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Original Article

Longitudinal Changes in Diffusion Properties in White Matter Pathways of Children With Tuberous Sclerosis Complex



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ABSTRACT

BACKGROUND: Abnormal white matter development in patients with tuberous sclerosis complex, a multisystem hamartomatous disorder caused by aberrant neural proliferation and axonal maturation, may be associated with poorer neurocognitive outcomes. The purpose of this study is to identify predictors of longitudinal changes in diffusion properties of white matter tracts in patients with tuberous sclerosis complex. **METHODS:** Diffusion magnetic resonance imaging was carried out in 17 subjects with tuberous sclerosis complex (mean age, 7.2 ± 4.4 years) with at least two magnetic resonance imaging scans (mean number of days between scans, 419.4 ± 105.4). There were 10 males; 5 of 17 had autism spectrum disorder and 10 of 17 had epilepsy. Regions of interest were placed to delineate the internal capsule/corona radiata, cingulum, and corpus callosum. The outcomes were mean change in apparent diffusion coefficient and fractional anisotropy. Data were analyzed using Pearson's correlation and multiple linear regression analyses. **RESULTS:** Gender was a significant predictor of mean change in apparent diffusion coefficient in the left internal capsule, right and left cingulum bundles, and corpus callosum and a significant predictor of mean change in fractional anisotropy in the corpus callosum. Epilepsy was a significant predictor of mean change in apparent diffusion coefficient in the left internal capsule. Autism spectrum disorder was not predictive of diffusion changes in any of the studied pathways. **CONCLUSION:** Clinical variables, including gender and epilepsy, have an effect on the development of white matter pathways. These variables should be taken into consideration when counseling tuberous sclerosis complex patients and in future imaging studies in this population.

Keywords: tuberous sclerosis complex, white matter, development, human, diffusion imaging, tractography, epilepsy, gender
Pediatr Neurol 2015; 52: 615–623

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Article History:

Received November 30, 2014; Accepted in final form February 4, 2015

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Introduction

Tuberous sclerosis complex (TSC) is a multisystem hamartomatous disorder that affects 1 in 6000 people. Most cases of TSC are linked to mutations in the *TSC1* and *TSC2* genes. The translated protein products from these genes help regulate cell growth and proliferation by inhibiting the mammalian target of the rapamycin cascade. In the developing brain, this aberrant proliferation leads to the

formation of cortical tubers, subependymal nodules, and subependymal giant cell astrocytomas. Cells in the central nervous system express TSC1/2 proteins not only during early development, but also throughout adulthood. These proteins are thought to help regulate functions such as axon guidance and myelination, dendritic arborization, and synaptic formation and function.^{1,2}

Despite a growing understanding of the molecular changes occurring in TSC, correlating cellular changes with clinical phenotype remains challenging. This challenge is due in part to the wide spectrum of neurological outcomes manifested by TSC patients. For example, 80%–90% of the TSC patients develop epilepsy,^{3,4} 50%–60% show some degree of cognitive limitation,^{3,5,6} and approximately 15%–50% develop autism spectrum disorder (ASD).^{6–8} However, to date, no consistent relationship between tuber location, burden, or genotype with cognitive outcomes has been reported.^{9,10}

Efforts have thus turned to diffusion tensor imaging to assess for any imaging correlates in subjects with TSC. Diffusion tensor imaging measures the diffusion of water molecules in the brain tissue. The magnitude of diffusion (apparent diffusion coefficient [ADC]) and the directionality of water movement (fractional anisotropy [FA]) provide information about tissue microstructure, including the degree of myelination and axonal membranes.¹¹ Interestingly, diffusion tensor imaging studies show differences in diffusion properties not only in tubers^{12,13} but also in normal appearing white matter in TSC patients when compared with control subjects.^{14–17} Abnormal diffusion characteristics in normal-appearing white matter in TSC subjects have been speculated to arise from diffuse abnormal neuronal and axonal organization and hypomyelination.¹⁸ Alternatively, a recent study proposed that radial migration streams that represent discrete but multifocal pathology may also cause the diffusion changes.¹⁹

Recent imaging studies have examined correlations between diffusion tensor imaging parameters of normal-appearing white matter and neurocognitive outcomes in TSC. Although region of interest analysis looking at ADC and FA in normal-appearing white matter in TSC subjects^{12,20} has not demonstrated an association between diffusion properties with neurological outcomes, tractography studies analyzing complete white matter tracts, such as the corpus callosum¹⁸ and arcuate fasciculus,²¹ report significant differences not only in ADC and FA between TSC subjects and controls but also between TSC subjects with and without comorbid ASD. These findings suggest that abnormal white matter tract development in TSC patients may be associated with poorer neurocognitive outcomes. However, it remains unknown which white matter tracts are affected and how these affected tracts evolve over time.

To better understand the development of this disease process as it relates to white matter microstructural integrity, a subset of TSC patients who were serially scanned was identified and the change in ADC and FA of selected white matter tracts between scans (mean change) was measured. This outcome represents the magnitude of change of the diffusion parameter during the developmental period and provides insight about the neurological evolution of the disease process. Because prior studies suggest that neurocognitive outcomes are correlated with white matter microstructure, TSC subjects were categorized by

neurocognitive outcomes, such as epilepsy and ASD. First, diffusion parameters were correlated with age to evaluate trends and changes in ADC and FA. Second, using multiple linear regression analyses, predictors of the magnitude of longitudinal change of the diffusion properties among the internal capsule/corona radiata, cingulum bundle, and corpus callosum were identified using demographic characteristics and neurocognitive outcomes as variables. These tracts were selected as sample tracts from major commissural, projection, and association pathways. These tracts have been associated with changes in diffusion or anisotropic properties in children with epilepsy^{22–24} or autism spectrum disorder^{18,25–27} and were thus selected for the study.

Materials and Methods

Participants

The source population included patients who fulfilled the clinical criteria for TSC set forth by the Tuberous Sclerosis Consensus Conference^{28,29} and participated in the Boston Children's Hospital Multidisciplinary Tuberous Sclerosis Program. Eligible participants from this population were retrospectively identified by the following inclusion/exclusion criteria: greater than 1 year of age, two brain magnetic resonance imaging (MRI) scans obtained at least 300 days apart, no neurosurgical interventions, not enrolled in everolimus trials, no hydrocephalus on imaging, and adequate image quality without motion degradation. The Institutional Review Board at Boston Children's Hospital deemed this an exempt project because the research involved existing data with no risk to patient confidentiality.

Thirty-six subjects with two MRI scans were identified from the Tuberous Sclerosis Complex database. Nineteen subjects did not meet the eligibility criteria and were excluded, leaving 17 subjects with TSC ($n = 9$, excluded if <1 year old, prior neurosurgical intervention, or enrollment in therapeutic drug [everolimus] trial; $n = 10$, excluded because of poor quality of the MRI scans secondary to motion artifact). The mean age was 7.2 ± 4.4 years (range: 2.0–17.5 years); 59% ($n = 10$) were male and 41% ($n = 7$) were female. The mean number of days that lapsed between the two MRI scans was 419 ± 105 (range: 309–741 days). Five subjects had ASD and 10 had epilepsy.

Imaging

High-angular resolution diffusion imaging (HARDI) is a technique that enables identification of complex crossing tissue coherence in mature³⁰ and immature brains with less myelin.^{31–35} T1-weighted magnetization-prepared rapid-acquisition gradient-echo, T2-weighted turbo spin-echo, and a three-dimensional diffusion-weighted spin-echo echo-planar imaging were performed. Thirty diffusion-weighted measurements ($b = 1,000$ seconds/mm²) and five non-diffusion-weighted measurements ($b = 0$ seconds/mm²) were acquired from a 3T Siemens MR system with repetition time = 10 seconds; echo time = 88 milliseconds; $\delta = 12.0$ milliseconds; $\Delta = 24.2$ milliseconds; field of view = 22 cm; slice thickness = 2.0 mm; matrix size = 128 × 128; integrated parallel acquisition techniques = 2.

Diffusion data reconstruction for tractography

Diffusion Toolkit and TrackVis (<http://trackvis.org>) were used to reconstruct and visualize HARDI tractography pathways. HARDI detects multiple local maxima on an orientation distribution function. Using all the orientation distribution function local maxima to produce HARDI tractography pathways permitted identification of crossing pathways within a voxel. Trajectories were propagated by consistently pursuing the orientation vector of least curvature. Tracking was terminated when the angle between two consecutive orientation vectors was greater than the given threshold (45°).³⁶

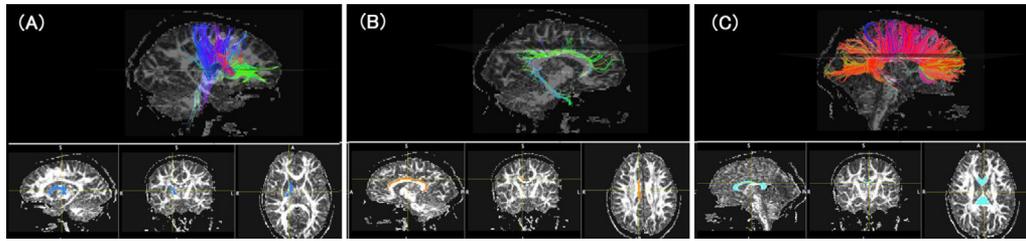


FIGURE 1.

Examples of tractography pathway identification. The left and right internal capsules (A), the left and right cingulum bundles (B), and the corpus callosum (C) were identified using atlas-based region of interest (ROI) approach (examples of ROIs are shown in the lower row). The color-coding of tractography pathways is based on a standard red-green-blue code applied to the vector between the end-points of each fiber (red: left-right, green: anteroposterior, blue: dorsal-ventral).

Tract delineation

A coordinate-based tractography atlas³⁷ was used to guide region of interest placement to delineate white matter pathways on MRI datasets for each subject at the first scan (scan 1) (Fig 1). We used T1, T2, and diffusion images for region of interest placements. Regions of interest were then coregistered using FLIRT software (<http://fsl.fmrib.ox.ac.uk/fsl/fsl-4.1.9/flirt/overview.html>) to each subject's second scan. Segmentation of each white matter tract pathway for the second scan was then checked manually to ensure accuracy and adjusted as needed. Mean ADC and mean FA for each tract were calculated. The corpus callosum, cingulum bundles, and bilateral internal capsule/corona radiata tracts were segmented.

Statistical analysis

Descriptive data for continuous variables are presented as mean \pm standard error and categorical variables as percentages and counts. Outcomes include the paired change in ADC and FA for each tract. Paired changes were calculated by subtracting the diffusion measurements at the time of the second scan (scan 2) from those at the time of the first scan (scan 1). Except for change in ADC for the corpus callosum, all outcomes were normally distributed per the Shapiro-Wilk test. Visual inspection of a Q-Q plot and a stem-and-leaf plot identified a single outlier in the distribution for change in ADC of the corpus callosum. Without this outlier, the remaining data were normally distributed, and a sensitivity analysis with and without this outlier showed consistent results.

Comparison of each outcome with each categorical variable was performed with the Student *t* test. Correlations between continuous variables were assessed with Pearson's correlation coefficient (*r*). Because of the small sample size, nonparametric tests (Mann-Whitney *U* test and Spearman's correlation coefficient) were also performed to confirm the results. Only parametric results are shown because the results were consistent with the nonparametric tests.

Multiple linear regressions identified predictors of mean change in ADC or FA. Each model included the ADC or FA measure at scan 1 (baseline scan value) and one other predictor variable (age, gender, ASD, or epilepsy). Regression diagnostics, including residual plots and Q-Q plots, were used to evaluate assumptions of constant variance and normality. One influential outlier was identified; however, review of patient history and imaging was unremarkable from a clinical standpoint, and the subject was retained. Statistical significance was set at $P \leq 0.05$, and all tests were two-sided. All statistical analyses were performed with SPSS version 19.0 (IBM SPSS, Chicago, IL, USA).

Results

Mean changes in ADC and FA

Visual representations of absolute measures of FA and ADC in single subjects are shown in Figs 2–4. Figure 2 illustrates segmented white matter pathways from subjects with the lowest ADC (Fig 2A) and highest ADC (Fig 2B). Figure 3 illustrates male (Fig 3A,C) and female (Fig 3B,D) subjects, and Fig 4 illustrates subjects with (Fig 4A,C) and without epilepsy (Fig 4B,D).

Comparisons of the mean change in ADC and FA by gender, epilepsy, and ASD are shown in Table 1. A gender effect was seen in several of the white matter tract pathways. An increase in the mean change ADC was seen in males compared with a decrease in females in the left internal capsule ($3.78 \pm 6.17 \text{ mm}^2/\text{second}$ versus $-20.2 \pm 7.92 \text{ mm}^2/\text{second}$; $P = 0.03$) and left cingulum bundle ($3.65 \pm 3.21 \text{ mm}^2/\text{second}$ versus $-19.8 \pm 5.98 \text{ mm}^2/\text{second}$; $P = 0.002$). A significant difference in the mean change in FA between males and females in the corpus callosum was also seen (-0.02 ± 0.006 versus 0.003 ± 0.009 ; $P = 0.04$).

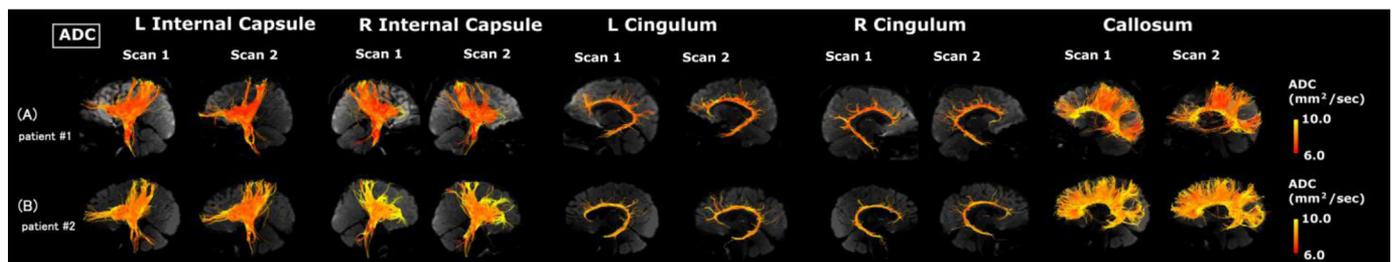


FIGURE 2.

Examples of tractography pathways from a patient having lower apparent diffusion coefficient (ADC) at scan 1 (A) and a patient having higher ADC at scan 1 (B). The color-coding of tractography pathways indicate the magnitude of ADC, as shown by the scale bar on the right. L, left; R, right.

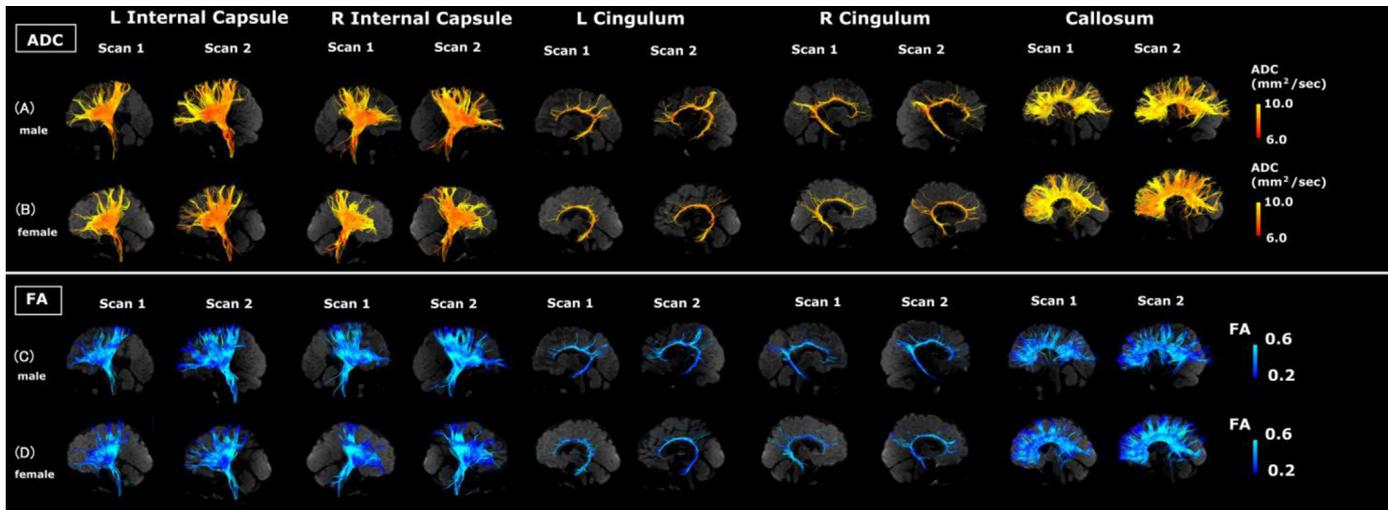


FIGURE 3.

Examples of tractography pathways showing apparent diffusion coefficient (ADC) measures in a male subject (A, C) and a female subject (B, D) at similar ages (male: 2.5 years old at scan 1 and 3.6 years old at scan 2; female: 2.0 years old at scan 1 and 3.0 years old at scan 2). Please note this figure shows representative images from subjects. The color-coding of tractography pathways indicate the magnitude of ADC, as shown by the scale bar on the right. FA, fractional anisotropy.

Correlations analyses

Table 2 reports correlations between age and ADC and FA. Age negatively correlated with ADC for both the right and left internal capsules at scan 1 (right: $r = -0.62$ [$P = 0.007$], left: $r = -0.56$ [$P = 0.02$]) and at scan 2 (right: $r = -0.52$ [$P = 0.03$], left: $r = -0.59$ [$P = 0.01$]). Age positively correlated with FA for both the right and left internal capsules at scan 1 (right: $r = 0.48$ [$P = 0.05$], left: $r = 0.52$ [$P = 0.03$]) and at scan 2 (right: $r = 0.47$ [$P = 0.06$], left: $r = 0.59$ [$P = 0.01$]). Age positively correlated with mean

change ADC at scan 2 for the right internal capsule was also seen ($r = 0.49$, $P = 0.05$).

The left cingulum bundle demonstrated significant correlations between age and ADC (scan 1: $r = -0.51$ [$P = 0.04$], scan 2: $r = -0.55$ [$P = 0.02$]) and FA (scan 1: $r = 0.53$ [$P = 0.03$], $r = 0.57$ [$P = 0.02$]). At scan 2, age positively correlated with FA in the right cingulum bundle ($r = 0.51$, $P = 0.04$).

Neither ADC nor FA correlated with age in the corpus callosum. Age positively correlated with mean change in ADC at scan 2 ($r = 0.55$, $P = 0.02$).

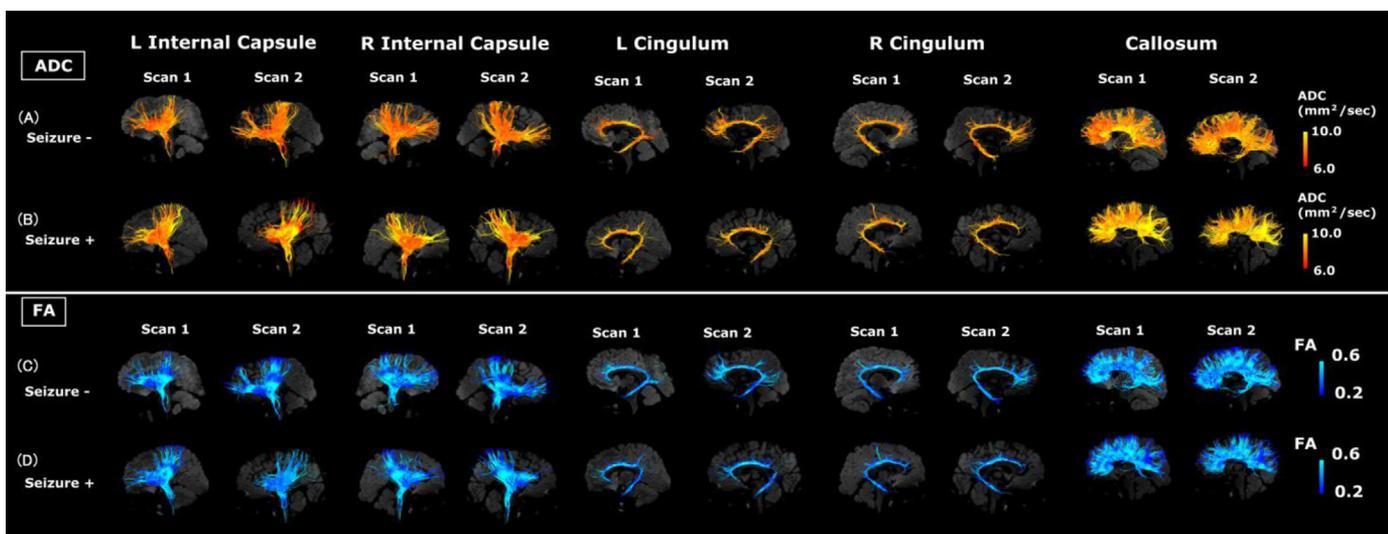


FIGURE 4.

Examples of tractography pathways showing the apparent diffusion coefficient (ADC) values in a subject without epilepsy (A, C) and a patient with epilepsy (B, D) at similar ages (without epilepsy: 4.9 years old at scan 1 and 5.9 years old at scan 2; with epilepsy: 4.5 years old at scan 1 and 5.3 years old at scan 2). This figure shows representative images from subjects. The color-coding of tractography pathways indicate the magnitude of ADC, as shown by the scale bar on the right. FA, fractional anisotropy.

TABLE 1.
Comparison of Mean Change in ADC and FA From Scan 1 to Scan 2

	Δ ADC (mm ² /second)			Δ FA		
	Mean	Standard Error	P	Mean	Standard Error	P
Right internal capsule						
Gender			0.30			0.57
Male	8.51	6.07		-0.02	0.007	
Female	-3.72	10.7		-0.01	0.01	
Epilepsy			0.51			0.75
No	8.10	6.74		-0.02	0.010	
Yes	0.24	8.54		-0.01	0.007	
ASD			0.24			0.24
No	7.88	5.81		-0.01	0.006	
Yes	-7.11	13.2		0.01	0.013	
Left internal capsule						
Gender			0.03*			0.40
Male	3.78	6.17		-0.01	0.005	
Female	-20.2	7.92		0.01	0.009	
Epilepsy			0.15			0.61
No	-15.8	4.67		-0.02	0.006	
Yes	0.76	8.42		-0.01	0.008	
ASD			0.89			0.27
No	-6.75	5.33		-0.02	0.005	
Yes	-4.45	15.2		-0.003	0.012	
Right cingulum						
Gender			0.07			0.17
Male	2.40	5.95		0.003	0.003	
Female	-17.0	8.12		0.02	0.010	
Epilepsy			0.70			0.89
No	-8.15	6.96		0.01	0.005	
Yes	-3.78	7.74		0.01	0.007	
ASD			0.94			0.18
No	-5.86	5.93		0.004	0.004	
Yes	-4.91	12.0		0.02	0.012	
Left cingulum						
Gender			0.002*			0.83
Male	3.65	3.21		0.01	0.003	
Female	-19.8	6.00		0.02	0.009	
Epilepsy			0.35			0.93
No	-10.8	3.79		0.01	0.006	
Yes	-2.61	6.51		0.01	0.006	
ASD			0.98			0.25
No	-6.08	8.94		0.01	0.004	
Yes	-5.80	17.9		0.02	0.01	
Corpus callosum						
Gender			0.21			0.04*
Male	-4.60	6.40		-0.02	0.006	
Female	-29.2	20.71		0.003	0.009	
Epilepsy			0.67			0.61
No	-9.73	7.29		-0.01	0.016	
Yes	-18.3	15.5		-0.01	0.028	
ASD			0.36			0.20
No	-5.88	5.61		-0.02	0.006	
Yes	-36.0	29.0		0.001	0.013	

Abbreviations:
ADC (mm²/second) = Apparent diffusion coefficient
FA = Fractional anisotropy
* P ≤ 0.05.

Predictors of mean change in ADC measures

For the left internal capsule, when controlling for the baseline scan ADC measure, the mean ADC change in males was 23.3 ± 8.29 mm²/second greater than in females (P = 0.01) and 22.5 ± 8.74 mm²/second greater in subjects with epilepsy than those without (P = 0.02) (Table 3).

TABLE 2.
Correlation Analyses With Age

	Age 1* (Time Point 1)		Age 2* (Time Point 2)	
	Pearson's r	P	Pearson's r	P
Left internal capsule				
ADC scan 1	-0.56 [†]	0.02 [†]	-0.59 [†]	0.01 [†]
FA scan 1	0.47	0.06	0.59 [†]	0.01 [†]
Change in ADC	0.10	0.70	0.11	0.69
Change in FA	-0.03	0.90	-0.14	0.59
Right internal capsule				
ADC scan 1	-0.62 [†]	0.007 [†]	-0.52 [†]	0.03 [†]
FA scan 1	0.48	0.05	0.52 [†]	0.03 [†]
Change in ADC	0.46	0.06	0.49	0.05 [†]
Change in FA	-0.12	0.67	-0.18	0.50
Left cingulum				
ADC scan 1	-0.51 [†]	0.04 [†]	-0.55 [†]	0.02 [†]
FA scan 1	0.53 [†]	0.03 [†]	0.57 [†]	0.02 [†]
Change in ADC	0.01	0.96	-0.06	0.82
Change in FA	-0.20	0.44	-0.30	0.27
Right cingulum				
ADC scan 1	-0.47	0.06	-0.33	0.20
FA scan 1	0.45	0.07	0.51	0.04 [†]
Change in ADC	0.42	0.09	0.40	0.11
Change in FA	-0.08	0.77	-0.10	0.70
Corpus callosum				
ADC scan 1	-0.34	0.18	-0.88	0.74
FA scan 1	0.26	0.32	0.28	0.28
Change in ADC	0.45	0.07	0.55	0.02 [†]
Change in FA	-0.13	0.62	-0.19	0.47

Abbreviations:
ADC (mm²/second) = Apparent diffusion coefficient
FA = Fractional anisotropy
* Correlation analyses are performed with age and the scan measures at the respective time point (i.e., age 1 and corresponding scan measure at time point 1; age 2 and corresponding scan measure at time point 2).
[†] P ≤ 0.05.

Relationships among gender, initial ADC scan measure, and mean ADC changes are illustrated in Fig 5.

The baseline ADC scan measure was a significant predictor of mean change in ADC for the right internal capsule when controlling for each subject characteristic (P ≤ 0.05 for gender, epilepsy, and ASD). However, adjusting for baseline ADC scan measure, none of the subject characteristics emerged as significant predictors in the right internal capsule.

For the left and right cingulum bundles, only gender emerged as a significant predictor of the mean change in ADC (Table 3), controlling for the initial ADC scan measure. The mean change in ADC for males was 23.8 ± 6.4 mm²/second greater than for females in the left cingulum (P = 0.002) and 20.1 ± 9.0 mm²/second greater in the right cingulum (P = 0.04). Neither epilepsy, ASD, nor the baseline ADC scan measure emerged as significant predictors in these models.

For the corpus callosum, because age and the baseline ADC scan measures were not significantly correlated (Table 2), an additional model including both independent variables was used to assess mean change in ADC. The baseline ADC scan measure was a significant predictor of the mean change in ADC after controlling for age (β = -0.24 ± 0.11, P = 0.05), gender (β = -0.32 ± 0.10, P = 0.005), epilepsy (β = -0.32 ± 0.12, P = 0.02), and ASD (β = -0.26 ± 0.13, P = 0.05). By contrast, when controlling for the baseline ADC scan measure, only gender emerged

TABLE 3.
Multiple Regression Analysis for Identifying Predictors of Mean Change ADC

Model	Variable	Unstandardized Coefficients B	Standard error	P
Left internal capsule				
1	ADC scan 1*	-0.43	0.17	0.03*
	Age (years)	-1.36	1.35	0.33
2	ADC scan 1*	-0.32	0.12	0.02*
	Gender	-23.3	8.29	0.01*
3	ADC scan 1*	-0.40	0.12	0.006*
	Epilepsy*	22.5	8.74	0.02*
4	ADC scan 1*	-0.40	0.15	0.02*
	ASD	13.3	11.5	0.27
Right internal capsule				
1	ADC scan 1*	-0.36	0.13	0.02*
	Age (years)	0.21	1.27	0.87
2	ADC scan 1*	-0.37	0.94	0.001†
	Gender	13.1	8.14	0.13
3	ADC scan 1*	-0.40	0.11	0.003*
	Epilepsy	6.23	9.56	0.53
4	ADC scan 1*	-0.37	0.12	0.006*
	ASD	0.55	10.69	0.96
Left cingulum				
1	ADC scan 1	-0.03	0.16	0.88
	Age (years)	-0.05	1.17	0.97
2	ADC scan 1	-0.06	0.10	0.56
	Gender*	-23.8	6.44	0.002*
3	ADC scan 1	-0.09	0.15	0.54
	Epilepsy	10.7	9.51	0.28
4	ADC scan 1	-0.20	0.14	0.87
	ASD	0.59	9.91	0.95
Right cingulum				
1	ADC scan 1	-0.13	0.13	0.36
	Age (years)	1.45	1.27	0.27
2	ADC Scan 1	-0.21	0.11	0.07
	Gender	-20.1	9.02	0.04
3	ADC scan 1	-0.23	0.13	0.08
	Epilepsy	10.2	10.6	0.35
4	ADC scan 1	-0.22	0.127	0.11
	ASD	6.34	11.64	0.60
Corpus callosum				
1	ADC scan 1*	-0.24	0.111	0.05*
	Age (years)	2.42	1.88	0.22
2	ADC scan 1*	-0.32	0.096	0.005*
	Gender	-31.8	14.64	0.05*
3	ADC scan 1*	-0.32	0.12	0.02*
	Epilepsy	12.0	18.13	0.52
4	ADC scan 1	-0.26	0.125	0.05*
	ASD	-9.32	20.48	0.66

Abbreviations:

ADC (mm²/second) = Apparent diffusion coefficient

ASD = Autism spectrum disorder

Reference: gender, 0 = male; epilepsy

0 = no epilepsy

0, ASD = no ASD.

* $P \leq 0.05$; † $P \leq 0.001$.

as a significant predictor; the mean change in males was 31.8 ± 14.6 mm²/second greater than in females ($P = 0.05$).

Predictors of mean change in fractional anisotropy measures

For the left and right internal capsules and bilateral cingulum bundles, only the baseline FA scan measure emerged as a significant predictor of the mean change in FA when controlling for gender, epilepsy, and ASD in each

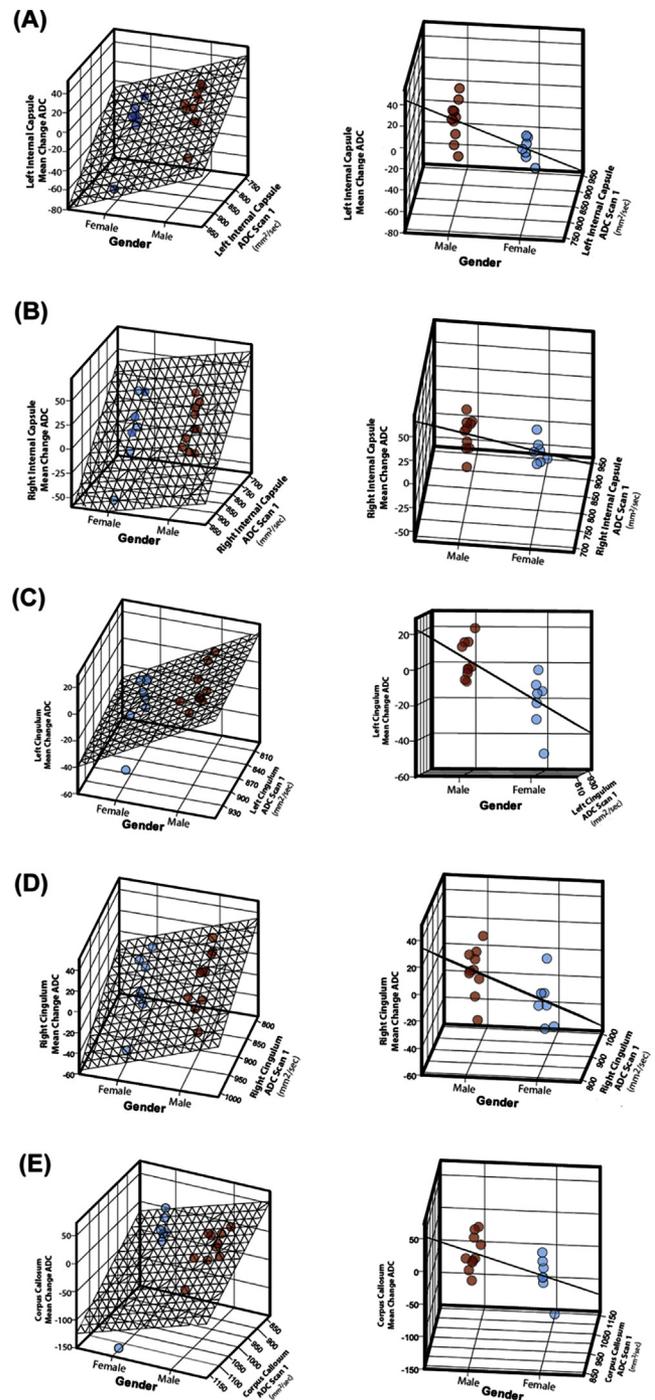


FIGURE 5.

Relationships among gender, baseline apparent diffusion coefficient (ADC) scan measure, and mean ADC changes are illustrated for the left and right internal capsule tracts (A, B), the left and right cingulum bundles (C, D), and the corpus callosum (E). The left and right columns show the same three-dimensional graphs but at different angles to illustrate the distribution (left column) and standard error (right column) of the mean ADC change data by gender. Blue dots indicate females; red dots indicate males.

respective model (Table 4). Controlling for the initial scan FA measure, gender emerged as a significant predictor of mean change in FA ($\beta = 0.02 \pm 0.007$, $P = 0.003$) in the corpus

TABLE 4.
Multiple Regression Analysis for Identifying Predictors of Mean Change FA

Model	Variable	Unstandardized Coefficients B	Standard Error	P
Left internal capsule				
1	FA scan 1*	-0.59	0.16	0.002*
	Age (years)	0.002	0.001	0.12
2	FA scan 1*	-0.44	0.14	0.006*
	Gender	0.014	0.008	0.09
3	FA scan 1*	-0.52	0.14	0.003*
	Epilepsy	-0.01	0.008	0.15
4	FA scan 1*	-0.45	0.16	0.01*
	ASD	0.005	0.009	0.62
Right internal capsule				
1	FA scan 1*	-0.59	0.18	0.005*
	Age (years)	0.001	0.001	0.30
2	FA scan 1*	-0.50	0.16	0.009*
	Gender	0.006	0.009	0.54
3	FA scan 1*	-0.51	0.17	0.009*
	Epilepsy	-0.003	0.010	0.74
4	FA scan 1*	-0.47	0.01	0.014*
	ASD	0.007	0.17	0.49
Left cingulum				
1	FA scan 1*	-0.313	0.11	0.02
	Age (years)	0.001	0.001	0.52
2	FA scan 1*	-0.27	0.10	0.01
	Gender	-0.002	0.007	0.77
3	FA scan 1*	-0.33	0.100	0.005
	Epilepsy	-0.010	0.007	0.18
4	FA scan 1*	-0.26	0.101	0.02
	ASD	0.005	0.008	0.53
Right cingulum				
1	FA scan 1*	-0.37	0.13	0.01*
	Age (years)	0.001	0.001	0.34
2	FA scan 1*	-0.31	0.11	0.01*
	Gender	0.013	0.008	0.12
3	FA scan 1*	-0.41	0.12	0.004*
	Epilepsy	-0.02	0.008	0.11
4	FA scan 1*	-0.29	0.13	0.04*
	ASD	0.005	0.01	0.61
Corpus callosum				
1	FA scan 1*	-0.46	-0.67	0.007*
	Age (years)	0.0002	0.04	0.84
2	FA scan 1†	-0.46	0.10	0.0004†
	Gender	0.02	0.007	0.003*
3	FA scan 1*	-0.50	0.13	0.002*
	Epilepsy	-0.01	0.009	0.14
4	FA scan 1*	-0.43	0.15	0.01*
	ASD	0.004	0.01	0.73

Abbreviations:
 ASD = Autism spectrum disorder
 FA = Fractional anisotropy
 Reference: gender, 0 = male; epilepsy = no epilepsy
 ASD, 0 = no ASD.

* $P \leq 0.05$; † $P \leq 0.001$.

callosum. Relationships among gender, baseline FA scan measure, and mean FA changes are illustrated in Fig 6.

Discussion

This study is the first to describe evolving changes in the diffusion properties of developing white matter tracts in TSC subjects and the first to report tractography results for the complete internal capsule and cingulum in TSC subjects. Understanding the natural evolution of diffusion changes in patients with TSC is particularly timely and relevant as

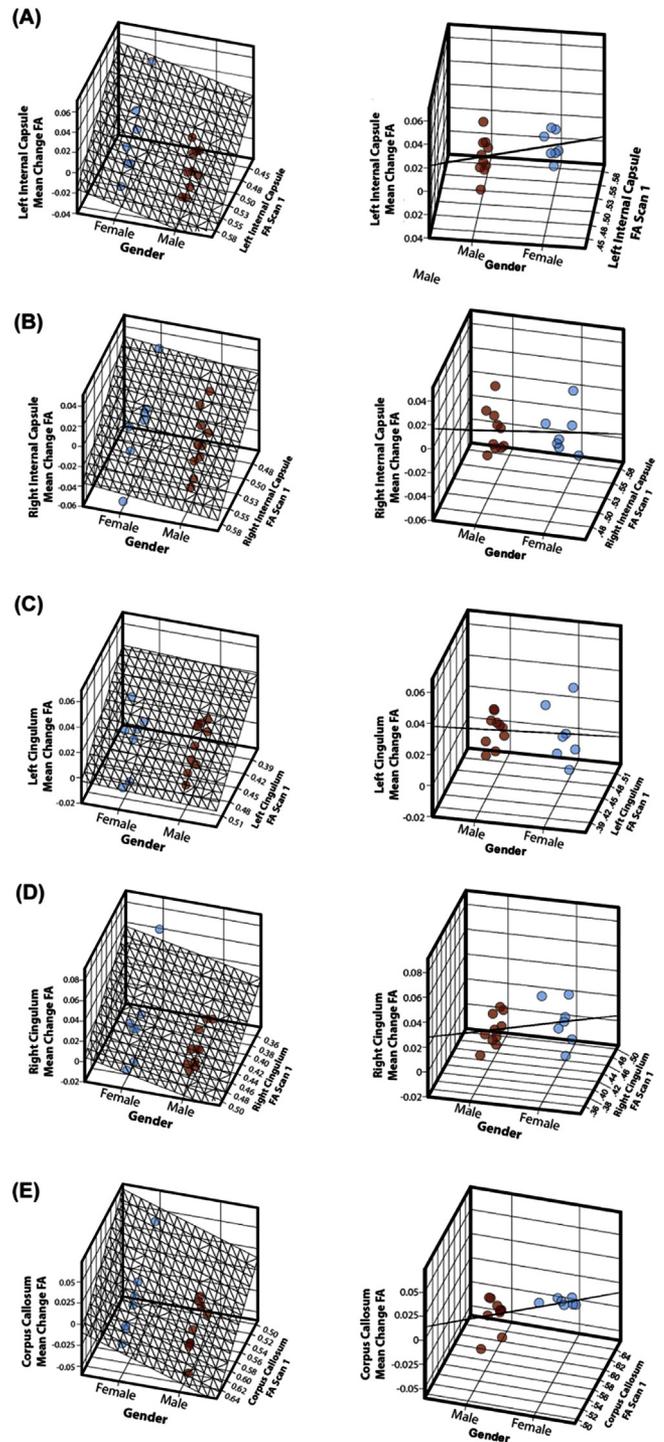


FIGURE 6. Relationships among gender, baseline fractional anisotropy (FA) scan measure, and mean FA changes are illustrated for the left and right internal capsule (A, B), the left and right cingulum bundles (C, D), and the corpus callosum (E). The left and right columns show the same three-dimensional graphs but at different angles to illustrate the distribution (left column) and standard error (right column) of the mean apparent diffusion coefficient change data by gender. Blue dots indicate females; red dots indicate males.

patients are currently undergoing therapeutic trials with the mammalian target of the rapamycin inhibitors to investigate these parameters. Tillema et al³⁸ analyzed serial MRI scans in

subjects with TSC and growing subependymal giant cell astrocytomas. They reported that during the 12- to 18-month study period, subjects who received everolimus showed an increase in FA and a decrease in radial diffusivity in the corpus callosum, internal capsule, and geniculate-calcarine regions compared with no change in diffusion parameters in subjects who did not undergo this treatment. The authors speculated that the improvement in white matter structural integrity was due to the drug effect, but did not report on other clinical variables that could have an impact—such as the severity of epilepsy or presence of autism. Moving forward, it will be important to understand what other clinical factors have an impact on white matter organization before interpreting these treatment trials.

In this study, a significant gender effect in the mean change ADC, but not mean change FA, emerged in several white matter tracts. Our results also show that the mean change ADC in males is significantly smaller, by nearly 35- to 100 fold, than in females (Table 3). Interestingly, no gender effect was seen in the mean change in FA in any of the white matter tracts. In comparison, serial MRI studies of healthy subjects have not found a difference between genders in the magnitude of change of ADC or FA over time.³⁹⁻⁴¹ These results suggest that, on a microstructural level, the development of white matter pathways of the left internal capsule, bilateral cingulum bundles, and corpus callosum is delayed or decreased in males with TSC compared with females. Only a few studies have examined gender differences in TSC in the context of neurocognitive phenotypes. Studies report a significantly increased neurological morbidity in males than females, as manifested by more cortical tubers and subependymal nodules and a higher incidence of epilepsy and intellectual disability.^{42,43} Therefore, the more significant neurological problems experienced by males with TSC are likely not only explained by macrostructural disruptions, such as tubers but also by microstructural disruptions in white matter pathways. It has been hypothesized that gender-specific manifestations of TSC may be related to the effects of modifier genes on the X-chromosome and/or the influence of sex hormones; future studies should seek to clarify this mechanism.

In this study, epilepsy emerged as a significant predictor of the left internal capsule's mean change in ADC, but epilepsy had no effect in either the mean change of ADC or FA properties of any of the white matter tracts. We expected that a history of epilepsy would be correlated with microstructural abnormalities in TSC subjects because early seizure onset is the only clinical risk factor known to be associated with poorer cognitive outcomes in children with TSC,⁴⁴⁻⁴⁷ and it was a surprise that this was only significant in the left internal capsule (Table 3). There have been several interesting but inconclusive studies documenting differences between the right and left hemispheres in subjects with TSC, with reports of poorer cognition associated independently with both left¹⁰ and right^{45,48} hemisphere tuber burden. Left hemisphere MRI findings have not been previously associated with epilepsy in this population. Because our study did not control for intelligence and early-onset epilepsy has been correlated with lower cognition in TSC in prior studies, epilepsy may be serving as a proxy for IQ in our group. Alternatively, because seizures are known to affect myelination and tissue microstructure,⁴⁹ epilepsy itself or one of the

treatments used for it may have a modifying effect on diffusion parameters in subjects with TSC. If so, the left-sided findings described previously may be explained by either more frequent left-sided seizures or by an increased susceptibility of the left hemisphere to the injury caused by seizures. Clearly, a larger study with detailed phenotyping of epilepsy in each subject would be required to answer such questions.

Unlike epilepsy, ASD was not a significant predictor of mean change ADC or FA in any of the white matter tracts investigated. Recent studies report a significant difference in the absolute diffusion parameters for subjects with TSC with and without comorbid ASD in the corpus callosum¹⁸ and arcuate fasciculus.²¹ Our results suggest that although the absolute measures may significantly differ, the magnitude of change in FA and ADC over time is not significantly different between TSC subjects with and without ASD. Given that the absolute values of these diffusion parameters are affected but their trajectory of change is not, we hypothesize that white matter in TSC subjects with ASD may suffer from a more static, early insult but thereafter continue to develop along a more normal trajectory. National cohorts investigating imaging findings in newborns and infants with TSC will help test this hypothesis (ClinicalTrials.gov NCT01780441).

A limitation of the study is the short interval (approximately 1 year) between MRI scans for these TSC subjects. As the Boston Children's Hospital Multidisciplinary TSC program expands its enrollment and follows subjects via MRI scans for extended periods, the number of subjects will increase and additional time points can be added to track evolving changes in diffusion parameters.

Conclusion

This study adds to the literature because it uses HARDI tractography to characterize diffusion properties in the corpus callosum, internal capsule, and cingulum bundles in subjects with TSC. Moreover, this study is the first to look at TSC subjects longitudinally over time to gain insight about the developmental trajectory of complete white matter tracts. The development of white matter microstructural diffusivity parameters was more affected in males than in females in the left internal capsule, right and left cingulum bundles, and corpus callosum. Additionally, diffusion parameters were more affected in TSC subjects with epilepsy in the left internal capsule than in subjects without epilepsy. This information will help with the interpretation of imaging changes seen in subjects enrolled in therapeutic trials with mammalian target of the rapamycin inhibitors.

MS was supported by NIH U01NS082320, U54 NS092090-01, P30HD018655 and BCH Translational Research Program, and ET was supported by NIH R01HD078561 and R21HD069001. Keh-Li Sheng provided technical assistance.

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