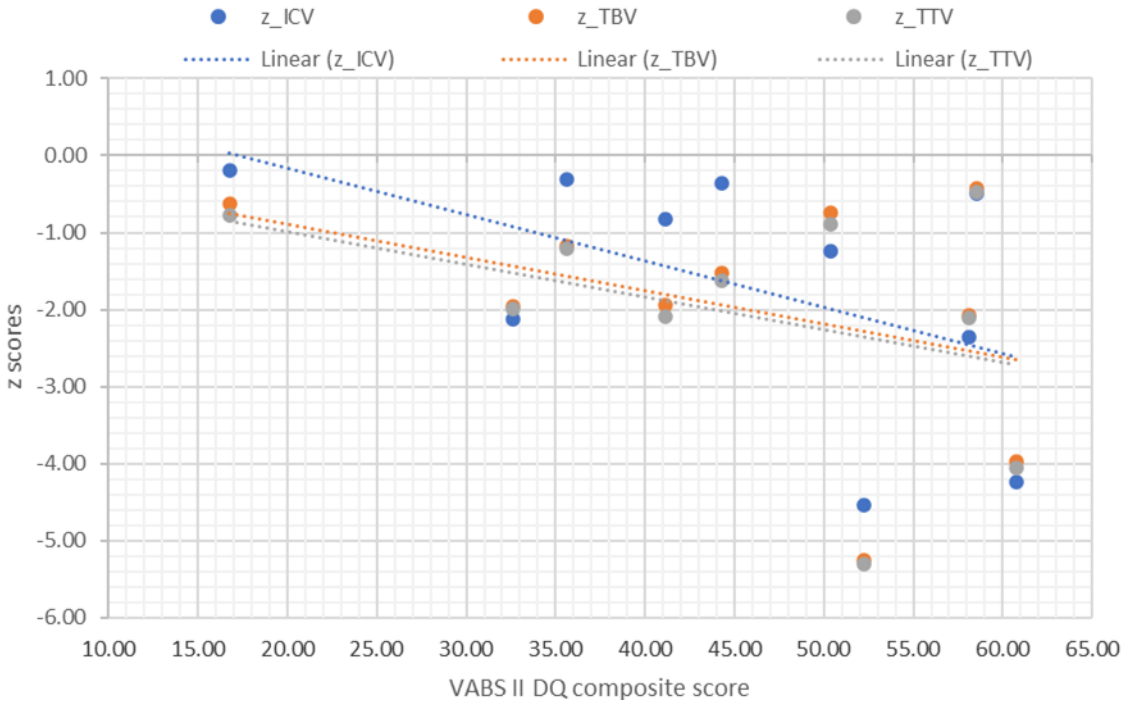
Figure 4.10 *Scatterplot showing relationship between overall brain volume (ICV, TBV and TTV) z scores and VABS II AE composite score.*



A Spearman correlation was also performed to analyse the relationship between overall brain volume z scores (ICV, TBV and TTV) and the Vineland DQ composite score. As opposed to VABS II AE composite score, there were no significant correlations between VABS II DQ composite score and any of the overall brain volumes (ICV r_s= -.600, p= .067; TBV, r_s= -.309, p= .385; TTV r_s= -.333, p= .347). And although the effect sizes ranged from medium to large (ICV d= 1.572; TBV d=.650; TTV, d=.706), the follow up Bayesian correlations confirmed there was weak evidence in favour of any of the alternate hypotheses (ICV, BF_{10}= 1.103; TBV, BF_{10}= 0.666; TTV, BF_{10}= 0.656). As with all overall brain volume correlations, the there was a negative relationship between overall brain volumes and VABS II DQ composite score, meaning that higher scores were related to smaller brain volumes, or a bigger negative deviation from the norm. However, the data pattern shown in Figure 4.11, more precisely the spread of the data, as well as the Bayesian

correlation results, suggests that this negative pattern might be driven by noise, rather than by a true effect.

Figure 4.11 *Scatterplot showing relationship between overall brain volume (ICV, TBV and TTV) z scores and VABS II DQ composite score.*

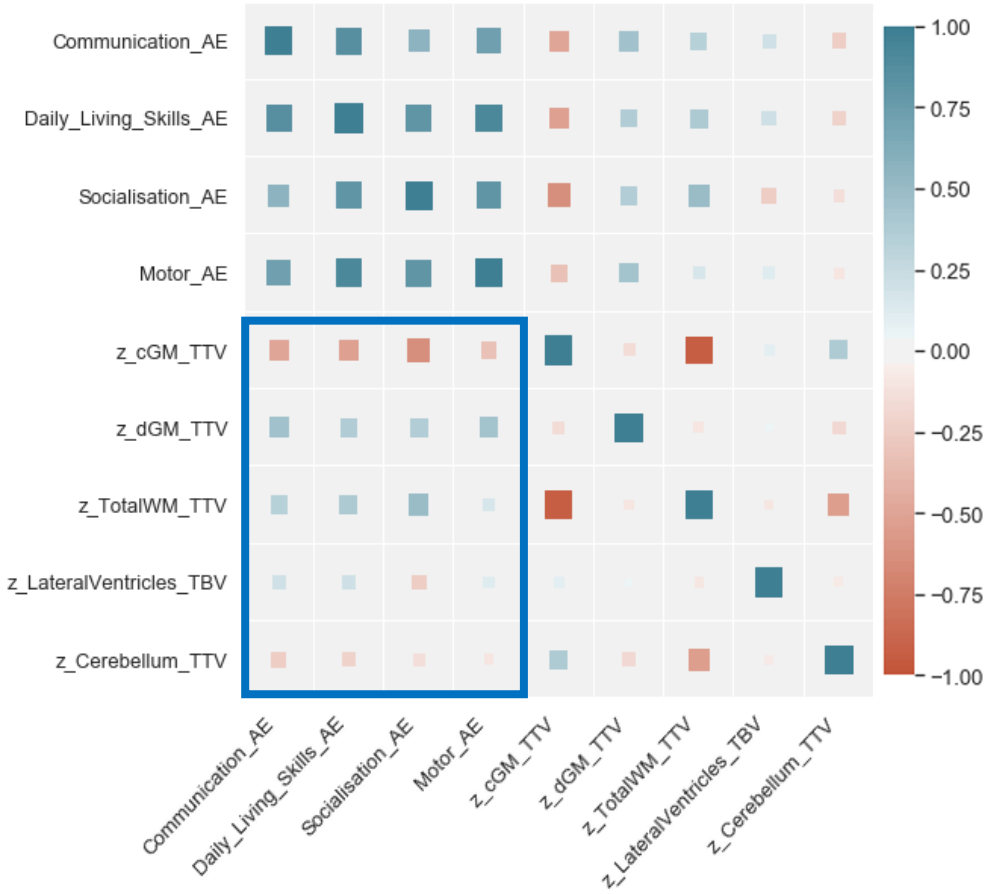


Relative regional brain volumes

A Spearman correlation was also performed to explore the relationship between regional brain volumes and Vineland domain AE scores. The results showed significant correlations between cGM and Communication (r_s= -.636, p= .048), and Socialisation (r_s= -.721, p= .019) domain AE scores before multiple comparison correction. No other correlations were significant before correction. Regarding effect sizes, there were medium to large effect sizes between cGM and Daily living skills (r_s= -.610) and Motor (r_s= -.467) domains (Figure 4.12). There were also medium effect sizes between dGM and all the Vineland domain AE scores (Communication, r_s= .418; Daily living skills, r_s= .305;

Socialisation, r_s= .321; Motor, r_s=.467). The effect sizes for all the other correlations were small (Figure 4.12).

Figure 4.12 *Correlation matrix heatmap showing the relationship between Vineland domain AE scores and regional relative brain volume z-scores. Spearman's rho coefficients represented, where larger squares represent larger correlation coefficients.*



Bayesian correlations confirmed weak evidence for a relationship between any of the regional brain volume z scores, and VABS II domain AE scores (see Table 4.8).

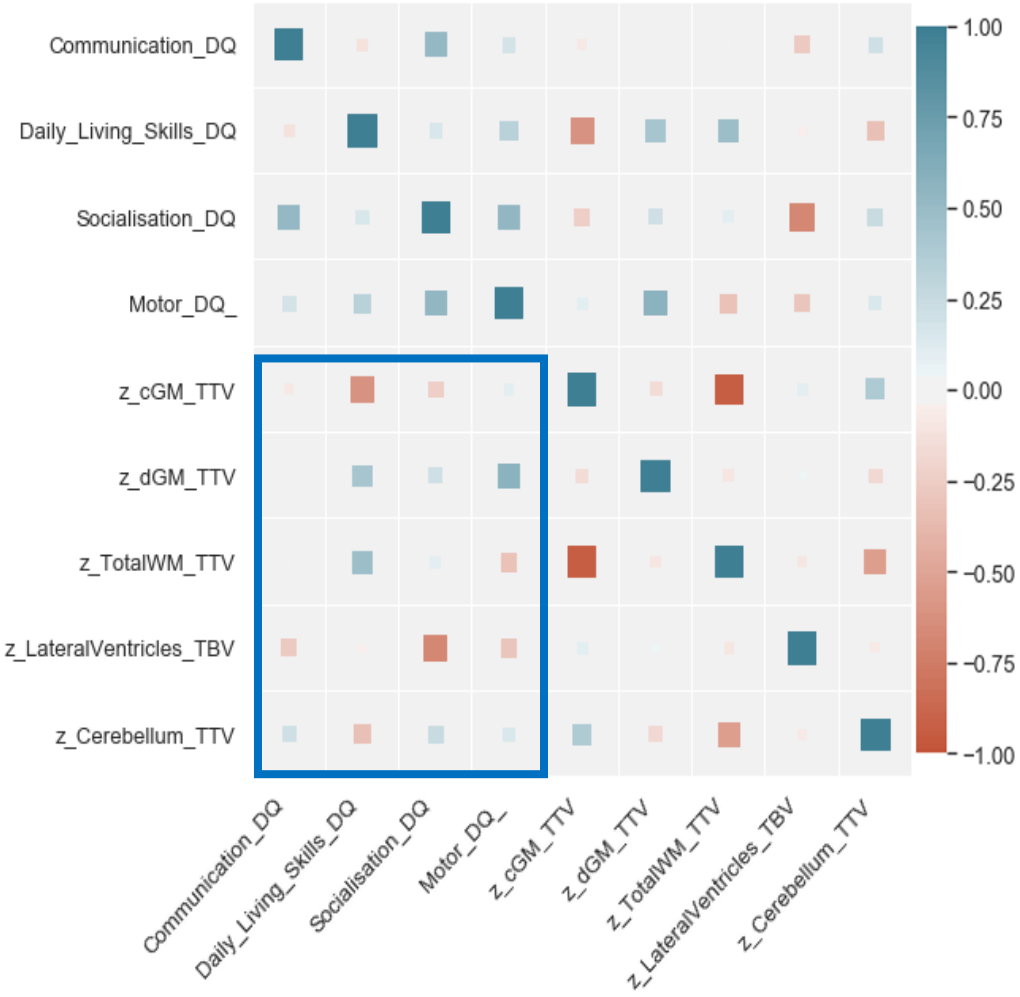
Table 4.8 *Bayes factors for correlations between Vineland domain AE scores and relative regional brain volume z scores.*

	Comm	**DLS**	**Soc**	**Motor**
Bayes factor	BF_{10}	BF_{10}	BF_{10}	BF_{10}
cGM	0.984	1.119	2.173	0.553
dGM	0.861	0.643	0.625	0.819
Total WM	0.591	0.690	1.047	0.427
Lateral Ventricles	0.450	0.457	0.482	0.408
Cerebellum	0.478	0.449	0.415	0.400

Note. cGM= cortical grey matter; Comm= Communication domain score; dGM= deep grey matter; DLS= Daily Living Skills domain score; Soc= Socialisation domain score; WM= White matter.

A Spearman correlation was also performed to explore the relationship between regional brain volumes and Vineland domain DQ scores. There was a significant negative correlation between relative cGM volumes and Daily living skills domain DQ scores (r_s= -.644, p= .044) before multiple comparison correction. No other correlations were significant before correction. As seen in Figure 4.13, there were medium to large effect sizes between Motor domain and dGM (r_s=.568), Total WM (r_s=.-.463), and lateral ventricle (r_s= -.370), as well as between Socialisation domain and lateral ventricle volumes (r_s= -.553) and finally, between Daily living skills domain and dGM (r_s= .337) and Total WM (r_s= .301) volumes.

Figure 4.13 *Correlation matrix heatmap showing the relationship between Vineland domain DQ scores and regional relative brain volume z-scores. Spearman's rho coefficients represented, where larger squares represent larger correlation coefficients.*



Follow up Bayesian correlation analysis confirmed that there was weak evidence for a relationship between all VABS II domain DQ scores and all relative regional brain volumes, expect for one (see Table 4.9). In fact, the Bayesian analysis showed moderate evidence for the relationship between Socialisation domain DQ scores and lateral ventricles volumes (BF_{10}= 3.14).

Table 4.9 *Bayes factors for correlations between Vineland domain DQ scores and relative regional brain volume z scores.*

	Comm	**DLS**	**Soc**	**Motor**
Bayes factor	BF_{10}	BF_{10}	BF_{10}	BF_{10}
cGM	0.396	1.693	0.457	0.414
dGM	0.388	0.763	0.453	1.506
Total WM	0.387	0.954	0.398	0.582
Lateral Ventricles	0.492	0.389	**3.141**	0.542
Cerebellum	0.451	0.574	0.485	0.410

Note. cGM= cortical grey matter; Comm= Communication domain score; dGM= deep grey matter; DLS= Daily Living Skills domain score; Soc= Socialisation domain score; WM= White matter.

On the whole, correlations between Vineland scores and brain measures were stronger than those for MSEL scores. Besides, overall intracranial volume was the strongest predictor of adaptive functions and cognitive abilities, and the strongest regional associations were for relative cortical and deep grey matter, though these associations were in opposite directions (i.e., negative for cortical grey matter and positive for deep grey matter). However, results from Bayesian correlations suggest there is currently not enough evidence in favour for any of the alternative hypotheses. Regarding DQ scores, although there was no difference in findings for overall brain volumes when compared to AE score analysis, it seems that neonatal lateral ventricle relative volumes might be good predictors for MSEL Receptive language DQ scores later in development and potential VABS II Socialisation domain scores.

4.3.4 Case studies

Four of the 12 participants in the sample had a second neurodevelopmental assessment 18 to 36 months after the first one, enabling developmental trajectories to be considered. Table 4.10 shows clinical and demographic information for the case studies. Their AE MSEL scores were used to create individual developmental trajectories. To contextualise the results for the four children, their MSEL developmental trajectories were

plotted against the larger sample of infants and children with DS and TD controls tested as part of the LonDownS cohort (D'Souza et al., 2021) and their volumetric data against the larger cohort of neonates with DS reported in Fukami-Gartner et al. (2021) and controls. The relationships between the individual developmental MSEL trajectories and the relative position of each child's brain volumes were explored.

Table 4.10 *Demographic and clinical details for case studies who had two behavioural assessments.*

	DS1	**DS2**	**DS4**	**DS5**
Sex	Male	Female	Male	Female
GA at birth	38+3	37+5	36+4	36+3
GA at scan	41+6	43+3	39+1	43+0
Days between birth and scan	24	40	18	47
Age at visit 1 (months)	6.13	11.53	11.93	14.90
Age at visit 2 (months)	42.8	35.87	29.90	32.40
Months between assessments	36.67	24.34	17.97	17.5
Birth weight (kg)	2.25	2.25	2.58	2.58
Weight at scan (kg)	2.6	3.16	3.19	3.31
CHD	AVSD	PDA and PFO	none	AVSD
Other conditions	none	none	Duodenal atresia	none
APGAR scores (1 minute/5 minute)	9/9	NR	9/10	7/6
Ventilation after birth	Yes	Yes	Yes	Yes

Note. CHD= Congenital heart defect; AVSD= Atrioventricular Septal Defect; NR= not reported; PFO= Patent foramen ovale; PDA= Patent ductus arteriosus; APGAR= Appearance, Pulse, Grimace, Activity, Respiration.

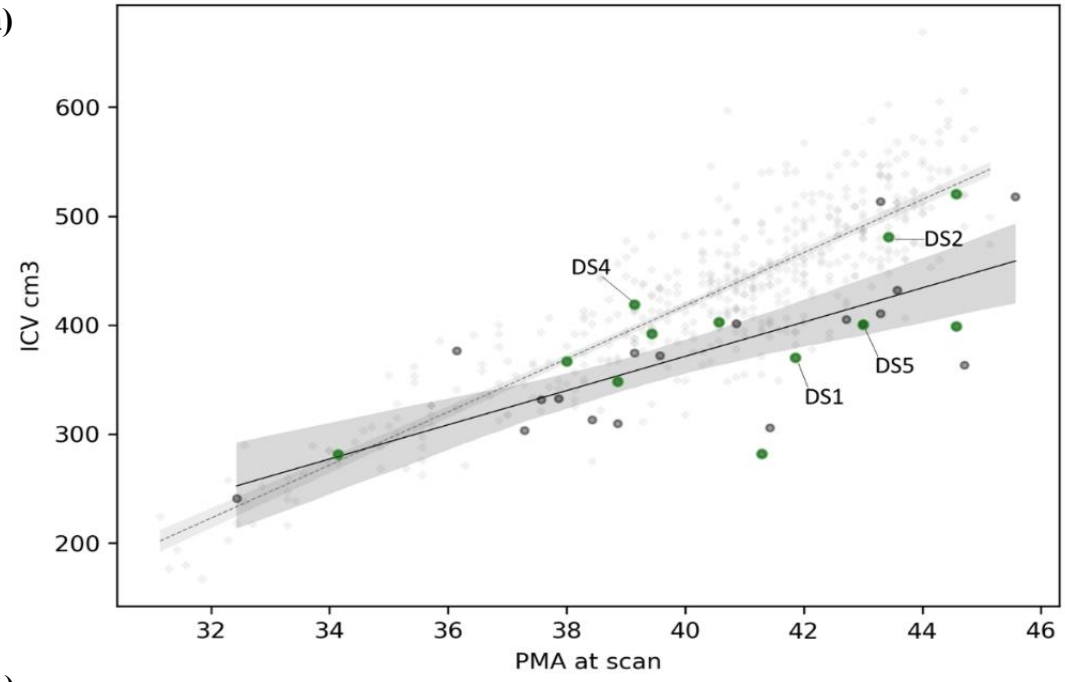
Figure 4.14 shows (a) the position of each case's ICV relative to the larger imaged DS and TD cohorts, and (b) individual MSEL composite score developmental trajectories for each of the case studies. Cases DS2 and DS4 both have ICV measures that are not only above the DS cohort average, but also within the TD cohort range. Similarly, the slopes for both of their developmental trajectories seem steeper than for the other two cases, and slightly steeper than the DS cross-sectional developmental trajectory. A linear regression equation predicting MSEL AE composite scores from CA was generated from the DS and the TD cohorts' cross-sectional developmental trajectories (see Table 4.3) to explore this

further, and the slope for each case's trajectory was calculated. Case DS2's and DS4's AE composite trajectories' slopes (β_1= 2.51 and β_1= 2.34, respectively) were indeed higher than the DS cross-sectional trajectory's slope (β_1= 2.23), and close or above of the 95% CI's upper bound [2.02-2.45]. This suggests that these two cases had a developmental rate faster than the average DS child. On the contrary, cases DS1 and DS5's ICV measures are below the DS cohort average, and the same happened with their individual composite AE trajectories' slopes (DS1 β_1= 1.20; DS5 β_1= 1.26), suggesting a slower development of overall cognition.

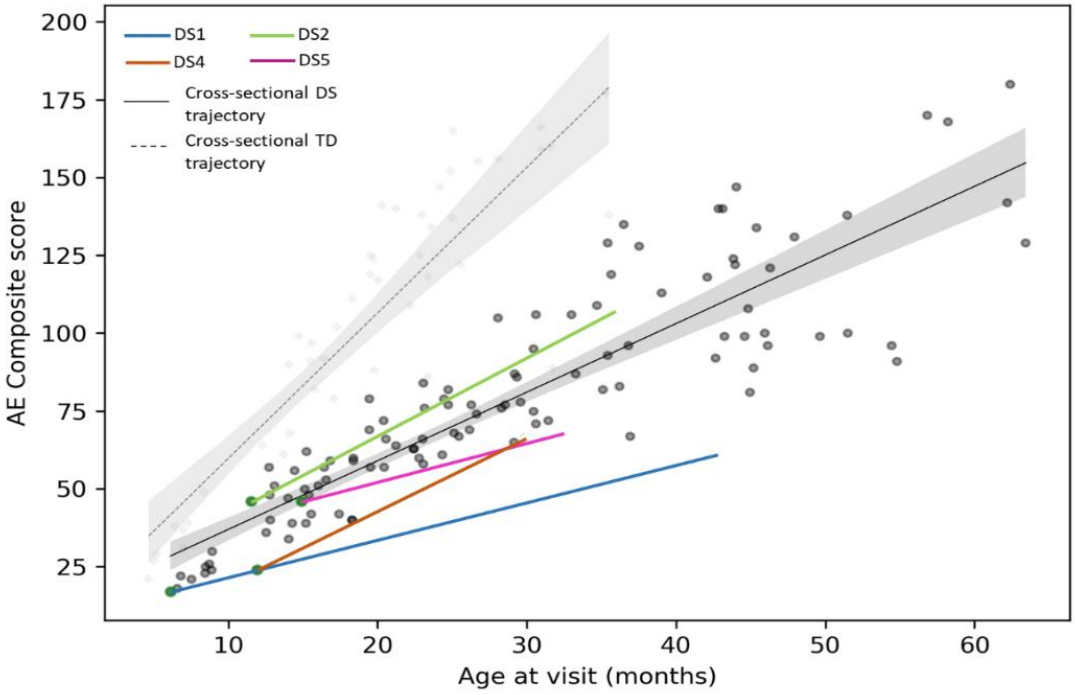
When compared to the TD control group, the slope of the developmental trajectories of all four cases were well below the TD cross-sectional trajectory (β_1= 4.67) and outside the 95% CI [3.94-5.41]. This suggests a gross neuroanatomical measure, intracranial volume, may be associated with differential rates of cognitive and motor development within this, albeit very small, DS cohort.

Figure 4.14 *ICV (a) and MSEL composite score (b) scatterplots for the four case studies. Volumetric data plotted against the larger cohort of neonates with DS (dark grey) and TD controls (light grey) reported in Fukami-Gartner et al. (2021) (PMA at scan is measured in weeks). MSEL data plotted against cross-sectional trajectories created from a larger sample of infants and children with DS and TD controls tested as part of the LonDownS cohort (D'Souza et al., 2021).*

a)



b)

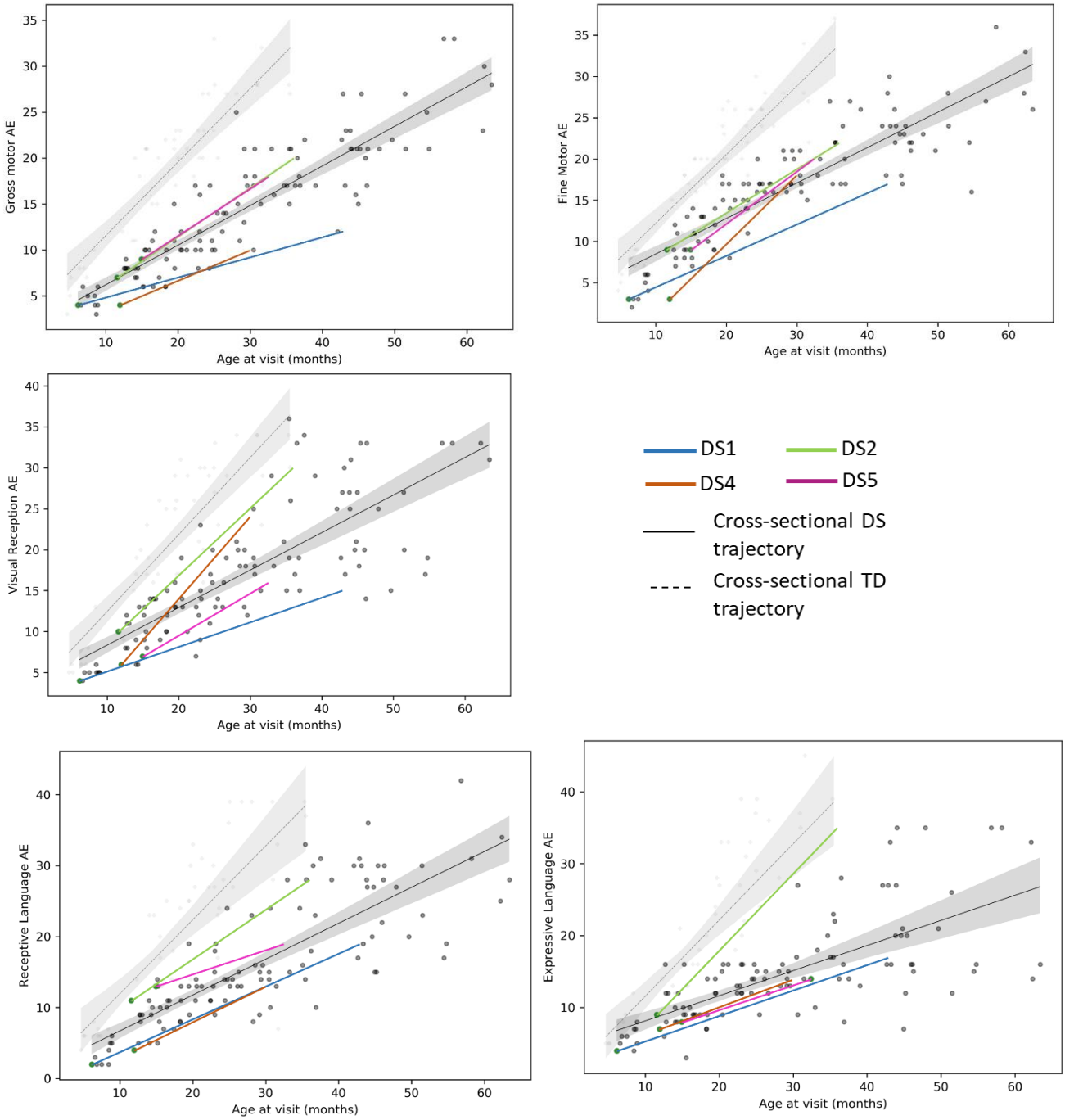


Developmental trajectories for each of the MSEL subscales are presented in Figure 4.15, and statistics extracted from separate linear regression equations predicting each of the 5 MSEL subscale AE scores from CA for the DS and the TD cohorts' cross-sectional developmental trajectories are presented in Table 4.3.

The different slopes of the individual trajectories for the four cases represent different patterns of development and different developmental delays for each of the cases. Moreover, when looking at the development of the five subscales for each of the cases, different patterns emerge.

For instance, case DS2's developmental trajectories are above the cross-sectional DS trajectory in all the subscales (GrM β_1= .53; FM β_1=.53; RL β_1= .69), but the trajectories for Visual reception (β_1= .82) and Expressive language (β_1= 1.07) were steeper than for the rest of the subscales, suggesting a faster development. In fact, DS2's VR and EL trajectory slopes fall within the TD cross-sectional trajectories' slopes 95% CI (VR [.77-1.11] and EL [.81-1.30], suggesting that the development of these cognitive abilities is as expected in TD, but delayed.

Figure 4.15 *Individual developmental trajectories for MSEL subscales for the four participants with two developmental assessments. (DS and TD group data taken from D'Souza et al., 2021).*



A similar pattern appears in DS4, but in this case, Visual Reception (β_1= 1.00) and Fine motor (β_1=.84) development were developing faster than the rest of the cognitive abilities (GrM β_1= .33; RL β_1= .50; EL β_1= .39). In fact, although this child's VR and FM scores were below the cohort mean when they were first assessed, they were above the DS cohort average 18 months later. Case DS1 seems to have a similar developmental trajectory in all the subscales (FM β_1= .38; RL β_1= .46; ELβ_1= .36), but when compared to the cross-sectional DS developmental trajectory, this child's development in Gross motor (β_1=. 22) and Visual reception abilities (β_1= .30) appears to be slower, as represented by a trajectory moving away from the cross-sectional one. Finally, for DS5, while the development for most of the subscales was either equal or faster (GrM β_1= .51; FM β_1= .63; VR β_1= .51; EL β_1= .34) than the cross-sectional DS trajectory, Receptive language development was much slower (β_1= .34) when compared to the group's trajectory. In fact, although the child had RL scores above the cohort average in the first and second visits, the developmental trajectory suggest that this child's RL score might eventually end well below the cohort average.

Overall, case DS1 and DS5, whose ICV volumes were below the DS average, showed a developmental pattern expected from DS, albeit DS1 having a slower GrM and VR development and DS5 a slower RL development. Case DS2's neonatal ICV volume was above the DS average, and so were all the outcome developmental trajectories, with the cognitive trajectories being within the expected range for TD. Case DS4, also with an ICV above the DS mean, had a more uneven developmental profile, with language abilities developing as expected from DS, but GrM abilities developing slower, and VR

and FM abilities starting below the mean, and then exponentially developing, so much so that the trajectories fall within the expected range for TD.

These results suggest that ICV differences might not be suitable to predict the more fine-grained differences found in the cognitive development profiles in DS and exploring differences in regional brain volumes might be more appropriate. Figure 4.16 shows a bar graph representing the differences in deviation from the norm (represented by 0 and matched for sex and age at scan for each case) in lateral ventricle, cGM, dGM, total WM and cerebellar relative volumes for the four case studies. Whilst case DS1 shows a more inconsistent pattern of deviation (i.e., lateral ventricle volumes being 4 z-scores above the mean but total WM and cGM being around ±1), case DS5's deviation for all regional brain volumes is within the same range (i.e., ~ ±1), showing a more consistent pattern of deviation. It should also be noted that the direction of the deviation from the norm is inconsistent between the cases, suggesting that the relationship between relative regional brain volumes (e.g., having a proportionally larger cGM is related to having a proportionally larger dGM) is not the same for all individuals.

Figure 4.16 *Bar graph comparing relative volume z scores for regional volumes for the four case studies. The 0 represents the sex and age matched normative TD mean.*

4.4 Discussion

The current study was the first time the relationship between neonatal brain volumes and later cognitive outcomes was explored in DS. After correction for multiple comparisons, no significant correlations were found between overall or regional brain volumes, and any of the AE or DQ outcome measures, except for lateral ventricle volumes and MSEL Receptive Language DQ scores. As seen in Table 4.2, few previous studies conducted with atypical neonatal populations have chosen to focus on ventricular volumes when investigating predictors of later cognitive development (only 2 out of 9 presented studies) and the findings have been mixed. Whilst Bonthrone et al.'s (2021) study found no significant relationship between absolute ventricular volume z score and cognitive or motor outcomes in 18-month-old children with CHDs, Keunen et al. (2016) found that both absolute and relative ventricular volumes were significantly related to cognitive, fine motor and gross motor outcomes in preterm born children.

Nevertheless, findings from studies focused on investigating the impact of ventriculomegaly in later cognitive outcome have been more consistent. More precisely, in line with the current results, perinatal enlarged ventricles have been found to be, specifically, related to delays in language development (Fox et al., 2014; Lockwood Estrin et al., 2016). One of the theories behind this relationship highlights the possibility that the enlargement of the lateral ventricles might lead to the atypical development of WM tracts involved in language and cognition (Lockwood Estrin et al., 2016), like the superior and inferior longitudinal fasciculi or the corpus callosum, which had been found to have atypical DTI measures in neonates with DS (see Chapter 3 for more details). The results from Bayesian analysis also showed moderate evidence in favour of a relationship

between neonatal lateral ventricle volumes and VABS II Socialisation domain DQ scores. This, however, should be further investigated, as previous literature has consistently found no relationship between perinatal enlarged ventricles and childhood VABS scores (e.g., Bar-Yosef et al., 2017; Meyer et al., 2018).

The lack of any other significant results or evidence for any of the alternative hypotheses might be due to the study being under powered, and correlation analysis not being robust to outliers, as there were some medium to large effect sizes. More precisely, there were medium effect sizes in the correlations between intracranial brain volume and relative cortical GM (corrected for TTV), and MSEL and Vineland composite scores and subscales/domains. These results are consistent with previous literature, as presented in Table 4.2, where cGM (*d*= .27-.87) and TBVs (*d*= .25-.87) have been previously found to be predictive of cognitive outcomes in other atypical populations (e.g., Hansen-Pupp et al., 2013; Kelly et al., 2020; Keunen et al., 2016).

These previous studies have all focused on assessing brain-cognition relations, potentially assuming that static snapshots of cognitive abilities are representative of cognitive development in the population under investigation. However, as shown by the presented case studies, and as found in previous literature (e.g., D'Souza et al., 2021) there is a wide variability in the developmental trajectories of cognitive abilities between infants with DS, and of different cognitive abilities within an individual with DS, and overall brain volumes measures do not appear to capture the complexity of brain-cognition relationships in DS. Perhaps exploring the individual differences in scaling of brain regions (e.g., larger cortex, smaller cerebellum) and how this relates to the different

cognitive developmental trajectories in DS might better capture the complex phenotype in DS.

Another interesting finding in the current study's exploratory analysis was the opposite direction of the cGM and dGM associations to outcomes. The positive association between dGM and outcome (larger dGM related to higher scores) was as expected. In fact, this was also found in Bonthrone et al.'s (2021) study, where dGM was divided into its subregions (i.e., left and right lentiform, caudate nucleus and thalamus), which were found to be significantly positively related to Bayley III cognitive composite scores. It should be noted that not all dGM subregions were bilaterally predictive of cognitive outcome. For instance, only the left lentiform nucleus was significantly related to cognition (Bonthrone et al., 2021). This suggests that laterality might be of relevance when exploring dGM-cognition relationships, which has not been considered in the current study.

The negative association between cGM and outcome (larger volumes relate to lower scores), however, was not expected. In fact, most of the previous studies have found a positive associations between cortical volumes and cognitive abilities (Bonthrone et al., 2021; Kelly et al., 2020; Meuwly et al., 2019; Peterson et al., 2003). There has been, however, a previous study where negative associations have been found between neonatal cortical volumes and cognitive outcome in a sample of preterm born toddlers. Keunen et al.'s (2016) study explored the relationship between neonatal brain volumes and Bayley III cognitive, fine motor, and gross motor outcomes at 24 months old in 112 preterm born participants and found that smaller cGM volumes were related to higher Bayley III cognition and GrM scores. The authors visually inspected a posteriori the anatomical MRI

image of participants whose cortex was larger and found that their WM-cGM boundary was less defined than in those participants with smaller cortex, which they believed it could be an indication of neuronal migration disruption and possible vestiges of the subplate (Keunen et al., 2016). They then argued that different cellular organisation was likely to influence MRI signal intensities in the grey- white matter border and have an impact in the subsequent image segmentation (i.e., identifying voxels as part of the cortex when they are not). It could be that the same blurred WM-cortex boundary as in Keunen et al. (2016) is present in the current study's sample. In fact, evidence from histological studies shows that there are reduced radial glial progenitor cells in the brain of fetuses with DS, which potentially has an impact in neuronal migration and cortical lamination (Baburamani et al., 2020; Golden & Hyman, 1994).

Cortical volumes larger than in the TD population have also been found in other populations with neurodevelopmental conditions (NDCs), like autistic children (Courchesne et al., 2001; Donovan & Basson, 2017) and ventriculomegaly (enlarged ventricles) (Gilmore et al., 2008; Kyriakopoulou et al., 2014), both of them comorbidities found in the population with DS. The current results and previous literature seem to suggest that the quality and connectivity of the cortical tissue might be more important for typical cognitive development, rather than its volume, and are in line with the neuroconstructivist idea that functional development of brain regions occurs in the context of other regions (Johnson, 2005). The mechanisms involved in cortical volume variability in DS should be further explored in vivo (e.g., if larger ventricles are related to larger cortical volumes) as well as the reverse association between cGM and dGM and outcome. It would also be of interest to further explore the developmental relations between dGM

and cGM to see if a pattern arises (e.g., smaller dGM leads to lager cGM volumes) and what the potential mechanisms behind this could be.

It should also be noted that the results suggest that grey matter, overall, seems to be a stronger predictor than WM. There have been mixed results in the past literature regarding the predictive power of neonatal WM volumes and cognitive outcome. For instance, Bonthrone et al.'s (2021) study also found no significant association between neonatal total WM volumes and Bayley III cognitive and language scores in a sample of toddler with CHD. Conversely, Meuwly et al. (2019) found that neonatal total WM was significantly positively associated to Bayley III cognition composite scores, but not with language composite scores in a sample of toddlers born with a CHD. Previous studies that did find a significant association between neonatal WM volumes and cognitive outcome either focused on regional WM volumes (e.g., frontal lobe, temporal lobe) (Kelly et al., 2020; Meuwly et al., 2019) or differed between myelinated and unmyelinated WM (Hansen-Pupp et al., 2013; Keunen et al., 2016). So, perhaps, total WM is too broad a measure for any cognitive phenotype significance, and exploration of other MRI derived WM properties, such as diffusivity, might lead to a more appropriate predictor of cognitive abilities. Due to the small sample size, this was not possible to do in Chapter 3, however, the presented case studies (see Section 3.3.3) suggest that variability in FA values in neonates with DS do indeed relate to variability in cognitive outcomes, although the specific pattern of this relationship is yet to be determined with a larger sample size.

Contrary to our hypothesis, there was no significant relationship between cerebellar volumes and motor abilities. In fact, out of all the regional volumes, the correlations between cerebellum and outcome measures were some of the weakest, with

small effect sizes. Considering that the cerebellum is one of the regions consistently found to be significantly smaller in DS (Contestabile et al., 2007; Guidi et al., 2011; Haydar & Reeves, 2012; Patkee et al., 2020) and that in previous literature small to large effect sizes have been found between cerebellar volume and cognition and motor abilities (Hansen-Pupp et al., 2013; Keunen et al., 2016; Kooij et al., 2012; Meuwly et al., 2019), the lack of any effect was unexpected. However, the cerebellum is the most architecturally complex region of the CNS (Butts et al., 2014), so maybe gross cerebellar anatomy is not best predictor of cognitive outcome, and cerebro-cerebellar connections (e.g., WM tract microstructure) should be explored instead. Finally, the effect sizes of the correlations were also larger, overall, when relating brain volumes to Vineland scores than to MSEL ones. This might be a reflection of better sensitivity of the Vineland scales' measure than the MSEL when studying NDCs. In fact, the MSEL scores are based on the observations of the researcher within a limited period of time, whereas the scores extracted from Vineland encompass the amalgamation of the parent's knowledge of the child's abilities (Sparrow et al., 2005).

Although the current study sample was small, it seems to be representative of an age-matched sample recruited in the UK. There are, however, some other limitations. For instance, previous studies have found different results when controlling for prematurity in the sample (Bonthrone et al., 2021). Although half of our imaged DS cohort were born prematurely (<37 weeks GA), this was not controlled for in our analysis due to being underpowered. Aside from prematurity, sex, and the presence of a CHD have been previously found to have an effect not only in brain development (Bonthrone et al., 2021; Giedd & Rapoport, 2010; Rose et al., 2009), but also cognitive development (Aoki et al.,

2018). Although sex was controlled for in our brain volumes, this was not the case for either the cognitive outcomes, or the presence of a CHD. Moreover, regarding the brain volume data, although PMA at scan was controlled for when the z-score extraction was done, a posteriori examination of the data showed a relationship between z-scores and PMA at scan in some of the volumes (e.g., ICV), where the older babies had higher z scores (i.e., a larger deviation from the age and sex-matched norm). This could have potentially had an impact in the brain-outcome correlations and should be further investigated. Finally, overall cognitive ability or adaptive functioning was not controlled for when exploring the relationship between brain volumes and specific cognitive abilities or adaptive domains (e.g., FM, language), which should be done to correctly assess individual differences in cognitive profile.

Finally, no significant correlations and small effect sizes were found between brain volumes and outcome in the TD population in the current study, which is in line with some previous studies (e.g., Meuwly et al., 2019). Therefore, it should be considered that brain volumes are not the best level of neuroanatomical description to explain cognition, even though they are a more readily accessed early clinical marker. Future research should focus on exploring how different early brain mechanisms, like brain function or brain diffusivity, might be associated to the cognitive variability in DS. In fact, there is evidence showing that anomalous functional connectivity (Pujol et al. 2015) or inhibitory imbalance (Kleschevnikov et al. 2012, de San Martin et al. 2017) is also part of the DS neurophenotype.

4.4.1 Conclusion

The current study sought to investigate whether brain volumes in neonates with DS could help predict later cognitive performance. A significant negative relationship was found between lateral ventricle volumes and receptive language DQ scores, but the initial data were too few to draw any robust conclusions on any other brain volumes and assessment of a larger cohort may be informative. It is possible that cGM and ICV might be predictors of static measures of cognitive abilities but focus on more detailed regional volume variabilities may be more appropriate when seeking to predict the profile of developmental trajectories. Similarly, parental reports of adaptive skills seem to capture different abilities of infants and children with DS than lab-based observations (e.g., Mattie et al., 2023), and individual longitudinal trajectories of cognitive development may be better outcome measures in the population with DS than single time snapshot measures, as supported by the neuroconstructivist approach (discussed in 2.2.2).

Regardless, the mechanisms by which brain volumes relate to cognitive outcomes should be further investigated, as this may be driven by several factors, such as volumes of cellular bodies, neural synapses, white matter tracts or even extracellular matrix. Further research should also be conducted to assess whether other neural mechanisms, such as functional networks, might be better predictors of cognition in DS.

Chapter 5: Main Discussion

The work presented in the current thesis sought to improve the understanding of early brain alterations in neonates with DS and investigate whether they might be associated to the individual variability found in their cognitive phenotype in the first five years of life.

5.1 Summary of findings

5.1.1 Diffusion MRI study

An important feature of the body of work presented in this thesis was the novel application of diffusion MRI techniques and analyses to assess white matter (WM) microstructure in neonates with DS. To the best of our knowledge, there are no previous studies investigating WM so early in brain development in DS. A study investigating WM alterations in neonates with DS was presented in Chapter 3. The results from the Tract-based spatial statistic (TBSS) analysis showed reduced fractional anisotropy (FA), mean diffusivity (MD), radial diffusivity (RD) and axial diffusivity (AD) in the anterior parts of the brain (e.g., genu of the corpus callosum (CC), anterior internal capsule, anterior commissure, anterior external capsule), the cingulum and the splenium of the CC, and in the cerebral and superior cerebellar peduncles. Similar FA reductions have been reported in children and the aging population with DS (Fenoll et al., 2017; Gunbey et al., 2017; Lee et al., 2020; Powell et al., 2014) suggesting that these are WM alterations already present at birth, and persistent throughout life. The reduction in the other TBSS measures (i.e., MD, AD and RD), however, were inconsistent with previous findings from Romano et al. (2018), who found increased MD, RD and AD in anterior WM regions in a sample of adolescent and adults with DS.

The finding of WM alteration this early in brain development has implications for the theoretical framework that considers dementia as a developmental condition, as individual variability in these alterations could be further investigated to determine whether they could be early biomarkers reflecting risk or protective factors for later Alzheimer's (Karmiloff-Smith et al., 2016; Thomas et al., 2020). The findings also have

implications for the research conducted in the population with DS investigating WM alteration preceding any clinical signs of Alzheimer's, as they highlight the importance of considering the baseline WM differences in the DS population.

A more novel diffusion data analysis approach, that is Fixel-based analysis (FBA), was also used to investigate whether fibre bundle density (FD) and fibre bundle cross-section (FC) differed between neonates with DS and matched controls. The results showed increased FC and fibre density and cross-section (FDC) in the CC, cingulum and anterior limb or the internal capsule. However, there were some anatomically implausible results when FD differences between groups were analysed, and post hoc analysis of the images led to the conclusion that the findings had been affected by an error in the processing pipeline, specifically in image registration. The error seemed to be caused by the underlying anatomical differences between groups, which the used processing pipeline could not overcome. As all the measures were extracted following the same registration process, no reliable inferences could be drawn from any of the current results. Nevertheless, efforts to improve the processing pipeline are underway. More precisely, different approaches to create the study-specific template are being considered, such as utilising the signal from the cerebrospinal fluid (CSF) as well as the WM (i.e., a multi-contrast template), in hopes that the alignment of the images will be more accurate during the registration step (see section 5.2.1 for methodological considerations).

5.1.2 Neonatal volumes and cognitive outcomes study

In Chapter 4 the association between neonatal brain volumes (corrected for age, sex, and overall brain size) and later cognitive performance was investigated in a cohort with DS. To the best of our knowledge, this is the first time these associations have been

explored in DS. Although the small sample size (*n*= 12) limited the ability to draw any robust inferences, the results suggested that, amongst all the investigated brain volumes, cortical grey matter (cGM) and intracranial volume (ICV) might be best at predicting cognitive outcome in DS. However, the use of AE scores as the outcome measure might have distorted the findings, considering the disadvantages of using these scores with populations with NDCs (see Section 2.2.4). This notion was supported by the significant findings found when using an alternative score, that is, developmental quotient (DQ). More precisely, there was a significant strong negative correlation between lateral ventricle volume z scores and MSEL receptive language DQ scores, suggesting that enlarged lateral ventricles might predict language outcomes later in life.

The associations between cGM volumes and outcomes were negative, suggesting that a larger cortical volume may be associated with lower cognitive scores. This reversed correlation might be in line with findings from other neurodevelopmental conditions (NDCs), such as autism, where a larger cortex has been reported when compared to typically developing (TD) controls (Courchesne, 2004; Donovan & Basson, 2017). However, evidence has consistently shown smaller cerebral cortical volumes in DS when compared to the TD population (e.g., Patkee et al., 2020). Therefore, the mechanisms underlying this reverse correlation should be further explored, as it could be that a larger cortical volume within the DS population is a result of differences at the cytoarchitectural level, like in cortical layering organisation, rather than simply containing more cells.

Moreover, contrary to predictions, cerebellar volumes were not related to outcome measures. Even though the study was potentially underpowered to detect significant effects, the effect sizes of the correlations between cerebellar volumes and outcome

measures were still the smallest effects, so it might be unlikely any significant associations will show in a larger sample. Considering the cerebellum is one of the most impacted area in the brain in the DS population (Contestabile et al., 2007; Guidi et al., 2011; Haydar & Reeves, 2012; Patkee et al., 2020) the lack of larger effect sizes between brain volumes and outcomes gives rise to the theoretical consideration of the role of the cerebellum in cognitive functioning and whether coarse macro measures of it, such as whole cerebellar volumes, are the most appropriate measures (see section 5.2.2 for further discussion).

The findings also showed that the effect sizes of the correlations were also larger, overall, when brain volumes were related to parent reports of adaptive functioning, than when they were related to the cognitive ability scores extracted from lab-based researcher observations. This could be a reflection of better validity of the former measurements when assessing a population with an NDC, as caregiver scores tend to encompass the amalgamation of the caregiver's knowledge of the child, rather than single snapshots of their abilities. Or it could be because, as suggested by Mattie et al. (2023), adaptive and cognitive abilities, although related, are separate skills, and their relation to brain development and functioning might also be different. These findings also add to the methodological considerations discussed in Chapter 2 (discussed in section 2.2.4) about the need to accept caregiver reports as well as direct observations when scoring an item in an standardised test, and raise the need for future research to investigate the validity and reliability of lab-based, single-session measures of cognitive abilities in DS, and potentially other NDCs where there might be an instability in performance (Wishart & Duffy, 1990). Finally, the presented case studies illustrated not only wide individual variability in the cognitive profile of infants, toddlers and children with DS, but also that

overall brain volumes might lack the necessary detail to understand their cognitive developmental pattern. However, larger sample sizes are needed to determine whether these findings replicate.

5.1.3 Result interpretation from the neuropsychological perspective

The work presented in this thesis and the interpretation of the results has been conducted based on the neuroconstructivist approach (see Section 2.2.2), which suggests that cognitive outcome is the result of multilevel, multisystem interaction and has development itself in its core (Karmiloff-Smith, 2006). The neuropsychological approach, on the other hand, favours a more modular approach, where specific domains, or brain regions are related to specific outcomes. The presented results, although preliminary, do suggest that specific brain regions (e.g., lateral ventricle or CC) are related to specific cognitive domains, such as language in the population with DS, in line with what research conducted form the neuropsychological framework would have predicted. Therefore, exploring brain-behaviour relationships at a more regional level could be an alternative analysis approach in our future research.

In fact, some of the WM tracts that have been found to have altered microstructure in our neonatal DS sample (i.e., CC, internal capsule and anterior commissure), have been regions of interest in studies that have found relationships between WM and language (Northam, Liégeois, Tournier, et al., 2012), oromotor control and speech (Northam, Liégeois, Chong, et al., 2012) in preterm born adolescents. Considering that within the cognitive profile of the population with DS language, especially expressive language, has consistently been found to be a relative weakness compared to other abilities (Abbeduto et

al. 2001; Fiddler et al., 2006; Grieco et al., 2015; Mundy et al., 1995; Vicari, 2006; Wang, 1996), this is indeed an important research avenue to pursue.

5.2 Main contributions of this work

The contributions of this body of work to the understanding of the DS phenotype can be divided into two main aspects: methodological and theoretical. The following section will present several considerations for each.

5.2.1 Methodological considerations

MRI to assess brain anatomy

Different neuroimaging techniques are commonly used in research to study the brain in NDCs, such as electroencephalography (EEG), functional near infrared spectroscopy (fNIRS), MRI or Magnetoencephalography (Karmiloff-Smith, 2010; Lloyd-Fox, 2014). Each of them measures different aspects of the brain, and each of them has limitations and advantages regarding their temporal and spatial accuracy, which should be taken into consideration when selecting what measure would be more appropriate to answer the research question at hand. Out of all these measures, MRI has the best spatial resolution, as it is the optimal way to visualise the anatomy of the brain in vivo (Horga et al., 2014). The work presented in this thesis is a testament to the usefulness and versatility of this technique to investigate different levels of measurement in the brain of a population with an NDC. However, the challenges with the diffusion data analysis described in Chapter 3, and the inconsistencies in brain segmentations and findings presented in section 4.1 have highlighted some of the aspects in which MRI approaches need to be improved in cohorts with atypical neuroanatomical features.

Anatomical accuracy

In fact, MRI is still only a tool, and has its limitations as with any other tool. In essence, MRI is, as with all in vivo techniques, an indirect method of assessing brain anatomy. The images and the subsequent measurements extracted from them are simply reconstructions of the signal acquired from the body (and signal registration thereof), only indirectly reflecting the underlying brain anatomy and processes, (Dubois et al., 2021) so caution needs to be taken when interpreting the results and drawing inferences from the data.

Diffusion MRI

For instance, although diffusion MRI (dMRI) is an important non-invasive tool that offers quantitative measures related to brain tissue microstructure and white matter architecture, the anatomical accuracy of the WM tracts is limited (Schilling et al., 2019; Thomas et al., 2014). In fact, regardless of the improvement on data quality and the implementation of sophisticated processing pipelines, the independent knowledge of the true anatomical WM connections in the human brain is still limited, rendering the assessment of dMRI extracted tract accuracy difficult (Bach et al., 2014; Thomas et al., 2014). Considering that very few tracts are myelinated at the time of birth (e.g., those involved in motor and sensory systems) (Stiles 2008b), and how this affects the signal acquired from the scanner (i.e., it is very similar to the grey matter one), the anatomical inaccuracies of dMRI extracted WM tracts might be even larger than in adults. Therefore, inferences pertaining to the alterations of single WM bundles in neonates should be cautious, and in accordance with the chosen data acquisition (e.g., acquisition in a 7T scanner would allow more anatomical accuracy than in a 3T) and analysis approaches.

Anatomical MRI

Measures extracted from anatomical images (e.g., volumes) might seem more reliable and anatomically accurate. However, this might only be true in cases where little processing has been necessary to extract those volumes, that is, when little to no segmentation has been done (e.g., ICV). The aim of MRI image segmentation is to divide the brain into anatomically meaningful nonoverlapping regions, and there are different approaches to do so, such as manual, automatic or hybrid, among others (Despotović et al., 2015; Devi et al., 2015). Manual segmentation has often been the preferred approach as it allows more accurate brain segmentations (i.e., it allows to separately define brain regions that automatic segmentation might define as single regions) (Akudjedu et al., 2018; Morey et al., 2009). However, manual segmentation relies on substantial anatomical knowledge, it is time consuming, and it is very subjective, often prone to user variability (Akudjedu et al., 2018; Devi et al., 2015). For that reason, automated segmentations have been favoured in research, as they produce faster reproducible results (Akudjedu et al., 2018; Devi et al., 2015; Morey et al., 2009). However, they are not without flaws. In fact, automated segmentations may not only often fail to replicate the anatomical intricacies and accuracy of manual segmentations, but they also struggle with structural variation between subjects, particularly where there is a large inter individual variability (Devi et al., 2015). It is for this reason that fine editing is essential following most automatic segmentation approaches. Therefore, unless automatic segmentations have been developed in collaboration with and revised by people with extensive neuro anatomical knowledge (e.g., with some form of clinical training), results from studies using more detailed regional segmentations may need to be interpreted with caution.

Inter-user variability

Moreover, before performing the segmentation of the brain or the extraction of diffusion measures, several processing steps are also needed to prepare the MRI images and extract the desired measures, which are subject to user variability. Each decision taken in these steps might have an impact on the final image intensity and hence the segmentation, the extracted brain volumes, and the final diffusion measures (Despotović et al., 2015). This hinders the reproducibility and generalisation of the results (Nichols et al., 2017), and should be considered when reviewing the literature.

MRI on atypical brain

Finally, aside from the effect that different processing steps and tools might have on the final outcome, structural anomalies can have an impact on the results of MRI measures, which is why working with MRI images acquired from atypical populations is a challenge (Devi et al., 2015; Horga et al., 2014). This was clearly illustrated by the difficulties with diffusion data analysis discussed in Chapter 3. The inability to draw any conclusions from the fixel-based analysis (FBA) was symptomatic of the limitations of the current MRI approaches to address these issues. Therefore, when conducting research with NDCs and deciding what neuroimaging tools will be used to investigate the brain, this shortcoming of MRI should be considered and addressed during the analysis pipeline. Besides, further efforts should be made to optimise existing processing and analysis pipelines to accommodate populations with atypical neuroanatomy (discussed in section 3.4.2).

Multilevel description of the DS brain: focused on methods

The body of work presented in this thesis has focused on macrostructural and microstructural measures of the brain in neonates with DS as possible correlates for their cognitive and behavioural phenotype. Although these describe two different aspects of the brain, they both relate to neuroanatomy, only one of the possible descriptions of the brain. To understand the underlying mechanisms related to intellectual disability and the cognitive profile in individuals with DS, a multilevel description of the brain should be implemented to have a more comprehensive overview of their phenotype (Karmiloff-Smith, 2010). In fact, there is evidence suggesting that the chromosomal trisomy leads to alterations at all levels of description of the brain (see Vacca et al., 2019 for a review).

Neurochemistry

At the neurochemistry level, there is extensive evidence suggesting GABAergic and glutamatergic transmission alterations in the brain of the DS population (Kleschevnikov et al., 2012; Vacca et al., 2019), as well as reduced levels of serotonin, γ-aminobutyric acid, taurine, and dopamine in brain tissue of fetuses with DS, all critical for brain development (Whittle et al., 2007). A recent study from our research team investigated *in vivo* the neurochemistry in neonates with DS using magnetic resonance spectroscopy, and found altered metabolite (e.g., Myo-inositol) levels in the thalamus (Patkee et al., 2021). Altered metabolite levels have also been found to be related to poorer cognitive performance and dementia status (i.e., having dementia or not) in adult with DS (Lin et al., 2016), so future research should investigate whether individual differences in metabolite levels in neonates with DS are associated with their cognitive outcomes later on. Moreover, considering that myo-inositol levels were found to be altered in both

neonates and adults with dementia with DS, it would be interesting to investigate differences in metabolite levels throughout development, to ascertain whether this might be one of the possible protective or resilience factors that lead to some adults with DS not developing dementia later on in life (Thomas et al., 2020). Applying a developmental framework when investigating neurochemistry in DS would also allow to illustrate whether observed differences in neurochemistry in adults are primary or secondary effects (i.e., if they are the cause of cognitive differences in DS, or if they are the result of the compensatory mechanisms to deal with atypical development).

Cytoarchitecture

At the cytoarchitectural level, or the cellular composition level, aside from reduced cell numbers and altered WM as found in Chapter 3, there is also evidence from histological and mouse model studies showing altered dendrite arborisation and spines within the cells of the cortex (Becker et al., 1986; Uguagliati et al., 2021), as early as 19 weeks GA (Weitzdoerfer et al., 2001). Appropriate dendrite morphology is crucial for normal nervous system functioning, as it allows neurons to communicate with each other. In fact, dendritic abnormalities have been found in many neuropsychiatric conditions, such as autism or schizophrenia, and have been found to explain cortical volume reductions where there was no differences in neuron or axonal numbers (Kulkarni & Firestein, 2012). Neurite orientation dispersion and density imaging (NODDI) is a diffusion MRI technique that allows one to assess dendritic arborisation in vivo (Zhang et al., 2012), and it has been successfully used in neonatal dMRI data to investigate cortical maturation *in vivo* (Batalle et al., 2019). The inclusion of this analysis in the body of work presented in this thesis was originally discussed, but the time constrains resulting from

the COVID-19 pandemic left little time to acquire all the necessary skills. Therefore, future research could use the NODDI technique to investigate cortical dendritic morphology in neonates with DS, and whether they are associated to cortical volume differences, on one hand, and cognitive outcomes in childhood on the other hand.

Functional systems

Although the research conducted has been limited (Carbó-Carreté et al., 2020), in vivo studies in humans have shown alterations in brain synchrony in adolescents and adults with DS using both EEG and fMRI methods (e.g., Anderson et al., 2013; Babiloni et al., 2010), which has been related to adaptive behaviour, specifically, communication skills (Pujol et al., 2015). Using fMRI Anderson et al. (2013) found widespread increased synchrony between brain regions whilst watching cartoons in 15-year-olds teenagers with DS when compared to TD controls. Functional network alterations have also been found using resting state fMRI. Figueroa-Jimenez et al. (2021) found that individuals with DS had increased complexity within the default mode network when compared to age-matched TD controls, and that this complexity had increased variability within the group with DS. There is also evidence suggesting that the resting-state functional connectivity alterations in DS are syndrome specific (Vega et al., 2015).

However, it is still not clear how early in development these alterations in brain functioning arise, or how they might be relevant to cognitive development in DS. So, future research could seek to investigate the development of the functional networks in the brain of babies with DS from an early stage. To do so, different neuroimaging methods could be used and even combined for more comprehensive data (Karmiloff-Smith, 2012). For instance, although task based fMRI is not feasible, the advance in MRI techniques

now allows one to assess resting state networks in neonates (Cusack et al., 2015; Eyre et al., 2021; Fitzgibbon et al., 2020), which emerge as early as the third trimester of gestation (Doria et al., 2010). Alternatively, although less anatomically accurate, fNIRS or EEG, or a combination of both could be used to measure early task-based activation (e.g., whilst looking at a screen). The investigation of early functional systems and their development in infants with DS would complement the existing knowledge on early brain alterations, to which the work in this thesis has contributed.

5.2.2 Theoretical considerations surrounding studying variability in the DS phenotype

Taking development into consideration

The case studies presented in Chapter 4 are a clear illustration of how focusing on development, understood as the change of a system or ability through time, is essential when studying individuals with DS. However, in the current body of work, it has only been possible to apply this longitudinal approach to a small subset of participants, and only regarding their outcome cognitive scores. That is, all neural measures investigated in the current project have been based on static MRI snapshots of a rapidly changing system. In fact, during the neonatal period, both brain size and the water content in the brain rapidly change, which has an impact on the acquired signal and subsequent image processing (Devi et al., 2015; Pietsch et al., 2019). For this reason, the time elapsed between birth and the time of the scan might have an influence on the measured signal from the brain. This time gap was inconsistent in the samples included in this body of work, but the small number of participants did not allow to include it as a covariate. Similarly, some of the neonates included in the studies also had anatomical fetal MRI

scans, so a longitudinal study could be done to investigate whether changes in brain volumes might be better predictors of outcome than the static measures currently used. This work was not conducted for this thesis due to the lower number of cases for which both fetal and neonatal volumetric data were available.

Similarly, it has been shown that myelination follows a systematic progression within the brain, starting in caudal regions, following central regions, and finishing in anterior and posterior poles (Stiles 2008b). Considering that the results in Chapter 3 suggest that most of the WM alterations in neonates with DS were in anterior regions of the brain, a developmental approach would be needed to discern whether these group differences are indeed due to a fixed abnormal or atypical phenotype.

The cerebellum as a region of interest

The cerebellum is the most architecturally complex region of the central nervous system (CNS), representing 10% of the brain's total volume, but containing more than half of the neurons (Butts et al., 2014; Wang & Zoghbi, 2001). In fact, the cerebellar cortex is much more tightly packed than the cerebral cortex accounting for the 78% of the total surface area of the human neocortex, as opposed to the 33% of the area of the macaque monkey (Sereno et al., 2020). This suggests that the cerebellum might have a prominent role in the evolution of distinctively human behaviour and cognition. Yet, the findings in Chapter 4 raise the theoretical consideration of 1) the role of the cerebellum in cognitive abilities in DS, and 2) what level of cerebellar description should be investigated in relation to it.

The role of the cerebellum in human cognition

Once considered a region principally involved in planning and execution of movements and coordination, the cerebellum is increasingly being associated to other higher order, non-motor cognitive functions, and neuropsychological and neuropsychiatric conditions (O'Halloran et al., 2012; Stoodley & Schmahmann, 2018). This has been supported by several functional MRI (fMRI) studies conducted with healthy adults. For instance, Stoodley and colleagues (2013) reported cerebellar activation during a wide range of tasks, from motor execution (finger tapping, motor learning, smooth pursuit eye movements) to higher-level cognitive tasks (Tower of London, working memory paradigms, verbal fluency) in which motor responses were eliminated or controlled for.

In a more recent study, King et al. (2019) used a multi-domain task battery to map the functional organisation of the human cerebellum and derive a functional parcellation of the cerebellar cortex. Based on the activation, they defined 10 functional regions, which were independent from the anatomical lobular boundaries. Similarly results were found by Buckner et al. (2011), who used resting state fMRI from 1000 healthy adults to produce a detailed map of cerebellar organisation based on cortically defined networks extracted from an independent study. These finding are not surprising, considering that the majority of the human cerebellum is associated with cerebral networks involved in cognition. In fact, the cortico-cerebellar system is one of the most prominent networks in the human brain, with around 40 million axons exiting the neocortex and traversing the cerebral peduncles (Diedrichsen et al., 2019), where distinct output channels within the cerebellar

nuclei target multiple nonmotor areas in the prefrontal and posterior parietal cortex, as well as the cortical motor areas.

The cerebellum in NDCs

The role of the cerebellum in NDCs has also been of interest for the last few decades. In fact, early cerebellar damage is often associated with poorer outcomes than cerebellar damage in adulthood, suggesting that the cerebellum is particularly important during development (Stoodley, 2016). For instance, Messerschmidt et al. (2008) found that disrupted cerebellar development in preterm-born neonates was associated to poorer Bayley scores at 24 months-old. Similarly, there is growing evidence of cerebellar dysfunction in different NDCs, such as autism, ADHD or dyslexia (Sathyanesan et al., 2019). It has been suggested that differences in cerebellar development and/or early cerebellar damage could impact a wide range of behaviours via the closed-loop circuits connecting the cerebellum with multiple cerebral cortical regions (Stoodley, 2016).

The cerebellum in DS

The growing evidence showing the role of the cerebellum in higher cognitive abilities in adults (e.g., Buckner et al., 2011; King et al., 2019), and the existence of altered cerebellar development and function in several NDCs (e.g., Sathyanesan et al., 2019; Stoodley, 2016), together with the well-known cerebellar alterations in DS (e.g., Haydar & Reeves, 2012; Patkee et al., 2020) highlight the importance of investigating how the cerebellum contributes to the cognitive phenotype in DS. The work presented in this thesis has provided a stepping stone from which to direct this future research.

From a theoretical point of view, the potential importance of the cerebro-cerebellar network in cognitive outcome and development might explain why the effect sizes of

correlations between cerebellar volumes and outcomes presented in Chapter 4 were small. That is, perhaps overall cerebellar volumes is too coarse a measure to capture the cerebellar properties and mechanisms influencing cognition and behaviour in DS, especially when considering how architecturally complex the cerebellum is (Butts et al., 2014; Wang & Zoghbi, 2001). This notion is further supported by King et al.'s (2019) findings showing functional regions within the adult human cerebellum that were independent from anatomical lobular boundaries. That is, the macrostructural level of description in the cerebellum (e.g., whole cerebellar volume or cerebellar lobe volumes) might not be appropriate to understand functional systems and their association to different cognitive abilities. Similarly, the prominence of the cortico-cerebellar system within the brain and its importance in NDCs (Stoodley, 2016) also raises the theoretical importance to better understand whether this system is altered in the DS population and how it might be associated to their cognitive development. Hence, the WM alterations found in the cerebellar and cerebral peduncles in the study presented in Chapter 3 should be further investigated, and their potential association with cognitive abilities explored.

Future research should also focus on further investigating whether other levels of description (rather than macrostructural and microstructural ones) of the cerebellum, are altered in the population with DS (e.g., functional connectivity), and whether individual differences might be associated with the variability found in later cognitive abilities (see section 5.2.1 for further discussion in multilevel description of the brain). Perhaps a resting state neonatal fMRI study could be conducted, as in Buckner et al. (2011), to investigate (1) whether cerebellar functional boundaries differ between neonates with DS and TD neonates, and (2) whether variability in cerebellar function is associated with

cognitive outcomes. This would contribute to the theoretical understanding of how the different descriptions of the cerebellum relate to cognition and behaviour in DS.

The Multilevel approach

The importance of the multilevel approach when studying NDCs has been highlighted when the neuroconstructivist framework was introduced (discussed in section 2.2.2). The behavioural, cognitive and brain anatomy levels have been investigated in this project. However, to fully understand the causal factors underlying the variability in the cognitive phenotype in DS, the genetic and environmental levels of description should be considered in future research too.

Genetics

Although it is still unknown in practice how genetic variation might result in uneven cognitive profiles via the influence of genes on brain development (e.g., Grasby et al., 2020), at least from a theoretical perspective, the genetic level needs to be considered when identifying specific factors contributing to the individual variability in the DS phenotype. It could be that the phenotypic variability in DS results from a difference between individuals in the expression of the triplicated genes. Or it could also be the result of the effect of genes other than those on chromosome 21. DNA samples have been collected from those neonates with DS that have been scanned as part of the eBiDS project, so the next step in untangling the factors contributing to the variability in cognitive phenotype in DS will be to investigate the impact of genetic variability.

Environmental effect consideration

Environmental influences should be considered when trying to understand the causal mechanisms underlying the cognitive and behavioural phenotype in DS. However,

no environmental effects have been controlled for in the work presented in this thesis due to the low sample sizes. At least two different aspects of environmental influences could be taken into consideration for larger samples: (1) the in-utero influences of maternal environment in brain development, and (2) the ex-utero influence of the participant's environment in cognitive development.

In-utero influences

In fact, there is evidence showing that prenatal maternal socioeconomic status (SES) might have an impact in fetal (Lu et al., 2021) and neonatal brain morphology, as well as in later cognitive abilities, like language (Spann et al., 2020). Similarly, maternal prenatal stress is associated with WM microstructure alterations in preterm neonates (Lautarescu et al., 2020) but appears to have no effect in brain volumes (Lautarescu et al., 2021). This suggests that not all environmental aspects have the same effect on the brain in-utero, so the variable to control for should be carefully selected. Prenatal maternal stress has also been related to maternal malnutrition (over and undernutrition), which has been related to neurocognitive development (Marques et al., 2015; Monk et al., 2013).

Ex-utero influences

The impact of the ex-utero environment in brain and cognitive development has been widely researched too. For instance, evidence suggests that timing and quality of early experiences and environmental stimulation and parental nurturance might gave an impact on the development of brain architecture (S. E. Fox et al., 2010) and cognitive outcomes (Farah et al., 2008). The importance of parental emotional state on the development of cognitive abilities was also highlighted in a recent study by D'Souza et al. (2020). In a cross-sectional developmental trajectory study, they found that children

with DS whose parents reported depression had a slower rate of expressive language development than children with DS whose parents did not report depression. Interestingly, this effect was not found in any of the other measured cognitive abilities (i.e., gross motor, fine motor, receptive language and visual reception).

The effect of childhood SES on brain and cognitive development has also been consistently found. For instance, in a recent study, Tooley et al. (2021) found that higher childhood SES was associated with more efficient cortical networks in adulthood, driven by protracted structural brain development and a prolonged trajectory of functional network segregation. One of the issues when studying the effect of SES in cognitive outcome, is that there are many different SES indicators that can be chosen. Wong and Edwards (2013) conducted a systematic review to illustrate the strength and consistency of the effect of SES across thirteen different SES indicators. They found that maternal education level was the most frequently investigated SES indicator and was also most consistently associated with cognitive outcome. This effect was replicated in a recent multi-cohort European study. Sentenac et al. (2021) used data from 15 population-based cohorts of preterm-born children from 13 countries and found that low maternal education was associated with low cognitive scores in preterm-born toddlers, children and adolescents. So, when further investigating the brain-cognition relationship in infants and children with DS, at least maternal educational level should be controlled for as an indicator of ex-utero environmental influences.

Embodied cognitive development

The infant's body could also be considered as part of the environment in which the brain and cognitive abilities develop (D'Souza & D'Souza, 2019; Mareschal et al.,

2007). This means that if there is multisystem involvement (e.g., cardiac anomaly, disordered muscle tone, low birth weight), this will impact on the child's cognitive development and even on the development of the brain itself. For instance, due to the disruption in hemodynamics and lack of compensatory mechanisms to meet the high oxygen demands of the brain, there is growing evidence showing that congenital heart defects (CHD) have an impact in brain development (e.g., Bonthrone et al., 2021; von Rhein et al., 2015). More precisely, there is consistent evidence showing reduced hippocampal volumes not only in neonates with CHD compared to TD neonates (e.g., Jo & Limperopoulos, 2019) but also in older children that had corrective surgery soon after birth (Naef et al., 2023) suggesting long term effects, and have been related to poorer cognitive outcomes in toddlerhood (Bonthrone et al., 2021) and lower IQ and memory scores in childhood (Naef et al., 2023). Low birth weight has also been consistently associated with poorer outcomes. It has been associated with reduced FA values throughout the brain in neonates (e.g., Dudink et al., 2007; Kim et al., 2023; Zhu et al., 2021), as well as reduced brain volumes, enlarged lateral ventricles, and reduced corpus callosum surface when compared to TD controls in teenagers (Taylor et al., 2011) and young adults (Bjuland et al., 2014) associated with poorer cognitive outcomes.

Considering the high prevalence of CHD, low birth weight and many other clinical and psychiatric comorbidities in the population with DS (Haydar & Reeves, 2012; Lagan et al., 2020; Startin et al., 2020) further research should be conducted to investigate whether they have any further impact in brain and cognitive development, and whether they could help explain the wide individual variability found in their cognitive phenotype.

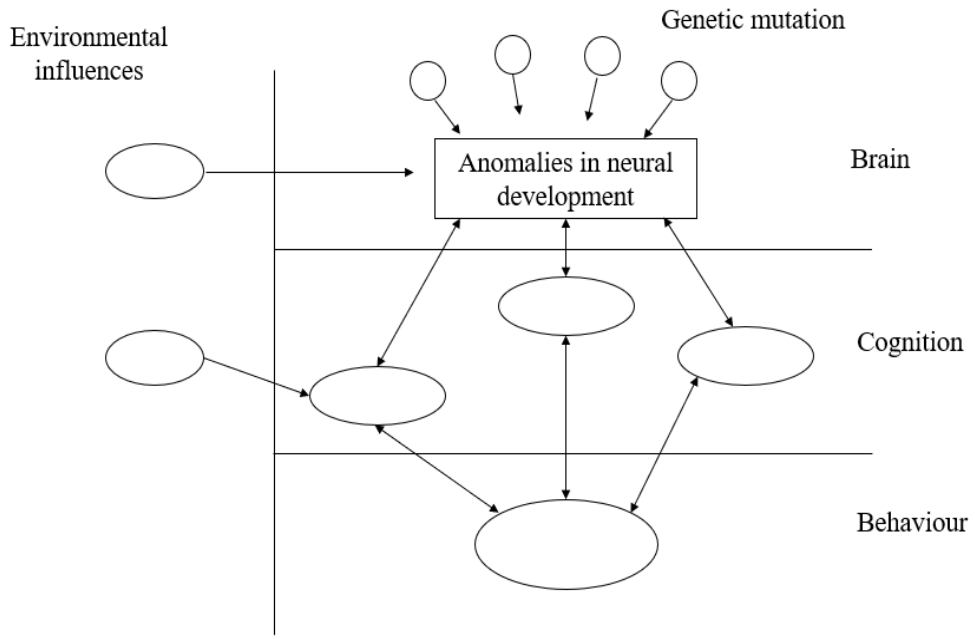
5.3 Updated causal model

The work presented in this thesis has added to the theoretical understanding of the causal model underlying the cognitive-behavioural phenotype in DS and has highlighted the importance of considering the different levels of description of the brain. In light of this, and considering the current findings and past literature (Bjuland et al., 2014; Fox et al., 2014; Lockwood Estrin et al., 2016; Naef et al., 2023; Taylor et al., 2011; Vandormael et al., 2019; Zhu et al., 2021), we believe that the causal model presented in Chapter 2 should be modified (see Figure 5.1) to include the complexities found in the neural phenotype in DS.

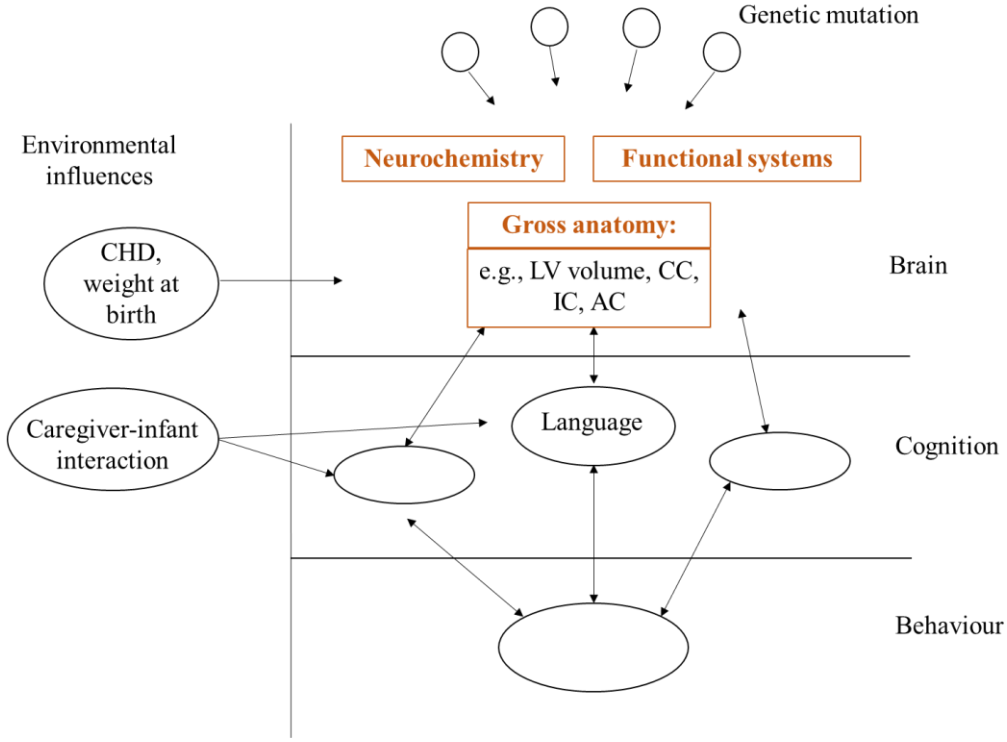
The studies in this thesis have focused on the gross anatomy level of the brain, using brain volumes as a measure of macrostructure, and on lower-level microstructure by using diffusion MRI. However, from a theoretical point of view it should be recognised that coarse measures of the brain may give limited insights into the brain properties and mechanisms influencing cognition and behaviour (Thomas et al., 2020).

Figure 5.1 *Original (a) and updated (b) possible causal model of the cognitive and behavioural phenotype in DS, including the different descriptive levels of the brain that should be considered (in orange) (modified from Morton, 2008). AC=anterior commissure; CC=corpus callosum; IC= internal capsule; LV= lateral ventricle.*

a)



b)



5.4 The impact of COVID-19 on the project

The novelty of my PhD project lay indrawing together two measures of early development (neuropsychological assessment and early MRI brain imaging) and the demonstration that diffusion MRI could potentially be used as an early biomarker of atypical development in a population with DS. The COVID-19 pandemic that started in March 2020 had an impact on several aspects of the project.

Testing facilities at Birkbeck were closed during the time when not only had I planned to continue the recruitment and follow-up assessment of infants and children with DS that had an MRI scan around time of birth in St. Thomas' Hospital, but I had also planned to invite back some of the already recruited families for a second follow up assessment. This would have allowed not only to increase the sample size, but also to provide more robust analysis using longitudinal follow-up data.

In parallel the Perinatal Imaging and Health department at King's College London was essentially closed at this time, which meant that I had limited access to resources necessary (e.g., the access to colleagues and mentors to learn the necessary skills for image processing and analysis) to advance with my dMRI data processing and analysis. This resulted in a delay and time constraint, that led to the inability to include the diffusion data from a subsample of neonates (n= 14) with DS that had been scanned following a protocol preceding the Developing Human Connectome Project (dHCP) one used to acquire the data presented in this thesis. The quality of these older data was very poor, which made the pre-processing very challenging, requiring knowledge and skills that I did not possess. Experts in the topic were consulted, and they concluded that the

optimisation of the pre-processing pipeline for these data might be feasible, but it would be very time consuming, which made the inclusion of the data in this thesis impossible.

Due to time constraints resulting from the COVID-19 pandemic, it was also not possible to investigate any possible relationships between the different MRI measures (i.e., brain volumes and diffusion metrics). For example, the volumetric alterations found in individuals with DS could be the result of many underlying mechanisms, including reduced cell numbers or reduced myelination. Therefore, it would be interesting to investigate whether there is an association between diffusion metrics and brain volumes as was investigated in Gunbey et al., (2017), and perhaps especially focusing in those areas where diffusion metrics were found to be altered (e.g., frontal, temporal lobe volumes).

5.5 Relevance of the presented work: bringing it all together

The work presented in this thesis has implication for the scientific community, as well as the clinical setting, the families of children with DS and the general public.

Relevance for the scientific community.

Here I found WM microstructure differences in neonates with DS for the first time. This is crucial because it has potential to inform treatment targets, future research into interventions and helps understand the neuroanatomical foundations of the development in DS. I also found no associations between neonatal brain volumes and cognitive outcome in DS. Should these results be replicated in a bigger sample, it would further support the need to re-evaluate the causal models of cognitive and behavioural abilities in DS, but also in other NDCs and even TD.

The complex, multi-system alterations resulting from the trisomy highlights the need for a multi-disciplinary team of researcher to truly understand the genotype-phenotype association, and the relationships between the different levels of description (e.g., MRI measures of the brain and underlying cellular composition). To alleviate the methodological difficulties found in the diffusion data analysis in Chapter 3, collaboration with engineers and physicists would be beneficial to ensure that the MRI data analysis pipelines are optimised to study the population with DS. Similarly, if the differences found in the TBSS analysis in Chapter 3 are indeed real, or if there is indeed an association between cortical volumes and cognitive outcome as investigated in Chapter 4, collaboration with researchers working with human tissue could allow to determine what are the mechanisms underlying the WM microstructure differences, or the individual variability in cortical volumes. That is, whether they are driven by differenced in myelin, differences in fibre density, differences in the extra cellular environment, or differences in cellular organisation.

One of the challenges found throughout the presented work is the small number of participants with available data, limiting the ability to draw any robust conclusions. This is a reflection of the hardship in recruitment in the population with DS, an issue that may not be surmounted without national or international multi-centre collaboration. This illustrates the need for the academic community to collaborate with industry and government research policies to collect the resources necessary for such collaboration.

Relevance for the clinical setting and the families of children with DS

Should my results be confirmed with a bigger sample, they would have great implications for the clinical setting. A large enough sample could potentially allow to

standardise brain measures in neonates with DS, which could then be used to predict where within the wide spectrum each child would fall and potentially be used for the prediction of future cognitive and behavioural abilities. This would then allow to provide more information to the parents of babies with DS on how their child would develop, which would allow to support them in providing the best environment for optimal development. The ability to predict the cognitive development of children with DS would also be invaluable for developing and designing individualised early interventions targeted to those motor or cognitive skills that might be more delayed.

5.6 Overall conclusion

This doctoral research provided new insights into early brain anatomy in the population with DS and assessed its association with later cognitive outcome, focusing specifically on brain volumes and WM microstructure. The results revealed, firstly, that WM alterations found in adults, adolescents and children with DS are present from birth. These WM alterations should be further examined, perhaps using other analytical techniques, and their association to later cognitive abilities considered. Secondly, the presented results suggest that regional neonatal brain volumes, although readily available, might lack the necessary intricacies needed to predict cognitive outcome and its variability. This, however, might be a consequence of the limited sample size. Future work should therefore focus on, first increasing the sample size, and second, investigating whether other neural descriptive levels of the brain, such as functional networks, might be better at predicting later outcome. Moreover, following the neuroconstructivist approach, the association between genetic variation and variation in early brain development should be investigated too. This future body of work is worth pursuing as it would allow not only to

improve the understanding of the causal pathways in DS, but also to further disentangle the complex neural mechanisms underlying the variable cognitive phenotype found in the DS population. This could in turn advance the knowledge necessary not only for potential clinical interventions, but also to guide and support caregivers of babies with DS during the first years of life and provide information on potential cognitive outcomes.

References

Abbeduto, L., Pavetto, M., Kesin, E., Weissman, M., Karadottir, S., O'Brien, A., & Cawthon, S. (2001). The linguistic and cognitive profile of Down syndrome: Evidence from a comparison with fragile X syndrome. *Down Syndrome Research and Practice*. https://doi.org/10.3104/reports.109

Ábrahám, H., Vincze, A., Veszprémi, B., Kravják, A., Gömöri, É., Kovács, G. G., & Seress, L. (2012). Impaired myelination of the human hippocampal formation in Down syndrome. *International Journal of Developmental Neuroscience*, *30*(2), 147–158. https://doi.org/10.1016/j.ijdevneu.2011.11.005

Adamson, L. B., Bakeman, R., Deckner, D. F., & Romski, M. (2008). Joint Engagement and the Emergence of Language in Children with Autism and Down Syndrome. *Journal of Autism and Developmental Disorders*, *39*(1), 84. https://doi.org/10.1007/s10803-008-0601-7

Akudjedu, T. N., Nabulsi, L., Makelyte, M., Scanlon, C., Hehir, S., Casey, H., Ambati, S., Kenney, J., O'Donoghue, S., McDermott, E., Kilmartin, L., Dockery, P., McDonald, C., Hallahan, B., & Cannon, D. M. (2018). A comparative study of segmentation techniques for the quantification of brain subcortical volume. *Brain Imaging and Behavior*, *12*(6), 1678–1695. https://doi.org/10.1007/s11682-018-9835-y

Alexander, M., Petri, H., Ding, Y., Wandel, C., Khwaja, O., & Foskett, N. (2016). Morbidity and medication in a large population of individuals with Down syndrome compared to the general population. *Developmental Medicine & Child Neurology*, *58*(3), 246–254. https://doi.org/10.1111/dmcn.12868

Alexandrou, G., Mårtensson, G., Skiöld, B., Blennow, M., Ådén, U., & Vollmer, B. (2014). White matter microstructure is influenced by extremely preterm birth and neonatal respiratory factors. *Acta Paediatrica*, *103*(1), 48–56. https://doi.org/10.1111/apa.12445

Allyse, M., Minear, M. A., Berson, E., Sridhar, S., Rote, M., Hung, A., & Chandrasekharan, S. (2015). Non-invasive prenatal testing: A review of international implementation and challenges. *International Journal of Women's Health*, *7*, 113–126. https://doi.org/10.2147/IJWH.S67124

REFERENCES

Anderson, C. L., & Brown, C. E. L. (2009). Fetal Chromosomal Abnormalities: Antenatal Screening and Diagnosis. *American Family Physician*, *79*(2), 117–123.

Anderson, J. S., Nielsen, J. A., Ferguson, M. A., Burback, M. C., Cox, E. T., Dai, L., Gerig, G., Edgin, J. O., & Korenberg, J. R. (2013). Abnormal brain synchrony in Down Syndrome. *NeuroImage: Clinical*, *2*, 703–715. https://doi.org/10.1016/j.nicl.2013.05.006

Aoki, S., Yamauchi, Y., & Hashimoto, K. (2018). Developmental trend of children with Down's syndrome – How do sex and neonatal conditions influence their developmental patterns? *Brain and Development*, *40*(3), 181–187. https://doi.org/10.1016/j.braindev.2017.10.001

Arber, S. (2004). Subplate neurons: Bridging the gap to function in the cortex. *Trends in Neurosciences*, *27*(3), 111–113. https://doi.org/10.1016/j.tins.2004.01.005

Armstrong, N. M., An, Y., Shin, J. J., Williams, O. A., Doshi, J., Erus, G., Davatzikos, C., Ferrucci, L., Beason-Held, L. L., & Resnick, S. M. (2020). Associations between cognitive and brain volume changes in cognitively normal older adults. *NeuroImage*, *223*, 117289. https://doi.org/10.1016/j.neuroimage.2020.117289

Aslin, R. N. (2012). Infant Eyes: A Window on Cognitive Development. *Infancy*, *17*(1), 126–140. https://doi.org/10.1111/j.1532-7078.2011.00097.x

Babiloni, C., Albertini, G., Onorati, P., Muratori, C., Buffo, P., Condoluci, C., Sarà, M., Pistoia, F., Vecchio, F., & Rossini, P. M. (2010). Cortical sources of EEG rhythms are abnormal in down syndrome. *Clinical Neurophysiology*, *121*(8), 1205–1212. https://doi.org/10.1016/j.clinph.2010.02.155

Baburamani, A. A., Patkee, P. A., Arichi, T., & Rutherford, M. A. (2019). New approaches to studying early brain development in Down syndrome. *Developmental Medicine & Child Neurology*, *0*(0). https://doi.org/10.1111/dmcn.14260

Baburamani, A. A., Vontell, R. T., Uus, A., Pietsch, M., Patkee, P. A., Wyatt-Ashmead, J., Chin-Smith, E. C., Supramaniam, V. G., Donald Tournier, J., Deprez, M., & Rutherford, M. A. (2020). Assessment of radial glia in the frontal lobe of fetuses

with Down syndrome. *Acta Neuropathologica Communications*, *8*(1), 141. https://doi.org/10.1186/s40478-020-01015-3

Bach, M., Laun, F. B., Leemans, A., Tax, C. M. W., Biessels, G. J., Stieltjes, B., & Maier-Hein, K. H. (2014). Methodological considerations on tract-based spatial statistics (TBSS). *NeuroImage*, *100*, 358–369. https://doi.org/10.1016/j.neuroimage.2014.06.021

Ball, G., Pazderova, L., Chew, A., Tusor, N., Merchant, N., Arichi, T., Allsop, J. M., Cowan, F. M., Edwards, A. D., & Counsell, S. J. (2015). Thalamocortical Connectivity Predicts Cognition in Children Born Preterm. *Cerebral Cortex*, *25*(11), 4310–4318. https://doi.org/10.1093/cercor/bhu331

Bartolini, G., Ciceri, G., & Marín, O. (2013). Integration of GABAergic Interneurons into Cortical Cell Assemblies: Lessons from Embryos and Adults. *Neuron*, *79*(5), 849–864. https://doi.org/10.1016/j.neuron.2013.08.014

Bar-Yosef, O., Barzilay, E., Dorembus, S., Achiron, R., & Katorza, E. (2017). Neurodevelopmental outcome of isolated ventriculomegaly: A prospective cohort study. *Prenatal Diagnosis*, *37*(8), 764–768. https://doi.org/10.1002/pd.5078

Basser, P. J., & Ozarslan, E. (2010). Anisotropic diffusion: From the apparent diffusion coefficient to the apparent diffusion tensor. In D. Jones (Ed.), *Diffusion MRI: Theory, Methods, and Applications. Oxford University Press, Oxford* (pp. 79–91). Oxford University Press.

Batalle, D., Edwards, A. D., & O'Muircheartaigh, J. (2018). Annual Research Review: Not just a small adult brain: understanding later neurodevelopment through imaging the neonatal brain. *Journal of Child Psychology and Psychiatry*, *59*(4), 350–371. https://doi.org/10.1111/jcpp.12838

Batalle, D., Hughes, E. J., Zhang, H., Tournier, J.-D., Tusor, N., Aljabar, P., Wali, L., Alexander, D. C., Hajnal, J. V., Nosarti, C., Edwards, A. D., & Counsell, S. J. (2017). Early development of structural networks and the impact of prematurity on brain connectivity. *NeuroImage*, *149*, 379–392. https://doi.org/10.1016/j.neuroimage.2017.01.065

Batalle, D., O'Muircheartaigh, J., Makropoulos, A., Kelly, C. J., Dimitrova, R., Hughes, E. J., Hajnal, J. V., Zhang, H., Alexander, D. C., Edwards, A. D., & Counsell, S. J. (2019). Different patterns of cortical maturation before and after 38 weeks gestational age demonstrated by diffusion MRI in vivo. *NeuroImage*, *185*, 764–775. https://doi.org/10.1016/j.neuroimage.2018.05.046

Bayley, N. (1993). *Bayley Scales of Infant Development: Manual.* (Second Edition). The Psychological Corporation.

Bayley, N. (2006). *Bayley Scales of Infant and Toddler Development* (Third Edition). Harcourt Assessment.

Beare, R. J., Chen, J., Kelly, C. E., Alexopoulos, D., Smyser, C. D., Rogers, C. E., Loh, W. Y., Matthews, L. G., Cheong, J. L. Y., Spittle, A. J., Anderson, P. J., Doyle, L. W., Inder, T. E., Seal, M. L., & Thompson, D. K. (2016). Neonatal Brain Tissue Classification with Morphological Adaptation and Unified Segmentation. *Frontiers in Neuroinformatics*, *10*. https://www.frontiersin.org/article/10.3389/fninf.2016.00012

Becker, L. E., Armstrong, D. L., & Chan, F. (1986). Dendritic atrophy in children with Down's syndrome. *Annals of Neurology*, *20*(4), 520–526. https://doi.org/10.1002/ana.410200413

Bendlin, B. B., Fitzgerald, M. E., Ries, M. L., Xu, G., Kastman, E. K., Thiel, B. W., Rowley, H. A., Lazar, M., Alexander, A. L., & Johnson, S. C. (2010). White Matter in Aging and Cognition: A Cross-Sectional Study of Microstructure in Adults Aged Eighteen to Eighty-Three. *Developmental Neuropsychology*, *35*(3), 257–277. https://doi.org/10.1080/87565641003696775

Bennett, I. J., & Madden, D. J. (2014). Disconnected aging: Cerebral white matter integrity and age-related differences in cognition. *Neuroscience*, *276*, 187–205. https://doi.org/10.1016/j.neuroscience.2013.11.026

Bisdas, S., Bohning, D. E., Bešenski, N., Nicholas, J. S., & Rumboldt, Z. (2008). Reproducibility, Interrater Agreement, and Age-Related Changes of Fractional Anisotropy Measures at 3T in Healthy Subjects: Effect of the Applied b-Value. *American Journal of Neuroradiology*, *29*(6), 1128–1133. https://doi.org/10.3174/ajnr.A1044

Bjuland, K. J., Rimol, L. M., Løhaugen, G. C. C., & Skranes, J. (2014). Brain volumes and cognitive function in very-low-birth-weight (VLBW) young adults. *European Journal of Paediatric Neurology*, *18*(5), 578–590. https://doi.org/10.1016/j.ejpn.2014.04.004

Blasi, A., Mercure, E., Lloyd-Fox, S., Thomson, A., Brammer, M., Sauter, D., Deeley, Q., Barker, G. J., Renvall, V., Deoni, S., Gasston, D., Williams, S. C. R., Johnson, M. H., Simmons, A., & Murphy, D. G. M. (2011). Early Specialization for Voice and Emotion Processing in the Infant Brain. *Current Biology*, *21*(14), 1220–1224. https://doi.org/10.1016/j.cub.2011.06.009

Bonthrone, A. F., Dimitrova, R., Chew, A., Kelly, C. J., Cordero-Grande, L., Carney, O., Egloff, A., Hughes, E., Vecchiato, K., Simpson, J., Hajnal, J. V., Pushparajah, K., Victor, S., Nosarti, C., Rutherford, M. A., Edwards, A. D., O'Muircheartaigh, J., & Counsell, S. J. (2021). Individualized brain development and cognitive outcome in infants with congenital heart disease. *Brain Communications*, *3*(2), fcab046. https://doi.org/10.1093/braincomms/fcab046

Bos, A. F. (2013). Bayley-II or Bayley-III: What do the scores tell us? *Developmental Medicine & Child Neurology*, *55*(11), 978–979. https://doi.org/10.1111/dmcn.12234

Bozzo, M. T., & Zecca, G. M. (1993). *Adattamento italiano della scala d'intelligenza Stanford-Binet forma LM nella revisione Terman-Merril: Manuale*. OS.

Brindley, N. (2019). Antenatal Screening/Prenatal Diagnosis. In R. Carachi & S. H. E. Doss (Eds.), *Clinical Embryology: An Atlas of Congenital Malformations* (pp. 69–72). Springer International Publishing. https://doi.org/10.1007/978-3-319-26158-4_7

Brown, J. H., Johnson, M. H., Paterson, S. J., Gilmore, R., Longhi, E., & Karmiloff-Smith, A. (2003). Spatial representation and attention in toddlers with Williams syndrome and Down syndrome. *Neuropsychologia*, *41*(8), 1037–1046. https://doi.org/10.1016/S0028-3932(02)00299-3

Bruckert, L., Travis, K. E., Mezer, A. A., Ben-Shachar, M., & Feldman, H. M. (2020). Associations of Reading Efficiency with White Matter Properties of the

Cerebellar Peduncles in Children. *The Cerebellum*, *19*(6), 771–777. https://doi.org/10.1007/s12311-020-01162-2

Bubb, E. J., Metzler-Baddeley, C., & Aggleton, J. P. (2018). The cingulum bundle: Anatomy, function, and dysfunction. *Neuroscience & Biobehavioral Reviews*, *92*, 104–127. https://doi.org/10.1016/j.neubiorev.2018.05.008

Buckner, R. L., Krienen, F. M., Castellanos, A., Diaz, J. C., & Yeo, B. T. T. (2011). The organization of the human cerebellum estimated by intrinsic functional connectivity. *Journal of Neurophysiology*, *106*(5), 2322–2345. https://doi.org/10.1152/jn.00339.2011

Burack, J. A., Iarocci, G., Flanagan, T. D., & Bowler, D. M. (2004). On Mosaics and Melting Pots: Conceptual Considerations of Comparison and Matching Strategies. *Journal of Autism and Developmental Disorders*, *34*(1), 65–73. https://doi.org/10.1023/B:JADD.0000018076.90715.00

Busciglio, J., & Yankner, B. A. (1995). Apoptosis and increased generation of reactive oxygen species in Down's syndrome neurons in vitro. *Nature*, *378*(6559), 776–779. https://doi.org/10.1038/378776a0

Butts, T., Green, M. J., & Wingate, R. J. T. (2014). Development of the cerebellum: Simple steps to make a 'little brain'. *Development*, *141*(21), 4031–4041. https://doi.org/10.1242/dev.106559

Caeyenberghs, K., Leemans, A., Geurts, M., Taymans, T., Linden, C. V., Smits-Engelsman, B. C. M., Sunaert, S., & Swinnen, S. P. (2010). Brain-behavior relationships in young traumatic brain injury patients: DTI metrics are highly correlated with postural control. *Human Brain Mapping*, *31*(7), 992–1002. https://doi.org/10.1002/hbm.20911

Caplan, B., Neece, C. L., & Baker, B. L. (2015). Developmental Level and Psychopathology: Comparing Children with Developmental Delays to Chronological and Mental Age Matched Controls. *Research in Developmental Disabilities*, *37*, 143–151. https://doi.org/10.1016/j.ridd.2014.10.045

Carducci, F., Onorati, P., Condoluci, C., Di Gennaro, G., Quarato, P. P., Pierallini, A., Sarà, M., Miano, S., Cornia, R., & Albertini, G. (2013). Whole-brain voxel-based

morphometry study of children and adolescents with Down syndrome. *Functional Neurology*, *28*(1), 19–28.

Carruthers, P. (2006). *The architecture of the mind*. Oxford University Press.

Catani, M., & Thiebaut de Schotten, M. (2008). A diffusion tensor imaging tractography atlas for virtual in vivo dissections. *Cortex; a Journal Devoted to the Study of the Nervous System and Behavior*, *44*(8), 1105–1132. https://doi.org/10.1016/j.cortex.2008.05.004

Chanraud, S., Zahr, N., Sullivan, E. V., & Pfefferbaum, A. (2010). MR Diffusion Tensor Imaging: A Window into White Matter Integrity of the Working Brain. *Neuropsychology Review*, *20*(2), 209–225. https://doi.org/10.1007/s11065-010-9129-7

Clarke, J. M., & Zaidel, E. (1994). Anatomical-behavioral relationships: Corpus callosum morphometry and hemispheric specialization. *Behavioural Brain Research*, *64*(1), 185–202. https://doi.org/10.1016/0166-4328(94)90131-7

Contestabile, A., Benfenati, F., & Gasparini, L. (2010). Communication breaks-Down: From neurodevelopment defects to cognitive disabilities in Down syndrome. *Progress in Neurobiology*, *91*(1), 1–22. https://doi.org/10.1016/j.pneurobio.2010.01.003

Contestabile, A., Fila, T., Ceccarelli, C., Bonasoni, P., Bonapace, L., Santini, D., Bartesaghi, R., & Ciani, E. (2007). Cell cycle alteration and decreased cell proliferation in the hippocampal dentate gyrus and in the neocortical germinal matrix of fetuses with down syndrome and in Ts65Dn mice. *Hippocampus*, *17*(8), 665–678. https://doi.org/10.1002/hipo.20308

Courchesne, E. (2004). Brain development in autism: Early overgrowth followed by premature arrest of growth. *Mental Retardation & Developmental Disabilities Research Reviews*, *10*(2), 106–111. https://doi.org/10.1002/mrdd.20020

Courchesne, E., Karns, C. M., Davis, H. R., Ziccardi, R., Carper, R. A., Tigue, Z. D., Chisum, H. J., Moses, P., Pierce, K., Lord, C., Lincoln, A. J., Pizzo, S., Schreibman, L., Haas, R. H., Akshoomoff, N. A., & Courchesne, R. Y. (2001). Unusual brain growth patterns in early life in patients with autistic disorder: An MRI study. *Neurology*, *57*(2), 245–254. https://doi.org/10.1212/wnl.57.2.245

Couzens, D., Cuskelly, M., & Jobling, A. (2004). The Stanford Binet fourth edition and its use with individuals with Down Syndrome: Cautions for clinicians. *International Journal of Disability, Development and Education, 51*(1), 39–56. https://doi.org/10.1080/1034912042000182193

Couzens, D., Haynes, M., & Cuskelly, M. (2012). Individual and Environmental Characteristics Associated with Cognitive Development in Down Syndrome: A Longitudinal Study. *Journal of Applied Research in Intellectual Disabilities*, *25*(5), 396–413. https://doi.org/10.1111/j.1468-3148.2011.00673.x

Cusack, R., Wild, C., Linke, A. C., Arichi, T., Lee, D. S. C., & Han, V. K. (2015). Optimizing Stimulation and Analysis Protocols for Neonatal fMRI. *PLOS ONE*, *10*(8), e0120202. https://doi.org/10.1371/journal.pone.0120202

Dale, B. M., Brown, M. A., & Semelka, R. C. (2015a). Concepts of magnetic resonance. In *MRI: Basic Principles and Applications* (pp. 10–16). John Wiley & Sons, Incorporated. http://ebookcentral.proquest.com/lib/bbk/detail.action?docID=4040525

Dale, B. M., Brown, M. A., & Semelka, R. C. (2015b). Principles of magnetic resonance imaging – 1. In *MRI: Basic Principles and Applications* (pp. 26–38). John Wiley & Sons, Incorporated. http://ebookcentral.proquest.com/lib/bbk/detail.action?docID=4040525

Dale, B. M., Brown, M. A., & Semelka, R. C. (2015c). Principles of magnetic resonance imaging – 2. In *MRI: Basic Principles and Applications* (pp. 39–64). John Wiley & Sons, Incorporated. http://ebookcentral.proquest.com/lib/bbk/detail.action?docID=4040525

Dale, B. M., Brown, M. A., & Semelka, R. C. (2015d). Pulse sequences. In *MRI: Basic Principles and Applications* (pp. 65–85). John Wiley & Sons, Incorporated. http://ebookcentral.proquest.com/lib/bbk/detail.action?docID=4040525

Dale, B. M., Brown, M. A., & Semelka, R. C. (2015e). Relaxation. In *MRI: Basic Principles and Applications* (pp. 17–25). John Wiley & Sons, Incorporated. http://ebookcentral.proquest.com/lib/bbk/detail.action?docID=4040525

Dean, D. C., O'Muircheartaigh, J., Dirks, H., Waskiewicz, N., Lehman, K., Walker, L., Han, M., & Deoni, S. C. L. (2014). Modeling healthy male white matter and

myelin development: 3 through 60months of age. *NeuroImage*, *84*, 742–752. https://doi.org/10.1016/j.neuroimage.2013.09.058

del Hoyo Soriano, L., Rosser, T., Hamilton, D., Wood, T., Abbeduto, L., & Sherman, S. (2020). Gestational age is related to symptoms of attention-deficit/hyperactivity disorder in late-preterm to full-term children and adolescents with down syndrome. *Scientific Reports*, *10*(1), Article 1. https://doi.org/10.1038/s41598-020-77392-5

Despotović, I., Goossens, B., & Philips, W. (2015). MRI Segmentation of the Human Brain: Challenges, Methods, and Applications. *Computational and Mathematical Methods in Medicine*, *2015*, e450341. https://doi.org/10.1155/2015/450341

Devi, C. N., Chandrasekharan, A., Sundararaman, V. K., & Alex, Z. C. (2015). Neonatal brain MRI segmentation: A review. *Computers in Biology and Medicine*, *64*, 163–178. https://doi.org/10.1016/j.compbiomed.2015.06.016

Diamandopoulos, K., & Green, J. (2018). Down syndrome: An integrative review. *Journal of Neonatal Nursing*, *24*(5), 235–241. https://doi.org/10.1016/j.jnn.2018.01.001

Diedrichsen, J., King, M., Hernandez-Castillo, C., Sereno, M., & Ivry, R. B. (2019). Universal Transform or Multiple Functionality? Understanding the Contribution of the Human Cerebellum across Task Domains. *Neuron*, *102*(5), 918–928. https://doi.org/10.1016/j.neuron.2019.04.021

Dierssen, M. (2012). Down syndrome: The brain in trisomic mode. *Nature Reviews Neuroscience*, *13*(12), 844–858. https://doi.org/10.1038/nrn3314

Dierssen, M., Martí, E., Pucharcós, C., Fotaki, V., Altafaj, X., Casas, K., Solans, A., Arbonés, M. L., Fillat, C., & Estivill, X. (2001). Functional genomics of Down syndrome: A multidisciplinary approach. In G. Lubec (Ed.), *Protein Expression in Down Syndrome Brain* (pp. 131–148). Springer Vienna. https://doi.org/10.1007/978-3-7091-6262-0_11

Dimitrova, R., Pietsch, M., Christiaens, D., Ciarrusta, J., Wolfers, T., Batalle, D., Hughes, E., Hutter, J., Cordero-Grande, L., Price, A. N., Chew, A., Falconer, S., Vecchiato, K., Steinweg, J. K., Carney, O., Rutherford, M. A., Tournier, J.-D., Counsell, S. J., Marquand, A. F., … O'Muircheartaigh, J. (2020). Heterogeneity

in Brain Microstructural Development Following Preterm Birth. *Cerebral Cortex (New York, N.Y.: 1991)*, *30*(9), 4800–4810. https://doi.org/10.1093/cercor/bhaa069

Donovan, A. P. A., & Basson, M. A. (2017). The neuroanatomy of autism – a developmental perspective. *Journal of Anatomy*, *230*(1), 4–15. https://doi.org/10.1111/joa.12542

Doria, V., Beckmann, C. F., Arichi, T., Merchant, N., Groppo, M., Turkheimer, F. E., Counsell, S. J., Murgasova, M., Aljabar, P., Nunes, R. G., Larkman, D. J., Rees, G., & Edwards, A. D. (2010). Emergence of resting state networks in the preterm human brain. *Proceedings of the National Academy of Sciences*, *107*(46), 20015–20020. https://doi.org/10.1073/pnas.1007921107

D'Souza, D., & D'Souza, H. (2019). Emergent and constrained: Understanding brain and cognitive development. *Journal of Neurolinguistics*, *49*, 228–231. https://doi.org/10.1016/j.jneuroling.2018.04.011

D'Souza, D., D'Souza, H., Johnson, M. H., & Karmiloff-Smith, A. (2015). Concurrent Relations between Face Scanning and Language: A Cross-Syndrome Infant Study. *PLOS ONE*, *10*(10), e0139319. https://doi.org/10.1371/journal.pone.0139319

D'Souza, D., D'Souza, H., Johnson, M. H., & Karmiloff-Smith, A. (2016). Audio-visual speech perception in infants and toddlers with Down syndrome, fragile X syndrome, and Williams syndrome. *Infant Behavior and Development*, *44*, 249–262. https://doi.org/10.1016/j.infbeh.2016.07.002

D'Souza, H., Brady, D., Wiseman, F. K., Good, M. A., & Thomas, M. S. C. (2020). Aligning cognitive studies in mouse models and human infants/toddlers: The case of Down syndrome. In *Taking Development Seriously: A Festschrift for Annette Karmiloff-Smith*. Routledge.

D'Souza, H., & Karmiloff-Smith, A. (2017). Neurodevelopmental disorders. *Wiley Interdisciplinary Reviews. Cognitive Science*, *8*(1–2). https://doi.org/10.1002/wcs.1398

D'Souza, H., Karmiloff-Smith, A., Mareschal, D., & Thomas, M. S. C. (2021). The Down syndrome profile emerges gradually over the first years of life

[Manuscript submitted for publication]. *Centre for Brain and Cognitive Development, Birbeck, University of London*.

D'Souza, H., Lathan, A., Karmiloff-Smith, A., & Mareschal, D. (2020). Down syndrome and parental depression: A double hit on early expressive language development. *Research in Developmental Disabilities*, *100*, 103613. https://doi.org/10.1016/j.ridd.2020.103613

Dubois, J., Alison, M., Counsell, S. J., Hertz-Pannier, L., Hüppi, P. S., & Benders, M. J. N. L. (2021). MRI of the Neonatal Brain: A Review of Methodological Challenges and Neuroscientific Advances. *Journal of Magnetic Resonance Imaging*, *53*(5), 1318–1343. https://doi.org/10.1002/jmri.27192

Dubois, J., Dehaene-Lambertz, G., Perrin, M., Mangin, J.-F., Cointepas, Y., Duchesnay, E., Bihan, D. L., & Hertz-Pannier, L. (2008). Asynchrony of the early maturation of white matter bundles in healthy infants: Quantitative landmarks revealed noninvasively by diffusion tensor imaging. *Human Brain Mapping*, *29*(1), 14–27. https://doi.org/10.1002/hbm.20363

Dudink, J., Lequin, M., van Pul, C., Buijs, J., Conneman, N., van Goudoever, J., & Govaert, P. (2007). Fractional anisotropy in white matter tracts of very-low-birth-weight infants. *Pediatric Radiology*, *37*(12), 1216–1223. https://doi.org/10.1007/s00247-007-0626-7

Dykens, E., Hodapp, R., & Evans, D. (2006). Profiles and development of adaptive behavior in children with Down syndrome. *Down Syndrome Research and Practice*, *9*(3), 45–50. https://doi.org/10.3104/reprints.293

Edgin, J. O. (2013). Cognition in Down syndrome: A developmental cognitive neuroscience perspective. *Wiley Interdisciplinary Reviews: Cognitive Science*, *4*(3), 307–317. https://doi.org/10.1002/wcs.1221

Engidawork, E., & Lubec, G. (2003). Molecular changes in fetal Down syndrome brain. *Journal of Neurochemistry*, *84*(5), 895–904. https://doi.org/10.1046/j.1471-4159.2003.01614.x

Eyre, M., Fitzgibbon, S. P., Ciarrusta, J., Cordero-Grande, L., Price, A. N., Poppe, T., Schuh, A., Hughes, E., O'Keeffe, C., Brandon, J., Cromb, D., Vecchiato, K., Andersson, J., Duff, E. P., Counsell, S. J., Smith, S. M., Rueckert, D., Hajnal, J.

V., Arichi, T., … Edwards, A. D. (2021). The Developing Human Connectome Project: Typical and disrupted perinatal functional connectivity. *Brain*, *144*(7), 2199–2213. https://doi.org/10.1093/brain/awab118

Fame, R. M., Cortés-Campos, C., & Sive, H. L. (2020). Brain Ventricular System and Cerebrospinal Fluid Development and Function: Light at the End of the Tube. *BioEssays*, *42*(3), 1900186. https://doi.org/10.1002/bies.201900186

Fan, C. C., Brown, T. T., Bartsch, H., Kuperman, J. M., Hagler, D. J., Schork, A., Searcy, Y., Bellugi, U., Halgren, E., & Dale, A. M. (2017). Williams syndrome-specific neuroanatomical profile and its associations with behavioral features. *NeuroImage: Clinical*, *15*, 343–347. https://doi.org/10.1016/j.nicl.2017.05.011

Farah, M. J., Betancourt, L., Shera, D. M., Savage, J. H., Giannetta, J. M., Brodsky, N. L., Malmud, E. K., & Hurt, H. (2008). Environmental stimulation, parental nurturance and cognitive development in humans. *Developmental Science*, *11*(5), 793–801. https://doi.org/10.1111/j.1467-7687.2008.00688.x

Fenchel, D., Dimitrova, R., Seidlitz, J., Robinson, E. C., Batalle, D., Hutter, J., Christiaens, D., Pietsch, M., Brandon, J., Hughes, E. J., Allsop, J., O'Keeffe, C., Price, A. N., Cordero-Grande, L., Schuh, A., Makropoulos, A., Passerat-Palmbach, J., Bozek, J., Rueckert, D., … O'Muircheartaigh, J. (2020). Development of Microstructural and Morphological Cortical Profiles in the Neonatal Brain. *Cerebral Cortex*, *30*(11), 5767–5779. https://doi.org/10.1093/cercor/bhaa150

Feng, K., Rowell, A. C., Andres, A., Bellando, B. J., Lou, X., Glasier, C. M., Ramakrishnaiah, R. H., Badger, T. M., & Ou, X. (2019). Diffusion Tensor MRI of White Matter of Healthy Full-term Newborns: Relationship to Neurodevelopmental Outcomes. *Radiology*, *292*(1), 179–187. https://doi.org/10.1148/radiol.2019182564

Fenoll, R., Pujol, J., Esteba-Castillo, S., de Sola, S., Ribas-Vidal, N., García-Alba, J., Sánchez-Benavides, G., Martínez-Vilavella, G., Deus, J., Dierssen, M., Novell-Alsina, R., & de la Torre, R. (2017). Anomalous White Matter Structure and the Effect of Age in Down Syndrome Patients. *Journal of Alzheimer's Disease*, *57*(1), 61–70. https://doi.org/10.3233/JAD-161112

Ferrazzi, G., Kuklisova Murgasova, M., Arichi, T., Malamateniou, C., Fox, M. J., Makropoulos, A., Allsop, J., Rutherford, M., Malik, S., Aljabar, P., & Hajnal, J. V. (2014). Resting State fMRI in the moving fetus: A robust framework for motion, bias field and spin history correction. *NeuroImage*, *101*, 555–568. https://doi.org/10.1016/j.neuroimage.2014.06.074

Fidler, D., Hepburn, S., & Rogers, S. (2006). Early learning and adaptive behaviour in toddlers with Down syndrome: Evidence for an emerging behavioural phenotype? *Down Syndrome Research and Practice*, *9*(3), 37–44. https://doi.org/10.3104/reports.297

Fidler, D. J., Schworer, E., Will, E. A., Patel, L., & Daunhauer, L. A. (2018). Correlates of early cognition in infants with Down syndrome. *Journal of Intellectual Disability Research*, *0*(0). https://doi.org/10.1111/jir.12566

Filley, C. M. (2005). White Matter and Behavioral Neurology. *Annals of the New York Academy of Sciences*, *1064*(1), 162–183. https://doi.org/10.1196/annals.1340.028

Finlay, B. L. (2019). Generic Homo sapiens and Unique Mus musculus: Establishing the Typicality of the Modeled and the Model Species. *Brain, Behavior and Evolution*, *93*(2–3), 122–136. https://doi.org/10.1159/000500111

Finlay, B. L., Hinz, F., & Darlington, R. B. (2011). Mapping behavioural evolution onto brain evolution: The strategic roles of conserved organization in individuals and species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *366*(1574), 2111–2123. https://doi.org/10.1098/rstb.2010.0344

Finlay, B. L., & Uchiyama, R. (2015). Developmental mechanisms channeling cortical evolution. *Trends in Neurosciences*, *38*(2), 69–76. https://doi.org/10.1016/j.tins.2014.11.004

Finlay, B., & Uchiyama, R. (2017). *The Timing of Brain Maturation, Early Experience, and the Human Social Niche* (pp. 123–148). https://doi.org/10.1016/B978-0-12-804042-3.00134-2

Figueroa-Jimenez, M. D., Carbó-Carreté, M., Cañete-Massé, C., Zarabozo-Hurtado, D., Peró-Cebollero, M., Salazar-Estrada, J. G., & Guàrdia-Olmos, J. (2021). Complexity Analysis of the Default Mode Network Using Resting-State fMRI in Down Syndrome: Relationships Highlighted by a Neuropsychological

Assessment. *Brain Sciences*, *11*(3), Article 3. https://doi.org/10.3390/brainsci11030311

Fitzgibbon, S. P., Harrison, S. J., Jenkinson, M., Baxter, L., Robinson, E. C., Bastiani, M., Bozek, J., Karolis, V., Cordero Grande, L., Price, A. N., Hughes, E., Makropoulos, A., Passerat-Palmbach, J., Schuh, A., Gao, J., Farahibozorg, S.-R., O'Muircheartaigh, J., Ciarrusta, J., O'Keeffe, C., … Andersson, J. (2020). The developing Human Connectome Project (dHCP) automated resting-state functional processing framework for newborn infants. *NeuroImage*, *223*, 117303. https://doi.org/10.1016/j.neuroimage.2020.117303

Flanagan, T., Russo, N., Flores, H., & Burack, J. (2008). *The developmental approach to the study of Down syndrome: Contemporary issues in historical perspective*. https://library.down-syndrome.org/en-us/research-practice/online/2008/developmental-approach-down-syndrome-contemporary-issues-historical-perspective

Fogliarini, C., Chaumoitre, K., Chapon, F., Fernandez, C., Lévrier, O., Figarella-Branger, D., & Girard, N. (2005). Assessment of cortical maturation with prenatal MRI. Part I: Normal cortical maturation. *European Radiology*, *15*(8), 1671–1685.

Fox, L. M., Choo, P., Rogerson, S. R., Spittle, A. J., Anderson, P. J., Doyle, L., & Cheong, J. L. Y. (2014). The relationship between ventricular size at 1 month and outcome at 2 years in infants less than 30 weeks' gestation. *Archives of Disease in Childhood - Fetal and Neonatal Edition*, *99*(3), F209–F214. https://doi.org/10.1136/archdischild-2013-304374

Fox, S. E., Levitt, P., & Nelson III, C. A. (2010). How the Timing and Quality of Early Experiences Influence the Development of Brain Architecture. *Child Development*, *81*(1), 28–40. https://doi.org/10.1111/j.1467-8624.2009.01380.x

Fryer, S. L., Frank, L. R., Spadoni, A. D., Theilmann, R. J., Nagel, B. J., Schweinsburg, A. D., & Tapert, S. F. (2008). Microstructural integrity of the corpus callosum linked with neuropsychological performance in adolescents. *Brain and Cognition*, *67*(2), 225–233. https://doi.org/10.1016/j.bandc.2008.01.009

Fukami-Gartner, A., Baburamani, A. A., Dimitrova, R., Patkee, P. A., Ojinaga Alfageme, O., Bonthrone, A. F., Uus, A., Hughes, E. J., Deprez, M., Counsell, S. J., Hajnal, J. V., Edwards, A. D., O'Muircheartaigh, J., & Rutherford, M. A. (2021). Individualised assessment of regional brain volumes in neonates with Down syndrome reveals extreme deviation in white matter and cerebellum [Manuscript in preparation]. *Centre for the Developing Brain, School of Biomedical Engineering and Imaging Sciences, King's College London*.

Gao, J., Li, X., Hou, X., Ding, A., Chan, K. C., Sun, Q., Wu, E. X., & Yang, J. (2012). Tract-based spatial statistics (TBSS): Application to detecting white matter tract variation in mild hypoxic-ischemic neonates. *2012 Annual International Conference of the IEEE Engineering in Medicine and Biology Society*, 432–435. https://doi.org/10.1109/EMBC.2012.6345960

Giedd, J. N., & Rapoport, J. L. (2010). Structural MRI of Pediatric Brain Development: What Have We Learned and Where Are We Going? *Neuron*, *67*(5), 728–734. https://doi.org/10.1016/j.neuron.2010.08.040

Gilmore, J. H., Smith, L. C., Wolfe, H. M., Hertzberg, B. S., Smith, J. K., Chescheir, N. C., Evans, D. D., Kang, C., Hamer, R. M., Lin, W., & Gerig, G. (2008). Prenatal Mild Ventriculomegaly Predicts Abnormal Development of the Neonatal Brain. *Biological Psychiatry*, *64*(12), 1069–1076. https://doi.org/10.1016/j.biopsych.2008.07.031

Glass, T. J. A., Chau, V., Gardiner, J., Foong, J., Vinall, J., Zwicker, J. G., Grunau, R. E., Synnes, A., Poskitt, K. J., & Miller, S. P. (2017). Severe retinopathy of prematurity predicts delayed white matter maturation and poorer neurodevelopment. *Archives of Disease in Childhood - Fetal and Neonatal Edition*, *102*(6), F532–F537. https://doi.org/10.1136/archdischild-2016-312533

Golden, J. A., & Hyman, B. T. (1994). Development of the Superior Temporal Neocortex Is Anomalous in Trisomy 21. *Journal of Neuropathology & Experimental Neurology*, *53*(5), 513–520. https://doi.org/10.1097/00005072-199409000-00011

Grieco, J., Pulsifer, M., Seligsohn, K., Skotko, B., & Schwartz, A. (2015). Down syndrome: Cognitive and behavioral functioning across the lifespan. *American*

Journal of Medical Genetics Part C: Seminars in Medical Genetics, *169*(2), 135–149. https://doi.org/10.1002/ajmg.c.31439

Gui, L., Loukas, S., Lazeyras, F., Hüppi, P. S., Meskaldji, D. E., & Borradori Tolsa, C. (2018). Longitudinal study of neonatal brain tissue volumes in preterm infants and their ability to predict neurodevelopmental outcome. *NeuroImage*. https://doi.org/10.1016/j.neuroimage.2018.06.034

Guidi, S., Bonasoni, P., Ceccarelli, C., Santini, D., Gualtieri, F., Ciani, E., & Bartesaghi, R. (2008). RESEARCH ARTICLE: Neurogenesis Impairment and Increased Cell Death Reduce Total Neuron Number in the Hippocampal Region of Fetuses with Down Syndrome. *Brain Pathology*, *18*(2), 180–197. https://doi.org/10.1111/j.1750-3639.2007.00113.x

Guidi, S., Ciani, E., Bonasoni, P., Santini, D., & Bartesaghi, R. (2011). Widespread Proliferation Impairment and Hypocellularity in the Cerebellum of Fetuses with Down Syndrome. *Brain Pathology*, *21*(4), 361–373. https://doi.org/10.1111/j.1750-3639.2010.00459.x

Guidi, S., Giacomini, A., Stagni, F., Emili, M., Uguagliati, B., Bonasoni, M. P., & Bartesaghi, R. (2018). Abnormal development of the inferior temporal region in fetuses with Down syndrome. *Brain Pathology*, *28*(6), 986–998. https://doi.org/10.1111/bpa.12605

Guihard-Costa, A.-M., Khung, S., Delbecque, K., Ménez, F., & Delezoide, A.-L. (2006). Biometry of Face and Brain in Fetuses with Trisomy 21. *Pediatric Research*, *59*(1), 33–38. https://doi.org/10.1203/01.pdr.0000190580.88391.9a

Gunbey, H. P., Bilgici, M. C., Aslan, K., Has, A. C., Ogur, M. G., Alhan, A., & Incesu, L. (2017). Structural brain alterations of Down's syndrome in early childhood evaluation by DTI and volumetric analyses. *European Radiology*, *27*(7), 3013–3021. https://doi.org/10.1007/s00330-016-4626-6

Hammill, D. D., Pearson, N. A., & Voress, J. K. (1994). *TPV. Test di percezione visiva e integrazione visuo-motoria* (Vol. 12). Edizioni Erickson.

Hansen-Pupp, I., Hövel, H., Löfqvist, C., Hellström-Westas, L., Fellman, V., Hüppi, P. S., Hellström, A., & Ley, D. (2013). Circulatory insulin-like growth factor-I and

brain volumes in relation to neurodevelopmental outcome in very preterm infants. *Pediatric Research*, *74*(5), 564–569. https://doi.org/10.1038/pr.2013.135

Haydar, T. F. (2020). Neurogenesis, Myelination, and Circuitry: The Case for a Distributed Therapeutic Regimen in Down Syndrome. *American Journal on Intellectual and Developmental Disabilities*, *125*(2), 100–102. https://doi.org/10.1352/1944-7558-125.2.100

Haydar, T. F., & Reeves, R. H. (2012). Trisomy 21 and early brain development. *Trends in Neurosciences*, *35*(2), 81–91. https://doi.org/10.1016/j.tins.2011.11.001

Horga, G., Kaur, T., & Peterson, B. S. (2014). Annual Research Review: Current limitations and future directions in MRI studies of child- and adult-onset developmental psychopathologies. *Journal of Child Psychology and Psychiatry*, *55*(6), 659–680. https://doi.org/10.1111/jcpp.12185

Hoshino, M. (2016). Specification of Cerebellar Neurons. In D. L. Gruol, N. Koibuchi, M. Manto, M. Molinari, J. D. Schmahmann, & Y. Shen (Eds.), *Essentials of Cerebellum and Cerebellar Disorders: A Primer For Graduate Students* (pp. 143–147). Springer International Publishing. https://doi.org/10.1007/978-3-319-24551-5_15

Hughes, E. J., Winchman, T., Padormo, F., Teixeira, R., Wurie, J., Sharma, M., Fox, M., Hutter, J., Cordero-Grande, L., Price, A. N., Allsop, J., Bueno-Conde, J., Tusor, N., Arichi, T., Edwards, A. D., Rutherford, M. A., Counsell, S. J., & Hajnal, J. V. (2017). A dedicated neonatal brain imaging system. *Magnetic Resonance in Medicine*, *78*(2), 794–804. https://doi.org/10.1002/mrm.26462

Hüppi, P. S., & Dubois, J. (2006). Diffusion tensor imaging of brain development. *Seminars in Fetal and Neonatal Medicine*, *11*(6), 489–497. https://doi.org/10.1016/j.siny.2006.07.006

Hutter, J., Tournier, J. D., Price, A. N., Cordero-Grande, L., Hughes, E. J., Malik, S., Steinweg, J., Bastiani, M., Sotiropoulos, S. N., Jbabdi, S., Andersson, J., Edwards, A. D., & Hajnal, J. V. (2018). Time-efficient and flexible design of optimized multishell HARDI diffusion. *Magnetic Resonance in Medicine*, *79*(3), 1276–1292. https://doi.org/10.1002/mrm.26765

Jarrold, C., & Brock, J. (2004). To Match or Not to Match? Methodological Issues in Autism-Related Research. *Journal of Autism and Developmental Disorders*, *34*(1), 81–86. https://doi.org/10.1023/B:JADD.0000018078.82542.ab

Jeong, J.-W., Lee, M.-H., Fernandes, N., Deol, S., Mody, S., Arslanturk, S., Chinnam, R. B., & Tan, S. (2021). Neonatal encephalopathy prediction of poor outcome with diffusion-weighted imaging connectome and fixel-based analysis. *Pediatric Research*, 1–11. https://doi.org/10.1038/s41390-021-01550-2

Jeurissen, B., Tournier, J.-D., Dhollander, T., Connelly, A., & Sijbers, J. (2014). Multi-tissue constrained spherical deconvolution for improved analysis of multi-shell diffusion MRI data. *NeuroImage*, *103*, 411–426. https://doi.org/10.1016/j.neuroimage.2014.07.061

Jiang, S., Xue, H., Counsell, S., Anjari, M., Allsop, J., Rutherford, M., Rueckert, D., & Hajnal, J. V. (2009). Diffusion tensor imaging (DTI) of the brain in moving subjects: Application to in-utero fetal and ex-utero studies. *Magnetic Resonance in Medicine*, *62*(3), 645–655. https://doi.org/10.1002/mrm.22032

Jo, A., & Limperopoulos, C. (2019). Impaired Amygdala and Hippocampal Growth in Neonates with Congenital Heart Disease. *GW Research Days 2016 - 2020*. https://hsrc.himmelfarb.gwu.edu/gw_research_days/2019/CNHS/6

Johansen-Berg, H. (2010). Behavioural relevance of variation in white matter microstructure. *Current Opinion in Neurology*, *23*(4), 351–358. https://doi.org/10.1097/WCO.0b013e32833b7631

Johnson, M. H. (2005). Sensitive periods in functional brain development: Problems and prospects. *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology*, *46*(3), 287–292.

Johnson, M. H., & de Haan, M. (2015). Methods and Populations. In *Developmental Cognitive Neuroscience: An introduction* (Fourth, pp. 17–31). Wiley Blackwell.

Johnson, S., & Marlow, N. (2006). Developmental screen or developmental testing? *Early Human Development*, *82*(3), 173–183. https://doi.org/10.1016/j.earlhumdev.2006.01.008

Jones, D. K., Knösche, T. R., & Turner, R. (2013). White matter integrity, fiber count, and other fallacies: The do's and don'ts of diffusion MRI. *NeuroImage*, *73*, 239–254. https://doi.org/10.1016/j.neuroimage.2012.06.081

Kaller, M. S., Lazari, A., Blanco-Duque, C., Sampaio-Baptista, C., & Johansen-Berg, H. (2017). Myelin plasticity and behaviour—Connecting the dots. *Current Opinion in Neurobiology*, *47*, 86–92. https://doi.org/10.1016/j.conb.2017.09.014

Kanold, P. O., & Luhmann, H. J. (2010). The Subplate and Early Cortical Circuits. *Annual Review of Neuroscience*, *33*(1), 23–48. https://doi.org/10.1146/annurev-neuro-060909-153244

Kaplan, E., Goodglass, H., Weintraub, S. L., & Goodglass, H. L. (1983). *Boston Naming Test*. L & Febiger.

Karmacharya, S., Gagoski, B., Ning, L., Vyas, R., Cheng, H. H., Soul, J., Newberger, J. W., Shenton, M. E., Rathi, Y., & Grant, P. E. (2018). Advanced diffusion imaging for assessing normal white matter development in neonates and characterizing aberrant development in congenital heart disease. *NeuroImage: Clinical*, *19*, 360–373. https://doi.org/10.1016/j.nicl.2018.04.032

Karmiloff-Smith, A. (2006). The tortuous route from genes to behavior: A neuroconstructivist approach. *Cognitive, Affective, & Behavioral Neuroscience*, *6*(1), 9–17. https://doi.org/10.3758/CABN.6.1.9

Karmiloff-smith, A. (2009). Nativism Versus Neuroconstructivism: Rethinking the Study of Developmental Disorders. *Developmental Psychology*, *45*(1), 56–63. https://doi.org/10.1037/a0014506

Karmiloff-Smith, A. (2010). Neuroimaging of the developing brain: Taking "developing" seriously. *Human Brain Mapping*, *31*(6), 934–941. https://doi.org/10.1002/hbm.21074

Karmiloff-Smith, A. (2012). Brain: The neuroconstructivist approach. In E. K. Farran & A. Karmiloff-Smith (Eds.), *Neurodevelopmental Disorders Across the Lifespan: A Neuroconstructivist Approach* (pp. 37–58). Oxford University Press, Incorporated.

Karmiloff-Smith, A. (2018). Neuroimaging of the developing brain: Taking "developing" seriously. In *Thinking Developmentally from Constructivism to Neuroconstructivism*. Routledge.

Karmiloff-Smith, A., Al-Janabi, T., D'Souza, H., Groet, J., Massand, E., Mok, K., Startin, C., Fisher, E., Hardy, J., Nizetic, D., Tybulewicz, V., & Strydom, A. (2016). The importance of understanding individual differences in Down syndrome. *F1000Research*, *5*. https://doi.org/10.12688/f1000research.7506.1

Karmiloff-Smith, A., Scerif, G., & Ansari, D. (2003). Double Dissociations in Developmental Disorders? Theoretically Misconceived, Empirically Dubious. *Cortex*, *39*(1), 161–163. https://doi.org/10.1016/S0010-9452(08)70091-1

Karmiloff-Smith, A., Scerif, G., & Thomas, M. (2002). Different approaches to relating genotype to phenotype in developmental disorders. *Developmental Psychobiology*, *40*(3), 311–322. https://doi.org/10.1002/dev.10035

Kelly, C. E., Thompson, D. K., Spittle, A. J., Chen, J., Seal, M. L., Anderson, P. J., Doyle, L. W., & Cheong, J. L. (2020). Regional brain volumes, microstructure and neurodevelopment in moderate–late preterm children. *Archives of Disease in Childhood - Fetal and Neonatal Edition*, *105*(6), 593–599. https://doi.org/10.1136/archdischild-2019-317941

Kennedy, K. M., & Raz, N. (2005). Age, Sex and Regional Brain Volumes Predict Perceptual-Motor Skill Acquisition. *Cortex*, *41*(4), 560–569. https://doi.org/10.1016/S0010-9452(08)70196-5

Keunen, K., Counsell, S. J., & Benders, M. J. N. L. (2017). The emergence of functional architecture during early brain development. *NeuroImage*, *160*, 2–14. https://doi.org/10.1016/j.neuroimage.2017.01.047

Keunen, K., Išgum, I., van Kooij, B. J. M., Anbeek, P., van Haastert, I. C., Koopman-Esseboom, C., Fieret-van Stam, P. C., Nievelstein, R. A. J., Viergever, M. A., de Vries, L. S., Groenendaal, F., & Benders, M. J. N. L. (2016). Brain Volumes at Term-Equivalent Age in Preterm Infants: Imaging Biomarkers for Neurodevelopmental Outcome through Early School Age. *The Journal of Pediatrics*, *172*, 88–95. https://doi.org/10.1016/j.jpeds.2015.12.023

Kim, S. Y., Kim, E.-K., Song, H., Cheon, J.-E., Kim, B. N., Kim, H.-S., & Shin, S. H. (2023). Association of Brain Microstructure and Functional Connectivity With Cognitive Outcomes and Postnatal Growth Among Early School–Aged Children Born With Extremely Low Birth Weight. *JAMA Network Open*, *6*(3), e230198. https://doi.org/10.1001/jamanetworkopen.2023.0198

King, M., Hernandez-Castillo, C. R., Poldrack, R. A., Ivry, R. B., & Diedrichsen, J. (2019). Functional boundaries in the human cerebellum revealed by a multi-domain task battery. *Nature Neuroscience*, *22*(8), 1371–1378. https://doi.org/10.1038/s41593-019-0436-x

Kleschevnikov, A. M., Belichenko, P. V., Faizi, M., Jacobs, L. F., Htun, K., Shamloo, M., & Mobley, W. C. (2012). Deficits in Cognition and Synaptic Plasticity in a Mouse Model of Down Syndrome Ameliorated by GABAB Receptor Antagonists. *Journal of Neuroscience*, *32*(27), 9217–9227. https://doi.org/10.1523/JNEUROSCI.1673-12.2012

Knowland, V. C. P., Purser, H., & Thomas, M. S. C. (2015). Cross-Sectional Methodologies in Developmental Psychology. In *International Encyclopedia of the Social & Behavioral Sciences* (pp. 354–360). Elsevier. https://doi.org/10.1016/B978-0-08-097086-8.23235-2

Kolasinski, J., Takahashi, E., Stevens, A. A., Benner, T., Fischl, B., Zöllei, L., & Grant, P. E. (2013). Radial and tangential neuronal migration pathways in the human fetal brain: Anatomically distinct patterns of diffusion MRI coherence. *NeuroImage*, *79*, 412–422. https://doi.org/10.1016/j.neuroimage.2013.04.125

Kontis, D., Catani, M., Cuddy, M., Walshe, M., Nosarti, C., Jones, D., Wyatt, J., Rifkin, L., Murray, R., & Allin, M. (2009). Diffusion tensor MRI of the corpus callosum and cognitive function in adults born preterm. *Neuroreport*, *20*(4), 424–428. https://doi.org/10.1097/WNR.0b013e328325a8f9

Kooij, B. J. M. V., Benders, M. J. N. L., Anbeek, P., Haastert, I. C. V., Vries, L. S. D., & Groenendaal, F. (2012). Cerebellar volume and proton magnetic resonance spectroscopy at term, and neurodevelopment at 2 years of age in preterm infants. *Developmental Medicine & Child Neurology*, *54*(3), 260–266. https://doi.org/10.1111/j.1469-8749.2011.04168.x

Kooij, B. J. M. van, Vries, L. S. de, Ball, G., Haastert, I. C. van, Benders, M. J. N. L., Groenendaal, F., & Counsell, S. J. (2012). Neonatal Tract-Based Spatial Statistics Findings and Outcome in Preterm Infants. *American Journal of Neuroradiology*, *33*(1), 188–194. https://doi.org/10.3174/ajnr.A2723

Korzh, V. (2018). Development of brain ventricular system. *Cellular and Molecular Life Sciences*, *75*(3), 375–383. https://doi.org/10.1007/s00018-017-2605-y

Kostovic, I., & Rakic, P. (1990). Developmental history of the transient subplate zone in the visual and somatosensory cortex of the macaque monkey and human brain. *Journal of Comparative Neurology*, *297*(3), 441–470. https://doi.org/10.1002/cne.902970309

Kostović, I., Sedmak, G., Vukšić, M., & Judaš, M. (2014). The Relevance of Human Fetal Subplate Zone for Developmental Neuropathology of Neuronal Migration Disorders and Cortical Dysplasia. *CNS Neuroscience & Therapeutics*, *21*(2), 74–82. https://doi.org/10.1111/cns.12333

Kulkarni, V. A., & Firestein, B. L. (2012). The dendritic tree and brain disorders. *Molecular and Cellular Neuroscience*, *50*(1), 10–20. https://doi.org/10.1016/j.mcn.2012.03.005

Kunz, N., Zhang, H., Vasung, L., O'Brien, K. R., Assaf, Y., Lazeyras, F., Alexander, D. C., & Hüppi, P. S. (2014). Assessing white matter microstructure of the newborn with multi-shell diffusion MRI and biophysical compartment models. *NeuroImage*, *96*, 288–299. https://doi.org/10.1016/j.neuroimage.2014.03.057

Kyriakopoulou, V., Vatansever, D., Elkommos, S., Dawson, S., McGuinness, A., Allsop, J., Molnár, Z., Hajnal, J., & Rutherford, M. (2014). Cortical Overgrowth in Fetuses With Isolated Ventriculomegaly. *Cerebral Cortex*, *24*(8), 2141–2150. https://doi.org/10.1093/cercor/bht062

Lagan, N., Huggard, D., Mc Grane, F., Leahy, T. R., Franklin, O., Roche, E., Webb, D., O' Marcaigh, A., Cox, D., El-Khuffash, A., Greally, P., Balfe, J., & Molloy, E. J. (2020). Multiorgan involvement and management in children with Down syndrome. *Acta Paediatrica*, *109*(6), 1096–1111. https://doi.org/10.1111/apa.15153

Larsen, K. B., Laursen, H., Græm, N., Samuelsen, G. B., Bogdanovic, N., & Pakkenberg, B. (2008). Reduced cell number in the neocortical part of the human fetal brain in Down syndrome. *Annals of Anatomy - Anatomischer Anzeiger*, *190*(5), 421–427. https://doi.org/10.1016/j.aanat.2008.05.007

Lautarescu, A., Hadaya, L., Craig, M. C., Makropoulos, A., Batalle, D., Nosarti, C., Edwards, A. D., Counsell, S. J., & Victor, S. (2021). Exploring the relationship between maternal prenatal stress and brain structure in premature neonates. *PLOS ONE*, *16*(4), e0250413. https://doi.org/10.1371/journal.pone.0250413

Lautarescu, A., Pecheva, D., Nosarti, C., Nihouarn, J., Zhang, H., Victor, S., Craig, M., Edwards, A. D., & Counsell, S. J. (2020). Maternal Prenatal Stress Is Associated With Altered Uncinate Fasciculus Microstructure in Premature Neonates. *Biological Psychiatry*, *87*(6), 559–569. https://doi.org/10.1016/j.biopsych.2019.08.010

Lebel, C., Gee, M., Camicioli, R., Wieler, M., Martin, W., & Beaulieu, C. (2012). Diffusion tensor imaging of white matter tract evolution over the lifespan. *NeuroImage*, *60*(1), 340–352. https://doi.org/10.1016/j.neuroimage.2011.11.094

Lee, N. R., Nayak, A., Irfanoglu, M. O., Sadeghi, N., Stoodley, C. J., Adeyemi, E., Clasen, L. S., & Pierpaoli, C. (2020). Hypoplasia of cerebellar afferent networks in Down syndrome revealed by DTI-driven tensor based morphometry. *Scientific Reports*, *10*(1), 5447. https://doi.org/10.1038/s41598-020-61799-1

Leto, K., Hawkes, R., & Consalez, G. G. (2016). Cerebellar Neurogenesis. In D. L. Gruol, N. Koibuchi, M. Manto, M. Molinari, J. D. Schmahmann, & Y. Shen (Eds.), *Essentials of Cerebellum and Cerebellar Disorders: A Primer For Graduate Students* (pp. 127–135). Springer International Publishing. https://doi.org/10.1007/978-3-319-24551-5_13

Lin, A.-L., Powell, D., Caban-Holt, A., Jicha, G., Robertson, W., Gold, B. T., Davis, R., Abner, E., Wilcock, D. M., Schmitt, F. A., & Head, E. (2016). 1H-MRS metabolites in adults with Down syndrome: Effects of dementia. *NeuroImage: Clinical*, *11*, 728–735. https://doi.org/10.1016/j.nicl.2016.06.001

Lloyd-Fox, S. (2014). Researching the brain in neurodevelopmental disorders. In J. Van Herwegen & D. Riby (Eds.), *Neurodevelopmental Disorders* (pp. 48–69). Psychology Press.

Lloyd-Fox, S., Blasi, A., Mercure, E., Elwell, C. E., & Johnson, M. H. (2012). The emergence of cerebral specialization for the human voice over the first months of life. *Social Neuroscience*, *7*(3), 317–330. https://doi.org/10.1080/17470919.2011.614696

Lockwood Estrin, G., Kyriakopoulou, V., Makropoulos, A., Ball, G., Kuhendran, L., Chew, A., Hagberg, B., Martinez-Biarge, M., Allsop, J., Fox, M., Counsell, S. J., & Rutherford, M. A. (2016). Altered white matter and cortical structure in neonates with antenatally diagnosed isolated ventriculomegaly. *NeuroImage: Clinical*, *11*, 139–148. https://doi.org/10.1016/j.nicl.2016.01.012

Lowe, J. R., Erickson, S. J., Schrader, R., & Duncan, A. F. (2012). Comparison of the Bayley II Mental Developmental Index and the Bayley III Cognitive Scale: Are we measuring the same thing? *Acta Paediatrica (Oslo, Norway: 1992)*, *101*(2), e55–e58. https://doi.org/10.1111/j.1651-2227.2011.02517.x

Lowery, L. A., & Sive, H. (2009). Totally tubular: The mystery behind function and origin of the brain ventricular system. *BioEssays*, *31*(4), 446–458. https://doi.org/10.1002/bies.200800207

Lu, J., Esposito, G., Scuderi, C., Steardo, L., Delli-Bovi, L. C., Hecht, J. L., Dickinson, B. C., Chang, C. J., Mori, T., & Sheen, V. (2011). S100B and APP Promote a Gliocentric Shift and Impaired Neurogenesis in Down Syndrome Neural Progenitors. *PLoS ONE*, *6*(7). https://doi.org/10.1371/journal.pone.0022126

Lu, Y.-C., Kapse, K., Andersen, N., Quistorff, J., Lopez, C., Fry, A., Cheng, J., Andescavage, N., Wu, Y., Espinosa, K., Vezina, G., du Plessis, A., & Limperopoulos, C. (2021). Association Between Socioeconomic Status and In Utero Fetal Brain Development. *JAMA Network Open*, *4*(3), e213526. https://doi.org/10.1001/jamanetworkopen.2021.3526

Madden, D. J., Bennett, I. J., & Song, A. W. (2009). Cerebral White Matter Integrity and Cognitive Aging: Contributions from Diffusion Tensor Imaging.

Neuropsychology Review, *19*(4), 415–435. https://doi.org/10.1007/s11065-009-9113-2

Malamateniou, C., Malik, S. J., Counsell, S. J., Allsop, J. M., McGuinness, A. K., Hayat, T., Broadhouse, K., Nunes, R. G., Ederies, A. M., Hajnal, J. V., & Rutherford, M. A. (2013). Motion-Compensation Techniques in Neonatal and Fetal MR Imaging. *American Journal of Neuroradiology*, *34*(6), 1124–1136. https://doi.org/10.3174/ajnr.A3128

Maloney, E. S., & Larrivee, L. S. (2007). Limitations of age-equivalent scores in reporting the results of norm-referenced tests. *Contemporary Issues in Communication Science and Disorders*, 34(Fall), 86–93.

Makropoulos, A., Aljabar, P., Wright, R., Hüning, B., Merchant, N., Arichi, T., Tusor, N., Hajnal, J. V., Edwards, A. D., Counsell, S. J., & Rueckert, D. (2016). Regional growth and atlasing of the developing human brain. *NeuroImage*, *125*, 456–478. https://doi.org/10.1016/j.neuroimage.2015.10.047

Makropoulos, A., Gousias, I. S., Ledig, C., Aljabar, P., Serag, A., Hajnal, J. V., Edwards, A. D., Counsell, S. J., & Rueckert, D. (2014). Automatic Whole Brain MRI Segmentation of the Developing Neonatal Brain. *IEEE Transactions on Medical Imaging*, *33*(9), 1818–1831. https://doi.org/10.1109/TMI.2014.2322280

Makropoulos, A., Robinson, E. C., Schuh, A., Wright, R., Fitzgibbon, S., Bozek, J., Counsell, S. J., Steinweg, J., Vecchiato, K., Passerat-Palmbach, J., Lenz, G., Mortari, F., Tenev, T., Duff, E. P., Bastiani, M., Cordero-Grande, L., Hughes, E., Tusor, N., Tournier, J.-D., … Rueckert, D. (2018). The developing human connectome project: A minimal processing pipeline for neonatal cortical surface reconstruction. *NeuroImage*, *173*, 88–112. https://doi.org/10.1016/j.neuroimage.2018.01.054

Mandelli, M. L., Caverzasi, E., Binney, R. J., Henry, M. L., Lobach, I., Block, N., Amirbekian, B., Dronkers, N., Miller, B. L., Henry, R. G., & Gorno-Tempini, M. L. (2014). Frontal White Matter Tracts Sustaining Speech Production in Primary Progressive Aphasia. *Journal of Neuroscience*, *34*(29), 9754–9767. https://doi.org/10.1523/JNEUROSCI.3464-13.2014

Marchal, J. P., Maurice-Stam, H., Houtzager, B. A., Rutgers van Rozenburg-Marres, S. L., Oostrom, K. J., Grootenhuis, M. A., & van Trotsenburg, A. S. P. (2016). Growing up with Down syndrome: Development from 6 months to 10.7 years. *Research in Developmental Disabilities*, *59*, 437–450. https://doi.org/10.1016/j.ridd.2016.09.019

Mareschal, D., Johnson, M. H., Sirois, S., Spratling, M., Thomas, M. S. C., & Westermann, G. (2007). *Neuroconstructivism - I: How the brain constructs cognition*. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780198529910.001.0001

Marques, A. H., Bjørke-Monsen, A.-L., Teixeira, A. L., & Silverman, M. N. (2015). Maternal stress, nutrition and physical activity: Impact on immune function, CNS development and psychopathology. *Brain Research*, *1617*, 28–46. https://doi.org/10.1016/j.brainres.2014.10.051

Mattie, L. J., Loveall, S. J., Channell, M. M., & Rodgers, D. B. (2023). Perspectives on adaptive functioning and intellectual functioning measures for intellectual disabilities behavioral research. *Frontiers in Psychology*, 14. https://www.frontiersin.org/articles/10.3389/fpsyg.2023.1084576

McCarron, M., McCallion, P., Reilly, E., & Mulryan, N. (2014). A prospective 14-year longitudinal follow-up of dementia in persons with Down syndrome. *Journal of Intellectual Disability Research: JIDR*, *58*(1), 61–70. https://doi.org/10.1111/jir.12074

Menghini, D., Costanzo, F., & Vicari, S. (2011). Relationship Between Brain and Cognitive Processes in Down Syndrome. *Behavior Genetics*, *41*(3), 381–393. https://doi.org/10.1007/s10519-011-9448-3

Messerschmidt, A., Fuiko, R., Prayer, D., Brugger, P. C., Boltshauser, E., Zoder, G., Sterniste, W., Weber, M., & Birnbacher, R. (2008). Disrupted cerebellar development in preterm infants is associated with impaired neurodevelopmental outcome. *European Journal of Pediatrics*, *167*(10), 1141–1147.

Meuwly, E., Feldmann, M., Knirsch, W., von Rhein, M., Payette, K., Dave, H., Tuura, R. O. G., Kottke, R., Hagmann, C., Latal, B., & Jakab, A. (2019). Postoperative brain volumes are associated with one-year neurodevelopmental outcome in

children with severe congenital heart disease. *Scientific Reports*, *9*(1), 10885. https://doi.org/10.1038/s41598-019-47328-9

Meyer, R., Bar-Yosef, O., Barzilay, E., Hoffman, D., Toussia-Cohen, S., Zvi, E., Achiron, R., & Katorza, E. (2018). Neurodevelopmental outcome of fetal isolated ventricular asymmetry without dilation: A cohort study. *Ultrasound in Obstetrics & Gynecology*, *52*(4), 467–472. https://doi.org/10.1002/uog.19065

Mithani, K., Davison, B., Meng, Y., & Lipsman, N. (2020). The anterior limb of the internal capsule: Anatomy, function, and dysfunction. *Behavioural Brain Research*, *387*, 112588. https://doi.org/10.1016/j.bbr.2020.112588

Moayedi, M., & Hodaie, M. (2019). Trigeminal nerve and white matter brain abnormalities in chronic orofacial pain disorders. *PAIN Reports*, *4*, e755. https://doi.org/10.1097/PR9.0000000000000755

Modo, M., & Bulte, J. W. M. (Eds.). (2011). *Magnetic Resonance Neuroimaging* (Vol. 711). Humana Press. https://doi.org/10.1007/978-1-61737-992-5

Monje, M. (2018). Myelin Plasticity and Nervous System Function. *Annual Review of Neuroscience*, *41*(1), 61–76. https://doi.org/10.1146/annurev-neuro-080317-061853

Monk, C., Georgieff, M. K., & Osterholm, E. A. (2013). Research Review: Maternal prenatal distress and poor nutrition – mutually influencing risk factors affecting infant neurocognitive development. *Journal of Child Psychology and Psychiatry*, *54*(2), 115–130. https://doi.org/10.1111/jcpp.12000

Morey, R. A., Petty, C. M., Xu, Y., Hayes, J. P., Wagner, H. R., Lewis, D. V., LaBar, K. S., Styner, M., & McCarthy, G. (2009). A comparison of automated segmentation and manual tracing for quantifying hippocampal and amygdala volumes. *NeuroImage*, *45*(3), 855–866. https://doi.org/10.1016/j.neuroimage.2008.12.033

Morton, J. (2008). *Understanding developmental disorders: A causal modelling approach*. John Wiley & Sons.

Morton, J., & Frith, U. (1995). *Causal modeling: A structural approach to developmental psychopathology.*

Mount, C. W., & Monje, M. (2017). Wrapped to Adapt: Experience-Dependent Myelination. *Neuron*, *95*(4), 743–756. https://doi.org/10.1016/j.neuron.2017.07.009

Mulkey, S. B., Ou, X., Ramakrishnaiah, R. H., Glasier, C. M., Swearingen, C. J., Melguizo, M. S., Yap, V. L., Schmitz, M. L., & Bhutta, A. T. (2014). White Matter Injury in Newborns With Congenital Heart Disease: A Diffusion Tensor Imaging Study. *Pediatric Neurology*, *51*(3), 377–383. https://doi.org/10.1016/j.pediatrneurol.2014.04.008

Mullen, E. M. (1995). *Mullen Scales of Early Learning: Manual* (AGS). Pearson.

Mundy, P., Kasari, C., Sigman, M., & Ruskin, E. (1995). Nonverbal Communication and Early Language Acquisition in Children With Down Syndrome and in Normally Developing Children. *Journal of Speech, Language, and Hearing Research*, *38*(1), 157–167. https://doi.org/10.1044/jshr.3801.157

Naef, N., Ciernik, A., Latal, B., & Liamlahi, R. (2023). Hippocampal volume and cognitive performance in children with congenital heart disease. *Pediatric Research*, *94*(1), Article 1. https://doi.org/10.1038/s41390-022-02457-2

Nadarajah, B., & Parnavelas, J. G. (2002). Modes of neuronal migration in the developing cerebral cortex. *Nature Reviews Neuroscience*, *3*(6), 423–432. https://doi.org/10.1038/nrn845

Nagy, Z., Westerberg, H., & Klingberg, T. (2004). Maturation of White Matter is Associated with the Development of Cognitive Functions during Childhood. *Journal of Cognitive Neuroscience*, *16*(7), 1227–1233. https://doi.org/10.1162/0898929041920441

Neale, N., Padilla, C., Fonseca, L. M., Holland, T., & Zaman, S. (2018). Neuroimaging and other modalities to assess Alzheimer's disease in Down syndrome. *NeuroImage: Clinical*, *17*, 263–271. https://doi.org/10.1016/j.nicl.2017.10.022

Nichols, T. E., Das, S., Eickhoff, S. B., Evans, A. C., Glatard, T., Hanke, M., Kriegeskorte, N., Milham, M. P., Poldrack, R. A., Poline, J.-B., Proal, E., Thirion, B., Van Essen, D. C., White, T., & Yeo, B. T. T. (2017). Best practices in data analysis and sharing in neuroimaging using MRI. *Nature Neuroscience*, *20*(3), 299–303. https://doi.org/10.1038/nn.4500

Northam, G. B., Liégeois, F., Chong, W. K., Baker, K., Tournier, J.-D., Wyatt, J. S., Baldeweg, T., & Morgan, A. (2012). Speech and Oromotor Outcome in Adolescents Born Preterm: Relationship to Motor Tract Integrity. *The Journal of Pediatrics*, *160*(3), 402-408.e1. https://doi.org/10.1016/j.jpeds.2011.08.055

Northam, G. B., Liégeois, F., Tournier, J.-D., Croft, L. J., Johns, P. N., Chong, W. K., Wyatt, J. S., & Baldeweg, T. (2012). Interhemispheric temporal lobe connectivity predicts language impairment in adolescents born preterm. *Brain*, *135*(12), 3781–3798. https://doi.org/10.1093/brain/aws276

Nowakowski, T. J., Pollen, A. A., Sandoval-Espinosa, C., & Kriegstein, A. R. (2016). Transformation of the Radial Glia Scaffold Demarcates Two Stages of Human Cerebral Cortex Development. *Neuron*, *91*(6), 1219–1227. https://doi.org/10.1016/j.neuron.2016.09.005

O'Halloran, C. J., Kinsella, G. J., & Storey, E. (2012). The cerebellum and neuropsychological functioning: A critical review. *Journal of Clinical and Experimental Neuropsychology*, *34*(1), 35–56. https://doi.org/10.1080/13803395.2011.614599

Olmos-Serrano, J. L., Kang, H. J., Tyler, W. A., Silbereis, J. C., Cheng, F., Zhu, Y., Pletikos, M., Jankovic-Rapan, L., Cramer, N. P., Galdzicki, Z., Goodliffe, J., Peters, A., Sethares, C., Delalle, I., Golden, J. A., Haydar, T. F., & Sestan, N. (2016). Down Syndrome Developmental Brain Transcriptome Reveals Defective Oligodendrocyte Differentiation and Myelination. *Neuron*, *89*(6), 1208–1222. https://doi.org/10.1016/j.neuron.2016.01.042

O'Rahilly, R., & Müller, F. (1990). Ventricular system and choroid plexuses of the human brain during the embryonic period proper. *American Journal of Anatomy*, *189*(4), 285–302. https://doi.org/10.1002/aja.1001890402

Osborne, L. R. (2012). Genes: The gene expression approach. In E. K. Farran & A. Karmiloff-Smith (Eds.), *Neurodevelopmental Disorders Across the Lifespan: A Neuroconstructivist Approach* (pp. 59–81). Oxford University Press, Incorporated.

Pangelinan, M. M., Zhang, G., VanMeter, J. W., Clark, J. E., Hatfield, B. D., & Haufler, A. J. (2011). Beyond age and gender: Relationships between cortical and

subcortical brain volume and cognitive-motor abilities in school-age children. *NeuroImage*, *54*(4), 3093–3100. https://doi.org/10.1016/j.neuroimage.2010.11.021

Pannek, K., Guzzetta, A., Colditz, P. B., & Rose, S. E. (2012). Diffusion MRI of the neonate brain: Acquisition, processing and analysis techniques. *Pediatric Radiology*, *42*(10), 1169–1182. https://doi.org/10.1007/s00247-012-2427-x

Paquette, L. B., Wisnowski, J. L., Ceschin, R., Pruetz, J. D., Detterich, J. A., Castillo, S. D., Nagasunder, A. C., Kim, R., Painter, M. J., Gilles, F. H., Nelson, M. D., Williams, R. G., Blüml, S., & Panigrahy, A. (2013). Abnormal Cerebral Microstructure in Premature Neonates with Congenital Heart Disease. *American Journal of Neuroradiology*, *34*(10), 2026–2033. https://doi.org/10.3174/ajnr.A3528

Patkee, P. A., Baburamani, A. A., Kyriakopoulou, V., Davidson, A., Avini, E., Dimitrova, R., Allsop, J., Hughes, E., Kangas, J., McAlonan, G., & Rutherford, M. A. (2020). Early alterations in cortical and cerebellar regional brain growth in Down Syndrome: An in vivo fetal and neonatal MRI assessment. *NeuroImage: Clinical*, *25*, 102139. https://doi.org/10.1016/j.nicl.2019.102139

Patkee, P. A., Baburamani, A. A., Long, K. R., Dimitrova, R., Ciarrusta, J., Allsop, J., Hughes, E., Kangas, J., McAlonan, G. M., Rutherford, M. A., & De Vita, E. (2021). Neurometabolite mapping highlights elevated myo-inositol profiles within the developing brain in down syndrome. *Neurobiology of Disease*, *153*, 105316. https://doi.org/10.1016/j.nbd.2021.105316

Peterson, B. S., Anderson, A. W., Ehrenkranz, R., Staib, L. H., Tageldin, M., Colson, E., Gore, J. C., Duncan, C. C., Makuch, R., & Ment, L. R. (2003). Regional Brain Volumes and Their Later Neurodevelopmental Correlates in Term and Preterm Infants. *Pediatrics*, *111*(5), 939–948. https://doi.org/10.1542/peds.111.5.939

Pierpaoli, C., Jezzard, P., Basser, P. J., Barnett, A., & Di Chiro, G. (1996). Diffusion tensor MR imaging of the human brain. *Radiology*, *201*(3), 637–648. https://doi.org/10.1148/radiology.201.3.8939209

Pietsch, M., Christiaens, D., Hutter, J., Cordero-Grande, L., Price, A. N., Hughes, E., Edwards, A. D., Hajnal, J. V., Counsell, S. J., & Tournier, J.-D. (2019). A framework for multi-component analysis of diffusion MRI data over the neonatal period. *NeuroImage*, *186*, 321–337. https://doi.org/10.1016/j.neuroimage.2018.10.060

Pinter, J. D., Eliez, S., Schmitt, J. E., Capone, G. T., & Reiss, A. L. (2001). Neuroanatomy of Down's Syndrome: A High-Resolution MRI Study. *American Journal of Psychiatry*, *158*(10), 1659–1665. https://doi.org/10.1176/appi.ajp.158.10.1659

Porter, E. J., Counsell, S. J., Edwards, A. D., Allsop, J., & Azzopardi, D. (2010). Tract-Based Spatial Statistics of Magnetic Resonance Images to Assess Disease and Treatment Effects in Perinatal Asphyxial Encephalopathy. *Pediatric Research*, *68*(3), Article 3. https://doi.org/10.1203/PDR.0b013e3181e9f1ba

Powell, D., Caban-Holt, A., Jicha, G., Robertson, W., Davis, R., Gold, B. T., Schmitt, F. A., & Head, E. (2014). Frontal white matter integrity in adults with Down syndrome with and without dementia. *Neurobiology of Aging*, *35*(7), 1562–1569. https://doi.org/10.1016/j.neurobiolaging.2014.01.137

Preda, C. (2000). VMI developmental test of visual motor integration. *Italian Version. Florence: Organizzazioni Speciali*.

Pujol, J., del Hoyo, L., Blanco-Hinojo, L., de Sola, S., Macià, D., Martínez-Vilavella, G., Amor, M., Deus, J., Rodríguez, J., Farré, M., Dierssen, M., & de la Torre, R. (2015). Anomalous brain functional connectivity contributing to poor adaptive behavior in Down syndrome. *Cortex*, *64*, 148–156. https://doi.org/10.1016/j.cortex.2014.10.012

Purger, D., Gibson, E. M., & Monje, M. (2016). Myelin plasticity in the central nervous system. *Neuropharmacology*, *110*, 563–573. https://doi.org/10.1016/j.neuropharm.2015.08.001

Purser, H., & Van Herwegen, J. (2016). Standardised and experimental psychological tasks: Issues and solutions for research with children. In J. Prior & J. Van Herwegen (Eds.), *Practical Research with Children* (p. 70). Psychology Press.

Raffelt, D. A., Smith, R. E., Ridgway, G. R., Tournier, J.-D., Vaughan, D. N., Rose, S., Henderson, R., & Connelly, A. (2015). Connectivity-based fixel enhancement: Whole-brain statistical analysis of diffusion MRI measures in the presence of crossing fibres. *NeuroImage*, *117*, 40–55. https://doi.org/10.1016/j.neuroimage.2015.05.039

Raffelt, D. A., Tournier, J.-D., Smith, R. E., Vaughan, D. N., Jackson, G., Ridgway, G. R., & Connelly, A. (2017). Investigating white matter fibre density and morphology using fixel-based analysis. *Neuroimage*, *144*(Pt A), 58–73. https://doi.org/10.1016/j.neuroimage.2016.09.029

Ravitsky, V., Roy, M.-C., Haidar, H., Henneman, L., Marshall, J., Newson, A. J., Ngan, O. M. Y., & Nov-Klaiman, T. (2021). The Emergence and Global Spread of Noninvasive Prenatal Testing. *Annual Review of Genomics and Human Genetics*, *22*(1), 309–338. https://doi.org/10.1146/annurev-genom-083118-015053

Reiche, L., Küry, P., & Göttle, P. (2019). Aberrant Oligodendrogenesis in Down Syndrome: Shift in Gliogenesis? *Cells*, *8*(12), 1591. https://doi.org/10.3390/cells8121591

Reynolds, T. (2010). The triple test as a screening technique for Down syndrome: Reliability and relevance. *International Journal of Women's Health*, *2*, 83–88.

Ritchie, S. J., Booth, T., Valdés Hernández, M. del C., Corley, J., Maniega, S. M., Gow, A. J., Royle, N. A., Pattie, A., Karama, S., Starr, J. M., Bastin, M. E., Wardlaw, J. M., & Deary, I. J. (2015). Beyond a bigger brain: Multivariable structural brain imaging and intelligence. *Intelligence*, *51*, 47–56. https://doi.org/10.1016/j.intell.2015.05.001

Rodríguez-Barrera, J. C., & Chaves-Castaño, L. (2017). ¿ Cuál es el nivel de desarrollo esperable para un niño con síndrome de Down en sus primeros años de vida? *Universitas Psychologica*, *16*(4).

Romano, A., Moraschi, M., Cornia, R., Bozzao, A., Rossi-Espagnet, M. C., Giove, F., Albertini, G., & Pierallini, A. (2018). White matter involvement in young non-demented Down's syndrome subjects: A tract-based spatial statistic analysis. *Neuroradiology*, *60*(12), 1335–1341. https://doi.org/10.1007/s00234-018-2102-5

Rose, J., Butler, E. E., Lamont, L. E., Barnes, P. D., Atlas, S. W., & Stevenson, D. K. (2009). Neonatal brain structure on MRI and diffusion tensor imaging, sex, and neurodevelopment in very-low-birthweight preterm children. *Developmental Medicine & Child Neurology*, *51*(7), 526–535. https://doi.org/10.1111/j.1469-8749.2008.03231.x

Rustioni, D. (1994). Prove di Valutazione della Comprensione Linguistica [Test of Linguistic Comprehension]. *Firenze, Italy: Giunti OS*.

Rutherford, M., Srinivasan, L., Dyet, L., Ward, P., Allsop, J., Counsell, S., & Cowan, F. (2006). Magnetic resonance imaging in perinatal brain injury: Clinical presentation, lesions and outcome. *Pediatric Radiology*, *36*(7), 582–592. https://doi.org/10.1007/s00247-006-0164-8

Sasson, E., Doniger, G., Pasternak, O., Tarrasch, R., & Assaf, Y. (2013). White matter correlates of cognitive domains in normal aging with diffusion tensor imaging. *Frontiers in Neuroscience*, *7*. https://www.frontiersin.org/article/10.3389/fnins.2013.00032

Sathyanesan, A., Zhou, J., Scafidi, J., Heck, D. H., Sillitoe, R. V., & Gallo, V. (2019). Emerging connections between cerebellar development, behaviour and complex brain disorders. *Nature Reviews Neuroscience*, *20*(5), 298–313. https://doi.org/10.1038/s41583-019-0152-2

Schilling, K. G., Nath, V., Hansen, C., Parvathaneni, P., Blaber, J., Gao, Y., Neher, P., Aydogan, D. B., Shi, Y., Ocampo-Pineda, M., Schiavi, S., Daducci, A., Girard, G., Barakovic, M., Rafael-Patino, J., Romascano, D., Rensonnet, G., Pizzolato, M., Bates, A., … Landman, B. A. (2019). Limits to anatomical accuracy of diffusion tractography using modern approaches. *NeuroImage*, *185*, 1–11. https://doi.org/10.1016/j.neuroimage.2018.10.029

Sentenac, M., Benhammou, V., Aden, U., Ancel, P.-Y., Bakker, L. A., Bakoy, H., Barros, H., Baumann, N., Bilsteen, J. F., Boerch, K., Croci, I., Cuttini, M., Draper, E., Halvorsen, T., Johnson, S., Källén, K., Land, T., Lebeer, J., Lehtonen, L., … Zeitlin, J. (2021). Maternal education and cognitive development in 15 European very-preterm birth cohorts from the RECAP Preterm platform. *International Journal of Epidemiology*, *50*(6), 1824–1839. https://doi.org/10.1093/ije/dyab170

Seo, Y. (2013). Effects of different field strengths, gradient directions, and acquisitions on fractional anisotropy in diffusion tensor imaging: A tract-based spatial statistics study. *Concepts in Magnetic Resonance Part B: Magnetic Resonance Engineering*, *43B*(1), 41–48. https://doi.org/10.1002/cmr.b.21230

Sereno, M. I., Diedrichsen, J., Tachrount, M., Testa-Silva, G., d'Arceuil, H., & Zeeuw, C. D. (2020). The human cerebellum has almost 80% of the surface area of the neocortex. *Proceedings of the National Academy of Sciences*, *117*(32), 19538–19543. https://doi.org/10.1073/pnas.2002896117

Seunarine, K. K., & Alexander, D. C. (2014). Chapter 6 - Multiple Fibers: Beyond the Diffusion Tensor. In H. Johansen-Berg & T. E. J. Behrens (Eds.), *Diffusion MRI (Second Edition)* (pp. 105–123). Academic Press. https://doi.org/10.1016/B978-0-12-396460-1.00006-8

Shapiro, B. L. (2001). Developmental instability of the cerebellum and its relevance to Down syndrome. In G. Lubec (Ed.), *Protein Expression in Down Syndrome Brain* (pp. 11–34). Springer Vienna. https://doi.org/10.1007/978-3-7091-6262-0_2

Shany, E., Inder, T. E., Goshen, S., Lee, I., Neil, J. J., Smyser, C. D., Doyle, L. W., Anderson, P. J., & Shimony, J. S. (2017). Diffusion tensor tractography of the cerebellar peduncles in prematurely born 7-year-old children. *The Cerebellum*, *16*(2), 314–325

Shim, S.-Y., Jeong, H.-J., Son, D. W., Jeong, J. S., Oh, S. H., Park, S.-Y., Ryu, T.-H., Kim, Y.-B., & Cho, Z.-H. (2012). Altered Microstructure of White Matter Except the Corpus Callosum Is Independent of Prematurity. *Neonatology*, *102*(4), 309–315. https://doi.org/10.1159/000341867

Śmigielska-Kuzia, J., Boćkowski, L., Sobaniec, W., Sendrowski, K., Olchowik, B., Cholewa, M., łukasiewicz, A., & łebkowska, U. (2011). A volumetric magnetic resonance imaging study of brain structures in children with Down syndrome. *Neurologia i Neurochirurgia Polska*, *45*(4), 363–369. https://doi.org/10.1016/S0028-3843(14)60107-9

Smith, R., Dhollander, T., & Connelly, A. (2019). On the regression of intracranial volume in fixel-based analysis. *Proc Int Soc Magn Reson Med Sci Meet Exhib*.

Smith, R. E., Tournier, J.-D., Calamante, F., & Connelly, A. (2013). SIFT: Spherical-deconvolution informed filtering of tractograms. *NeuroImage*, *67*, 298–312. https://doi.org/10.1016/j.neuroimage.2012.11.049

Smith, S. M., Jenkinson, M., Johansen-Berg, H., Rueckert, D., Nichols, T. E., Mackay, C. E., Watkins, K. E., Ciccarelli, O., Cader, M. Z., Matthews, P. M., & Behrens, T. E. J. (2006). Tract-based spatial statistics: Voxelwise analysis of multi-subject diffusion data. *NeuroImage*, *31*(4), 1487–1505. https://doi.org/10.1016/j.neuroimage.2006.02.024

Smith, S. M., Jenkinson, M., Woolrich, M. W., Beckmann, C. F., Behrens, T. E. J., Johansen-Berg, H., Bannister, P. R., De Luca, M., Drobnjak, I., Flitney, D. E., Niazy, R. K., Saunders, J., Vickers, J., Zhang, Y., De Stefano, N., Brady, J. M., & Matthews, P. M. (2004). Advances in functional and structural MR image analysis and implementation as FSL. *NeuroImage*, *23*, S208–S219. https://doi.org/10.1016/j.neuroimage.2004.07.051

Soukup-Ascençao, T., D'Souza, D., D'Souza, H., & Karmiloff-Smith, A. (2016). Parent-child interaction as a dynamic contributor to learning and cognitive development in typical and atypical development / Influencia dinámica entre la interacción padre/madre-hijo y el aprendizaje y el desarrollo cognitivo en el desarrollo típico y atípico. *Infancia y Aprendizaje*, *39*(4), 694–726. https://doi.org/10.1080/02103702.2016.1221054

Spann, M. N., Bansal, R., Hao, X., Rosen, T. S., & Peterson, B. S. (2020). Prenatal socioeconomic status and social support are associated with neonatal brain morphology, toddler language and psychiatric symptoms. *Child Neuropsychology*, *26*(2), 170–188. https://doi.org/10.1080/09297049.2019.1648641

Sparrow, S. S., Cicchetti, D. V., & Balla, D. A. (2005). *Vineland Adaptive Behavior Scales, Second Edition (Vineland-II)*. Pearson.

Sperber, D. (2001). In defense of massive modularity. *Language, Brain and Cognitive Development: Essays in Honor of Jacques Mehler*, *7*, 47–57.

Stagni, F., Giacomini, A., Emili, M., Guidi, S., & Bartesaghi, R. (2018). Neurogenesis impairment: An early developmental defect in Down syndrome. *Free Radical*

Biology and Medicine, *114*, 15–32. https://doi.org/10.1016/j.freeradbiomed.2017.07.026

Startin, C. M., D'Souza, H., Ball, G., Hamburg, S., Hithersay, R., Hughes, K. M. O., Massand, E., Karmiloff-Smith, A., Thomas, M. S. C., Strydom, A., Fisher, E., Nizetic, D., Hardy, J., Tybulewicz, V., Karmiloff-Smith, A., Thomas, M., Mareschal, D., Al-Janabi, T., Aggarwal, N., … LonDownS Consortium. (2020). Health comorbidities and cognitive abilities across the lifespan in Down syndrome. *Journal of Neurodevelopmental Disorders*, *12*(1), 4. https://doi.org/10.1186/s11689-019-9306-9

Startin, C. M., Hamburg, S., Hithersay, R., Al-Janabi, T., Mok, K. Y., Hardy, J., Strydom, A., Fisher, E., Nizetic, D., Hardy, J., Tybulewicz, V., Karmiloff-Smith, A., Aggarwal, N., Davies, A., Fodor-Wynne, L., Lowe, B., Rodger, E., Checkley, L., Gallagher, P., … Strydom, A. (2019). Cognitive markers of preclinical and prodromal Alzheimer's disease in Down syndrome. *Alzheimer's & Dementia*, *15*(2), 245–257. https://doi.org/10.1016/j.jalz.2018.08.009

Stella, G., Pizzoli, C., & Tressoldi, P. (2000). Peabody picture vocabulary test. Italian adaptation. *Omega Edizioni, Torino*.

Stiles, J. (2008a). Formation of the Neural Tube. In *The fundamentals of brain development: Integrating nature anad nurture*. Harvard University Press.

Stiles, J. (2008b). Late prenatal and postnatal changes in human brain structure. In *The fundamentals of brain development: Integrating nature anad nurture*. Harvard University Press.

Stiles, J. (2008c). Neuronal migration and neuronal differentiaiton. In *The fundamentals of brain development: Integrating nature anad nurture*. Harvard University Press.

Stiles, J. (2008d). The production of brain cells: Neurons and Glia. In *The fundamentals of brain development: Integrating nature anad nurture*. Harvard University Press.

Stiles, J., & Jernigan, T. L. (2010). The Basics of Brain Development. *Neuropsychology Review*, *20*(4), 327–348. https://doi.org/10.1007/s11065-010-9148-4

Stoodley, C. J., Desmond, J. E., & Schmahmann, J. D. (2013). Functional topography of the human cerebellum revealed by functional neuroimaging studies. *Tijdschrift. Jaartal*.

Stoodley, C. J., & Schmahmann, J. D. (2018). Chapter 4—Functional topography of the human cerebellum. In M. Manto & T. A. G. M. Huisman (Eds.), *Handbook of Clinical Neurology* (Vol. 154, pp. 59–70). Elsevier. https://doi.org/10.1016/B978-0-444-63956-1.00004-7

Sullivan, J. R., Winter, S. M., Sass, D. A., & Svenkerud, N. (2014). Assessing Growth in Young Children: A Comparison of Raw, Age-Equivalent, and Standard Scores Using the Peabody Picture Vocabulary Test. *Journal of Research in Childhood Education, 28*(2), 277–291. https://doi.org/10.1080/02568543.2014.883453

Tafreshi, D., Thompson, J. J., & Racine, T. P. (2014). An Analysis of the Conceptual Foundations of the Infant Preferential Looking Paradigm. *Human Development*, *57*(4), 222–240. https://doi.org/10.1159/000363487

Takahashi, M., Iwamoto, K., Fukatsu, H., Naganawa, S., Iidaka, T., & Ozaki, N. (2010). White matter microstructure of the cingulum and cerebellar peduncle is related to sustained attention and working memory: A diffusion tensor imaging study. *Neuroscience Letters*, *477*(2), 72–76. https://doi.org/10.1016/j.neulet.2010.04.031

Takeuchi, H., Taki, Y., Nouchi, R., Yokoyama, R., Kotozaki, Y., Nakagawa, S., Sekiguchi, A., Iizuka, K., Yamamoto, Y., Hanawa, S., Araki, T., Miyauchi, C. M., Shinada, T., Sakaki, K., Sassa, Y., Nozawa, T., Ikeda, S., Yokota, S., Daniele, M., & Kawashima, R. (2017). Global associations between regional gray matter volume and diverse complex cognitive functions: Evidence from a large sample study. *Scientific Reports*, *7*(1), 10014. https://doi.org/10.1038/s41598-017-10104-8

Taylor, H. G., Filipek, P. A., Juranek, J., Bangert, B., Minich, N., & Hack, M. (2011). Brain Volumes in Adolescents With Very Low Birth Weight: Effects on Brain Structure and Associations With Neuropsychological Outcomes. *Developmental Neuropsychology*, *36*(1), 96–117. https://doi.org/10.1080/87565641.2011.540544

Tcw, J., & Goate, A. M. (2017). Genetics of β-Amyloid Precursor Protein in Alzheimer's Disease. *Cold Spring Harbor Perspectives in Medicine*, *7*(6), a024539. https://doi.org/10.1101/cshperspect.a024539

Telford, E. J., Cox, S. R., Fletcher-Watson, S., Anblagan, D., Sparrow, S., Pataky, R., Quigley, A., Semple, S. I., Bastin, M. E., & Boardman, J. P. (2017). A latent measure explains substantial variance in white matter microstructure across the newborn human brain. *Brain Structure and Function*, *222*(9), 4023–4033. https://doi.org/10.1007/s00429-017-1455-6

ten Donkelaar, H. J., Lammens, M., Wesseling, P., & Hori, A. (2014). Development and Developmental Disorders of the Human Cerebellum. In H. J. ten Donkelaar, M. Lammens, & A. Hori (Eds.), *Clinical Neuroembryology: Development and Developmental Disorders of the Human Central Nervous System* (pp. 371–420). Springer. https://doi.org/10.1007/978-3-642-54687-7_8

ten Donkelaar, H. J., Lammens, M., Wesseling, P., Thijssen, H. O., & Renier, W. O. (2003). Development and developmentaldisorders of the human cerebellum. *Journal of Neurology*, *250*(9), 1025–1036. https://doi.org/10.1007/s00415-003-0199-9

Thomas, A. R., Lacadie, C., Vohr, B., Ment, L. R., & Scheinost, D. (2017). Fine Motor Skill Mediates Visual Memory Ability with Microstructural Neuro-correlates in Cerebellar Peduncles in Prematurely Born Adolescents. *Cerebral Cortex*, *27*(1), 322–329. https://doi.org/10.1093/cercor/bhw415

Thomas, C., Ye, F. Q., Irfanoglu, M. O., Modi, P., Saleem, K. S., Leopold, D. A., & Pierpaoli, C. (2014). Anatomical accuracy of brain connections derived from diffusion MRI tractography is inherently limited. *Proceedings of the National Academy of Sciences*, *111*(46), 16574–16579. https://doi.org/10.1073/pnas.1405672111

Thomas, M. S. C., Annaz, D., Ansari, D., Scerif, G., Jarrold, C., & Karmiloff-Smith, A. (2009). Using Developmental Trajectories to Understand Developmental Disorders. *Journal of Speech Language and Hearing Research*, *52*(2), 336. https://doi.org/10.1044/1092-4388(2009/07-0144)

Thomas, M. S. C., Ojinaga Alfageme, O., D'Souza, H., Patkee, P. A., Rutherford, M. A., Mok, K. Y., Hardy, J., & Karmiloff-Smith, A. (2020). A multi-level developmental approach to exploring individual differences in Down syndrome: Genes, brain, behaviour, and environment. *Research in Developmental Disabilities*, *104*, 103638. https://doi.org/10.1016/j.ridd.2020.103638

Thomas, M. S., & Karmiloff-Smith, A. (2002). Modelling typical and atypical cognitive development. *Handbook of Childhood Development*, 575–599.

Thompson, D. K., Wood, S. J., Doyle, L. W., Warfield, S. K., Lodygensky, G. A., Anderson, P. J., Egan, G. F., & Inder, T. E. (2008). Neonate hippocampal volumes: Prematurity, perinatal predictors, and 2-year outcome. *Annals of Neurology*, *63*(5), 642–651. https://doi.org/10.1002/ana.21367

Tocchio, S., Kline-Fath, B., Kanal, E., Schmithorst, V. J., & Panigrahy, A. (2015). MRI evaluation and safety in the developing brain. *Seminars in Perinatology*, *39*(2), 73–104. https://doi.org/10.1053/j.semperi.2015.01.002

Toffalini, E., Buono, S., Zagaria, T., Calcagnì, A., & Cornoldi, C. (2019). Using Z and age-equivalent scores to address WISC-IV floor effects for children with intellectual disability. *Journal of Intellectual Disability Research, 63*(6), 528–538. https://doi.org/10.1111/jir.12589

Tooley, U. A., Bassett, D. S., & Mackey, A. P. (2021). Environmental influences on the pace of brain development. *Nature Reviews Neuroscience*, *22*(6), 372–384. https://doi.org/10.1038/s41583-021-00457-5

Tournier, J.-D., Calamante, F., & Connelly, A. (2007). Robust determination of the fibre orientation distribution in diffusion MRI: Non-negativity constrained super-resolved spherical deconvolution. *NeuroImage*, *35*(4), 1459–1472. https://doi.org/10.1016/j.neuroimage.2007.02.016

Tournier, J.-D., Calamante, F., Gadian, D. G., & Connelly, A. (2004). Direct estimation of the fiber orientation density function from diffusion-weighted MRI data using spherical deconvolution. *NeuroImage*, *23*(3), 1176–1185. https://doi.org/10.1016/j.neuroimage.2004.07.037

Tournier, J.-D., Hughes, E., Tusor, N., Sotiropoulos, S. N., Jbabdi, S., Andersson, J., Rueckert, D., Edwards, A. D., & Hajnal, J. V. (2015). Data-driven optimisation of multi-shell HARDI. *Proc ISMRM*, *20*(2426).

Tournier, J.-D., Mori, S., & Leemans, A. (2011). Diffusion Tensor Imaging and Beyond. *Magnetic Resonance in Medicine*, *65*(6), 1532–1556. https://doi.org/10.1002/mrm.22924

Tournier, J.-D., Smith, R., Raffelt, D., Tabbara, R., Dhollander, T., Pietsch, M., Christiaens, D., Jeurissen, B., Yeh, C.-H., & Connelly, A. (2019). MRtrix3: A fast, flexible and open software framework for medical image processing and visualisation. *NeuroImage*, *202*, 116137. https://doi.org/10.1016/j.neuroimage.2019.116137

Travis, K. E., Leitner, Y., Feldman, H. M., & Ben-Shachar, M. (2015). Cerebellar white matter pathways are associated with reading skills in children and adolescents. *Human Brain Mapping*, *36*(4), 1536–1553. https://doi.org/10.1002/hbm.22721

Tsao, R., & Kindelberger, C. (2009). Variability of cognitive development in children with Down syndrome: Relevance of good reasons for using the cluster procedure. *Research in Developmental Disabilities*, *30*(3), 426–432. https://doi.org/10.1016/j.ridd.2008.10.009

Uguagliati, B., Al-Absi, A.-R., Stagni, F., Emili, M., Giacomini, A., Guidi, S., Nyengaard, J. R., & Bartesaghi, R. (2021). Early appearance of developmental alterations in the dendritic tree of the hippocampal granule cells in the Ts65Dn model of Down syndrome. *Hippocampus*, *31*(4), 435–447. https://doi.org/10.1002/hipo.23303

Urbina, S. (2014). *Essentials of Psychological Testing*. John Wiley & Sons.

Vacca, R. A., Bawari, S., Valenti, D., Tewari, D., Nabavi, S. F., Shirooie, S., Sah, A. N., Volpicella, M., Braidy, N., & Nabavi, S. M. (2019). Down syndrome: Neurobiological alterations and therapeutic targets. *Neuroscience & Biobehavioral Reviews*, *98*, 234–255. https://doi.org/10.1016/j.neubiorev.2019.01.001

van Kooij, B. J. M., van Pul, C., Benders, M. J. N. L., van Haastert, I. C., de Vries, L. S., & Groenendaal, F. (2011). Fiber Tracking at Term Displays Gender Differences

Regarding Cognitive and Motor Outcome at 2 Years of Age in Preterm Infants. *Pediatric Research*, *70*(6), 626–632. https://doi.org/10.1203/PDR.0b013e318232a963

Vasung, L., Huang, H., Jovanov-Milošević, N., Pletikos, M., Mori, S., & Kostović, I. (2010). Development of axonal pathways in the human fetal fronto-limbic brain: Histochemical characterization and diffusion tensor imaging. *Journal of Anatomy*, *217*(4), 400–417. https://doi.org/10.1111/j.1469-7580.2010.01260.x

Vender, C., Borgia, R., Bruno, S., Freo, P., & Zardini, G. (1981). Un test di ripetizione di frasi. Analisi delle performances di bambini normali (A test of sentence repetition. Analysis of performance in normal children). *Neuropsichiatria Infantile*, *243*, 819–831.

Vega, J. N., Hohman, T. J., Pryweller, J. R., Dykens, E. M., & Thornton-Wells, T. A. (2015). Resting-State Functional Connectivity in Individuals with Down Syndrome and Williams Syndrome Compared with Typically Developing Controls. *Brain Connectivity*, *5*(8), 461–475. https://doi.org/10.1089/brain.2014.0266

Vicari, S. (2006). Motor Development and Neuropsychological Patterns in Persons with Down Syndrome. *Behavior Genetics*, *36*(3), 355–364. https://doi.org/10.1007/s10519-006-9057-8

Vicari, S. (2007). *PROMEA: Prove di memoria e apprendimento per l'età evolutiva*. Giunti. Organizzazioni speciali.

von Rhein, M., Buchmann, A., Hagmann, C., Dave, H., Bernet, V., Scheer, I., Knirsch, W., Latal, B., Bernet, V., Bürki, C., Dave, H., Prêtre, R., Hagmann, C., Knirsch, W., Kretschmar, O., Kellenberger, C., Scheer, I., Latal, B., Liamlahi, R., … Sennhauser, F. H. (2015). Severe Congenital Heart Defects Are Associated with Global Reduction of Neonatal Brain Volumes. *The Journal of Pediatrics*, *167*(6), 1259-1263.e1. https://doi.org/10.1016/j.jpeds.2015.07.006

Wang, P. P. (1996). A neuropsychological profile of Down syndrome: Cognitive skills and brain morphology. *Mental Retardation and Developmental Disabilities Research Reviews*, *2*(2), 102–108. https://doi.org/10.1002/(SICI)1098-2779(1996)2:2<102::AID-MRDD8>3.0.CO;2-V

Wang, P. P., & Bellugi, U. (1993). Williams Syndrome, Down Syndrome, and Cognitive Neuroscience. *American Journal of Diseases of Children*, *147*(11), 1246–1251. https://doi.org/10.1001/archpedi.1993.02160350120019

Wang, V. Y., & Zoghbi, H. Y. (2001). Genetic regulation of cerebellar development. *Nature Reviews Neuroscience*, *2*(7), 484–491. https://doi.org/10.1038/35081558

Warren, J. E., Crinion, J. T., Lambon Ralph, M. A., & Wise, R. J. S. (2009). Anterior temporal lobe connectivity correlates with functional outcome after aphasic stroke. *Brain*, *132*(12), 3428–3442. https://doi.org/10.1093/brain/awp270

Webb, S. J., Monk, C. S., & Nelson, C. A. (2001). Mechanisms of Postnatal Neurobiological Development: Implications for Human Development. *Developmental Neuropsychology*, *19*(2), 147–171. https://doi.org/10.1207/S15326942DN1902_2

Weis, S., Weber, G., Neuhold, A., & Rett, A. (1991). Down syndrome: MR quantification of brain structures and comparison with normal control subjects. *American Journal of Neuroradiology*, *12*(6), 1207–1211.

Weitzdoerfer, R., Dierssen, M., Fountoulakis, M., & Lubec, G. (2001). Fetal life in Down Syndrome starts with normal neuronal density but impaired dendritic spines and synaptosomal structure. In G. Lubec (Ed.), *Protein Expression in Down Syndrome Brain* (pp. 59–70). Springer Vienna. https://doi.org/10.1007/978-3-7091-6262-0_5

Whittle, N., Sartori, S. B., Dierssen, M., Lubec, G., & Singewald, N. (2007). Fetal Down Syndrome Brains Exhibit Aberrant Levels of Neurotransmitters Critical for Normal Brain Development. *Pediatrics*, *120*(6), e1465–e1471. https://doi.org/10.1542/peds.2006-3448

Wiseman, F. K., Al-Janabi, T., Hardy, J., Karmiloff-Smith, A., Nizetic, D., Tybulewicz, V. L. J., Fisher, E. M. C., & Strydom, A. (2015). A genetic cause of Alzheimer disease: Mechanistic insights from Down syndrome. *Nature Reviews Neuroscience*, *16*(9), 564–574. https://doi.org/10.1038/nrn3983

Wishart, J. G., & Duffy, L. (1990). Instability of Performance on Cognitive Tests in Infants and Young Children with Down's Syndrome. *British Journal of*

Educational Psychology, *60*(1), 10–22. https://doi.org/10.1111/j.2044-8279.1990.tb00918.x

Wong, H. S., & Edwards, P. (2013). Nature or Nurture: A Systematic Review of the Effect of Socio-economic Status on the Developmental and Cognitive Outcomes of Children Born Preterm. *Maternal and Child Health Journal*, *17*(9), 1689–1700. https://doi.org/10.1007/s10995-012-1183-8

Zhang, H., Schneider, T., Wheeler-Kingshott, C. A., & Alexander, D. C. (2012). NODDI: Practical in vivo neurite orientation dispersion and density imaging of the human brain. *NeuroImage*, *61*(4), 1000–1016. https://doi.org/10.1016/j.neuroimage.2012.03.072

Zhang, H., Yushkevich, P. A., Alexander, D. C., & Gee, J. C. (2006). Deformable registration of diffusion tensor MR images with explicit orientation optimization. *Medical Image Analysis*, *10*(5), 764–785. https://doi.org/10.1016/j.media.2006.06.004

Zhu, D., Wang, M., Feng, F., Nan, N., Liu, Y., Shi, J., & Mao, B. (2021). Application of Diffusion Tensor Imaging in the Evaluation of Brain Injury in Premature Infants with Low and Very Low Birth Weight. *Iranian Journal of Radiology*, *18*(4), Article 4. https://doi.org/10.5812/iranjradiol.112648

Zorrilla de San Martin, J., Delabar, J.-M., Bacci, A., & Potier, M.-C. (2018). GABAergic over-inhibition, a promising hypothesis for cognitive deficits in Down syndrome. *Free Radical Biology and Medicine*, *114*, 33–39. https://doi.org/10.1016/j.freeradbiomed.2017.10.002