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Simulating disease propagation across white matter connectome reveals anatomical substrate for neuropathology staging in amyotrophic lateral sclerosis

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ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease, characterized by progressive loss of motor function. While the pathogenesis of ALS remains largely unknown, recent histological examinations of Brettschneider and colleagues have proposed four time-sequential stages of neuropathology in ALS based on levels of phosphorylated 43 kDa TAR DNA-binding protein (pTDP-43) aggregation. What governs dissemination of these aggregates between segregated regions of the brain is unknown. Here, we cross-reference stages of pTDP-43 pathology with in vivo diffusion weighted imaging data of 215 adult healthy control subjects, and reveal that regions involved in pTDP-43 pathology form a strongly interconnected component of the brain network ($p = 0.04$) likely serving as an anatomical infrastructure facilitating pTDP-43 spread. Furthermore, brain regions of subsequent stages of neuropathology are shown to be more closely interconnected than regions of more distant stages ($p = 0.002$). Computational simulation of disease spread from first-stage motor regions across the connections of the brain network reveals a pattern of pTDP-43 aggregation that reflects the stages of sequential involvement in neuropathology ($p = 0.02$), a pattern in favor of the hypothesis of pTDP-43 pathology to spread across the brain along axonal pathways. Our findings thus provide computational evidence of disease spread in ALS to be directed and constrained by the topology of the anatomical brain network.

Introduction

Investigations of the brain's wiring architecture are a powerful approach in the examination of the pathogenic mechanisms of neurological and neuropsychiatric disorders (Pievani et al., 2014; Rowe, 2010). In amyotrophic lateral sclerosis (ALS) – a devastating, rapidly progressive neurodegenerative motor disorder – anatomical connectivity studies have revealed clear white matter impairments, with tracts directly linking to the motor cortex being the most affected (Douaud et al., 2011; Schmidt et al., 2014; Verstraete et al., 2010, 2013). While these observations of macroscopic disease effects allow for important insights regarding the ultimate consequences on the brain, the underlying pathogenic mechanism of ALS remains largely unknown. Histological examinations have identified phosphorylated 43 kDa TAR DNA-binding protein (pTDP-43) aggregates to form a hallmark of disease pathology in sporadic ALS (Brettschneider et al., 2012, 2014; Neumann et al., 2006), potentially a key factor in the pathogenesis. Recently, Brettschneider and colleagues importantly reported a sequential distribution of pTDP-43 aggregates across multiple, anatomically widespread cortical and subcortical brain areas and defined four sequential stages of neuropathology corresponding to disease burden (Table 1) (Brettschneider et al., 2013). How these pTDP-43 aggregates spread, however, remains elusive.

Mechanisms have been proposed for spread of neuropathology including contiguous models, describing cell-to-cell transfer between neighboring cells, and non-contiguous models describing spread between distant cells either via axonal transport (trans-synaptic) or through blood or cerebrospinal fluid (non-synaptic) (Aguzzi and Rajendran, 2009; Braak et al., 2013; Brundin et al., 2010). In the neurodegenerative diseases of Creutzfeldt–Jacob, Alzheimer, Parkinson and Huntington, aggregates of misfolded proteins have been noted to trigger misfolding of the corresponding healthy protein in newly affected regions (Costanzo and Zurzolo, 2013), thus initiating a feedback loop leading to the accumulation and propagation of pathogenic aggregates in a "prion-like" fashion (Lee et al., 2010). In Alzheimer disease and frontotemporal dementia "prion-like" trans-synaptic transmission of

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Table 1
Staging of pTDP-43 implicated regions described by Brettschneider and colleagues and the mapping to the human and macaque brain as subjects for anatomical connectivity investigation.

Stage	Implicated regions	Human brain nodes	Macaque cortical nodes
I	Primary motor cortex	Precentral gyrus	B4, B6
	Supplementary motor area	Superior frontal gyrus	SMA, SEF
	Brainstem motor nuclei	Brain stem	–
	Spinal cord	–	–
II	Middle frontal gyrus	Caudal and rostral middle frontal gyri	FEF, B46, B45, B12
	Brainstem reticular formation	–	–
	Precerebellar nuclei	–	–
	Red nucleus	–	–
III	Gyrus rectus	Medial orbitofrontal gyrus	B14
	Orbital gyrus	Lateral orbitofrontal gyrus	B11, B13
	Postcentral neocortex	Postcentral gyrus	B3a[a], B3b[a]
	Striatum	Caudate, putamen, accumbens area	–
IV	Anteromedial temporal lobe	Entorhinal cortex	ER, B35
	Hippocampal formation	Hippocampus	TH

"–" indicates that the region was not covered by the parcellation scheme.
[a] 3a and 3b (postcentral gyrus) were excluded due to insufficient reports on the presence/absence of corticocortical connections to and from these regions in the macaque.

pathogens along connections of the macroscale brain network formed by white matter pathways has been simulated in a recent study (Raj et al., 2012), showing that patterns of cortical atrophy can be predicted by network diffusion models.

Concerning ALS, in vitro studies have revealed aggregates of pTDP-43 to exhibit "prion-like" behavior (Furukawa et al., 2011) and there is accumulating evidence of active pTDP-43 transport in axons of somatomotor neurons (Fallini et al., 2012). Combining post-mortem observations of Brettschneider and colleagues of pTDP-43 to aggregate in multiple cortical and subcortical regions of the brain (Brettschneider et al., 2013) with recent in vivo connectivity studies showing an expanding network of affected white matter connections with disease progression (Verstraete et al., 2013), has led to the hypothesis of misfolded pTDP-43 to spread along axonal pathways of the brain.

To computationally test this hypothesis, we combined the microscopic histological observation of the four stages of the disease by Brettschneider and colleagues with information on macroscopic wiring of the mammalian brain as derived from ultra-high resolution in vivo diffusion weighted imaging (DWI) data of the Human Connectome Project (Van Essen et al., 2013). Using in silico simulations we show evidence of sequential pTDP-43 spread to be directed by the topological structure of the anatomical white matter pathways of the human macroscale connectome. First, we show a dense level of anatomical connectivity between the regions of the four stages of the disease. Second, we show a natural ordering of anatomical connectivity within this subnetwork, an organization that follows the sequential order of pTDP-43 involvement. And third using computational modeling, we show simulated spread from primary motor regions along the anatomical pathways of the human connectome to significantly overlap with the spread of pTDP-43 aggregates as empirically observed by Brettschneider and colleagues. Mapping regions of the four stages to the macaque cortex – allowing for the inclusion of information on directed anatomical pathways as reconstructed from gold standard tract-tracing data – we verify the natural ordering of anatomical connectivity in the connectome to 'guide' or 'direct' disease spread, giving rise to the empirically observed sequential neuropathological stages. Our results illustrate the value of computational simulations in examining and testing potential disease mechanisms.

Materials and methods

Human connectome reconstruction

The human macroscale connectome – a comprehensive map describing all neural connections between large-scale brain regions – was constructed from diffusion weighted MRI of 215 adult healthy control subjects of the Human Connectome Project (HCP, release Q3) (Van Essen et al., 2013). Tissue segmentation was performed on T1 images (voxel size: 0.7 mm isotropic) using Freesurfer (Fischl et al., 2004), followed by a parcellation of the left hemisphere into 42 distinct brain regions, including 34 cortical regions, 7 subcortical structures and the brain stem (Verstraete et al., 2013). For additional cortex-only analyses, parcellation schemes of respectively 68 and 219 cortical regions- both hemispheres- were used (De Reus and van den Heuvel, 2014). Processing of HCP high-resolution DWI data (1.25 mm isotropic, TR/TE = 5520/89.50 ms, multiple b-values, 270 directions, 18 b0 volumes) (Van Essen et al., 2013) included motion, eddy current and susceptibility distortion corrections (Glasser et al., 2013). Anatomical pathways were reconstructed with generalized q-sampling imaging (GQI) and streamline tractography (De Reus and van den Heuvel, 2014), forming a connectome graph of *edges* (reflecting anatomical pathways) and *nodes* (representing brain regions). Numbers of reconstructed streamlines (NOS) were rescaled to a Gaussian distribution (Honey et al., 2009) and were taken as the connectivity strengths (edge weights) of reconstructed pathways. The group-averaged connectome map used for connectivity analyses contained edges present in at least 60% of the subjects (De Reus and van den Heuvel, 2013).

pTDP-43 stages

Investigating white matter connectivity in relation with pTDP-43 neuropathology, anatomical locations of pTDP-43 aggregation as described by Brettschneider (Brettschneider et al., 2013) were mapped to the Desikan-Killiany brain atlas (Cammoun et al., 2012) consisting of 42 distinct brain regions per hemisphere plus the brain stem as used for connectome reconstruction. Brettschneider and colleagues defined four stages of neuropathology based on sequential pTDP-43 involvement, with stage I referring to the regions implicated in ALS cases with least extensive patterns of pTDP-43 pathology: the precentral gyrus, superiorfrontal gyrus and the brain stem. Cases with higher pathological burden also showed pTDP-43 inclusions in stage II regions including the caudal middle frontal and rostral middle frontal gyri followed by stage III regions: medial and lateral orbitofrontal cortex, postcentral gyrus, caudate nucleus, putamen and nucleus accumbens. In patients with most extensive patterns of neuropathology, inclusions extended to stage IV regions including the entorhinal cortex and the hippocampus (Table 1, Fig. 1a).

Anatomical connectivity of pTDP-43 subnetwork

Strengths of connections between nodes of the pTDP-43 subnetwork were tested against connection strengths of connections linking the pTDP-43 component to the rest of the brain using two sample *t*-tests, to examine whether anatomical connectivity may contribute to confinement of spread within the pTDP-43 subnetwork. In addition, to test the significance of the internal wiring strength of the pTDP-43 subnetwork (i.e. the mean strength of connections between pTDP-43 nodes) in an *absolute* sense, instead of *relative* to connectivity strength with the rest of the brain (see above), a null distribution of internal wiring strengths was computed for 1000 random subnetworks. Each random