

Prediction of neurodevelopmental outcome after hypoxic–ischemic encephalopathy treated with hypothermia by diffusion tensor imaging analyzed using tract-based spatial statistics

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INTRODUCTION: Objective biomarkers are needed to assess neuroprotective therapies after perinatal hypoxic–ischemic encephalopathy (HIE). We tested the hypothesis that, in infants who underwent therapeutic hypothermia after perinatal HIE, neurodevelopmental performance was predicted by fractional anisotropy (FA) values in the white matter (WM) on early diffusion tensor imaging (DTI) as assessed by means of tract-based spatial statistics (TBSS).

METHODS: We studied 43 term infants with HIE. Developmental assessments were carried out at a median (range) age of 24 (12–28) mo.

RESULTS: As compared with infants with favorable outcomes, those with unfavorable outcomes had significantly lower FA values ($P < 0.05$) in the centrum semiovale, corpus callosum (CC), anterior and posterior limbs of the internal capsule, external capsules, fornix, cingulum, cerebral peduncles, optic radiations, and inferior longitudinal fasciculus. In a second analysis in 32 assessable infants, the Griffiths Mental Development Scales (Revised) (GMDS-R) showed a significant linear correlation ($P < 0.05$) between FA values and developmental quotient (DQ) and all its component subscale scores.

DISCUSSION: DTI analyzed by TBSS provides a qualified biomarker that can be used to assess the efficacy of additional neuroprotective therapies after HIE.

Perinatal hypoxic–ischemic encephalopathy (HIE) leading to brain injury remains an important cause of neurologic disability, accounting for 15–28% of children with cerebral palsy (CP) (1). A recent meta-analysis found that therapeutic hypothermia increases survival with normal neurologic function, and it is now the standard care for infants affected by acute perinatal hypoxia–ischemia. However, even after this treatment ~50% of the infants have an abnormal outcome (2).

Intensive research is focused on preclinical and early clinical studies of agents that, when used alongside hypothermia treatment, may improve intact survival after perinatal hypoxia–ischemia (3–5). An effective bench-to-bedside pipeline requires

the ability to evaluate the efficacy of a treatment by means of early biomarkers. Tract-based spatial statistics (TBSS) analysis of diffusion tensor imaging (DTI) data offers the potential to serve as an early objective imaging biomarker after treatment for perinatal HIE. DTI exploits the random motion of water in tissue to make inferences regarding the underlying microstructure. Objective and reproducible measures, such as the apparent diffusion coefficient (ADC) and fractional anisotropy (FA), can be derived from DTI. TBSS allows voxel-wise analysis of DTI data (6); we have previously shown that there are differences in the extent of white matter (WM) injury in infants who were treated with therapeutic hypothermia as compared with an untreated group (7).

The aim of this study was to determine whether DTI analyzed by means of TBSS could be a qualified biomarker to predict outcomes in infants treated with therapeutic hypothermia after HIE. For this purpose, we tested the hypothesis that early neurodevelopmental performance is correlated with FA values in the WM soon after birth.

RESULTS

A total of 70 term infants with perinatal asphyxia underwent therapeutic hypothermia at Queen Charlotte's and Chelsea Hospital between June 2007 and June 2010. Of the 70 infants, 27 could not be enrolled in the study for the following reasons: no magnetic resonance imaging (MRI) scan ($n = 12$), imaging carried out outside the study period ($n = 2$), poor quality DTI data ($n = 6$), DTI data available in 15 directions only ($n = 1$), and no outcome data available ($n = 6$). Of the 12 infants for whom an MRI scan was not available, 7 infants died within the first week after birth and their discharge summaries documented that they were too unstable to undergo an MRI scan. The other 5 infants had been transferred to another hospital without undergoing MRI. There were no significant differences between the developmental quotient (DQ) or subscale scores for the 5 infants who were discharged to another hospital and those of infants who underwent an MRI scan.

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The clinical characteristics of the infants, including those of the infants who could not be enrolled in the study are shown in [Table 1](#).

Conventional MRI Findings

No congenital abnormality was demonstrated on conventional MRI. Lesions in the basal ganglia and thalami (BGT), consistent with acute hypoxia-ischemia, were detected in 27 infants; these were classified as severe in 12 of the infants. The posterior limb of the internal capsule (PLIC) was equivocal in regard to 5 infants and abnormal in 15 infants. WM abnormalities were present in all except 2 infants, being severe in 12 infants. The cortex showed changes typical of acute hypoxic-ischemic injury in 21 infants.

Neurodevelopmental Outcome

Of the total group, 18 infants had unfavorable outcomes, including 9 who died. Of the surviving infants, six had DQ values of >2 SDs below the mean on the Griffiths Mental Development Scales (Revised) (GMDS-R) scale; two of these infants developed CP (Gross Motor Function Classification System (GMFCS) levels III–IV) and two others were diagnosed with CP (GMFCS level V). The children who developed CP were assessed at a median age of 24.5 (range: 24–28) mo. One infant had a DQ of 87 and developed a right hemiplegia. Of the seven children assessed between 12 and 17.9 (median: 12) mo, four had started to walk independently and the other three were walking with minimal support. One had a mild increase in limb tone on the right side, and another had a mild asymmetry of tone, but neither was judged as having CP. None of the children developed severe cortical visual impairment, but one child required hearing aids.

Neurodevelopmental assessments using the GMDS-R were carried out on 32 of the 34 surviving children, including 2 with CP, at a median age of 24 mo (range: 12–28). The mean \pm SD DQ was 91 ± 19 and the mean \pm SD quotients for the subscales were as follows: locomotor, 92 ± 23 ; personal-social, 100 ± 24 ; hearing and language, 87 ± 24 ; eye-hand coordination, 86 ± 18 ; and performance, 85 ± 17 .

Correlation Between Severity of Encephalopathy and Outcome

There was no correlation between clinical severity of encephalopathy, as assessed by Thompson score (8) before cooling and DQ or any of the subscale scores (DQ, $P = 0.121$; locomotor score, $P = 0.231$; personal-social score, $P = 0.149$; hearing-language score, $P = 0.102$; eye-hand coordination score, $P = 0.265$; performance score, $P = 0.290$).

Correlation Between Conventional MRI Findings and Outcome

[Table 2](#) shows the location and severity of abnormalities observed on conventional MRI and indicates the outcomes. On the basis of conventional MRI, 19 infants were predicted to have favorable outcomes, and 18 of these did have favorable outcomes. Also, 24 infants were predicted to have unfavorable outcomes, and 17 of these infants had unfavorable outcomes. The sensitivity of MRI to detect an abnormal outcome was 94% and specificity was 72%. The positive predictive value of MRI was 71% and negative predictive value was 95%. Of note, five of the seven infants with abnormal MRI and DQ value >76 had WM injury (including two infants with parasagittal infarction and one with a right-sided middle cerebral artery infarction), with normal BGT. All of these infants had a DQ value <90 and all had head circumferences <10 th percentile at assessment.

Prediction of Outcome by TBSS

The mean FA skeleton consisted of 21,357 voxels. We found no correlation between FA values and the number of days after birth. Significantly lower FA values were seen in infants who went on to have unfavorable outcomes as compared with those who had favorable outcomes. These lower values of FA were seen in several regions of the brain, including the right centrum semiovale, splenium, isthmus and genu of the corpus callosum (CC), anterior limb of the internal capsule, PLIC, external capsules, optic radiations, cerebral peduncles, fornix, cingulum, and inferior longitudinal fasciculus ([Figure 1](#)). [Figure 2](#) shows the FA values in the two groups in the regions that were identified as significantly different on the basis of TBSS.

Table 1. Clinical characteristics of the study infants

Clinical characteristic	Total (n = 43)	Died/CP (n = 11)	Griffiths (n = 32)	Excluded (n = 27)
Male sex, n (%)	30 (70)	7 (64)	23 (72)	15 (55)
Postmenstrual age at birth (wk) ^a	39 ⁺⁴ (36 ⁺¹ –42 ⁺³)	39 ⁺⁵ (36 ⁺⁴ –42 ⁺⁰)	39 ⁺⁴ (36 ⁺¹ –42 ⁺³)	39 ⁺⁶ (36 ⁺⁰ –41 ⁺⁵)
Postmenstrual age at scan (wk) ^a	40 ⁺³ (36 ⁺⁵ –43 ⁺¹)	41 ⁺⁶ (37 ⁺² –43 ⁺¹)	40 ⁺⁶ (37 ⁺² –43 ⁺⁰)	40 ⁺⁶ (36 ⁺³ –43 ⁺³)
Postnatal days at scan ^a	6 (2–14)	6 (2–11)	7 (4–14)	6 (3–14)
Birth weight (kg) ^a	3.2 (2.0–5.3)	3.2 (2.0–5.3)	3.1 (2.2–4.1)	3.2 (2.2–4.7)
Apgar score at 1 min ^a	1 (0–5)	0 (0–3)	1 (0–5)	1 (0–6)
Apgar score at 5 min ^a	3 (0–8)	1 (0–4)	3 (0–8)	4 (0–7)
Apgar score at 10 min ^a	5 (0–9)	4 (0–6)	5 (0–9)	5 (0–9)
pH within 1 h of birth ^a	6.8 (6.6–7.1)	6.9 (6.7–7.0)	6.8 (6.6–7.1)	6.80 (6.6–7.2)
Base deficit within 1 h of birth ^a	19.9 (9.7–34.5)	22.1 (16.0–27.5)	19.5 (9.7–34.5)	20.0 (10–28.4)
Thompson score before cooling ^a	11 (1–20)	14 (6–20)	8 (1–18)	12 (1–19)

The superscripts (+ and numbers) used in table 1 are a standard way to express gestational age. CP, cerebral palsy; Griffiths, assessed using Griffiths Mental Development Scales (Revised).

^aMedian (range).

Table 2. Location of lesions on MRI and outcome

Region	Pattern	n (43)	Outcome	
			Favorable	Unfavorable
Basal ganglia and thalami	0	16	15	1
	1	9	8	1
	2	6	2	4
	3	12	0	12
Posterior limb of the internal capsule	Normal	21	20	1
	Equivocal	5	3	2
	Abnormal	15	1	14
	Unable to assess	2	1	1
White matter	Normal	2	2	0
	1	4	3	1
	2	25	14	11
	3	12	6	6
Cortex	0	22	15	7
	1	9	6	3
	2	7	4	3
	3	5	0	5

MRI, magnetic resonance imaging.

The nine infants who died and two of those who developed CP could not be assessed using the GMDS-R scale and, therefore, their data were excluded from the linear regression analysis of FA and DQ/subscale scores. TBSS demonstrated a significant linear correlation between scores for DQ, and personal-social, hearing and language, eye-hand coordination, and performance subscales, as well as in FA values in a number of voxels throughout the WM, including the centrum semiovale, CC, PLIC, external capsules, anterior limb of the internal capsule, optic radiations, frontal WM, cerebral peduncles, fornix, uncinate fasciculus, and cingulum. Across the whole brain, the number of voxels in which FA correlated significantly with DQ was 12,112; personal-social, 7,174; hearing and language, 13,243; eye-hand coordination, 13,195; and performance, 8,006. A significant relationship between FA and locomotor subscale scores was observed in the PLIC, centrum semiovale, CC, left cerebral peduncle, and brain stem (3,140 voxels) (**Figure 3**). These relationships remained significant even after the data of the two infants with CP were removed from the analysis. The associations between FA in the most significant voxel (corrected for postmenstrual age at the time of imaging) and DQ/subscale scores are shown in **Figure 4**.

DISCUSSION

Neuroimaging using magnetic resonance techniques reliably and noninvasively assesses anatomy and pathology in infants after perinatal hypoxia-ischemia. Conventional MRI provides excellent details of brain lesions characteristic of perinatal hypoxic-ischemic injury; these lesions can be graded, and

the pattern of involvement can be related to outcome (9). On visual inspection, injury is often detected in the central gray matter and PLIC. Bilateral BGT lesions are strongly associated with the development of motor impairment, and the extent of the lesions is closely related to the severity of such impairment (10). The predictive value of conventional MRI regarding subsequent neurological impairment is not affected by hypothermia treatment (11).

However, WM injury is often also observed after HIE and may be associated with cognitive and memory difficulties that may not be apparent until school age, even in the absence of motor deficits (12,13). School-age children with a history of moderate to severe perinatal asphyxia show delays in reading, spelling, and arithmetic (14); have poorer narrative memory, memory for names, and everyday memory (15); and demonstrate severe impairment of episodic memory (16) and visual recall (17).

DTI utilizes the random Brownian motion of water molecules. In WM, water preferentially diffuses along the axons, producing anisotropic diffusion. Objective measures such as ADC values (a measure of the overall magnitude of water diffusion) and FA (the fraction of diffusion that can be attributed to anisotropic diffusion) can be derived from DTI. The utility of ADC measurements in HIE is limited due to pseudonormalization: typically, ADC values are lower during the first week after HIE, then return to normal, and increase further after ~2 wk; therefore postnatal age at scanning must be taken into account (18–23). Although in several studies an ADC value of $<0.78\text{--}0.85 \times 10^{-3} \text{ mm}^2/\text{s}$ in the PLIC was associated with poor outcome (22,24–26), a recent meta-analysis of MRI biomarkers in asphyxia showed that ADC has poor specificity (64%) and sensitivity (66%) in predicting outcome (27). Consistent with previous reports, we found that FA values do not pseudonormalize after HIE (28).

Malik *et al.* reported a correlation between FA in the corticospinal tract and DQ at 3 mo of age, both in control infants and those who had experienced HIE, but no correlation was found in any other regions (29). Brissaud *et al.* found that FA values in the cerebral peduncles and PLIC correlate with early neurologic function during the first week after birth (26). However, these studies used manually defined regions of interest, an approach that can potentially introduce observer error. Such problems can be avoided by using TBSS, which is an objective, observer-independent tool, to assess WM microstructure across multisubject DTI data (6). In our recent study (7), widespread WM abnormalities were revealed through TBSS assessment in infants who had experienced perinatal asphyxia. Those who had received hypothermia treatment exhibited much less extensive WM injury. However, our previous TBSS study did not examine correlations between outcome and neonatal FA measures.

In this study, we investigated subjects all of whom had received hypothermia treatment as standard care for HIE. TBSS demonstrated significantly lower FA values in infants who went on to have unfavorable outcomes, including in those who died or developed such severe CP that formal neurodevelopmental

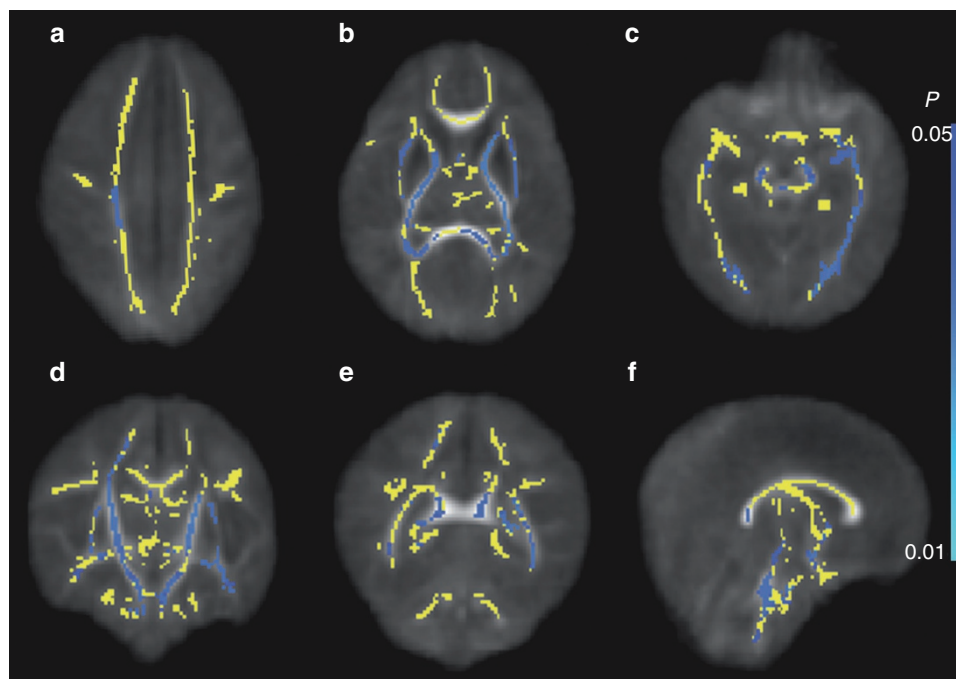


Figure 1. Mean FA skeleton (yellow) overlaid on mean FA image. Voxels wherein infants with unfavorable outcome had significantly lower FA are shown in blue. (a) Axial view at the level of the centrum semiovale. (b) PLIC. (c) Cerebral peduncles. (d) Coronal view at the level of the PLIC and mid CC. (e) Splenium of the CC and the cerebellum. (f) Midsagittal view showing the CC. CC, corpus callosum; FA, fractional anisotropy; PLIC, posterior limb of the internal capsule.

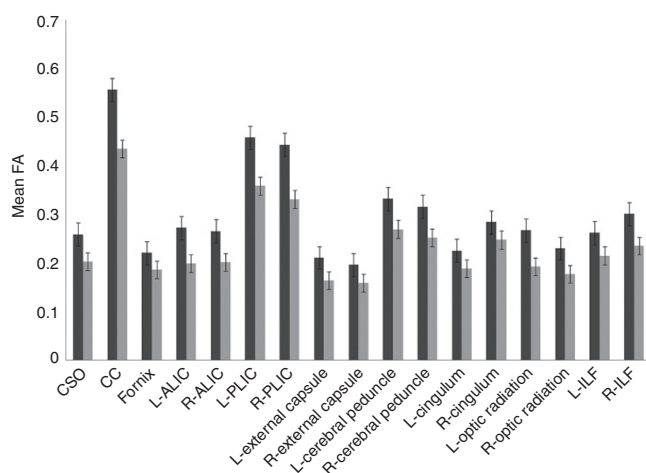


Figure 2. Graph showing FA values extracted from the most significant voxel in regions wherein FA was significantly higher in infants with a favorable (indicated in black) outcome as compared with infants with an unfavorable (indicated in gray) outcome. ALIC, anterior limb of the internal capsule; CC, corpus callosum; CSO, centrum semiovale; FA, fractional anisotropy; ILF, inferior longitudinal fasciculus; L, left; PLIC, posterior limb of the internal capsule; R, right.

assessment using GMDS-R could not be performed. We accept that defining CP in children of <18 mo of age may be difficult, but all the children we saw at 12–18 mo were either already walking independently or requiring only minimal support. We observed a correlation between locomotor function and FA values in the CC and corticospinal tracts, as reported previously (30). We also observed a correlation between DQ scores subscales and FA values in the fornix, cingulum, and uncinate

fasciculus—structures associated with cognition and memory in adults (30). We found that FA values in the CC correlated with overall DQ and with certain subscale scores. Although the CC is not typically found to be abnormal on visual inspection of early MRI scans after HIE, a recent study found reduced callosal dimensions at 2 y of age in children who suffered moderate asphyxia; these were predictive of unfavorable outcome (25). A DTI study of adolescents with a history of moderate neonatal encephalopathy showed significantly lower FA values in the CC in association with cognitive impairment (31). Our results suggest that microstructural abnormalities in the CC are present in the perinatal period after HIE.

We accept that the number of infants excluded from this study because of lack of follow-up or absence of DTI data is a limitation. However, TBSS proved to be a more accurate predictor of outcome than the severity of encephalopathy. Also, although the sensitivity of conventional MRI to predict abnormal outcome was high, the specificity was only 72%, largely because infants with extensive WM injury but relatively spared BGT scored >76 on the GMDS-R scale. Previous studies have observed poor outcomes at 30 mo of age in infants who had experienced WM infarction after HIE (32). Therefore, later follow-up assessments would be of interest in these cases.

Meta-analyses of the cooling trials based on 18-mo follow-up data indicate that therapeutic hypothermia is beneficial in term infants after perinatal asphyxia (2,33,34). The meta-analyses report significant reductions in the rate of composite outcome of deaths or disability, with number needed to treat of 9, as well as significant reduction in CP, cognitive developmental delay,

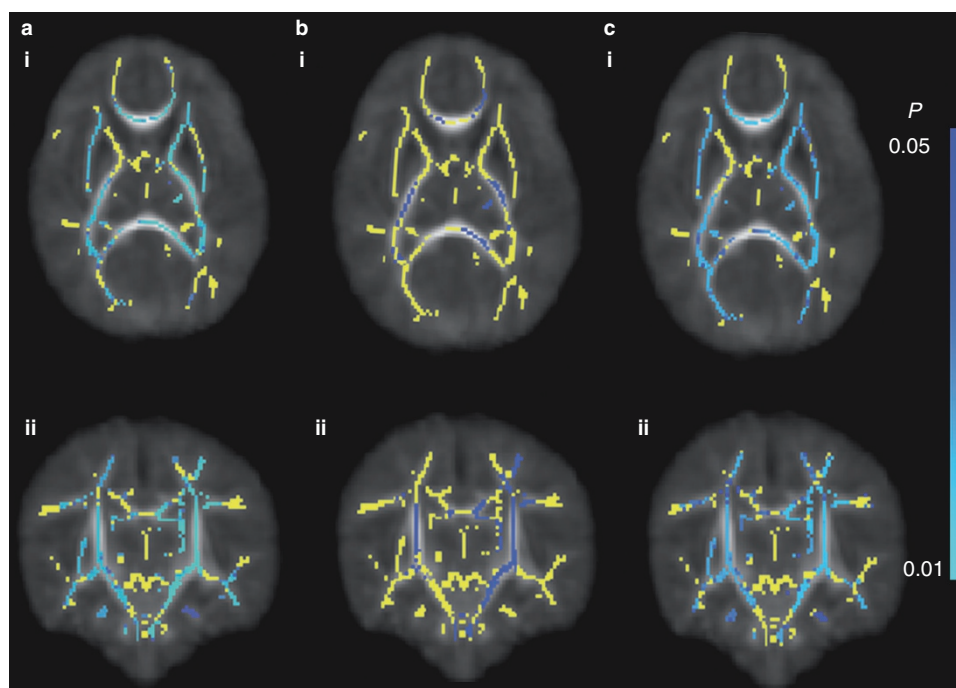


Figure 3. Mean FA skeleton (yellow) overlaid on mean FA image. Voxels wherein FA is significantly correlated to GMDS-R scores are shown in blue. (a) DQ. (b) Locomotor. (c) Hearing and language in the (i) axial view at the level of the PLIC and (ii) coronal view at the level of the PLIC and corpus callosum. DQ, developmental quotient; FA, fractional anisotropy; GMDS-R, Griffiths Mental Development Scales (Revised); PLIC, posterior limb of the internal capsule.

and blindness, with numbers needed to treat of 8, 9, and 17, respectively (2). These results suggest that infants with a history of perinatal HIE would benefit from additional neuroprotective therapies. The availability of qualified biomarkers would facilitate translation of potential therapies into clinical trials (35). These data suggest that TBSS can be used as a qualified biomarker to study additional neuroprotective interventions in infants with HIE who received therapeutic hypothermia.

METHODS

The study was approved by the West London Research Ethics Committee, and written consent was obtained from the parents of all the infants before scanning.

Subjects

We studied infants with HIE who fulfilled the criteria for therapeutic hypothermia (36). Infants were eligible to be enrolled into this study if (i) they had received hypothermic neural rescue therapy at the Queen Charlotte's and Chelsea Hospital neonatal intensive care unit; (ii) cerebral MRIs with good-quality DTI data were obtained within the first 3 wk after birth; and (iii) outcome data were available at least at 12 mo after birth. Demographic data were collected with respect to all the infants from the hospital records.

Imaging

MRI was performed on a 3 Tesla Philips system (Philips, Best, The Netherlands). Three-dimensional MPRAGE and T2-weighted images were acquired for clinical evaluation. Single-shot echo planar DTI was acquired in 32 noncollinear directions with b value 750 s/mm².

MRI scans were performed under chloral hydrate sedation unless sedatives had already been administered for clinical reasons. Before screening, all the infants were assessed by an experienced pediatrician to ensure that they were clinically stable enough to undergo scanning. Physiologic parameters (heart rate, oxygen saturation, and temperature) were monitored before and throughout the scan. Ear protection was used during scanning; individually molded earplugs made of

silicone-based putty (President Putty; Coltene/Whaledent, Mahwah, NJ) were placed into the external ear, and neonatal earmuffs (Natus MiniMuffs; Natus Medical, San Carlos, CA) were also used. All the examinations were supervised by a neonatologist experienced in MRI procedures.

Visual analysis of the conventional images was carried out by a perinatal radiologist blinded as to the outcome data. The patterns of injury in the WM, BGT, PLIC, and cortex were classified as described previously (11).

Neurodevelopmental Assessment

Most of the study infants were brought to the follow-up clinic at our institution and were assessed using a standardized neurologic examination (37,38). With respect to the few infants examined at other hospitals, the information was obtained from their local pediatric neurodevelopmental team. Neurodevelopmental assessments were carried out by pediatricians trained in performing GMDS-R (39) testing. The GMDS-R scores were used to provide an overall DQ, with subscales assessing different skill areas (locomotor, personal-social, hearing and language, eye-hand coordination, and performance). The presence of CP was defined and classified from information collected from hospital notes and letters, using the data from Surveillance of Cerebral Palsy in Europe (40). The GMFCS was used to grade functional impairment (41).

TBSS

DTI data were analyzed using tools implemented in FMRIB's Software Library (<http://www.fmrib.ox.ac.uk/fsl>). DTI data were affine-registered to the b0 image to minimize distortions caused by eddy currents. Nonbrain tissue was removed, and FA maps were generated. Voxel-wise preprocessing of the FA data was carried out using an optimized protocol, which we have modified to improve its reliability in neonatal DTI analysis (42). Two linear registration steps were performed before nonlinear registration (6 and 12 degrees of freedom), in order to register every subject's FA map to each other. We selected the target with minimum mean warp displacement score as our chosen target. Each infant's FA map was aligned in the target space, and the mean FA map was created. A second set

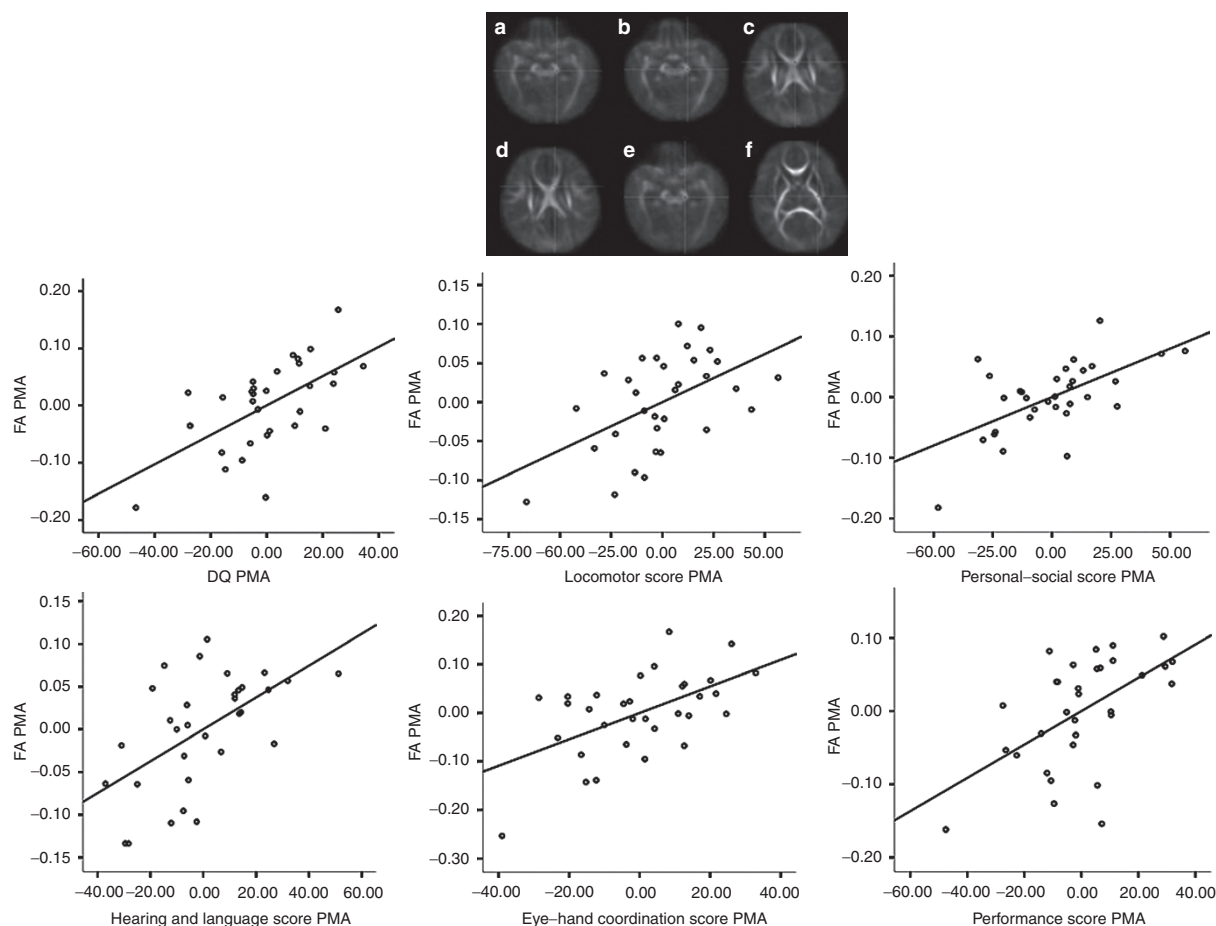


Figure 4. Graphs showing associations between FA in the most significant voxel (crosshairs), corrected for age at imaging, and (a) DQ ($R^2 = 0.417$), and (b) locomotor ($R^2 = 0.277$), (c) personal-social ($R^2 = 0.326$), (d) hearing and language ($R^2 = 0.301$), (e) eye-hand coordination ($R^2 = 0.311$), and (f) performance ($R^2 = 0.365$) subscale scores. Key: FA PMA = residuals of FA given the model; DQ PMA = residuals of DQ given the model; locomotor score PMA = residuals of locomotor scores given the model; personal-social score PMA = residuals of personal-social scores given the model; hearing and language score PMA = residuals of hearing and language scores given the model; eye-hand coordination score PMA = residuals of eye-hand coordination scores given the model; performance score PMA = residuals of performance scores given the model. DQ, developmental quotient; FA, fractional anisotropy; PMA, postmenstrual age.

of registrations was performed to register each individual FA map to the mean FA map. The aligned images were used to create the final mean FA map, and a mean FA skeleton representing the centers of all the tracts that were common to the group. This was thresholded to FA ≥ 0.15 to include the major WM pathways but exclude peripheral tracts where there was significant inter-subject variability and/or partial volume effects with gray matter. Each subject's aligned FA data were projected onto this skeleton.

TBSS was used to assess the relationship between FA and outcome. As a first step, the infants were divided into two groups: (i) infants with favorable outcomes and (ii) infants with unfavorable outcomes. Unfavorable outcome was defined as death or the presence of at least one of the following impairments (36): (i) DQ value of ≥ 2 SD below the mean on the GMDS-R scale (<76); (ii) GMFCS levels 3–5; and (iii) bilateral cortical visual impairment with no useful vision. The FA values of the two groups of infants were compared using voxel-wise cross-subject statistics. As a next step, infants who died or who developed such severe CP or global developmental delay that neurodevelopmental assessment could not be performed using GMDS-R were excluded before performing voxel-wise cross-subject linear regression to assess the relationship between FA, corrected for PMA at scan, and neurodevelopmental performance. In both analyses, the results were corrected for multiple comparisons by controlling family-wise error rate after threshold-free cluster enhancement; $P < 0.05$ was considered significant. To explore whether the age of the infant (number of days after birth) at the time of the scan had

an impact on FA, we performed linear regression analysis to assess the relationship between FA and the number of days elapsed since birth.

To demonstrate the results of these analyses graphically, FA values were extracted from the most significant voxel in the regions in which FA was higher in the group with the favorable outcome. For the purpose of exploring the relationship between tissue microstructure and neurodevelopmental performance scores in the regions wherein a significant linear relationship was observed, the FA value (adjusted for postmenstrual age at scan) in the most significant voxel was extracted and plotted against the DQ/subscale scores.

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