

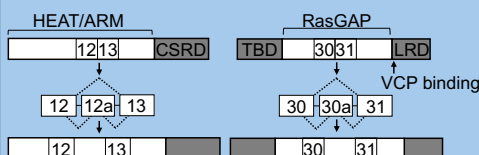
The Role of NF1 in Frontotemporal Dementia

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Introduction

Frontotemporal dementia (FTD) presents with overlapping clinical changes in memory, language, behavior, and motor abilities. FTD is characterized pathologically by an accumulation of abnormal proteins due to a dysfunctional proteostasis network. Valosin-containing protein (VCP) is a calcium-dependent ATPase that functions as a master regulator of protein homeostasis by modulating protein clearance, ER and mitochondria function, and protein synthesis. Neurofibromin (NF1) is a critical component of the VCP complex which promotes VCP localization to pre- and post-synaptic sites where it regulates dendritic spine density by modulating local protein synthesis and autophagy. The functional role of this interaction has not been explored in human cases.

NF1 isoforms expressed in the brain



Schematic of alternatively spliced NF1 exons, functional domains and VCP binding domain. CSRD (cysteine-serine rich domain), TBD (tubulin binding domain), LRD (leucine rich binding domain), HEAT (huntingtin, elongation factor 3, PR65/A, TOR), ARM (armadillo), RasGAP (Ras GTPase activating protein).

Methods

Formalin fixed paraffin embedded control and FTLT brain tissue was obtained from the Carroll A. Campbell, Jr. Neuropathology Laboratory. Samples were tested with ACD designed BaseScope probes for in situ hybridization and commercially available VCP, NF1, MAP2, and amyloid beta antibodies for immunohistochemical staining using standard protocols (Pathology HistoCore). Sections were analyzed with an Olympus BX53 microscope under 20X, 40X and 60X magnification. Bulk RNA-Seq and differentially expressed genes were identified using DESeq2 and subsequent GSEA analysis was performed on NF1-null glial cells compared to wild type cells.

Results

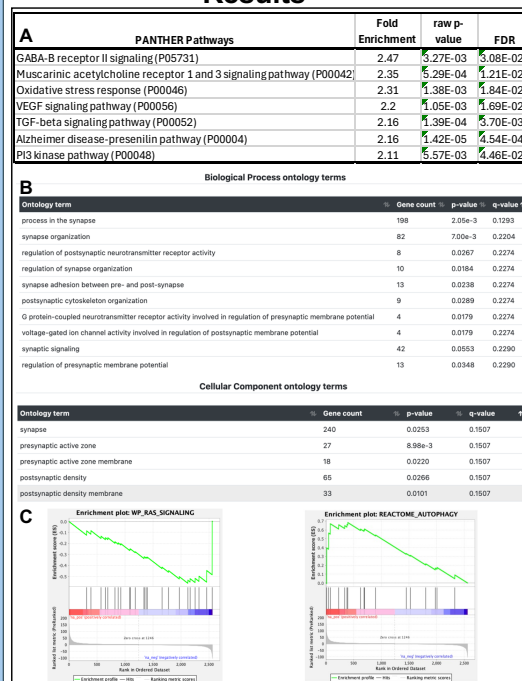


Figure 1. Bioinformatic analysis of DEGs identified from bulk RNA-Seq and DESeq2 analysis from NF1 null glial cells. (A) Top disrupted Panther pathways identified from an overrepresented gene set. (B) Gene Ontology cellular compartment terms identified via SynGo analysis. (C) GSEA enrichment plot of expected pathways to be disrupted by NF1 loss.

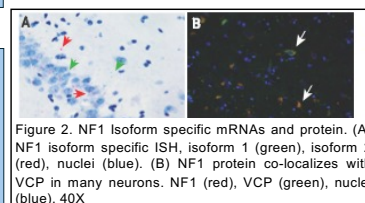


Figure 2. NF1 isoform specific mRNAs and protein. (A) NF1 isoform specific ISH, isoform 1 (green), isoform 2 (red), nuclei (blue). (B) NF1 protein co-localizes with VCP in many neurons. NF1 (red), VCP (green), nuclei (blue). 40X

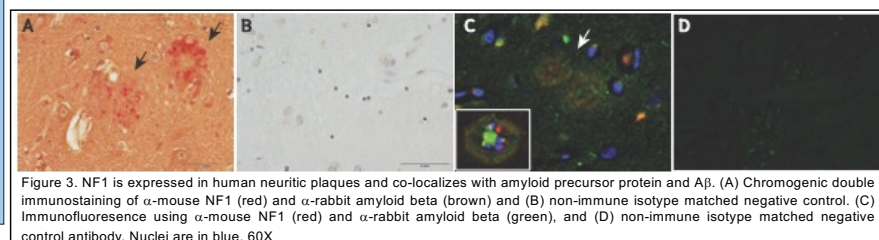


Figure 3. NF1 is expressed in human neuritic plaques and co-localizes with amyloid precursor protein and Aβ. (A) Chromogenic double immunostaining of α-mouse NF1 (red) and α-rabbit amyloid beta (brown) and (B) non-immune isotype matched negative control. (C) Immunofluorescence using α-mouse NF1 (red) and α-rabbit amyloid beta (green), and (D) non-immune isotype matched negative control antibody. Nuclei are in blue. 60X

Conclusion

In FTD/AD patients there is a mislocalization of neurofibromin in neurons. Understanding how this contributes to failures in ER-associated proteostasis provides a promising avenue for therapeutic development. We have found that:

1. Loss of NF1 disrupts:
 - synaptic function and signaling
 - oxidative stress response pathways
 - APP and Aβ processing
 - PI3K and Ras signaling
 - autophagy
2. NF1 isoforms are expressed in distinct cell types
3. NF1 and VCP co-localized in some neurons
4. NF1, Aβ & APP are co-expressed in neuritic plaques.

References

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