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Atypical Alzheimer's disease in an elderly US resident with amyotrophic lateral sclerosis and pathological tau in spinal motor neurons

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Introduction

Pathological 43-kDa transactive responsive DNA-binding protein (TDP-43) has been identified as the major, i.e. disease defining, pathological protein in the majority of patients with amyotrophic lateral sclerosis (ALS) and frontotemporal degeneration (FTD) or frontotemporal lobar degeneration (FTLD) with ubiquitin-positive inclusions (FTLD-U) with or without motor neuron disease (MND), which is known now as FTLD-TDP (1,2). ALS has been linked to FTLD through TDP-43 pathology that may be widespread in the central nervous system (CNS), thereby creating a new concept of TDP-43 proteinopathy as a disease continuum that encompasses ALS and FTLD as well as rarer conditions such as progressive muscular atrophy or primary lateral sclerosis (3, for review see (4)). TDP-43 pathology in addition, or 'secondary', to disease defining inclusion pathologies may occur in other neurodegenerative diseases such as tauopathies or α -synucleinopathies (4). A subgroup of ALS and FTLD cases with neuronal inclusions composed of an ubiquitinated protein, negative for pathological TDP-43, tau, or α -synuclein, was shown to be characterized by the pathological aggregation of a protein called fused in sarcoma (FUS), which shows functional homology to TDP-43 (5–7). Previous to the identification of these two disease defining proteins, there have been reports of single cases of sporadic or familial ALS or FTLD cases showing pathological tau aggregations (or neurofibrillary tangles) as

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their pathological hallmark feature with (8) or without (9–14) the presence of additional ubiquitinated inclusions, published by research groups in continental Europe and Asia. The tau pathology in these cases is in part reminiscent of the Parkinsonism-dementia complex and ALS (ALS/PDC) occurring in geographic isolates such as the Kii Peninsula of Japan (15) or Guam in the Mariana Islands (16–18). These are tauopathies that differ from Alzheimer's disease (AD) in terms of their topographical distribution of tau pathology and the lower level of amyloid- β depositions (19). We here report a patient with sporadic ALS and eye closure apraxia from the continental United States showing multisystem tau pathology atypical for AD by morphology in the brain and spinal cord. There also were robust cortical amyloid- β deposits, but no pathognomonic TDP-43, FUS or α -synuclein pathology. We conclude that tau pathology alone is sufficient to cause ALS and FLTD-MND-like neurodegeneration in the continental United States suggesting that, in part, similar pathogenetic mechanisms might be involved in the patient described in this report, as in ALS occurring in the Kii Peninsula and Guam.

Material and methods

Study subject

The patient was longitudinally followed in the Penn ALS Center. Central nervous system (CNS) samples including tissues from the patient and tissue for positive controls came from the Penn Center for Neurodegenerative Disease Research (CNDR) Brain Bank following neuropathology diagnostic assessment as described (2,3). Informed consent for autopsy was obtained in accordance with the local institutional review board.

Immunohistochemistry

The case was fully examined by routine diagnostic techniques. Briefly, fresh tissues from multiple CNS areas were fixed in 10% neutral buffered formalin or 70% ethanol with 150 mmol NaCl, paraffin-embedded, and cut into 6- μ m sections. Sections were stained with hematoxylin and eosin. Immunohistochemistry was performed as previously described using (1) the avidin-biotin complex detection method (Vectastain ABC kit, Vector Laboratories, Burlingame, CA, USA) with 3,3-diaminobenzidine (DAB) as the chromogen. In an attempt to screen broadly for neurodegenerative lesions, the following primary antibodies were used: mouse monoclonal anti-paired helical filament-1 (PHF1 from Peter Davies; 1:1000), mouse monoclonal anti-ubiquitin (1510, Chemicon, Temecula, CA, USA; 1:100,000; Sigma-Aldrich St. Louis, MO, USA; 1:600), rabbit polyclonal anti-TDP-43 (Protein-Tech Group, Chicago, IL, USA; 1:4500), mouse monoclonal anti- α -synuclein (synuclein 303, generated at the CNDR); rabbit polyclonal anti-FUS antibody (Sigma-Aldrich, Saint Louis, MO, USA; 1:400); mouse monoclonal anti-amyloid- β NAB228 (1:15,000; generated at CNDR); rat monoclonal anti-glial fibrillary acidic protein (2.2B10, 1:5000, generated at CNDR). Sections stained for ubiquitin and TDP-43 were boiled in citrate antigen unmasking solution (Vector Laboratories Burlingame, CA, USA) using a pressure cooker and microwave. Formic acid (88%) pretreatment was employed for sections stained for GFAP. A positive control drawn from a specimen with known reactivity to the antibody in question was included with all IHC studies. Thioflavin-S staining was used for detection of AD-like plaque and tangle pathology.

Evaluation of relative topographical severity of pathology and neuronal loss

For assessing the relative severity of lesions linked to pathological proteins (i.e. inclusion pathology and neuronal loss), a 5-point ordinal scale (i.e. 0 to 4) was used by one of the authors (FG, see Table I). Neuronal and glial inclusion pathologies were rated, and the presence or absence of significant neuronal loss was considered in addition. We chose the assessment of pathology by means of an ordinal scale rather than by using numeric image analysis based quantification tools, as the former acknowledges the sequential nature of stages of increasing severity, ultimately corresponding to a spread of pathology throughout the CNS as shown for all major neurodegenerative diseases. Ordinal data provide information about a relation of severity stages rather than being a measurement acknowledging that one stage follows continuously into the other, which, therefore, represent sequential classes rather than values on a numerical scale.

Sequencing of MAPT, TARDBP, and SOD1 genes

Mutations in the *MAPT*, *TARDBP*, and *SOD1* genes were excluded by DNA sequencing. DNA was extracted from brain tissue using an automated extractor according to manufacturer protocols (Qiagen). DNA sequencing of the coding regions of the *MAPT*, *TARDBP*, and *SOD1* genes was performed as previously described (20–22). Genotyping for a C9orf72 hexanucleotide repeat expansion was performed using repeat-primed PCR plus fluorescent sizing PCR with detection by capillary electrophoresis (23). Two single nucleotide polymorphisms (rs7412 and rs429358) were genotyped with TaqMan allelic discrimination assays on an ABI7500 (Life Technologies) to determine the apolipoprotein E (*APOE*) genotype.

Results

Clinical characteristics

Past medical history—This 65-year-old right handed female was first examined on 28 December 2005. She had a prior history of osteoporosis, sensorineural hearing loss and chronic sinusitis. Her family reported that she had never travelled outside of the United States and lived all her life in New Jersey. She had no unusual dietary habits. The patient developed progressive dysarthria that began in May 2004. This was followed within 1–2 months by progressive dysphagia that led to a five-pound weight loss as well as increasing difficulty with coughing and choking on liquids and solids and difficulty handling oropharyngeal secretions by the time of her initial presentation. In November 2004 these symptoms were accompanied by inappropriate and uncontrollable crying. She was treated with citalopram with improvement. In January 2005 she noted the onset of progressive weakness of the arms (left > right) that caused increasing difficulty with activities of daily living over the ensuing months. In May 2005 she also noted the onset of progressive weakness of the legs (right > left) that caused her to fall frequently and require the use of a cane in June 2004. The weakness was accompanied by muscle atrophy, cramps and twitching in the upper and lower limbs. In November 2004 she first noted increasing exertional dyspnea and two-pillow orthopnea. She had no bowel or bladder difficulty, pain, sensory disturbance, involuntary movements, ptosis or diplopia. The patient denied cognitive difficulty but her family noted a personality change manifested as apathy and

disengagement from family activities unusual for her. The family history was negative for neurodegenerative diseases.

Physical examination on 28 December 2005—Forced vital capacity was 30% predicted with poor technique. There was no evidence of tachypnea, abdominal paradox or accessory muscle use. The Amyotrophic Lateral Sclerosis Functional Rating Score-revised was 33. She was awake and alert. Mini-Mental Status was 29/30 with one error in short-term recall. The patient was only able to produce three words beginning with the letter ‘F’ in 1 min. The oral trails exam was correct only to F-6. Forward digit span was 6; reverse was 4. Cranial nerve examination demonstrated normal ocular motility, mild symmetric facial diparesis and slow tongue movements without atrophy or fasciculations. She was unable to maintain eye closure on command. Speech was dysarthric with a spastic quality but understandable with hypophonia. Muscle wasting, fasciculation, and spasticity were more severe in the upper than lower limbs. Power was Medical Research Council 4 in the right deltoid, biceps, triceps and finger extensors but 2 in the interossei and abductor pollicis brevis. Power was Medical Research Council 5 in the left arm apart from 4 in the finger extensors and 3 in the interossei and abductor pollicis brevis. There was no ataxia or involuntary movements. Sensation was normal. Reflexes were 1/4 at the jaw, 3/4 in the arms (left > right) with note of distal spread to finger flexors. A Hoffman’s sign was elicited on the left. Reflexes were 3 at the knees, 2 at the adductors with bilateral crossing, and 2 at the ankles. There was a Babinski sign with dorsiflexion of the ankle, as well as flexion of the knee and hip bilaterally. Gait was narrow based with moderate spasticity bilaterally.

Diagnostic testing—Magnetic resonance imaging of the brain demonstrated mild generalized atrophy and non-specific periventricular white matter hyperintensity on T2 weighted protocol. Degenerative changes but no root compression and normal spinal cord signal were demonstrated on magnetic resonance imaging of the cervical spine. Sensory and motor nerve conduction study was normal in the arms and in the legs apart from mildly reduced sural sensory responses at 5 microvolts bilaterally. Electromyography demonstrated fibrillations, positive sharp waves and fasciculations in multiple arm, hand and leg muscles bilaterally and unilaterally in the mid-thoracic paraspinal muscles. Motor unit morphology demonstrated increased amplitude and duration with an increased number of polyphasic motor units in all muscles tested including the sternocleidomastoid, and recruitment was reduced in all muscles tested. Laboratory testing was normal apart from a creatine kinase of 350 micromoles per milliliter.

Clinical course—The diagnosis of clinically definite ALS with FTD and eye closure apraxia was made. She refused to take riluzole. Non-invasive positive pressure ventilation was instituted and she successfully used this during sleep. Over the ensuing six months her limb weakness worsened. The patient required increasing assistance with activities of daily living and use of a wheelchair. Her speech worsened requiring use of writing for communication. Her dyspnea worsened. Forced vital capacity declined to 21% predicted. The ALSFRS-R declined to 26. She entered a nursing home and did not return for further follow-up. Because of increasing dyspnea she eventually required use of non-invasive

positive pressure ventilation for much of the day. She died of respiratory failure four years from symptom onset and three years after diagnosis.

Neuropathological examination

The brain weight was 1185 g and gross examination did not show significant pathological findings. TDP-43 and FUS immunostaining did not reveal pathognomonic inclusion pathology as described previously for ALS or FTLTDP/FTLD-FUS (2–5). However, in the spinal cord, there was an up to moderate density of tau-positive neuronal inclusions (and dystrophic cellular processes) combined with significant atrophy of the anterior horn and cell loss (Table II, Figure 1a). The lower medulla transitioning to the spinal cord showed focally round inclusions, which were also found in the basal ganglia. Other brainstem or subcortical brain areas showed less pathology (Table II). While the spinal cord and rhombencephalon were free from amyloid-beta deposition, in the basal ganglia and thalamus senile plaques were present focally up to a moderate degree, but no unequivocal signs of amyloid angiopathy were found. However, the allo- and neo-cortex showed an overall moderate to severe density of senile plaques consistent with an amyloid-beta phase 3 according to Thal et al. (24), and an amyloid angiopathy. Hematoxylin and eosin stained sections of the hippocampus showed granulovacuolar degeneration in the CA1/CA2 (subiculum) areas. The dentate gyrus of the hippocampus and the basal ganglia showed an up to moderate density of crescent-shaped or roundish tau immunoreactive cytoplasmic neuronal inclusions. Severe tau pathology (including neurofibrillary tangles, neuronal cytoplasmic roundish or sickle shaped inclusions/neuronal cytoplasmic punctuate or granular tau immunoreactivity, dystrophic cellular processes, neuropil grains, neuritic plaques) was present in the CA1–CA4/subiculum of the hippocampus (Figure 2a) and (peri-)amygdaloid areas, approximating a bi-layer formation in the latter with the superficial layers showing a higher level of pathology than the deeper ones. Similar tau pathology was also present in the temporal cortex (Figure 2b), albeit to a slightly lesser degree. Other neocortical areas, i.e. the angular gyrus, frontal cortex, and cingulate cortex had an even lesser degree of tau pathology mostly in superficial layers (layer II). Neuronal tau pathology in the motor cortex was robust and predominantly found in layer II and mainly had the appearance of round or crescent-shaped tau cytoplasmic aggregations and smaller cytoplasmic punctuate or granular tau immunoreactivity (Figure 3). These proteinaceous changes were accompanied by a disorganization of the laminar cortical architecture. White matter tau inclusion pathology was less conspicuous than grey matter tau pathology and consisted mainly of dystrophic neurites and rare coiled bodies. There was also a periventricular (and perivascular arrangement) of tau-immunoreactive glial cells, some of them probably corresponding to astrocytes (Figure 4). Neuronal drop-out in the grey matter was variable accompanied by gliosis especially conspicuous in immunohistochemistry for glial acidic fibrillary protein (Figure 1b).

Genetic analysis

Genetic analysis of the genes most commonly associated with familial and/or sporadic forms of FTLTDP and ALS (*MAPT*, *TARDBP*, *SOD1*, and *C9orf72*) did not identify any pathogenic mutations in these genes. The *APOE* genotype was E3/E3.

Discussion

On examination, this patient met criteria for clinically definite ALS Plus according to El Escorial criteria (25), with evidence of upper motor neuron dysfunction in the cranial, cervical and lumbosacral segments; lower motor neuron dysfunction in the cervical and lumbosacral segments; negative imaging of the brain and cervical spine; electromyography and nerve conduction studies consistent with a diffuse lower motor neuron process; a family history negative for neurodegenerative disease; and a negative laboratory evaluation apart from mild elevation of creatine kinase. There was also no evidence of involuntary movements, autonomic dysfunction, objective sensory loss, or cerebellar abnormalities. She had no known relationship with an identified geographic cluster of ALS. She did have a reported personality change and evidence of executive dysfunction on mental status testing consistent with the socioexecutive form of FTD commonly associated with ALS (26). The patient also showed eye closure apraxia. She had a relentlessly progressive course and expired from respiratory failure three years after diagnosis.

Tau pathology (or neurofibrillary tangles) may be the main or ‘primary’ pathological finding in diseases clinically characterized by various combinations of motor neuron disease, dementia and Parkinsonism (9–14). Furthermore, a recent consensus study reported on different subtypes of a distinct disease entity called ‘globular glial tauopathy’ (27). It is also known that tau pathology in addition, or ‘secondary’, to the disease defining inclusion pathology in ALS with or without dementia occurs (28,29). However, the neuropathology of the present case was unusual for several reasons. First, glial cytoplasmic inclusions pathology – in the form of rare white matter coiled bodies and foci of periventricular inclusion pathology – was a relatively infrequent finding, and, in keeping with this, the present case does not show the pathognomonic features of ‘globular glial tauopathy’ (27). Secondly, there was no evidence of pathognomonic ALS-like or FTLTDP-like TDP-43 or FUS pathology (for review see (4)). Rather there was evidence of widespread brain and spinal cord tau pathology. The relative higher degree of tau inclusion pathology in the motor gyrus compared to the spinal cords possibly could denote a more ‘active’, ongoing degeneration in the former at the time of death with, rather, atrophy being present in the latter. Thirdly, laminar distribution of tau inclusion pathology in neocortical brain areas (especially the motor cortex) was atypical for classic AD since the superficial layers were affected the most. In allocortical brain areas, this pattern was more similar to typical AD. In the latter, tau neurofibrillary pathology affects the cortex in a bi-laminar pattern with a predominant involvement of deep cortical layers. Hence, the pattern of neocortical tau pathology resembles that typically associated with Guam ALS which is often associated with Parkinsonism and dementia wherein tau pathology is the predominating lesion (16,30). However, her family confirmed that she was born in and resided in New Jersey her entire life and had never travelled outside of the continental United States. Non-AD-like tau pathology is also known to occur in chromosome 170-associated FTLTDP caused by mutations in the *MAPT* gene (31). In our case, testing for *MAPT* gene mutations – as for mutations in the *TDP43*, *FUS*, and *C9orf72* genes – was negative. However, our paper strengthens the connection between tau pathology and MND, which is also supported by tau transgenic mouse models (32,33). Spinal cord tau pathology is not exclusively present in MND on

geographic isolates (such as Guam) or elsewhere in the world, but has also been reported to occur in AD (34–37). Of note, granulovacuolar degeneration has been shown in a range of neurodegenerative diseases including ALS/PDC on Guam and ALS/AD (38,39). However, the case presented here is also distinct from Guam ALS/PDC because it showed robust cortical A β plaque deposits as well as amyloid angiopathy. Senile plaques are infrequent in Guam ALS/PDC, but if present they do not have a strong neuritic tau-positive component (19,40). The biochemical properties of amyloid plaques in the Guamanian disease are similar to AD (40). Finally, unlike this case, many Guam ALS/PDC cases show concomitant TDP-43 and α -synuclein pathology (35,41,42).

In conclusion, we here present evidence that tau pathology, atypical for AD, may underlie ALS-FTLD in the continental U.S. similar in some aspects to cases of ALS-PDC in Guam and the Kii Peninsula.

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Figure 1.

Sacral spinal cord. a. Neuronal loss and tau-positive inclusions in residual motor neurons (example arrow, anti-paired helical filament-1 immunohistochemistry). b. Anti-glial fibrillary acidic immunohistochemistry reveals significant astrogliosis (example arrow). The same low power magnification for both a. and b.

Figure 2. Immunohistochemistry for PHF1 revealing neuronal cytoplasmic inclusions predominantly in layer II of the motor cortex (example arrow, anti-paired helical filament-1 immunohistochemistry).

Figure 3.

Immunohistochemistry for PHF1 showing temporal lobe structures with severe neurofibrillary pathology in CA1/subiculum (a. example arrow) and neurofibrillary pathology (b. example long arrow) as well as dystrophic neuritic pathology, either diffuse (examples, short arrow) or arranged in form of plaques (examples, asterisk). a. and b. same low power magnification.

Figure 4. Immunohistochemistry for PHF1 revealing periventricular glial pathology (example given, arrow) in the periamygdaloid area.

Table I

Five-point ordinal grading scheme.

0 No INC	and no NL
1 Rare INC	and no significant or no NL
2 Mild INC	and no significant or no NL
3 Moderate INC	and significant or no significant NL
4 a. Severe INC	and significant or no significant NL
b. Less than severe INC	and significant neuronal loss

INC: inclusions (cytoplasmic aggregates, dystrophic cellular processes); NL: neuronal loss.

Table II

Tau pathology in amyotrophic lateral sclerosis with dementia.

Corticoid	Allocortex					
	Periamygdala			Hippocampus		
	Amygdala	GM	WM	Dentate gyrus	CA1-4/Subiculum	
4a, F	4a, D	4, PVE, PVA	3, D	4a, D		
Neocortex						
Motor cortex	Midfrontal cortex		Angular cortex		Temporal cortex	
	GM	WM	GM	WM	GM	WM
4a, D	3, D	3, D	2, D	2	4a, D	3, D
						2
Deep brain nuclei						
Rhombencephalon						
Lower medulla						
Lentiform nucleus	Thalamus	Dorsal motor plate	Inferior olive	Elsewhere	Cerebellum	Spinal cord
	3, D	1	2	1	3, F	0
						4b, D

GM: grey matter; WM: white matter.

Pattern of moderate and severe pathologies. D: diffuse, i.e. not restricted to a focus, but still follows topographical pattern if described in the main text; F: focal; PVE: periventricular; PVA: perivascular.