Abnormal Cerebral Structure Is Present at Term in Premature Infants

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ABSTRACT. Background. Long-term studies of the outcome of very prematurely born infants have clearly documented that the majority of such infants have significant motor, cognitive, and behavioral deficits. However, there is a limited understanding of the nature of the cerebral abnormality underlying these adverse neurologic outcomes.

Aim. The overall aim of this study was to define quantitatively the alterations in cerebral tissue volumes at term equivalent in a large longitudinal cohort study of very low birth weight premature infants in comparison to term-born infants by using advanced volumetric 3-dimensional magnetic resonance imaging (MRI) techniques. We also aimed to define any relationship of such perinatal lesions as white matter (WM) injury or other potentially adverse factors to the quantitative structural alterations. Additionally, we wished to identify the relationship of the structural alterations to short-term neurodevelopmental outcome.

Methods. From November 1998 to December 2000, 119 consecutive premature infants admitted to the neonatal intensive care units at Christchurch Women’s Hospital (Christchurch, New Zealand) and the Royal Women’s Hospital (Melbourne, Australia) were recruited (88% of eligible) after informed parental consent to undergo an MRI scan at term equivalent. Twenty-one term-born infants across both sites were recruited also. Postacquisition advanced 3-dimensional tissue segmentation with 3-dimensional reconstruction was undertaken to estimate volumes of cerebral tissues: gray matter (GM; cortical and deep nuclear structures), WM (myelinated and unmyelinated), and cerebrospinal fluid (CSF).

Results. In comparison to the term-born infants, the premature infants at term demonstrated prominent reductions in cerebral cortical GM volume (premature infants [mean ± SD]: 178 ± 41 mL; term infants: 227 ± 26 mL) and deep nuclear GM volume (premature infants: 10.8 ± 4.1 mL; term infants: 13.8 ± 5.2 mL) and an increase in CSF volume (premature infants: 45.6 ± 22.1 mL; term infants: 28.9 ± 16 mL). The major predictors of altered cerebral volumes were gestational age at birth and the presence of cerebral WM injury. Infants with significantly reduced cortical GM and deep nuclear GM volumes and increased CSF volume volumes exhibited moderate to severe neurodevelopmental disability at 1 year of age.

Conclusions. This MRI study of prematurity born infants further defines the nature of quantitative cerebral structural abnormalities present as early as term equivalent. The abnormalities particularly involve cerebral neuronal regions including both cortex and deep nuclear structures. The pattern of cerebral alterations is related most significantly to the degree of immaturity at birth and to concomitant WM injury. The alterations are followed by abnormal short-term neurodevelopmental outcome. Pediatrics 2005;115:286–294; magnetic resonance imaging, prematurity, neurodevelopmental outcome.

ABBREVIATIONS. VLBW, very low birth weight; WM, white matter; GM, gray matter; SPGR, spoiled gradient recalled; MR, magnetic resonance; DE, spin-echo sequence; CSF, cerebrospinal fluid; ICV, intracranial volume; IVH, intraventricular hemorrhage; IUGR, intrauterine growth restriction.

Throughout the last decade there has been an increasing prevalence of surviving very low birth weight (VLBW) premature infants. The improvements in survival have been greatest in the most immature infants but have been accompanied by an increasing awareness of subsequent neurodevelopmental deficits. Approximately 10% of VLBW infants (~4000 yearly in the United States) develop cerebral palsy, and fully 50% (~20 000 yearly) have impaired academic achievement and/or behavioral disorders requiring additional educational resources. The nature of the cerebral abnormalities that underlie these common and serious developmental disabilities is not entirely understood. To improve outcome for these infants, it is crucial to understand both the nature of the cerebral abnormalities and any modifiable perinatal factors involved in pathogenesis.

Studies based on neuropathological analysis and conventional brain imaging of premature infants have provided evidence primarily for injury to cerebral white matter (WM). The principal lesions include periventricular hemorrhagic infarction and periventricular leukomalacia. The latter of these 2 forms of WM injury seems to be more common and more important in the genesis of the subsequent neurologic deficits. Periventricular leukomalacia at its most severe results in focal necroses with subsequent cyst formation, readily detected by cranial ultrasonography. The clinical importance of the finding of cystic WM injury by neonatal cranial ul-
trasonography is illustrated by the frequent demonstration of a greatly increased risk of subsequent neurologic deficits observable on follow-up. However, recent studies by conventional magnetic resonance imaging (MRI) have shown that noncystic WM injury, manifested by signal abnormalities in cerebral WM, often accompanied by ventricular dilation, is much more common than cystic WM injury. In our preliminary study of this cohort, we documented in 94 premature infants that diffuse cerebral WM injury was common and associated with proven sepsis and inotrope use. Unfortunately, the principal method for imaging the premature brain, cranial ultrasonography, has a low sensitivity for detection of noncystic WM injury identified by either MRI or neuropathological analysis. The clinical correlate of the noncystic WM abnormality has not been established yet, although it is noteworthy that the high frequency of this noncystic disorder is similar to the high frequency (ie, ~50%) of later cognitive and behavioral deficits in VLBW infants. Taken together, these data lead to the postulate that the relatively high frequency of cognitive and related neurodevelopmental deficits documented on follow-up of premature infants who do not exhibit overt WM abnormalities (ie, cystic injury) on neonatal cranial ultrasonography is related to noncystic WM injury.

Recent observations in the premature infant suggest that the basis for the cognitive and related deficits may not relate directly to the WM injury per SE. Qualitative appearances of alteration in gray matter (GM) development have been undertaken in this cohort and shown to have a relationship between WM injury and delayed GM gyral development. However, qualitative appreciation of both cortical and deep nuclear GM development are extremely limited in comparison to more advanced MRI analysis tools such as 3-dimensional volumetric assessment. Thus, several studies of prematurely born infants evaluated by volumetric MRI in late childhood and adolescence have shown reductions in either regional total cerebral volumes (GM and WM) or cortical GM volumes and, importantly, correlations of these reductions with impaired IQ, memory, calculation, and global cognitive functioning. Of particular note is that the affected structures have been principally GM, especially cerebral cortical GM. A recent small study of premature infants reported regional reductions in GM and WM volumes that correlated with measures of neurodevelopment at 18 months. However, this small set of premature infants may not be truly representative, because 9 of 10 had bronchopulmonary dysplasia and 6 of 10 received postnatal steroids. Additionally, the relationship of the presence of neonatal cerebral injury to WM, GM, or both to the subsequent cortical GM alterations was not assessed. The possibility that cerebral cortical changes could reflect alterations in cortical development directly related to the presence of noncystic WM injury was suggested by our recent volumetric MRI study of premature infants at term.

However, the number of infants studied without WM injury in that series was small (n = 10), and the issue of the relation of reduced cerebral cortical GM volume at term to neonatal factors other than WM injury was not resolved conclusively. Resolution of this issue is critical, because detection of reductions in cortical GM as early as term has implications for pathogenesis of the disturbance and, perhaps, for early detection of infants at risk for subsequent cognitive deficits.

Thus, the aim of this study was, first, to utilize quantitative volumetric 3-dimensional MRI at term in a large sample of premature infants to determine the presence of alterations in specific cerebral GM and WM structures reflecting altered cerebral structural development. Second, we aimed to define the relationship of any such alterations to such perinatal lesions as WM injury and to other potentially adverse perinatal factors. Third, we also aimed to define the relationship of any cerebral volumetric alterations to neurodevelopmental outcome at 1 year of age.

METHODS

Subjects

One hundred nineteen premature infants with birth weight <1500 g and gestation <32 weeks' postconceptional age were recruited into the study to undergo an MRI scan at term equivalent. These infants were recruited from 2 geographical sites: Christchurch Women's Hospital neonatal intensive care unit (New Zealand) and the Royal Women's Hospital neonatal intensive care unit (Melbourne, Australia). One hundred consecutive premature infants were recruited from Christchurch between November 1998 and March 2001 (90% of those eligible), and 29 consecutive premature infants were recruited from Melbourne between July 2001 and September 2001 (80% of those eligible). With the recruitment of the premature infants, 21 healthy term-born infants were recruited from the 2 sites (10 from Christchurch and 11 from Melbourne) and were also scanned within 1 week of due date. The term infants were recruited randomly from the labor-and-delivery wards of the same public hospitals in which the premature infants were delivered. The recruitment rate for term infants was 38% of those eligible. An extensive perinatal database was collected regarding the pregnancy, labor, and neonatal course of the infants. The characteristics of the premature infants are shown in Table 1.

Informed parental consent was obtained for all infants. The study was approved by the relevant ethics committees (ie, the Canterbury Health Ethics Committee and the Royal Women's Hospital Ethics Committee).

MRI

MRI scanning was undertaken on all recruited infants between the 39th and 41st gestational weeks (term equivalent) based on the most accurate method of gestational assessment available for the infant, usually fetal ultrasound assessment at ~18 weeks' gestational age. Premature infants were scanned at identical postconceptual times (mean for preterm infants: 40.2 ± 0.3 weeks; mean for term infants: 40.3 ± 0.4 weeks). The infants were fed and gently wrapped in a Vac Fix bean bag (S&S Radiograph Products, Brooklyn, NY), and the MRI scan was undertaken without sedation. MRI scanning was performed with a 1.5-T General Electric Signa System (GE-Medical Systems, Milwaukee, WI). For the acquisition of the primary magnetic resonance (MR) data, 2 different imaging modes were applied: a 3-dimensional Fourier-transform spoiled gradient recalled (SPGR) sequence (1.5-mm coronal slices; flip angle: 45°; repetition time: 35 milliseconds; echo time: 5 milliseconds; field of view: 18 cm; matrix: 256 × 256; 124 slices) and a double-echo (echo power and T2-weighted) spin-echo sequence (DE) (3-mm axial slices; repetition time: 3000 milliseconds; echo times: 36 and 162 milliseconds; field of view: 18 cm; matrix: 256 × 256, interleaved acquisition; 68 slices).
MRI Analysis

For quantitative volumetric analyses, postacquisition processing was conducted on workstations (Sun Microsystems, Mountain View, CA). For the 129 infants that were recruited, there were 10 infants with images that could not be processed due to motion artifact (n/H11005 5), sequence errors (n/H11005 3), or registration difficulties (n/H11005 2). Thus, 119 (92%) infants were able to have image processing and volumetric analysis. A sequence of established image-processing algorithms was used to segment each of the MRI slices into separate tissue classes: cerebral cortical GM, deep nuclear GM, unmyelinated WM, myelinated WM, and cerebrospinal fluid (CSF) (including ventricular and extra-axial CSF; Fig 1).26,27 These algorithms were designed to reduce imaging-system noise, identify a linear transformation, and resample the DE spin-echo images according to this transform, to align the axial DE spin-echo images with the coronal SPGR images to form a 3-channel data set, classify tissue types on the basis of the MR intensity in the 3 channels, and identify tissue-class surfaces for 3-dimensional visualization. Absolute volumes of each cerebral tissue were determined, and these absolute volumes also were referenced to the total intracranial volume (ICV) and thereby reported as relative percentage of each cerebral tissue within the intracranial cavity. These segmentation and volumetric analyses for this study were undertaken by a single investigator (H.W.) and repeated on every fifth case in a systematic fashion, with <5% variance in volumes.

For qualitative structural assessment, MR images were graded for WM abnormality in a blinded manner by an experienced neuroradiologist and by a second independent scoring (T.E.I.) for WM and GM abnormality and development. The scoring system was adapted from previously published systems.28,29 WM was graded with a score between 1 and 3 for 5 variables including WM signal abnormality (shortening on T1-weighted imaging), qualitative reduction in WM volume, apparent cystic abnormality, lateral ventricular size, and the combination of corpus callosum size and maturation of cerebral myelination, particularly in the posterior limb of the internal capsule. WM abnormality then was categorized further by the composite scores of these 5 variables into no WM abnormality, mild WM abnormality, moderate-severe non-cystic WM abnormality, or moderate-severe cystic WM abnormality.12,14 Concordance between assessors was 95%, with 5% requiring consensus on second review. A repeat blinded reading resulted in identical scores for 19 of 20 cases.

Neurodevelopmental Evaluation

At 12 months’ corrected age, 112 of 119 premature study infants underwent an evaluation that included a pediatric physical examination by an experienced neurologist or neonatologist and/or a developmental examination based on observational data from parent report and examination with the Denver Developmental Screening tool. Children were classified as exhibiting severe disability if they had clinical evidence of severe abnormality on neurologic motor examination (eg, marked spasticity, weakness, and developmental delay of >6 months); moderate disability if they had clinical evidence of moderate abnormality on neurologic

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**TABLE 1.** Characteristics of the Total Cohort of Premature Infants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of Infants in Cohort (%) Total Cohort n = 119 Infants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, g, mean ± SD (range)</td>
<td>1040 ± 272 (440–1505)</td>
</tr>
<tr>
<td>Gestational age, wk, mean ± SD (range)</td>
<td>27.7 ± 2.2 (23–32)</td>
</tr>
<tr>
<td>Male/female</td>
<td>99.60</td>
</tr>
<tr>
<td>Singleton</td>
<td>91 (76%)</td>
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<tr>
<td>IUGR*</td>
<td>26 (22%)</td>
</tr>
<tr>
<td>Prolonged rupture of membranes†</td>
<td>26 (22%)</td>
</tr>
<tr>
<td>Antenatal steroids administered</td>
<td>103 (87%)</td>
</tr>
<tr>
<td>Positive pressure ventilation, d, mean ± SD (range)</td>
<td>5.7 ± 12 (0–62)</td>
</tr>
<tr>
<td>Oxygen therapy, d, mean ± SD (range)</td>
<td>26 ± 37 (0–138)</td>
</tr>
<tr>
<td>Bronchopulmonary dysplasia (oxygen requirement at 36 wk)</td>
<td>25 (21%)</td>
</tr>
<tr>
<td>Pneumothorax</td>
<td>5 (4%)</td>
</tr>
<tr>
<td>Postnatal steroid therapy‡</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>Patent ductus arteriosus (confirmed by echocardiography)</td>
<td>38 (32%)</td>
</tr>
<tr>
<td>Age of full enteral feeding, wk, mean ± SD (range)</td>
<td>10.3 ± 9.2 (0–50)</td>
</tr>
<tr>
<td>IVH, any grade</td>
<td>20 (17%)</td>
</tr>
<tr>
<td>IVH, grade III/IV§</td>
<td>7 (6%)</td>
</tr>
</tbody>
</table>

* z score > 2 SD below weight for gestational age.
† Proven rupture of membranes for >24 hours.
‡ Postnatal dexamethasone, 0.15 mg/kg per day, reducing over 14 days.
§ Based on Papile classification.

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**Fig 1.** Tissue segmentation (A) from the coronal T2-weighted (B) and coronal SPGR (C) MR images. The tissues are segmented into cortical GM (gray), unmyelinated WM (red), myelinated WM (yellow), deep nuclear GM (white), and CSF (blue).
motor examination and developmental delay between 4 and 6 months; or mild disability if they had only mild spasticity and/or motor deficit and developmental delay of 2 and 4 months.

Statistical Analysis

Statistical analyses were undertaken with SPSS for Windows (SPSS Inc, Chicago, IL) to compare the volume of cerebral tissues between the premature and the term-born infant groups by using Student’s independent t test with unequal variance assumed. For group analysis within the premature infant groups of the effect of perinatal factors, such as grade of intraventricular hemorrhage (IVH) or WM injury, an analysis of variance (1-way) with pairwise multiple comparison procedures was used. To isolate the group or groups that differ from the others, a multiple comparison procedure using the Bonferroni t test with unequal variance assumed. For continuous variables of outcome (cerebral volumes) and predictors (perinatal factors such as length of gestation and birth weight). Among the premature infants, cerebral volumes and relative cerebral volumes were analyzed for potential confounding variables by multiple linear regressions. Because of the collinearity in certain variables of interest, a single variable was selected based on its strength of primary significance with the dependent variable (ie, for gestational age and birth weight, gestational age was selected; for ventilation days and days of oxygen therapy, days of oxygen therapy was selected). The major variables included in the multiple regression models with cerebral volumes were gestational age, days of oxygen therapy, grade of IVH, grade of WM injury, and intrauterine growth restriction (IUGR).

RESULTS

Subjects

One hundred nineteen premature infants were included with a mean (±SD) gestational age of 27.7 ± 2.2 weeks (range: 23–32 weeks) and a mean (±SD) birth weight of 1040 ± 227 g (range: 440–1505 g). The characteristics of the population of premature infants are described in Table 1. There were 21 term-born infants recruited over the same study period. The premature and term infants had no history of prenatal illicit drug use and did not differ in maternal socioeconomic status or highest level of education. There was no significant difference in head circumference (term infants [mean ± SD]: 35.6 ± 0.9 cm; premature infants: 34.9 ± 1.3 cm [P > .1]) or weight (term infants: 3.5 ± 0.5 kg; premature infants: 3.05 ± 0.6 kg [P > .1]) at MRI between the premature and term-born infants.

Cerebral Tissue Volumes of Premature Versus Term-Born Infants

Prematurely born infants had prominently decreased volumes of cerebral tissue classes at term equivalent in comparison to term-born infants. Thus, when compared with the term-born infants, premature infants had significantly reduced absolute cerebral volumes of cortical GM (P = .001), deep nuclear GM (P = .02), and myelinated WM (P = .02) (Table 2). The absolute volumes of both cortical GM and deep nuclear GM were reduced by 22% and the absolute volume of myelinated WM was reduced by 35% for premature infants in comparison to term-born infants. Notably the absolute volumes of myelinated WM at this age are relatively small. There was a prominent increase in the volume of CSF in the premature versus the term-born infants (P = .02) associated with the clear reduction in the total absolute volume of cerebral tissue within the cranial cavity (Table 2). The absolute cerebral tissue volumes are represented as the percentage of the total ICV in both premature and term infants (Table 2), demonstrating significant reductions in percentage of cortical GM (P = .01), myelinated WM (P = .05), and brain tissue (P = .001) and significant increase in percentage of CSF volume (P = .001).

Perinatal Factors Associated With Altered Cerebral Tissue Volumes

Cerebral Injury

Twenty-one infants (18% of all premature infants) exhibited qualitative MRI evidence for moderate or severe WM injury. Of these infants, only 5 had cystic WM injury, and 16 had noncystic diffuse WM injury. The latter consisted principally of diffuse signal changes in the cerebral WM with moderate reduction in WM volume and ventriculomegaly (n = 8) or with marked reduction in WM volume with ventriculomegaly, delayed cerebral myelination, and thinning of the corpus callosum (n = 8).

Table 2. Cerebral Tissue Volumes (Mean ± SD as absolute volume [mL] and relative to ICV) for All Premature (n = 119) and Term-Born (n = 21) Infants

<table>
<thead>
<tr>
<th>Tissue Class</th>
<th>Premature Infants (n = 119)</th>
<th>Term-Born Infants (n = 21)</th>
<th>P Value (t Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical GM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute volume, mL</td>
<td>178 ± 41</td>
<td>227 ± 26</td>
<td>.001</td>
</tr>
<tr>
<td>% ICV</td>
<td>39.4 ± 7.6</td>
<td>48.2 ± 9.5</td>
<td>.01</td>
</tr>
<tr>
<td>Deep nuclear GM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute volume, mL</td>
<td>10.8 ± 4.2</td>
<td>13.8 ± 5.2</td>
<td>.02</td>
</tr>
<tr>
<td>% ICV</td>
<td>2.4 ± 1.0</td>
<td>2.9 ± 0.9</td>
<td>.09</td>
</tr>
<tr>
<td>Myelinated WM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute volume, mL</td>
<td>13.5 ± 5.8</td>
<td>20.8 ± 12.2</td>
<td>.02</td>
</tr>
<tr>
<td>% ICV</td>
<td>3.4 ± 1.1</td>
<td>4.2 ± 2.2</td>
<td>.05</td>
</tr>
<tr>
<td>Unmyelinated WM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute volume, mL</td>
<td>20.2 ± 4.1</td>
<td>206.5 ± 78.9</td>
<td>.9</td>
</tr>
<tr>
<td>% ICV</td>
<td>42.6 ± 5.9</td>
<td>41.4 ± 9.9</td>
<td>.9</td>
</tr>
<tr>
<td>Cerebral tissue, total</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute volume, mL</td>
<td>40.6 ± 57</td>
<td>457 ± 67</td>
<td>.003</td>
</tr>
<tr>
<td>% ICV</td>
<td>89.9 ± 4.4</td>
<td>94.1 ± 3.1</td>
<td>.001</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute volume, mL</td>
<td>45.6 ± 22.1</td>
<td>28.9 ± 16.8</td>
<td>.01</td>
</tr>
<tr>
<td>% ICV</td>
<td>10.1 ± 3.8</td>
<td>5.2 ± 3.0</td>
<td>.001</td>
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</table>
the day of the MR scan did not alter the significance of the reductions in cortical GM and myelinated WM volumes or the increase in CSF volume.

There were 20 infants with IVH. Ten infants had grade I IVH, 3 infants had grade II IVH, 5 infants had grade III IVH, and 2 infants had grade IV IVH (with parenchymal hemorrhagic infarction). There was overlap between the presence of IVH and cerebral WM injury, with 3 of 13 (23%) infants with grades I/II IVH displaying moderate-severe WM injury in comparison to 3 of 7 (43%) infants with grades III/IV IVH (Fisher’s exact test: \( P < .05 \)). However, on univariate analysis, CSF volume was the only cerebral volume significantly altered with IVH. Absolute CSF volume was increased significantly (IVH \( \text{mean} \pm \text{SD} \): 51.9 \pm 23.6 mL; no IVH: 41.6 \pm 19.6 mL; \( P = .04 \)), although CSF volumes relative to total ICV were not significantly different between premature infants with IVH and premature infants without IVH. The higher grades of IVH (grades III and IV) had a trend toward higher CSF volumes in comparison to the lower grades of IVH, but this did not reach statistical significance.

Fig 2. Cortical GM and CSF volumes at term in premature infants with \( (n = 21) \) and without \( (n = 98) \) evidence of moderate-severe WM injury (expressed as medians, with 25th/75th centile box, 10th/90th centile error bars, and outliers).

Fig 3. Deep nuclear GM volumes as a function of gestational age at birth. The entire range for all the term-born control infants is shown in the shaded box at the right. All infants were scanned on the week of their expected date of delivery. Note that the majority of infants born at \(< 27\) weeks had volumes below the normal term range.
Immaturity

Alterations in cerebral tissue volumes in premature infants at term were found in the most immature infants. The infants were subdivided into 2 gestational age groups based on our previous qualitative MRI observations of the impact of immaturity.14 On analysis of the infants into 2 groups (ie, extreme immaturity [‡26 weeks’ gestational age at birth] and more mature [‡27 weeks’ gestational age at birth]), the extremely immature infants exhibited significant reductions in deep nuclear GM (Fig 3) and total cerebral tissue volumes and an increase in CSF volumes (Table 3). By contrast, there was no significant influence of immaturity on cerebral myelinated or unmyelinated WM volumes. For percent ICV there remained a significant reduction in deep nuclear GM in the more immature infants (immature infants: 1.6% ± 0.7% ICV; mature infants: 2.7% ± 0.9% ICV; P < .01), although the statistical significance in relation to CSF volumes and cerebral tissue volumes was lost (P = .1 [not significant]).

Respiratory Illness

There was a significant relationship between the severity of respiratory illness and the deep nuclear GM volumes and percent ICV deep nuclear GM volumes. Thus, there was a negative correlation between absolute deep nuclear GM volumes and the number of days of ventilator support (r = −0.312; P = .005) and the number of days of oxygen therapy (r = −0.272; P = .01). These findings were similarly significant for volumes of deep nuclear GM relative to ICV (P < .01 for all).

Other Influences on Cerebral Tissue Volumes

There was no effect of gender, multiple pregnancy, assisted conception, in utero use, or infant sepsis on cerebral tissue volumes at term equivalent. Antenatal steroid therapy had no effect on cerebral tissue volumes, although no mother received >2 doses of steroids. Only 3 infants received postnatal steroids, and thus there was an insufficient number of infants for analysis. IUGR resulted in a significant reduction in the absolute volumes of cerebral cortical GM only (IUGR [mean ± SD] [n = 26]: 177.2 ± 44 mL; no IUGR [n = 87]: 192.6 ± 31.9 mL; P = .01).

Multivariate Model

The major variables included in the multiple regression models with cerebral tissue volumes were gestational age, days of oxygen therapy, IVH grade, grade of WM injury, and IUGR. After adjusting the relationship of gestational age at birth in the premature infants with volumes of deep nuclear GM and of cortical GM for days of oxygen therapy, IVH grade, grade of WM injury, and IUGR, there persisted a significant effect of immaturity on prediction of volumes of both deep nuclear GM (r = 0.29; R² = 0.130; P = .02), and CSF (r = −0.21; R² = 0.11; P = .04). With this model, the gestational age regression coefficient (B) was 0.18.

Neurodevelopmental Outcome at 1 Year

Neurodevelopmental outcome was assessed in 112 of the 119 premature infants (94%) at 1 year of age (2 infants died before follow-up at 1 year of age, and 7 were unable to be tracked). Of these infants, 11 exhibited moderate to severe disability. The infants who had moderate to severe disability did not differ in gestational age in comparison to those without (Table 4). However, of these 11 infants, 7 (64%) were classified on qualitative MRI as having moderate-severe WM injury. Premature infants with moderate-severe disability had significant reductions in GM volumes, both cortical GM and deep nuclear GM volumes (Table 4). CSF volumes were significantly increased in the infants with moderate-severe disability (Table 4).

DISCUSSION

This study used advanced MR techniques to document in vivo impaired cerebral development, present by term equivalent, in a large unselected cohort of prematurely born infants. The impairments

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**TABLE 3. Absolute Cerebral Tissue Volumes for Premature Infants‡26 Weeks (n = 37) in Comparison With Premature Infants‡27 Weeks (n = 82)**

<table>
<thead>
<tr>
<th>Tissue Class</th>
<th>Premature Infants‡26 wk (Range: 23–26 wk; n = 37)</th>
<th>Premature Infants‡27 wk (Range: 27–32 wk; n = 82)</th>
<th>P Value (t Test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical GM, mL</td>
<td>166 ± 42</td>
<td>178 ± 41</td>
<td>.9</td>
</tr>
<tr>
<td>Deep nuclear GM, mL</td>
<td>7.4 ± 3.7</td>
<td>12.3 ± 4.2</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Cerebrospinal fluid, mL</td>
<td>53.2 ± 26.1</td>
<td>38.8 ± 18.8</td>
<td>.01</td>
</tr>
<tr>
<td>Total ICV, mL</td>
<td>448 ± 61</td>
<td>463 ± 59</td>
<td>.7</td>
</tr>
<tr>
<td>Cerebral tissues, total, mL</td>
<td>386 ± 60</td>
<td>419 ± 57</td>
<td>.06</td>
</tr>
</tbody>
</table>

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**TABLE 4. Absolute Cerebral Tissue Volumes for Premature Infants With Moderate-Severe Disability (n = 11) Versus Premature Infants With No or Minimal Disability (n = 107 at 1 Year of Age)**

<table>
<thead>
<tr>
<th>Tissue Class</th>
<th>Premature Infants With Moderate-Severe Disability (n = 11)</th>
<th>Premature Infants With No or Minor Disability (n = 101)</th>
<th>P Value (Kruskal-Wallis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical GM, mL</td>
<td>157 ± 41</td>
<td>181 ± 41</td>
<td>.04</td>
</tr>
<tr>
<td>Deep nuclear GM, mL</td>
<td>8.4 ± 3.6</td>
<td>11.3 ± 4.2</td>
<td>.03</td>
</tr>
<tr>
<td>Cerebrospinal fluid, mL</td>
<td>59.3 ± 28.8</td>
<td>44.3 ± 21.1</td>
<td>.04</td>
</tr>
<tr>
<td>Gestational age at birth, wk</td>
<td>27.1 ± 1.6</td>
<td>28.0 ± 2.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS indicates not significant.
in cerebral development include, particularly, highly significant reductions in cerebral cortical and deep nuclear GM volumes in comparison to volumes in term-born infants. These reductions in cerebral GM are accompanied by a near doubling of the ICV of CSF. These cerebral structural changes would not be detectable by conventional cranial ultrasonography and would be difficult to discern by qualitative MR techniques. The decrease in cerebral cortical GM volume was strongly related to the presence of cerebral WM injury, confirming our previous findings with a smaller and selected series of premature infants. However, in contrast to this previous study, in this much larger cohort we demonstrate that just being born prematurely, without any evidence of overt cerebral WM injury, also results in a significant alteration in cerebral structure observable by term equivalent. We also document that these alterations in cerebral structure are related to adverse neurodevelopmental outcome by 1 year of age.

Qualitative MR and Quantitative MR Techniques
Qualitative MR studies of prematurely born infants in both the neonatal period and childhood have not defined a consistent relationship between the presence of qualitative WM abnormalities defined by conventional MRI and the more common cognitive deficits identified subsequently in these infants. This inconsistency may reflect the lack of sensitivity of qualitative MR for the more subtle alterations in cerebral volumetric structure that may underlie these deficits. We previously reported 94 infants from this cohort with qualitative analysis of the nature of cerebral abnormality in the WM and GM. This study assisted in the definition of cerebral injury, particularly that of diffuse cerebral WM injury, alongside the risk factors of proven sepsis and inotrope support. In contrast, qualitative MR analysis is limited in the detection of subtle alterations in the sequence of cerebral structural development, particularly in structures such as the deep nuclear GM. Postacquisition MR-processing techniques, using tissue-segmentation methods with 3-dimensional renderings, have been applied by our group to quantitate the major cerebral tissue classes (cortical GM, deep nuclear GM, unmyelinated WM, myelinated WM, and CSF) in the neonatal period and to thereby define normal maturation of these structures between 29 and 41 weeks’ gestation. The work has also shown the impact of postnatal dexamethasone administration and periventricular leukomalacia on cerebral volumetric development by term in relatively small selected groups of infants. More recent studies using volumetric cerebral analysis in adolescence and later childhood of subjects born prematurely have demonstrated reductions in specific regional cerebral volumes that correlate with impaired cognitive functions. A recent smaller study of a group of sick premature infants (n = 10) showed reductions in cerebral WM and GM volumes. Notably, these infants had severe respiratory illness with significant postnatal steroid exposure, and comparisons of MRI between premature and term infants were based on somewhat different time points for MRI (premature infants at 35 weeks versus term infants at 41 weeks). Nevertheless, our current findings in a much larger and more representative group of premature infants are consistent with these pilot findings and support the conclusion that important alterations in cerebral structure in the premature infant occur in the cerebral GM, involving both cortical and deep nuclear GM, and are present by term equivalent. The involvement of GM structures in our study is consistent with the GM involvement documented by volumetric MRI at later ages by others (see earlier), but our findings suggest that potential interventions must be applied before term, perhaps during the neonatal intensive care unit course, to optimize long-term cerebral structural development.

Insights Into the Nature and Pathogenesis of the Cerebral Lesions Associated With Prematurity
This study suggests that disruption of cerebral GM structures is a major feature in prematurely born infants and occurs in relation to 2 critical factors: cerebral WM injury and immaturity. Although the precise nature of the neuropathological abnormalities associated with this reduced GM volume is unknown, the major possibilities are either neuronal loss or impaired neuronal differentiation with a reduction in dendritic and axonal development.

Relationship to Cerebral WM Injury
A role for cerebral WM injury in the genesis of the reductions in cerebral cortical GM at term is clear from the current data. We previously documented in a small case series of premature infants with periventricular leukomalacia a significant reduction in cerebral GM volumes by term. The reduction in cortical GM volumes in premature infants with cerebral WM injury could reflect blunted neuronal differentiation caused by destruction of ascending and descending axons (corticopetal, corticofugal, and corticocortical association fibers) in WM with resulting input deprivation and output isolation of the overlying cortical GM. As a consequence of this cortical GM isolation, there may be impaired GM differentiation. These conclusions are based on the work by Marin-Padilla with newborn brain using Golgi stains of autopsy or neurosurgical specimens of the cortical GM overlying destructive WM lesions; the neuronal changes included impaired development of neuropil. Supportive of the notion of axonal injury in cerebral WM injury are the immunocytochemical findings of axonal injury (β-amyloid precursor protein immunoreactivity) within and surrounding areas of necrosis in periventricular leukomalacia. However, whether prominent axonal injury is present in noncystic WM injury is unknown. Diffusion-imaging studies in vivo in the premature infant have shown that there seems to be an alteration of axonal fiber development in association with periventricular leukomalacia, whether cystic or noncystic in nature. The relationship of adverse neurodevelopmental outcome to the level of immaturity at birth in the preterm infant is well documented.
Relationship to Immaturity

Our data also show a potent effect of gestational age at birth on volumetric measures of cerebral deep nuclear GM at term equivalent. Controlling for many other potential confounders did not alter the significance of this association. There are several potential pathogenetic pathways that may be interactively responsible for the association of immaturity with altered deep nuclear GM volumes including direct injury to neurons of cortex and deep nuclear structures or secondary atrophy after sublethal axonal injury, or injury to the vulnerable subplate neurons. The subplate neuronal cell population is particularly abundant in superficial WM of human brain from 22 to 34 weeks of gestation and is crucial for development of overlying corticothalamic connectivity, including the proper guidance of the “waiting” thalamocortical afferents to their final cortical targets. Injury within the subplate zone during this early period therefore could have a highly significant effect on subsequent connectivity and development of the deep nuclear GM.

Relationship to Other Perinatal Factors

There are many potentially adverse perinatal medical, nutritional, and environmental factors that could be postulated to cause altered cerebral development in the premature infant. In our cohort, severity of respiratory illness seemed to have a consistent impact on deep nuclear GM structure. Interestingly, infants with bronchopulmonary dysplasia have been noted to exhibit a movement disorder, with neuropathological evidence of neuronal loss in the caudate, putamen, and globus pallidus.

It is interesting that many other factors that we had hypothesized a priori to be of potential importance, such as gender, multiple pregnancy, fertility-assisted conception, poor postnatal growth, patent ductus arteriosus and its treatment, neonatal sepsis, and other markers of severity of medical illness had little or no influence on cerebral structures at term equivalent. It is important to note that our sample, although the largest published series, may lack adequate power to detect smaller independent effects from such perinatal variables.

High-dose postnatal dexamethasone therapy has been shown by our group to be associated with significantly reduced cortical GM and cerebral tissue volumes. This observation is consistent with clinical data from longitudinal follow-up studies that show an adverse neurodevelopmental outcome after such therapy, and together these findings have led to a reduced use of this potentially harmful drug. This change in practice is reflected in our cohort, because too few infants received postnatal steroids to analyze this effect further. Repeated courses of antenatal steroids administered to women at risk of premature delivery also have been hypothesized to have a detrimental effect on brain development, including a reduction in cerebral gyral development. Our study documented no adverse effect of a partial or completed course of antenatal steroids on cerebral structure. It is important to note that no woman received >2 completed courses of steroids, but in this large unselected series our findings provide support for a lack of any adverse effect from a single or double prenatal steroid course.

Relationship of Altered Cerebral Volumes to Neurodevelopmental Outcome

The relationship of our measures to functional performance in our children at 1 year of age suggests that the MR findings are clinically relevant. Thus, the reduction in cerebral cortical GM and deep nuclear GM volumes seemed sensitive as predictive markers for disability at 1 year. Neurologic outcome at 1 year is acknowledged to be of limited predictive capacity, and thus we will be assessing the functional performance in our cohort more extensively at later ages. Additionally, regional brain volumetric data combined with the potency of later MR evaluation with functional MR imaging may also provide critical insights into the structural bases for plasticity and changing cognitive abilities in these prematurely born infants.

CONCLUSIONS

This study has further defined alterations in cerebral structure in the largest published cohort of prematurely born infants at term equivalent by using in vivo advanced MR techniques. Cerebral cortical and deep nuclear GM were particularly affected. The nature of these changes cannot be visualized by cranial ultrasound or conventional qualitative MR techniques. The major risk factors for the alterations in cerebral structure were immaturity and MR-defined cerebral WM injury. These findings of altered cerebral structure were associated with adverse short-term neurodevelopmental outcome. This study shows that alterations in cerebral structure, similar to those found in volumetric studies in older premature infants, are present by term equivalent. Thus, attention must be focused on this critical neonatal period to optimize cerebral structural development.

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