Imaging biomarkers of outcome in the developing preterm brain

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The neurodevelopmental disabilities of those who were born prematurely have been well described, yet the underlying alterations in brain development that lead to these changes remain poorly understood. Processes that are vulnerable to injury in the developing brain include maturation of oligodendrocyte precursors and genetically programmed changes in cortical connectivity; recent data have indicated that diffuse injury of the white matter accompanied by neuronal and axonal disruption is common in prematurely born infants. Recent advances in MRI include diffusion tensor imaging and sophisticated image analysis tools, such as functional connectivity, voxel-based morphometry, and mathematical morphology-based cortical folding strategies. These advanced techniques have shown that white matter structure is dependent on gestational age and have started to provide important information about the dynamic interactions between development, injury, and functional recovery in the preterm brain. Identification of early biomarkers for outcome could enable physicians and scientists to develop targeted pharmacological and behavioural therapies to restore functional connectivity.

Introduction

Preterm birth is among the leading public health problems in the USA and Europe.1 There are more than 4 million livebirths in the USA every year, and more than 1–5% of these infants weigh less than 1500 g (ie, have a very low birthweight). Survival for these medically fragile neonates is about 70–80%, but the annual new perinatal care costs are also being recognised at school age and beyond.1–11

Preterm infants are at high risk of brain injury, yet the dynamic interactions between the genetically determined programme of development, injury, and functional recovery remain poorly understood. MRI has enabled non-invasive high-resolution evaluation of the developing brain, and many studies have documented the macrostructural sequelae of premature birth, including delayed cerebral grey-white matter differentiation and diffuse excessive hyperintense signal in the developing white matter.12–17 Furthermore, when compared with term control infants, preterm infants have global and regional decreases in cortical grey and deep grey matter, less myelinated white matter, smaller corpus callosal areas, and substantial ventriculomegaly, yet the long-term neurodevelopmental predictive value of these relatively large global and regional volumetric changes remains limited.18–19

Cerebral white matter injury, or the periventricular leukomalacia (PVL) complex, has long been recognised as the most common neuropathology in prematurely born infants, and one crucial MRI finding is that of decreased white matter volumes at term-equivalent age. White matter injury in preterm neonates is accompanied by diffuse neuronal and axonal disease affecting not only cerebral white matter but also deep grey matter, cortical, and cerebellar areas.20 During this crucial period of human brain development, cortical folding occurs, and little is known about the effect of cerebral white matter injury on this important process.21,22

Imaging strategies that provide data on both connectivity and cortical development might reveal important early biomarkers that are predictive of later development. Two relatively new techniques, diffusion tensor imaging (DTI) and functional MRI connectivity (fMRI), combined with advanced image analysis tools, such as voxel-based morphometry (VBM) and mathematical morphology-based analysis of cortical folding, provide complementary data for understanding development, injury, and recovery in the developing brain. These non-invasive imaging techniques might guide our understanding of alterations in brain development and their correlation with functional outcome in prematurely born infants.

Preterm birth and disability

Data from studies from the USA, Europe, and Australia have all revealed poorer educational outcomes in preterm children than in term controls. At school entry, minor developmental impairment is diagnosed in 30–40% of preterm children and major disabilities in almost 20% of preterm children.23,28,29 More than half of these children require special assistance in the classroom, 20% are in special education, and 15% have repeated at least one grade in school.26,27 In France, 42% of children born at 24–28 weeks of gestation and 31% of those born at 29–31 weeks needed special health-care support owing to neurological sequelae, compared with only 16% of those born at 39–40 weeks.4 The intellectual deficits of preterm subjects can persist through adolescence and young adulthood.22 In a study by the Victorian Infant Collaborative Study Group, 14% of extremely low birthweight preterm children (900–999 g) were classified as severely disabled and 15% were classified as moderately disabled at the age of 14 years, compared with 2% of controls who were classified as severely disabled at this age.41 Similarly, when 1097 Dutch preterm children with a gestational age of less than 33 weeks and/or a birthweight of 1500 g were assessed at the age of 14 years, more
than half had disorders in learning, attention, and social–emotional skills. Finally, when compared with healthy controls, fewer prematurely born young adults continue their education after high school, and fewer have full-time employment or live outside the parental home as adults.

Injury in the developing preterm brain
Injury is common in the preterm brain. Although parenchymal involvement of intraventricular haemorrhage and focal PVL have been viewed as the major forms of brain injury that cause cerebral palsy after preterm birth, intraventricular haemorrhage with periventricular haemorrhagic infarction now occurs in about 5% of infants with very low birthweight, whereas cases of focal PVL have decreased in recent years. This is not the case for the more diffuse injury in the periventricular white matter of neonates with very low birthweight. Results from conventional MRI studies indicate that up to 50% of neonates with very low birthweight have signs of such diffuse white matter injury and accompanying neuronal or axonal disease.

First described by Banker and Larroche, PVL is a unique pattern of neonatal brain injury involving not only the cerebral white matter but other areas as well. PVL is characterised by both focal macroscopic and microscopic regions of necrosis, loss of premyelinating oligodendrocytes, and marked astrogliosis and microgliosis of the white matter; for many neonates, cerebral ventriculomegaly detected with cerebral ultrasonography is the only biomarker of this cascade of events.

The axonal and neuronal changes found in neonates with diffuse white matter injury are less well described. MRI studies of prematurely born subjects from the neonatal period to late adolescence show decreased volumes of cortical grey matter and alterations in the thalamus, basal ganglia, and cerebellum. As the late second and third trimesters are the time of both axonal ingrowth and elaboration of synaptic connections, and oligodendroglia produce guidance molecules in addition to myelin, microstructural changes in connectivity have been postulated.

In addition to MRI findings and neuropathological results, preclinical studies indicate the occurrence of both oligodendrogial and neuro-axonal injury. Both hypoxic–ischaemia and chronic hypoxia cause acute degeneration of late oligodendroglia precursors, followed by a regenerative response of the surviving preoligodendroglia. However, the surviving cells have arrested maturation with failure to generate myelin. Several authors have noted regional vulnerability of the developing brain on imaging studies, and, in these preclinical studies, the regional extent of white matter damage coincides with the presence of susceptible populations of late oligodendroglia.

In vivo imaging strategies
DTI and blood oxygen level dependent (BOLD)-based functional MRI, combined with advanced image analysis tools such as automatic voxel-based analysis methods for whole-brain comparisons, provide complementary in vivo data for understanding the effect of preterm birth on the connectivity and functional competence of the developing brain. Additionally, recent assessments suggest that primary cortical folding might provide an early marker of later behavioural development and might indicate microstructural changes that affect functional cortical field development and neural connectivity. These strategies are briefly reviewed in the panel and are more extensively described in the webappendix.

DTI measures water diffusion in biological tissues at a microstructural level and is a powerful technique to study the structural basis of white matter development. As such, this technique provides a unique imaging tool to assess important microstructural changes in brain development, such as axon calibre changes and premyelination and myelination stages, all likely to be affected by prematurity. DTI parameters include the three diffusion tensor eigenvalues ($\lambda_1, \lambda_2, \lambda_3$), which represent diffusion along the three tensor principal axes, the mean diffusivity ($D_m$) or apparent diffusion coefficient (ADC), and a mathematical measure of anisotropy, which describes the degree to which water diffusion is restricted in one direction relative to all others (panel and webappendix).

Fibre tracking uses each voxel’s primary eigenvector of the diffusion tensor to follow an axonal tract in three dimensions from voxel to voxel through the brain, thus enabling the delineation of specific cerebral white matter tracts and connectivity. Different tractography tools have been developed; the most common are streamline deterministic and probabilistic fibre tracking. There are several approaches to analysing DTI data, including region of interest approaches, VBM approaches, histogram analyses, and tract-based analysis (see webappendix and elsewhere for reviews). It is likely that the functional deficits associated with prematurity are partly due to alterations in cortical connectivity. The different image analysis tools that enable the three-dimensional visualisation of brain fibre tracts (tractography) provide the opportunity to study and understand the structural alterations underlying functional deficits.

Diffusion imaging enables the study of the establishment of brain connectivity and plasticity during a time period of extreme importance for the structural and functional integrity of the brain. New diffusion-based approaches (such as AxCaliberTM for the measurement of axonal diameter and diffusion spectrum imaging (DSI)) to characterise establishment of corticocortical connections during development will further refine image-based knowledge on brain development and integrity (figure 1, webvideo).

Although there are only a few studies using fcMRI, a method to assess both neural processing and resting state connectivity, in newborns, this technique might also offer insights into the microstructural changes in brain development that occur in preterm infants. In resting state fcMRI, bandpass-filtered (0-0.012–0.1 Hz) BOLD signal intensity time courses show coherent spontaneous
oscillations between specific areas of the brain, and this new technique has been applied to study interactions between spatially distinct regions in typically developing children and adolescents.\textsuperscript{44,45} Research into resting states suggests that functional connectivity is at least partially anatomically determined in typically developing subjects. This finding is of particular importance to preterm infants in whom white matter development might be compromised.\textsuperscript{46–48} There can be several design problems with fMRI, including registration of infants’ brains, selection of BOLD signal significance values, and analysis strategies (see webappendix and elsewhere for review).\textsuperscript{49}

Finally, computational approaches using mathematical morphology, a novel technique for shape processing and analysis, can be used to investigate primary cortical folding in the preterm brain. Through three-dimensional reconstruction of the interface between the cortex and white matter, this approach quantifies both surface area and gyration through curvature measurements.\textsuperscript{50,51} To accurately define the cortical surface, one would ideally identify the cortical grey matter–CSF interface, but partial-volume averaging precludes this with current image resolution. Instead, the interface between the cortical grey matter and the white matter is reliably identified by use of a mathematical morphology approach (see webappendix and elsewhere for review).\textsuperscript{52} figure 2).

As the incidence of handicap increases among those who are prematurely born, the need for early biomarkers of injury and outcome is crucial to the care of this vulnerable population. These strategies have the potential to provide important early information about the effect of preterm birth on structure and function in the developing brain. Nonetheless, there are many challenges faced by those who use and interpret these strategies that might affect reliability of the data. Understanding the difficulties that are unique to each technology is crucial to our understanding of the data that they produce (webappendix).

Imaging the preterm brain: the neonatal period

During the late second and third trimesters of gestation, cerebral development is characterised by sequential periods of cellular proliferation, migration of glia and neurons into appropriate cortical positions, axonal ingrowth, and the elaboration of synaptic connections. Although most cortical neurons have migrated before this time, neurogenesis continues in the superior ventricular zone during the second trimester and the population of subplate neurons attains maximal development at 24–32 weeks. Finally, maturation of the oligodendroglial lineage and the initiation of myelination are the hallmark features of the third trimester.

Microstructural development of the white matter

The diffusion and anisotropy parameters of developing white matter have been well described.\textsuperscript{75–85} ADC values of white matter are directly associated with gestational age\textsuperscript{86} and, during the third trimester of gestation, ADC values of cerebral white matter decrease, whereas those for anisotropy increase.\textsuperscript{77,79} Decreases in diffusion are observed principally in λ2 and λ3, which reflect alterations in water diffusion perpendicular to white matter fibres and indicate changes due to both pre-myelination and myelination.\textsuperscript{88,86} Changes during pre-myelination have been attributed to
an increase in the number of axonal microtubule-associated proteins or calibre, as well as to the presence of oligodendroglia.

When healthy preterm infants were compared at term-equivalent age with term controls, there was no difference in ADC values between the groups (table 1). By contrast, fractional anisotropy (FA) values for central white matter regions, including the posterior limb of the internal capsule, the centrum semiovale, and genu of the corpus callosum, were all significantly lower in the preterm group. These infants with the lowest gestational ages (<28 weeks) had additional decreases in FA. Data from both fibre-tracking investigations of preterm neonates of varying gestational ages and serial tractography studies indicate that FA values in corticospinal tracts are significantly correlated with postmenstrual age (ie, gestational age plus time elapsed after birth). As these tractography methods use MRI indices from whole-brain white matter, without inter-subject registration, the anatomical localisation is expected to be more accurate than in studies that use automatic voxel-based comparisons.

By contrast, recent data suggest that in certain regions of the brain, FA values for preterm infants at term-equivalent age might exceed those for term control infants. In infants who had neonatal ultrasound free of major structural lesions, Gimenez and colleagues noted higher values for FA in the inferior frontal occipital fascicle, stria terminalis, fornix, optic radiations, inferior longitudinal fascicle, and lateral geniculate nucleus in preterm infants at term-equivalent age when compared with term controls. These areas correspond to fibre tracts of the neurosensory pathways of vision and hearing, functions that mature more rapidly in preterm infants owing to early experience. Similarly, Rose and colleagues found significantly higher FA values in the corticospinal tract at the level of the cerebral peduncles of preterm infants (25–29 weeks’ gestational age) studied at term-equivalent age when compared with term control infants. Rose and co-workers examined term infants at less than 3 days after birth and found that their T2 measurements had higher water content than those of preterm infants, possibly consistent with physiological data suggesting increased neonatal water content at birth. These authors concluded that, in an area of similar fibre tract maturation between preterm and term infants (ie, the cerebral peduncle), higher water content caused altered diffusion parameters in term infants compared with preterm infants at term-equivalent age.

Effects of injury on neonatal white matter microstructure

The early assessment of white matter in preterm infants with diffusion imaging can reveal bilateral periventricular diffusion restriction similar to the typical distribution of focal PVL when ultrasound and conventional MRI show no or non-specific abnormalities. Histological changes in the acute phase of PVL alter white matter microstructure and water diffusivity, and a reduced ADC in the periventricular white matter in an otherwise normal preterm brain is considered an early indicator of white matter injury.

Anisotropy of white matter also changes after focal and diffuse injury. In the chronic stage of PVL, decreased relative anisotropy can be present and vector maps might show disruption of white matter tracts distant from focal lesions detected on conventional imaging. In this case, changes in anisotropy are found not only near the site of primary injury, but also in the posterior limb of the internal capsule, indicating a disturbance of developing fibres that project through this area. In this way, anisotropy and vector maps show injury that is not detectable by more conventional means.

For preterm infants with moderate white matter injury, the expected progression of ADC and FA values at term-equivalent age is not seen. Miller and colleagues noted...
increasing ADC values with gestational age in both frontal white matter and occipital visual regions in preterm infants with white matter injury, although the expected changes in anisotropy values with maturation did not occur, indicating disruption of normal brain maturation. Finally, in preterm neonates with no evidence of white matter injury on traditional MRI at term-equivalent age, Anjari and colleagues recently reported that, independent of the degree of prematurity, respiratory disease was associated with cerebral white matter abnormalities.

**White matter injury in subcortical structures**

Boardman and colleagues tested the hypothesis that preterm infants with diffuse white matter injury would have substantial cerebral morphological alterations when compared with preterm infants without white matter injury and term controls. All infants were studied at term-equivalent age, and the data indicated reduced thalamic and lentiform volumes in preterm infants compared with term controls. These changes increased with decreasing gestational age for infants who were born at less than 28 weeks of gestational age and were more common in infants with diffuse white matter injury. There was no a priori hypothesis about the specific regions affected and the methods were based on a whole-brain approach similar to VBM techniques. These data provided an estimate of voxel-wise volume change for all transformed images compared with a standardised control template image; this sophisticated rater-independent method was subsequently confirmed in a second study by manual segmentation.

**Cortical folding**

Several authors have investigated the effect of preterm birth on primary cortical folding because white matter connectivity has the potential to partly determine gyriﬁcation in the developing brain. Several hypotheses have been suggested to explain the processes that underlie gyriﬁcation in the developing brain, including genetic control, active growth of convolutions during gyrogenesis, differential growth of inner and outer cortical layers, cortical growth, and tension from white matter axonal ﬁbres. All these factors might be affected by preterm birth and associated injury.

Biagioni and colleagues suggested that, for preterm neonates, cortical folding signiﬁcantly increases with postmenstrual age. Both Dubois and colleagues and Vasileiadis and co-workers found smaller cortical and white matter volumes but equivalent sulcation in female preterm infants compared with male preterm infants. Preterm neonates with intrauterine growth restriction had

<table>
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<tr>
<th>Number</th>
<th>Birthweight/ GA</th>
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<th>Age at MRI</th>
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<th>Results</th>
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<tbody>
<tr>
<td>Hüppi et al76</td>
<td>17 preterm, 7 term</td>
<td>NA</td>
<td>25-35 weeks</td>
<td>Serial studies over first 2 weeks after birth and at term</td>
<td>ADC and RA, ROI: RA directly associated with gestational age for preterm infants who were born in internal capsule (p&lt;0.01) and central WM (p&lt;0.01) than did term infants</td>
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<tr>
<td>Hüppi et al76</td>
<td>10 preterm with WM disease or injury, 10 preterm controls</td>
<td>NA</td>
<td>29 weeks</td>
<td>Serial studies over first 3 weeks after birth and at term</td>
<td>ADC, RA of central, anterior frontal, occipital, and temporal WM, and PLIC, ROI: No difference in ADC between groups at term-equivalent age (p&gt;0.03) and PLIC (p&gt;0.2) than did control infants</td>
</tr>
<tr>
<td>Miller et al78</td>
<td>11 preterm normal, 7 preterm with mild WM disease or injury, 5 preterm with moderate WM disease or injury</td>
<td>Congenital infection, malformations, periventricular haemorhage and infarctions</td>
<td>25-34 weeks</td>
<td>Serial studies over 2-6 weeks after birth and at term</td>
<td>ADC and anisotropy, ROI: In normal infants, ADC decreased and anisotropy increased with age. In infants with moderate WM disease or injury, ADC did not decline and anisotropy did not increase</td>
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<tr>
<td>Partridge et al72</td>
<td>14 preterm</td>
<td>WM injury on MRI, grade 2 IVH, congenital infection, malformations</td>
<td>25-34 weeks</td>
<td>First scan at 28-39 weeks’ post-menstrual age; 8 of 14 were serially imaged at near-term age</td>
<td>Tract-speciﬁc maturation of WM: deterministic and probabilistic tractography: Commissural tracts of CC and internal capsule matured earlier than in subcortical projection and association pathways. Maturation characterised by increasing FA and decreasing mean diffusivity</td>
</tr>
<tr>
<td>Berman et al79</td>
<td>37 preterm</td>
<td>IVH, ventriculomegaly (patients with minimal WM lesions included)</td>
<td>28-43 weeks’ post-conceptual age; 10 with serial scans</td>
<td>FA, transverse diffusion of motor and sensory tracts: deterministic and probabilistic tractography</td>
<td>All tract-speciﬁc diffusion parameters signiﬁcantly correlated with age. Motor tracts had higher FA and lower diffusivity than sensory tracts</td>
</tr>
<tr>
<td>Anjari et al80</td>
<td>26 preterm with normal MRI, 6 term controls</td>
<td>Focal lesions on MRI</td>
<td>25-33 weeks</td>
<td>Term</td>
<td>TBISS and FA: Preterm infants had lower FA in centrum semiovale, frontal WM, and genu of CC (p&lt;0.001 for all) Infants with &lt;28 weeks’ gestational age had additional decreases in FA in external capsule, PLIC, isthmus, and midbody of CC (p&lt;0.05 for all)</td>
</tr>
<tr>
<td>Dudink et al81</td>
<td>28 preterm with normal neonatal MRI and normal development at 1-3 years of age</td>
<td>Congenital infections and malformations, WM injury, IVH, or ventriculomegaly on MRI</td>
<td>25-32 weeks</td>
<td>First 4 days after birth</td>
<td>ADC and FA of WM tracts, ROI and seed point tractography: Significant correlation between gestational age and FA of PLIC (r=0.495; p&lt;0.01)</td>
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a more pronounced decrease in volume in relation to surface area and increased sulcation; these values correlated with impaired behavioural functions.72

Additionally, preterm infants with early white matter lesions were more likely to have increased gyrification in overlaying cortex that might lead to overt polymicrogyria or increased sulcation.73,96 These abnormalities in cortical folding have been associated with functional development in preterm infants at term-equivalent age; they might be a marker for those early changes in cortical development that give rise to the large percentage of preterm infants with cognitive impairment in the absence of focal cerebral lesions.71

Ramenghi and colleagues88 quantitatively assessed brain development in preterm infants with MRI-diagnosed white matter injury and matched preterm controls with a normal MRI appearance at term-equivalent age. Myelination and cortical folding were significantly delayed in infants with white matter injury. Therefore, similar to FA and thalamic volume measurements, cortical folding is altered by white matter injury.

Structure–function relationships
Central white matter DTI parameters obtained in preterm neonates at term-equivalent age might provide useful prognostic data as cerebral palsy and deficits in gross motor function have long been attributed to alterations in central white matter development in preterm infants (table 2).96,97 Arzoumanian and colleagues96 evaluated DTI studies at term-equivalent age and follow-up neurological examinations at a corrected age of 18–24 months (age corrected for prematurity) on 137 preterm neonates, and found that FA values in the right posterior limb of the internal capsule were significantly lower for preterm infants with cerebral palsy compared with those with normal examinations. For preterm children who underwent developmental testing at 18–24 months’ corrected age, the Bayley psychomotor developmental index correlated with FA values in the internal capsule at 30 weeks’ gestational age.96 Krishnan and colleagues97 reported a significant negative correlation between ADC values in the centrum semiovale and the Griffiths mental scale score at the age of 2 years, and several other investigators have found highly significant correlations between FA values in both the posterior limb of the internal capsule98 and the splenium of the corpus callosum99 and neuro-developmental outcome measures in preterm children who were scanned at term-equivalent age and examined in early childhood.

Table 1: Effects of preterm birth on WM maturation in neonates—DTI studies

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<tr>
<th>Number</th>
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<tr>
<td>Gimenez et al82</td>
<td>27 preterm, 10 term</td>
<td>28–34 weeks</td>
<td>Neonatal ultrasound with major lesions (2 preterm with germinal matrix haemorrhage and 1 with ventriculomegaly included)</td>
<td>Term</td>
<td>FA of WM tracts and voxel-based morphometry</td>
</tr>
<tr>
<td>Rose et al83</td>
<td>12 very preterm, 11 preterm, 10 term</td>
<td>25–29 weeks</td>
<td>Cortical or WM injury or haemorrhage</td>
<td>Term</td>
<td>FA of WM tracts and TBSS</td>
</tr>
<tr>
<td>Cheong et al84</td>
<td>111 preterm</td>
<td>&lt;1250 g and/or gestational age &lt;30 weeks</td>
<td>NA (13.5% infants had IVH, 4.5% had cystic periventricular leukomalacia)</td>
<td>Term</td>
<td>ADC, FA, and axial and radial diffusivity, ROI</td>
</tr>
<tr>
<td>Anjari et al85</td>
<td>53 preterm</td>
<td>24+2 to 32+4 weeks</td>
<td>Focal lesions on MRI</td>
<td>Term</td>
<td>TBSS and FA</td>
</tr>
</tbody>
</table>

ADC=apparent diffusion coefficient. CC=corpus callosum. DTI=diffusion tensor imaging. FA=fractional anisotropy. GA=gestational age. IVH=intraventricular haemorrhage. NA=not available. PLIC=posterior limb of internal capsule. ROI=region of interest. TBSS=tract-based spatial statistics. WM=white matter.
Correlations between DTI values and visual assessment scores have also been found. The FA values in the optic radiations increase with gestational age, and FA values from both tract-based spatial statistics analyses and probabilistic tractography indicate that visual function is directly associated with FA in the optic radiations of preterm infants at term-equivalent age.

Functional connectivity

Several resting state networks are reported to be present in preterm infants at term-equivalent age. Similar to term controls, preterm neonates had resting state networks that encompassed the primary visual cortex, bilateral sensorimotor areas, bilateral auditory cortex, a network including the precuneus, lateral parietal cortex and the cerebellum, and an anterior region that incorporated the medial and dorsolateral prefrontal cortex. Several preliminary reports have indicated the presence of multiple resting state networks in preterm infants as early as 30 weeks’ postmenstrual age. Whether these networks are intrinsic to the developing brain or mature across the third trimester remains an area of active investigation.

Imaging the preterm brain: childhood and adolescence

Although many progressive and regressive events that are crucial for cerebral development occur during the third trimester of gestation and in the newborn period, both postmortem examinations and MRI studies of typically developing subjects suggest that dynamic changes in brain composition and connectivity continue during childhood, adolescence, and early adulthood. In typically developing subjects, there are linear increases in white matter of 12–25% between the ages of 4 and 20 years, while synapses are pruned and grey matter decreases in size.

Structure-function relationships

DTI abnormalities have been reported in prematurely born children during childhood and adolescence (table 3 and figure 3). Similar to neonatal data, these abnormalities appear to cluster in central white matter and the regions to which it connects. In 33 children who were born

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<tbody>
<tr>
<td>137 preterm</td>
<td>&lt;1800 g</td>
<td>Congenital malformation; gross structural abnormalities on MRI</td>
<td>Term</td>
<td>Neurological examination, cerebral palsy at 28–24 months, ROI</td>
<td>Children with cerebral palsy had lower FA of right PLIC than did healthy infants (p=0.006)</td>
</tr>
<tr>
<td>30 preterm</td>
<td>28–33 weeks</td>
<td>Congenital abnormalities and infections; abnormal MRIs</td>
<td>2 weeks’ CA</td>
<td>Early intervention study; FA at 2 weeks; behaviour at 9 months, ROI</td>
<td>Experimental group had increased relative anisotropy in left PLIC compared with control group (p=0.03)</td>
</tr>
<tr>
<td>24 preterm</td>
<td>&lt;32 weeks</td>
<td>Congenital anomalies</td>
<td>Serial 30 and 36 weeks’ GA</td>
<td>No injury, grade 1–2 IVH, and grade 3–4 IVH groups; ADC, FA, Bayley PDI at 18–24 months’ CA, ROI</td>
<td>Decreased FA and increased ADC in infants with grade 3–4 IVH at second MRI compared with preterm infants with no IVH</td>
</tr>
<tr>
<td>38 preterm</td>
<td>&lt;33 weeks</td>
<td>Overt pathology on conventional MRI</td>
<td>Term</td>
<td>Griffiths mental developmental scale; ADC of centrum semiovale</td>
<td>Significant negative correlation between mean ADC and developmental scores</td>
</tr>
<tr>
<td>24 preterm</td>
<td>&lt;1800 g</td>
<td>Congenital anomalies and infections; blindness; quadruplets</td>
<td>Term</td>
<td>GMFS, cerebral palsy, ROI</td>
<td>Children with low neonatal FA had negative correlation between FA in left and right PLIC and normalcy index for severity of gait deficits (p=0.001; r=-0.59) and GMFS (p=0.06; r=-0.65)</td>
</tr>
<tr>
<td>27 very low birthweight</td>
<td>27 6±2 5 weeks, birthweight 1021±335 g</td>
<td>Congenital anomalies and infections</td>
<td>37 weeks’ postmenstrual age</td>
<td>Bayley PDI scales and GMFS at 18 months’ CA, ROI</td>
<td>Male infants had lower FA and higher ADC than did female infants (p&lt;0.05)</td>
</tr>
<tr>
<td>27 preterm</td>
<td>24–32 weeks</td>
<td>Congenital anomalies</td>
<td>2 weeks’ CA</td>
<td>Visual assessment score; TBSS, probabilistic fibre tracking</td>
<td>Logistic regression predicted development with 94% accuracy; only the right PLIC was a significant logistic component (p=0.015)</td>
</tr>
<tr>
<td>36 preterm</td>
<td>21–49 weeks</td>
<td>NA</td>
<td>29–41 weeks’ postmenstrual age</td>
<td>DTI fibre tracking of optic radiations; visual fixation task; probabilistic fibre tracking</td>
<td>Correlation between optic radiation FA and visual fixation tracking scores (p=0.001)</td>
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ADC=apparent diffusivity coefficient. CA=corrected age. DTI=diffusion tensor imaging. FA=fractional anisotropy. GA=gestational age. GMFS=good motor function scale. IVH=intraventricular haemorrhage. NA=not available. PDI=psychomotor developmental index. PLIC=posterior limb of internal capsule. ROI=region of interest. TBSS=tract-based spatial statistics. WM=white matter.

Table 2: Relations between WM alterations and developmental outcome in prematurely born subjects—DTI studies
prematurely at 2 years’ corrected age, the developmental quotient was linearly associated with FA in parts of the corpus callosum, whereas performance subscores were correlated with FA values in both the corpus callosum and the right cingulum.181

DTI parameters in prematurely born school-aged children, adolescents, and young adults have also been shown to significantly correlate with behavioural and motor outcomes.182,183 Nagy and colleagues183 noted alterations in FA values for both internal capsules and the corpus callosum in preterm males with attention-deficit hyperactivity disorder and ten matched controls at 11 years’ corrected age. Vangberg and colleagues190 reported decreased FA values in several white matter regions, including the internal capsule, corpus callosum, and superior fasciculus, in preterm subjects compared with term controls at the age of 15 years. Preterm adolescents with low intelligence scores had low FA values in the thalamocortical connections; furthermore, visuomotor deficits correlated with low FA values in the posterior limb of the internal capsule, and inferior fasciculus (p=0·05 for all).
Similarly, Constable and colleagues\(^{117}\) reported widespread decreases in FA in preterm children compared with term controls at the age of 12 years. Changes were found in intrahemispheric association fibres subserving language skills (ie, the external capsule and the anterior portions of the uncinate fasciculi bilaterally) and the deep white matter regions to which they project, as well as the splenium of the corpus callosum. Finally, after studying prematurely born adolescents and term control subjects at 19 years of age, Kontis and colleagues\(^{119}\) found that the D\(_V\) in the body of the corpus callosum was significantly correlated with verbal memory skills in the preterm group.

VBM studies
VBM has been extensively used to examine the effects of preterm birth on the developing brain at adolescence and in early adulthood (table 4 and figure 4).\(^{121,122}\) Gimenez and colleagues\(^{123,124}\) examined preterm and term control subjects at ages 12–18 years using both modulated (volume based) and unmodulated (density) analyses. The unmodulated data showed regions in the periventricular white matter and longitudinal fascicles that were significantly smaller in the preterm subjects than in term controls, whereas in the modulated analyses there were white matter decreases in regions distant from the ventricles at the origins and ends of the longitudinal fascicles. For many regions throughout the brain, the lower the prematurely born subjects’ gestational age or birthweight, the lower the white matter volume found.

Isaacs and colleagues\(^{121}\) identified a region in the left parietal lobe where there was less grey matter in prematurely born children with calculation deficits than in those without this disability. In the same population, absolute IQ scores were associated with changes in areas in both the parietal and temporal lobes.\(^{122}\) Similarly, Gimenez and colleagues\(^{125}\) noted significant decreases in thalamic nuclei when prematurely born subjects were compared with term controls at 14 years of age; there were significant correlations between thalamic nuclei volumes and tests of verbal fluency for the preterm group. These findings confirm the potential for decreases in thalamic volumes to be used as a biomarker of injury in preterm infants. New techniques such as automatic labelled brain atlases combined with conventional image analysis will further refine the structural assessment of the effects of prematurity.\(^{129}\)

VBM studies also indicate widespread decreases in white matter at adolescence.\(^{55}\) Soria-Pastor and colleagues\(^{126}\) reported significant decreases in white matter in 80% of adolescents who were born prematurely (<32 weeks’ gestational age) when compared with term controls at the age of 14 years. There were significant alterations in the centrum semiovale in 41% of the preterm subjects, while more than one-fifth had lower periventricular white matter volumes than term controls. Nosarti and colleagues\(^{56}\) have studied the largest cohort of prematurely born adolescents and term controls, matched by age and socioeconomic status. They noted widespread alterations in both grey and white matter volumes throughout the cerebral hemispheres (table 4). All areas where there were between-group white and grey matter differences were structurally associated, and subjects with ventriculomegaly had the most significant white matter changes. Several white matter regions, including those within the corpus callosum, were linearly associated with gestational age for the preterm group, and decreases in white matter volume in the middle

![Image](https://www.thelancet.com/neurology)
<table>
<thead>
<tr>
<th>Number</th>
<th>Major exclusion criteria</th>
<th>Birthweight/ GA</th>
<th>Age at MRI</th>
<th>VBM template, outcome measures</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isaacs et al(^{121}) 12 preterm with deficits in numerical operations, 12 preterm with deficits in mathematical reasoning, 12 preterm controls for each group</td>
<td>NA</td>
<td>&lt;30 weeks</td>
<td>15 years</td>
<td>VBM: template from 20 normal children; IQ, WM</td>
<td>Subjects who had deficits in numerical operations had less GM in left intraparietal sulcus (p=0.001) than those with no numerical operation deficits No differences for group who had deficits in numerical operations compared with control group</td>
</tr>
<tr>
<td>Isaacs et al(^{122}) 82: 4 groups (verbal IQ and performance IQ, small and large declines)</td>
<td>Neuromotor or neurosensory impairment</td>
<td>&lt;30 weeks</td>
<td>15 years</td>
<td>VBM: template from 20 normal children; IQ at 7 and 15 years</td>
<td>Negative correlation between performance IQ decline and GM in hippocampi (p=0.03) Volumetric data confirmed hippocampi were 4–5% smaller in group with large decline in performance IQ compared with those without change in IQ</td>
</tr>
<tr>
<td>Gimenez et al(^{123}) 50 preterm, 50 term</td>
<td>Evidence of WM injury on T2 MRI</td>
<td>≤2 weeks</td>
<td>12–18 years</td>
<td>Modulated and unmodulated VBM analyses; T3 SPM2 template with WM-specific template Unmodulated data identified regions in the periventricular WM and longitudinal fascicles that had significantly lower volume in preterms compared with term controls Modulated data showed WM decreases in regions distant from the ventricles at the origin and end of the longitudinal fascicles For several regions throughout the brain, the lower the GA and/or birthweight, the lower the WM volume</td>
<td></td>
</tr>
<tr>
<td>Gimenez et al(^{124}) 22 preterm, 22 term</td>
<td>Focal TBI, CP, neurological diagnosis, MRI abnormalities</td>
<td>≤2 weeks</td>
<td>14-15 years</td>
<td>VBM: SPAM normalised brain, sulcal measurements In preterm subjects, VBM indicated reduced GM volume in the orbital region compared with term controls Preterm subjects had significant decrease in the secondary sulci depth but not in primary sulci depth compared with term controls</td>
<td></td>
</tr>
<tr>
<td>Gimenez et al(^{125}) 30 preterm, 30 term</td>
<td>Focal TBI, CP, neurological diagnosis, MRI abnormalities</td>
<td>≤2 weeks</td>
<td>14 years</td>
<td>VBM: T1 SPM template; phonetic and semantic fluency tasks Preterm subjects had worse scores for both phonetic (p=0.044) and semantic fluency (p=0.001) than did term controls Preterm subjects had lower thalamic volumes than did term controls (left: p=0.001; right: p=0.002) Significant correlations between thalamic nuclei volume changes and verbal fluency (p=0.05) Semantic fluency was more highly correlated with thalamic nuclei GM than was phonetic fluency</td>
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</tr>
<tr>
<td>Kesler et al(^{126}) 29 preterm with no brain injury on ultrasound, 22 term</td>
<td>Congenital infection or anomalies; IVH, PVL, or ventriculomegaly on neonatal ultrasound</td>
<td>600–1250 g</td>
<td>12 years</td>
<td>VBM: customised template from all study subjects; complete paediatric neuropsychological battery Preterm males had lower WM in bilateral cingulum, CC, corticospinal tracts, prefrontal cortex, and superior and inferior longitudinal fasciculus than did term subjects (p&gt;0.001), no difference for females Preterm males had less GM volume in prefrontal cortex, basal ganglia, and temporal lobe (p=0.001) than did term males; no difference for females No correlation between VBM and perinatal or cognitive data</td>
<td></td>
</tr>
<tr>
<td>Sonia-Pastor et al(^{127}) 44 preterm, 43 term</td>
<td>TBI, CP, seizures, MRI abnormalities</td>
<td>≤2 weeks</td>
<td>14 years</td>
<td>VBM: T1 SPM2 template; full scale IQ, verbal IQ, performance IQ and digit symbol subtest Reduced global WM volume in preterm compared with term subjects (p=0.05) Performance IQ correlated with whole-brain WM volume (r=0.34; p=0.026) 80% preterm subjects had significant WM abnormalities; 41% had abnormal centrum semiovale, and 21% had abnormal periventricular WM Low scores on digit symbol subtest correlated with decreases in WM</td>
<td></td>
</tr>
<tr>
<td>Nosarti et al(^{128}) 218 preterm, 128 term</td>
<td>Neurological diagnosis, meningitis, TBI, cerebral infection</td>
<td>≤33 weeks</td>
<td>14-15 years</td>
<td>VBM of GM and WM: customised template from all study subjects; measures of reading, spelling, IQ, executive function, visuomotor integration test Widespread alterations in GM and WM throughout brain for preterm subjects All areas where between-group WM and GM differences were observed were structurally associated Preterm subjects with IVH and ventriculomegaly had greatest WM and GM alterations Preterm subjects had lower scores on language and executive function and were more likely to show cognitive impairment compared with term controls Several GM (sensorimotor, middle, superior temporal gyrus) and WM (precentral and postcentral gyrus, longitudinal fasciculus, CC) areas were linearly associated with GA; middle temporal gyrus (both WM and GM) associated with cognitive impairment in preterm group (p&lt;0.001)</td>
<td></td>
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<tr>
<td>Narberhaus et al(^{129}) 21 preterm, 22 term</td>
<td>History of CP, grade 3–4 IVH, PVL</td>
<td>≤33 weeks</td>
<td>20 years</td>
<td>fMRI: visual paired associates task; VBM of hippocampus Preterm and term subjects had differential BOLD signal during both encoding and recognition Preterm subjects had decreased hippocampal GM compared with term controls</td>
<td></td>
</tr>
<tr>
<td>Nosarti et al(^{130}) 28 preterm, 26 term</td>
<td>Neurological impairment</td>
<td>≤33 weeks</td>
<td>20–21 years</td>
<td>fMRI, VBM, phonological verbal fluency fMRI task Significant differences in BOLD signal for both “easy” and “hard” words between the groups Altered GM distribution in the preterm infants compared with control infants fMRI results only partly explained by volumetric differences</td>
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</tbody>
</table>


Table 4: Relations between GM and WM alterations and outcome in prematurely born children and adolescents—VBM studies
temporal gyrus were associated with cognitive impairment in the preterm group (table 5).

Finally, studies in typically developing children have documented the correlation between structure and function.10,11 Narberhaus and colleagues12 investigated the neural substrates of visual memory using a visual paired associates test in young adults born prematurely. Despite good task performance, preterm subjects had activation of different neural networks during mnemonic processing of visuo-perceptual material; additionally, the preterm group had decreased hippocampal grey matter bilaterally. Nosarti and colleagues13 used the same strategies on preterm subjects and term controls to detect alterations in brain activation during completion of a letter fluency task and compared this with regional VBM data. BOLD signals differed significantly between groups, but fMRI results were only partly explained by structural changes.

Functional connectivity

Over time, the connections in spatially remote regions in the developing brain are strengthened in an anterior to posterior direction, weaving distal brain areas into highly cohesive and connected circuits, while the strength of connections between contralateral homologues is reduced.14,15 When Gozzo and colleagues16 used a passive language task to study fcMRI in preterm children and term controls at the age of 8 years, those born prematurely had significantly stronger neural circuits between Wernicke’s area and alternative language regions including the right inferior frontal gyrus and both left and right supramarginal gyri. These data suggest that either prematurely born children use alternative networks for neural processing of language, or that there is a delay in maturation in the preterm group; additional studies are needed to verify this hypothesis.

Conclusions and future perspectives

Preterm birth is a common event, and injury to the developing brain occurs far too often in the prematurely born. Imaging technologies that enable exploration of acute injury and post-lesional alterations in structural and functional connectivity associated with preterm birth are crucially important—not only for assessing long-term neurodevelopmental risk but also for planning both individualised treatment and therapeutic intervention trials. The outcome of future neuroprotective treatments ranging from pharmacological drugs to stem cell therapies and environmental enrichment will require the routine monitoring of anatomic integrity, microstructural connectivity, and functional performance.

DTI-based imaging technologies are being developed to better examine the effect of preterm birth on axonal development. Future work is needed to investigate both strategies for automatic standardised segmentation of the preterm brain as well as the generation of a neonatal neuroimaging atlas that would provide uniform nomenclature among investigators. Longitudinal studies will provide important information about the dynamic effect of preterm birth at adolescence and beyond, and the refinement of a combined structure–function approach, such as use of the recently developed technique of diffusion spectrum imaging with the establishment of high-resolution connection matrix and resting state fcMRI.

Table 5: Effects of preterm birth on grey and white matter in adolescence—voxel-based morphometry studies

<table>
<thead>
<tr>
<th>Regions of interest</th>
<th>Effect of gestational age</th>
<th>Effect of injury</th>
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<tbody>
<tr>
<td>Grey matter</td>
<td>Regions linearly associated with gestational age include the middle and superior temporal gyrus, inferior frontal gyrus, medial frontal gyrus, fusiform gyrus, and thalamus</td>
<td>Decreased volume in the thalamus and pulvinar</td>
</tr>
<tr>
<td>White matter</td>
<td>Regions linearly associated with gestational age include the middle temporal gyrus, medial frontal gyrus, fusiform gyrus, anterior corpus callosum, parahippocampal gyrus, and supramarginal gyrus</td>
<td>Decreased volume in the middle temporal gyrus, cingulum, and posterior corpus callosum</td>
</tr>
</tbody>
</table>

Figure 4: Optimised voxel-based morphometry group by sex effect (ie, term males minus preterm males)

Decreased grey matter volume was seen in the left inferior frontal gyrus, or Broca’s region, at 12 years of age in preterm males (p<0·001; data from Kesler and colleagues17). Figure courtesy of Shelli Kesler (Stanford University School of Medicine, Palo Alto, CA, USA).

Search strategy and selection criteria

References for this Review were identified through searches of PubMed for MRI studies in prematurely born individuals published between January, 1985, and August, 2009. We used combinations of the following search terms: “infant”, “neonate”, “child”, “adolescent”, “adult”, “preterm”, “premature”, “prematurely born”, “brain”, “magnetic resonance imaging”, “MRI”, “functional magnetic resonance imaging”, “fMRI”, “functional connectivity”, “fcMRI,” “resting state connectivity”, “diffusion tensor imaging”, “DTI”, “tractography”, “probabilistic tractography”, “TBSS (tract-based spatial statistics)”, “volumetric”, “voxel-based morphometry”, “VBM”, “cortical folding”, “gyrification”, and “cortical surface area”. We reviewed the published studies and selected those that we judged to have most relevance to the topic. Only papers published in English were included. Preclinical studies, case reports, unblinded analyses, and topic reviews were excluded.
will inform both physicians and scientists about the processes of ongoing plasticity in vivo.

Preterm birth is now a major paediatric public health problem. If the goal of neonatal intensive care is survival without handicap for prematurely born infants, then identifying those early biomarkers of injury and outcome is crucial for our understanding of the developing brain. If connectivity can be altered and plasticity understood, targeted pharmacological and behavioural therapies could be developed to restore functional connectivity.

Contributors
All authors contributed equally to this paper.

Conflicts of interest
We have no conflicts of interest.

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Ashburner J, Friston KJ. Why voxel-based morphometry should be used. Neuroimage 2001; 14: 1238–43.


