Globally impaired network of anatomical connectivity in tuberous sclerosis complex patients with autism spectrum disorders

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Background. Tuberous Sclerosis Complex (TSC) is a neurocutaneous autosomal dominant disorder involving mutations of the TSC1 or TSC2 genes. It is characterized by the presence of benign tumors throughout the body, including the brain where they are known as cortical tubers. TSC symptoms may take time to develop and vary considerably from minimally affected people with normal intelligence and no seizures to severely affected people with profound retardation. Particularly, between 17% and 61% of children with TSC exhibit autism spectrum disorder (ASD) symptoms [1]. This makes TSC a unique population to study the development of autism. However, there remains an unclear association between tuber load, tuber location and neurological outcomes. Other mechanisms than the tubers are likely to contribute to the pathogenesis of autism in individuals with TSC. Particularly, differences in normal-appearing white matter [2] and miswiring of neuronal connections that are independent of the tubers may actually be the root cause of neurological symptoms in TSC.

Objective. The purpose of this study was to investigate with diffusion tensor imaging and whole brain tractography the network of anatomical connectivity in a cohort of patients with TSC, and its relationship to autism spectrum disorder.

Methods. A group of 41 patients (ages 0.5-25 years), diagnosed as 'definite TSC' as described by the Tuberous Sclerosis Consensus Conference, 12 of whom had autism spectrum disorders (ASD), and 41 healthy age-matched controls participated in this study. All subjects were imaged on a Siemens 3T Trio MRI scanner. The imaging protocol was based on routine clinical imaging including a T1-weighted MPRAGE and a T2-weighted TSE. The protocol was extended with a diffusion tensor imaging acquisition utilizing a twice-refocused spin echo sequence and the following parameters: FOV=220mm, matrix=128x128, in-plane resolution=1.7x1.7mm^2, slice-thickness=2mm, 5 b=0s/mm^2, 30 directions at b=1000s/mm^2, TE=88ms, TR=10s. The diffusion weighted images were affine-registered to the T1-weighted MPRAGE to compensate for remnant eddy currents and to correct for possible patient motion. The intracranial cavity was segmented following the structural T1-weighted and T2-weighted images [3] and the obtained brain mask applied to the diffusion weighted images. The brain grey-matter was parcellated in 114 regions with a multiple-templates fusion approach. We considered 18 templates, constructed by combining the labels of the cortical and the subcortical manual segmentations provided by the Center for Morphometric Analysis at Massachusetts General Hospital (IBSR, [4]). The templates were non-linearly aligned to each subject and the consensus label map computed with STAPLE [5] for each subject. We found this approach to be more robust in presence of tubers compared to the Freesurfer parcellation (see Fig. 1). Whole brain stochastic tractography was performed by initializing tracts in voxels with high fractional anisotropy (above 0.4). Streamlines were generated with sequential steps through the tensor field by using sub-voxel resolution steps with log-Euclidean interpolation. Inertia momentum and tensor deflection were employed to improve the results in regions of crossing fibers. Conventional stopping criteria for each streamline were checked, including streamline curvature (angle>45 degrees) and fractional anisotropy (FA<0.15). Only streamlines starting and ending in grey-matter voxels were preserved. Streamlines touching a grey-matter voxel along their path were split in multiple individual streamlines. The connectivity between each pair of regions was synthesized in a so-called connectivity matrix, which is a symmetric matrix with each element (i,j) representing a connectivity feature between the regions i and j. We considered for each element (i,j) the mean fractional anisotropy in the region-of-interest defined by the streamlines connecting i and j, characterizing microstructural white-matter properties of the pathways between each region. Here we focused on investigating *global* properties of the network of connectivity. Using weighted graph theory analysis, we assessed the overall weighted transitivity of the network, which is an established global scalar measure describing the likelihood that neighbors of nodes are interconnected in a network. The weighted transitivity measure was considered response variable in a regression model with Age and Group status. Three groups were examined: Controls, TSC patients with ASD (TSC+ASD), and TSC patients without ASD (TSC-ASD). log(Age) was chosen rather than Age based on a visual examination of the data (see Fig.2). Consequently, the effects of age and were assessed using linear regression models with an intercept, and two predictors: log(age) and group. We developed models for 3-way interactions (all three group comparisons) and 2-way interactions (group-pair comparisons, which included controls/TSC-ASD, controls/TSC+ASD, and TSC-ASD/TSC+ASD).
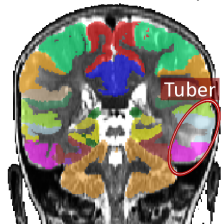

Fig. 1. Parcellation in 114 regions of a TSC patient. The parcellation was found to be robust to the presence of tubers.
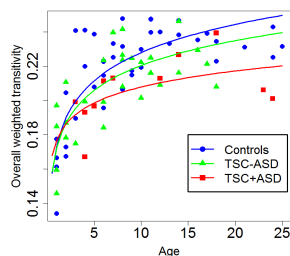

Fig.2. Graphical display of the fit linear regression model.

	3-way interaction		Controls TSC+ASD		Controls TSC-ASD		TSC+ASD TSC-ASD	
P-values:								
intercept:	3.8e-47	(***)	1.1e-32	(***)	1.5e-34	(***)	4.0e-17	(***)
log(age):	1.2e-16	(***)	4.6e-11	(***)	2.2e-15	(***)	8.7e-08	(***)
group:	1.2e-03	(**)	1.4e-03	(**)	1.4e-01		6.2e-02	(*)
Coefficients								
intercept:	0.19		0.19		0.18		0.2	
log(age):	0.021		0.022		0.022		0.019	
group:	-0.0086		-0.0094		-0.0062		-0.011	
Confidence Intervals								
log(age):	[0.01702, 0.02498]		[0.01675, 0.02717]		[0.01814, 0.0682]		[0.01349, 0.02511]	
group:	[-0.0137, -0.0035]		[-0.01495, -0.00385]		[-0.01428, 0.00208]		[-0.02302, 0.00042]	
R^2	0.60		0.60		0.62		0.53	

Table 1. Numerical results of the linear regression (**=p<0.01 ; ***=p<0.001)

Results. Based on the p-values and 95% confidence intervals (see Table 1) for estimated regression coefficients and their respective values, we found a strong positive effect between the overall weighted transitivity and log(age), as well as a weaker yet significant negative group effect (p=1.2e-3). In addition, when assessing two-way interactions, there was a significant group effect between normals and TSC+ASD subjects (p=1.4e-3), but no significant effect between Controls and TSC-ASD or TSC-ASD and TSC+ASD. Although developed models may not be optimal (based on the estimated R^2 values), the results suggest that global network connectivity measured by the overall weighted transitivity in patients with TSC+ASD is significantly different that normals, and there is a consistently significant group effect when all groups are compared.

Conclusion. This study is to our knowledge the first to investigate the network of anatomical connectivity in TSC patients and its relationship to ASD. We achieved whole brain stochastic tractography in 41 healthy controls and 41 TSC patients, 12 of whom had ASD, and built a weighted network of connectivity by considering the average fractional anisotropy along the tract streamlines connecting each pair of regions. Using graph theory analysis, we focused on globally characterizing this network. Our results suggest that the overall transitivity of the network is significantly affected in TSC patients with ASD. This is consistent with previous studies suggesting impaired connectivity in ASD [6]. Future work will focus on identifying local differences in the connectivity network of TSC patients, and investigating their relationship to autism.

References.
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