Clinicopathologic Correlations in a Family with a *TBK1* Mutation Presenting as Primary Progressive Aphasia and Primary Lateral Sclerosis.

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Abstract

Mutations in the TANK binding kinase 1 gene (*TBK1*) are associated with amyotrophic lateral sclerosis and/or frontotemporal dementia; however, the range of clinical phenotypes and neuropathological changes associated with these mutations have not yet been completely elucidated. We present the detailed clinical, neuroimaging and neuropathological features of two brothers carrying the *TBK1* p.Gly272_Thr331del mutation. Both presented with very similar and unusual clinical features including primary progressive aphasia and asymmetric-onset primary lateral sclerosis (PLS). Repeated electrophysiological studies failed to reveal any lower motor neuron involvement. Neuropathological evaluation of both cases revealed frontotemporal lobar degeneration with TDP-43 proteinopathy type B and selective involvement of upper motor neurons with TDP-43 inclusions. The stereotypical clinical presentation and neuropathological findings in these cases widen the phenotypic spectrum of *TBK1* mutations and provide insights into the pathogenesis of PLS.

Keywords

TBK1; primary progressive aphasia; primary lateral sclerosis; TDP-43; neuropathology

Introduction

Frontotemporal dementia (FTD) is clinically characterized by abnormalities in personality, behaviour and/or language. Some patients with FTD also develop prominent extrapyramidal movement disorders, motor neuron disease (MND) or both. The neuropathological substrate of FTD is most often selective atrophy of the frontal and temporal lobes, termed

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frontotemporal lobar degeneration (FTLD). Four major subtypes of FTLD are recognized based on the identity of the protein that abnormally aggregates in neurons and glia. Of these, FTLD with inclusions of TAR DNA binding protein 43 (FTLD-TDP) has been suggested to be on a spectrum with MND, in particular amyotrophic lateral sclerosis (ALS). ALS is characterized by progressive neurodegeneration of upper and lower motor neurons (UMN and LMN, respectively) and results in spasticity, hyper-reflexia, weakness, muscle atrophy and fasciculations. A related MND, primary lateral sclerosis (PLS), affects only UMN and does not lead to muscle atrophy or fasciculations. PLS is relatively rare, representing less than 5% of all MND (1), and is usually not associated with other major neurological deficits. However, both ALS and PLS have some clinical overlap with FTD: approximately half of ALS and 22% of PLS patients develop signs of frontotemporal dysfunction and approximately 15% of ALS and 3% of PLS patients develop full-blown FTD (2-5). Although half of FTD patients develop signs of MND and approximately 15% develop full-blown ALS (6), typical PLS is uncommon.

Controversy exists as to whether PLS is simply a 'forme fruste' of ALS or a distinct entity with a different molecular basis. Both are characterized neuropathologically by degeneration of UMN in the primary motor cortex, corticobulbar and corticospinal tracts, but only ALS shows LMN degeneration in the brainstem and spinal cord. In addition, the vast majority of ALS cases show TDP-43 immunoreactive (-ir) inclusions in UMN, LMN and glia (7,8); whereas the molecular signature of PLS is less established. At least some cases described after the discovery of TDP-43 have been shown to display this pathology (5,9,10).

Strengthening the concept of an FTLD/ALS-TDP disease spectrum are genetic variants that may result in FTD, ALS or FTD and ALS, in members of the same family. Recently, loss-of-function (LoF) mutations in the TANK binding kinase 1 gene (*TBK1*) have been added to the list of ALS/FTD causing mutations (11). Initial reports estimated the prevalence of *TBK1* mutations in ALS/FTD somewhere between 2 to 10% (12-14). A more recent meta-analysis reported that *TBK1* mutations may be less common than originally thought (1.0% and 1.8% for *TBK1* LoF and missense mutations, respectively), but that they are strong risk factors for both ALS and FTD (15). The full range of clinical and neuropathological phenotypes associated with these mutations is being elucidated.

Here we report two brothers who carried the c.992+1G>A *TBK1* mutation (p.G272_T331del) and developed remarkably similar and unusual clinical features. Both presented with prominent language dysfunction and asymmetric UMN signs associated with strikingly asymmetric, left greater than right (left > right) cerebral atrophy without evidence of LMN involvement. Neuropathological evaluation of both cases demonstrated TDP-ir pathology with an anatomical distribution that correlated strongly with their primary progressive aphasia (PPA) and PLS phenotypes.

Methods

Genetics

The mutation was initially identified through whole-genome sequencing (WGS) of the proband as described elsewhere (16). Variant calling was performed according to the GATK

best practice recommendations (17,18). To validate and extend these results, *TBK1* sequencing of exons 2-21 was performed on three siblings. Each *TBK1* exon was amplified and Sanger sequenced using Big Dye chemistry (Applied Biosystems, Foster City, CA, USA). All PCR and sequence products were purified using Mag-Bind bead technology (Omega Bio-tek, Norcross, GA, USA) and run on ABI3730 DNA Analyzer.

cDNA was prepared from frontal cortex available from the proband (Case 1) and a neuropathological normal individual (Invitrogen, Carlsbad, CA, USA). cDNA was amplified using primers located in *TBK1* exons 7 and 9 and migrated on an 1% agarose gel (primers available upon request). RT-PCR products were gel extracted using the Gel extraction kit MinElute (Qiagen, Hilden, Germany) and Sanger sequenced.

Neuropathology

Formalin-fixed, paraffin-embedded brain and spinal cord tissue blocks were stained with standard histochemical, silver and Congo red stains. Immunohistochemistry (IHC) was performed for the following; alpha-synuclein (Thermo Scientific; 1:10,000), beta amyloid (DAKO; 1:100), hyperphosphorylated tau (clone AT-8; Innogenetics; 1:2000), phosphorylation-independent TDP-43 (ProteinTech; 1:1000), ubiquitin (DAKO; 1:500), FUS (Sigma-Aldrich; 1:1000) and SOD1 (monoclonal against misfolded SOD1, gift from Dr. N. Cashman; 1:500 dilution).

Results

Genetics

This family of European ancestry showed a suspected autosomal dominant pattern of involvement [Figure 1]. WGS revealed the presence of a c.992+1G>A *TBK1* mutation in the proband (Case 1). Sanger sequencing confirmed the presence of this mutation in an affected sibling (Case 2) and its absence in an unaffected sibling [Figure 2A]. No other mutations in known neurodegenerative disease genes with a minor allele frequency of less than 5% in GnomAD non-Finnish European ancestry were observed. RT-PCR led to the identification of two products: one at the expected size (359bp) and a shorter one (179bp; Figure 2B). Sanger sequencing of the smaller product indicated the absence of exon 8, suggesting that c. 992+1G>A leads to aberrant exon 8 splicing.

Clinical presentation and imaging

Case 1—This right-handed male developed word-finding difficulties, impaired naming, and slow hesitant speech at age 68. He also showed mild difficulties in visuospatial function, depressed mood, irritability, and lack of motivation. His assessment at age 69 showed markedly reduced verbal fluency, word-finding pauses, semantic paraphasias, impaired naming, and reduced phonemic and semantic fluency with preserved repetition, comprehension, reading, writing, grammar and syntax. His memory and orientation were relatively preserved. He performed poorly on tests of abstraction and executive functioning. His initial neurological examination only showed subtle, asymmetric spasticity and hyperreflexia affecting predominantly his right upper extremity. Strength and muscle bulk were normal and there were no fasciculations. Electrophysiological studies revealed normal

nerve conduction and only mildly abnormal electromyography with no sustained fibrillation potentials or positive sharp waves and normal motor unit morphology and recruitment. Magnetic resonance imaging (MRI) of his brain showed asymmetrical, left > right, temporal lobe atrophy [Figure 3]. SPECT and FDG-PET scans revealed decreased perfusion and glucose metabolism (respectively) in the left temporal and parietal lobes.

Over the next year, his language and other cognitive functions deteriorated, but his auditory comprehension and reading remained relatively preserved. His behavioural and personality changes remained stable. His right spasticity and weakness progressed to right-sided pyramidal hemiparesis with only mild involvement of the left side. He also developed bulbar symptoms. He remained free of clinical or electrophysiological signs of LMN involvement. He died at the age of 71 with clinical diagnoses of unclassifiable PPA and PLS.

Case 2—The 62 year-old, right-handed brother of the proband reported abrupt-onset slurred speech, word-finding and naming difficulties, clumsiness in his right hand, writing difficulties, and poor dexterity. His initial assessment revealed slow and effortful speech, word-finding pauses, severely impaired naming, spastic dysarthria and apraxia of speech. Other language components were normal. He also had poor delayed short-term memory and difficulty planning constructional tasks but tested normally in calculation, right-left orientation, graphesthesia, attention, and executive functioning. His initial neurological examination showed flattening of the right nasolabial fold, asymmetric (right > left) increased tone and hyperreflexia and mild right pyramidal weakness but no LMN signs. Extrapyramidal signs including decreased blink rate, prominent stare, and limited facial expression were also present. His gait was slow and narrow-based with reduced right arm swing. He had normal eye movements and no sensory or cerebellar deficits. Brain MRI showed asymmetric (left > right) atrophy of the temporal lobe, insula and inferior frontal lobe [Figure 3].

One year later he was anarthric with relative preservation of other language skills. He was perseverant and showed signs of executive dysfunction, but his judgment and recent memory were relatively intact. His motor weakness progressed to right hemiparesis and bulbar dysfunction by age 64 and later to spastic quadriplegia with generalized muscle atrophy but without fasciculations. Electrophysiological studies performed on three separate occasions showed no significant LMN involvement. He had no behavioural symptoms throughout his disease. He died at the age of 67 with clinical diagnoses of non-fluent variant PPA and PLS.

Clinical summary—Both brothers presented in their 60s with progressive speech, cognitive and motor symptoms. Their speech difficulties were due to a combination of dysarthria and aphasia, with the latter characterized by impaired fluency and anomia. The motor presentation was strikingly similar with asymmetric UMN impairment of the right upper limb which subsequently progressed to include all limbs and bulbar cranial nerves but with no significant LMN involvement. Brain imaging in both cases showed asymmetric (left > right) temporal lobe degeneration.

Minor differences included mild behavioural changes, more prominent semantic dysfunction and earlier executive dysfunction in Case 1, while Case 2 showed more problems with verbal fluency and additional extrapyramidal features.

Neuropathology

Case 1—The post mortem brain showed moderate-to-severe cerebral atrophy significantly greater on the left side and preferentially involving the temporal lobe and the precentral gyrus. The ventricles were asymmetrically dilated and the left hippocampus was slightly atrophic.

Microscopic examination revealed chronic neurodegenerative changes that were most prominent in the left primary motor and temporal neocortices and both hippocampi [Figure 4, Table 1]. The basal ganglia, thalamus and substantia nigra showed mild degeneration without Lewy bodies. At all levels, the corticospinal tracts (CST) showed reduced myelin staining and numerous lipid-laden macrophages [Figure 4]. A normal complement of LMN without Bunina bodies was present in the hypoglossal nucleus and spinal cord.

TDP-ir pathology was diffuse but most severe in the left hemisphere [Figure 4, Table 1]. The primary motor cortex (bilateral) and left temporal cortex were the most severely affected neocortical regions and showed TDP-ir neuronal cytoplasmic inclusions (NCI) in all cortical layers, with few neuritic profiles and no neuronal intranuclear inclusions. This pattern was most consistent with FTLD-TDP type B, with the unusual feature that the NCI were mostly compact (rather than diffuse granular) and included tangle, crescentic and annular morphologies. The hippocampi showed TDP-ir NCI and CA1 neurites, greater on the left. The striatum also showed abundant TDP-ir pathology. There were no TDP-ir NCI in LMN of the hypoglossal nucleus or spinal cord and no TDP-ir glial inclusions in the CST.

Moderate Alzheimer's type pathology (CERAD moderate neuritic senile plaques and Braak IV/VI neurofibrillary pathology) and rare Lewy bodies restricted to the transentorhinal cortex were the only co-pathologies. Neuropathological diagnoses were (i) FTLD-TDP type B, (ii) PLS, (iii) moderate left hippocampal sclerosis with TDP proteinopathy and (iv) moderate Alzheimer-type pathology.

Case 2—The brain weighed 1235 grams. Macroscopically, there was striking asymmetric (left > right) atrophy of the frontal and temporal lobes [Figure 4]. The precentral gyri and hippocampi were asymmetrically atrophic (left > right). The deep grey nuclei appeared normal. The substantia nigra showed mild paleness.

Microscopic examination showed chronic neurodegenerative changes that were most severe in the primary motor cortex bilaterally, the left temporal neocortex and left hippocampus. There was severe degeneration of the CST at all levels, greater on the right. A normal complement of LMN was present in the hypoglossal nucleus and spinal cord with no Bunina bodies [Figure 4].

TDP-ir pathology was most abundant in the left temporal, frontal and bilateral primary motor cortices, hippocampi and basal ganglia. NCI were present in all neocortical layers

without significant neuritic pathology or intranuclear inclusions; consistent with FTLD-TDP type B. Similar to case 1, most of the NCI were compact and included unusual annular, tangle and crescentic morphologies. A single filamentous TDP-ir inclusion was identified in one LMN of the lumbar spinal cord; otherwise, there was no TDP-ir pathology in the brainstem or spinal cord.

This case also showed moderate Alzheimer's pathology (CERAD moderate senile neuritic plaques and Braak III/VI neurofibrillary pathology). Neuropathological diagnoses were (i) FTLD-TDP type B, (ii) PLS and (iii) moderate Alzheimer-type pathology.

Clinicopathologic correlations

Both cases presented with PPA. Imaging studies and neuropathological assessment showed striking left > right temporal lobar atrophy and temporal > frontal TDP-ir pathology. The asymmetric UMN signs and symptoms correlated with greater degeneration and TDP-ir pathology in the left primary motor cortex. The absence of clinical LMN features was explained by the preservation of LMN without significant TDP-ir pathology. The extrapyramidal symptoms in Case 2 may be explained by the greater involvement of the basal ganglia compared to his brother.

Discussion

TBK1 is a multifunctional kinase involved in the regulation of various cellular pathways, including immune response, inflammation, insulin signalling, cell proliferation, and autophagy (19). Mutations in *TBK1* have been reported to cause ALS and FTD via a loss-of-function mechanism by either reducing mRNA or protein levels, or leading to reduced TBK1 function (13,14,20-22). The previously reported c.992+1G>A *TBK1* mutation (14,23) identified in this family results in an in-frame deletion of the *TBK1* transcript (p.Gly272_Thr331del) with loss of exon 8 (see results section), which forms part of the ubiquitin-like and kinase domains, likely resulting in loss of TBK1 function (20). In addition, this mutation was previously shown to reduce TBK1 protein levels by approximately 50% in *in vitro* and *in vivo* (14).

The full clinical spectrum of patients carrying *TBK1* mutations has not been explored in detail. Most *TBK1* mutation-associated phenotypes have been reported in the context of large genetic studies, in which clinical details are scant, and have been variably associated with bvFTD, PPA and/or ALS (bulbar and/or spinal) (13,14). More recent reports have widened that spectrum to include extrapyramidal movement disorders, such as PSP, and cerebellar manifestations (24). A report of 16 patients carrying several different *TBK1* mutations described a range of phenotypes including bvFTD (N=5), PPA (N=1), bvFTD-ALS (N=1), unspecified dementia (N=2) and ALS (N=7). No definite cases of PLS were described. Imaging studies revealed varying patterns of atrophy (including none, frontotemporal and generalized) and either symmetric or asymmetric (usually right > left) involvement (23). Detailed case reports include four patients presenting with semantic dementia or non-fluent variant PPA, all of which later developed ALS, with striking asymmetric (left > right) anterior temporal atrophy (25); six affected family members carrying the p.Arg573Gly *TBK1* mutation that developed either PPA or bvFTD (N=4) or

bulbar or spinal PLS (N=2) (26); and a single patient carrying the p.Ala705fs* *TBK1* mutation who developed bvFTD with subsequent cognitive impairment and anterior right temporal lobe atrophy (27). From these reports it is difficult to determine whether patients with *TBK1* mutations have a distinct phenotype compared with other FTD/ALS-associated mutations. It is also difficult to establish (and beyond the scope of this report) a genotype/ phenotype correlation for *TBK1* mutations given the relative lack of detailed clinical information in most of the reported cases, the known clinical variability in familial cases with the same *TBK1* mutation (26) and the fact that all *TBK1* mutations result in loss of protein function at either the transcriptional, mRNA or protein levels.

Neuropathological reports of patients carrying mutations in *TBK1* are relatively scant. Changes compatible with FTLD-TDP type B (p.Gly272_Thr331del, p.Val97Phefs*2, p.Ala417* and p.Thr79del) and ALS-TDP (c.1551_1552insTT) have been reported, sometimes in association with tau-ir pathology (14,21,23). A single patient with the p.Thr79del *TBK1* mutation has been described as having ring-like TDP-43 ir inclusions (21) similar to the NCI found in our cases. FTLD-TDP type A with unusual neuritic structures at the cortical/white matter junction was described in the patient carrying the p.Ala705fs* *TBK1* mutation (27).

To our knowledge, the only other reported case with a c.992+1G>A *TBK1* mutation was a male who presented in his late 40s with a two-year duration of FTD. Neuropathological examination showed mild neuronal loss in the frontal cortex with moderate numbers of TDP-43 and p62-ir NCI and short dystrophic neurites but no neuronal intranuclear inclusions, interpreted as most compatible with FTLD-TDP type B (14,23). These features are different from those of our two cases, making a mutation-specific effect an unlikely explanation for the homogenous presentation of our brothers.

One striking clinical aspect in our cases is that their motor syndrome fulfilled criteria for PLS rather than ALS. Although PLS was anecdotally reported in two patients carrying the p.Arg573Gly *TBK1* mutation, most cases with *TBK1* mutations present as ALS. The cases described herein had a mutation in a gene known to cause ALS (*TBK1*), clinical features of FTD (commonly associated with ALS), FTLD-TDP subtype B (FTLD-TDP subtype most often present in patients with concomitant ALS) and TDP-ir inclusions in UMN. These findings support the interpretation that their pyramidal system degeneration was part of the ALS spectrum. However, the exquisitely selective UMN degeneration and nearly complete absence of TDP-ir pathology in LMN, after as much as five years of disease progression, suggests that some uncommon (likely genetic) modifying factor may act to protect LMN from degeneration. Moreover, this or other modulating factor(s) may also be responsible for the unusual and strikingly similar clinical presentation, neuroimaging and neuropathology of these brothers, which stands in contrast with the wider phenotypic and neuropathological variability described in other patients carrying the same or other *TBK1* mutations. Identification of this factor would require a much larger cohort of patients with the same mutation. Candidate factors would include transcriptional regulators and interacting partners of TBK1 and relative levels of proteins with TBK1-like functions. In addition, to complete the assessment, the overall functionality of all TBK1-dependent cellular pathways would need to be compared between cases with the same mutation but different phenotypes.

Acknowledgments

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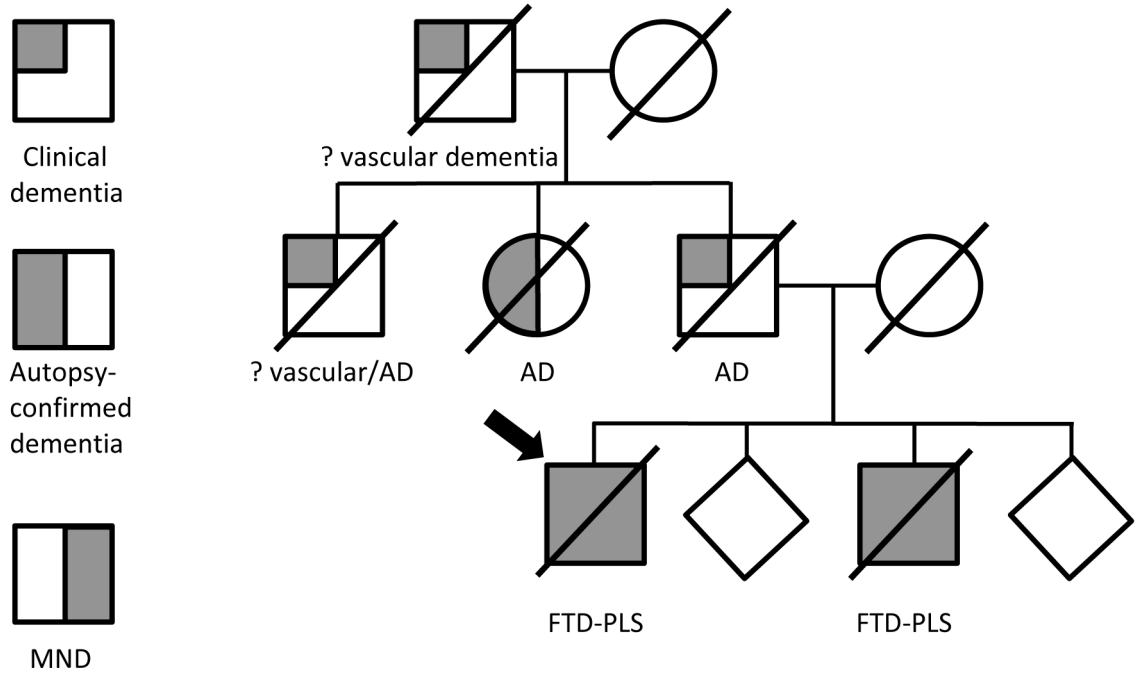
Figure 1.
Family pedigree. Proband (case 1) is marked with an arrow. Autopsy of the proband's aunt confirmed only Alzheimer-type pathology.
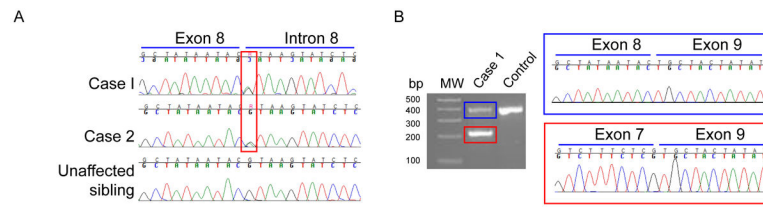
Figure 2.
Confirmation of the *TBK1* c.992+1G>A mutation and effect on *TBK1* splicing. Presence of the c.992+1G>A mutation was confirmed by Sanger sequencing in Case 1 and Case 2 (red rectangle). c.992+1G>A is absent from the genomic DNA of an unaffected sibling (A). RT-PCR using primers specific for *TBK1* exons 7 and 9 in Case 1 shows the presence of a product representing the wild-type cDNA (blue rectangle) and a shorter product (red rectangle). Sanger sequencing revealed that the shorter product lacks *TBK1* exon 8. MW = molecular-weight size DNA marker.
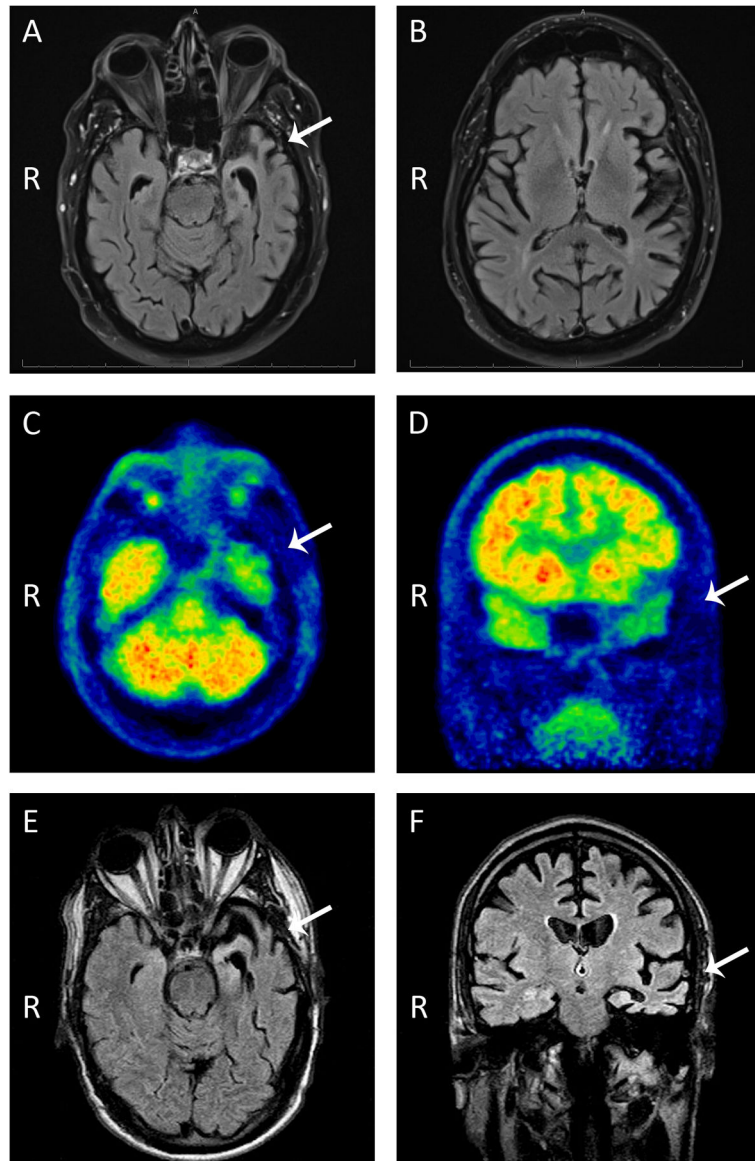
Figure 3.
MRI scan (A, B) and FDG-PET (C, D) on case 1 and MRI scan on case 2 (E, F) showed asymmetric (left greater than right) temporal lobe atrophy (white arrows) and hypometabolism, with relative preservation of frontal lobes. R= right side.
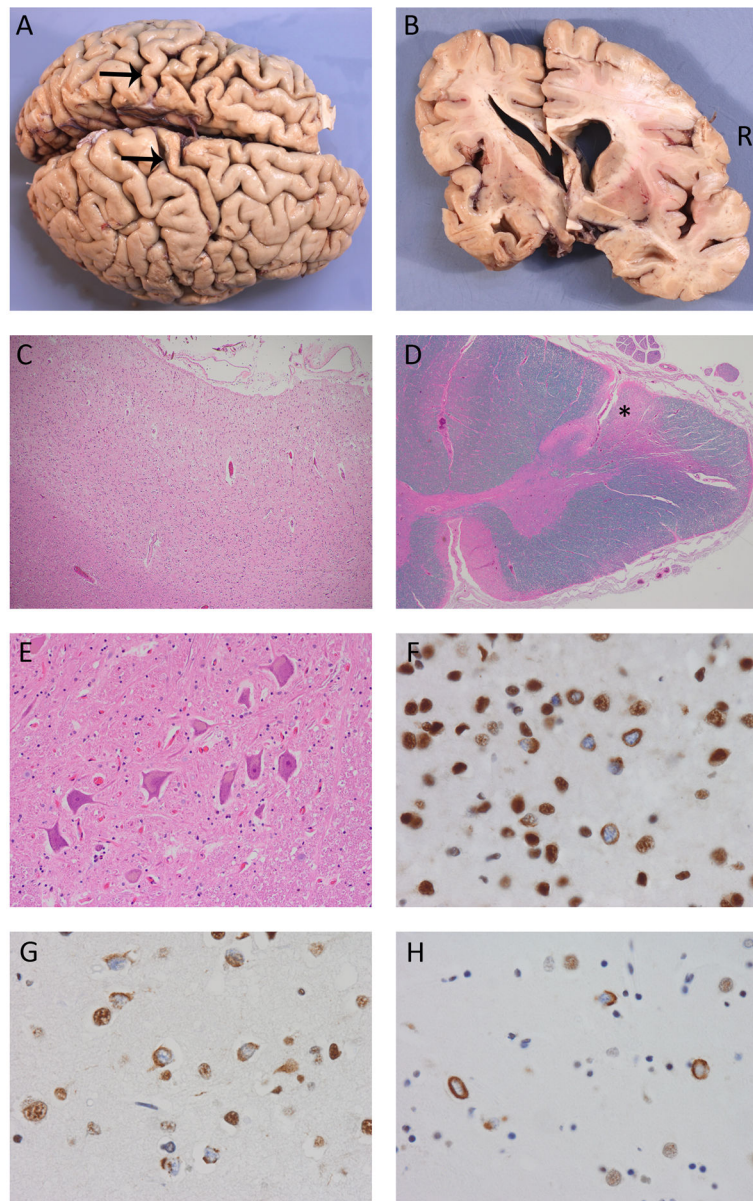
Figure 4.
Pathological findings. Both cases showed similar gross and microscopic findings, including cerebral atrophy that was more severe on the left and preferentially affected the temporal lobes and primary motor cortex (arrows, Case 2) (A, B). There was moderate-to-severe chronic degeneration of the motor cortex (C) and corticospinal tracts (*, D) with a normal number of healthy-appearing lower motor neurons (E). TDP immunohistochemistry demonstrated neuronal cytoplasmic inclusions (NCI) in all cortical laminae of the primary motor (F), prefrontal (G) and temporal neocortex with relatively few dystrophic neurites and no intranuclear inclusions, consistent with FTLD-TDP type B. Many of the NCI had an unusual crescentic, annular or tangle-like morphology. NCI were also abundant in the basal ganglia (H). Magnifications: C:100x; D:20x; E:200x; F,G and H:600x.

Table 1.

Semiquantitative rating of degeneration (deg) and TDP-43 immunoreactive pathology burden (TDP) by region: – (white), none;+ (yellow), mild; ++ (orange), moderate; +++ (red), severe. CA,hippocampal pyramidal layre; cer, cerebellum; CST,=corticospinal tracts; ctx,cortex; GP,globus pallidus; HC,hippocampus; LMN, lower motor neurons; med, medulla; par,parietal; SN, substantia nigra; temp, temporal; XII, hypoglossal nucleus.

	Case 1				Case 2			
	Left		Right		Left		Right	
	deg	TDP	deg	TDP	deg	TDP	deg	TDP
motor ctx	++	+++	+/++	+++	+++	++/+++	++/+++	++/+++
prefrontal ctx	+/++	++	+	+/++	++	++/+++	+	++
temp ctx	++	+++	+	–/+	+++	+++	+	++
par ctx	+	++	+	–/+	+	++	+	+
HC – dentate	+	+++	–	++	++	+++	–	+++
HC – CA	++	++	+	+	+++	+	++	+
	deg		TDP		deg		TDP	
caudate	+		++		+		+++	
putamen	+		+++		++		+++	
GP	+		+		+		+++	
thalamus	+		+		+		+	
SN	+		+		+		+	
basis pons	–		+		–		+	
med – CST	+++		–		+++		–	
med – XII	–		–		–		–	
cord – CST	++		–		+++		–	
cord – LMN	–		–		–		single	
cer – ctx	–		–		+		–	
cer - dentate	–		–		–		–	