A common neonatal image phenotype predicts adverse neurodevelopmental outcome in children born preterm


Abstract

Diffuse white matter injury is common in preterm infants and is a candidate substrate for later cognitive impairment. This injury pattern is associated with morphological changes in deep grey nuclei, the localization of which is uncertain. We test the hypotheses that diffuse white matter injury is associated with discrete focal tissue loss, and that this image phenotype is associated with impairment at 2 years. We acquired magnetic resonance images from 80 preterm infants at term equivalent (mean gestational age 29+6 weeks) and 20 control infants (mean GA 39+2 weeks). Diffuse white matter injury was defined by abnormal apparent diffusion coefficient values in one or more white matter region (frontal, central or posterior white matter at the level of the centrum semiovale), and morphological difference between groups was calculated from 3D images using deformation based morphometry. Neurodevelopmental assessments were obtained from preterm infants at a mean chronological age of 27.5 months, and from controls at a mean age of 31.1 months.

We identified a common image phenotype in 66 of 80 preterm infants at term equivalent comprising: diffuse white matter injury; and tissue volume reduction in the dorsomedial nucleus of the thalamus, the globus pallidus, periventricular white matter, the corona radiata and within the central region of the centrum semiovale. The abnormal image phenotype is associated with reduced median developmental quotient (DQ) at 2 years (DQ=92) compared with control infants (DQ=112), p<0.001. These findings indicate that specific neural systems are susceptible to maldevelopment after preterm birth, and suggest that neonatal image phenotype may serve as a useful biomarker for studying mechanisms of injury and the effect of putative therapeutic interventions.

Keywords: Diffuse white matter injury; Apparent diffusion coefficient; Neurodevelopmental outcome; Diffusion tensor imaging; Deformation based morphometry

Introduction

The prevalence of preterm birth is increasing in resource-rich countries (Goldenberg et al., 2008), and this presents a growing burden to education services, as well as affected individuals and their families, because neurocognitive impairment occurs in 40–50% of children born extremely preterm (Bhutta et al., 2002; Larroque et al., 2001; Marlow et al., 2005). The neural substrates that underlie impairment are not fully understood, but diffuse white matter injury on magnetic resonance (MR) imaging is seen commonly among preterm infants at term equivalent age, and is likely to contribute to the substrate for impairment because it occurs with a similar prevalence, and is associated with short term measures of adverse neurodevelopmental outcome (Dyet et al., 2006; Krishnan et al., 2007). Previous imaging studies suggest that white matter injury does not occur in isolation, but rather, it is associated with maldevelopment of remote grey matter structures, which suggests that developing neural systems are affected by preterm birth (Inder et al., 1999; Lin et al., 2001).

We previously carried out a group-wide comparison of volume change maps from preterm infants and term-born controls with respect to a common template, and detected discreet areas of volume reduction in the thalamus and lentiform nuclei in the preterm group, which was most apparent among those infants with diffuse non-cystic white matter abnormalities (Boardman et al., 2006). However, the children were too young for functional correlates of the neonatal image phenotype to be assessed, and the random field family-wise error rate (FWER) correction for multiple tests was used, which is now known to be a less powerful approach compared with techniques...
that control the false discovery rate (the proportion of false rejections of the null hypothesis among the total number of rejections), when relatively small groups are studied (Benjamini and Hochberg, 1995; Genovese et al., 2002; Laird et al., 2005; Langers et al., 2007). In this study we test the hypothesis that after premature birth there is an imaging phenotype comprised of white matter abnormality and focal morphological change which predicts neurodevelopmental impairment in early childhood.

Materials and methods

Participants

The MR images of 80 preterm infants at term equivalent age (33 males and 47 females), and 20 term-born control infants (12 males and 8 females) were analyzed. The infants were recruited at Hammersmith Hospital between February 2001 and November 2003. Preterm infants were eligible for recruitment if they were born at ≤ 34 completed weeks of gestation and had no congenital malformation or congenital central nervous system infection. Infants with cystic periventricular leukomalacia, hemorrhagic parenchymal infarction or post-hemorrhagic ventricular dilation were excluded. All control infants were considered healthy and recruited from the post-natal wards.

The preterm group underwent MR imaging at a mean of 40 ± 3 weeks post-natal age (range 37–43 weeks), and the control group underwent MR imaging at a mean age of 40 ± 3 weeks PMA (range 36–43 weeks). Demographic and anthropometric details are shown in Table 1. Twenty-six of the preterm infants were growth restricted [birth weight < 10th centile for age, Child Growth Foundation software (Freeman et al., 1995)], and five required supplemental oxygen at 36 weeks post-natal age. No infant received post-natal steroids. Preterm infants were sedated for the MR examination with chloral hydrate, and control infants were examined in natural sleep. Pulse oximetry, electrocardiographic and televisial monitoring were used throughout the examination. Ear protection was used (Natus Mini-Ucl.ac.uk/spm/) to identify regions of apparent diffusion coefficient (ADC) values were calculated by positioning ROIs in frontal, central and posterior white matter at the level of the centrum semiovale on the reference image (b = 0) and on the read, phase and slice DWIs, using published methods (Cournell et al., 2003). Infants were classified as having white matter injury if one or more white matter region had an ADC value greater than 2 standard deviations above the mean of that measured in a group of normal term controls: >1.566 × 10⁻³ mm²/s (frontal white matter); >1.378 × 10⁻³ mm²/s (central white matter); >1.474 × 10⁻³ mm²/s (posterior white matter) (Boardman et al., 2006).

Deformation-based morphometry

A high dimensional non-rigid registration algorithm which uses a free form deformation model (cubic B-spline) and normalised mutual information as the maximised similarity measure was used to bring all subjects into alignment with a reference template, chosen as the 3D image of an infant born at term (Rueckert et al., 1999; Rueckert et al., 2003; Boardman et al., 2006). The goal of the registration process is to achieve precise spatial correspondence between all subjects and the anatomy of the reference subject. The output of the registration process is a deformation field from which voxel-wise spatially resolved scalar measurements of volume change are extracted (Davatzikos et al., 1996; Studholme et al., 2004). A qualitative evaluation of the accuracy of anatomical alignment of the transformed images with the template was made before deformation fields were used to calculate volume changes.

Statistical analysis

Volume change maps for each subject relative to the reference anatomy were analyzed using a two-sample t-test implemented in SPM5 (http://www.fil.ion.ucl.ac.uk/spm/) to identify regions of statistically significant difference in tissue volume between groups. Analyses were carried out using control of the false discovery rate with a significance threshold of p < 0.001. Only voxel clusters > 10 were considered in the model. The effect of quantitatively defined diffuse white matter injury on tissue volume was assessed by comparing preterm infants with and without white matter injury to term-born controls.

Neurodevelopmental outcome

Neurodevelopmental progress was assessed using the Griffiths Mental Development Scales (Revised) (Huntley, 1996) which provide an overall developmental quotient (DQ) with subscales assessing skill areas (locomotor, personal–social, hearing and language, eye and hand coordination, performance, and additionally practical reasoning for children ≥ 2 years of age). The developmental and subscale quotients from the Griffiths scales are calculated taking into account the exact age of the child at the time of the assessment. Scores are presented for chronological age because the majority of infants were over 2 years at

Table 1

<table>
<thead>
<tr>
<th>Preterm infants at term equivalent age</th>
<th>Term-born controls n = 20</th>
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<tbody>
<tr>
<td>Group A n = 66</td>
<td>Group B n = 14</td>
</tr>
<tr>
<td>PMA at birth/weeks (mean and range)</td>
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<tr>
<td>29.6 ± 6 (24.1–33.3)</td>
<td>30 (24.6–34)</td>
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<tr>
<td>Birth weight/g (mean and range) [SD]</td>
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<tr>
<td>1259 (610–2226) (−0.70 [1.06])</td>
<td>1307 (640–2190) (−0.81 [1.21])</td>
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<tr>
<td>Occipitofrontal head circumference at birth/cm [SD]</td>
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<tr>
<td>27.0 (22.0–32.5) (−0.64 [1.05])</td>
<td>27.4 (24.8–30.6) (−0.67 [1.67])</td>
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<tr>
<td>Weight at time of image acquisition/g (mean and range) [SD]</td>
<td></td>
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<tr>
<td>3144 (1700–4380) (−0.94 [1.36])</td>
<td>3095 (2170–3560) (−1.34 [0.86])</td>
</tr>
<tr>
<td>Occipitofrontal head circumference at scan/cm (mean and range) [SD]</td>
<td></td>
</tr>
<tr>
<td>35.2 (31.2–38.0) (0.08 [1.49])</td>
<td>34.8 (33.0–38.0) (−0.43 [1.21])</td>
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</tbody>
</table>
the time of assessment, and because the Griffiths scores calculated on chronological age are more predictive of functional outcome in later childhood than scores corrected for prematurity (van den Hout et al., 1998; Miller et al., 1984). All children were examined neurologically looking specifically for evidence of cerebral palsy (Bax et al., 2005). All assessments were carried out by experienced developmental paediatricians unaware of the quantitative MRI findings.

Weight and head circumference were measured and the standard deviation scores (SDS) with respect to the age and sex adjusted means were calculated with the Child Growth Foundation software (Freeman et al., 1995).

The relationship between developmental quotient and gestational age at birth was examined by estimating the linear regression controlling for age at time of assessment. Parametric tests were used for all analyses, except the analysis of variance of the DQ scores at 2 years because these values were not normally distributed, and required a non-parametric rank based method. All analyses were carried out using SPSS v16.0.

Results

Computational anatomic phenotype associated with diffuse white matter injury

Of the 80 preterm infants that had DWI images free of movement artefact and suitable for quantitative analysis, 66 had abnormally high ADC values in at least one white matter region at the level of the centrum semiovale (group A). Fourteen had ADC values within normal limits throughout the white matter (group B). There was no significant difference between preterm groups A and B with respect to gestational age at birth (p = 0.66) or birth weight (p = 0.68).

Group A had discrete tissue loss within the dorsomedial complex of the thalami, the globus pallidi, and focally within the posterior periventricular white matter, the corona radiata and the centrum semiovale compared with term controls compared with controls (r = 4.42 p < 0.001, FDR). No morphological changes were detected in group B at the same significance threshold (Fig. 1) compared to controls. There were no significant volumetric differences between preterm groups A and B when multiple tests were controlled.

Neurodevelopmental outcome

Sixty-nine percent (55/80) of the preterm group were assessed at a mean chronological age of 27.5 months (range 24.0–31.0). The mean weight of the preterm group at follow-up was 12.94 kg (range 9.18–17.64) with mean SDS (weight) 0.29 (SD 1.12); the mean OFC of the preterm group was 49.1 cm (44.8–54.0), with mean SDS (OFC) −0.45 (SD 1.24). There was no significant difference in gestational age at birth, prevalence of supplemental oxygen dependency or IUGR between those who were seen at 2 years and those who were not. Eighty-five percent of the term control group (17/20) were assessed at a mean age of 31.1 months (range 23.5–40.0). None of the children had cerebral palsy.

The DQ values were not normally distributed: the median DQ for all preterm infants was 101 (range 68–141). The median DQ of the term-born control group was 112 (range 94–129). There was a dose-dependent relationship between DQ and gestational age at birth (B = 1.9, 95% CI 0.353–2.026, p = 0.006) (Fig. 2). Age at time of assessment was not significant in the model.

The DQ and subscale quotients for preterm group A, preterm group B, and for the controls are shown in Table 2: there was significant inequality in the median DQ between preterm group A, preterm group B and term controls, Table 3 (H = 18.825, p = 0.001 Kruskal–Wallis).

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Post-hoc tests show that this is accounted for by a highly significant difference in DQ between preterm group A \((p = 0.01)\) and controls, whereas the difference in DQ scores between preterm group B and controls \((p = 0.054)\), and between group A and group B \((p = 0.354)\) did not reach statistical significance (Mann–Whitney test with Bonferroni correction for multiple tests).

The significant effect attributable to neonatal image phenotype remained when DQ scores were corrected for degree of prematurity at birth \(H = 8.85, p = 0.012\).

**Discussion**

We identified a common highly significant anatomic phenotype consisting of diffuse white matter injury and focal tissue loss localised to the dorsomedial nucleus of the thalamus, the globus pallidus, and within the white matter of the corona radiata, posterior periventricular white matter, and the central region of the centrum semiovale. The abnormal neonatal image phenotype was associated with a lower DQ at 2 years of age compared to preterm infants without this pattern of injury and to term controls. Our data support the hypothesis that white matter injury impacts upon the development of basal ganglia and thalami, and that this disturbance to the connectivity of developing neural systems has important functional consequences. This pattern of injury in surviving infants is consistent with neuropathological studies of infants who died, which show involvement of both grey and white matter structures (recently labelled the ‘encephalopathy of prematurity’), and give confidence that these post-mortem data truly reflect the effects of prematurity rather than the events associated with mortality (Volpe, 2009). We have shown previously that these structural changes are not attributable to a global failure in brain growth (Boardman et al., 2007).

Focal tissue volume changes were not detected in preterm infants without white matter injury (group B) with respect to the controls, but neither was difference detected between the two preterm groups. This may be attributable to type 2 error because preterm group B consisted of only 14 subjects. Further investigation of a larger group of preterm infants with normal diffusion parameters in white matter would be required to address this issue. Unfortunately, prior specification of the required sample size is not possible in the absence of established methods for power calculations in this type of morphometric study. However it is predictable that very large groups would be needed because the general effect of prematurity (as seen in Tables 2 and 3, and Fig. 2) on neural function means that even those infants with less severe white matter injury not detected as an abnormal ADC might have some lesser element of deep grey matter change. A design that included region of interest (ROI) measurements and prior hypotheses about effect size could enable accurate sample size calculations, but robust tools to segment the ROIs identified in this study are not yet available.

We present neurodevelopmental outcome scores calculated on chronological age at time of assessment because most of the children were older than 24 months, and although there is no universal agreement there is some consensus to cease correction at this age (Johnson and Marlow, 2006). Specifically, with respect to the Griffiths scales, uncorrected DQ scores are probably more predictive of cognitive performance at 5.5 years (van den Hout et al., 1998). One limitation of the study is that outcome data were only available for 69% of the preterm group. It is possible that we saw children who were not representative, but there was no significant difference in known neonatal risk factors for adverse outcome between the two preterm groups.

The dorsomedial nucleus of the thalamus consists of three morphologically distinct regions (magnocellular, parvocellular, and densocellular portions), and is the principal thalamic relay nucleus for the prefrontal cortex, as well as receiving afferent connections from the striatum, basal ganglia and limbic system. It has reciprocal projections to the prefrontal cortex and also projects to anterior cingulate, anterior insular and dorsomedial frontal cortices (Goldman-Rakic and Porrino, 1985; Jones, 1997; Armstrong, 1990). This connectivity distribution implies a role in integrating cognition with affective experience, and neuronal number in the nucleus appears to be associated with behavioural function and disease in adults (Jelsing et al., 2006; Young et al., 2004).

| Table 3 |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                | Median DQ (range) | Mean rank |
| Preterm group A | 92 (59–131)      | 29.18         |
| Preterm group B | 102 (64–112)     | 35.69         |
| Term controls   | 112 (94–129)     | 54.59         |

Kruskal–Wallis one way analysis of variance for median DQ at 2 years based on preterm image phenotype and control patients \((H = 18.825, df. 2, p = 0.001)\).

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The nucleus has a unique developmentally regulated pattern of neuronal death and survival in that there is a relative excess in neuronal number in the human newborn compared with the adult (Abitz et al., 2007). This could be explained by a second wave of neuronal migration from the telencephalic ganglionic eminence to the dorsal thalamus after the earlier migration of neurones from the ventricular zone (Leticin and Rakic, 2001; Bystron et al., 2008). The loss of volume in this region that we detected in association with prematurity birth and diffuse white matter injury could represent an attenuation of the second wave of neurones from the telencephalon, deafferentation secondary to white matter injury or cortical abnormalities, or dysregulation of the apoptotic mechanisms that govern thalamic development (Volpe, 2009). It is relevant that in a post-mortem series of infants who died with cystic PVL the dorsomedial nucleus was especially affected by neuronal loss over and above other thalamic nuclei (Ligam et al., 2008).

The globus pallidus consists of an internal (GPI) and external (GPe) segment separated by the mediadl mediulary lamina. The main afferent input is from the dorsal striatum and substantia nigra, and the GPi sends inhibitory connections to the thalamus. The globus pallidus has been identified as an essential substrate for controlling access to working memory (McNab and Klingberg, 2008), which underpins a variety of cognitive functions including general intelligence, attention and reasoning (Suss et al., 2002; Conway et al., 2003), and which is impaired among children born preterm (Woodward et al., 2005). Our data are consistent with a disturbance to this system contributing to later functional impairment in preterm infants.

We identified tissue contraction within white matter among children with abnormally high ADC values. Volume reduction was distributed within the posterior periventricular white matter, and is consistent with the relative enlargement of the adjacent posterior horns of the lateral ventricles seen in the preterm population (Peterson et al., 2003; Boardman et al., 2006). Other foci of reduced white matter volume were detected in the corona radiata and the middle region of the centrum semiovale (p<0.001). Since these are among the first white matter regions to myelinate (Counsell et al., 2002), it is possible that focal reductions in tissue volume may reflect reduced or delayed myelination in these tracts compared with infants born at term. Maldevelopment of posterior periventricular white matter would be expected to result in motor impairment, and although none of the children in this study had cerebral palsy, changes in this region could explain the reduction in locomotor scores seen in the preterm group compared with controls (Table 2), and contribute to the substrate to minor motor impairment that is common among children born preterm (Marlow et al., 2007).

Cortical volume reduction at term equivalent age has been reported when global tissue classification methods are used (Inder et al., 2005). We did not expect to detect global cortical changes in this application of DBM because it is sensitive to local change that is spatially consistent across the whole group, rather than summated changes detected by methods that use tissue classification. We did not detect cerebellar volume loss; this might be because cerebellar growth appears to be preserved in the absence of major destructive supratentorial lesions (Limeropoulos et al., 2005; Srivinasa et al., 2006) and we excluded such infants from our study.

A potential limitation of the study was classification of diffuse white matter injury based on ADC values. We chose this metric because it provides an objective measure of diffuse white matter injury (Counsell et al., 2003), but measures of white matter microstructure that take account of anisotropy, such as fractional anisotropy (FA), may provide a more refined metric of white matter integrity. Regional or tract based FA is likely to be helpful for investigating the growth of specific structures with respect to their connectivity patterns.

Identifying a statistically significant neonatal MR phenotype with functional correlates suggests that early image acquisition with computational analysis is valuable for providing objective data on neurodevelopmental prognosis for preterm infants. Morphometry advances understanding of the disturbances to neural systems that occur in association with premature birth and it may be a useful biomarker for investigating mechanisms of injury and the efficacy of interventions designed to reduce the burden of cognitive impairment following preterm birth.

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References


