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Linking tuberous sclerosis complex, excessive mTOR signaling, and age-related neurodegeneration: a new association between *TSC1* mutation and frontotemporal dementia

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A 52-year-old right-handed man presented for the evaluation of progressive emotional flattening, social withdrawal, and loss of empathy. Prior to symptom onset, his wife of 21 years described him as “easy going, loving, kind, and generous.” As symptoms progressed, the subject began to alienate coworkers with unwelcome and persistent practical jokes. These disinhibited behaviors were accompanied by new, compulsive Internet usage. No language, visuospatial, memory, or motor symptoms were reported.

Past medical history included chronic headaches and multiple concussions but no seizures, psychiatric disorders, or drug use. He denied cutaneous, dental, or renal abnormalities. His childhood development was

unremarkable. He completed 13 years of formal education and worked in the upper echelons of management at a professional firm.

The subject’s family history (Supplementary Fig. S1a) was notable for a father with multiple concussions and late-life seizures, alcoholism, impulsiveness, and poor decision-making. An older sibling with left arm hemihypertrophy (Parkes-Weber Syndrome) was deceased after post-operative stroke. A younger sibling developed epilepsy at age 19 and underwent frontal lobe resection surgery at age 43; this individual experienced progressive difficulty with concentration and multitasking, qualifying for a diagnosis of mild cognitive impairment (MCI) in the executive domain.

On exam, the proband was 72 inches tall and weighed 220 lb (BMI of 29.8). Skin exam revealed unguinal fibromas and a shagreen patch. His neurological exam was normal except for an expansive mood, intrusive commentary, and jocularity verging on the inappropriate. Neuropsychological testing revealed impaired confrontational naming with mild deficits in semantic knowledge. Visual episodic memory, executive functioning, and information processing speed were also impaired. Other cognitive domains were within normal limits for age. Deficits localized to the right greater than left temporal and frontal lobes. Magnetic resonance imaging (MRI) revealed corresponding areas of atrophy and white matter lesions (Fig. 1a). Electroencephalogram (EEG) was negative for epileptiform activity.

The proband met criteria for probable behavioral variant frontotemporal dementia (bvFTD), a sub-type of frontotemporal lobar degeneration (FTLD), based on early behavioral disinhibition, loss of empathy, perseverative behaviors, neuropsychological profile, and MRI findings [12]. Given his family history, he was screened for known genetic causes of FTLD with no observed pathogenic variants. Therefore, the

Nicholas T. Olney and Carolina Alquezar have contributed equally to this work.

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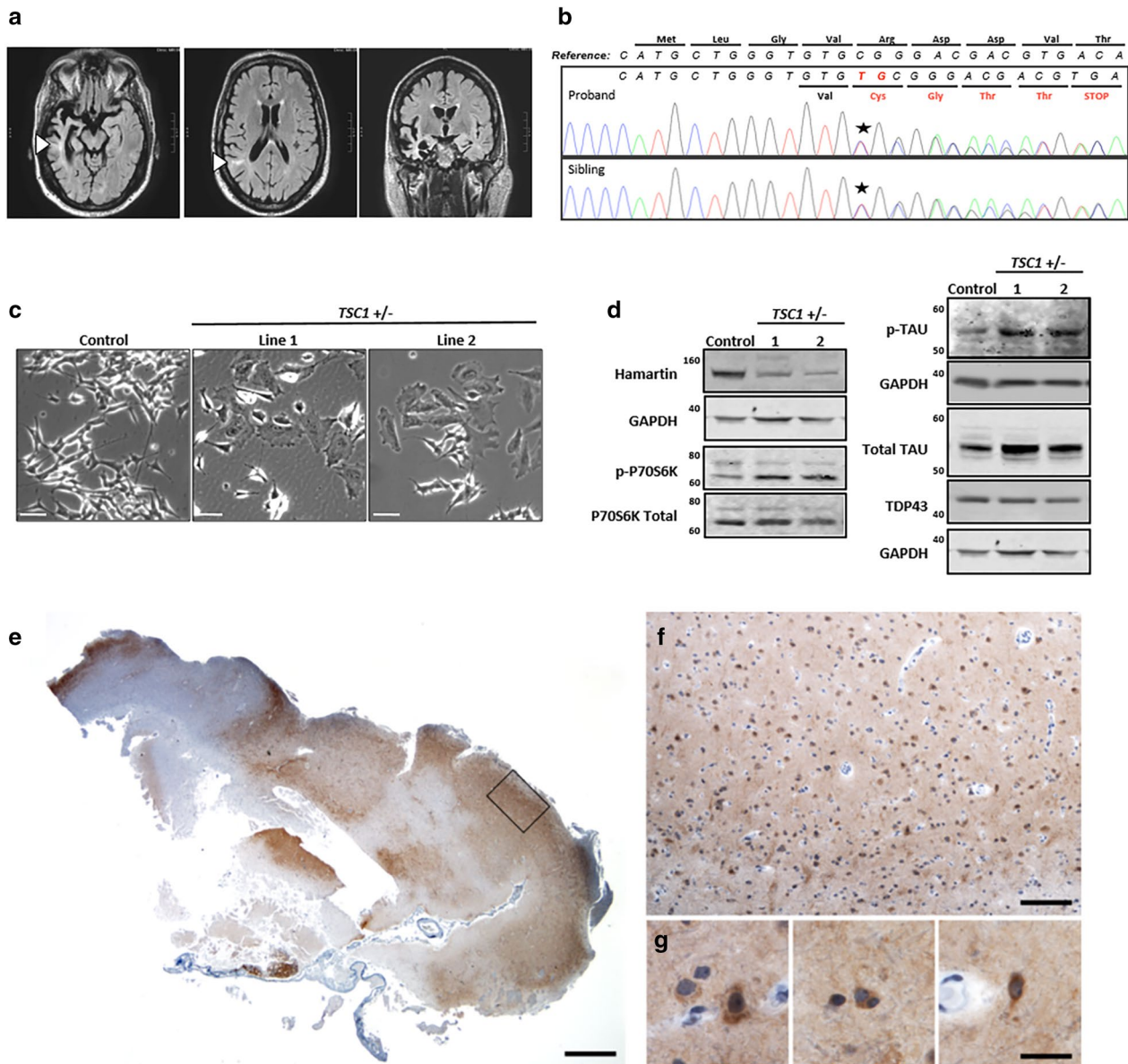


Fig. 1 Proband clinical MRI, novel *TSC1* mutation, cell model of *TSC1*/hamartin haploinsufficiency, and neuropathology of a *TSC1* mutation carrier. **a** Magnetic resonance imaging (MRI) FLAIR sequences revealed severe atrophy of the right anterior temporal lobe, particularly the entorhinal/perirhinal cortices and inferior temporal gyrus, as well as the hippocampus and amygdala. There was also involvement of the left anterior/mesial temporal lobe and the bilateral frontal and right parietal lobes. T2/FLAIR hyperintensities are seen within the subcortical white matter, some of which extended to the superolateral aspects of the lateral ventricles (arrowheads). These lesions can be seen in tuberous sclerosis or focal cortical dysplasias as a transmantle sign. **b** Sanger sequencing con-

firmed a novel frameshift variant in the *TSC1* gene (NM_000368.4: c.62_63insTG; p. Arg22CysfsTer5) that segregated with the proband and affected sibling. **c** Representative micrographs of the enlarged cell body in the undifferentiated *TSC1* heterozygous mutant SH-SY5Y lines. Scale bars represent 50 microns. **d** Representative western blots showing decreased *TSC1*/hamartin and increased phospho-P70S6K^{thr389}, total tau, and phospho-tau levels in retinoic acid differentiated *TSC1* +/- SH-SY5Y cells. TDP-43 levels are unchanged. **e–g** Fragment of frontal cortex resection from the proband's sibling was received and stained for hyperphosphorylated tau, revealing patchy staining in neurons, glia, and surrounding neuropil. Scale bars represent 1000 (**e**), 250 (**f**), and 25 (**g**) microns

proband and his affected sibling underwent whole-exome sequencing. Among variants identified, a novel frameshift mutation in one allele of the *TSC1* gene, predicted to result in early termination of the *TSC1*/hamartin protein, was

shared by the proband and his younger sibling (Fig. 1b, Supplementary Fig. S1b, c; Table S1).

TSC1/hamartin is an upstream inhibitor of mTOR, which regulates cell growth and protein degradation [8].

To determine the significance of the *TSC1* loss of function variant, CRISPR/Cas9 genome editing [6] was used to generate a cellular model of *TSC1*/hamartin deficiency in SH-SY5Y cells, a human neuroblastoma cell line that can be differentiated into neuronal-like cells [7] (Supplementary Fig. S2a). We confirmed that these lines exhibited decreased *TSC1*/hamartin, increased cell size, and elevated P70S6K phosphorylation, consistent with mTOR over activity (Fig. 1c, Supplementary Fig. S2b, c). After retinoic acid (RA) differentiation, *TSC1* +/- cells maintained lower levels of *TSC1*/hamartin and increased mTOR activity. Interestingly, the RA-differentiated *TSC1* +/- lines accumulated phospho-tau and total tau. However, levels of TDP-43, another protein that aggregates in FTLD [2], were unchanged compared to control (Fig. 1d, Supplementary Fig. S2d–h).

Histological samples were obtained from the partial frontal lobe resection of the affected sibling, also a *TSC1* mutation carrier. Similar to SH-SY5Y model of *TSC1* heterozygosity, immunostaining of the resected tissue for phosphorylated tau revealed patchy neuropil staining as well as diffuse cytoplasmic staining of scattered neurons and glia, with variable intensity (Fig. 1e–g). No neurofibrillary tangles, Pick bodies, glial inclusions, or other features of an inherited or sporadic tauopathy were observed. Sections were also stained for TDP-43, phospho-TDP-43, amyloid beta (A β), α -synuclein, and ubiquitin. Surprisingly, they did not demonstrate elevated immunoreactivity for any other neurodegenerative disease proteins (data not shown).

In this case report, we describe the first case of bvFTD in an adult with sub-clinical tuberous sclerosis complex (TSC) due to a novel *TSC1* frameshift variant. Although TSC has been called an “infantile tauopathy” [13], *TSC1* mutations have not previously been implicated in age-associated neurodegenerative disease. This report, therefore, potentially adds *TSC1* to the list of genes that are implicated in both a juvenile-onset lysosomal storage disease and adult-onset neurodegeneration. Others include *GBA* and *ATP13A2* in Parkinson’s disease, *CTSD* in Alzheimer’s disease, and *PGRN* in FTLD [10, 15]. The *TSC1*/*TSC2* complex normally inhibits mTOR activity, with loss of function mutations resulting in overactive mTOR and consequential increased protein synthesis and decreased protein degradation [3, 8, 9]. Strikingly, the decreased protein degradation seems selective for tau, as evidenced from both our cell-based and neuropathological data. This case now suggests that with age, accumulated tau can precipitate neuron loss and neurodegeneration. It also raises the intriguing possibility that tau metabolism is selectively regulated by the mTOR pathway. Our data suggest that *TSC1* loss of heterozygosity, which is necessary for TSC-related tumors [11], is not required for *TSC1*-related tau accumulation and neurodegeneration.

Our findings could be highly significant to the care of TSC patients. Many features of tuberous sclerosis associated neuropsychiatric disorders (TAND) [4, 5], including obsessive behavior, attention deficits, altered eating, impulsivity, memory loss, and language dysfunction, overlap with bvFTD criteria. Thus, these two clinical entities may represent overlapping disorders precipitated by progressive tau pathology. Additional sequencing of the *TSC1/2* genes in FTLD cohorts, use of new tau-based PET imaging, and review of neuropathological specimens from older TSC patients may further support the association between TSC, TAND, and FTLD.

This study extends the relationship between mTOR and tau metabolism [1, 16] to include a potential age-related component. How does aging alter the *TSC1*/mTOR axis to potentially contribute to the development of neurodegeneration? Why do tau and phospho-tau accumulate, while related proteins that undergo chaperone mediated autophagy, such as TDP-43, do not? Do *TSC2* mutations also link to age-related tauopathies? These questions and others are stimulated by our report. In the meantime, this study supports trials of rapamycin analogs which are already underway for neurodegeneration [14], and moves us towards a personalized medicine future in which subpopulations of tauopathy patients may be selectively responsive to this therapeutic approach.

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Author contributions A.W.K., B.L.M., G.C., and W.W.S. designed the study. N.T.O., C.A., E.M.R., A.L.N., J.C.F., A.M.K., J.B.T., M.L.S., M.D.D., A.R.A., V.A.v.B., D.R.D., E.H.S., G.A.J., W.P.D., R.S.D., B.L.M., A.W.K., G.C., and W.W.S. were responsible for data acquisition and analysis. N.T.O., C.A., E.M.R., J.C.F., J.B.T., R.S.D., and A.N.L. created the figures. N.T.O., C.A., W.W.S., G.C., B.L.M., and A.W.K. wrote the manuscript.

Compliance with ethical standards

Conflict of interest Dr. Seeley reports personal fees from Merck, Inc., personal fees from Bristol Myers-Squibb, outside the submitted work. Dr. Miller has served as an Advisor/Director to The Tau Consortium, The John Douglas French Foundation, The Larry L. Hillblom Foundation, Medical Advisory Board, National Institute for Health Research, Cambridge Biomedical Research Centre and its subunit, the Biomedical Research Unit in Dementia (UK); he has served as an External Advisor to University of Washington ADRC, Stanford University

ADRC, and University of Pittsburgh ADRC; he receives royalties from Cambridge University Press, Guilford Publications, Inc., and Neurocase. All other authors have no conflicts of interest to report.

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