

Partners in crime: CYLD interactions with FTD-ALS proteins

Eilish Nesbitt,¹ Brynn Dowon Kim,^{1,2} Oriana Xing,¹ Jennifer Fifita,¹ Lauren Fitzpatrick,¹ Marianne Hallupp,¹ Albert Lee,³ Roger Chung,³ John B Kwok,¹ Carol Dobson-Stone¹

1. The University of Sydney, Brain and Mind Centre and School of Medical Sciences, Faculty of Medicine and Health, Camperdown; 2. Kids Neuroscience Centre, Kids Research, Children's Hospital at Westmead, Sydney, NSW 2145, Australia; 3. Centre for Motor Neuron Disease Research, Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, North Ryde; Australia

Introduction

Frontotemporal dementia (FTD) and amyotrophic lateral sclerosis (ALS) are clinically and pathologically overlapping disorders with shared genetic causes. We identified mutation of CYLD as a cause of disease in a European Australian family with FTD-ALS.¹ CYLD binds FTD-ALS proteins TBK1, OPTN & SQSTM1/p62, and proteomic analyses show several other FTD-ALS proteins as putative CYLD interactors.

We sought to investigate these interactions and identify whether known FTD- and/or ALS-causative mutations affect binding to CYLD.

Materials and methods

We curated a list of putative CYLD-interactor proteins through literature review.

We identified additional neuronal CYLD interactors by overexpression of FLAG-tagged CYLD in SH-SY5Y cells, followed by anti-FLAG immunoprecipitation (IP) and label-free mass spectrometry quantitative proteomic analysis.

Putative CYLD interaction partners were validated, and binding of wild-type versus mutant partners to CYLD was assessed, by co-IP in HEK293 cells overexpressing FLAG- and HA-tagged cDNA constructs.

Results

114 proteins were >1.5-fold enriched in the SH-SY5Y CYLD IP relative to control, including two known CYLD binding partners. From these data and those from two other large-scale CYLD IP studies,^{2,3} we prioritised 11 putative CYLD interactors with high relevance to FTD and/or ALS for further analysis. We verified CYLD binding to six proteins encoded by genes that have been reported with FTD- and/or ALS-causative mutations: VCP, MATR3, TUBA4A, DCTN1, DNAJB6, ERLIN2, plus key TDP-43-regulated axonal maintenance protein STMN2 (Fig 1).

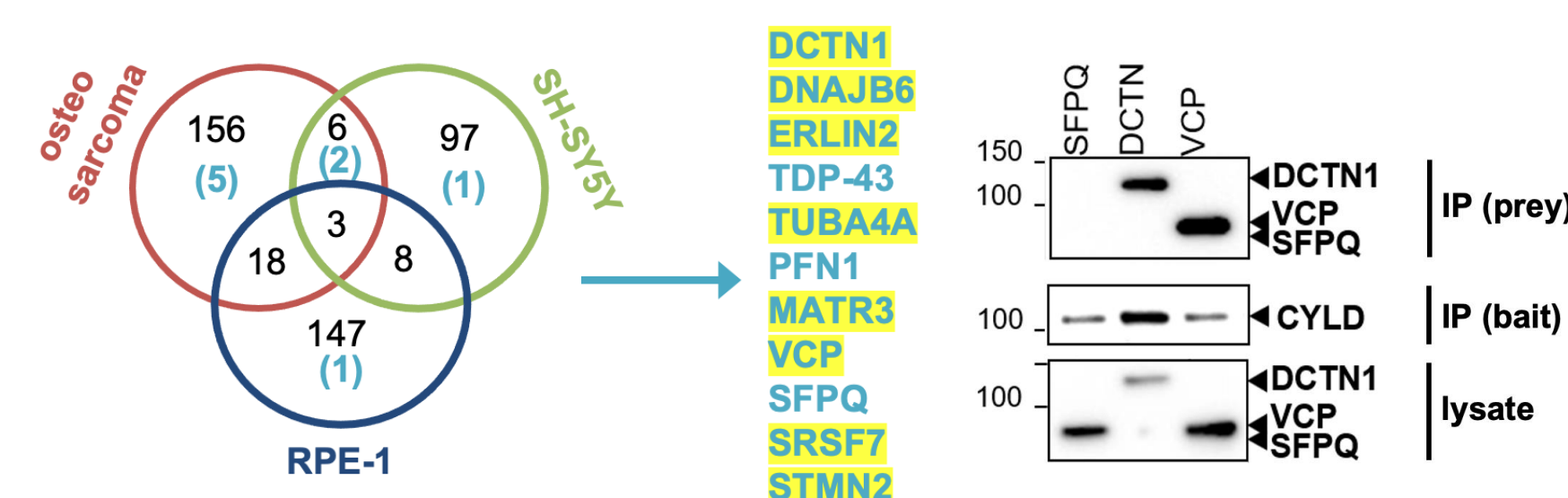


Fig 1. CYLD interacts with multiple FTD-ALS proteins. Left: overlap between CYLD IP studies conducted by us, Douanne *et al.*,² or Elliott *et al.*,³ indicating numbers of known FTD-ALS proteins. Right: coIP verifying binding of DCTN1 & VCP to CYLD.

Gene	Mutation
DCTN1	p.G59S
DNAJB6	p.P93L
ERLIN2	p.V168M
OPTN	p.E478G, p.H486R
SQSTM1	p.P392L
TBK1	p.E696K
TUBA4A	p.R320C
VCP	p.R155H

Table 1. Known pathogenic missense mutations selected for analysis of CYLD binding.

Of the nine known pathogenic missense mutations in eight FTD-ALS disease genes selected for analysis (Table 1), two led to a significant decrease in CYLD binding relative to wild-type: **TBK1_{E696K}** and **ERLIN2_{V168M}** (Fig 2).

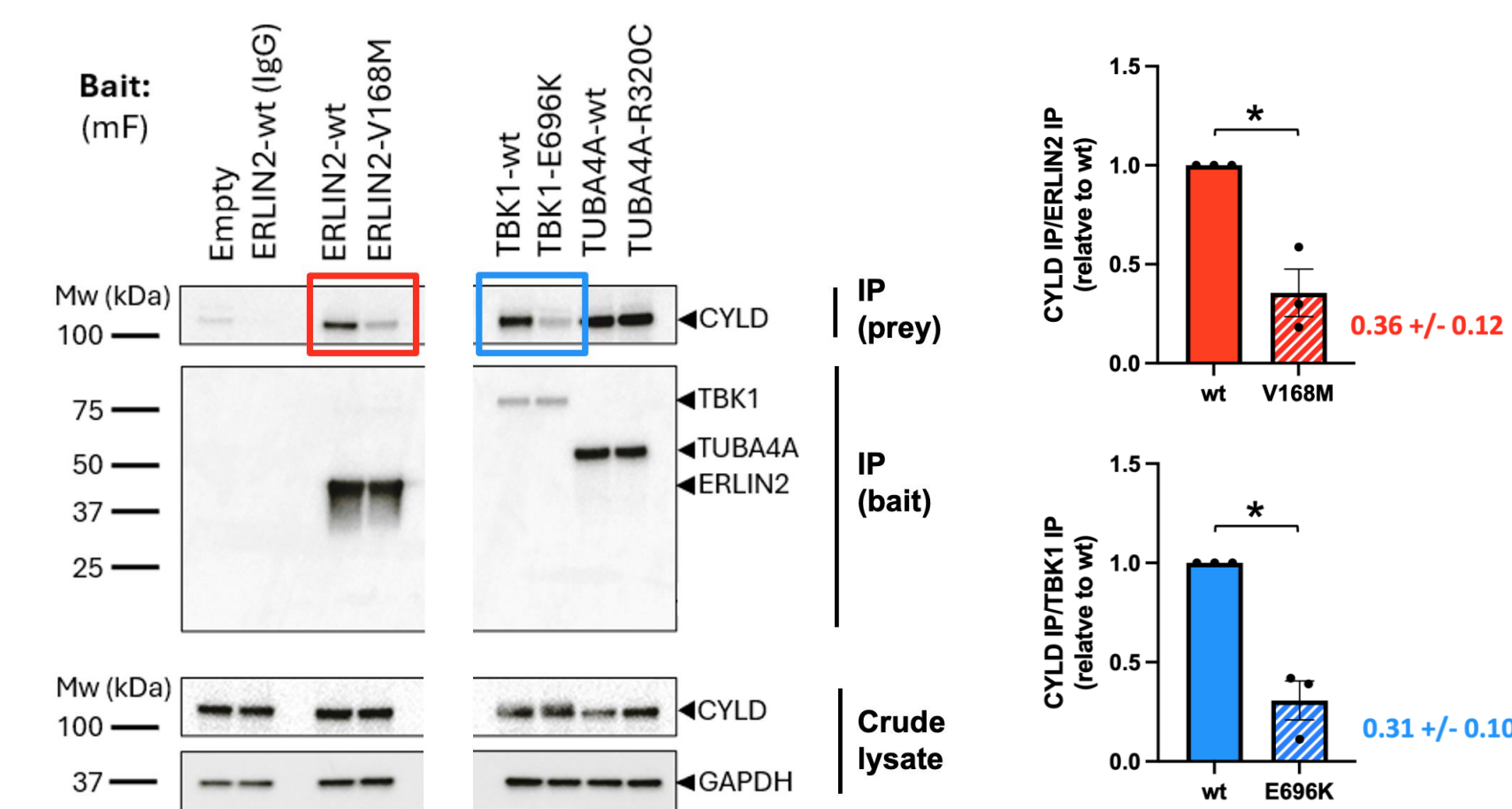


Fig 2. TBK1 E696K and ERLIN2 V168M show reduced binding to CYLD. Left: coIP analysis using FLAG-tagged partner proteins as bait and HA-tagged CYLD as prey. Right: quantitation of IP blots from n = 3 independent experiments. *, p < 0.05

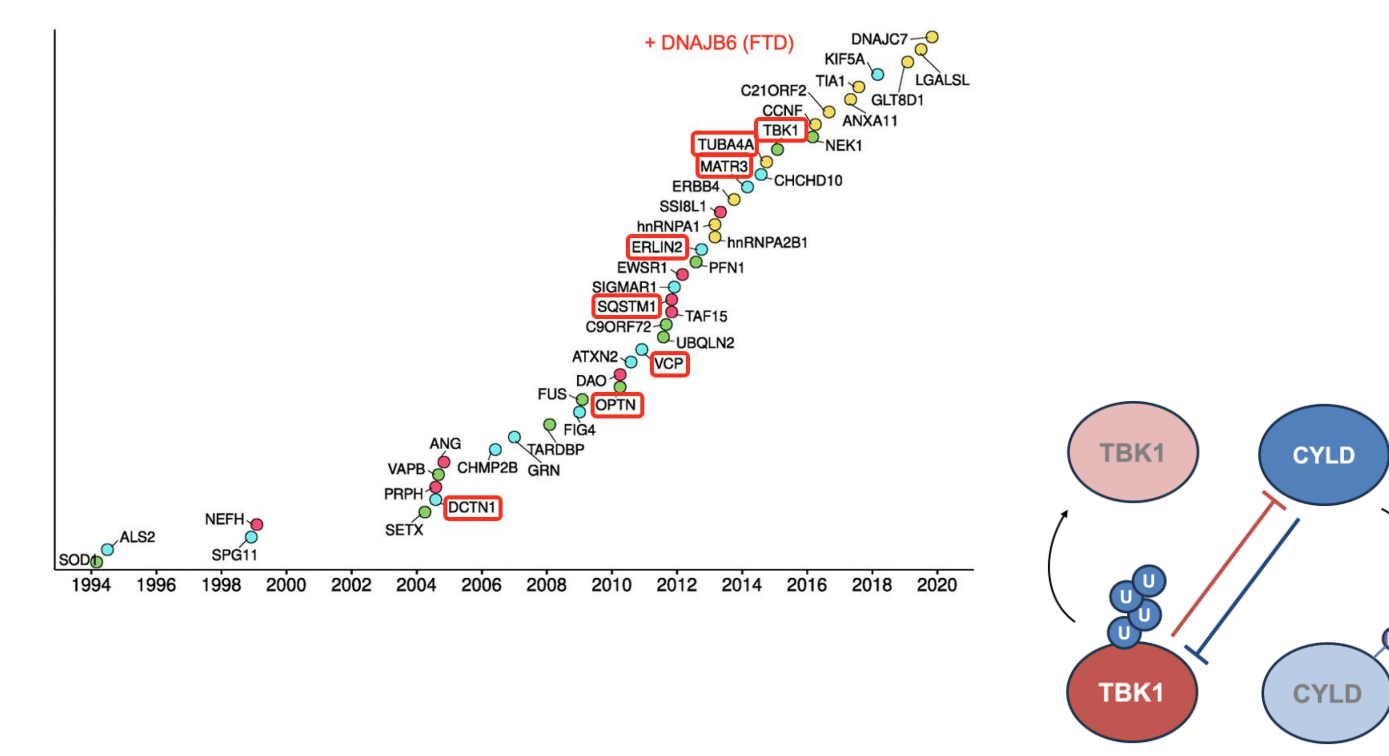


Fig 3. CYLD as a major interactor with FTD-ALS proteins. Left: Adapted from Ref 4. Right: Schematic of proposed reciprocal regulation of CYLD and TBK1 via deubiquitination and phosphorylation

Conclusions

Our study has identified a physical interaction between CYLD and several other FTD-ALS proteins (Fig 3), indicating that CYLD-partner protein binding may play a central role in FTD/ALS pathogenesis. Altered CYLD binding in two ALS-causative mutations lends further support to the idea that CYLD's interactions with these proteins may act as a mechanism of disease.

Most known pathogenic **TBK1** variants are null mutations: p.E696K is one of few known pathogenic missense mutations. CYLD & TBK1 inactivate each other (Fig 3), and therefore reduced binding could interrupt this negative feedback loop. TBK1 p.E696K also shows reduced binding to OPTN,⁵ implying that disruption of a CYLD/TBK1/OPTN complex may be important for pathogenesis.

ERLIN2 mutations usually cause 'pure' hereditary spastic paraplegia (HSP), but p.V168M causes HSP evolving to ALS. Common pathways for CYLD & ERLIN2 (and possible clues for pathogenic role) include cell-cycle regulation and microtubule binding.

CYLD is a lysine 63 deubiquitinase and the FTD-ALS-causative CYLD_{M719V} mutant shows a significant increase in deubiquitinase activity.¹ Future work should investigate whether these FTD/ALS-relevant partner proteins are targets for CYLD deubiquitination, which could suggest therapeutic possibilities.

References

- Dobson-Stone C *et al* Brain 2020;143:783-799.
- Douanne T *et al* Cell Rep 2019;27:1657-1665.e4.
- Elliott PR *et al* Mol Cell 2016;63:990-1005.
- Gregory JM *et al* Curr Genet Med Rep 2020;8:121-131.
- Freischmidt A *et al* Nat Neurosci 2015;18:631-636.

Acknowledgments

This work was funded by:
NHMRC Project Grant 1140708
Alzheimer's Association Research Grant 1143627



Australian Government
National Health and Medical Research Council

ALZHEIMER'S ASSOCIATION®

Further information

Please contact **Carol Dobson-Stone** at

carol.dobson-stone@sydney.edu.au

<https://www.linkedin.com/in/carol-dobson-stone-375a573/>