Navigating the neuropathologic landscape of ANXA11 proteinopathies

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Background

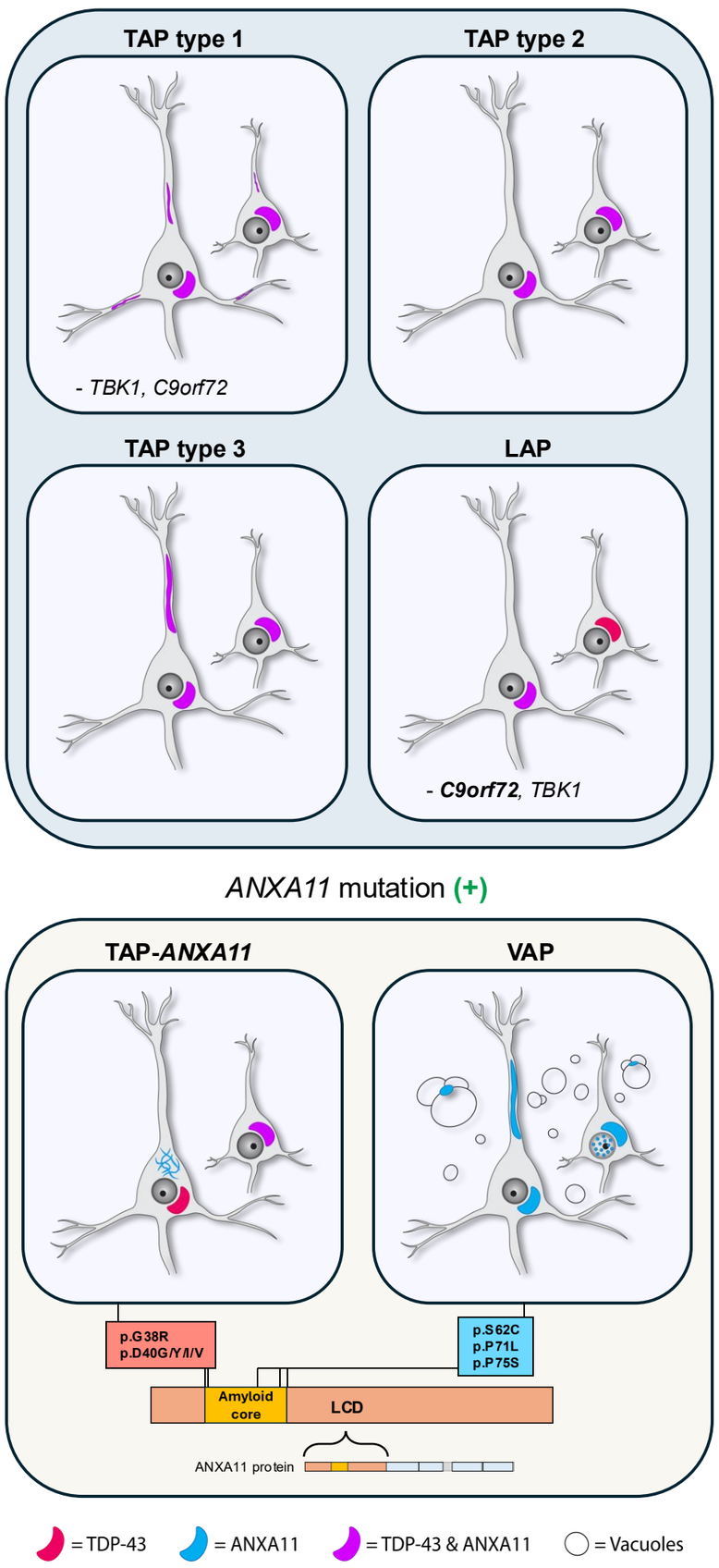
- Pathogenic *ANXA11* variants (G38R, D40G/Y/I/V, Q362ins24) were first associated with TDP-43 and ANXA11 proteinopathies in ALS and have been described clinically in svFTD and myopathy patients.
- Recently, ANXA11 was shown to colocalize with TDP-43 wherein both co-assemble to form amyloid filaments in FTLD-TDP type C.
- Spongiform change, ANXA11 proteinopathy, and an *ANXA11* variant (p.P75S) was described in one patient who presented with PSP/FTD.

ANXA11 variant carriers

Table 1 Clinical, pathologic, and genetic features of 4 ANXA11 variant carriers evaluated at the Mayo Clinic (red = negative, green = positive).

#	PathDx	Sex	AAO	AAD	ClinDx	aINX	TDP-43	ANXA11	Genetic variants
1	VAP	F	46	49	PSP-CBS				*ANXA11* p.Ser62Cys
2	VAP	M	64	67	MSA-P				*ANXA11* p.Pro71Leu
3	VAP	M	62	65	FTD-ALS				*ANXA11* p.Pro71Leu
4	AD/DLBD/LATE	F	68	83	CBS				*ANXA11* p.Gly38Arg

ANXA11 disease family



Novel TDP-43 & ANXA11 proteinopathies (TAP) in FTLD-PLS

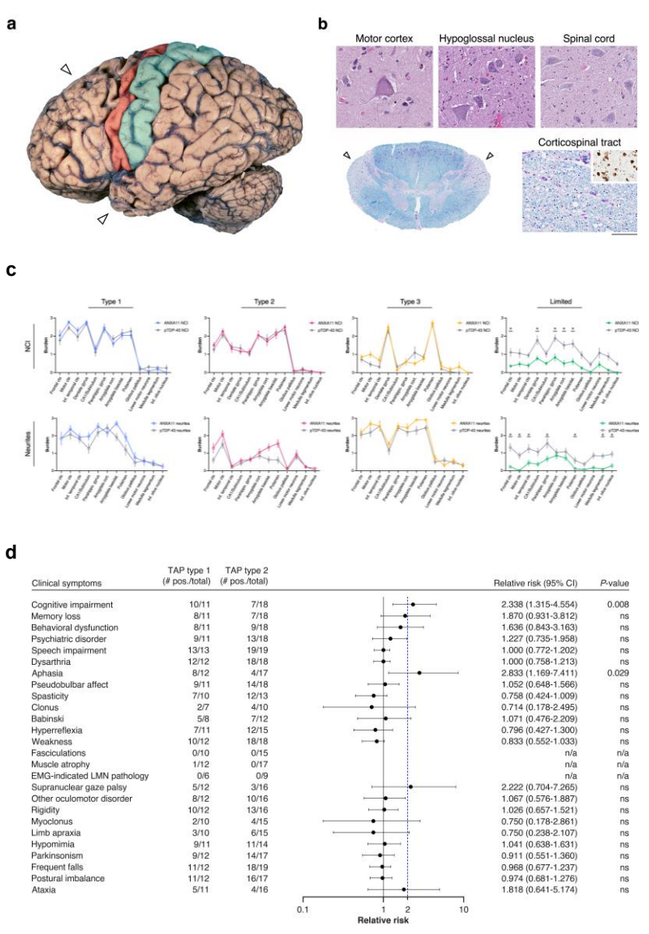


Figure 1 **a)** Macroscopic hallmarks of FTLD-PLS include degeneration of the frontal, anterior temporal, (arrowheads), and primary motor cortices (red), corticospinal tract myelin loss and microgliosis (arrowheads), and relative preservation of the primary sensory cortex (cyan) and lower motor neurons in the hypoglossal nucleus and spinal cord. **b)** Neuronal cytoplasmic inclusions and neuritic pathologies are abundant and widespread in TAP type 1, while TAP type 2 affects predominantly neurons and TAP type 3 affects predominantly neurites. ANXA11 inclusions are relatively infrequent compared to TDP-43 in patients with limited ANXA11 proteinopathy (LAP). **c)** Clinically, TAP type 1 patients more often experienced cognitive impairment and aphasia than TAP type 2 patients, who trended towards having relatively increased relative risk of developing UMN signs. Relative risk of developing supranuclear gaze palsy and ataxia were notably high in TAP type 1 patients. **d)** Pathologically, TAP type 1 features include abundant short and globular cortical neurites (arrowheads) and annular NCIs. TAP type 2 has more variable NCI morphology and less neurites in extramotor cortices. TAP type 3 features long, thick neurites and infrequent Pick-body-like NCIs. **e)** In all TAP types, ANXA11 colocalized nearly entirely with pTDP-43 in both neuronal and neuritic inclusions. Occasional ANXA11 neurites were not labeled by pTDP-43 in TAP type 1 and prominent immunoreactivity for either protein was observed at the ends of long neurites in TAP type 3 cases. Scale bar(s) = 100µm for panel a images, 30µm for insets, 50µm for IHC images, and 25µm for IF images.

Vacuolar ANXA11 proteinopathy in FTLD-MND-UPS

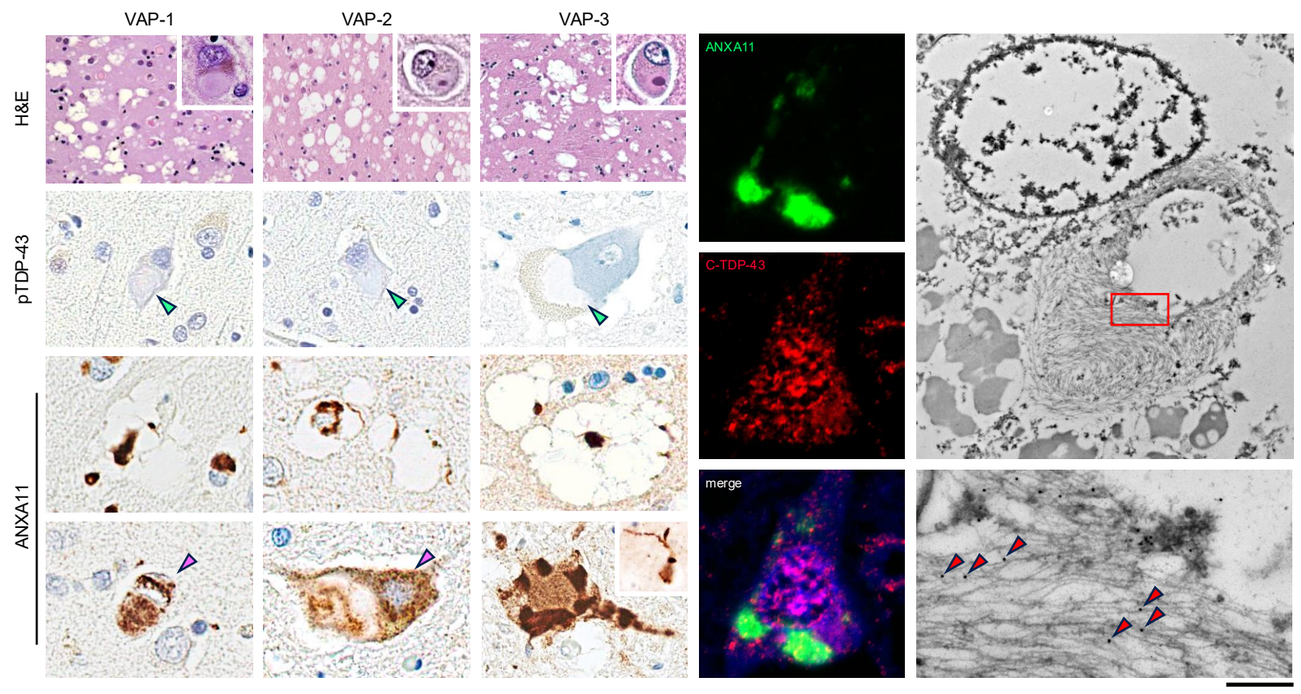


Figure 2 Light and immunogold-electron microscopic (IEM) findings in vacuolar ANXA11 proteinopathy. Spongiform change is most prominent in the putamen and hyaline inclusions are frequent in the neocortex of all three cases. All pathologic inclusions are negative for pTDP-43 (green arrows). ANXA11 neurites are observed within and surrounding vacuoles. Granular and vermiform neuronal intranuclear inclusions can be seen in neurons with granulofilamentous cytoplasmic inclusions in the motor cortex, red nucleus, and anterior horn of the lumbar spinal cord (pink arrowhead). ANXA11 neurites (inset) resembling barbed wire are characteristic in neocortical and subcortical brain regions. Immunofluorescence for ANXA11 and C-terminal TDP-43 revealed predominantly physiologic cytoplasmic and nuclear TDP-43 localization and sparse colocalization with ANXA11 cytoplasmic aggregates. ANXA11-IEM depicts ANXA11 labeling (10nm-particles) of a fibrillar inclusion surrounding an intracellular vacuole. Bars = 30um for IHC and insets, 50um for spongiform H&E, 25um for IF, and 2um and 500nm for IEM images.

ANXA11 p.G38R with AD/LBD/LATE-NC polyproteinopathy

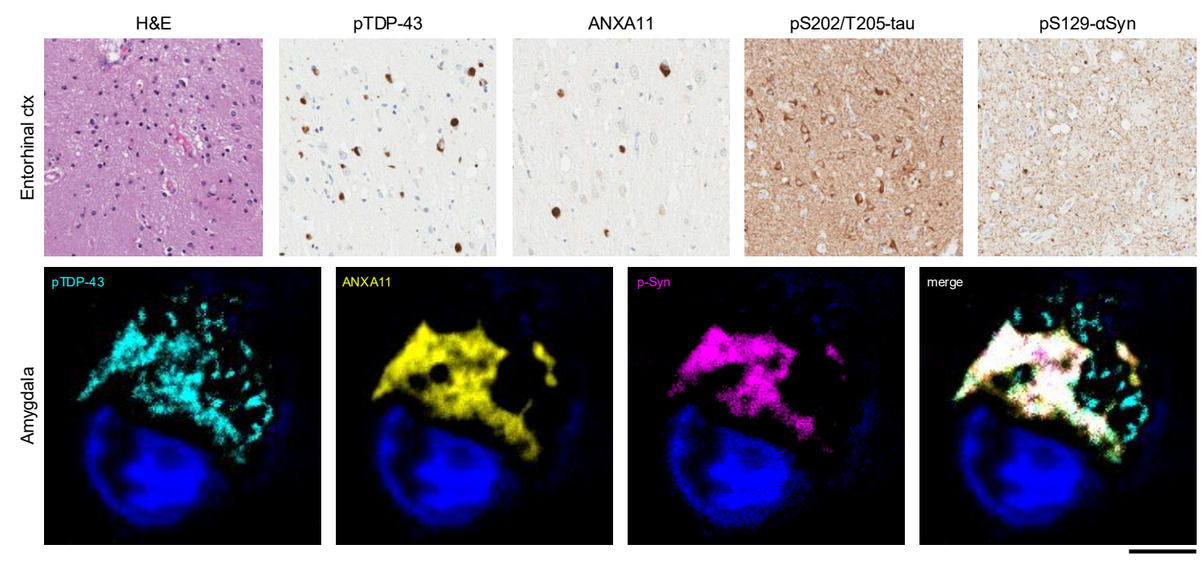


Figure 3 H&E demonstrated severe hippocampal sclerosis and cotton-wool plaque-like amyloid deposits in cortical regions. Tissue microvacuolation was observed; however, this most often occurred in proximity to plaque-like deposits and was rarely observed distal to these lesions. Notably, spongiform change was not observed in the putamen. TDP-43 pathology was predominantly limbic and qualified as LATE-NC stage 2. ANXA11 proteinopathy affected the same regions seen with pTDP-43, but was notably less frequent. In these limbic regions, pS202/T205-tau (p-tau) and pS129-α-synuclein (p-Syn) was severe. In cotton wool plaques, neuritic immunoreactivity was observed stains for p-tau, p-Syn, pTDP-43, but not for ANXA11. Triple immunofluorescence staining for pTDP-43, ANXA11, and p-Syn was revealing for neurons with immunoreactivity for all three antibodies. In these neurons, pTDP-43 was not consistently colocalized with ANXA11. On the other hand, ANXA11 occasionally colocalized with p-Syn but pTDP-43 was infrequently colocalized in these aggregates. Generally, p-Syn was most prominent; however, single immunoreactive inclusions were only observed for pTDP-43 and p-Syn. Bars = 30um for H&E and IHC, and 25um for IF images.

Methods

Case selection & genetic screening

379 cases in the Mayo Clinic Brain Bank with a primary TDP-43 proteinopathy, 959 cases with LATE-NC, and 15 TDP-43 negative FTLD-U cases (4 NIFID-TAF15, 4 aFTLD-U, 4 BIBD, and 3 FTLD-MND-UPS) were screened for ANXA11. All coding exons of the *ANXA11* gene were screened in 379 primary TDP-43 proteinopathy cases, 951 LBD cases, and the 15 FTLD-U cases (all autopsy confirmed).

Immunohistochemistry

All immunohistochemical studies were performed using a rabbit ANXA11 antibody (10479-2-AP) and IEM studies were performed with a mouse ANXA11 antibody (68089-1-Ig). TDP-43 antibodies included pTDP-43 (CAC-TIP-PTD-M01A) and a C-terminal TDP-43 antibody from Dr. Len Petrucelli (MC2085).

Conclusions

- Colocalization of TDP-43 and ANXA11 occurs in three clinically and neuropathologically distinct subtypes (TAP types 1, 2, 3).
- ANXA11 can cause neurodegeneration independently of TDP-43 mislocalization and phosphorylation.
- *ANXA11* variant associated proteinopathies can be dichotomized into two main subtypes, based on the presence/absence of TDP-43, striatal-predominant spongiform change, and distinct *ANXA11* variants.
- The p.G38R *ANXA11* variant may cause a broader spectrum of pathology than first anticipated when it was detected in ALS and FTD.
  - These findings should encourage screening for *ANXA11* variants in elderly clinical cohorts and ANXA11 proteinopathy in autopsy-confirmed LATE-NC.
- There are many different subtypes of ANXA11 proteinopathies that are associated with a broad age range, from young-onset to late-onset.

References

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