Article:
Ugbode, C. and West, R.J.H. orcid.org/0000-0001-9873-2258 (2021) Lessons learned from CHMP2B, implications for frontotemporal dementia and amyotrophic lateral sclerosis. Neurobiology of Disease, 147. 105144. ISSN 0969-9961

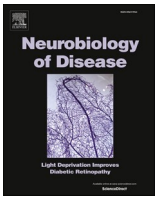
https://doi.org/10.1016/j.nbd.2020.105144

Neurobiology of Disease 147 (2021) 105144

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi



Review

Lessons learned from CHMP2B, implications for frontotemporal dementia and amyotrophic lateral sclerosis

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ARTICLE INFO

Keywords:
Neurodegeneration
Therapeutics
Immunity
Proteostasis
FTD
ALS
ESCRT
Motor neurone disease
CHMP2B

ABSTRACT

Frontotemporal dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS) are two neurodegenerative diseases with clinical, genetic and pathological overlap. As such, they are commonly regarded as a single spectrum disorder, with pure FTD and pure ALS representing distinct ends of a continuum. Dysfunctional *endo*-lysosomal and autophagic trafficking, leading to impaired proteostasis is common across the FTD-ALS spectrum. These pathways are, in part, mediated by CHMP2B, a protein that coordinates membrane scission events as a core component of the ESCRT machinery. Here we review how ALS and FTD disease causing mutations in CHMP2B have greatly contributed to our understanding of how endosomal-lysosomal and autophagic dysfunction contribute to neurodegeneration, and how *in vitro* and *in vivo* models have helped elucidate novel candidates for potential therapeutic intervention with implications across the FTD-ALS spectrum.

1. Introduction

Frontotemporal Dementia (FTD) is a common cause of early-onset dementia with a typical age of onset under 65 years. FTD is frequently used as an umbrella term referring to a heterogeneous group of neurodegenerative disorders associated with Frontotemporal lobar degeneration (FTLD), a progressive atrophy of the frontal and temporal cortices. These include behavioural variant FTD (bvFTD), primary progressive aphasia and semantic dementia. Amyotrophic Lateral Sclerosis (ALS) is the most common form of motor neurone disease and is characterised by the progressive atrophy and dysfunction of upper and lower motor neurons. FTD and ALS show significant genetic, neuropathological and clinical overlap with approximately 14% of FTD patients displaying concomitant motor neurone disease (Phukan et al., 2012). This has led to the general consensus that FTD and ALS represent a continuum of a single spectrum disorder. Impaired proteostasis and dysfunctional endosomal-lysosomal and autophagic trafficking are common across the FTD-ALS spectrum. Here we review how FTD and ALS disease causing mutations in Charged Multivesicular Body Protein 2B (CHMP2B), have greatly contributed to our understanding of how endosomal-lysosomal and autophagic dysfunction contribute to neurodegeneration, with implications across the FTD-ALS spectrum.

2. CHMP2B structure and function

CHMP2B is an evolutionary conserved, 213 amino acid protein encoded by the *CHMP2B* gene (Fig. 1A-B). It is an essential component of the Endosomal Sorting Complex Required For Transport III (ESCRT-III), which with ESCRTs 0, I and II play a fundamental role in membrane scission events during the biogenesis of multivesicular bodies (MVBs) and sorting of endosomal cargos (Fig. 1C). The ESCRT machinery also controls other fundamental cellular processes including cytokinesis (Bhutta et al., 2014; Caballe and Martin-Serrano, 2011), viral exocytosis (Lata et al., 2009), endo/lysosomal repair (Radulovic et al., 2018) and autophagy (Rusten and Stenmark, 2009). CHMP2B is a core component of the ESCRT-III complex, which plays a part in all these processes. It has been shown to be expressed in multiple human tissues, in all major regions of the brain (Skibinski et al., 2005) and in every cell type in the central nervous system (CNS) (Cahoy et al., 2008; Zhang et al., 2014).

CHMP2B contains two N-terminal coiled coil domains (1–50 and 120–150) (Fig. 1B), peptide repeats found throughout all kingdoms of life (Liu and Rost, 2001). Coiled coil domains are purported to function

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https://doi.org/10.1016/j.nbd.2020.105144
Received 17 August 2020; Received in revised form 16 October 2020; Accepted 23 October 2020
Available online 2 November 2020

as molecular scaffolds, mediating events including vesicle tethering (Truebestein and Leonard, 2016; Witkos and Lowe, 2015) and playing an integral role in communicating conformational change within a cell (Liu et al., 2006; Redwine et al., 2012). Though limited in sequence homology, the 11 human CHMP proteins show various commonalities. They are all components of the ESCRT-III complex (Morita, 2012; Olmos and Carlton, 2016), are of a similar size, have predicted coiled-coil domains and importantly display highly asymmetric charge distribution, with negatively charged basic residues at the N-terminus and acidic positively charged residues at the C-terminus (Carlton and Martin-Serrano, 2009; Howard et al., 2001; Lata et al., 2009). The C-terminal region of CHMP2B contains a MIT-interacting motif (MIM) (Fig. 1B) which is essential for interaction with proteins containing corresponding microtubule interacting and transport (MIT) domains. Vps4 was identified as a putative binding partner for CHMP2 proteins (Babst et al., 1998). Mutations in Vps4 (Finken-Eigen et al., 1997) and genetic silencing of CHMP genes in yeast disrupted endosomes, identifying profound deficits in vesicle trafficking (Howard et al., 2001). This regulation was originally thought to be mediated by the N-terminal coiled coil regions of the CHMP proteins, however GST-pulldown assays showed that Vps4 interacts with the acidic C-terminus of CHMP proteins (Scott et al., 2005). The MIM domain of CHMP2B is composed of a single helix that binds between two of the 3 helices in the MIT domain of Vps4 and is essential for ESCRT function (Krasniak and Ahmad, 2016).

The most well described role of CHMP2B is during its regulation of MVB biogenesis (Fig. 1C). Invagination and budding off of the endosomal limiting membrane allows the formation of MVB intraluminal vesicles, into which ubiquitinated proteins are internalised and sorted for degradation *via* the lysosome, or trafficking back to the plasma membrane (Babst, 2006; Lata et al., 2009; Schmidt and Teis, 2012). This

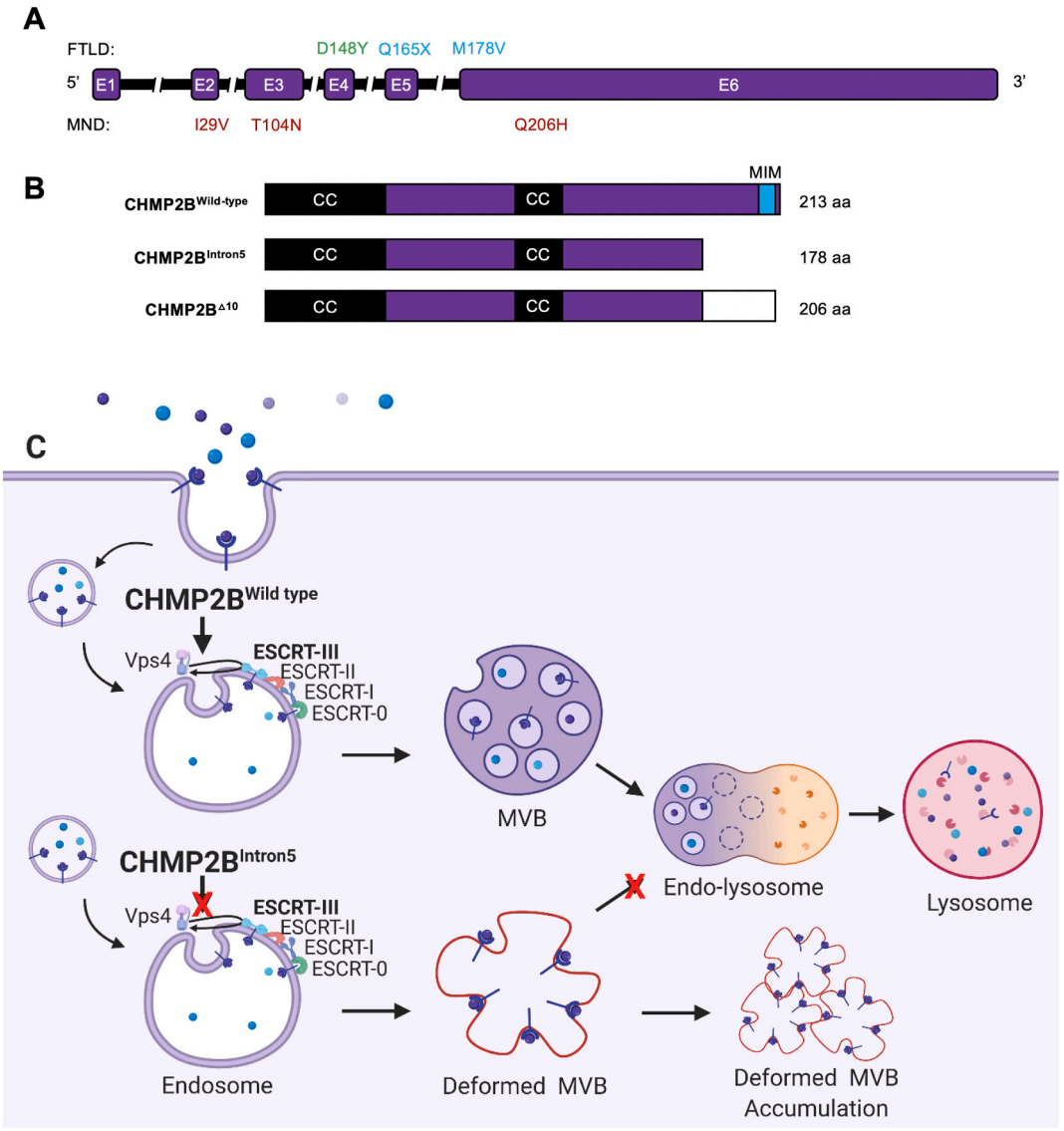

Fig. 1. CHMP2B Structure and Function.
A. CHMP2B gene schematic showing some of the mutations associated with FTLD (D148Y (Semantic Dementia, Green), Q165X and M178V (CHMP2B^{Intron5}) (bvFTD, Blue)) and Motor Neurone Disease (I29V, T104N and Q206H (Primary Muscular Atrophy, Red)). B. CHMP2B is a 213 amino acid protein containing two N-terminal coiled coil domains (CC) (1–50 and 120–150) and a C-terminal MIT-interacting motif (MIM) essential for interacting with Vps4. The FTLD mutation M178V results in two C-terminally truncated proteins, CHMP2B$^{\Delta 10}$ and CHMP2B^{Intron5}, resulting in a loss of the MIM domain and acidic alpha helices, reducing auto inhibition.
C. CHMP2B is a core component of ESCRT-III, which acts sequentially with other ESCRTs in the biogenesis of multivesicular bodies (MVB's) and the sorting of ubiquitinated cargo into intraluminal vesicles during endosomal-lysosomal trafficking. The altered C-terminus of CHMP2B^{Intron5} prevents membrane scission, leading to accumulation of deformed MVB's containing ubiquitinated proteins, incapable of fusing with the lysosome. Adapted from "Generic Endocytic Pathway", by BioRender.com (2020). Retrieved from https://app.biorender.com/biorender-templates.

process requires coordinated activation of ESCRTs 0-III and the Vps4 complex. Briefly, ESCRT-0, composed of HGS (Vps27) and STAM (HSE) heterodimers, localizes to endosomes through an interaction with phosphatidylinositol-3-phosphate (PI3P) (Morita, 2012; Olmos and Carlton, 2016; Schmidt and Teis, 2012). When localized to endosomes, ESCRT-0 binds to ubiquitinated proteins initiating the first stage of the process. The presence of ESCRT-0 on endosomes triggers the recruitment of the cytosolic ESCRT-I, a soluble hetero-tetramer consisting of TSG101 (Vps23), Vps28, Vps37(A–D) and MVB12. ESCRT-I also binds ubiquitinated proteins and then interacts with ESCRT-II. ESCRT-II, another hetero-tetrameric complex consisting of Vps36, SNF8 (Vps22) and two Vps25 molecules, causes membrane invagination and triggers the stepwise assembly of the core regulators of this process, ESCRT-III and the Vps4 complex. ESCRT-0-II represent stable cytoplasmic complexes however the assembly of ESCRT-III is transient and coordinated *via* sequential activity of ESCRT-III proteins (Morita, 2012; Schmidt and Teis, 2012). Firstly, CHMP6 (Vps20) is activated by ESCRT-II, triggering the oligomerization of CHMP4 (Snf7) (Schmidt and Teis, 2012). Recent work from Bertin et al. (2020) demonstrated that CHMP4B filaments, in the absence of other ESCRT-III proteins, bind preferentially to flat membranes (Bertin et al., 2020). CHMP3/Vps24 then terminates the assembly of ESCRT-III filaments on endosomal membranes and recruits CHMP2A and B, which complete the assembly of the core components of the ESCRT-III complex (Schmidt and Teis, 2012). In contrast to CHMP4B, CHMP2A/B and CHMP3 prefer positively curved membranes (Bertin et al., 2020).

Activation of CHMP2A/B encourages the recruitment of the Vps4 complex. This complex consists of the AAA-(ATPases Associated with diverse cellular Activities) ATPase Vps4, and its cofactor Vta1. During unstimulated conditions, CHMP2B exists in an auto-inhibited state, with the acidic C-terminus bound to the basic N-terminus, masking the CHMP2B domains that interact with members of the ESCRT-III complex (Krasniak and Ahmad, 2016; Schmidt and Teis, 2012). Upon activation, the N-terminal MIT domain of Vps4 binds the C-terminal MIM domain of CHMP2B, which begins the process of recruiting the Vps4 complex to ESCRT-III. When Vps4 assembles on ESCRT-III, it forms a dodecamer, a ring shaped oligomer with a central pore. The binding of Vta1 to Vps4 activates its ATPase activity and *via* the physical interaction with CHMP2B, using energy generated from ATP, Vps4 constricts the neck of intraluminal vesicles, coordinating a scission event, freeing vesicle cargoes into the lumen of the MVB (Krasniak and Ahmad, 2016; Morita, 2012; Olmos and Carlton, 2016; Schmidt and Teis, 2012). As such, CHMP2B and Vps4 are critical for this process. Vps4 then regulates ESCRT-III disassembly, which dissociates into inactive protomers (Schmidt and Teis, 2012) in order to coordinate the next round of MVB biogenesis.

ESCRT-III has also been identified as a core regulator of endo/lysosomal repair. L-leucyl-L-leucine *O*-methyl ester (LLOME) is commonly used to rupture endolysosomes (Aits et al., 2015; Maejima et al., 2013; Thiele and Lipsky, 1990). LLOME is cell permeable and accumulates in the lumen of acidified organelles, where through a reaction with the lysosomal enzyme cathepsin C, is condensed into a membranolytic polymer. The damage to lysosomal membranes triggers the rapid recruitment of ESCRT proteins, which accumulate on damaged lysosomes and facilitate their repair. This protective response to lysosomal disruption precedes lysophagy and promotes cell survival (Radulovic et al., 2018).

Along with regulating lysosomal repair, ESCRT-III has also been described as a regulator of macroautophagy (Feng et al., 2014; Krasniak and Ahmad, 2016; Lee et al., 2007; Lu et al., 2013). This cellular event, where autophagosomes coordinate the bulk degradation and recycling of cytoplasmic material and organelles through fusion with the lysosome, represents a fundamental process across species (Feng et al., 2014). Loss of ESCRT genes has been shown to cause accumulation of autophagosomes in yeast (Roudier et al., 2005), *Drosophila* (Rusten et al., 2007) and mammals (Hara et al., 2006; Komatsu et al., 2006) suggesting that autophagosomal fusion with the lysosome requires ESCRT subunits. This has been further demonstrated in mammalian neurons, where loss of CHMP4B/Snf7-2 causes autophagosome accumulation, as well as dendritic retraction and neuronal cell death (Lee et al., 2007). This implicates ESCRT function in the regulation of autophagy in neurons and shows that basal levels of autophagy are essential for the maintenance of neuronal health.

Consistent with the regulation of these fundamental cellular processes, ESCRT proteins have been demonstrated to regulate key processes within both the developing and adult nervous system. This includes sorting of synaptic proteins, neurite outgrowth and dendrite and axon pruning (reviewed in Sadoul et al., 2018). Of particular importance to neurodegenerative disease, is the observation that CHM2PB and its association with CHMP4B, is critical to the maintenance of mushroom shaped spines. Depletion of CHMP2B decreases dendrite length and spine volume, resulting in a concomitant reduction of synaptic efficacy, in a chemical model of long-term potentiation (LTP) (Chassefeyre et al., 2015; Sadoul et al., 2018). As previously mentioned, genetic silencing of CHMP proteins also disturbs endosomal generation and trafficking. Defective endosomal-lysosomal signalling has been described in many neurodegenerative diseases, including ALS and FTD (Burk and Pasterkamp, 2019; Lee et al., 2007; Nixon, 2005; Sasaki, 2011). Most recently in Alzheimer's disease, where knockdown of CHMP6 and double knockdown of CHMP2A + B accelerated the propagation of pathological tau *in vitro* (Chen et al., 2019). Here, we focus on mutations in CHMP2B, how these mutations affect CHMP2B function and what this means in the context of FTD and ALS.

3. CHMP2B in FTD and ALS

The identification of CHMP2B as a causative FTD loci came in 2005, when a Danish cohort diagnosed with a familial pre-senile dementia, distinct from Alzheimer's (Gydesen et al., 1987), were shown to have a rare autosomal dominant heterozygous mutation in the CHMP2B gene (Skibinski et al., 2005). The disease causing mutation was identified as a G-to-C transition within the splice acceptor site of the 6th, and final, exon of CHMP2B. The resultant, aberrant mRNA splicing was shown to produce two novel transcripts; CHMP2B$^{\Delta 10}$ and CHMP2B$^{\text{Intron5}}$, identified in the brains of FTD patients at approximately 10% and 35% of wild-type CHMP2B transcript levels, respectively. Translation of these transcripts leads to the formation of two C-terminally truncated proteins with the terminal 36 amino acids replaced with either a valine residue (CHMP2B$^{\text{Intron5}}$ (M178V)) or a 29 amino acid nonsense sequence (CHMP2B$^{\Delta 10}$) (Fig. 1B). Subsequent C-terminal truncating mutations identified include a novel missense mutation, CHMP2B$^{\text{Q165X}}$, in a Belgian patient (van der Zee et al., 2008) and CHMP2B$^{\text{R186X}}$ in two asymptomatic siblings of a familial, autosomal dominant FTD patient (Momeni et al., 2006). A rare sporadic mutation, CHMP2B$^{\text{D148Y}}$, has also been identified in semantic dementia (Skibinski et al., 2005). Prior to the identification of specific CHMP2B mutations, the disease-associated gene in the original Danish family was mapped to the pericentromeric region of chromosome 3, leading to the designation of CHMP2B-related FTD as chromosome 3-linked frontotemporal dementia (FTD3) (Brown et al., 1995; Gydesen et al., 2002). Mutations in CHMP2B as a cause of FTD-ALS are uncommon, however different mutations have now been identified across the FTD-ALS spectrum, including FTD (Ghanim et al., 2010; Skibinski et al., 2005; van der Zee et al., 2008), ALS (Cox et al., 2010; Narain et al., 2018; Parkinson et al., 2006; van Blitterswijk et al., 2012) and FTD with ALS (Parkinson et al., 2006). A number of CHMP2B mutations (I29V, T104N and Q206H) have been identified in progressive muscular atrophy (PMA), a rare form of motor neuron disease (Fig. 1A) (Cox et al., 2010). Further mutations have been observed in related neurodegenerative diseases falling outside the FTD-ALS spectrum, including corticobasal degeneration (van der Zee et al., 2008) and Alzheimer's disease (Hooli et al., 2014).

Although the mutations in CHMP2B are rare, the importance of

CHMP2B to the FTD-ALS spectrum does not lie in the number of cases, but instead in the distinctive pathology observed. In contrast to the majority of FTD causative loci, mutations in CHMP2B are associated with TDP-43 negative, ubiquitin and/or p62 positive inclusions (Holm et al., 2007). As such, they are commonly categorised as a distinct pathological subtype, FTLD-UPS (FTLD-Ubiquitin Proteasome System). Despite the lack of TDP-43 positive inclusions in CHMP2B^{Intron5} patients or models, the ESCRT machinery and MVBs have been implicated in the clearance of TDP-43 (Filimonenko et al., 2007). Both TSG101 and CHMP3 knockdown result in TDP-43 accumulations colocalising with ubiquitin and p62 positive cytoplasmic inclusions in HeLa cells (Filimonenko et al., 2007). This was not observed in CHMP2B^{Intron5} or CHMP2B$^{\Delta 10}$ cells. Furthermore, a recent study demonstrated that knockdown of CHMP2B was sufficient to alleviate toxicity associated with overexpression of TDP-43 in both *Drosophila* and mammalian cell models (Sun et al., 2020). CHMP2B knockdown alleviated the perturbed, rough eye phenotype observed when human TDP-43 (hTDP-43) was expressed in the fly eye, as well as reduced longevity and impaired climbing ability when hTDP-43 was pan-neuronally expressed (Sun et al., 2020). In contrast to previous findings, Sun et al., also demonstrated increased TDP-43 hyperphosphorylation and insolubility in CHMP2B^{Intron5} models. This was associated with reduced turnover of Casein Kinase 1 alpha 1 (CSNK1A1/CK1), which is known to phosphorylate TDP-43 (Hasegawa et al., 2008; Kametani et al., 2009; Sun et al., 2020). The presence of hyperphosphorylated TDP-43 in cellular CHMP2B^{Intron5} models is a distinct parallel to the absence of TDP-43 staining in CHMP2B^{Intron5} patient tissue. Nonetheless, given that endocytosis regulates TDP-43 toxicity and turnover (Liu et al., 2017), CHMP2B^{Intron5} perturbs endocytic trafficking (Urwin et al., 2010) and knockdown of CHMP2B prevents TDP-43 toxicity; it is clear that there is a complex relationship between CHMP2B and TDP-43 which warrants further investigation. Furthermore, these observations suggest potentially independent mechanisms by which the ESCRT machinery may contribute towards both TDP-43 positive and negative forms of FTD-ALS, highlighting the importance of understanding the role of the ESCRT machinery in neurodegeneration across the FTD-ALS spectrum.

CHMP2B mutations are largely characterised by perturbations to normal endosomal-lysosomal and autophagic trafficking, resulting in the aberrant accumulation of enlarged endosomes and autophagic organelles (Lee et al., 2007; Nielsen et al., 2012; Skibinski et al., 2005; Urwin et al., 2010; West et al., 2020; Zhang et al., 2017). However, an increasing body of evidence highlights the importance of CHMP2B in interconnected signalling cascades linking proteostasis, immune signalling, redox biology and neuronal homeostasis. These observations have been determined by a number of *in vitro* and *in vivo* models that have provided insights into the mechanisms underpinning CHMP2B-related neurodegeneration, with relevance across the FTD-ALS spectrum.

4. Endosomal-lysosomal dysfunction

The pathogenic CHMP2B^{Intron5} mutation has been extensively modelled *in vitro*. The earliest reports monitored the expression of N-terminal myc-tagged CHMP2B^{Intron5} and CHMP2B$^{\Delta 10}$ in PC12 cells (Skibinski et al., 2005). Transient transfection of PC12 cells revealed that wild type CHMP2B shows a diffuse cytosolic distribution (Skibinski et al., 2005). In contrast CHMP2B^{Intron5} and CHMP2B$^{\Delta 10}$ induced aberrant punctate cytoplasmic inclusions. Large cytoplasmic inclusions in CHMP2B^{Intron5} cells stained positive for the endosomal/lysosomal marker CD63. Fluorescent dextran, used as an exogenous marker of the endosomal pathway, also accumulated in the larger inclusions of CHMP2B^{Intron5} positive cells. CHMP2B^{Intron5} cells also displayed smaller inclusions, however these showed minimal dextran or CD63 co-localisation (Skibinski et al., 2005). In contrast, only small inclusions partially colocalizing with dextran or CD63 were observed in CHMP2B$^{\Delta 10}$ cells (Skibinski et al., 2005). Accumulation of large endosomes has been observed in a number of CHMP2B FTD models (Table 1) including rat primary neurons transfected with CHMP2B^{Intron5} (Lee et al., 2007; West et al., 2020), patient derived fibroblasts and cortical tissue (Urwin et al., 2010). The accumulation of aberrant endosomes associated with CHMP2B^{Intron5} is due to its failure to dissociate from CHMP4B during MVB biogenesis (Lee et al., 2007). Immunoprecipitation of CHMP4B from HEK293 cells revealed 3.5× more CHMP2B^{Intron5} bound to CHMP4B than wild type CHMP2B (Lee et al., 2007). Ectopically expressed CHMP2B^{Intron5} sequestered CHMP4B into Rab7 positive vesicular structures, indicating that dysfunctional ESCRT-III assembles on late endosomes (Lee et al., 2007). Expression of a dominant negative Vps4 induced a similar phenotype, indicating that dysfunctional assembly and disassembly of ESCRT-III may contribute to neuronal cell loss (Lee et al., 2007).

In primary neurons CHMP2B^{Intron5} aggregates also co-localise with Rab4, Rab5 and Rab7, markers of recycling, early and late endosomes (West et al., 2020). The localisation of these aggregates is similar to other CHMP2B mutations (T104N) which, although less toxic, also co-localise with Rab5 and Rab7 positive endosomes (Han et al., 2012). Accumulation of recycling, early and late endosomes was also observed in *Drosophila* models pan-neuronally expressing CHMP2B^{Intron5} under the control of the pan-neuronal driver, neuronal *synaptobrevin* (*nSyb*) Gal4 (West et al., 2020). In both rat primary neuron and *Drosophila* models, aberrant accumulation of ubiquitinated proteins, a hallmark of FTD-3, was also observed (Lee et al., 2007; West et al., 2020).

The first CHMP2B^{Intron5} mouse model was described by Ghazi-Noori et al. in 2012. Expression of human CHMP2B^{Intron5} using the hamster prion promoter, driving strong expression in the hippocampus, thalamus and cerebellum Purkinje cells, recapitulated aspects of FTD3, including accumulation of ubiquitin/p62 positive aggregates, negative for TDP-43 (Ghazi-Noori et al., 2012). Subsequent studies identified a significant accumulation of autofluorescent aggregates, distinct from p62 aggregates, in the thalamus (significant *vs* CHMP2B wild type and non-transgenic controls at 12 months) and cortex (significant *vs* CHMP2B wild type and non-transgenic controls at 18 months)(Clayton et al., 2015). Autofluorescent aggregates were identified in neurons and microglia and were shown to be surrounded by LAMP-1 and LAMP-2 positive membranes, suggesting they are endosomal in origin. Accumulation of ubiquitin positive inclusions and an increase in insoluble p62 was also observed in a mouse model described by Gascon et al., 2014, in which CHMP2B^{Intron5} was expressed within forebrain neurons, using a tetracycline inducible Calcium/Calmodulin Dependent Protein Kinase II Alpha (CamK2a) promoter system (Gascon et al., 2014). A third CHMP2B^{Intron5} mouse model was described by Vernay et al. (2016) (Vernay et al., 2016). This mouse expressed human CHMP2B^{Intron5} under the control of the mouse Thy1.2 promoter, a promoter proposed to express strongly in central and peripheral neurons, including both cortical and motor neurons. Whilst heterozygous expression of CHMP2B^{Intron5} showed mRNA levels comparable to endogenous CHMP2B, mRNA levels were 3.4-fold higher in homozygotes. Semi-quantitative analysis demonstrated that both hetero and homozygous mice show an age-dependent accumulation of ubiquitin and p62 aggregates with the nervous system (Frontal cortex, corpus callosum, thalamus, brainstem and spinal cord). It must be noted that in contrast to previous studies, in which CHMP2B^{Intron5} mice were compared to mice overexpressing CHM2B$^{Wild\ type}$ at the same genomic locus, this study looked at both heterozygous and homozygous CHMP2B^{Intron5} *versus* non-transgenic controls.

5. Autophagy

Accumulation of aggregated proteins in neurodegenerative disorders has been suggested to be caused, at least in part, by the decline of cellular degradative processes, notably autophagy (Monaco and Fraldi, 2020). Autophagosome accumulation has been identified as a common feature of CHM2B-related FTD models (Table 1). Microtubule-associated

Table 1
Phenotypes observed in different CHMP2B^{Intron5} models.

Pathway	Phenotype	Model	Reference
Endosomal-Lysosomal and Autophagic Dysfunction	Ubiquitin and/or p62 aggregates	HeLa	Filimonenko et al., 2007
		Fly & Rat Primary Neurons	West et al., 2020
		Rat Primary Neurons	Lee et al., 2007
		Rat Primary Neurons	Lee and Gao, 2009
		Mouse	Ghazi-Noori et al., 2012
		Mouse	Gascon et al., 2014
		Mouse	Vernay et al., 2016
	Accumulation of Enlarged Autophagosomes	Rat Primary Neurons	Lee et al., 2007
		Rat Primary Neurons	Lee and Gao, 2009
		HeLa	Filimonenko et al., 2007
		Fly & Rat Primary Neurons	West et al., 2020
	Accumulation of Enlarged Endosomes	Rat Primary Neurons	Lee et al., 2007
		Patient Fibroblasts	Nielsen et al., 2012
		Rat Primary Neurons	Lee and Gao, 2009
		PC12	Skibinski et al., 2005
		Patient fibroblasts & cortex	Urwin et al., 2010
		iPSC	Zhang et al., 2017
		Fly & Rat Primary Neurons	West et al., 2020
		Mouse	Ghazi-Noori et al., 2012
	dendritic retraction	Rat primary neurons	West et al., 2020
Neuronal Homeostasis and Cell DeathDeath	dendritic retraction	Rat primary neurons	Lee et al., 2007
	decrease the number of mushroom spines	Rat primary neurons	Belly et al., 2010
	elevated p53	iPSC	Zhang et al., 2017
	elevated p53	Fly	West et al., 2020
	elevated apoptosis markers	Fly and Rat GPNT	West et al., 2018, West et al., 2020
	Perturbed Neuronal Growth/Moprhology	Fly	West et al., 2015
	neuronal cell loss / cortical volume	Mouse	Clayton et al., 2017
	increase in immature spines and dysregulation of AMPAR composition	Mouse	Gascon et al., 2014
	Impaired neuromuscular function and distal axonopathy	Mouse	Vernay et al., 2016
Immunity	Increase in Toll signalling	Fly	Ahmad et al., 2009
		Fly & Mouse	
	Accumulation of SH3RF1/POSH		West et al., 2018
	Genetic Interaction between CHMP2B and TBK1	Fly	Lu et al., 2020
	gliosis and proinflamatory cytokine elevation	Mouse	Clayton et al., 2017
	gliosis	Mouse	Gascon et al., 2014
	gliosis	Mouse	Vernay et al., 2016
	motor / behavioural dysfunction	Fly	West et al., 2020
Other	motor / behavioural dysfunction	Mouse	Clayton et al., 2017
	motor / behavioural dysfunction	Mouse	Gascon et al., 2014
	motor / behavioural dysfunction	Mouse	Vernay et al., 2016
	redox dysfunction	iPSC	Zhang et al. (2017)
	redox dysfunction	Fly and Rat neuroepithelial	West et al., 2020
	circadian dysfunction	Fly	Lee et al., 2019
	developmental dysfunction	Fly	Cheruiyot et al., 2014
	developmental dysfunction	Fly	Wilson et al. (2019)
	Impaired retroviral silencing	Fly and Rat primary neurons	Fort-Aznar et al., 2020

protein 1A/1B-light chain 3 (LC3) positive autophagosomes were observed in CHMP2B^{Intron5} transfected rat cortical neurons using GFP-LC3 (Lee et al., 2007; Lee and Gao, 2009). These GFP-LC3 positive autophagosomes were shown to form after the initial appearance of ubiquitylated-protein-containing vesicles (Lee et al., 2007). Monitoring autophagic flux, using the pH-sensitive tandem tagged GFP-mCherry-LC3 dual colour construct (Pankiv et al., 2007), also revealed expression of CHMP2B^{Intron5} in rat primary neurons resulted in an aberrant accumulation of large GFP-mCherry-LC3 double labelled puncta, suggesting an impairment of autophagosome-lysosome fusion (West et al., 2020). In contrast, neurons expressing wild type CHMP2B rarely showed GFP positive puncta, indicating normal autophagosome-lysosome fusion leading to quenching of the pH-sensitive GFP tag. These observations were recapitulated *in vivo* using *Drosophila*, identifying autophagosome accumulation and dysfunctional phagosomal-lysosomal fusion as a conserved feature of CHMP2B^{Intron5} pathology (West et al., 2020).

Autophagosome accumulation in CHMP2B^{Intron5} neurons has been proposed to be a contributing factor in neuronal cell death (Filimonenko et al., 2007; Lee et al., 2007; Lee and Gao, 2008). Pharmacological inhibition of autophagy, using 3-Methyladenine, or genetic knockdown of ATG5 and ATG7 was shown to delay CHMP2B^{Intron5} induced neuronal cell loss (Lee and Gao, 2009). It is important to note however, that despite the prevention of cell death, inhibition of autophagy does not rescue the endosomal hallmarks associated with the mutation, suggesting that deficits in endo-lysosomal trafficking may cause neurodegeneration independent of the neurons autophagic status (Lee and Gao, 2009). Recent evidence demonstrated the FDA approved compound Ursodeoxycholic Acid (UDCA) as a potent neuroprotectant in *Drosophila* and primary neuronal models of CHMP2B^{Intron5}. UDCA was shown to act downstream of defective endosomal/lysosomal and autophagosomal trafficking, suggesting novel potential therapeutic targets also exist downstream of endosomal-lysosomal and autophagic dysfunction (West et al., 2020).

6. Neuronal homeostasis and cell death

In addition to impaired proteostasis associated with perturbations to the endosomal-lysosomal and autophagic trafficking pathways, CHM2PB has also been implicated in neuronal homeostasis (Table 1). The importance of CHMP2B in neuronal homeostasis became evident when CHMP2B^{Intron5} and CHMP2B$^{\Delta 10}$ mutations were expressed in primary cortical neurons. Ectopic expression of CHMP2B^{Intron5} caused severe dendritic retraction after three days in culture (Lee et al., 2007). Concomitant experiments where neurons expressed CHMP2B$^{\Delta 10}$ or CHMP2B^{D148Y} showed minimal dendrite loss (Lee et al., 2007). CHMP2B^{Intron5} has also been demonstrated to decrease the number of mushroom spines in primary neurons (Belly et al., 2010).

Supporting a role for CHMP2B^{Intron5} in perturbed neuronal homeostasis *in vivo*, an increase in the number of immature spines was observed in pyramidal neurons in the medial prefrontal cortex of CHMP2B^{Intron5} mice (Gascon et al., 2014). This was accompanied by an age-dependent downregulation of miR-124 and a concomitant dysregulation of AMPAR subunit composition, correlating with the age of onset of behavioural phenotypes (Gascon et al., 2014). miR-124 downregulation and concomitant increase in AMPARs was also shown in iPSC-derived cortical neurons and in the frontal cortex of bvFTD patients (Gascon et al., 2014). Electrophysiological analysis suggests social deficits in CHMP2B^{Intron5} mice was linked to an increase in Ca^{2+}-impermeable AMPARs at excitatory synapses of pyramidal neurons in the prefrontal cortex. Dysregulation of AMPAR subunit composition has been implicated in a number of ALS variants including C9orf72 and SOD1, as well as sporadic ALS cases (Gregory et al., 2020; Kawahara et al., 2003; Kwak et al., 2010). Variation in which AMPAR subunits are up- or down-regulated may provide insight into both converging and diverging mechanisms in different FTD and ALS variants. Endosomal-lysosomal trafficking and sorting of AMPARs in MVBs plays a critical role in AMPAR dynamics and synaptic homeostasis (Parkinson and Hanley, 2018). Ubiquitination and efficient sorting of AMPARs allows targeted recycling, sorting and degradation of AMPARs within neurons. Endosomal-lysosomal dysfunction therefore underpins a number of neurodegenerative diseases, resulting in aberrant AMPAR recycling and degradation. Approaches to rectify the altered trafficking of AMPARs as a result of endosomal-lysosomal dysfunction are therefore a significant target for therapeutic intervention. As such elucidating the role of CHMP2B and ESCRT machinery in AMPAR dynamics in CHMP2B-related FTD-ALS represents an important area of research with implications across the disease spectrum.

In addition to perturbed neuronal structure and function, expression of CHMP2B^{Intron5} has been implicated in neuronal death and upregulation of apoptotic cascades in both *in vitro* and *in vivo* models. *Drosophila* pan-neuronally expressing CHMP2B^{Intron5} display increased levels of p53 and cleaved DCP-1, the fly homolog of caspase 3 (West et al., 2020; West et al., 2018). Elevated levels of *p53* mRNA have also been shown in patient-derived iPSC neurons (Zhang et al., 2017). This was shown to be reduced when the CHMP2B^{Intron5} mutation is isogenised with CRISPR/Cas9 (Zhang et al., 2017). Furthermore, neuroepithelial cells expressing CHMP2B^{Intron5} display increased levels of cleaved caspase 3, a key regulator of apoptosis, suggesting that neuronal cell death may be regulated by the p53/caspase 3 pathway (West et al., 2018). Neuronal cell death has also been observed in mouse models. At 18 months, mice showed a significant loss of thalamic and cortical volume, coupled with a significant reduction in the number of neurons present within the thalamus (Clayton et al., 2017). Aberrant accumulation of POSH/SH3RF1, a known regulator of JNK-dependent apoptotic cascades was also observed in the Gascon mouse model and in *Drosophila* pan-neuronally expressing CHMP2B^{Intron5} (West et al., 2018). POSH/SH3RF1 has also been implicated in the regulation of calcium homeostasis and immune signalling cascades, providing a potential link between neuronal homeostasis, apoptosis and immune signalling in FTD/ALS.

7. Immunity

The first *in vivo* model of FTD3 was the *Drosophila* CHMP2B^{Intron5} model reported by the Gao and Sweeney labs (Ahmad et al., 2009). This model used the UAS/Gal4 system to allow cell/tissue specific control of *CHMP2B^{Intron5}* expression in *Drosophila* and has formed the foundation for a number of significant studies elucidating the molecular mechanisms underpinning CHMP2B^{Intron5} associated toxicity, *in vivo* (Ahmad et al., 2009; Cheruiyot et al., 2014; Fort-Aznar et al., 2020; Lee et al., 2019; Lu et al., 2020; Lu et al., 2013; West et al., 2015; West et al., 2020; West et al., 2018; Wilson et al., 2020). Many of these studies utilised the genetic power of *Drosophila* for genome wide, *in vivo*, modifier screens, followed by genetic dissection of identified modifier loci. Having previously demonstrated, *in vitro*, that CHMP2B^{Intron5} toxicity resulted from a failure of CHMP2B to dissociate from CHMP4B (Lee et al., 2007). Ahmad et al. (2009) showed that expression of a GFP tagged Shrub (the *Drosophila* CHMP4B orthologue), which acts as a dominant negative, phenocopied CHMP2B^{Intron5} (Ahmad et al., 2009). An unbiased, dominant genome-wide (covering ~75% of the *Drosophila* genome), modifier screen using expression of *shrub-GFP* in the fly eye identified 29 potent modifier loci. Secondary screens tested strong shrub-GFP enhancers against CHMP2B^{Intron5} expression. The initial screen employed chromosomes carrying large defined deletions (Ryder et al., 2007), followed by more refined deletions and finally individual gene mutations to identify genes that, when heterozygous, dominantly enhanced or suppressed the CHMP2B^{Intron5} eye phenotype. Analysis of individual loss-of-function alleles covered by the strongest enhancer (*Df(3R)ED5664)*, a deletion on the right arm of chromosome 3 covering 55 genes (2 partially), demonstrated loss of function of the serine protease inhibitor Serpin 88Ea (Serpin 88Ea/Serpin5) as a potent enhancer of CHMP2B^{Intron5} toxicity. Spn88Ea overexpression partially rescued CHMP2B^{Intron5} toxicity (Ahmad et al., 2009). Characterisation of Spn88Ea identified it to be a negative regulator of Toll signalling pathways, leading to the observation of a 3.4 fold increase in Toll (Both the toll receptor and the toll ligand cleaved-spaetzle) in the heads of CHMP2B^{Intron5} flies. This increase was coupled with an elevation of the *Drosophila* antimicrobial peptide, drosomycin, a transcriptional target of Toll. Together these findings identified Toll signalling as a major signalling pathway disrupted by CHMP2B^{Intron5} *in vivo* (Ahmad et al., 2009).

As part of the same genome wide modifier screen that identified Spn88Ea, West et al. (2015) identified that mutations in the small GTPase Rab8 significantly potentiated CHMP2B^{Intron5} toxicity (West et al., 2015). Rab8 is a known interactor of Optineurin (OPTN) and was subsequently identified as a downstream target of C9orf72 GDP/GTP exchange factor activity, identifying potential common pathways in ALS disease pathology (Corbier and Sellier, 2017; Hattula and Peranen, 2000). Analysis of synaptic morphology at the *Drosophila* 3rd instar larval neuromuscular junction, revealed synaptic overgrowth in Rab8 mutants was driven by aberrant JNK and TGFβ signalling (West et al., 2015). Elevated JNK signalling was mediated by TAK1 and POSH/SH3RF1, which together form part of the *Drosophila* Immune deficiency (IMD) signalling pathway, a component of the *Drosophila* innate immune system homologous to mammalian TNFR signalling (Tsuda et al., 2005). POSH/SH3RF1 was subsequently shown to accumulate within the nervous system of both *Drosophila* and mouse CHMP2B^{Intron5} models, mediating aberrant JNK-dependent apoptotic cascades (West et al., 2018). Knockdown of POSH/SH3RF1 was sufficient to alleviate aberrant synaptic growth at the *Drosophila* larval NMJ, in larvae pan-neuronally expressing CHMP2B^{Intron5}, as well as early pupal/pharate lethality. POSH/SH3RF1 shRNA knockdown could also alleviate dendritic collapse in primary mammalian neurons transfected with CHMP2B^{Intron5}. Studies have also shown that POSH/SH3RF1 directly interacts with and can ubiquitinate PDCD6IP/ALIX, which in turn has been shown to associate with and regulate ESCRT assembly (Christ et al., 2016; Votteler et al., 2009).

In a more recent targeted screen looking at the genetic interaction between known FTD and ALS loci, I-kappaB kinase ε (IKKε/IK2), the *Drosophila* orthologue of Tank-binding kinase 1 (TBK1), was identified as a potent modifier of CHMP2B^{Intron5} (Lu et al., 2020). TBK1, an IKK kinase, has been implicated in the regulation of both immune signalling cascades and autophagy, in part through phosphorylation of OPTN (Richter et al., 2016). As such, genetic interaction between CHMP2B^{Intron5} and TBK1 further underlines potential interplay between autophagic and immune signalling pathways in FTD-ALS spectrum disorders. These findings also contribute to a developing framework of known FTD-ALS loci and interactors, including TBK1, OPTN, CHMP2B, Rab8, Ubiquilin-2 and C9Orf72 involved in these pathways.

All three CHMP2B^{Intron5} mice models have been reported to display gliosis, supporting a conserved role of immune signalling cascades in neurodegeneration (Gascon et al., 2014; Ghazi-Noori et al., 2012; Vernay et al., 2016). At just 3 months of age the mouse model reported by Ghazi-Noori et al. (2012) showed a significant increase in Iba-1 positive microglia throughout the hippocampus and thalamus. By 18 months, gliosis was accompanied by a significant increase in pro-inflammatory cytokines (Clayton et al., 2017).

Taken together these studies elucidate novel components of immune signalling cascades that interact with, or are regulated by, the ESCRT machinery and contribute towards neurodegeneration in CHMP2B^{Intron5} models of FTD-ALS. Perturbed immune signalling pathways, neuroinflammation and microgliosis are common across the FTD-ALS spectrum (McCauley and Baloh, 2019). Unravelling the mechanisms by which CHMP2B^{Intron5} elicits aberrant immune activation may identify conserved regulators of neurodegeneration and potential therapeutic targets.

8. Other mechanisms

Studying CHMP2B from the perspective of basic biology and disease, has demonstrated the profound effect CHMP2B has in the regulation of neuronal homeostasis, proteostasis and immune signalling cascades. Models of CHMP2B^{Intron5} have also identified more diverse, overlapping, biological mechanisms at play, with broad relevance across the FTD-ALS spectrum.

The majority of genome wide screens in *Drosophila* used the eye specific driver *GMR*-Gal4 to express CHMP2B^{Intron5} in the fly eye. A recent study demonstrated *GMR*-Gal4 also drives expression in a subset of timeless expressing neurons in the *Drosophila* optic lobe (Lee et al., 2019). Expression of CHMP2B^{Intron5} in these neurons causes a reduction of timeless transcripts during light periods and a shortening of locomotor free running during dark:dark cycles (Lee et al., 2019). These findings suggest CHMP2B^{Intron5} can disrupt the function of the circadian clock. While classical ALS and FTD symptoms may directly perturb sleep, a growing body of evidence suggests that circadian dysfunction is not merely a symptom and may actually drive pathogenesis early in the course of neurodegeneration. This has been observed in a number of neurodegenerative disorders where disturbances have been shown to precede classical symptoms (Musiek and Holtzman, 2016; Videnovic et al., 2014). Recent studies have also shown a critical, bidirectional interplay between the circadian clock and biological mechanisms, including inflammation, proteostasis, metabolism and redox homeostasis during neurodegeneration (reviewed in Musiek and Holtzman (2016) and Videnovic et al. (2014)).

Few groups have monitored the oxidative stress burden in CHMP2B^{Intron5} cells, however oxidative stress has long been linked to neurodegeneration in FTD and ALS. Deficits in redox homeostasis have been identified by Zhang et al. (2017) in iPSC derived neurons from CHMP2B^{Intron5} patients. This is an important and useful tool for the field, given the rare nature of the mutation and subsequent lack of patient tissue. This *in vitro* model identified aberrant endosomes and mitochondria in CHMP2B^{Intron5} neurons compared to CHMP2B$^{Wild\ type}$ controls. CHMP2B^{Intron5} mitochondria in patient neurons had substantial deficits in mitochondrial capacity, including significant reductions in basal and maximum respiration as well as spare respiratory capacity. These deficits were alleviated when the CHMP2B^{Intron5} mutation was repaired using CRISPR/Cas9. Transcriptomic analysis of CHMP2B^{Intron5} neurons, compared to isogenic controls, identified a cluster of genes known to be involved in iron homeostasis. It was posited that deficits in iron homeostasis may increase intracellular ferrous ions (Fe^{2+}), contributing to the ongoing endosomal pathology (Zhang et al., 2017). Consistent with this hypothesis, a significant increase in intracellular Fe^{2+} was identified in CHMP2B^{Intron5} neurons, compared to isogenic controls. A significant increase in the protein level of transient receptor potential channel 6 (TRPC6), a channel implicated in iron and zinc uptake in PC12 cells and primary cortical neurons, was also observed (Knutson, 2019; Mwanjewe and Grover, 2004; Zhang et al., 2017). Impaired iron metabolism and homeostasis has been implicated in a number of neurodegenerative disorders including Parkinson's disease, Huntington's disease and ALS (Muller and Leavitt, 2014; Petillon et al., 2018; Yu et al., 2018). Furthermore, lysosomal degradation and autophagy have been shown to play an important role in the regulation of iron levels and iron metabolism (Jacomin et al., 2019; Kurz et al., 2008). In a more recent study it was found that *Drosophila* models pan-neuronally expressing *CHMP2B^{Intron5}* exhibited a significant increase in the Glutathione S-Transferase D1-GFP reporter (West et al., 2020). Furthermore, overexpression of the catalytic and modifying subunits of glutamate cysteine ligase (GCL) prevents dendritic retraction in *CHMP2B^{Intron5}* expressing primary rat neurons, suggesting that dendrite pathology may be linked to defective redox homeostasis (West et al., 2020). Pan-neuronal co-expression of the catalytic subunit of GCL (GCLC) with *CHMP2B^{Intron5}* was also sufficient to rescue perturbed neuronal morphology at the *Drosophila* third instar neuromuscular junction, as well as impaired locomotor function in these larvae. Impaired locomotor function, characterised both by reduced crawling velocity and directional indecision is a well characterised phenotype in CHMP2B^{Intron5} fly models (West et al., 2020; West et al., 2018). Behavioural deficits representative of both FTD and ALS phenotypes have also been reported in all three *CHMP2B^{Intron5}* mouse models. Mice reported by Ghazi-Noori et al. (2012) displayed social and motor deficits at 18 months of age, correlating with a significant loss of thalamic and cortical volume, coupled with a significant reduction in the number of neurons present within the thalamus (Clayton et al., 2017). The Gascon et al. (2014) mice displayed a significant, age-dependent, selective impairment in sociability with roughly 20% of mice also showing a compulsive grooming phenotype, suggesting additional behavioural deficits. Age-dependent motor impairment and behavioural perturbations, as well as a significant reduction in longevity, compared to non-transgenic litter-mates, was also observed in mice described by Vernay et al. (2016).

In addition to neurodegenerative phenotypes *CHMP2B^{Intron5}* models have also highlighted a functional role for CHMP2B in developmental processes. In 2014 Cheruiyot et al., modified the previously described UAS-CHMP2B^{Intron5} construct (Ahmad et al., 2009) to introduce an N-terminal Flag tag, which was subsequently injected into *Drosophila* to generate a new transgenic line (UAS-Flag-CHMP2B^{Intron5}). As both this and the original UAS-CHMP2B^{Intron5} fly lines were generated through random integration into the genome, as opposed to site-directed approaches, the UAS-Flag-CHMP2B^{Intron5} and original UAS-CHMP2B^{Intron5} will be located in different regions of the genome. These lines will therefore have potential variances in expression. Indeed Cheruiyot et al. (2014) showed Flag tagged CHMP2B^{Intron5} was expressed more strongly than the untagged CHMP2B^{Intron5} line. Expression of both models under the control of the *eyeless*-gal4 driver, which drives earlier in development and in a distinct population of cells to the *GMR*-gal4 driver used in previous studies, revealed developmental perturbations to the fly eye and photoreceptor patterning. Further characterisation revealed these phenotypes were mediated by increased Notch signalling, with notch accumulating in Rab5 and Rab7 positive endosomes. In a subsequent

study, Wilson et al. (2019) demonstrated neurodevelopmental defects associated with ectopic expression of CHMP2B^{Intron5}, revealing Notch dependent defects in cell fate determination (Wilson et al., 2020). In addition to supporting previous observations that CHMP2B^{Intron5} mediated perturbations to endosomal-lysosomal trafficking significantly impairs normal receptor homeostasis, these findings contribute to an increasing number of studies implicating altered Notch signalling in FTD-ALS neurodegeneration (Gomez-Pinedo et al., 2019; Liu et al., 2020; Nonneman et al., 2018; Wang et al., 2015; Yang et al., 2015). Further studies will be important in elucidating the contribution of the many pathways regulated by Notch, including cell fate, growth, migration, synaptic plasticity and neuronal survival, in neurodegenerative cascades in FTD-ALS spectrum disorders.

9. Pharmacological rescue of CHMP2B^{Intron5} and implications for FTD-ALS spectrum disorders

Since the initial identification of CHMP2B^{Intron5} as a causative FTD locus, a combination of *in vitro* and *in vivo* models have greatly contributed to our understanding of the molecular mechanisms underpinning CHMP2B^{Intron5}-related neurodegeneration. Although a rare cause of FTD and ALS, the fundamental role of CHMP2B and the ESCRT machinery in the regulation of neuronal proteostasis, endosomal-lysosomal and autophagic trafficking events, means that models of CHMP2B have also contributed to our understanding of the mechanisms driving neurodegeneration across the FTD-ALS spectrum more broadly. Identification of novel therapeutics that prevent or delay neurodegeneration associated with CHMP2B^{Intron5} mutations are therefore of potential significance to other monogenic and sporadic forms of FTD-ALS.

Two recent studies combining the use of *Drosophila* and mammalian primary neuron models have identified compounds acting on distinct molecular pathways to alleviate toxicity in CHMP2B^{Intron5} models. The first of these studies identified a potential role for antiretroviral compounds in the treatment of CHMP2B^{Intron5} FTD-ALS. Using their unbiased genetic screen in *Drosophila*, Fort-Aznar et al. (2020) identified mutations in genes functioning in retrovirus (RV) repression as dominant modifiers of CHMP2B^{Intron5} (Fort-Aznar et al., 2020). RV's are genetically mobile elements, present in most eukaryotic genomes that accumulate in heterochromatic regions (Saito et al., 2006). Their ability to replicate, transpose and infect other cells is controlled by a complex cellular network of RV repressors, which prevent RV activation. Deficits in RV silencing machinery are thought to occur in different neurodegenerative pathologies, particularly FTD-ALS, where increases in RV expression have been observed in patient tissue (Douville and Nath, 2017; Li et al., 2015). Furthermore, an increase in the reverse transcriptase activity of human endogenous RV (HERV)-K, has been identified in cerebrospinal fluid from ALS patients (Andrews et al., 2000; MacGowan et al., 2007; McCormick et al., 2008; Steele et al., 2005; Viola et al., 1975). For these reasons, nucleoside reverse transcriptase inhibitors have been used as potential therapeutic treatments for ALS (Gold et al., 2019). In this study the authors found expression of CHMP2B^{Intron5} led to an increase in RV translocation events and an accumulation of the gypsy RV in the *Drosophila* brain (Fort-Aznar et al., 2020). Furthermore they found that pharmacological inhibition of viral reverse transcriptase activity (using Stavudine or Lamivudine) was sufficient to alleviate degenerative phenotypes in rat primary neuron models of CHMP2B^{Intron5} (Fort-Aznar et al., 2020). This study was the first to demonstrate that RV de-repression occurs in CHMP2B^{Intron5} models. However a similar study found that glial expression of hTDP-43 in *Drosophila* resulted in de-repression and activation of gypsy, leading to glial secretion of toxic factors driving DNA damage and apoptotic cascades in adjacent neurons (Chang and Dubnau, 2019). Taken together these studies highlight convergent mechanisms that may underpin spreading pathology in FTD-ALS and other neurodegenerative diseases. This data also suggests that nucleoside reverse transcriptase inhibitors may represent novel treatments for CHMP2B^{Intron5} and other FTD-ALS spectrum diseases. Future work on this topic will likely benefit from data monitoring the effects of these drugs in patients across the FTD-ALS spectrum. A phase 2a trial in Australia determined the safety and tolerability of Triumeq, a combination therapy consisting of dolutegravir (50 mg) abacavir (600 mg) and lamivudine (300 mg). Overall, this combination therapy was well tolerated, with no drug related adverse effects described (Gold et al., 2019). A significant decrease in HERV-K expression was observed accompanied by a decline in the ALS functioning rating scale-revised (ALSFRS-R) progression rate of 21.8%. Based on these reports, a phase 3 trial will be planned. Similarly, a phase 1 proof of concept trial is now recruiting, testing the efficacy of four antiretroviral compounds (darunavir, ritonavir, dolutegravir, tenofovir alafenamide) in ALS patients (see ClinicalTrials.gov Identifier: NCT02437110) .

In a second study it was demonstrated that the FDA approved compound, UDCA and Ursocholanic acid (UCA), a structurally related compound, prevent neuronal cell death in *Drosophila* and mammalian models of CHMP2B^{Intron5}. UDCA is a natural hydrophilic bile acid produced in the pancreas as tauro-ursodeoxycholic (TUDCA), and processed in the gut to UDCA and has been shown to ameliorate mitochondrial dysfunction in Parkinson's and Alzheimer's patient fibroblasts, albeit through an unknown mechanism (Bell et al., 2018; Mortiboys et al., 2015). UDCA and UCA ameliorated aberrant NMJ morphology and crawling deficits in *Drosophila* larvae pan-neuronally expressing CHMP2B^{Intron5} (West et al., 2020). Both compounds also prevented dendritic collapse phenotypes associated with overexpression of CHMP2B^{Intron5} in rat primary neurons. Interestingly, UDCA did not affect any of the endo-lysosomal or autophagic hallmarks associated with the mutation, indicating it may act downstream of perturbed endosomal dysfunction and proteostasis. UDCA has previously been shown to negatively regulate p53 transcription and assembly, ultimately preventing apoptosis (Amaral et al., 2007; Amaral et al., 2010). UDCA prevented p53 and cleaved caspase 3 accumulation in CHMP2B^{Intron5} *Drosophila* models. RNA sequencing of CHMP2B^{Intron5} larvae revealed that UDCA positively regulates GCLC, the rate limiting enzyme in glutathione synthesis, suggesting that along with reducing p53, UDCA may influence neuronal redox homeostasis. UDCA has also been shown to exhibit anti-inflammatory activity further supporting the hypothesis that neurodegeneration in CHMP2B^{Intron5}, and FTD-ALS spectrum disorders more broadly, may be mediated by dynamic interplay between redox homeostasis, immune signalling, endosomal-lysosomal and autophagic trafficking and apoptotic cascades.

The identification that UDCA appears to act downstream of endosomal-lysosomal dysfunction and the classical hallmarks of CHMP2B^{Intron5} suggests it may have a broad neuroprotective function. As such, therapeutics similar to UDCA may have broader relevance across the FTD-ALS spectrum and other neurodegenerative pathologies. This is evidenced by a number of clinical trials monitoring UDCA and it's taurine conjugate form, TUDCA in ALS (Trial Number NCT00877604) and Parkinson's Disease (Trial Number NCT03840005) patients.

10. Conclusion

Since its identification as an FTD-ALS locus over 15 years ago, the study of mutations in CHMP2B have helped define its role in endo-lysosomal and autophagic proteostasis, immune signalling cascades and neuronal homeostasis, in both health and disease. Although mutations in CHMP2B are rare, deploying CHMP2B as a model to understand why these defects in endosomal trafficking and proteostasis cause disease, has provided insights into the complex mechanistic biology that causes neuronal cell loss. Furthermore, *in vitro* and *in vivo* models have identified a number of potential therapeutic targets and novel small molecules that ameliorate neurodegeneration. The CHMP2B chapter is far from closed, and as we uncover more about the functions of CHMP2B and ESCRT-III, we enhance our understanding of both FTD-ALS

spectrum disorders and mechanisms underpinning neurodegeneration.

Funding

This work was supported by The Alzheimer's Society [AS-JF-16b-004 (510)], awarded to RJHW and Wellcome Trust [Grant number 204829], awarded to CU.

Acknowledgements

We thank Dr. Sean T. Sweeney for comments on the manuscript.

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