Quantitative diffusion tensor imaging and intellectual outcomes in spina bifida

Laboratory investigation

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**Object.** Patients with spina bifida (SB) have variable intellectual outcomes. The authors used diffusion tensor (DT) imaging to quantify whole-brain volumes of gray matter, white matter, and cerebrospinal fluid (CSF), and perform regional quantitative microstructural assessments of gray matter nuclei and white matter tracts in relation to intellectual outcomes in patients with SB.

**Methods.** Twenty-nine children with myelomeningoceles and 20 age- and sex-matched children with normal neural tube development underwent MR imaging with DT image acquisition and assessments of intelligence. The DT imaging–derived metrics were the fractional anisotropy (FA), axial (parallel), and transverse (perpendicular) diffusivities. These metrics were also used to segment the brain into white matter, gray matter, and CSF. A region-of-interest analysis was conducted of the white and gray matter structures implicated in hydrocephalus.

**Results.** The amount of whole-brain gray matter was decreased in patients with SB, with a corresponding increase in CSF (p < 0.0001). Regional transverse diffusivity in the caudate nucleus was decreased (p < 0.0001), suggesting reduced dendritic branching and connectivity. Fractional anisotropy in the posterior limb of the internal capsule increased in the myelomeningocele group (p = 0.02), suggesting elimination of some divergent fascicles; in contrast, the FA in several white matter structures (such as the corpus callosum genu [p < 0.001] and arcuate fasciculus) was reduced, suggesting disruption of myelination. Diffusion tensor imaging–metrics involving gray matter volume and the caudate nucleus, but not other structures, predicted variations in IQ (r = 0.37–0.50; p < 0.05).

**Conclusions.** Diffusion tensor imaging–derived metrics provide noninvasive neuronal surrogate markers of the pathogenesis of SB and predict variations in general intellectual outcomes in children with this condition.

(DOI: 10.3171/PED/2008/2/7/075)

**Key Words • caudate nucleus • diffusion tensor imaging • IQ • myelomeningocele • neurodevelopment • spina bifida**

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**Abbreviations used in this paper: CSF = cerebrospinal fluid; DT = diffusion tensor; FA = fractional anisotropy; ROI = region of interest; SB = spina bifida; SD = standard deviation; SNR = signal-to-noise ratio; WASI = Wechsler Abbreviated Scale of Intelligence; WISC = Wechsler Intelligence Scale for Children.**
nonverbal IQ. Authors of more recent studies with larger, more diverse patient cohorts have not found this difference in verbal and nonverbal IQ impairment, but have continued to find relatively low rates of mental retardation. A variety of factors have been associated with variations in IQ, including the level of the lesion, number of shunt revisions and infections, and environmental factors. An obvious source of variation is the extent to which the brain is congenitally malformed and further damaged by hydrocephalus. In addition to older studies based on pneumomegalo- and cerebral CT, more recent studies have used MR imaging quantitative volumetry methods to assess regional brain volumes. These have shown reductions in gray and white matter volumes with corresponding increases in CSF volume in posterior brain areas in patients with SB. The authors of these studies did not assess specific structures other than the corpus callosum, and were restricted to large subdivisions of the brain. Total cerebral volumes were comparable between children with SB and those in the control group, but cerebellar volumes were significantly lower in the SB group, especially in children with upper level (thoracic and above) spinal lesions. There were weak correlations between intellectual and motor performance outcomes with increased CSF in the posterior areas of the brain and in area measurements of the corpus callosum. Few studies have assessed ROIs or brain microstructure using newer MR imaging methods such as DT imaging.

There are 2 preliminary DT imaging studies in patients with SB. Vachha and colleagues reported that qualitative DT imaging fiber tracking assessments of the fornices and cingulate yielded abnormal results in most of their 13 patients, and that only those with abnormal fornices showed lower memory performance on cognitive assessments. The present study is a larger and extended account of our preliminary quantitative DT imaging report. We used a novel application of optimized whole-brain DT imaging at a high SNR to quantify white matter, gray matter, and CSF volumes in children with SB and in a control group. Regional metrics involving FA and axial and transverse diffusivities were obtained using a landmark-driven methodology. The DT imaging global and regional measures were correlated with IQ scores. We hypothesized that DT imaging assessments would show global reductions in gray and white matter with increased CSF volume comparable to those obtained with traditional MR imaging volumetric methods. Region-of-interest measurements would be consistent with reduced white matter development and would correlate with lower IQ scores, a general measure of outcome in patients with SB.

Study Participants

Twenty-nine children with SB (mean age ± standard error of the mean) 13.2 ± 2.9 years, range 8.7–18 years) born with myelomeningocele (verified on medical record review and neurosurgical operative reports) and who underwent shunt placement for hydrocephalus were compared with a control group of 20 children with typical spinal cord development (mean age 11.8 ± 2.9 years, range 8–16.7 years). The age difference between the groups was not statistically significant (p > 0.1). The SB cohort included 13 girls and 16 boys of mixed ethnicities; 21 of 29 children were right-handed. The control group included 8 girls and 12 boys; 16 of 20 children were right-handed. The groups did not differ by age (p = 0.10, 2-tailed t-test), sex (p = 0.09, chi-square test), or handedness (p = 0.07, chi-square test). All children were primarily English speakers and in stable medical condition at the time of assessment. Written informed consent was obtained from guardians and adolescents, and assent from the participating children, in accordance with the University of Texas Health Science Center at Houston regulations for the protection of human research subjects.

Although DT imaging results can be influenced by edema and inflammatory lesions, no participants had edema or focal inflammatory lesions in any of the regions reported in this study as confirmed by a board-certified radiologist (L.A.K.) who reviewed the anatomical and fluid-attenuated images obtained in all participants. Additional coding of the qualitative characteristics of the MR images for expected features of SB revealed that 27 of 29 patients showed the characteristic Chiari malformation Type II of the hindbrain and cerebellum. The 2 patients who did not show this malformation had sacral lesions. Shunts were placed on the right side in 25 patients and on the left in 4. Consistent with findings in previous studies, no child had a normal corpus callosum, with 15 showing dysgenesis of either the rostrum, splenium/posterior body, or both; in the other 14 thinning of some of the corpus callosum structure was evident. No child had undergone > 8 shunt revisions, and most children (71%) had undergone ≤ 2 revisions. The rate of shunt infections was 23%, with most revisions due to obstruction. One patient had an actively treated seizure disorder, 2 had received previous treatment for seizures, and the others had no history of seizures. There were 9 patients with thoracic, 16 with lumbar, and 4 with sacral level spinal lesions. Altogether, this group was similar to the 268-patient cohort we reported on previously.

Intelligence Testing

Intelligence was assessed at the time of imaging with the Stanford–Binet Intelligence Test-IV in the group with SB, and the WASI in the control group. The children in the control group were recruited from another study in which the same imaging sequences were used; the 2 measures are probably highly correlated because the WASI and WISC-III strongly correlate (r = 0.92), and the Stanford–Binet-4 and WISC-III also strongly correlate (r = 0.81). As expected, the average IQ score in the control group (mean ± standard error of the mean) 106.4 ± 15.6, range 83–126) was significantly higher (and less variable) than in the children with SB (mean 79.5 ± 13.7, range 47–108; p < 0.0001, Mann–Whitney test). There were no significant differences between verbal and nonverbal IQ (p > 0.10).

Imaging Methods

We acquired whole-brain data using a Philips 3.0-T Intera system with a SENSE parallel imaging head coil. The MR imaging protocol included conventional imaging (3D spoiled gradient echo and 3D fast spin echo) in the coronal plane, phase-sensitive MR imaging in the sagittal and axial planes, and matching axial diffusion-encoded data.

Acquisition. The diffusion weighted data were acquired using a single-shot spin echo diffusion sensitized echo pla
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The slice thickness was 3.0 mm with 44 axial slices covering the entire brain (foramen magnum to vertex), a square field of view of 240 × 240 mm2, and an image matrix of 256 × 256. The number of non–diffusion weighted or b-factor, ~ 0 magnitude image averages was 8; in addition, each encoding was repeated twice and magnitude-averaged to enhance the SNR.7,16,38–40,57 Thus, 50 DT images were acquired for each of the 44 axial sections to cover the entire brain. The total DT imaging acquisition time was ~ 7 minutes, and resulted in SNR-independent DT imaging-metric estimation. The entire MR imaging data acquisition scan time was kept < 30 minutes to accommodate children.38

Processing. Although the raw DT images were acquired with fat suppression, all images were semiautomatically stripped to remove nonparenchymal tissue. Diffusion weighted data were distortion–corrected using the mutual information maximization approach.34 The details of DT image processing are described elsewhere.7,38–40

Diffusion Tensor Imaging–Derived Metrics. Fractional anisotropy is a measure of the intravoxel directionality of water translational random motion in the presence of barriers and is expressed as a ratio ranging from 0 to 1 (0 = isotropic or no predilection for any particular direction, and 1 = unidirectional). Although the underlying histological processes leading to anisotropy changes in white matter detected on DT imaging are not completely understood,8 factors that contribute to FA other than intravoxel orientation of the fibers include regional differences in fiber packing density, degree of myelination, fiber diameter, and density of the neuronal glial cells. The mean diffusivity provides the overall magnitude of water diffusion (expressed in mm²/s−1) and is a sensitive indicator of maturational changes in the brain independent of anisotropy measures. There are 2 specific diffusivity measures that can be derived from the DT data. The axial (parallel) (λ1 = λ2) diffusivity is the magnitude of water movement along the long axis of axons, while the transverse (perpendicular) (λ1 = λ2) diffusivity is the magnitude of water movement perpendicular to the long axis of the axons. The transverse diffusivity was defined as the mean of the minor (third) and medium (second) eigenvalues (λ1 = (λ2 + λ3)/2).8 The axial and transverse diffusivities have been shown in animal models of human disease, and in DT imaging applications to human brain neurodevelopment and neurodegeneration to provide a more specific interpretation of DT imaging results than that provided by tensor anisotropy and mean diffusivity values.8,34,35,40,65 Specifically, the axial diffusivity is more related to the intrinsic characteristics of the axons or changes in the extracellular/extracellular space, while the transverse diffusivity provides a more specific surrogate of changes associated with myelination.8,40,53,65

Tissue Segmentation. The collected DT imaging metrics and their corresponding errors were further used in a multidimensional, supervised, and trained feature space–initiated clustering procedure to segment the entire brain (cerebrum, brainstem, and cerebellum) into white matter, gray matter, and CSF. This new method uses the contrast in FA maps between CSF, white matter, and gray matter, and the cluster separability and discriminability of white matter and gray matter based on the principal diffusivity indices.38 The CSF was segmented based on its high diffusivity and low anisotropy.38,57 Advantages of the DT imaging approach to multimodal conventional MR imaging include: 1) the use of high SNR and uniformly distributed and rotationally invariant diffusion encoding scheme;3,39,40 2) the use of reduced image distortions with the adoption of parallel imaging;42,60 3) the utility of self–registered DT imaging metrics;42 and 4) the fact that DT image processing involves decoupling of radio frequency inhomogeneity field–related intensity variations that are common in conventional MR imaging–based approaches.35,58 The entire brain white matter, gray matter, and CSF fractions found using this approach are consistent with published reports using multispectral conventional MR imaging approaches in normative samples.13,43,45,59 This is the first application of these DT imaging methods to a clinical sample.

Region-of-Interest Analysis. The ROI–derived from the analysis represented 50 “normal–appearing” white and gray matter structures, including the right–left (contralateral) caudate head, putamen, internal capsule (anterior, external, and posterior limb), corpus callosum segments (Fig. 1),38 corticospinal tract, forceps minor and major, inferior and superior longitudinal fasciculi, and the arcuate fasciculus and other gray matter regions (such as the cortex and hippocampus) and white matter structures (such as the cerebellar peduncles). The compact white matter structures belonged to 3 major categories: projection, commissural, and association fibers involved in inter- and intrahemispheric communication. The ROI placement procedure was supervised by a radiologist, and a 3D system was used that fused DT imaging maps with conventional MR images. This system has been shown to help reduce partial averaging artifacts due to CSF.37 A subset of these ROIs was selected because of the expected impact from hydrocephalus and is reported here (Fig. 1).

Data Quality and Reproducibility. Water phantom measurements were collected over the span of the data acquisition to assure the field uniformity and stability of the MR unit. The diffusion encoding (Icosa21b) provided 3 levels of SNR, and thus the SNR dependence of the DT imaging–metrics reported in this work were also studied on all participants.40 The ROI measurements were also examined for rater reproducibility.39,41 All results were reproduced at multiple SNRs and rating sessions (p > 0.9).

Statistical Analysis

Group mean comparisons were made using the t-test for unpaired groups; within group comparisons were conducted using paired t-tests. Alpha levels were adjusted for the number of dependent univariate tests M = 7 (p < 0.05/7 = 0.007). Correlations with IQ were based on the Spearman correlation coefficient.

Results

In Fig. 2a the percentages (mean ± SD) of total white matter, gray matter, and CSF volume fractions in the groups are compared. Notice the significant increase in whole–brain CSF and significant decrease in whole brain gray mat-
these data show different changes in the white matter and gray matter nuclei of children with SB, suggesting different mechanisms affecting the brain after early injury.

Within the group with SB (29 patients), IQ significantly correlated with the total gray matter/total brain volume percentage ($r = 0.50$, $p = 0.005$) (Fig. 3 left). In addition, IQ significantly correlated regionally with the transverse diffusivity of the right caudate head ($r = 0.42$, $p = 0.024$) (Fig. 3 right) and left caudate head ($r = 0.37$, $p = 0.046$; data not shown). There were no significant correlations with other DT imaging measures.

**Discussion**

This is the first quantitative DT imaging–based report documenting patterns of global and regional white and gray matter volumes with assessments of brain microstructure and corresponding correlations with outcomes in children with SB. Our DT imaging measurements were conducted at high SNR using optimized DT imaging encoding schemes to minimize well-documented biases in DT imaging–metric estimation. Because CSF volume/brain volume fraction is significantly larger in the SB group, one would expect a reduction in FA due to contamination with CSF bordering the atrophic caudate (Fig. 1); on the contrary, we identified significant bilateral increases in caudate FA in the group with SB.

It is well-established that hydrocephalus in humans with SB and in animals disrupts the development of myelination and the overall development of the white matter, consistent with our observation of decreased FA in several pathways (such as the corpus callosum genu and arcuate). These changes may be partially reversed by early shunt placement, which may lead to partial, but not complete, reconstruction of the cerebral mantle. Decreased FA of the white matter has also been reported in disorders not associated with severe hydrocephalus, including Krabbe disease, fragile X syndrome, velocardiofacial syndrome, and William syndrome and is interpreted as a marker of reduced myelination. Elevated white matter FA, as observed in this study in the posterior limb of the internal capsule, may reflect greater alignment of fibers in the voxel as a result of elimination of divergent fibers, developmental myelination, or increased microfilament density in the axons as a result of training, adaptation, and functional specialization.

In gray matter structures such as the caudate, increased FA and the commensurate increase in the axial diffusivity and reduction in the transverse diffusivity may be related to reduced dendritic branching. Experimental models in animals have shown that infantile hydrocephalus leads to neuronal damage that is progressive and not completely reversed by shunt placement. These changes affect neurotransmitter systems involved in learning and in cortical and subcortical connectivity in afferent and nonafferent pathways. There are also effects on dendritic formation that are only partially reversed by early shunt placement and in animal models are associated with impairments in learning, even after shunt insertion.

Based on the results of DT imaging, Assaf et al. reported that the compression of white matter as a result of acute hydrocephalus in humans reduces the FA of white matter structures, such as the genu of the corpus callosum. Assaf et
al. also reported an increase in FA along with reduced transverse diffusivity in the posterior limb of the internal capsule, hypothesizing elimination of some divergent fascicles traversing this region that subserves both sensory and motor functions. Our measurements of the posterior limb of the internal capsule in older children with SB are consistent with these observations. Altogether, the effects of hydrocephalus on brain parenchyma have been well-studied in animals and humans, and include disruption of both neurons and axons, and causing cellular death, migrational defects, and other problems.1,16,17 Our DT imaging measurements are sensitive to these changes and show continued effects of the early disruptive effects of hydrocephalus in these shunt-treated children.

Hydrocephalus has also been shown to affect the long-term potentiation of synaptic integration in experimental models.68 As the increase in FA for the caudate is observed in children with SB who are well beyond the infantile period, these results may reflect prolonged disruption of the processes that underlie synaptic connectivity in the late prenatal period and continuing through adolescence.43,45 Decreased dendritic arborization is the major mechanism underlying the reduction in gray matter volume that occurs in development and was recently interpreted as being responsible for individual differences in the relation of IQ and gray matter volumes in normal development.13,46,59 In adult-onset Huntington disease, greater extent of anisotropy of the caudate has been related to a loss of dendritic connectivity that also resulted in reduced caudate-thalamic-nigral and caudate–cortical connections resulting in loss of motor and cognitive function.4 In addition, DT imaging ROI studies of the basal ganglia of nor-

**FIG. 2.** Bar graphs. a: Whole-brain DT imaging of gray matter (GM), white matter (WM), and CSF fractions (% means ± SDs) in the age-matched SB group (29 children) and control group (20 children). b: Region-of-interest DT imaging comparisons of FA in both groups. The ROIs selected include the right caudate head (Rcau), right putamen (RPut), right forceps minor (Rfmin), genu of the corpus callosum (gCC), right anterior and posterior limbs of the internal capsule (Ralic and Rplic, respectively), and the right arcuate fasciculus (RArcF). c: Region-of-interest DT imaging group comparisons of transverse diffusivities (λ⊥) in both groups. d: Region-of-interest DT imaging group comparisons of axial diffusivities (λ∥) in both groups. The group means ± SDs are shown along with the corresponding probability values.
Brain maturation and healthy aging indicate that the diffusion tensor anisotropy of the putamen, caudate, and lenticular nuclei (combined putamen and globus pallidus) increases slowly with age, along with a steady decrease in caudate nuclei volume. Although the histological causes of this increase in diffusion anisotropy have not yet been isolated, hypotheses related to dendritic elimination along with neuronal loss have been previously invoked to explain the loss of basal ganglia neurons and their complex network of fiber connections, and are consistent with the negative effects of hydrocephalus on cortical development and dendritic branching.

An abnormal or “paradoxical” increase in caudate and putamen diffusion tensor anisotropy, along with a reduction in the mean diffusivity, has been reported in normal-appearing basal ganglia of patients with multiple sclerosis. The authors ruled out gliosis, which would have resulted in more disorganization (such as reduced anisotropy and mean diffusivity), attributing this finding to axonal degeneration due to fiber transection in remote focal multiple sclerosis lesions. A significant increase in caudate anisotropy, along with a slight decrease in mean diffusivity, has also been reported in benign intracranial hypertension, which rules out cerebral edema as an explanation.

The present study includes a relatively small number of patients that prevents analysis of relevant treatment factors (such as shunt placement) and we lack histological analyses using CSF metabolite analysis and postmortem data that would directly address the DT imaging–derived hypotheses about neuropathology. However, our results are consistent with studies of CSF in humans and in animal model studies of hydrocephalus, which itself results in tissue displacement, disruption, demyelination, and elimination of certain pathways. In the absence of comparisons with other origins of congenital hydrocephalus (such as aqueductal stenosis, which is even more rare than SB, and a cohort for which we did not sample), we cannot show that these findings are specific to SB or that they reflect more than the effects of hydrocephalus. The lack of significant findings involving white matter volumes is surprising since MR imaging morphometry studies have reported reductions in both gray and white matter in children with early hydrocephalus. However, the older MR imaging morphometry studies are not whole-brain analyses, with measurements of only the cortex. Finally, the cognitive assessments were restricted to IQ measures, which are a general proxy for overall outcomes, and not specifically related to the ROIs. Using MR imaging–based volumetry in children with SB, we have shown the relationship of variations in timing functions and cerebellar volumes, and focused attention with thinning of the posterior parietal region. Future studies should test hypotheses about specific cognitive and motor functions and DT imaging measurements in addition to factors such as shunt and revision status.

Conclusions

Diffusion tensor–derived metrics such as total-brain gray matter percentage and regional FA and transverse diffusivity in the caudate provide important noninvasive neuronal surrogate markers of the pathogenesis of SB, and can be used to predict variations in cognitive outcomes associated with this condition. Mechanisms of neural reorganization in SB thus may include disruption of myelination, reduced dendritic branching, reduced connectivity, and the targeted elimination of some fiber types. Diffusion tensor imaging provides noninvasive markers of brain development and disruption in neurodevelopmental disorders that may ultimately assist in the investigation of the effects of shunt placement and other interventions in patients with neurodevelopmental disorders.

Acknowledgment

We thank Vipul Kumar Patel for helping in data acquisition.

References

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35. Hasan KM: Diffusion tensor eigenvalues or both mean diffusivity and fractional anisotropy are required in quantitative clinical diffusion tensor MRI reports: fractional anisotropy alone is not sufficient. Radiology 239:611–613, 2006 (Letter)


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Manuscript submitted July 19, 2007. Accepted March 18, 2008. This work was funded by NIH-NICHD grants 01-019746 awarded to Dr. Fletcher, and grant NICHD-R01-NS046308 awarded to Dr. Ewing-Cobbs. Additional funding to Dr. Hasan is provided by NINDS-R01-NS052505-02. Address correspondence to: Jack M. Fletcher, Ph.D., Department of Psychology, University of Houston Texas Medical Center Annex, 2151 West Holcombe Boulevard, Suite 222, Houston, Texas 77204-5053. Email: jackfletcher@uh.edu.