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Perinatal risk factors altering regional brain structure in the preterm infant

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Neuroanatomical structure appears to be altered in preterm infants, but there has been little insight into the major perinatal risk factors associated with regional cerebral structural alterations. MR images were taken to quantitatively compare regional brain tissue volumes between term and preterm infants and to investigate associations between perinatal risk factors and regional neuroanatomical alterations in a large cohort of preterm infants. In a large prospective longitudinal cohort study of 202 preterm and 36 term infants, MR scans at term equivalent were undertaken for volumetric estimates of cortical and deep nuclear grey matter, unmyelinated and myelinated white matter (WM) and CSF within 8 parcellated regions for each hemisphere of the brain. Perinatal correlates analysed in relation to regional brain structure included gender, gestational age, intrauterine growth restriction, bronchopulmonary dysplasia, white matter injury (WMI) and intraventricular haemorrhage. Results revealed region-specific reductions in brain volumes in preterm infants compared with term controls in the parieto-occipital (preterm mean difference: −8.1%; 95% CI = −13.8 to −2.3%), sensorimotor (−11.6%; −18.2 to −5.0%), orbitofrontal (−30.6%; −49.8 to −11.3%) and premotor (−7.6%; −14.2 to −0.9%) regions. Within the sensorimotor and orbitofrontal regions cortical grey matter and unmyelinated WM were most clearly reduced in preterm infants, whereas deep nuclear grey matter was reduced mainly within the parieto-occipital and subgenual regions. CSF (ventricular and extracerebral) was doubled in volume within the superior regions in preterm infants compared with term controls. Cerebral WMI and intrauterine growth restriction were both associated with a more posterior reduction in brain volumes, whereas bronchopulmonary dysplasia was associated with a more global reduction across all regions. In contrast degree of immaturity was not related to regional brain structure among preterm infants. In summary, preterm birth is associated with regional cerebral tissue reductions, with the adverse pattern varying between risk factors. These findings add to our understanding of the potential pathways leading to altered brain structure and outcome in the preterm infant.

Keywords: cerebral development; preterm infant; volumetric MRI; white matter injury

Abbreviations: BPD = bronchopulmonary dysplasia; CGM = cortical grey matter; DNGM = deep nuclear grey matter; ICV = intracranial cavity volume; IUGR = intrauterine growth restriction; MWM = myelinated white matter; unMWM = unmyelinated white matter; WM = white matter; WMI = white matter injury

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Introduction

Mortality rates for very low birth weight preterm infants (<30 weeks or 1250 g) have fallen in the last decade, largely due to improvements in obstetric and neonatal intensive care in the early to mid 1990s (Horbar *et al.*, 2002). Surfactant therapy, new ventilator strategies and increasing use of pharmacological agents such as antenatal steroids and indomethacin have played a role in improving outcomes (Lorenz *et al.*, 1998; Stevenson *et al.*, 1998). However, there

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has been a growing concern over the persistence of high rates of adverse neurodevelopmental outcomes (Anderson and Doyle, 2003; Hack *et al.*, 1996). As many as 10–15% of very preterm infants develop cerebral palsy whilst up to 50% have significant neurobehavioural problems including lowered IQ, attention deficit hyperactivity disorder, anxiety disorders and learning disabilities (Hack and Fanaroff, 1999; Hack and Taylor, 2000; Perlman, 2001). These abnormalities lead to considerable educational burdens (Peterson, 2003), with economic and social implications (Doyle, 2004; Marlow *et al.*, 2005).

Cranial ultrasound is the most common neuro-imaging modality in the preterm infant used to assess the presence of cerebral injury. Cranial ultrasound is useful in the detection of intraventricular haemorrhage and cystic periventricular leucomalacia, but has poor sensitivity in the detection of diffuse white matter (WM) abnormalities that are detected by MR imaging (Maalouf *et al.*, 2001; Inder *et al.*, 2003*a*). Qualitative abnormalities in the WM have been described, in up to 70% of very preterm infants including WM signal abnormality, enlarged ventricles and delayed myelination (Maalouf *et al.*, 2001; Inder *et al.*, 2003*b*). In addition to evaluating injury, MRI techniques have been undertaken to define cerebral structure following preterm birth from the newborn period into later childhood. These studies have shown that preterm birth alters brain structure with reduced total cerebral tissue volumes when compared with term controls (Abernethy *et al.*, 2002; Nosarti *et al.*, 2002; Inder *et al.*, 2003*b*, 2005). Factors associated with reductions in total cerebral volumes have included the degree of immaturity at birth (Inder *et al.*, 2005), white matter injury (WMI; Olsen *et al.*, 1998; Inder *et al.*, 1999), intrauterine growth restriction (Tolsa *et al.*, 2004*a*) and exposure to postnatal dexamethasone therapy (Murphy *et al.*, 2001). During the third trimester, brain development proceeds in a systematic rostro-caudal progression (Rakic, 1988) with the pace of brain maturation varying by brain region (Huttenlocher and Dabholkar, 1997), suggesting that some brain regions may be more vulnerable to alteration with preterm birth. In the intrauterine environment these events are largely genetically controlled, but with preterm birth there may be disruption to this maturational sequence depending upon the nature and timing of the insult (Peterson, 2003). Such regional vulnerabilities have been previously demonstrated with reductions in brain volumes within the premotor, sensorimotor, midtemporal and parieto-occipital regions reported both near term (Peterson *et al.*, 2003) and at 8 years of age in infants born prematurely (Peterson *et al.*, 2000). There have been no previous studies on the impact of perinatal factors on alterations in specific brain regions rather than total brain volumes at term equivalent. In later childhood, preterm infants are reported to have reductions in GM reported within the temporal and frontal regions (Isaacs *et al.*, 2003, 2004; Reiss *et al.*, 2004) with intraventricular haemorrhage contributing to GM volume reductions with a gender effect noted. Lower birth weight, but not gestational age, was associated with reduced WM. The lack of insight of the impact of perinatal factors on the developing brain at the time of discharge from the neonatal intensive care environment limits our understanding of the pathway to altered brain structure in prematurely born infants.

Thus, this study aimed to investigate the impact of perinatal risk factors on regionally specific patterns of altered brain structure utilizing 3D MRI. These risk factors included those recognized for subsequent neurodevelopmental risk in the preterm infant including WMI, intraventricular haemorrhage, gestational age, intrauterine growth restriction and bronchopulmonary dysplasia. In addition, we aimed to compare regional brain tissue volumes between term and preterm infants.

Material and methods

Subjects

A prospective observational cohort study was conducted between 2001 and 2004 at the Royal Women's and Royal Children's Hospitals in Melbourne in which all preterm infants with birthweight <1250 g and/or gestational age <30 weeks surviving to term equivalent age were eligible for recruitment. Infants with congenital anomalies were excluded (3%). Over this study period 348 eligible preterm infants were admitted into the neonatal nurseries at these hospitals and 233 (67%) preterm infants were recruited. The most common reasons for failure to recruit were inability to obtain parental consent (22%) because of early hospital transfer or long distance from the hospital. There were no significant differences in the infants recruited in comparison to those not recruited in relation to gender, multiple birth, gestational age at delivery, intraventricular haemorrhage or bronchopulmonary dysplasia (BPD; defined as oxygen requirement at 36 weeks' corrected gestational age). From the postnatal wards of the Royal Women's Hospital and via response to advertisements in the recruiting hospitals 51 term control infants were recruited. These infants had an unremarkable antenatal course, labour and delivery at >37 weeks gestation and were in good health with no parental or medical concerns at the time of their study.

Perinatal data

Perinatal data were obtained by chart review. Birthweight *Z*-scores were computed relative to the British Growth Reference data (Cole *et al.*, 1998). Intrauterine growth restriction (IUGR) was defined as a birthweight *Z*-score <−2 SD. Cranial ultrasound scans were obtained serially throughout the neonatal intensive care course in all infants within the first 48 h and at ages 4–7 days and 4–6 weeks. If an abnormality was detected, weekly cranial ultrasound assessments were undertaken. All ultrasound scans were reported independently of clinical details and MR scans. The highest grade of intraventricular haemorrhage (IVH) was recorded.

MRI scanning

All infants were scanned at term or term equivalent (40 ± 1.7 weeks) without sedation. Infants were fed, swaddled, outfitted with earphones, and placed in a vacuum-fixation bean bag. Sleeping

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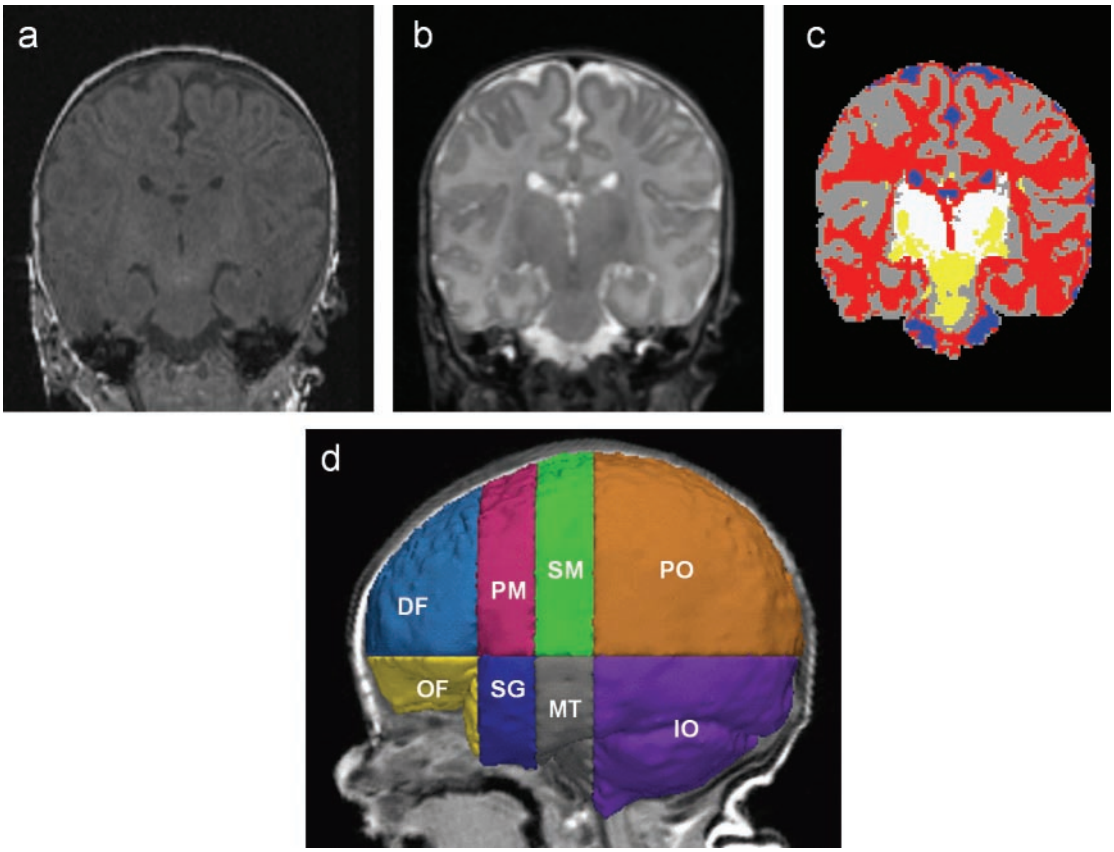

Fig. 1 Coronal SPGR (**A**) and coronal T2-weighted (**B**) MR images used to create tissue segmentation map representing CGM (grey), unMWM (red), CSF (blue), deep nuclear grey matter (white), and MWM (yellow) (**C**). Parcellated image of the infant brain illustrating cerebral regions (**D**). Region codes are: DF = dorsal prefrontal, OF = orbitofrontal, PM = premotor, SG = subgenual, SM = sensorimotor, MT = midtemporal, PO = parieto-occipital, IO = inferior occipital and cerebellum.

infants were scanned in a 1.5 tesla General Electric Signa System MR scanner (Milwaukee, WI, USA), located at the Royal Children's Hospital, Melbourne. Two different imaging modes were applied: 3D T1 spoiled gradient recalled (SPGR) [1.2 mm coronal slices; flip angle 45°; repetition time (TR) 35 ms; echo time (TE) 9 ms; field of view (FOV) 21 × 15 cm^2; matrix 256 × 192] and T2 dual echo (interleaved acquisition) fast recovery fast spin echo sequences (2 mm coronal; TR 4000 ms; TE 60/160 ms; FOV 22 × 16 cm^2; matrix 256 × 192, interpolated 512 × 512).

MR image analyses

Qualitative MR analysis

Images were analysed qualitatively for WMI by a single investigator (T.I.) blinded to the infant's clinical history. WMI was graded from 1–4 where Grade 1 was normal, Grade 2 was mild non-cystic abnormality, Grade 3 was moderate to severe non-cystic abnormality and Grade 4 was severe cystic abnormality (Inder *et al.*, 2003*b*). Of the MR scans 100 were randomly chosen to be re-read both by T.I. and independently by a second rater with intra-rater and inter-rater group assignment evaluated by cross tabulation with Cohen's kappa analysis. Intra-rater analysis revealed 95% concordance in group assignment with the 5% variation in Grades 1 and 2 WMI only. Inter-rater assignment revealed 94% concordance with 1% variation between Grades 2 and 3 and 5% variation in Grades 1 and 2 WMI. Differences in ratings were co-reviewed by both reviewers with a consensus reached on all cases.

Quantitative volumetric MR analysis

Due to problems with scan quality related mostly to imaging artefact, 238 infants (84% of those recruited) could be utilized for quantitative volumetric analysis, including 36 control infants (72% of those recruited) and 202 preterm infants (87% of those recruited). Analysis was undertaken on Sun Microsystems workstations (Palo Alto, CA). Linear transformation algorithms were applied in order to align the T2 images to the SPGR images for tissue classification. A non-parametric supervised estimator of tissue class conditional probability density functions was performed. This method utilizes the *k*-nearest neighbour density estimation, which is an optimal estimator that asymptotically approaches the minimum possible classification error rate R^* as $R^*(1+1/K)$. The optimal density function estimates were created by interactively selecting representative voxels as training points of CSF, cortical grey matter (CGM) and deep nuclear grey matter (DNGM), myelinated white matter (MWM) and unmyelinated white matter (unMWM), each of which differ in intensity (Fig. 1A–C). After iterative supervised training, a spatially varying model was identified through alignment with an anatomical atlas of

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a 40-week-old infant (Warfield *et al.*, 2000). Maximum likelihood estimation was used to select the most likely tissue class label for each voxel (Warfield, 1996; Warfield *et al.*, 2000). Tissue segmentation was carried out by a single operator (D.K.T.). Tissue segmentation was completed on five infants blinded for 10 segmentations with intra-observer reliability for D.K.T. on tissue segmentation of CSF 0.99, CGM 0.85, MWM 0.73, unMWM 0.83 and DNGM 0.61. In addition, the intracranial cavity volume (ICV) was measured by creating a brain versus non-brain tissue mask on the T1-weighted image. The ICV included all the grey matter, WM and CSF within the skull.

In order to make regional comparisons, the previously described and validated Talairach scheme was utilized (Peterson *et al.*, 2000, 2001). Each infant brain image was first registered into Talairach space (Talairach, 1988) by interactive rotation and translation in the left/right, anterior/posterior, and inferior/superior directions, followed by parcellation. The brain image was parcellated by dividing the brain into hemispheres along the mid-sagittal plane, and each hemisphere was further divided into eight anatomical regions (dorsal prefrontal, orbitofrontal, premotor, subgenual, sensorimotor, midtemporal, parieto-occipital and inferior occipital with cerebellum), using the axial plane passing through the anterior commissure and posterior commissure (AC–PC) line and three limiting coronal planes. The first coronal plane was positioned at the most anterior part of the genu of the corpus callosum, the second passed through the anterior border of the AC, and the third through the PC (Fig. 1D). Alignment and Talairach parcellation was carried out by a single operator (M.P.). Parcellation was completed five times on five separate infants with intraclass correlation providing intra-observer reliability for the regional volumes averaging 0.89 (range 0.77–0.98).

Statistical analyses

Statistical analysis was performed using SAS version 8 (SAS Institute Inc., Cary, NC). Exploratory analysis indicated that brain volumes were approximately normally distributed, whether considered in total or parcellated. Following the approach of Peterson *et al.* (2003) a mixed models analysis was used to assess differences between preterm and term infants, and to examine associations of preterm volumes with clinical factors, with all 16 brain regions analysed in the same model. Correlation among repeated measures (regions within the same infant) was modelled by including a random intercept for each infant. In addition to the random intercept variance, heteroscedasticity was modelled between regions of the brain by allowing different residual variances within each region.

Models included two within-subject factors, region (eight levels) and hemisphere (two levels). The primary between-subjects factor of interest was group (preterm and term), while further analysis within the preterm group examined effects due to gender, WMI grade (Grades 3/4 versus Grades 1/2), gestational age, BPD, any grade of IVH and IUGR. Further models included a term for total ICV of the brain, to allow for potential confounding due to differences in size. In addition, two- and three-way interaction effects between group, region and hemisphere were tested. To enhance interpretation of findings beyond the testing of null hypotheses relating to various overall effects and their interactions, estimates of contrasts of interest were constructed, in particular differences between preterm and term infants within each region, and, within the preterm infants, region-specific differences related to each covariate of interest.

Table 1 Characteristics of the total cohort

Characteristics	Preterm (n = 202)	Term (n = 36)
Gestational age at birth (weeks) [mean (SD)]	27.6 (2.0)	38.6 (1.5)
Gestational age at MRI (weeks) [mean (SD)]	40.1 (1.7)	40.2 (1.5)
Birth weight (g) [mean (SD)]	965 (239)	3224 (546)
Weight at MRI (g) [mean (SD)]	2990 (561)	3436 (499)
Head circumference at MRI (cm) [mean (SD)]	34.6 (3.3)	35.8 (1.5)
Male, n (%)	102 (51)	20 (56)
Multiple birth, n (%)	85 (42)	1 (3)
Bronchopulmonary dysplasia,[a] n (%)	69 (34)	0
Inotropic support, n (%)	80 (40)	0
Postnatal steroid therapy,[b] n (%)	18 (9)	0
Intrauterine growth restriction,[c] n (%)	23 (11)	2 (6)
Antenatal steroids administered, n (%)	175 (87)	1 (3)
Positive pressure ventilation, n (%)	153 (76)	1 (3)
Total parenteral nutrition (days), n (%)	169 (66)	2 (6)
Patent ductus arteriosus (indomethacin administered) n (%)	73 (36)	0
WMI, any grade, n (%)	131 (65)	1 (3)
WMI, grade 3/4, n (%)	34 (17)	0
IVH, any grade,[d] n (%)	25 (12)	0

[a]Required oxygen at 36 weeks gestational age; [b]postnatal dexamethasone, 0.15 mg/kg per day, reducing over 14 days; [c]Z score >2 SD below mean weight for gestational age; [d]three values missing.

Results

Subjects

MRI scans of 202 preterm infants and 36 term control infants were analysed. Table 1 shows the characteristics of the cohort. There was no difference in mean gestational age at MRI between the term and preterm infants (P = 0.9).

Total cerebral volumes: preterm versus term infants

Preterm infants had decreased mean whole brain volumes of all cerebral tissue types when compared with term controls (Table 2). Preterm infants had 25 ml (6%) less total cerebral tissue than term controls, which was principally the result of reduced volumes of CGM (−14 ml, 8%, P = 0.002) and DNGM (−2 ml, 13%, P < 0.001), with a trend toward reduced unMWM (−9 ml, 4%, P = 0.06). CSF was almost

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Table 2 Mean (and standard deviation) of tissue volumes (ml), for global and regional volumes with tissue classes, for preterm (P) and term (T) infants

	Total tissue (excl. CSF)				Tissue classes									
	Preterm		Term		CGM		unMWM		MWM		DNGM		CSF	
					P	T	P	T	P	T	P	T	P	T
Whole brain	395 (64)		420 (50)		159 (41)	173 (32)	212 (32)	221 (34)	9.8 (2.2)	10.3 (4.5)	13.6 (3.9)	15.6 (2.5)	46.0 (26.0)	26.3 (11)
Region	Left	Right	Left	Right	Right side only									
Dorsal prefrontal	21.7 (4.9)	24.3 (5.7)	22.6 (4.8)	23.9 (5.7)	5.3 (2.6)	5.9 (2.8)	18.7 (4.6)	17.8 (5.1)	0.2 (0.4)	0.2 (0.2)	0.01 (0.1)	0.01 (0.02)	5.5 (3.5)	1.98 (1.3)
Orbitofrontal	4.7 (2.8)	5.4 (3.0)	6.9 (4.2)	7.6 (4.3)	1.4 (0.8)	2.1 (1.5)	3.8 (2.4)	5.4 (3.1)	0.1 (0.3)	0.1 (0.2)	0.00 (0.00)	0.00 (0.0)	0.7 (0.7)	0.5 (0.4)
Premotor	23.4 (5.4)	25.0 (5.9)	25.7 (4.9)	26.7 (5.1)	7.4 (2.8)	8.3 (3.0)	15.5 (3.9)	15.9 (3.4)	0.2 (0.2)	0.2 (0.2)	1.9 (0.8)	2.2 (0.8)	4.0 (2.6)	2.1 (0.9)
Subgenual	9.0 (3.0)	9.8 (3.1)	9.7 (2.8)	10.5 (3.3)	3.4 (1.4)	3.6 (1.5)	5.9 (2.1)	6.3 (2.1)	0.2 (0.2)	0.2 (0.1)	0.3 (0.3)	0.4 (0.4)	2.0 (1.1)	2.0 (0.9)
Sensorimotor	28.4 (5.9)	29.4 (6.3)	32.4 (6.0)	33.1 (6.5)	12.5 (3.3)	14.3 (3.5)	12.2 (2.8)	13.6 (2.7)	1.2 (0.7)	1.3 (0.62	3.5 (1.2)	3.8 (1.0)	2.8 (2.0)	1.4 (0.6)
Midtemporal	13.8 (3.3)	14.4 (3.4)	14.4 (3.7)	14.6 (3.2)	5.6 (1.8)	5.8 (1.7)	6.8 (1.6)	6.9 (1.7)	1.6 (0.7)	1.3 (0.7)	0.5 (0.5)	0.7 (0.5)	1.3 (0.8)	1.6 (0.7)
Parieto-occipital	59.5 (12.0)	57.4 (11.0)	63.4 (12.0)	63.8 (10.0)	27.2 (8.0)	31.0 (7.6)	29.2 (6.6)	31.6 (6.6)	0.6 (0.6)	0.6 (0.7)	0.5 (0.4)	0.7 (0.3)	3.9 (3.3)	1.7 (1.0)
Inferior occipital	34.2 (9.0)	33.8 (6.0)	32.8 (10.0)	31.6 (8.9)	19.9 (7.3)	18.3 (8.4)	12.6 (4.1)	11.8 (5.2)	1.2 (0.9)	1.4 (0.9)	0.2 (0.2)	0.2 (0.2)	1.5 (1.7)	1.2 (0.7)

doubled with preterm infants having 19.7 ml (75%) more than full-term infants ($P < 0.001$). When infants with Grades 3/4 WMI (moderately to severely injured) were excluded from the analysis, the mean difference in CSF volume reduced to 15.7 ml ($P < 0.001$) with a mean reduction in total cerebral tissue volume of 19 ml ($P = 0.09$).

Regional analysis: comparison of tissue volumes between term and preterm infants

There was strong evidence that the differences between preterm and term brain structure varied according to the cerebral region for all cerebral tissue classes ($P < 0.001$, Table 3). Mean differences between term and preterm infants (group) were region-specific for all tissue classes ($P < 0.001$, Table 3). Similarly, mean volumes differed between hemispheres in a region-specific manner for all tissue types ($P \leqslant 0.001$) except DNGM ($P = 0.23$). There was no evidence that patterns of regional difference between preterm and term infants varied according to hemisphere (i.e. no three-way interaction).

Figure 2A shows the mean differences in absolute total tissue volumes (i.e. not adjusted for ICV) between preterm and term infants for each of the cerebral regions. The largest absolute volumetric reductions in preterm infants were evident in the parieto-occipital and sensorimotor regions. However, deficits were also significant in the orbitofrontal and premotor regions, while there was little evidence of a reduction in tissue volumes within the subgenual, dorsal prefrontal, midtemporal or inferior occipital regions in comparison to term infants.

The parcellated volumes in each of the eight cerebral regions differ greatly in size, with a difference of ~10-fold between the orbitofrontal and parieto-occipital regions. Therefore, we also display results as relative differences calculated by dividing the absolute mean difference of each tissue type by the mean volume of the parcel in the term control infants. Figure 2B highlights that the greatest relative reduction in total tissue occurred within the orbitofrontal region.

Figure 2B also elucidates differences in tissue class volumes within the regions. The most striking difference in relative volumes was the large excess of CSF in the four superior cerebral regions (dorsal prefrontal, premotor, sensorimotor, parieto-occipital) within the preterm infants. There were significant although relatively small deficits in CGM and unMWM in the sensorimotor region and larger relative reductions in both the CGM and unMWM in the orbitofrontal region. CGM was also reduced in the parieto-occipital and premotor areas, with a trend toward significance for reductions of unMWM within these regions. The inferior occipital region showed a clear deficit in MWM. There were deficits in DNGM within all regions, excluding the dorsal prefrontal and orbitofrontal regions, where DNGM was generally not present. Significant

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Table 3 Summary of results from fitting mixed model to tissue volumes, examining group (preterm versus term), region and hemisphere differences

Factor[a]	df		Total tissue (excl. CSF)	CGM	unMWM	MWM	DNGM[b]	CSF
Region	7	*F*	2038.7	1213.7	1311.8	330.2	816.8	201.3
		P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Hemisphere	1	*F*	10.2	43.8	8.9	32.4	6.8	21.9
		P	0.001	<0.001	0.003	<0.001	0.009	<0.001
Group	1	*F*	9.5	10.9	3.5	0.9	17	59.4
		P	0.002	0.001	0.061	0.35	<0.001	<0.001
Region × Hemisphere	7	*F*	3.7	9.3	36.6	3.4	1.4	12.5
		P	0.0006	<0.001	<0.001	0.001	0.23	<0.001
Region × Group	7	*F*	8	6.9	6.9	4.4	4.4	36.3
		P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

F = test statistic for null hypothesis that factor effect is zero, *P* = corresponding *P*-value; [a]There was no evidence for a Hemisphere × Group effect or for a three-way interaction ($P > 0.2$), therefore these terms were omitted; [b]The two frontal regions were excluded since they contained almost no tissue of this type for any infant.

decreases in DNGM were evident in the subgenual and parieto-occipital regions as well as the midtemporal and premotor regions.

These regional findings were not affected by adjustment for total brain volume, i.e. when a term for total ICV was included in the model.

Assessment of perinatal factors/factors predicting total tissue volume among preterm infants

Associations between perinatal risk factors and regional variation in total tissue volumes within the preterm group with adjustment for ICV are summarized in Table 4. These results show strong evidence for region-specific differences according to gender ($P < 0.0001$), WMI grade ($P < 0.0001$) and IUGR ($P < 0.0001$). There was somewhat weaker evidence of regionally specific volume differences in infants according to gestational age, entered into the model as a linear effect ($P = 0.017$). There was weak evidence for overall differences related to the presence of BPD ($P = 0.047$), but not regionally specific differences ($P = 0.163$). There was no evidence for differences related to the presence of IVH ($P = 0.11$ for interaction with region, $P > 0.5$ for overall mean difference).

These results can be best appreciated by examining estimated region-specific mean differences from the fitted model, according to covariate status (Fig. 3). All of these estimates relate to the combined model summarized in Table 4, so the effect of each factor is adjusted for the presence of all other factors. After adjustment for ICV, females had lower total tissue volumes in particular regions such as the inferior occipital [mean difference in favour of males (SE); 2.1 ml (0.7), $P = 0.004$] but also, with reducing strength of evidence, the dorsal prefrontal [1.2 ml (0.5), $P = 0.01$]. There was a trend to significance within the parieto-occipital [1.9 ml (1.0), $P = 0.055$], and sensorimotor [1.1 ml (0.6), $P = 0.058$] regions.

Grade 2 (mild) WMI was noted to occur in 97 infants (48%), while Grades 3 or 4 (moderate/severe) WMI was recorded in 34 infants (17%). Infants with Grade 3 or 4 WMI displayed reduction in total tissue in the dorsal prefrontal regions [−2.7 ml (0.6), $P < 0.0001$], as well as the four posterior regions; sensorimotor [−2.5 ml (0.8), $P = 0.0008$], midtemporal [−1.1 ml (0.4), $P = 0.008$], parieto-occipital [−5.2 ml (1.3), $P < 0.0001$] and inferior occipital [−3.6 ml (1.0), $P = 0.0002$] regions.

BPD occurred in 69 infants (34%). BPD was associated with an overall reduction, but with little evidence of a regional reduction in tissue volumes. Postnatal steroids were administered to 18 infants (9%) and did not appear to explain or modify the influence of BPD on brain structure.

IUGR occurred in 23 infants (11%) and was most strongly associated with deficits in the two occipital regions: inferior occipital [−3.9 ml (1.3), $P < 0.0001$] and parieto-occipital [−6.8 ml (1.7), $P = 0.002$].

Discussion

We have previously demonstrated that by utilizing advanced MRI techniques we can quantify the global cerebral volumetric differences associated with premature birth (Inder *et al.*, 2005; Inder *et al.*, 2003*b*). Consistent with these findings (Inder *et al.*, 2005), this study revealed significant reductions in total brain tissue, including cerebral CGM and DNGM when compared to term infants. There was a reciprocal increase in CSF as cerebral tissues decreased. The present study has extended these findings to demonstrate that preterm infant brains are affected in a regionally specific manner, and elucidates the perinatal variables contributing to these regional reductions in neural tissue types. This is the first study to combine information about tissue classes within regions and their relationship to perinatal variables in a large representative cohort of preterm infants.

Deficits in brain volumes in preterm infants appeared to be confined to the parieto-occipital, sensorimotor, orbitofrontal and premotor regions (in reducing order of

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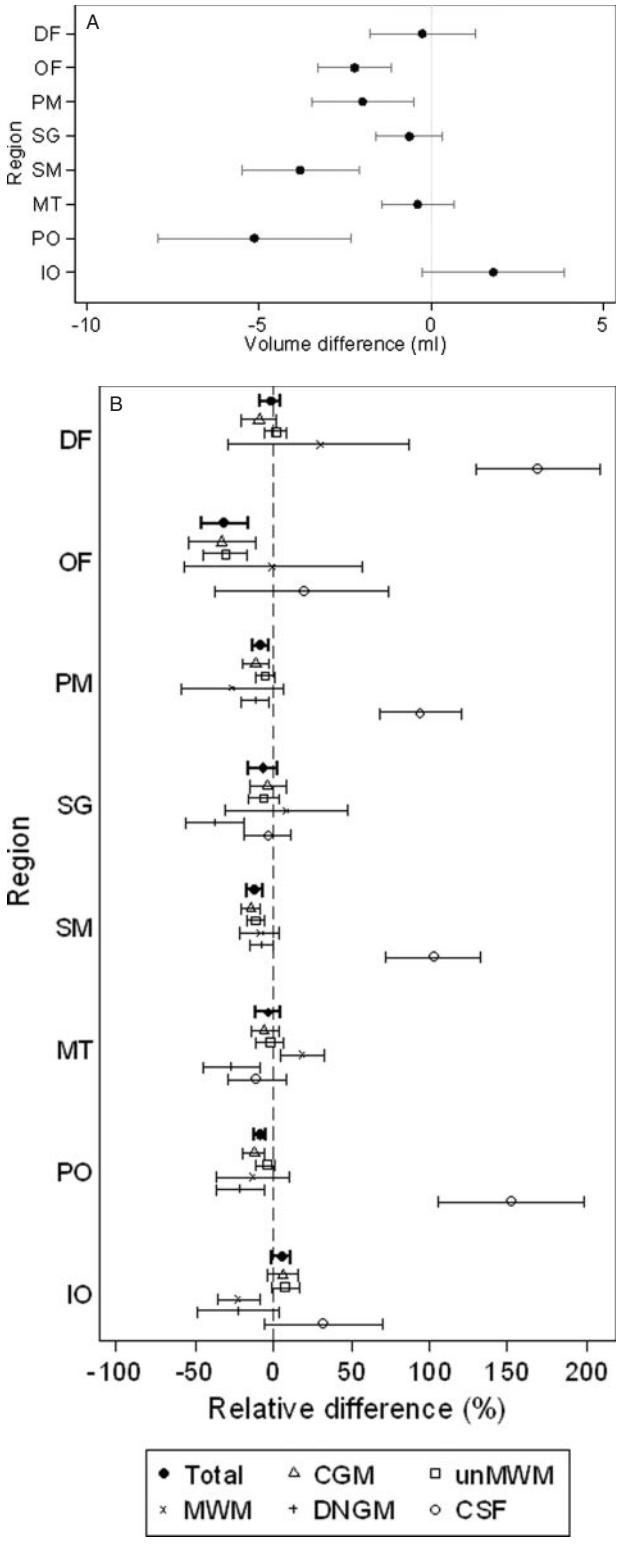

Fig. 2 Estimated differences (preterm minus term, with 95% confidence intervals) in mean absolute total tissue volume between preterm and term-born infants, by region. (**A**) Absolute differences in total tissue volume, preterm minus term. (**B**) Relative differences in tissue volume, calculated by dividing the absolute difference by the mean volume for term infants, for total tissue (excluding CSF) and for each tissue class, preterm minus term. (DNGM not present in DF or OF regions).

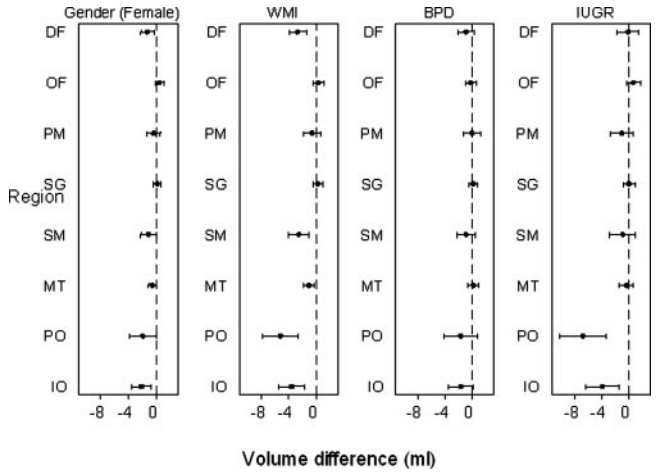

Fig. 3 Estimated differences in mean brain tissue volume (millilitres) for preterm infants by major clinical covariates, obtained from linear mixed model summarized in Table 4 (all effects are adjusted for each other, and also adjusted for total intracranial volume).

Table 4. Summary of results from mixed model fitted to examine association between clinical covariates and regional total tissue volumes within the preterm group

Factor[a]	DF	*F*	*P*
Total ICV	1	333	<0.0001
Region	7	10.8	<0.0001
Hemisphere	1	10.8	0.001
Gender	1	9.8	0.002
Gestational age (weeks)	1	4.9	0.027
WMI	1	30.0	<0.0001
BPD	1	3.9	0.047
IUGR	1	11.2	0.001
Region × Hemisphere	7	4.4	<0.0001
Region × gender	7	4.4	<0.0001
Region × gestational age	7	2.4	0.017
Region × WMI	7	9.1	<0.0001
Region × BPD	7	1.5	0.163
Region × IUGR	7	4.4	<0.0001

The table gives adjusted *F*-tests for each factor after including all other factors in the model, and with adjustment for total ICV.
[a]There was no evidence for differences in preterm infants overall tissue volume related to IVH, or for a Region × IVH effect, therefore these terms were omitted.

magnitude). The utilization of cerebral tissue segmentation in this study allowed comparison of regions according to cerebral tissue class. In relation to this, we found an increase in CSF volume in the superior regions, namely the dorsal prefrontal, premotor, sensorimotor and parieto-occipital regions in preterm infants compared with term-born infants. This corresponded with deficits in CGM and unMWM within the sensorimotor regions. CGM and unMWM were also greatly reduced within the orbitofrontal region in preterm infants, without a striking increase in CSF. DNGM volume was generally lower in preterm infants in all of the cerebral regions in which it was found.

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A major contributor to altered cerebral development in the preterm infant was the presence of WMI Grade 3 or 4 (moderate or severe), predominantly influencing the posterior cerebral regions. IUGR and BPD were also associated with reductions in total tissue volumes. IUGR had an effect on occipital volumes, whereas BPD-related deficits were more uniformly distributed across all regions. Males showed larger volumes across all regions, most notably posteriorly. The degree of immaturity had a small influence on regional brain structure within the preterm infants, but IVH had no significant effect.

There are potential limitations of this study worthy of note, particularly related to the post-acquisition image analysis techniques which we attempted to reduce. The first source of error relates to the image acquisition with infant motion artefact and/or image inhomogeneity rendering scans unable to be accurately segmented. Notably, many of the full-term infant scans were effected by motion artefact. All infants were firmly nested in a soft bean bag reducing motion artefact and researchers present at the time of the scan would request repeat images to be taken if there was motion artefact present. Image inhomogeneity was corrected for prior to image registration. The second source of error relates to image co-registration, which was carefully manually checked prior to tissue classification. The third and most significant source of error in the image segmentation relates to MR signal partial voluming resulting in misclassification of the tissue at boundary regions. This was most significant in our analysis at the boundary of the cerebral CGM with CSF where a single voxel containing both tissue types would be misinterpreted as unMWM. This error is minimized with the *k*-NN algorithm but misclassification does occur. Such an error was systematic across the entire cohort and thus, although this may influence the absolute values, it should not have influenced our comparative analysis. Finally there may be operator error in the tissue segmentation or in the variation of volumes measured within the parcellated regions. Operator inconsistency is possible while placing images into standard Talairach orientation and/or placing region divisions. Our intra-observer reliability measures were acceptable with values >0.8 for all regional analysis and >0.7 for segmentation results except for DNGM. The delineation of the DNGM from the unMWM in the immature brain is technically challenging and we continue to attempt to improve this. Therefore results relating to DNGM volumes utilizing this technique are more vulnerable to operator error and should be interpreted with care.

Despite these limitations, our findings are consistent with Peterson *et al.* (2003) who reported that CGM volumes were reduced in the parieto-occipital and sensorimotor regions in preterm infants at term equivalent. They also reported reductions in grey matter volumes within the inferior occipital region in the preterm infants, which we could not confirm. It is worthy of note that they studied a very small number of preterm infants (n = 10) with the majority of infants having BPD and/or receiving postnatal steroid therapy. The infants were of undocumented gestational age scanned over a broader post-menstrual age with statistical modelling attempting to correct for this. In contrast, our study population is larger and more representative of very preterm populations, and was scanned within a very tight post-menstrual age of 39–41 weeks.

The current study did identify a new finding of a cerebral region of major apparent vulnerability which has not been reported previously to be altered in the preterm infant at term (Peterson *et al.*, 2003)—that of the orbitofrontal region. We documented an approximate reduction in the volume of this region by one-third, resulting from equal contributions of reduced CGM and unMWM. This region is known to be sensitive to stress and mediates emotional responses (Rolls, 2002; Tranel, 2002). Patients with lesions within this region may display irritability, social inappropriateness, poor judgement, lack of persistence, poor frustration tolerance and inflexibility (Rolls, 2002; Tranel, 2002), behaviours which are commonly displayed by infants born prematurely. The orbitofrontal region also receives information from the object-processing visual stream, taste, olfactory and somatosensory inputs, as well as from the amygdala (Rolls, 2002; Tranel, 2002), and thus impairment in cerebral development in this region may contribute to the delayed sensory integration of preterm infants. The factors mediating disturbance in development in this cerebral region were not readily apparent in our analysis, with no clear association with any conventional perinatal factors, Cerebral WMI, gestational age at birth, IUGR and gender showed no association. This suggests that there may be important mediators of cerebral development, which are not currently recognized but which may include environmental stress and/or drug exposures. In further support of the potential contribution of environmental stress to frontal regional cerebral development in the preterm infant, interventions aimed at reducing stress in the preterm infant have been shown to improve frontal region cerebral WM development (Als *et al.*, 2004).

The current study also delineated the variation in the nature and the extent of the impact of perinatal factors on regional brain development in the preterm infant. Cerebral WMI had the largest influence on cerebral structure at term. We have previously documented the impact of cerebral WMI in relation to disturbances in cerebral structure in the preterm infant (Inder *et al.*, 1999, 2005), but this larger study defines the regional impact of this disturbance as influencing the posterior regions of the brain as well as the sensorimotor and dorsal prefrontal regions. Thus, such a regional impact of WMI would be predicted to influence visual functioning (Cioni *et al.*, 1997) and sensorimotor functioning (Holling and Leviton, 1999) as is commonly observed in neurodevelopmental outcomes for preterm infants with cystic periventricular leucomalacia.

The current study also demonstrates a gender effect on regional brain volumes within preterm infants, where males

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have greater volumes within the inferior occipital and cerebellum (IO) and DPF regions. This gender effect of increased regional brain volumes, despite controlling for total intracranial volume in males may be related to regional gonadal hormone influences during brain development (Nopoulos *et al.*, 2000) with greater sexual dimorphism having been found among brain areas with greater levels of sex steroid receptors (Goldstein *et al.*, 2001). Previous studies comparing regional brain structure between males and females have shown conflicting results. Our results reflect those found by Xu and colleagues (Xu *et al.*, 2000), who reported significantly larger adult male volumes in the occipital and cerebellum areas with a trend toward larger male volumes in the frontal lobes. Resnick and colleagues (Resnick *et al.*, 2000) also demonstrated larger volumes within the frontal regions in adult males. However, other studies have not demonstrated the same pattern of regional variation in relation to gender in either adults (Gur *et al.*, 2002; Carne *et al.*, 2006) or children (Caviness et al., 1996; Giedd *et al.*, 1996).

Huppi *et al.* (2004) highlighted the importance of IUGR on cerebral structural development with documented reductions in CGM volumes within growth restricted preterm infants (n = 28) compared with preterm infants without IUGR (Huppi *et al.*, 2004). Our data demonstrate a regional vulnerability of the parieto-occipital and inferior occipital (including the cerebellar region) to IUGR. The neurodevelopmental outcome for growth restricted preterm infants remains unclear, with the presence of IUGR appearing as a risk factor in some (Sweet *et al.*, 2003; Tolsa *et al.*, 2004*b*) but not all studies (Amin *et al.*, 1997). The factors contributing to the timing and nature of IUGR may well mediate the regional sensitivity and impact of this perinatal factor. IUGR is clearly worthy of further study given its impact on cerebral development in the preterm infant.

In contrast to the region-specific pattern of WMI and IUGR effects, there was a more uniform reduction in cerebral regional volumes in preterm infants suffering from prolonged oxygen requirement or BPD. The mechanisms by which BPD influences cerebral development with such a global impact are unclear, but may include hypoxia, stress, inflammation and drug therapies. It is clear that BPD is a significant risk factor for adverse neurodevelopmental outcome in the preterm infant (Gregoire *et al.*, 1998).

We found a weak effect of lower gestational age on regional cerebral structure at term after controlling for the major confounders, particularly BPD, WMI and IUGR. Previous work has noted an association between lower birth weight and reduced grey matter volumes in the parietal and occipital regions (Kesler *et al.*, 2004) but within that study there was no discussion of IUGR, which may explain some or all of the observed birth weight effect.

It is critical to determine whether MRI volumetric alterations at term are likely to persist through childhood and adulthood and whether such cerebral structural volumetric alterations have any functional significance. To address this issue, we are planning to undertake repeated MR volumetric analyses in early childhood for our cohort. Furthermore, correlation of neurodevelopmental outcome with brain volumes is of crucial importance, and has already been attempted in smaller cohorts (Peterson *et al.*, 2000, 2003; Inder *et al.*, 2005). Correlation of the volumetric results at term in the current study with neurodevelopmental outcomes will be valuable, and is being undertaken in this cohort.

In conclusion, there are severe regional disruptions to cerebral development in preterm infants by term equivalent, which vary in relation to perinatal exposures. WMI has the most significant impact together with IUGR, influencing more posterior cerebral structures. BPD has a global impact. Large disturbances in the orbitofrontal region in preterm infants require further investigation in order to define the contributing factors. For neuroprotective strategies to be fully realized, further understanding of how IUGR, BPD and especially WMI contribute to structural alterations in preterm infants is necessary requiring repeated serial MR imaging throughout the neonatal intensive care period. However, this current study has provided clear evidence of the potency of MR in defining regional cerebral vulnerability in this high risk population highlighting that such techniques could be used within randomized controlled trials to provide insight into the impact of therapies on regional brain structure. Gaining an improved understanding of the impact of intensive care therapies on brain development is likely to improve long-term outcomes.

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