Characteristics of Abnormal Diffusivity in Normal-Appearing White Matter Investigated with Diffusion Tensor MR Imaging in Tuberculous Sclerosis Complex

BACKGROUND AND PURPOSE: Although patients with tuberous sclerosis complex (TSC) manifest various structural abnormalities, we hypothesized that white matter (WM) structures that appear normal on conventional MR imaging may be accompanied by microstructural changes, such as gliosis and myelination defects. Our objective was to determine in vivo whether there was evidence for WM microstructural changes by using diffusion tensor imaging (DTI).

MATERIALS AND METHODS: We used DTI to evaluate diffusivity and anisotropy in normal-appearing WM (NAWM) of 6 children with TSC and 12 age-matched control subjects. The anterior and posterior limbs of the internal capsule, the external capsule, and the genu and splenium of the corpus callosum were assessed. We hypothesized that previously reported DTI abnormalities of NAWM in patients with TSC may not be equal in all diffusion directions as measured by the major, middle, and minor eigenvalues.

RESULTS: When combining NAWM regions in patients with TSC, we observed a significant increase in mean diffusivity ($P = .003$) and a decrease in anisotropy ($P = .03$) compared with those of controls. However, the increase in diffusivity was more pronounced in directions orthogonal to the axons measured by the minor and middle eigenvalues ($P = .005$) than by the major eigenvalue ($P = .02$).

CONCLUSION: Our findings revealed a decrease in anisotropy and an increase in longitudinal and radial diffusivities in NAWM beyond the location of TSC lesions seen on conventional MR imaging. The axonal microstructural changes suggested by our study may be related to changes in myelin packing due to giant cells accompanied by gliosis and myelination defects known to occur in TSC WM.
part of their clinical MR imaging protocol, were selected for this study. The age range was selected on the basis of availability of normal controls for comparison. Although we have DTI data on younger patients with TSC, younger controls were not available. Twelve normal control subjects (mean age, 10 ± 4 years; age range, 7–17 years) were included in this investigation. The MR imaging study in controls was approved by the Wayne State University Institutional Review Board (IRB), and written informed consent of parents/guardian and assent of children older than 13 years were obtained. The normal children were not sedated for the MR imaging. All MR images were interpreted by an experienced pediatric neuroradiologist to verify that the findings were normal. Qualitative isointense signal intensity in NAWM was reported in all of the 6 studied patients by using T1-weighted, fluid-attenuated inversion recovery (FLAIR), and T2-weighted imaging.

**MR Imaging Scanning Protocol and Image Analysis**

MR imaging studies were performed on a 1.5T Signa Excite (GE Healthcare, Milwaukee, Wis) and an 8-channel head coil. The clinical protocol included the following conventional sequences: 1) volumetric imaging performed using a 3D T1-weighted spoiled gradient-recalled sequence to cover the entire brain (voxel = 0.85 × 0.85 × 1.5 mm³), 2) coronal FLAIR images and a high-resolution morphologic axial T2-weighted fast spin-echo series (voxel = 1.75 × 1.0 × 1.0 mm³), and 3) a fast gradient-echo series to acquire T2* images in the coronal plane (voxel = 0.77 × 1.0 × 4.0 mm³). A contrast-enhanced gadolinium-diethylene-triaminepentaacetic acid axial T1-weighted series was performed only on patients with TSC.

Finally, diffusion-weighted dual spin-echo single-shot echo-planar MR imaging was performed by using the following parameters: TR/TE = 13 000/87 ms; image matrix, 128 × 128; FOV = 240 mm; 40 sections of 3-mm thickness covering the whole brain. The DTI sequence consisted initially of an image volume with no diffusion weighting (b = 0 [s/mm²]) followed by the acquisition of image volumes in 6 gradients in noncollinear directions with a b value of 1000 [s/mm²]. For each b value and gradient direction, 6 images were acquired, and magnitude averaging was used to reduce artifacts from subject motion. The overall acquisition time for the entire examination was less than 45 minutes.

Sedation was used only for patients with TSC if necessary, and no control subjects were sedated, in compliance with IRB procedure. The sedation protocol for children younger than 8 years of age included pentobarbital sodium (Nembutal, 3 mg/kg) followed by fentanyl (1 mg/kg) when needed. Older children were sedated with midazolam (Versed) (0.2 mg/kg) followed by fentanyl (1 mg/kg). Additional doses were administered at 5-minute increments, and all patients were monitored by pulse oximetry/end-tidal CO₂.

In this study, the 3 eigenvalues of the diffusion tensor were measured to provide a more direct assessment of the directional diffusion changes associated with WM. After identifying the structures listed in the following paragraph, we selected areas of interest over at least 3 sections that were averaged to obtain mean and SD values of all eigenvalues.

**Brain Regions Analyzed**

Five NAWM brain structures were selected for analysis: the genu of corpus callosum (GCC), the splenium of corpus callosum (SCC), the anterior limb of internal capsule (PLIC), the posterior limb of internal capsule (PLIC), and the external capsule (EC), delineated in Figs 2 and 3. DTI indices were measured separately for the GCC and SCC because previous studies reported a significantly higher anisotropy of the SCC compared with the GCC. The regions of interest (ROIs) of these structures were manually drawn on directionally color-encoded maps. Manually selected ROIs were carefully drawn at the edge of each structure to exclude pixels affected by TSC lesions (including NAWM), thus minimizing partial volume effects. Subsequently, these ROIs were copied and pasted on the 3 axial views of major (e₁), middle (e₂), and minor (e₃) eigenvector maps by using DTI Task Card software (Magnetic Resonance Center of Massachusetts General Hospital, Boston, Mass; under Syngo VB10I, Siemens Medical Solutions, Erlangen, Germany). The same procedure was performed by using the DTIStudio software package (H. Jiang and S. Mori; Department of Radiology, Johns Hopkins University, Baltimore, Md), and we found good precision between the 2 methods. The second measurement differed from the first one by a minimum of −(−2%) in the minor eigenvector of the corpus callosum and a maximum of −(−4%) in the minor eigenvector of the EC.
Statistical Analysis

The absolute mean percentage differences for the 2 repeated measurements of the DTI indices of the NAWM were assessed with the following relationship:

\[ \frac{2 \times (\text{Measure}_1 - \text{Measure}_2)}{(\text{Measure}_1 + \text{Measure}_2)} \times \% \]

To reduce the number of outcomes in the analyses, we initially assessed separately differences in \( \lambda_1 \), \( \lambda_2 \), and \( \lambda_3 \) and calculated the average radial diffusions \( \lambda_{2.3} = (\lambda_2 + \lambda_3)/2 \), the ADC, and the FA between the left and right hemispheres for the PLIC, ALIC, and EC regions. Separate 2 \( \times \) (2 \( \times \) 3) [ie, groups \( \times \) (sides \( \times \) regions)] mixed-design multivariate analyses of covariance were performed for each of the outcomes. For these analyses, the between-subjects factor had 2 levels (normal versus patient), and the 2 within-subjects factors were side with 2 levels (left versus right) and region with 3 levels (PLIC, ALIC, and EC). If the effect of side was not significant at \( P < .20 \), the left and right sides were combined, resulting in a single score for the \( \lambda_1, \lambda_{2.3}, \text{ADC}, \) and FA outcomes for each of the 3 regions. For these and all other analyses, the age of the subject was included as a covariate because previous studies have reported age-related increases in FA and decreases in ADC.

Results

For each of the 5 structures in the 2 groups, we performed a simple Pearson 2-tailed correlation test to compare middle
This means that overall the patient group had higher diffusivity in NAWM of patients with TSC is less restricted in the direction of the axons.

For each of the DTI indices, there was a significant effect across the regions between the TSC group and the normal control group, \([F(1,15) = 14.42, P < .001]\). In summary, these data show that though the average diffusion (measured by ADC) in patients with TSC was higher than that of normal controls in all selected NAWM structures, the greatest increase in diffusivity was in the directions perpendicular to the direction of the axons.

### Discussion

Measurements of diffusion and anisotropy make it possible to detect detailed structural characteristics of brain WM under pathologic conditions and, in the case of TSC, help in detecting microstructural abnormalities beyond the obvious lesions seen on conventional MR imaging sequences. In this study, DTI performed in a group of children diagnosed with TSC demonstrated significant changes in ADC in GCC, SCC, PLIC, ALIC, and EC when compared with a group of age-matched normal subjects. These WM tracts were not directly associated

### Table 1: DTI parameter values for NAWM in patients with TSC and the normal control group (NC)

| Region   | GCC | SCC | L-EC | R-EC |
|----------|-----|-----|------|------|
|          | Mean | SD  | Mean | SD  | Mean | SD  | Mean | SD  |
| \(\lambda_1\) | TSC | 1490 | 96  | 1437 | 171 | 1011 | 95  | 1007 | 80  |
|           | NC  | 1344 | 149 | 1360 | 134 | 963  | 104 | 919  | 81  |
| \(\lambda_2\) | TSC | 543  | 125 | 526  | 157 | 653  | 118 | 645  | 111 |
|           | NC  | 414  | 77  | 375  | 91  | 581  | 121 | 598  | 125 |
| \(\lambda_3\) | TSC | 366  | 98  | 395  | 98  | 452  | 45  | 474  | 59  |
|           | NC  | 275  | 78  | 270  | 104 | 373  | 60  | 373  | 60  |
| ADC      | TSC | 800  | 91  | 783  | 126 | 705  | 69  | 709  | 65  |
|           | NC  | 678  | 90  | 688  | 107 | 639  | 67  | 627  | 66  |
| FA       | TSC | 0.643 | 0.047 | 0.627 | 0.101 | 0.391 | 0.011 | 0.366 | 0.021 |
|           | NC  | 0.703 | 0.051 | 0.725 | 0.068 | 0.437 | 0.036 | 0.413 | 0.035 |

**Note:** DTI indicates diffusion tensor imaging; NAWM, normal-appearing white matter; TSC, tuberous sclerosis complex; GCC, genu of corpus callosum; SCC, splenium of corpus callosum; L-EC, left external capsule; R-EC, right external capsule; ADC, apparent diffusion coefficient; FA, fractional anisotropy; L-PLIC, left posterior limb of internal capsule; R-PLIC, right posterior limb of internal capsule.

**Table 2:** The 3 eigenvalues, \(\lambda_1\), \(\lambda_2\), and \(\lambda_3\) and ADC are expressed in \(10^{-6} \text{ mm}^2/\text{s}\).

The FA, the relative anisotropy, and the anisotropy index are dimensionless.

**Table 3:** The 3 eigenvalues separately on each side (Table). We found that \(\lambda_2\) and \(\lambda_3\) were highly correlated; therefore, the averaged values for \(\lambda_2\) and \(\lambda_3\) were used for the analyses. The major eigenvector \(\lambda_1\) can then be referred to as parallel diffusion (ie, along the axon direction).

For each of the DTI indices, there was a significant effect across the regions between the TSC group and the normal controls group: \([F(1,15) = 6.53, P = .022]\); \([F(1,15) = 10.90, P = .005]\); \([F(1,15) = 12.40, P = .003]\); \([F(1,15) = 5.75, P = .03]\) \([F(1,15) = 14.42, P < .001]\). These results indicate that the difference between \(\lambda_1\) and \(\lambda_2,3\) was related to group membership, partial \(\eta^2 = 0.34 \) with 95% confidence limits from 0.02 to 0.59. The larger significant difference between \(\lambda_1\) and \(\lambda_2,3\) was observed in the normal group. In each of the regions, \(\lambda_1\) was greater than \(\lambda_2,3\), and the amount of difference varied across the regions, multivariate \([F(4,12) = 14.42, P < .001]\). In summary, these data show that though the average diffusion (measured by ADC) in patients with TSC was higher than that of normal controls in all selected NAWM structures, the greatest increase in diffusivity was in the directions perpendicular to the direction of the axons.

For the analyses of the difference score between \(\lambda_1\) and \(\lambda_2,3\), there was a significant difference between the patient and normal groups, \([F(1,15) = 7.75, P = .014]\), and the group by region interaction was not a significant multivariate \([F(4,12) = 1.35, P = .308]\). These results indicate that the difference between \(\lambda_1\) and \(\lambda_2,3\) was related to group membership, partial \(\eta^2 = 0.34 \) with 95% confidence limits from 0.02 to 0.59. The larger significant difference between \(\lambda_1\) and \(\lambda_2,3\) was observed in the normal group. In each of the regions, \(\lambda_1\) was greater than \(\lambda_2,3\), and the amount of difference varied across the regions, multivariate \([F(4,12) = 14.42, P < .001]\). In summary, these data show that though the average diffusion (measured by ADC) in patients with TSC was higher than that of normal controls in all selected NAWM structures, the greatest increase in diffusivity was in the directions perpendicular to the direction of the axons.
with the structural lesions seen in TSC as demonstrable on conventional structural MR imaging sequences such as 3D T1-weighted or T2-FLAIR. Our results are consistent with those reported by Garaci et al.\textsuperscript{15} and Peng et al.,\textsuperscript{16} that additional abnormalities can be disclosed in patients with TSC by using DTI. However, Karadag et al.\textsuperscript{27} found no significant differences in FA or ADC in the NAWM of patients with TSC compared with that of controls. The findings of this group suggested that their study may have lacked sensitivity due to the large age range of the patients (2–20 years). Our study design and results differ from those of Karadag et al.\textsuperscript{27} because the ages of our patients (6–15 years) were matched with those of controls (7–17 years) and our statistical analyses controlled for age.

Diffusion tensor MR imaging offers increased sensitivity over conventional MR imaging in assessing microstructural malformation or damage in brain parenchyma,\textsuperscript{28,29} traumatic brain injury,\textsuperscript{30} and epilepsy.\textsuperscript{31–34} FA reflects the degree of alignment of cellular structures within the fiber tracts and their structural integrity. Because glial proliferation results in structurally disorganized tissue, gliosis results in decreased FA and increased ADC.

Factors that may increase the diffusivity in WM include the following: 1) wider packing of axons, 2) more permeable myelin sheaths, 3) more obliquely oriented axons, 4) altered radius of individual axons, 5) increased extracellular space, 6) the presence (or absence) of elements other than myelin sheaths within these structures that alter water diffusion, and 7) hypomyelination. However, because the degree of biologic membrane permeability is small, the main contribution of the diffusion coefficient comes from diffusion pathways that move around the cells rather than from those that cross cell membranes.\textsuperscript{14,15}

One possible mechanism for regional differences of ADC and FA is the myelination process, in agreement with previous studies in infancy and early childhood.\textsuperscript{35–38} Barkovich et al.\textsuperscript{39} proposed that water loss induced by the development of the hydrophobic inner layer of the myelin sheath contributes to decreases in ADC and changes in the anisotropy of diffusion. Thickening axonal diameter is another cause of decrease in water diffusion and increase of its anisotropy.\textsuperscript{30} Reduction in water content and increase of cohesiveness and compactness of the fiber tracts\textsuperscript{41} and a reduction in extra-axonal space\textsuperscript{42} are additional components of brain maturation affecting DTI parameters. ADC and FA can also be affected by changes in the organization of nerve fibers.\textsuperscript{43}

The present study provides some new observations. For example, it was evident that the increase in diffusivity was mainly due to a significant increase in directions perpendicular to the axons (as measured by the middle eigenvalues, $\lambda_2$, and minor eigenvalues, $\lambda_3$), and less so along the parallel direction of the axons (major eigenvalues, $\lambda_1$). This suggests that the changes in mean diffusivity are not merely explained by changes in brain water content and more likely reflect microstructural changes, causing increased water diffusion perpendicular to the direction of the axonal fibers.\textsuperscript{36–39}

Our findings of increased water diffusion in both perpendicular and parallel directions in NAWM of patients with TSC probably reflect the presence of disordered myelin sheaths and gliosis, suggestive of a relatively high extracellular water moi-
creased extracellular space. As a result, there may be less restriction of the diffusion process, especially perpendicular to the axons, suggestive of microstructural abnormalities such as myelinization defects. The increase in ADC in supratentorial NAWM of patients with TSC may also be related to astrogial proliferation within the WM of patients with TSC, similar to that observed in animal models of TSC.

References

1. Fryer AE, Chalmers A, Connor JM, et al. Evidence that the gene for tuberous sclerosis is on chromosome 9. Lancet 1987;1:659–61
2. Kandti RS, Haines JL, Smith M, et al. Linkage of an important gene locus for tuberous sclerosis to a chromosome 16 marker for polycystic kidney disease. Nat Genet 1992;2:37–41
3. Hunt A. Development, behaviour and seizures in 300 cases of tuberous sclerosis. J Intell Disabil Res 1993;37(pt 1):41–51
4. Hunt A, Shepherd C. A prevalence study of autism in tuberous sclerosis. J Autism Dev Disord 1993;23:323–39
5. Gillberg IC, Gillberg C, Ahlsen G. Autistic behaviour and attention deficits in tuberous sclerosis: a population-based study. Dev Med Child Neurol 1994;36:50–56
6. Curatolo P, Verdecella M, Bombardieri R. Tuberous sclerosis complex: a review of neurological aspects. Eur J Paediatr Neurol 2002;6:15–23
7. Ferrer I, Fabregues I, Coll J, et al. Tuberous sclerosis: a Golgi study of cortical astrocytes. J Neuropathol Exp Neurol 1984;43:87–51
8. Huttonlocher PR, Heydemann PT. Fine structure of cortical tubers in tuberous sclerosis: a Golgi study. Ann Neurol 1984;16:595–602
9. Machado-Salas JP. Abnormal dendritic patterns and aberrant spine development of Bournville's disease: a Golgi survey. Clin Neuropathol 1984;3:52–58
10. Kingsley DPE, Kendall BE, Fitz CR. Tuberous sclerosis: a clinicoradiological evaluation of 110 cases with reference of atypical presentation. Neuoradology 1980;28:38–46
11. Boesel CP, Paulson GW, Kosnik EL, et al. Brain hamartomas and tumors associated with tuberous sclerosis. Neurosurgery 2003;4:410–17
12. Braffman BH, Bilanidou LT, Naidich TP, et al. MR imaging of tuberous sclerosis: pathogenesis of this phakomatosis, use of gadopentetate dimeglumine, and literature review. Radiology 1992;185:227–38
13. Curatolo P, Casma R, Cortesi F, et al. Neuropsychiatric aspects of tuberous sclerosis. Ann N Y Acad Sci 1991;615:16–18
14. Griffiths PD, Bolton P, Verity C. White matter abnormalities in tuberous sclerosis complex. Acta Radiol 1998;39:482–86
15. Garaci FG, Floris R, Bozzao A, et al. Increased brain apparent diffusion coefficient in tuberous sclerosis. Radiology 2004;232:461–65. Erratum 2004 Jun 23
16. Peng SS, Lee WT, Yang YH, et al. Cerebral diffusion tensor images in children with tuberous sclerosis: a preliminary report. Pediatr Radiol 2004;34:387–92
17. Le Bihan D, Breton E, Lallemand D, et al. Diffusion and perfusion imaging in children and adolescents: reproducibility, hemispheric, and age-related differences. Neuroimage 2007;15:273–83. Epub 2006 Nov 26
18. Karasag D, Menteel HJ, Gulmar D, et al. Diffusion tensor imaging in children and adolescents with tuberous sclerosis. Pediatr Radiol 2005;35:980–83
19. Le Bihan D, Marguin JF, Poupon C, et al. Diffusion tensor imaging concepts and applications. J Magn Reson Imaging 2001;13:534–46
20. Aalop DC, Connelly A, Duncan JS, et al. Diffusion and perfusion MRI in epilepsy. Epilepsia 2002;43(suppl):69–77
21. Werring DJ, Clark CA, Barker GI, et al. The structural and functional mechanisms of motor recovery: complementary use of diffusion tensor and functional magnetic resonance imaging in a traumatic injury of the internal capsule. J Neurol Neurosurg Psychiatry 1998;65:863–69
22. Eriksson SH, Rugg-Gunn FJ, Symms MR, et al. Diffusion tensor imaging in patients with epilepsy and malformations of cortical development. Brain 2001;124:617–26
23. Rugg-Gunn FJ, Eriksson SH, Symms MR, et al. Diffusion tensor imaging of cryptogenic and acquired partial epilepsies. Brain 2003;126:273–37
24. Rugg-Gunn FJ, Eriksson SH, Symms MR, et al. Diffusion tensor imaging in refractory epilepsy. Lancet 2002;359:1748–51
25. Arfanakis K, Hermann BP, Rogers BP, et al. Diffusion tensor MRI in temporal lobe epilepsy. Magn Reson Imaging 2002;20:511–19
26. Brody BA, Kinney HC, Klamon AS, et al. Sequence of central nervous system myelination in human infancy. I. An autopsy study of myelination. J Neuropathol Exp Neurol 1986;45:283–301
27. Kinney HC, Brody BA, Klamon AS, et al. Sequence of central nervous system myelination in human infancy. II. Patterns of myelination in autopsied infants. J Neuropathol Exp Neurol 1988;47:217–34
28. Partridge SC, Mahjory P, Henry RG, et al. Diffusion tensor imaging: serial quantification of white matter tract maturity in premature newborns. Neuroimage 2004;22:1302–14
29. Hermoye L, Saint-Martin C, Connard G, et al. Pediatric diffusion tensor imaging: normal database and observation of the white matter maturation in early childhood. Neuroimage 2006;29:893–504
30. Barkovich AJ, Kos BO, Jackson DE, et al. Normal maturation of the neonatal and infant brain: MR imaging at 1.5 T. Radiology 1988;166:173–80
31. Sakuma H, Nomura Y, Takeda K, et al. Adult and neonatal human brain: diffusion anisotropy and myelination with diffusion-weighted MR imaging. Radiology 1991;180:229–33
32. McGraw P, Liang L, Provenzale JM. Evaluation of normal age-related changes in anisotropy during infancy and childhood as shown by diffusion tensor imaging. AJR Am J Roentgenol 2002;179:1515–22
33. Beaulieu C, Allen PS. Determinants of anisotropic water diffusion in nerves. Magn Reson Med 1994;31:394–400
34. Alexander AL, Hasan KM, Lazar M, et al. Analysis of partial volume effects in diffusion-tensor MRI. Magn Reson Med 2001;45:770–80
35. Jansen FE, Braun KP, van Nieuwenhuijzen O, et al. Diffusion-weighted magnetometric imaging and identification of the epileptogenic tuber in patients with tuberous sclerosis. Arch Neurol 2003;60:1580–84
36. Uhlmann EJ, Apicelli A, Baldwin RL, et al. Astrocyte-specific TSC1 conditional knockout mice exhibit abnormal neuronal organization and seizures. Ann Neurol 2002;53:285–93
37. Apicelli AJ, Uhlmann EJ, Baldwin RL, et al. Role of the Rap1 GTPase in astrocyte growth regulation. Glia 2003;42:225–34
38. Uhlmann EJ, Li W, Sheidzendek BM, et al. Loss of tuberous sclerosis complex 1 (TSC1) expression results in increased Rho/S6K pathway signaling important for astrocyte cell size regulation. Glia 2004;47:180–88
39. Uhlmann EJ, Apicelli A, Baldwin R, et al. Heterozygosity for the tuberous sclerosis complex (TSC) gene products results in increased numbers and decreased p27Kip1 expression in TSC2 4/4−/− cells. Oncogene 2002;21:4050–59
40. Bender BL, Yunis EI. Central nervous system pathology of tuberous sclerosis in children. Ultrastruct Pathol 1980;1:287–99
41. Tromley BK, Mirra SS. Ultrastructure of tuberous sclerosis: cortical tuber and subependymal tumor. Ann Neurol 1981;9:174–81

Diffusion tensor imaging of cerebral white matter from neonatal period to adolescence. Neuroradiology 2004;46:258–66

25. Snook L, Paulson LA, Roy D, et al. Diffusion tensor imaging of neurodevelopment in children and young adults. Neuroimage 2005;26:1164–73

26. Bonekamp D, Nagar LM, Degaonkar M, et al. Diffusion tensor imaging in children and adolescents: reproducibility, hemispheric, and age-related differences. Neuroimage 2007;15:34:733–8. Epub 2006 Nov 26

References