Novel pathologic and genetic associations of N-terminal TAF15 in FTLD/ALS

Nikhil B. Ghayal, BSc[1], Shanu F. Roemer, MD, PhD[1], Wouter De Coster, PhD[2], Matthew C. Baker[1], Sean Jiang, PhD[3]; Sara Alidadiani, BSc[2], Douglas Galasko, MD[4], David S. Eisenberg, PhD[3], Rosa Rademakers, PhD[1,2], Keith A. Josephs, MD, MST, MSc[5], Dennis W. Dickson, MD[1]

[1]Department of Neuroscience, Mayo Clinic, Jacksonville, FL; [2]Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium; [3]Molecular Brain Institute, University of California, Los Angeles, CA; [4]Department of Neurology, University of California, San Diego, CA; [5]Department of Neurology, Mayo Clinic Rochester, MN

Background

- Frontotemporal lobar degeneration/amyotrophic lateral sclerosis with FUS/EWS/TAF15 accumulation (FTLD/ALS-FET) is a group of clinically and pathologically heterogeneous disorders that lack distinct genetic associations.
- Recently, amyloid filaments composed of an N-terminal TAF15 fragment (7-99aa) were discovered in patients presenting as FTD and ALS who were neuropathologically diagnosed as atypical FTLD with ubiquitinated inclusions (aFTLD-U), a subtype of FTLD/ALS-FET.
- Accumulation of N-terminal TAF15 fragments has not been validated immunohistochemically or biochemically, as previous studies utilised antibodies to the middle and C-terminus of TAF15.
- Further, this finding raises questions about the role of N-terminal TAF15 in other subtypes of FTLD/ALS-FET, including neuronal intermediate filament inclusion disease (NIFID), basophilic inclusion body disease (BIBD), and ALS with basophilic inclusions (ALS-BI).

Methods

Immunohistochemistry

The medial temporal lobe and basal forebrain of seventeen FTLD-FET cases (five aFTLD-U, seven NIFID, three ALS-BI, and two BIBD) were immunostained with two TAF15 antibodies, one specific to the N-terminal core (N-TAF15) and another specific to the C-terminal RGG3/NLS domains (TAF15-C). Neuronal, glial, and neuritic pathologies were scored semiquantitatively (0-4). Subsequently, N-TAF15 was screened in another eighteen FTLD-FET cases.

Genetic screening

Exons 1 to 6 (1-161aa) of the *TAF15* gene were screened for coding variants in 27 FTLD-FET cases. Twelve cases were evaluated by Sanger sequencing (NIFID3,5-10; BIBD1-3, ALS-BI2-5), and whole-genome sequencing data was queried in fifteen aFTLD-U cases.

Protein biochemistry

Frozen sections of the frontal cortex and medulla were sampled from BIBD2, ALS-BI4, and the TAF15 variant carrier (BIBD3) and sequentially extracted in buffers of increasing strength to obtain a fraction of sarkosyl-insoluble proteins. These extracts were analyzed and immunoblotted with antibodies to N-TAF15, C-TAF15, FUS, EWS, transportin-1, and TMEM106B.

FTLD/ALS-FET cases

Table 1 Clinical, pathologic, and genetic features of 35 FTLD/ALS-FET cases evaluated at the Mayo Clinic (yellow = rare, green = positive).

Case #	Age/Sex	ClinDx	BIs	HyIs	aINX	FUS	TAF15	Genetic variants
ALS-*FUS*	47/F	ALS						*FUS* p.Arg521Gly
PLS-FUS	75/M	MSA-P						
ALS-BI1	62/M	ALS						
ALS-BI2	53/M	CBS/PSP						
ALS-BI3	74/F	ALS						
ALS-BI4	56/F	ALS						
ALS-BI5	68/F	ALS						
BIBD1	68/M	CBS/PPA						
BIBD2	78/M	AD						
BIBD3	50/M	bvFTD/PPA						*TAF15* p.Ser66Phe
NIFID1	47/M	PLS						
NIFID2	54/F	bvFTD						
NIFID3	51/M	PLS						
NIFID4	50/F	bvFTD						
NIFID5	49/M	CBS						
NIFID6	67/M	MSA-P						
NIFID7	41/M	bvFTD						
NIFID8	61/F	PSP						
NIFID9	39/M	FTD-NOS						
NIFID10	81/F	CBS						
aFTLD1	42/M	bvFTD						
aFTLD2	41/F	bvFTD						
aFTLD3	53/M	bvFTD						
aFTLD4	58/M	bvFTD						
aFTLD5	55/F	bvFTD						
aFTLD6	45/M	bvFTD						
aFTLD7	45/M	bvFTD						
aFTLD8	49/M	bvFTD/PPA						
aFTLD9	64/M	bvFTD						
aFTLD10	73/M	bvFTD						
aFTLD11	48/M	bvFTD						
aFTLD12	48/M	bvFTD						
aFTLD13	56/M	bvFTD						
aFTLD14	57/F	bvFTD						
aFTLD15	44/M	bvFTD						

Immunoblots

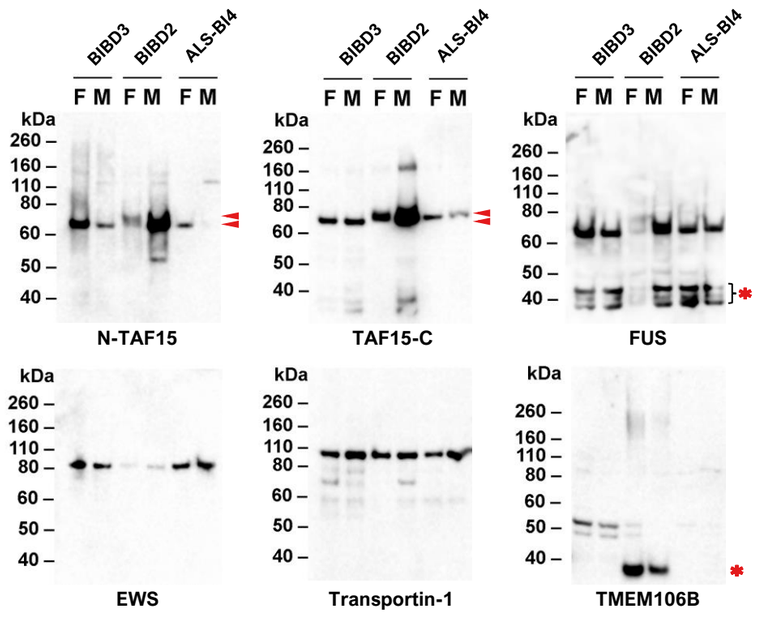

Figure 3 Western blots of sarkosyl-insoluble extracts of frontal cortex and medulla from BIBD3, BIBD2, and ALS-BI4 immunolabeled with N-TAF15, TAF15-C, FUS, EWS, Transportin-1, and TMEM106B [TAF15 full length bands denoted by red arrows; cleavage fragments = red asterisk].

TAF15 epitope-dependent regional vulnerability

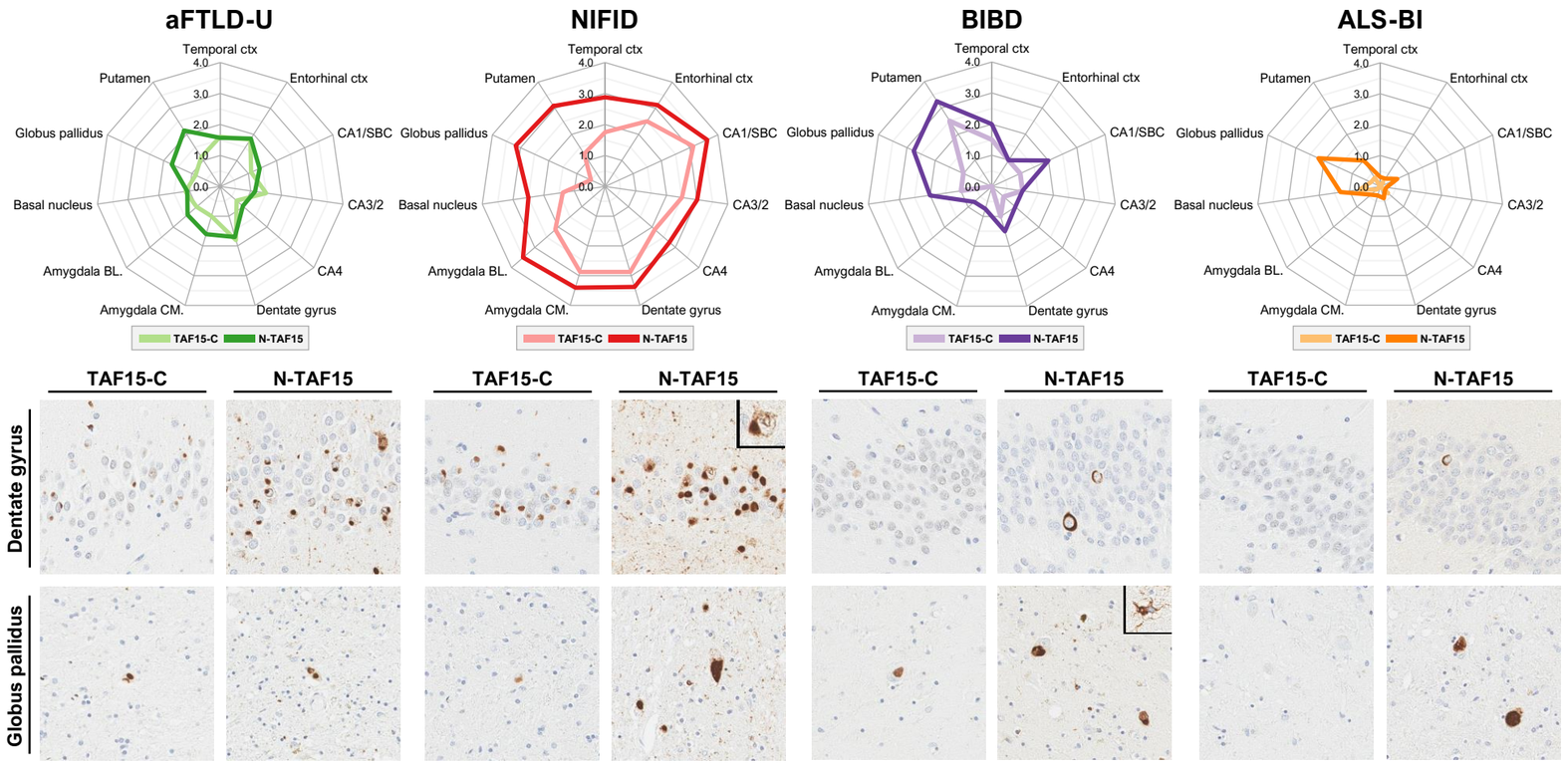

Figure 1 Radar chart visualization of the mean neuronal lesion burden (0-4) observed with C-TAF15 and N-TAF15 immunostains in corticolimbic and basal ganglionic regions of aFTLD-U, NIFID, BIBD, and ALS-BI cases. Representative images of the dentate gyrus and globus pallidus C-TAF15 and TAF15-N pathology observed within each FTLD-FET subtype (inset 1 = perinucleolar neuronal inclusion in NIFID; inset 2 = thorn-shaped astrocyte in BIBD).

Novel genetic variant in *TAF15*

Variant information

- ***TAF15* c.197C>T, p.Ser66Phe** was identified in BIBD case 3 and was absent in ExAC, 1000G, and GnomAD databases.
- *In silico* predictions: CADD score = 28.9; PolyPhen-2 = probably damaging; MutationTaster2021 = disease-causing.
- The variant alters an amino acid within the β-sheet-rich SGYS motif of the TAF15 amyloid core (variant labeled with red box and asterisk; SGYS motifs underlined in blue).

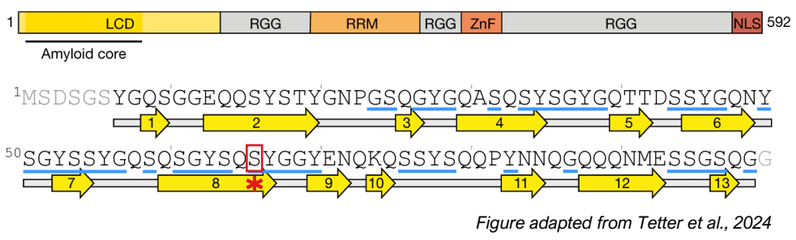

Figure adapted from Tetter et al., 2024

Clinical history of variant carrier

- The patient's family history included a maternal cousin with ALS; parents and children were neurologically normal.
- At age 44, he became withdrawn, apathetic, and hypomimic.
- In the next year, his short-term memory declined and he became aphasic, which progressed to complete mutism.
- At age 45, he began exhibiting criminal behaviors, including inviting himself into old friends' homes, abusing illegal drugs, shoplifting, hit and run, and grand theft auto, ultimately leading to multiple imprisonments and psychiatric hospitalization.
- At 46-years-old, he developed ideomotor apraxia, ritualistic behaviors, and binge-eating associated with rapid weight gain.
- His neurologic exam was normal for motor, reflexes, gait, and coordination; except for increased axial tone.
- He died at age 50.

Neuropathologic features of TAF15 p.Ser66Phe carrier

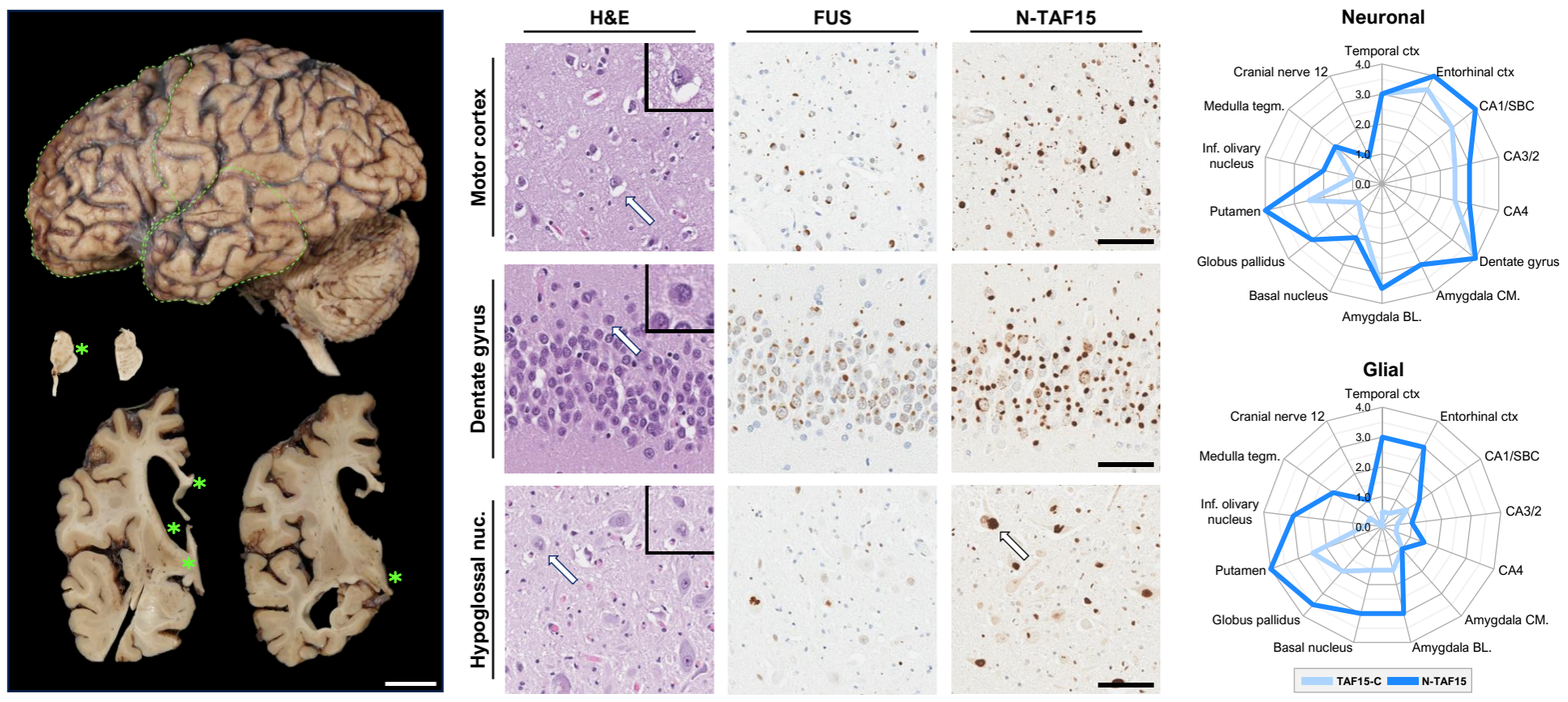

Figure 2 Macroscopic and microscopic pathology of BIBD3. Atrophy of the frontotemporal lobes [green dashed outline], corpus callosum, striatum, globus pallidus, and subthalamic nucleus/thalamus, as well as severe depigmentation of the substantia nigra were observed grossly [green asterisks, bar=2cm]. Abundant basophilic inclusions [white arrows/insets] were seen with haemotoxylin-eosin stains and IHC for FUS, N-TAF15, C-TAF15 revealed a greater neuronal lesion burden with N-TAF15 compared to FUS [scale bars=100um,200um,100um]. N-TAF15 revealed widespread glial pathology and a marginally greater neuronal lesion burden compared to TAF15-C.

Conclusions

- Overall, N-TAF15 revealed a greater burden of neuronal lesions than TAF15-C in all FTLD-FET subtypes; most markedly in NIFID.
- There was considerable overlap in the neuronal lesion burden observed with both TAF15 antibodies in aFTLD-U.
- Surprisingly, screening N-terminal exons of *TAF15* revealed a novel genetic variant in a BIBD patient who presented with bvFTD/PPA.
- The variant carrier had abundant basophilic inclusions and a burden of neuronal, glial, and neuritic pathology similar to NIFID cases.
- Similarly to aFTLD-U, there was little difference in the neuronal lesion burden observed with both TAF15 antibodies in several brain regions.
- Biochemically, both TAF15 antibodies labeled mostly full-length TAF15, and there were feint bands near 35-kDa with TAF15-C in both BIBD cases.
- Despite the immunohistochemical differences, it remains to be determined whether N-terminal TAF15 fragments can be observed biochemically.

References

1. Josephs KA, Holton JL, Rossor MN, Braendgaard H, Ozawa T, Fox NC, et al. Neurofilament inclusion body disease: a new proteinopathy? Brain. 2003;126(Pt 10):2291-303.
2. Mackenzie IR, Foti D, Woulfe J, Hurwitz TA. Atypical frontotemporal lobar degeneration with ubiquitin-positive, TDP-43-negative neuronal inclusions. Brain. 2008;131(Pt 5):1282-93.
3. Neumann M, Roeber S, Kretzschmar HA, Rademakers R, Baker M, Mackenzie IR. Abundant FUS-immunoreactive pathology in neuronal intermediate filament inclusion disease. Acta Neuropathol. 2009;118(5):605-16.
4. Munoz DG, Neumann M, Kusaka H, Yokota O, Ishihara K, Terada S, et al. FUS pathology in basophilic inclusion body disease. Acta Neuropathol. 2009;118(5):617-27.
5. Neumann M, Bentmann E, Dormann D, Jawaid A, DeJesus-Hernandez M, Ansorge O, et al. FET proteins TAF15 and EWS are selective markers that distinguish FTLD with FUS pathology from amyotrophic lateral sclerosis with FUS mutations. Brain. 2011;134(Pt 9):2595-609.
6. Chen J, Yuan X, Wei P, Wang D, Chen C, Guo Q, Luo SZ, Chen L. The SGYS motif of TAF15 prion-like domain is critical to amyloid fibril formation. Biophysical Journal. 2022 Jul 5;121(13):2613-23.
7. Tetter, S., Arseni, D., Murzin, A.G. et al. TAF15 amyloid filaments in frontotemporal lobar degeneration. Nature. 2004;625, 345–351.