NEURAL REGENERATION RESEARCH
www.nrronline.org

SCFD1 in amyotrophic lateral sclerosis: reconciling a genetic association with *in vivo* functional analysis

Ruben J. Cauchi*

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by progressive loss of upper and lower motor neurons, resulting in muscle weakness and spasticity, eventually leading to death due to respiratory failure. Analyses by our group of a case-control cohort from an isolated island population have found that genetics plays a significant role in disease etiology (Farrugia Wismayer et al., 2023). In addition to rare variants that cause familial monogenic forms of the disease, genetic variants that are commonly found in the population have also been associated with disease risk. To this end, the latest landmark cross-ancestry genome-wide association study (GWAS) identified multiple risk loci in patients with sporadic ALS or those without a family history of the disease (van Rheenen et al., 2021). Top-ranking loci identified in this study included the *Sec1 Family Domain Containing 1* (*SCFD1*) gene and the *uncoordinated 13 homolog A* (*UNC13A*) gene based on association with the rs229195 and rs12608932 variants, respectively. Interestingly, although proteins encoded by *SCFD1* and *UNC13A* have similar functions in vesicle transport and disruption of this pathway is well known to induce motor neuron degeneration (Mead et al., 2022), establishing a relationship between these risk genes and ALS pathophysiology has been challenging. This is nonetheless imperative because risk loci can be therapeutically targeted in a broad spectrum of ALS patients in addition to pre-symptomatic individuals with a higher ALS risk. Notably, recent studies have attempted to discover a potential link between major GWAS-identified risk loci and disease mechanism (Brown et al., 2022; Ma et al., 2022; Borg et al., 2023). For *UNC13A*, mis-splicing of its messenger RNA (mRNA) transcript in ALS patients was found to result in lower protein levels with serious consequences for synaptic maintenance (Brown et al., 2022; Ma et al., 2022). Making use of a pre-clinical model, we have shown that synaptic deficits and the resulting decline in neuromuscular function can also result from reduced levels of SCFD1 (Borg et al., 2023; **Figure 1**). Importantly, disease predisposition from loss of *UNC13A* or *SCFD1* function may be intimately linked to protein misfolding and aggregation which remains a hallmark feature of ALS (Mead et al., 2022). Risk variants in the *UNC13A* locus are thought to be consequential in the absence of functional nuclear TDP-43 (Brown et al., 2022; Ma et al., 2022), a main constituent of cytoplasmic protein aggregates in ALS patients (Mead et al., 2022). A general downregulation of protein folding pathways may explain why the loss of SCFD1 leads to a decline in neuromuscular function (Borg et al., 2023).

SCFD1 was identified in 1991 in the yeast *Saccharomyces cerevisiae* as a suppressor of the functional loss of Ypt1, a GTP-binding protein essential for endoplasmic reticulum (ER) to Golgi protein transport (Dascher et al., 1991). The SCFD1 yeast orthologue was thus termed Sly1 for suppressor of loss of Ypt1 function. A member of the Sec1/Munc18-like protein family, SCFD1 is a medium-sized protein with a molecular weight of around 72 kDa. Subsequent studies have delineated the precise role of SCFD1 in ER to Golgi anterograde transport (Peng and Gallwitz, 2002; Laufman et al., 2009; Lobingier et al., 2014). SCFD1 ensures the correct assembly in addition to opposing the disassembly of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex, itself required for the fusion of ER-derived vesicles with Golgi membranes (Peng and Gallwitz, 2002; Lobingier et al., 2014). A role for SCFD1 in intra-Golgi and Golgi-to-ER retrograde transport has also been proposed due to direct interaction with the conserved oligomeric Golgi tethering complex (Laufman et al., 2009). The function of SCFD1 as a crucial regulator of ER-Golgi trafficking is conserved in mammals with depletion of SCFD1 in mammalian cells found to induce an ER stress response that leads to autophagy (Renna et al., 2011). Controlled by both acetylation- and phosphorylation-dependent mechanisms, SCFD1 was also found to have a role in autophagosome-lysosome fusion in mammalian cell cultures (Huang et al., 2023). Nonetheless, studies on animal models in which SCFD1 function is disrupted either in the whole organism or, selectively, in specific tissues, which would be critical for assessing whether SCFD1 plays an important role in maintaining the motor system *in vivo*, have been limited.

We have recently made use of *Drosophila*, a genetically tractable model organism, to investigate the *in vivo* consequences of SCFD1 depletion (Borg et al., 2023). More than two-thirds of the genes implicated in human disease are conserved in *Drosophila*, so it is not surprising that *Drosophila* also has a highly conserved SCFD1 orthologue known as SLY-1 homologous or Slh. We made use of RNAi-mediated gene silencing to first induce *Slh* knockdown in the whole organism starting at an early developmental stage. This allowed us to reveal that Slh is an essential protein similar to its counterpart in yeast (Dascher et al., 1991). Next, we assessed the behavior of flies with a moderate depletion of Slh in either muscle or neurons making use of an RNAi transgene gene-switch system. Intriguingly, we found that during adulthood, flies exhibited obvious neuromuscular deficits that progressively worsened with age, an obvious overlap with phenotypes observed in ALS patients. A stronger knockdown of Slh in either component of the neuromuscular system did not lead to viable adult flies. However, it induced paralysis in larvae which eventually died a few hours later as uncontracted puparia. We succeeded in linking these motor deficits with defects in motor synapses, which are a known sign of ALS pathology. Through analysis of the neuromuscular junctions of motor neurons innervating body wall muscles that larvae use for locomotion, we uncovered a profound decrease in neuromuscular junction area and complexity on the loss of Slh function (**Figure 1**). To delve

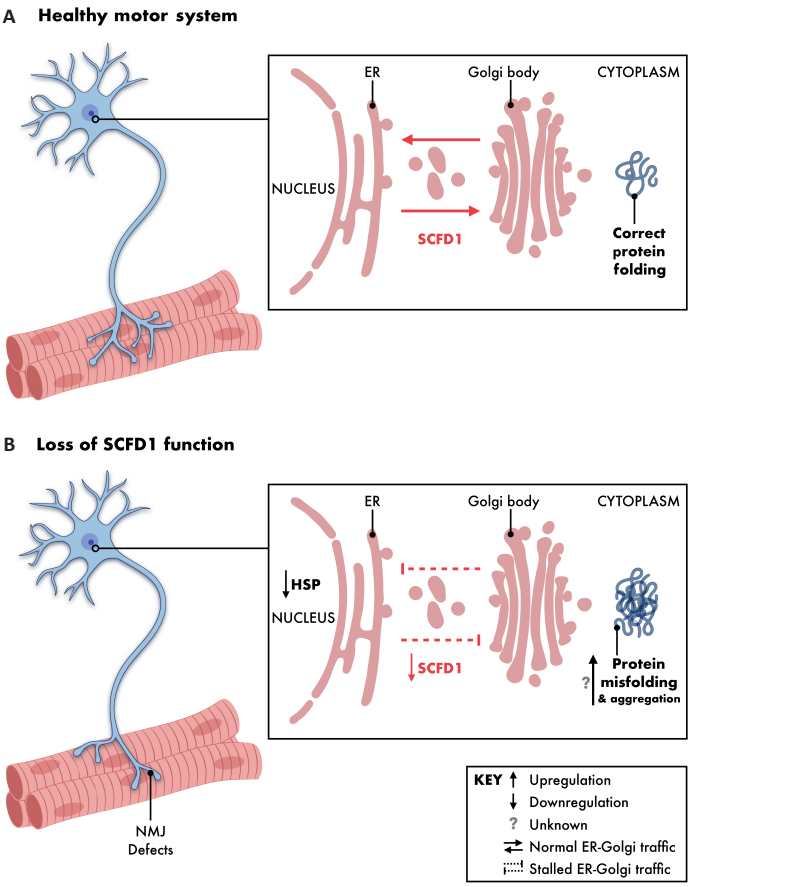

Figure 1 | **Loss of SCFD1 function leads to loss of neuromuscular function and erodes mechanisms protecting against misfolding and protein aggregation in an *in vivo* model.**
(A) In a healthy motor system, the function of SCFD1 in ER to Golgi vesicular trafficking ensures proteostasis. (B) In *Drosophila*, reduced SCFD1 levels lead to motor system dysfunction as manifested by NMJ defects and loss of neuromuscular performance. Decreased chaperoning activity due to low levels of HSPs is hypothesized to result in a cellular environment that is prone to the accumulation of malformed proteins. This can potentially increase ALS risk or aggravate pre-existing alterations in protein homeostasis. Created with BioRender.com. ALS: Amyotrophic lateral sclerosis; ER: endoplasmic reticulum; HSPs: heat shock proteins; NMJ: neuromuscular junction; SCFD1: Sec1 Family Domain Containing 1.

deeper into the molecular events triggering these phenotypes, we carried out RNA sequencing in these flies and surprisingly discovered that several genes encoding heat shock proteins (HSPs) were amongst the top-most downregulated genes. Hence, Gene Ontology enrichment analysis confirmed that protein folding or refolding were the most significantly downregulated pathways.

In addition to its identification as an ALS risk gene via GWAS (van Rheenen et al., 2021), *SCFD1* was also shown to be a top-most significant expression quantitative trait locus (eQTL) for ALS (Iacoangeli et al., 2021). To this end, for *SCFD1* eQTL rs8005942, SCFD1 expression was found increased in post-mortem motor cortex tissues of ALS patients compared to controls for homozygotes of the rarer genotype (AA) and this also correlated with a reduction in ALS survival (Iacoangeli et al., 2021). A recent proteome-wide association study (Gu et al., 2023) and two independent transcriptome-wide association studies also reported higher SCFD1 levels in ALS patient-derived brain tissue (*SCFD1* eQTL rs2070339) (Saez-Atienzar et al., 2021; Gu et al., 2023). At face value, a role for SCFD1 in ALS via a gain-of-function mechanism does not appear to concur with our *in vivo* data (Borg et al., 2023), which suggests just the opposite. Nonetheless, increased *SCFD1* expression detected post-mortem might reflect a protective response mechanism occurring during advanced stages of the disease. Loss of SCFD1 function during the early stages of the disease is therefore still plausible and this is in agreement with our functional results in the *Drosophila* model system (Borg et al., 2023) and, is also supported by a blood-based transcriptome-wide association study that reported an association between low SCFD1 expression (flagged by *SCFD1* eQTL rs7144204) and increased ALS risk (Saez-Atienzar et al., 2021).

Prior to our study, whether a factor with a housekeeping role in ER-Golgi trafficking is required for maintaining a functional motor system was unknown. Furthermore, how loss of SCFD1 expression might increase ALS risk was unclear. Our findings have addressed these questions, mostly underscoring that vesicle transport remains central to the physiology of the neuromuscular system as supported by an increasing number of ALS causing genes encoding proteins with a role in this pathway including *ALS2*, *SPG11*, *VABP*, *FIG4*, *CAV1*, *OPTN* and *NEK1* (Mead et al., 2022). Importantly, transcriptomic alterations downstream of *Slh* gene silencing, which point to a general downregulation of protein folding pathways, can serve as a plausible mechanism through which reduced SCFD1 levels can predispose to ALS.

Damaging variants or expansions in the major genes linked to familial ALS including *C9orf72*, *SOD1*, *TARDBP*, and *FUS* all lead to protein aggregates of different species. Despite an absence of mutations in *TARDBP*, its encoding gene, TDP-43 protein misfolding, and aggregation occurs in nearly all patients with sporadic ALS, which by far is the predominant form of the disease (Mead et al., 2022). Aggregated proteins cause damage to several processes that are essential for motor neuron function and survival (Mead et al., 2022). Misfolded proteins are either refolded or cleared by HSPs. Multiple lines of evidence indicate that chaperoning activity or its lack of it is therefore central to ALS pathophysiology. First, motor neurons and muscle cells are highly inefficient at mounting a heat shock stress response. Consequently, HSP downregulation is expected to further increase the vulnerability of the motor system to proteome stress, hence increasing ALS risk. Second, similar to our findings, reduced levels of HSPs have been reported in ALS animal models and patient-derived tissues or cells. Third, *DNAJC7*, which encodes HSP40 has been identified as a novel ALS-associated gene. Fourth, several studies are supportive of an amelioration of ALS symptoms by upregulation of HSPs. Most importantly, the sodium phenylbutyrate component in the recently FDA-approved AMX0035 (Relyvrio), is a histone deacetylase inhibitor that increases chaperone expression (Mead et al., 2022; Borg et al., 2023 and references therein).

Several lines of research are warranted to reconcile GWAS, proteome-wide association study, and transcriptome-wide association study findings with *in vivo* functional data with the aim of confirming a role for SCFD1 in ALS pathophysiology. It is presently unclear whether SCFD1 downregulation or upregulation or both are associated with an increase in ALS risk. Our findings in the *Drosophila* model system suggest that SCFD1 can lead to ALS through a loss-of-function mechanism and this may well be the case in the early stages of the disease. Studies that aim at defining temporal differences in SCFD1 expression in patient samples or cell culture models can provide crucial answers. Alternatively, the consequences of a gain of SCFD1 function on the motor system *in vivo* remains unknown and should be investigated. Work that defines the precise mechanism driving the transcriptome alterations in *Slh* flies and whether depressed expression of chaperones at the RNA level translates into increased protein aggregation is also crucially important. To this end, the impact of SCFD1 downregulation in established ALS animal models should also be investigated to confirm whether this worsens established phenotypes or leads to an earlier onset of symptoms. Importantly, it would be interesting to demonstrate whether the aggregate formation is exacerbated on the loss of SCFD1 function. Recent studies on the role of SCFD1 in ALS including ours provide new impetus to better define its contribution to the disease mechanism. This is an essential step that can potentially inform us on meaningful therapeutic strategies aimed at improving motor function in ALS patients.

This work was supported by the Malta Council for Science & Technology Fusion R&I Research Excellence Programme, the Malta Council for Science & Technology Internationalisation Partnership Award, and the Anthony Rizzo Memorial ALS Research Fund facilitated by the Research Trust (RIDT) of the University of Malta (to RJC).

Ruben J. Cauchi*
Centre for Molecular Medicine and Biobanking, Biomedical Sciences Building; Department of Physiology and Biochemistry, Faculty of Medicine and Surgery, University of Malta, Msida, Malta
***Correspondence to:** Ruben J. Cauchi, PhD, ruben.cauchi@um.edu.mt.
https://orcid.org/0000-0001-6150-1608 (Ruben J. Cauchi)

Date of submission: June 29, 2023
Date of decision: August 25, 2023
Date of acceptance: September 5, 2023
Date of web publication: October 2, 2023

https://doi.org/10.4103/1673-5374.386411
How to cite this article: *Cauchi RJ (2024) SCFD1 in amyotrophic lateral sclerosis: reconciling a genetic association with in vivo functional analysis. Neural Regen Res 19(6):1201-1202.*

References

Borg R, Purkiss A, Cacciottolo R, Herrera P, Cauchi RJ (2023) Loss of amyotrophic lateral sclerosis risk factor SCFD1 causes motor dysfunction in Drosophila. Neurobiol Aging 126:67-76.

Brown AL, Wilkins OG, Keuss MJ, Hill SE, Zanovello M, Lee WC, Bampton A, Lee FCY, Masino L, Qi YA, Bryce-Smith S, Gatt A, Hallegger M, Fagegaltier D, Phatnani H, Consortium NA, Newcombe J, Gustavsson EK, Seddighi S, Reyes, JF et al. (2022) TDP-43 loss and ALS-risk SNPs drive mis-splicing and depletion of UNC13A. Nature 603:131-137.

Dascher C, Ossig R, Gallwitz D, Schmitt HD (1991) Identification and structure of four yeast genes (SLY) that are able to suppress the functional loss of YPT1, a member of the RAS superfamily. Mol Cell Biol 11:872-885.

Farrugia Wismayer M, Farrugia Wismayer A, Borg R, Bonavia K, Abela A, Chircop C, Aquilina J, Soler D, Pace A, Vella M, Vassallo N, Cauchi RJ (2023) Genetic landscape of ALS in Malta based on a quinquennial analysis. Neurobiol Aging 123:200-207.

Gu XJ, Su WM, Dou M, Jiang Z, Duan QQ, Wang H, Ren YL, Cao B, Wang Y, Chen YP (2023) Identifying novel genes for amyotrophic lateral sclerosis by integrating human brain proteomes with genome-wide association data. J Neurol 270:4013-4023.

Huang H, Ouyang Q, Mei K, Liu T, Sun Q, Liu W, Liu R (2023) Acetylation of SCFD1 regulates SNARE complex formation and autophagosome-lysosome fusion. Autophagy 19:189-203.

Iacoangeli A, Fogh I, Selvackadunco S, Topp SD, Shatunov A, van Rheenen W, Al-Khleifat A, Opie-Martin S, Ratti A, Calvo A, Consortium UKBE, Van Damme P, Robberecht W, Chio A, Dobson RJ, Hardiman O, Shaw CE, van den Berg LH, Andersen PM, Smith BN, et al. (2021) SCFD1 expression quantitative trait loci in amyotrophic lateral sclerosis are differentially expressed. Brain Commun 3:fcab236.

Laufman O, Kedan A, Hong W, Lev S (2009) Direct interaction between the COG complex and the SM protein, Sly1, is required for Golgi SNARE pairing. EMBO J 28:2006-2017.

Lobingier BT, Nickerson DP, Lo SY, Merz AJ (2014) SM proteins Sly1 and Vps33 co-assemble with Sec17 and SNARE complexes to oppose SNARE disassembly by Sec18. Elife 3:e02272.

Ma XR, Prudencio M, Koike Y, Vatsavayai SC, Kim G, Harbinski F, Briner A, Rodriguez CM, Guo C, Akiyama T, Schmidt HB, Cummings BB, Wyatt DW, Kurylo K, Miller G, Mekhoubad S, Sallee N, Mekonnen G, Ganser L, Rubien JD, et al. (2022) TDP-43 represses cryptic exon inclusion in the FTD-ALS gene UNC13A. Nature 603:124-130.

Mead RJ, Shan N, Reiser HJ, Marshall F, Shaw PJ (2022) Amyotrophic lateral sclerosis: a neurodegenerative disorder poised for successful therapeutic translation. Nat Rev Drug Discov:1-28.

Peng R, Gallwitz D (2002) Sly1 protein bound to Golgi syntaxin Sed5p allows assembly and contributes to specificity of SNARE fusion complexes. J Cell Biol 157:645-655.

Renna M, Schaffner C, Winslow AR, Menzies FM, Peden AA, Floto RA, Rubinsztein DC (2011) Autophagic substrate clearance requires activity of the syntaxin-5 SNARE complex. J Cell Sci 124:469-482.

Saez-Atienzar S, Bandres-Ciga S, Langston RG, Kim JJ, Choi SW, Reynolds RH, International ALSGC, Italsgen, Abramzon Y, Dewan R, Ahmed S, Landers JE, Chia R, Ryten M, Cookson MR, Nalls MA, Chio A, Traynor BJ (2021) Genetic analysis of amyotrophic lateral sclerosis identifies contributing pathways and cell types. Sci Adv 7:eabd9036.

van Rheenen W, van der Spek RAA, Bakker MK, van Vugt J, Hop PJ, Zwamborn RAJ, de Klein N, Westra HJ, Bakker OB, Deelen P, Shireby G, Hannon E, Moisse M, Baird D, Restuadi R, Dolzhenko E, Dekker AM, Gawor K, Westeneng HJ, Tazelaar GHP, et al. (2021) Common and rare variant association analyses in amyotrophic lateral sclerosis identify 15 risk loci with distinct genetic architectures and neuron-specific biology. Nat Genet 53:1636-1648.

C-Editors: Zhao M, Liu WJ, Qiu Y; T-Editor: Jia Y