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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

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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

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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 callosum[18] 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 mature[30] 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/mm^2) and five non–diffusion-weighted measurements (b = 0 seconds/mm^2) were acquired from a 3T Siemens MR system with repetition time = 10 seconds; echo time = 88 milliseconds; ∂ = 12.0 milliseconds; Δ = 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]