# New approaches to studying early brain development in Down Syndrome

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**Abstract (150-200 words):**

Down Syndrome (DS) is the most common genetic developmental disorder in humans and is caused by partial or complete triplication of human chromosome 21 (Hsa21; Trisomy 21). It is a complex condition which results in multiple lifelong health problems, including varying degrees of intellectual disability and delays in speech, memory and learning. As both length and quality of life are improving for individuals with DS, attention is now being directed to understanding and potentially treating the associated cognitive difficulties and their underlying biological substrates. These have included imaging and post mortem studies which have identified decreased regional brain volumes and histological anomalies which accompany the early onset dementia. In addition, advances in genome-wide analysis and DS mouse models are providing valuable insight into potential targets for intervention that could improve neurogenesis and long term cognition. As little is known about early brain development in human DS, in this article we also review recent advances in MR imaging that allow non-invasive visualization of brain macro- and micro structure, even *in-utero*. It is hoped that together, these advances may enable DS to become one of the first genetic disorders to be targeted by antenatal treatments designed to "normalise" brain development.

**What this paper adds (1-5 points):**

1. Advances in MRI can now provide non-invasive characterisation of early brain development in DS
2. Mouse models of DS enables detailed study of the underlying pathology and testing of potential early intervention strategies
3. Potential therapies could modify brain structure and improve early cognitive levels
4. DS may be one of the first genetic disorders to be targeted with specific therapies aiming to alter early brain development during the antenatal period

## Introduction

Down Syndrome (DS) is caused by partial or complete triplication of human chromosome 21 (Hsa21; Trisomy 21) and is the most common genetic developmental disorder in humans. It is a complex condition which results in multiple lifelong health problems, including varying degrees of intellectual disability and delays in speech, memory and learning. Worldwide, DS affects 1 in 1000 - 1100 live births annually. Whilst there have been significant improvements in non-invasive prenatal screening[1], the prevalence of DS has remained relatively unchanged over the past 30 years, partly due to increasing maternal age[2]. In addition to cognitive difficulties, there is typically multi-system involvement, with comorbidities including congenital heart defects (CHD) (40 – 50%), hypothyroidism, hearing, vision and gastrointestinal (GI) complications. In early adulthood, cognitive decline is common with a high risk of early-onset dementia and Alzheimer's disease (AD). In recent years, increased research, education, health care and intervention programs have all contributed to people with DS now working and leading longer, healthier lives.

As DS is a multi-gene multi-system disorder, accurately predicting neurocognitive abilities through the lifespan and understanding the high degree of variability across functional phenotypes remains a significant challenge. As a result, the majority of clinical research in DS has been focused on understanding the pathogenesis of early onset dementia in adults; and clinical trials with pharmacological agents have been focussed on improving cognition and delaying the development of dementia in adolescents and adults. Here, neuroimaging has become an increasingly useful modality to understand the progression of the underlying brain abnormalities and monitor the effects of potential therapeutic intervention. In contrast, published brain phenotypes during the fetal and neonatal period have been limited to only a handful of small post-mortem case series. Therefore, whilst such studies have provided vital information about how early brain development is altered in DS, by nature, they cannot inform about the natural history of the abnormalities and crucially do not allow correlation of the identified brain phenotypes with subsequent outcome. In this review we describe how recent advances in developmental animal models of DS and non-invasive imaging methods can fill this gap in knowledge by enabling the first *in-vivo* studies in early human life.

## Individual Variability

Hsa21 contains 222 protein coding genes, and 325 non-protein encoding genes[3]. Studies of partial trisomy of Hsa21 have revealed that multiple regions of Hsa21 contribute to the observed physical and neurodevelopmental characteristics of DS[4,5]. The phenotype in DS is thought to arise from the overexpression and dysregulation of these genes and their associated pathways, together with global cellular stress responses and compensatory mechanisms early in development[6]. Epigenetic changes have also been observed in the fetal brain and blood from newborns with DS[7,8] which may further impact development and contribute to the range of observed cognitive outcomes. The neurological phenotype constantly changes over the life span of DS[9], with differences continuing into adulthood. From 20 – 40 years of age, the majority of individuals with DS appear to develop characteristic AD neuropathology such as amyloid-β (Aβ) plaques and neurofibrillary tangles, however not all will develop dementia, which has a clinical prevalence of 68 – 80% by 65 years of age[9-12]. Dementia is also strongly associated with early mortality in older (>36 years of age) adults with DS[13]. There are therefore multiple factors which may explain why individual differences exist across *all* levels of assessment; from gene expression, cellular responses and subsequent brain development, to cognitive, motor and behavioural phenotypes [6,12,14].

## Neurodevelopment in DS

Variable but atypical behavioural and cognitive functioning emerges throughout the lifespan in DS. IQ ranges from mild to severe disability (30 – 70; average IQ 50)[15], with females reported to have milder degrees of intellectual disability, compared to males[15,16]. Varying degrees of impairment in speech and language, memory, learning, and motor functions are also present [9,14,17-19].

In comparison to 'typically-developing controls', infants with DS may have only mild delays in learning and cognition during early infancy. Delayed or impaired cognitive and behavioural function then becomes more prominent from 2 years of age, with the rate of intellectual development slowing with increasing age[12,20]. Toddlers and young children with DS also have a higher prevalence (pooled prevalence of 16%) of autism spectrum disorder, which is further increased in those with greater cognitive impairment[21-23]. Epilepsy (and in particular West Syndrome) occurs at a higher incidence (1-13%) in children with DS[24,25]. Recent studies also suggest that pre-school age children with an associated CHD (typically atrioventricular septal defects (AVSD) and ventricular septal defects (VSD)) have poorer neurodevelopmental outcomes [26,27 28,29]. This wide spectrum of outcomes and limited understanding regarding how they relate to the underlying brain abnormalities therefore can significantly hamper antenatal parental counselling and undermine attempts to identify and assess potential treatment strategies.

## Neurological Phenotype – what is known from post-mortem and adult studies

It has been widely reported that children and adults with DS have smaller whole brain volumes, and a smoother, simplified gyral appearance[30]. Reduced cortical surface area and increased cortical thickness have also been observed in children and young adults with DS[31]. By middle adulthood, premature structural brain ageing can be detected[32]. This includes disproportionate volume reduction of the brain regions crucial for speech, learning and memory such as the prefrontal cortex, hippocampus and cerebellum[30,33].

Despite their small number of cases, post-mortem studies have provided important information about the range and high variability of the neuropathological features evident in DS across the life-span. These studies suggest that the known reductions in brain size (2D and 3D measures) and weight emerge during the fetal and newborn period[34,35]. During the second trimester, reduced cellular proliferation and increased cell death reflect the observation that fewer neurons are seen in the neocortex, hippocampus and cerebellum[36-40]. Fewer neurons in the ventricular zone and subventricular zone further suggest an underproduction of excitatory neurons, leading to enhanced inhibitory neural activity that may underlie some of the cognitive deficits observed in DS[37,40,41]. A reduction in serotonin (5-HT1) levels has also been described in fetal brains with DS[42]. In addition, there is growing evidence of a greater shift towards neural progenitor cells differentiating into glia (microglia, astrocytes and oligodendrocytes)[43,37], resulting in altered regional expression and cellular densities of glia and macrophages across gestation[44]. During late gestation, when neocortical expansion occurs, brains with DS also show delayed and disorganised patterns of cortical lamination[45,46].

Following birth (and described up to 14 years of age) there is a profound decrease in neuronal number (20-50%) and altered morphology of dendritic spines across the cortical layers[35,47,48]. From 3 months of age, more distinct deviations in brain growth and shape become evident, these include shorter anterior-posterior (A-P) diameter, flatter occipital poles and smaller frontal lobes, cerebellum and brainstem[35]. These are accompanied by reductions in synaptic density and length, and fewer

dendritic spines (that are thinner and shorter in length)[35,48-50]. Delays in myelination are also observed postnatally (from 2 months of age), which correlates with poorer psychomotor development[51]. Collectively, these observations are associated with over-expression of dosage-sensitive genes including (but not exclusively): DYRK1A, APP, S100β and Olig-2, all located on Hsa21[43,46,52,53]. In addition, superoxide dismutase (SOD-1) (also on Hsa21) is suggested to contribute to increased oxidative stress and mitochondrial dysfunction[46,54].

## Mouse Models of DS

Advances in genome-wide analysis and the development of animal models have provided valuable insight into understanding gene dosage imbalances in disorders such as DS[55]. Mouse models of DS have been crucial to help investigate the genetic and developmental origins of the DS phenotype and importantly to test therapies that have the potential to improve neurogenesis and long term cognition[56,57]. Hsa21 shares synteny with a large proportion of mouse chromosome (Mmu) 16 (~102 protein coding genes) and shorter regions of Mmu10 (37 protein coding genes) and Mmu17 (19 protein coding genes)[3]. These have all been key targets in generating mouse models of DS [for a comprehensive list of mouse models of DS see[57]]. Importantly, as in human post-mortem studies: an imbalance of excitatory and inhibitory neurons, impaired neurogenesis, synaptogenesis and altered dendritic development are also observed in mouse models of DS (for detailed reviews see [9,17-19,41,56,58,59]).

The Ts65Dn mouse (B6EiC3Sn *a*/*A*-Ts(17$^{16}$)65Dn/J) has historically been very important in the study of DS as it is trisomic for 90 protein coding genes on Mmu16 (~55% of orthologous genes to Hsa21). However, Ts65Dn mice contain an extra copy of 60 genes (35 protein coding) located on Mmu 17 (orthologous to Hsa6) that are not triplicated in people with DS and the resultant Ts65Dn phenotypes may be more severe than those seen in the human condition or possess spurious phenotypes not relevant to DS[3,60,61]. Other mouse strains have therefore been developed with partial trisomy of genes on Mmu16. The Ts1Cje strain (B6EiC3Sn-Ts(16C-tel)1Cje/DnJ) contains a partial trisomy of ~71-81 genes on Mmu16, but also monosomy of 7 genes on Mmu12, and has a milder phenotype compared to Ts65Dn mice[3,62]. Early studies of partial human trisomies suggested that the DS phenotype was due to the increased gene dosage of a smaller number of specific genes, known as the Down Syndrome Critical Region (DSCR) extending approximately 5.4Mb[63,64]. Using Cre-LoxP technology, the Ts1Rhr mouse strain (B6.129S6-Dp(16Cbr1-Fam3b)1Rhr/J) replicates trisomy of the DSCR (33 conserved and minimally conserved genes)[65]. However, additional studies into partial trisomies and advances in gene mapping strongly suggest that these genes alone are not sufficient to result in all DS phenotypes[4,5,65,66].

The Tc1 (B6129S-Tc(HSA21)1TybEmcf/J) transchromosomic (trans-species aneuploidy) mouse line, contains a freely segregated copy of Hsa21. Although some chromosomal rearrangement and deletions have been identified in the construction process, it has allowed exploration of the relationship between specific Hsa21 genes (including those not found in the mouse) and phenotype[3,57,67]. Whilst APP is known to significantly contribute to the early-onset of AD, recently it has been shown that triplication of other genes on Hsa21 (Tc1 mouse is 75% trisomic for Hsa21 genes) can exacerbate plaque formation and cognitive deficits in mice[68]. Advances in chromosomal engineering have facilitated the design of more specific mouse models which include duplications of entire syntenic segments of Mmu16 (Dp(16)1Yey [Dp16; B6.129S7-Dp(16Lipi-Zbtb21)1Yey/J] and Dp1Tyb; Dp(16Lipi-Zbtb21)1TybEmcf), Mmu17 (Dp(17)1Yey) and Mmu10 (Dp(10)Yey). This has led to the development of the most complete 'triple trisomic mouse' which develops DS-related neurological impairments[69]. The

Dp1Tyb and Dp16 contain the largest duplication of Mmu16, carrying an extra copy of 148 genes which is the entire region of Mmu16 that is orthologous to Hsa21 and does not perturb genes on any other chromosomes [57,69,70]. Whilst the triple trisomic mouse is incredibly labour intensive and costly to produce, assessing each individual trisomic mouse is providing further insight into the contribution of gene imbalance to DS phenotype.

Studies in mouse models have primarily focussed on understanding the pathology of the adult and ageing DS brain, despite knowledge that alterations in brain development are observed from fetal life. Comparison with human development can be challenging, as mice are postnatal brain developers with a gestational length of 19-21 days. The bulk of cortical neurogenesis occurs during the mouse embryonic period (corresponding to early fetal life in the human), but is ongoing into postnatal life in the hippocampus and cerebellum. The rate of cellular migration and maturation differ regionally, but around the time of (rodent) birth, postnatal day (P) 1 – P3, neural development is generally considered to be comparable to a preterm human infant of 23 – 32 weeks post-menstrual age (PMA). The brain growth spurt of rodents occurs at P7 – P10, which is comparable to a term human infant of 36-40 weeks PMA[56,71]. Alterations at both embryonic and postnatal ages have been reported in Ts65Dn and Ts1Cje mice [72] and reviewed in several recent publications[9,41,59]. More recently, the Dp16 strain did not show any forebrain defects embryonically (Embryonic day 13.5 – 18.5), but did show delayed growth, and delayed acquisition of milestones postnatally and a decrease in cortical excitatory and interneuron populations were observed at P15, but were not evaluated at earlier postnatal ages[73,74]. Comparisons of embryonic and adult gene expression, brain development and mouse behaviour have recently been done in the Ts65Dn, Ts1Cje and Dp16 mouse strains and suggest widespread differences between models[74]. This highlights the importance of assessing which mouse models best mimic the human phenotype of interest and then choosing a mouse model that is best suited for studying a specific outcome or genotype/phenotype relationship.

## Current therapeutic approaches

Studies are being conducted in mouse models of DS to target dosage sensitive genes that are involved in defects and delays in neurogenesis and neurotransmission, oxidative stress and neurodegeneration (comprehensive list reviewed in[18,57]). Pharmacological treatments in mouse models with DYRK1a inhibitors, selective serotonin reuptake inhibitors (SSRIs; fluoxetine) and sonic hedgehog agonists during the prenatal and/or postnatal period have provided promising evidence of improved cellular and behavioural outcomes[56,57]. Whilst the vast majority of these studies have been done in Ts65Dn mice, they provide crucial proof-of-concept that cognition can be improved and that aspects of brain structure can be restored, even if the drugs are administered well after periods of neuronal migration and maturation have ceased.

Current clinical trials in adolescents and adults with DS are aimed at improving cognition and delaying progression into AD. Two groups of common medications used to treat the symptoms of AD: acetylcholinesterase inhibitors (Aricept/Donepezil, Rivastigmine) and NMDA receptor antagonists (Memantine) are currently undergoing clinical trials in patients with DS [56,75]. With the ultimate aim of reducing amyloid toxicity and regulating myo-inositol levels, s*cyllo*-Inositol (ELND005) has recently also been shown to be well tolerated in a Phase II clinical trial in young adults with DS at risk of developing AD dementia[76]. In addition, further novel pharmacological interventions have also been developed based on the improved knowledge of the genes located on Hsa21 and their specific pathways, including

DYRK1a inhibitors (Epigallocatechin; ECGC), a selective GABA-A α5 receptor negative allosteric modulator (Basmisanil / RG1662; CLEMATIS Study), and antioxidant vitamin E [56].

***Searching for new therapeutic windows:*** Growing evidence suggests that alterations in key cellular processes result in permanent modifications in structure from a very early stage in brain development. It is therefore possible that an early life therapeutic window exists, during which atypical brain development could be potentially modified before the abnormalities and neurocognitive impairment are fully established. In current clinical practice, commonly used early interventions include physiotherapy, occupational therapy, and speech and language therapy which may help to improve the acquisition of developmental milestones in infants and children with DS in the absence of any known effective pharmacological intervention. Here it is important to consider that the identification of novel candidate agents is difficult, given the absence of detailed understanding of the very early neurobiological trajectory in DS.

### What we don't know

There is a lack of understanding about when deviations in brain development arise in DS, how these relate to subsequent function and whether they are further altered by additional congenital morbidities (e.g. cardiac defects). Such information is best monitored by *in-vivo* studies that provide opportunities to follow development longitudinally.

***Co-Morbidities:*** CHD (without DS) is generally associated with impaired clinical neurodevelopment and an underlying reduction in cortical grey matter volumes, gyrification index (indicative of less complex cortical folding) and abnormal cortical microstructure in the neonatal period. These changes were further associated with reduced cerebral oxygen delivery[77,78]. This therefore highlights the importance of understanding the additional and as yet unexplored, effects of a CHD on brain development in DS. Mouse models are also useful for this as the Ts65Dn[79], Ts1Cje[80], Tc1[81], Dp1Tyb[70] and the genetically similar Dp16 mouse strains all develop CHD, which are identifiable by embryonic day 14.5 (E14.5). Importantly, Tc1 (38% - 55% based on background strain) and Dp1Tyb mice (61.5% of embryos) share many of the specific features of AVSDs that are common in humans with DS [114,107]. Lana-Elola et al.,[70] have elegantly generated a mouse mapping panel using segmented duplications ranging in size to identify the location of a 4.9 Mb genomic critical region for CHD, which consists of 39 genes (two of which are required in triplication).

***Improve translation between Human studies and Mouse Models:*** Two studies in both human and mouse models of DS have utilised transcriptomic analysis to characterize the specific gene networks and associated biological processes which are altered during pre and postnatal development[6,82]. The identification of consistently disturbed signalling pathways could aid the recognition of novel pharmacological treatments[6,82]. However, to translate the findings from bench to bedside, an improved understanding of how molecular alterations impact on neurobiological development is needed through (i) better detailing of the human condition; and (ii) cross-species validation between DS mouse models and human DS.

### Advances in Fetal and Neonatal MRI

Whilst the aforementioned post-mortem studies and animal models have provided significant insights into the neuropathology of DS, a true understanding of the natural history of the human condition and how the pathology relates to neurodevelopmental outcome is only possible through *in vivo* studies.

Here, there is great potential for an enhanced understanding of *in utero* and neonatal brain development in DS through the application of recent advances in *non-invasive* imaging. Although ultrasound provides valuable insights into gross fetal body and brain development, it cannot provide detailed information about region and tissue specific brain development and growth trajectories.

Therefore Magnetic Resonance Imaging (MRI) is an attractive alternative which is safe and does not use ionizing radiation; and can provide more extensive, detailed biometric data across gestation including information about both brain macro- and micro structure [83,84]. Although there are MRI studies which have assessed structural brain volume in early childhood [59,85], very little work has been done with infants younger than 2 years of age with DS. Quantitative early MRI data could be related to data derived from clinical, cognitive and behavioural assessments, as well as genetic information, thus allowing a comprehensive understanding of the complex relationships which underpin the DS phenotype. Such studies are currently ongoing in adults with DS to assess the changes in brain structure and function associated with cognitive decline and progression into early onset AD [86].

***Fetal MR imaging*** is challenging due to fetal motion, size and position (relative to the surrounding maternal tissue). However, in comparison to ultrasound, it offers excellent soft tissue contrast and benefits from a wide field of view which allows the whole fetus to be imaged up until full term gestation. To combat the effects of fetal and maternal motion, significant advances have been made in acquisition and processing protocols (such as optimised fetal motion correction and image registration pipelines) which can now provide high resolution and high signal to noise volumetric image datasets [87-90] (Figure 1). This has now made it possible to obtain 3D MR structural and functional data within 30 minutes of image acquisition using snapshot to volume reconstruction (SVR) techniques[88,91]. These advances have led to increasing utilization of fetal MRI both in clinical practice and as a research tool to assess the fetal brain, heart and organs, as well as the placenta.

***Neonatal MRI***: Whilst imaging a newborn or young infant also presents technical and practical difficulties, there are now MR compatible incubators and population-specific processing pipelines to overcome these. Examples include a Neonatal Brain Imaging System developed for the Developing Human Connectome Project (http://www.developingconnectome.org/) for non-sedated sleeping infants, consisting of a neonatal head sized 32-channel receive array coil and positioning system which significantly improves signal to noise ratio, an MRI safe trolley to minimise disturbance of the sleeping infant, additional ear protection and a change in the start of MR sequences to reduce the abrupt noise at the start of an acquisition sequence that may wake the baby[92].

***Imaging infants and toddlers:*** During infancy and early childhood there is ongoing rapid growth of the cerebral cortex and maturation of white matter including myelination. Studying this population is therefore essential to provide a true characterization of these fundamental developmental processes and understand how a trajectory may deviate in pathological states. However, MR imaging of young children is associated with significant technical and practical challenges[93]. As a result, clinically indicated MR studies in children over 2 years of age are often done under general anaesthesia which would not be appropriate for research studies. Therefore in these children, other strategies have been explored to reduce anxiety, including mock-scanner training sessions or have a pre-meeting with the child and family to talk through the MRI process[94]. Although children under 2 years of age may settle with oral sedation, this is less commonly done for research MRI scans due to increasing concerns about possible neurotoxicity [95]. In this situation, co-ordinating with sleep, nap or feeding times and modifying the MR acquisition sequences to reduce sudden noise and/or volume may help avoid the use of sedation. Foam

padding around the head and vacuum immobilisation bags can also be used to reduce head motion[96]. Although such approaches make scanning feasible, success rates are often variable, particularly for the sequences which provide quantitative MR measures and are highly sensitive to motion artefact.

***Structural:*** Single shot T1-weighted and T2-weighted sequences are conventionally used to visualise the structure and composition of the fetal brain and can provide regional 2D measurements and 3D volumetric information. The recent development of detailed atlases of the fetal[97] (Figure 2) and neonatal (Figure 3)[98-100] brain now allow robust automated or semi-automated segmentation of brain regions and precise delineation of cortical sulcal and gyral development. This allows characterization of the normal trajectories of fetal brain growth and creation of population centile charts (available for 21-38 GW at: https://www.developingbrain.co.uk/fetalcentiles/; Figure 4)[101]. Comparison with these typically developing growth charts therefore provides an ideal approach with which to assess, quantify and identify when the deviations in regional and whole brain volumes seen in post-mortem and adult DS brains are established. Our preliminary findings from fetal MR images show enlargement of the fourth and lateral ventricles, as well as cerebellar vermis rotation in a fetus with DS (Figure 5).

***Diffusion MRI*** (dMRI) can provide quantitative information about tissue microstructure and structural connectivity by measuring the total and directional diffusion of water molecules (Figure 6). This can then provide specific measures which reflect white matter and cortical microstructure, such as fractional anisotropy (FA) which in high risk neonates significantly relates to later specific clinical neurodevelopmental impairment[102,103] and delays in cortical microstructural development[104]. More complex models of voxel-wise diffusion such as a fixel-based analysis (fixel refers to a fibre population in a given voxel) can also provide measures of white matter fiber density (FD), fiber cross-sectional area (FC) and the fibre orientation distribution (FOD)[105]. Other techniques, such as the neurite orientation distribution and density imaging (NODDI) model can also provide measures of the neurite density index (NDI) and orientation dispersion index (ODI) which may help explore the cortical synaptic and dendritic developmental abnormalities that are widely reported in DS[9,41].

Although dMRI has not yet been used to study white matter and cortical microstructure in fetuses and neonates with DS, regional reductions in white matter microstructural integrity have been seen in adults with DS, which are more severe in those with additional signs of dementia[106]. It has also been recently reported that children with DS (aged 2-4) have reduced FA in supratentorial white matter tracts[107] which mirrors both the reported delays in myelination in early childhood[51] and transcriptome studies in both DS human brain (fetal) and DS mouse models (embryonic) studies which describe defective oligodendrocyte differentiation and myelination[82].

***Functional MRI*** (fMRI) provides an indirect measure of neural activity by detecting dynamic variation of the Blood oxygen level-dependant (BOLD) contrast caused by locally coupled changes in cerebral blood flow and haemoglobin oxygenation [108]. This allows detailed mapping of functional activity which can be used to characterize the whole brain's large scale functional architecture. Studies in the neonate and more recently the fetus suggest that the perinatal period is of particular importance for the establishment of this architecture, as patterns of functional activity appear to rapidly increase in spatial complexity during this time [109-112].

In addition to analyzing BOLD signal changes when a subject is presented a specific stimulus or performs a task (known as task-based fMRI), data can also be collected and analyzed when a subject is at rest (known as resting state fMRI). The latter can be used to identify spatial patterns of temporal correlation

of intrinsic signal fluctuations (known as functional connectivity). Altered patterns of functional connectivity are seen in neuropsychiatric conditions and therefore may provide a suitable biomarker for abnormal brain function and predicting later adverse neurodevelopment in DS[113]. In keeping with this, impaired functional connectivity and a simplified network architecture has been described in adolescents and young adults with DS[114]. Functional connectivity could therefore potentially be used as a biomarker to monitor the outcome of clinical trials, as in a recent Phase 2 clinical trial in young adults with DS[115]. By combining dMRI and fMRI data, there is the potential to provide further insights into the complex relationship between the brain's structural connections and its activity patterns, and crucially how it is altered by different pathological states[116].

*Magnetic Resonance Spectroscopy* (MRS) can non-invasively quantify biochemical composition by sampling the resonant signal generated by hydrogen protons ($^{1}$H) (and less commonly other nuclei with an odd mass number (Sodium $^{23}$Na or phosphorus $^{31}$P)) from a voxel placed on a specific region of interest. $^{1}$H-MRS is most commonly used in human brain studies due to the abundance of hydrogen in human tissue. The resultant spectra demonstrate metabolite peaks at a specific frequency (parts per million, ppm). Specific brain metabolites that can be quantified include *myo*-Inositol (myo-ins; osmoregulation, glial cell marker), choline (Cho- cell membrane), creatine (Cr-energy metabolism), N-acetyl aspartate (NAA; neuronal marker and/or marker for mitochondrial function) and lactate (anaerobic glycolysis)[117]. The levels of different metabolites are often expressed as ratios rather than absolute metabolite quantification, particularly in cases with pathology where the detected changes may be subtle. Such measurements are of particular relevance in fetal and neonatal life as ongoing processes such as neuronal and glial proliferation, differentiation and maturation are associated with constant fluctuations in the levels of brain metabolites which can be measured using MRS (e.g. increases in NAA and decreases in measurable Cho with increasing brain maturation) [117]. Of interest, increased brain myo-ins has been reported both within the basal ganglia of children with DS[118] and in the hippocampus, occipital and parietal regions in adults with DS[119-121]. The correlation of altered brain metabolite levels, (such as NAA and NAA/myo-ins ratio) with cognitive function can also provide insight into the progression of dementia for adults with DS[122].

## Where to now: Future directions

Although current cognitive interventions target children and adults with DS, evidence suggests that deviations in brain development begin early in fetal life. However, to understand how to potentially intervene at this earlier time point, we need far greater knowledge about how the DS brain grows and develops, what causes the variability of neurodevelopmental outcomes and the genotypic/phenotypic relationship that occurs in DS.

Significant advances in fetal and neonatal MRI sequence acquisition, motion correction techniques and analysis methods now allow detailed characterization of the spectrum of early imaging phenotypes[84]. These essential developments are of both research and clinical importance. Such prognostic information can improve care, help to counsel parents, and could potentially identify new therapeutic windows for intervention early in development.

In addition, histological studies of human DS tissue at equivalent gestational ages can be used to determine the underlying neurobiological substrate for imaging phenotypes identified in the early developing brain in DS (Figure 7). This combined early human data can be compared with that from available mouse models to identify those which most closely mimic the human condition and would

therefore be suitable for use in interventional trials of early treatments designed to normalise brain development and improve cognition.

The combination of pre-clinical animal, human post-mortem and *in-vivo* imaging methods can therefore provide comprehensive and vital new insights into aberrant brain development in DS. This also has the potential to provide non-invasive imaging based surrogate markers to predict later neurodevelopmental outcome. In the future, this novel early human imaging data can also be used in clinical trials as biomarkers to monitor the effectiveness of new therapies intervening during antenatal or neonatal time-points.

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## Figure Legends

### Figure 1: T2 fetal image reconstruction

Top row: One loop of single shot T2 images acquired in the coronal plane (centre). Numerous black lines in the sagittal and axial images represent missing or motion corrupted data.
Bottom row: The reconstructed images have been obtained by registering several loops of single shot T2 images to provide high signal to noise, high resolution volumetric datasets[87-90]

### Figure 2: Segmentation of the brain from a fetus with Down Syndrome at $33^{+2}$ gestational weeks.

Semi-automated segmentation of T2-weighted volumetric MR images showing **(A)** whole brain; excluding cerebellum (red), **(B)** cortex (green), **(C)** lateral ventricles (dark blue), **(D)** extra cerebral cerebrospinal fluid (light blue), **(E)** cerebellar hemispheres (purple), cerebellar vermis (bright green), pons (yellow) and fourth ventricle (blue)[97].

### Figure 3: Automated segmentation of a brain from a neonate with Down Syndrome at $42^{+5}$ weeks post menstrual age.

T2 weighted neonatal brain volumetric images in axial, sagittal and coronal planes (left to right) segmented into multiple brain regions. **(A)** Raw T2 acquisition, **(B)** segmentation with 9 regions of interest and **(C)** segmentation with 87 regions of interest[98-100].

### Figure 4: Fetal brain development.

T2 weighted axial images from fetal ($23^{+6}$ – $38^{+2}$) and neonatal ($40^{+0}$) MRI showing development of the brain across gestation. Note is made of the marked increase in cortical complexity with increasing gestation (GA: gestational age expressed as weeks + days).

Figure 5: T2 weighted fetal MRI images in a control fetus and a fetus with Down Syndrome.
T2 weighted axial images (A, B) showing the fourth (A) and lateral ventricle (b) in Control ($34^{+1}$ GA; Ai, Bi) and fetus with DS ($33^{+2}$ GA; Aii, Bii). White arrows indicate enlarged fourth and lateral ventricles in a fetus with DS. T2 weighted sagittal (Ci, Di) and axial (Cii, Dii) images in a fetus with DS (30 weeks GA, D) compared to an age matched control (30 weeks GA, C). Red arrow indicates cerebellar vermis rotation (Di) and fourth ventricle enlargement (Dii). DS; Down Syndrome, GA; gestational age, as weeks+days.

Figure 6. Fetal diffusion tensor imaging (DTI).
Top row: Fiber orientations distributions per voxel
Bottom row: Tractography demonstrating major connections within the developing brain.
Courtesy of Dr Maria Deprez

Figure 7: Neuronal staining in the cortex of human fetal post-mortem tissue. HuC/HuD, a marker for all neurons in brain from Control fetus at $22^{+2}$ GA (Ai) and fetus with DS at $21^{+1}$ GA (Bi). In the fetal brain with DS (B), black arrow indicates evidence of aberrant cortical folding, a 'wavy' pattern which is in contrast to the control brain (A) [Research Ethics Committee UK: 07/H0707/139]. Scale bar = 500um. T2 weighted fetal MRI images in the axial plane show decreased cortical folding in a fetus with DS (Bii) compared to an aged matched control (Aii). DS; Down Syndrome, GA; gestational age, as weeks+days.

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