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Dysfunction of Autophagy and Endosomal-Lysosomal Pathways: Roles in Pathogenesis of Down Syndrome and Alzheimer's Disease

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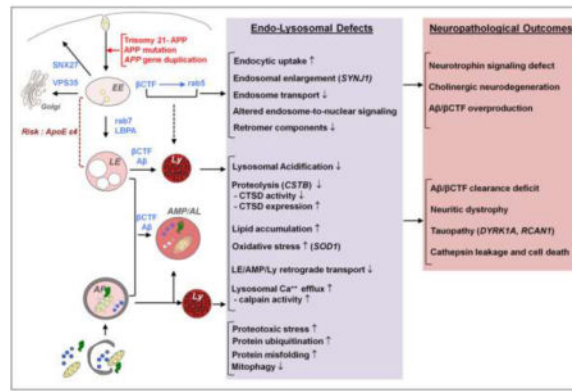
Abstract

Individuals with Down syndrome (DS) have an increased risk of early-onset Alzheimer's Disease (AD), largely owing to a triplication of the *APP* gene, located on chromosome 21. In DS and AD, defects in endocytosis and lysosomal function appear at the earliest stages of disease development and progress to widespread failure of intraneuronal waste clearance, neuritic dystrophy and neuronal cell death. The same genetic factors that cause or increase AD risk are also direct causes of endosomal-lysosomal dysfunction, underscoring the essential partnership between this dysfunction and APP metabolites in AD pathogenesis. The appearance of APP-dependent endosome anomalies in DS beginning in infancy and evolving into the full range of AD-related endosomal-lysosomal deficits provides a unique opportunity to characterize the earliest pathobiology of AD preceding the classical neuropathological hallmarks. Facilitating this characterization is the authentic recapitulation of this endosomal pathobiology in peripheral cells from people with DS and in trisomy mouse models. Here, we review current research on endocytic-lysosomal dysfunction in DS and AD, the emerging importance of APP/ β CTF in initiating this dysfunction, and the potential roles of additional trisomy 21 genes in accelerating endosomal-lysosomal impairment in DS. Collectively, these studies underscore the growing value of investigating DS to probe the biological origins of AD as well as to understand and ameliorate the developmental disability of DS.

Graphical abstract

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Keywords

Down Syndrome; Alzheimer's Disease; Endosomes; Lysosomes; Autophagy

INTRODUCTION

Arising from an extra copy of chromosome 21 (HSA21), Down syndrome (DS) is characterized by varying degrees of intellectual disability (learning, memory and language), heart defects, craniofacial abnormality and childhood leukemia (Lott and Dierssen, 2010; Wiseman et al., 2009). The average life expectancy of people with DS has increased to 60 years, as medical treatment has advanced and social acceptance has led to decreased institutionalization. Despite these positive developments, however, almost all individuals with DS reaching the age of 40 will develop progressive Alzheimer's Disease (AD) with characteristic neuropathological features including β -amyloid plaques, neurofibrillary tangles, and neuronal cell loss (Leverenz and Raskind, 1998; Wisniewski et al., 1985). Among DS individuals, 2–5% exhibit symptoms of AD dementia by the age of 40, while the occurrence of AD dementia approaches 100% in DS individuals by the age of 70 years (Lott and Dierssen, 2010). The extra copy of chromosome 21 is the fundamental source of this increased AD risk and can be mainly attributed to the gene encoding for amyloid precursor protein (APP), which is itself linked to familial AD (Tanzi, 2012). The observation that, in rare cases, a trisomy 21 state in which APP gene dosage is 2N rather than 3N, is associated with a DS phenotype but without an AD phenotype, implies that 3N APP is necessary, though not necessarily sufficient, for AD to develop in individuals with DS (Doran et al., 2017; Korbel et al., 2009; Prasher et al., 1998). Beyond APP, many of the triplicate genes on the HSA21 of DS individuals, such as superoxide dismutase 1 (SOD1), DYRK1A, BACE2, Down-Syndrome critical region (DSCR) and S100 β have been implicated in AD development (Lott and Dierssen, 2010). Common genetic and pathological factors operating in both AD and DS suggest commonalities in the underlying disease mechanisms. These shared pathological mechanisms are compounded by ageing in both conditions, but accelerated in DS relative to the disease progression observed in AD.

In AD, defects in endocytosis and autophagy have emerged as key drivers of accumulation of cellular waste products, progressive neuronal dysfunction, and neurodegeneration; which we have described previously (Nixon, 2017). Notably, many elements of endocytic and

autophagic dysfunction observed in AD are also found in DS brain (as summarized in Figure 1), suggesting common disease-associated pathogenic processes, and a prospect of gaining key insights into these processes by investigating the early onset of AD-related pathophysiological features in DS brain, peripheral tissues, and experimental models of DS. A distinct advantage of the analysis of early AD pathobiology is the authentic recapitulation of the endosomal-lysosomal phenotype and its APP gene dependence in primary skin fibroblasts, other peripheral cell elements, and in induced pluripotent stem (iPSC) neuronal cell lines derived from these cell sources. This has enabled increasingly detailed analyses of the molecular pathways underlying endosomal signaling defects linked to neurodegeneration (Jiang et al., 2010; Park et al., 2008; Weick et al., 2013). In this review, we will address the current state of research on endocytosis and autophagy defects in DS and the relevance of defects in these processes to the development of AD.

ENDOCYTIC DYSFUNCTION IN DS AND RELATIONSHIPS TO AD

Endosomal pathology in AD and DS and the role of specific APP metabolites

Early endosomes sort internalized and plasma membrane-associated cargoes that will be either recycled to the plasma membrane, transported to and from the Golgi via the retromer complex, or directed to lysosomes for degradation (Di Fiore and von Zastrow, 2014; Huotari and Helenius, 2011). Importantly, in addition, endosomes contain on their surface a signaling platform that transduces external or membrane receptor signals, via activation of intracellular signaling molecules, into multiple vesicular trafficking events controlling cargo fates, receptor occupancy on membranes, and endosome-nuclear translocation of transcriptional regulators like tropomyosin receptor kinases (Trks), receptor tyrosine kinases (RTKs), and cell cycle and inflammatory factors (Cendrowski et al., 2016; Cosker and Segal, 2014; Howe and Mobley, 2004; Kim et al., 2015; Miaczynska, 2013; Pyrzynska et al., 2009). The master regulator of endocytosis is the small Rab GTPase rab5, whose functions, similarly to other rabGTPases, depend on a) its membrane localization, regulated by GDP dissociation inhibitors (GDIs) (Felberbaum-Corti et al., 2005) and GDI displacement factors (GDFs) (Dirac-Svejstrup et al., 1997); and b) cycling between a GTP-bound/active and a GDP-bound/inactive state, via guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (RabGAPs) activities, respectively (Wandinger-Ness and Zerial, 2014).

Upon activation, Rab5 recruits effector proteins important for endosome maturation, trafficking and signaling. These effectors include: Rabaptin-5 (Stenmark et al., 1995), forming a complex with, and regulating the GEF activity of Rabex-5, a specific GEF for rab5 (Horiuchi et al., 1997; Zhang et al., 2014); phosphatidylinositol 3-kinase (PI3K)/Vps34 and its product PI(3)P, required for docking and fusion of incoming rab5 positive early endosomes via recruitment of EEA1 and SNAREs (Christoforidis et al., 1999; Simonsen et al., 1998). Rabenosyn-5, similarly to EEA1, specifically binds to PI(3)P via its FYVE finger motif and is also necessary for endosomal membrane targeting (Sonnichsen et al., 2000; Zerial and McBride, 2001). APPL1 (adaptor protein containing pleckstrin homology domain, phosphotyrosine binding (PTB) domain, and leucine zipper motif) (Miaczynska et al., 2004), enriched on a subpopulation of very early endosomes with distinct spatial distributions and cargo sorting activity (Kalaidzidis et al., 2015) has emerged as another

important rab5 regulatory factor highly relevant to emergence of early endosome dysfunction in AD. Owing to its BAR-PH and PTB domains (Li et al., 2007; Zhu et al., 2007), APPL1 binding to GTP-rab5 is key to mediate the signaling of growth factors (EGF, NGF, BDNF) (Fu et al., 2011; Lin et al., 2006; Miaczynska et al., 2004), NF- κ B (Hupalowska et al., 2012), adiponectin (Mao et al., 2006), insulin (Ryu et al., 2014) and phosphoinositides (Bohdanowicz et al., 2012).

In AD, acceleration of neuronal endocytosis (as measured directly in DS fibroblasts), is reflected in increased fusion and the swelling of rab5-positive early endosomes (Cataldo et al., 1997; Cataldo et al., 2008) and upregulated expression of rab5 and related rab effectors (Ginsberg et al., 2010a; Ginsberg et al., 2011; Ginsberg et al., 2010b) are the earliest AD neuronal pathology thus far identified. Our previous studies had linked the endosomal abnormality in DS to its extra copy of APP in neurons of the Ts65Dn and Ts2 mouse (Cataldo et al., 2000; Jiang et al., 2016), and also in fibroblasts derived from individuals with DS (Cataldo et al., 2008; Jiang et al., 2010). Notably for AD and DS, endosomes are sites of active APP processing due to the enrichment of β -site APP cleaving enzyme 1 (BACE1) and γ -site APP secretase (Israel et al., 2012; Nixon, 2013). Growing evidence shows that β -site cleavage of APP on endosomes (Kaether et al., 2006) to form APP- β CTF is actively modulated by the varied regulatory influences on BACE1 activity and localization, which include many AD risk factors.

APP- β CTF is elevated in both AD and DS human brains (Kim et al., 2015; Pera et al., 2013) and gene mutations in presenilin 1 (PSEN1) and APP that cause early-onset forms of AD accelerate APP- β CTF formation and/or accumulation (Cacquevel et al., 2012; Chang and Suh, 2005; De Jonghe et al., 2001; Deyts et al., 2016; van der Kant and Goldstein, 2015). APP- β CTF has been shown recently to pathologically activate rab5 by recruiting to early endosomes the adaptor protein APPL1, which binds both APP- β CTF and GTP-rab5, causing stabilization and further activation of rab5 (Figure 2A). This results in the upregulation of endocytosis, endosomal trafficking defects and abnormal endosome-mediated signaling (Kim et al., 2015). By crossing Ts2 mice with the BACE1^{+/-} line, we generated Ts2.BACE1^{+/-} mice that have reduced APP- β CTF but undetectable change in regional A β levels compared to Ts2.BACE1^{+/+} mice; this modulation rescued rab5-positive endosome size and normalized basal forebrain cholinergic neuron (BFCN) number (Jiang et al., 2016). Combined with findings from others (Choi et al., 2013; Lauritzen et al., 2012; Lauritzen et al., 2016; Salehi et al., 2006; Tamayev et al., 2012) our studies (Jiang et al., 2010; Jiang et al., 2016; Kim et al., 2015) implicate APP- β CTF in the pathogenesis of AD and DS, while also providing support for ongoing clinical study of BACE1 inhibitors as a therapy for AD. Genetic reduction of BACE1 has been shown to reduce amyloid burden, delay the onset BFCN degeneration, and improve cognitive function in several APP transgenic mouse models (McConlogue et al., 2007; Ohno et al., 2007; Singer et al., 2005).

In addition to APP, another gene from HSA21 that has been implicated in the observed endosomal dysfunction in DS is the synaptojanin1 (*SYNJ1*) gene (Cossec et al., 2012). *SYNJ1* encodes for a phosphatase, the activity of which is directed to phosphatidylinositol-4,5-bisphosphate PI(4,5)P₂, a signaling molecule involved in the regulation of clathrin-mediated endocytosis (Mani et al., 2007). *SYNJ1* levels are elevated in

the brain of human DS patients (Arai et al., 2002) and even greater in DS with AD, compared to sporadic AD and control brains (Martin et al., 2014). Moreover, elevated expression of SYNJ1 was shown to alter PI(4,5)P2 metabolism and cause cognitive deficits in the T65Dn mouse model of DS (Voronov et al., 2008).

Finally, inheritance of the APOE ϵ 4 allele, which is the strongest genetic risk factor for sporadic AD (Liu et al., 2013), promoted earlier appearance of endosomal enlargement at preclinical stages of AD (Cataldo et al., 2000). Notably, APOE ϵ 4 is a significant factor in the severity of AD pathology in DS. Several studies have shown that DS patients who carry APOE ϵ 4 exhibit increased risk of AD-DS, earlier dementia onset, and greater amyloid load; whereas APOE ϵ 2 has been shown to confer protective effects in DS (Lai et al., 1999; Prasher et al., 2008; Wiseman et al., 2015). Considering the shared genetics between AD and DS, it may be speculated that APOE ϵ 4 accelerates endosomal pathology in DS as well as in AD in the early development of disease.

Endosomal dysfunction in AD and DS: Consequences on downstream endocytic events relevant to AD pathogenesis

Endosome maturation defects—The conversion from rab5- to rab7-positive endosomes underlies the transition of cargos from early to late endosomes (Huotari and Helenius, 2011). Such transition is mediated by GTP-rab5, which recruits the SAND-1/Mon1 and Ccz1 complex (Poteryaev et al., 2007; Wang et al., 2003) and the class C vacuolar protein sorting/homotypic fusion and protein sorting (Class C VPS/HOPS) complex (Rink et al., 2005) for recruitment and activation of rab7 and suppression of rab5. The molecular link between the rab5 and rab7 machineries comes from the observation that expression of GTP hydrolysis-defective rab5 mutant or deletion of Vps39, one of the class C VPS/HOPS complex subunit known to be a GEF for rab7, caused enlargement and colocalization of rab5/EEA1 and rab7/LAMP1-positive vacuoles (Rink et al., 2005). As the endosome moves towards the nucleus, sorting complexes required for transport (ESCRT-0, ESCRT-I, ESCRT-II, ESCRT-III) regulate the sequestration of ubiquitinated cargoes (Raiborg and Stenmark, 2009) and initiate the lipid-driven formation of intra-luminal vesicles (ILVs), which are enriched in cholesterol, sphingolipids and PI(3)P in early phases and lyso-bisphosphatidic acid (LBPA) and phosphoinositol-3,5-bis phosphate (PI(3,5)P2) in later phases (Babst, 2011; Solinger and Spang, 2013).

As a result of the upregulation of endocytosis, DS fibroblasts (Cataldo et al., 2008), like neurons in DS/AD brain, exhibit late endosomal abnormalities, manifesting in increased numbers and size of rab7- and LBPA-positive perinuclear vacuoles. Microarray analysis of mild cognitive impairment (MCI) and AD hippocampal CA1 neurons revealed upregulation of both rab5 and rab7 GTPases (Ginsberg et al., 2010a). Subsequent analysis on rab5 and rab7 protein expression revealed a region-specific increase in their levels in basal forebrain, frontal cortex, hippocampus in MCI and AD (Ginsberg et al., 2011; Ginsberg et al., 2010b). Also, lipidomic analysis showed specific enrichment of LBPA, sphingomyelin, ganglioside GM3, and cholesterol esters in human AD brain entorhinal cortex (Chan et al., 2012). A similar increase in cholesterol esters and GM3 was also observed in PS1-APP mice, altogether suggesting a common collection of pathogenic mechanisms associated with endo-

lysosomal pathology in DS and AD (Chan et al., 2012). Disruption of the ESCRT machinery has also been shown to promote neuronal accumulation of ubiquitin-positive aggregates and autophagosomes (APs), as well as neurodegeneration (Kurashige et al., 2013; Lee et al., 2007; Tanikawa et al., 2012; Yamazaki et al., 2010), and causes an early accumulation of large lipid-related autofluorescent intraneuronal aggregates derived from the endolysosomal system (Clayton et al., 2015).

Aberrant endosomal signaling of neurotrophins leading to cholinergic neurodegeneration

—The coordinated action of Rab5 and rab7 is key to the regulation of axonal uptake and long-range retrograde transport of NGF-activated TrkA receptor within long-lived signaling endosomes (Howe and Mobley, 2004) (Deinhardt et al., 2006; Philippidou et al., 2011; Saxena et al., 2005) which play roles in the modulation of gene expression promoting neuronal growth, survival and synapse assembly and maintenance (Cosker and Segal, 2014; Lehigh et al., 2017; Sharma et al., 2010).

There are multiple lines of evidence supporting impaired endosomal signaling in AD and DS (Figure 2B). Activity-dependent release of NGF from cortical and hippocampal neurons and NGF signaling are crucial for the survival of BFCNs that are lost in DS and AD, contributing to cognitive decline (Grothe et al., 2012; Iulita and Cuello, 2016). Retrograde transport of NGF is disrupted and the cholinergic phenotype is lost in mouse models of AD and DS (Granholtm et al., 2000; Jiang et al., 2016; Salehi et al., 2006) and reversed by administration of NGF (Cooper et al., 2001; Granholtm et al., 2000) or by partial reduction of BACE-1 (Jiang et al., 2016). Additionally, microarray analysis of human AD hippocampal neurons and BFCN revealed an early down-regulation of Trk receptors accompanying the upregulation of early endocytic genes (Ginsberg et al., 2010a; Ginsberg et al., 2011).

APP- β CTF-mediated rab5 hyper-activation and endosomal enlargement (Kim et al., 2015; Xu et al., 2016) lead to BFCN atrophy (Xu et al., 2016) and disrupt retrograde transport and signaling causing a net slowing down of vesicle movements, which are prevented by reducing GTP-rab5 levels either by siAPPL1 (Kim et al., 2015) or by expression of dominant negative rab5 mutant (Xu et al., 2016). β CTF-mediated rab5 hyperactivation may also affect the biogenesis of signaling endosomes, which requires inactivation of Rab5 by rabGAP5, and constitutively active rab5 mutant blocks NGF-mediated neurite outgrowth (Haas et al., 2005; Liu et al., 2007). Finally, APPL1 recruitment to β CTF-GTP-rab5 endosomes may prevent APPL1-PTB binding to activated TrkA receptor mediating NGF signaling (Lin et al., 2006; Varsano et al., 2006) and contribute to slow down endosome transport by increasing rab5 activation and reducing TrkA signaling (Nixon, 2017). Aside from its effects on NGF, rab5 hyperactivation promotes activation of signaling pathways leading to cell death. APPL1-mediated nuclear delivery of transcription factors activates nuclear factor- κ B (NF- κ B) in human DS fibroblasts (Kim et al., 2015). Rab5 interaction with APP-BP1 induces cell cycle re-entry followed by apoptosis in London mutant APP (V642I)-FAD expressing primary rat cortical neurons (Laifenfeld et al., 2007).

Similarly to TrkA receptors (Ascano et al., 2009), also APP (Woodruff et al., 2016) and BACE1 (Buggia-Prevot et al., 2014) have been shown to require rab11-mediated transcytosis for their axonal localization. Following APP endocytosis into in rab5 positive early

endosomes, BACE1-mediated cleavage of APP redistributes a portion of full-length APP and β CTF into rab11 recycling endosomes where the γ -secretase-mediated cleavage of β CTF is the “signal” promoting transcytosis (Woodruff et al., 2016). fAD mutations that increase β CTF levels, either by enhancing BACE1 activity or impairing γ -secretase cleavage of APP, disrupt APP transcytosis, causing an accumulation of APP/ β CTF and rab11-positive endosomes in the soma and reduced levels in the axon (Woodruff et al., 2016). APP has also been proposed to influence TrkA anterograde transport via direct (Kamal et al., 2001; Stokin et al., 2005) or indirect (Inomata et al., 2003) binding to kinesins that may affect NGF signaling (Zhang et al., 2013). Therefore, decreased anterograde transport of TrkA due to defects in axonal transport of APP caused by overexpression or mutations of APP may also contribute to NGF retrograde transport defects.

Retromer defects—The sequential action of rab5 and rab7 has also been shown to regulate the retrograde transport from the sorting compartment to the trans-Golgi network (TGN), via recruitment of the retromer complex (Rojas et al., 2008), which also regulates the recycling of cargoes from endosomes back to the plasma membrane; a process which is necessary for synaptic remodeling and plasticity (Loo et al., 2014; Temkin et al., 2011; Zhang et al., 2012). Rab5 indirectly promotes the recruitment of the sorting nexin (SNX) subcomplex (different dimeric combinations of SNX1, SNX2, SNX5, and SNX6), likely via PI3K activation (Rojas et al., 2008). On the other hand, rab7, along with SNX3 and TBC1 domain family member 5 (TBC1DF5), also known as the “membrane-recruiting module” (Small and Petsko, 2015), directly interacts with the vacuolar protein sorting (Vps) subcomplex (a heterotrimer composed of Vps26, Vps29, and Vps35). Recently, another SNX, SNX27, was linked to retromer and its function (Steinberg et al., 2013; Temkin et al., 2011). SNX27 functions as an adaptor for binding to PDZ ligand-containing cargoes that are directed to the cell surface via the recycling pathway.

Defects in the retromer complex have been linked to neurodegenerative diseases, including AD and DS. While autosomal-dominant mutations in *VPS35* link late-onset PD with retromer dysfunction, AD brains are deficient in Vps35 and Vps26 components (Small and Petsko, 2015). Moreover, a recent study provides evidence for SNX27 deficiency in DS brains possibly due to an extra copy of a microRNA encoded by human chromosome 21, which causes decreased SNX27 expression (Wang et al., 2013). This resulted in disrupted glutamate receptor recycling in the hippocampus and led to dendritic dysfunction. Notably, upregulation of SNX27 in the hippocampus of Ts65Dn mice rescued both synaptic and cognitive deficits (Wang et al., 2013). It might be speculated that correcting the retromer-dependent trafficking from endosomes would also have a salutary effect on endosome enlargement and its consequences.

Altered endosome trafficking contributing to autophagic defects and neuritic pathology in AD and DS—Before fusing with lysosomes, late endosomes can fuse with autophagosomes (AP) to form prelysosomal compartments known as amphisomes (Nixon, 2013). Amphisome formation requires some of the components that regulate late endosome/multivesicular body (MVB) formation, such as HOPS and ESCRT protein complexes. Additionally, Rab7 is found on nascent autophagosomes and is required for AP maturation

(Gutierrez et al., 2004; Jager et al., 2004). In distal axons, late endosomes commonly fuse with APs and acquire retrograde motility by recruiting dynein-snapin complexes (Cheng et al., 2015). Snapin, originally implicated in vesicle fusion and synaptic transmission (Ilardi et al., 1999), has been shown to play a crucial role in regulating late endosome retrograde transport and maturation in neurons (Cai et al., 2010). Indeed, Snapin deletion causes accumulation of late endosomes and impairs lysosomal function.

Recent evidence indicates that amphisome retrograde transport is impaired in the mossy fiber tracts of the dentate gyrus in young 3×Tg-AD mice, compared to WT, as detected by increased colocalization of LC3-II with the late endosomal marker mannose-6-phosphate receptor (CI-MPR) in distal axons. Additionally, these axons were positive for ubiquitin, p62 and appeared swollen and dystrophic (Tammineni et al., 2017). APs have been shown to be key organelle for the degradation of β CTF via the MVB/lysosomal pathway, and inhibition of AP formation or fusion with the endolysosomal compartment, caused accumulation of β CTF in ILV/MVB (Gonzalez et al., 2017). Disrupted anterograde transport of β CTF-containing endosomes results in their accumulation in axons terminals along with autophagic vacuoles, presumably formed in an effort to facilitate clearance of endosomes, contributing to axonal swelling and neuritic dystrophy similar to that observed at early stages of AD (Stokin et al., 2005).

The majority of dystrophic neurites in human and mouse AD brains are characterized by the presence of autophagic vacuoles (Nixon, 2005). A sustained induction of autophagy and endocytosis paralleled by a progressive decline in lysosomal proteolysis and a slowing of retrogradely transported cargoes within vulnerable neuronal populations likely contributes to the extensive autophagic and neuritic pathologies implicated in AD (as illustrated in Figure 2C) (Bordi et al., 2016). Notably, even in the absence of APP- β CTF/amyloid, defective proteolysis or inhibition of endolysosomal acidification can induce the accumulation of vacuoles filled with incompletely degraded, ubiquitinated material that accumulates, causing neurite swelling and dystrophy (Boland et al., 2008; Lee et al., 2011). In DS fibroblasts, an increase in the number of large compartments with the structural features of early/late endosomal hybrids, MVB, autophagic vacuoles, late endosomes, or lysosomes, has also been reported (Cataldo et al., 2008), further highlighting the relationship between early endosomal dysfunction and downstream endocytic and proteolytic compartments. Extensive accumulation of misfolded and ubiquitinated proteins in the brain (Tramutola et al., 2016) and dystrophic neurites (as illustrated in Figure 2C) (Mattiace et al., 1991) of DS individuals suggests that global defects in protein quality control ultimately disrupt autophagic and lysosomal clearance (see following section), despite the lowered autophagy induction rate due to hyperactivation of the PI3K/Akt/mTOR axis (Perluigi et al., 2014). mTOR activation has also been reported to be elevated in inferior parietal lobule (Tramutola et al., 2015) and medial temporal cortex neurons bearing hyperphosphorylated tau in human AD brains (Li et al., 2005), as well as in non-neural cultured cells transfected with mutant APP (Caccamo et al., 2010) or exposed to A β oligomers (Bhaskar et al., 2009). On the other hand, a reduction in mTOR activity has been documented in AD hippocampus (Bordi et al., 2016), as well as murine neuroblastoma cells exposed to A β 42, PSEN1/APP mouse brain and lymphocytes from AD patients (Lafay-Chebassier et al., 2005). There is also evidence for an early transcriptional upregulation of genes promoting autophagy and downregulation of negative

regulators of autophagy flux in human AD entorhinal cortex (Lipinski et al., 2010) and in CA1 pyramidal neurons (Bordi et al., 2016). Taken together, these observations highlight the complexity in defining the net direction of autophagic signaling changes during the course of AD, due to the different experimental models used, the specific brain regions and cell populations examined at a given stage of AD pathology development and the different methods of analyzing mTOR activity itself and downstream effects on its substrates and autophagy.

LYSOSOMAL DYSFUNCTION IN DS AND RELATIONSHIPS TO AD

APP and APP β -C-terminal fragments (β CTF) and lysosomal dysfunction

The triplication of the *APP* gene in DS is of particular interest in the study of the AD-DS relationship, due to the role of *APP* mutations in fAD and the broader role of its metabolites in sAD (Selkoe and Hardy, 2016). In the context of lysosomal dysfunction, this attention towards APP is well-supported; APP holoprotein and its cleavage products are implicated in lysosomal defects in DS and AD (Nixon, 2007) (as described in Figure 3). Numerous lines of evidence connect APP and its products with lysosomal dysfunction in AD (Umeda et al., 2011; Xu et al., 2016; Xue et al., 2014; Yang et al., 2008; Yang et al., 2014), and the triplication of the APP gene in DS suggests that APP-mediated lysosomal defects occur in DS in a similar fashion. A recent study on an elderly DS individual lacking APP triplication due to partial trisomy 21 revealed a lack of AD pathology or dementia (Doran et al., 2017), providing human evidence for the role of APP in the development of AD pathology observed in older individuals with DS. This finding is supported by evidence from primary DS fibroblasts which shows that their AD-like endosomal phenotype is APP-dependent (Jiang et al., 2016). The relationship between lysosomal dysfunction and APP is thought to be bi-directional, as lysosomal failure permits formation of misfolded aggregates of APP products and other pathogenic proteins (Ivy et al., 1984; Mangieri et al., 2014), while both APP holoprotein and APP cleavage products (particularly β CTF) negatively impact lysosomal function (Ditaranto et al., 2001; Lauritzen et al., 2012; Lauritzen et al., 2016; Yang et al., 1998; Yang et al., 2011b; Yang et al., 2014). This relationship is observed in studies of the CRDN8 APP mutant mouse line, which exhibits age-related amyloid pathology and learning/memory deficits. These mice exhibit markers of lysosomal dysfunction, specifically swollen Cathepsin D (CTSD)-positive compartments, accumulation of amyloid products in the lysosome, and impaired lysosomal lipid processing (Yang et al., 2011b; Yang et al., 2014). Further, the amelioration of learning and memory defects in conjunction with reduced amyloid via rescue of lysosomal function in the CRDN8 model is compelling evidence for the centrality of lysosomal dysfunction in the progression of APP-associated disease phenotypes. Additional strong pathological evidence for autophagic-lysosomal dysfunction in APP mutant animal models has been reported in the 3xTG-AD mouse as well, specifically in regard to the role of β CTF (Lauritzen et al., 2012; Lauritzen et al., 2016).

While a great deal of research in AD has focused on $A\beta^{1-40/42}$ and the products of their aggregation, current studies indicate that the APP cleavage product β CTF exert significant pathological effects on lysosomal function, in addition to the demonstrated roles of β CTF in

endosomal dysfunction (described above). β CTF was found to promote neurodegeneration and formation of lesions *in vivo* following viral induction of β CTF expression (Lauritzen et al., 2012), and these pathological signs appear to be driven by impairments of lysosomal function. β CTF contributes to morphological changes in lysosomes, reduced lysosomal proteolysis, and accumulation of intracellular waste products (Lauritzen et al., 2016). Further studies of this phenomenon demonstrate that gamma-secretase inhibition, which blocks A β formation while increasing β CTF levels, significantly exacerbates the lysosomal dysfunction. These detrimental effects on endolysosomal function also occur independently of A β in models of DS (Jiang et al., 2010; Jiang et al., 2016). It is also shown that β CTF fragments can be found in the membranes of lysosomes and autophagic compartments, where they may impair the integrity of the membrane. This could impair acidification of lysosomes via loss of membrane stability and/or through the potential interactions of β CTF with any of the various membrane proteins of the lysosome which are essential to proper acidification. β CTF-induced membrane permeability could also promote cell death via release of lysosomal calcium and pro-apoptotic functions of leaked lysosomal enzymes (Guicciardi et al., 2004)(as described in Figure 3).

Amyloid- β

Additionally, there is also evidence that forms of A β can negatively impact lysosomes *en route* to promoting neuronal death. *In vitro* studies suggest that various forms of A β can permeabilize lysosomal membranes (Ditaranto et al., 2001; Kosenko et al., 2011; Song et al., 2011; Umeda et al., 2011; Yang et al., 1998), which is detrimental to the stability of the lysosome, permitting release of lysosomal enzymes and calcium into the cytoplasm, promoting apoptosis (Guicciardi et al., 2004; Nixon and Cataldo, 1993). Many of these studies tested the effects of intraneuronal amyloid peptides, which accumulate early in DS pathogenesis (Mori et al., 2002). Notably, in animal models of DS and AD, the identity of APP cleavage products is heterogeneous; β CTF and various forms of A β are generated, and A β peptides can be present as monomers or various stages of oligomerization. In the case of DS, increased expression of full-length APP is likely to increase the autophagic burden of clearing all types of APP cleavage products, but positive effects of BACE1 inhibition (Jiang et al., 2016) suggest potential benefit in targeting APP cleavage by BACE1 to reduce both β CTF and A β .

Cathepsin D (CTSD) defects and lysosomal pathology in DS and AD

Lysosomal CTSD-mediated proteolysis plays a very important role in maintaining neuronal cell homeostasis by degrading unwanted material delivered to lysosomes via autophagy or endocytosis. Many disease-associated aggregated proteins: A β / β CTF, α -synuclein, tau and huntingtin, abnormally accumulate in autophagic compartments when they are not efficiently degraded by CTSD and other lysosomal enzymes (Nixon, 2013). Numerous studies have shown that mice lacking CTSD have neurodevelopmental deficits and exhibit progressive neurodegeneration (Koike et al., 2003; Shacka et al., 2007; Tyynela et al., 2000; Yamasaki et al., 2007), demonstrating the essential functions of CTSD in efficient lysosomal function and subsequently, neuronal health. In human disease, a subtype of neuronal ceroid lipofuscinosis (NCL) is a lysosomal storage disease directly caused by mutations in CTSD (Ketterer et al., 2017; Stoka et al., 2016). Also, a rare form of Gaucher disease (another

lysosomal storage disease) is the result of lowered activity of lysosomal CTSD, caused by a pro-saposin gene mutation (Tatti et al., 2013). Abnormal CTSD protein levels and lowered specific enzyme activity, reflecting a accumulation of inactive or less active CTSD, are seen in the brains of individuals with either sporadic AD or PSEN-fAD (Cataldo et al., 2004). Moreover, also seen in AD is a) an elevated level of CTSD transcript in CA1 neurons of AD brains (Ginsberg et al., 2010a); b) increases in both mRNA expression and cellular content of CTSD in neocortical pyramidal neurons of AD brain (Cataldo et al., 1995); c) presence of CTSD immunoreactivity and active forms of other cathepsins and lysosomal enzymes within amyloid plaques of both DS and AD postmortem brains (Cataldo et al., 1996), reflecting the robust autophagy-lysosomal pathway pathology in neuritic plaques; and d) elevated level of CTSD of abnormally lowered specific enzyme activity in the cerebrospinal fluid of AD patients (Schwagerl et al., 1995). In addition, altered expression and subcellular localization of CTSD were found in both neurons and glia in aged brains (Nakanishi, 2003). CTSD has been associated with the clearance of A β , tau, and APOE, all of which are involved in the pathogenesis of AD (Kenessey et al., 1997; Zhou et al., 2006). Our recent studies in DS human fibroblasts show decreased lysosomal CTSD specific and *in situ* activities, despite an elevation of total CTSD activity (Nixon et al., 2017). Further research on CTSD dysfunction and related pathologic effects on DS and AD are needed to fully understand the mechanism underlying these changes, although impaired lysosomal acidification is a significant contributing factor (Nixon et al., 2017).

Lysosomal acidification defects in autophagic dysfunction during AD and DS

Lysosomal acidification is crucial to many functions of the lysosome, most notably proteolysis; as activities and maturation of multiple lysosomal hydrolases, including CTSD, are pH-dependent. But acidification is also critical for axonal transport of acidified vesicular compartments (Lee et al., 2011), fusion with AVs, lysosomal signaling functions via mTOR (Perera and Zoncu, 2016), lysosomal calcium release, and exocytosis (Wolfe et al., 2013). Acidification of lysosomes is fundamental to lysosomal function and autophagy in general throughout cell types, but is particularly important in neurons which are post-mitotic and need to exert quality control over large expanses of cytoplasm. The rapid constitutive rate of autophagy necessitates also having a highly efficient lysosomal processing of substrates (Boland et al., 2008). Lysosomal acidification defects have been implicated in a number of late-onset neurodegenerative diseases, including AD (Lee et al., 2010; Lee et al., 2015), and PD (Dehay et al., 2013), but also in a group of childhood genetic diseases affecting the CNS (Colacurcio and Nixon, 2016). These acidification-related diseases are often linked to mutation of a component of the vacuolar ATPase (v-ATPase) complex, the proton pump that is the primary driver of lysosomal acidification (McGuire et al., 2017). In these genetic disease contexts, a mutation of the v-ATPase or a v-ATPase associated protein leads to impaired lysosomal acidification, which can drive aggressive (i.e. childhood-onset) CNS pathology via progressive failure of lysosomal function in neurons.

While we have previously addressed the genetic and pathological overlap between AD and DS, it is noteworthy that lysosomal de-acidification causes another form of mental retardation in the case of Mental Retardation Hedera Type, during which a chaperone (ATP6AP2) of the lysosomal v-ATPase is mutated (Hedera et al., 2002; Kinouchi et al.,

2013; Korvatska et al., 2013). Combined with the observations of lysosomal de-acidification in AD and AD models (Avrahami et al., 2013; Lee et al., 2015), florid lysosomal pathology of APP mutant mice (Lauritzen et al., 2016; Yang et al., 2011a; Yang et al., 2014), and the overlapping role of APP in AD and DS, lysosomal de-acidification may contribute substantially to the lysosomal dysfunction in DS. Lysosomal de-acidification may also explain how the activities of CTSD and other lysosomal hydrolases become altered and why inactive enzyme forms accumulate. In this regard, our recent data demonstrate de-acidification of lysosomes, driven by loss of v-ATPase function in primary human DS fibroblasts relative to control cells (Nixon et al., 2017).

Also, recent proteomic studies have identified accumulation of ubiquitinated proteins in DS (Tramutola et al., 2016), indicative of aforementioned failures of both proteasomal function and autophagic clearance of proteins (Dephoure et al., 2014). In addition to a global increase in ubiquitinated proteins and loss of proteasomal function, this study (Tramutola et al., 2016) demonstrated progressive ubiquitination of certain proteins between brains of young DS individuals and older DS individuals with AD pathology compared to non-DS controls. For example, V1B2 subunits of the v-ATPase were increasingly ubiquitinated in the brains of DS individuals and further increased in DS individuals with AD pathology. Moreover, this ubiquitination increased with age in non-DS individuals. These changes in the v-ATPase could possibly impair the acidification of lysosomes. The correlation of v-ATPase ubiquitination with DS, DS-associated AD pathology, and ageing therefore further adds to previous evidence (Colacurcio and Nixon, 2016) that v-ATPase dysfunction and lysosomal de-acidification are significant contributors to a common theme of lysosomal dysfunction throughout neurodegenerative disease.

Downstream of lysosomal acidification defects, a number of pathological events can occur progressively throughout the lysosomal network, and ultimately impact the overall health of the cell as this progressive lysosomal corruption worsens (Guicciardi et al., 2004). De-acidification of lysosomes impairs maturation of cathepsins and their activity, leading to oxidative stress in conjunction with impaired clearance of oxidatively damaged proteins (Wolfe et al., 2013). Beyond this hydrolytic dysfunction, abnormal rise in lysosomal pH interferes with axonal trafficking of autophagic compartments (Lee et al., 2011), stalls clearance of defective mitochondria (mitophagy), causes release of lysosomal calcium (McBrayer and Nixon, 2013) leading to harmful downstream effects of calpain activation (Rao et al., 2014) and kinase activation. Many of these impairments overlap and synergize, such as mitophagy failure as an additional source of oxidative stress. The accumulated effects destabilize lysosomal membrane integrity, sending neurons into a “death spiral” (Malkus et al., 2009).

APP-independent lysosomal defects in DS and AD

While the aforementioned evidence from individuals with DS (Doran et al., 2017) and DS cells (Jiang et al., 2010; Jiang et al., 2016) implicates *APP* gene dosage with AD-like phenotypes in DS, a number of cellular abnormalities associated with DS may also contribute to lysosomal dysfunction. Without regard for specific Ts21 genes, aneuploidy alone has been shown to exert stress on the proteostatic network (Dephoure et al., 2014;

Oromendia et al., 2012; Torres et al., 2007). The abnormally high mRNA expression in trisomy 21 leads to proteotoxic stress, ER stress, activation of the unfolded protein response (UPR), oxidative stress, elevated ubiquitinated proteins, and decreased proteasomal degradation. This general disruption of the entire proteostatic network represents an increased burden on lysosomal clearance, especially considering the decrease in proteasomal function. Extensive accumulation of ubiquitinated proteins has also been observed in DS brain tissue (Di Domenico et al., 2013; Tramutola et al., 2016); further suggesting systemic defects in protein quality control and failed waste clearance by both proteasomal and lysosomal systems.

Beyond global defects in protein quality control, a number of individual genes triplicated in DS are linked to lysosomal dysfunction. Notably, the triplication of SOD1 contributes to an oxidative stress burden in DS, which has been shown in DS primary cells and in amniotic fluid samples from DS individuals (Butterfield et al., 2014; Lott and Dierssen, 2010; Pagano and Castello, 2012; Perluigi et al., 2011; Zana et al., 2007; Zana et al., 2006). The increased oxidative stress in DS drives accelerated senescence in DS cells (Rodriguez-Sureda et al., 2015). Oxidative stress is also shown pathologically in brain tissue from DS patients and correlates with amyloid pathology (Cenini et al., 2012). A proteomic study of oxidatively modified proteins in DS brain tissue showed that a number of lysosomal proteins (including v-ATPase, CTSD, GRP78, UCH-L1, and GFAP) become modified, in a manner that couples with decreased activity of the proteasome and AP formation (Di Domenico et al., 2013). The v-ATPase, which is carbonylated on the V1B2 subunit, may be impaired functionally by such changes, contributing to de-acidification (Porter et al., 2013; Wang and Floor, 1998). Oxidative modifications to other lysosomal proteins can also hamper the functions of the lysosome, as evidenced in models of acute oxidative stress (Pal et al., 2016; Zhang et al., 2017). The role of oxidative stress in DS is an intriguing area of research, and is addressed further in other reviews in this monograph.

Cystatin B functions as an endogenous lysosomal protease inhibitor, specifically found to inhibit the functions of cathepsins. Previous research from our group (Yang et al., 2011a; Yang et al., 2014) demonstrates *in vivo* that deletion of cystatin B stimulates lysosomal enzyme function, leading to improvement of lysosomal proteolysis. CRND8 *APP* mutant mice, when crossed with cystatin B-deficient mice, exhibit improved learning/memory function and amelioration of amyloid pathology compared to the CRND8 mice. These findings are highly relevant to the discussion of lysosomal dysfunction in AD and DS because cystatin B is located on chromosome 21 (Pennacchio and Myers, 1996), and the increased gene dosage (due to trisomy of chromosome 21) of Cystatin B in DS could impair cathepsin function, contributing to lysosomal dysfunction in DS.

As in AD and numerous other neurodegenerative diseases (Guo et al., 2017), neurofibrillary pathology is observed in DS (Head et al., 2016). Neurofibrillary pathology in DS does not appear until age 30–40, manifesting in a similar neuroanatomical pattern as in AD (Hof et al., 1995). Despite the timing of neurofibrillary pathology in relation to amyloid deposition, tau pathogenesis in DS may occur due to both APP-dependent and APP-independent causes. Conversely to this time frame, there is evidence for changes in tau phosphorylation in DS even at a prenatal stage, so initial intracellular changes in tau phosphorylation and

proteolysis could occur long before tangle pathology is observed (Milenkovic et al., 2017). Rather than changes in the *MAPT* gene, the triplication of the *Dyrk1A* gene in DS (Kay et al., 2016; Yin et al., 2017) is shown to increase the 3R forms of tau, which is associated with tau pathology (Cardenas et al., 2012; Wegiel et al., 2011). Concurrently, the neurofibrillary tangles in DS brain show increased amounts of 3R tau isoforms and Dyrk1A-mediated phosphorylation relative to AD, suggesting a specific role for Drk1A in DS tau pathology. Tau itself, particularly in aggregated form, is also a substrate of lysosomal degradation (Vincia et al., 2014), and so impairments in lysosomal function could favor the accumulation of Drk1A-phosphorylated and/or misfolded/aggregated tau. RCAN1 (Cardenas et al., 2012) is also implicated in tau pathology in DS, via the stimulation of the tau kinase GSK3 β and inhibition of calcineurin. The progression of tau pathology in DS has been reported to occur subsequently to early-life accumulation of intracellular APP metabolites (Mori et al., 2002) but on a more rapid time frame than in AD possibly due to excessive Drk1A tau phosphorylation and RCAN1 gain of function as contributing genetic factors. Additionally, progression of tauopathy in both DS and AD is potentially accelerated by lysosomal dysfunction via release of lysosomal calcium, which promotes tau pathology through activation of calpains and tau kinases (Rao et al., 2014) (as described in Figure 3).

DS CELL LINES AND TRISOMIC MICE: WINDOWS INTO THE EARLIEST STAGES OF AD AND THE MULTIFACTORIAL ROLES OF APP IN PATHOGENESIS

Beside human postmortem fixed or frozen brain tissue from control or DS individuals, both primary cell lines obtained from human and various trisomy 21 mice are widely used in studies of DS (Choong et al., 2015; Prandini et al., 2007). Human DS and age-matched control fibroblast cell lines and Epstein Barr virus (EBV)-transformed human lymphoblastoid cell lines (LCLs) are widely available. In addition, iPSC cells derived from fibroblast of multiple DS patients (Park et al., 2008; Weick et al., 2013), including fetal fibroblasts of monozygotic twins (Hibaoui et al., 2014), have been differentiated into neurons and astroglia (Chen et al., 2014) for further functional studies.

The most widely used DS mouse models is Ts65Dn [B6EiC3Sn a/A-Ts(17¹⁶)65Dn/J], which has triplicate *App* and other HSA21 analogous genes on mouse chromosome 16 (MMU16) (Davisson et al., 1993). Ts65Dn and other mouse models with various lengths of triplicate genes comparable to human HSA21, with or without *App* have been reviewed in detail (Choong et al., 2015). Therefore, we would like to focus on the Ts2 mouse (Ts[Rb(12.17¹⁶)]2Cje), a model of DS generated by a spontaneous Robertsonian fusion, was characterized by Villar and colleagues (Villar et al., 2005). Ts2 mice possess higher transmission rate of MMU16, and male fertility, resulting in a ~3-fold higher viable offspring compared to Ts65Dn mice (Davisson et al., 1993). Our study (Jiang 2016) showed that Ts2 mice develop a similar regional pattern of APP-dependent neuronal endosomal abnormality (Cataldo et al., 2003), and a similar loss of basal forebrain cholinergic neurons (BFCN) as seen in Ts65Dn mice at the similar age (Davisson and Costa, 1999; Davisson et al., 1990; Granholm et al., 2000; Holtzman et al., 1996). The evidence of endosomal pathology prior to the onset of substantial BFCN loss in Ts2 mice indicates that early

endosomal alterations are an early manifestation of pathology and precede BFCN neuron loss. Ts2 and Ts65Dn mice also show similar hippocampal glutamatergic neuronal deficits (Kaur et al., 2014) and similar MRI anomalies (Chen et al., 2009). The breeding advantages of Ts2 mouse over the more extensively used Ts65Dn model recommend it strongly for future studies on the role of APP and additional relevant genes in recapitulating the earliest pathobiology in AD.

Compared to AD mouse models, the triplication of APP in trisomy models of DS is a relatively precise genetic model of DS, as the key features of early pathology mirror the human disease authentically, and have been cross validated in primary cells from DS individuals (Jiang et al., 2016). The utility of DS model mouse systems makes them a valuable resource in addition to existing AD mouse models, which are typically based on the induced expression of mutant forms of APP, PSEN1 or MAPT, but are unfortunately narrow in their recapitulation of a full human AD phenotype. Models based on familial AD-associated genetic mutations are limited in their applicability to sporadic AD, and current APP overexpression models of AD may cause a range of artifactual responses in neurons that obscure the disease effects exerted at endogenous levels of a pathogenic protein (Nilsson et al., 2014). A frequent criticism of common AD mouse models is that high expression of mutant APP induces an accelerated amyloid phenotype which doesn't fully model the gradual onset of human AD in relationship with ageing, and can mask the detection of earlier changes within the endocytic and lysosomal systems in human AD (Cataldo et al., 2000) due to excessive amyloid deposition and widespread neuronal death early in life. Considering the relevance of endocytic and lysosomal dysfunction in the early stages of AD, and the commonality of these defects in the genetics and pathology of DS and AD, we suggest DS models as useful tools in the study of endocytic-lysosomal defects fundamental to AD pathogenesis.

CONCLUSIONS

Combined with the genetic and neuropathological similarities between AD and Down syndrome, emerging findings indicate that disruption of endocytosis and autophagy are fundamental factors in the pathogenesis of both AD and DS (as summarized in Figure 1). The same endosomal-lysosomal disruptions that occur at a rapid pace during DS (and familial early onset forms of AD) develop at a slower pace during sporadic AD when neuronal ageing becomes a key additional factor in the progressive corruption of endocytosis and autophagy. The ability to characterize these earliest endosomal anomalies in tractable models of the authentic disease (DS fibroblasts and trisomic mice) has greatly facilitated the elucidation of pathogenic APP-dependent mechanisms in the absence of massive amyloid deposition and secondary reactions to it. The continued use of DS cellular and animal models represents an appealing complement to the limitations of models often used in AD research.

Given that effective therapies for AD and DS remain elusive, expansion of drug development pursuits to include potential targets in the endosomal-lysosomal and autophagy pathways may lead to improved therapies for both DS and AD. For example, preclinical research has shown that stimulation of lysosomal CTSD function (via genetic deletion of cystatin B) in

mouse models of AD reverses autophagic-lysosomal pathology and improves learning and memory (Yang et al., 2011a). Our group and others also demonstrated improvement of lysosomal function in *in vitro* AD models using acidic nanoparticles to restore lysosomal acidification (Bourdenx et al., 2016; Lee et al., 2015; Xue et al., 2014). These studies, in conjunction with others (Menziez et al., 2017), demonstrate the potential of targeting endocytic-lysosomal function as a therapeutic avenue in neurodegenerative disease.

Additionally, modulation of β CTF generation has previously been ignored as a therapeutic target in AD and DS, even when clinical trials have been carried out with BACE1 inhibitors developed with the goal of lowering A β levels. The complex regulation of BACE1 compared with gamma-secretase, the worsening of cognition seen in clinical trials of gamma-secretase inhibitors that elevate β CTF levels as they lower A β levels, and the existence of a “protective mutation” of APP (Hashimoto and Matsuoka, 2014) that acts to reduce β CTF (not only A β), and the effects of mutation of PSEN1 and APP which alter β CTF as well as A β , are all consistent with the mounting data showing that β CTF is a regulator of endosomal signaling that becomes aberrant at the earliest stages of AD and in DS and is linked to early cholinergic neurodegeneration.

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Abbreviations

AD	Alzheimer Disease
DS	Down Syndrome
Aβ	amyloid- β
HSA21	Chromosome 21
APP	Amyloid precursor protein
BACE1/2	β -site APP cleaving enzyme 1/2
βCTF	β carboxyl-terminal fragment
PSEN1	presenilin 1
APPL1	adaptor protein containing pleckstrin homology domain, phosphotyrosine binding domain, and leucine zipper motif
GEF	guanine nucleotide exchange factor
GAP	GTPase-activating protein
GDI	GDP dissociation inhibitor
GDF	GDI displacement factor

PI3K/vps34	phosphoinositide 3-kinase
SYN1	synaptojanin1
Class C VPS/HOPS	class C vacuolar protein sorting/homotypic fusion and protein sorting
ESCRT	sorting complexes required for transport
LBPA	lyso-bisphosphatidic acid
BFCN	basal forebrain cholinergic neurons
NGF	nerve growth factor
Trk	tropomyosin receptor kinase
ILV	intra-lumenal vesicles
MVB	multivesicular body
SNX	sorting nexin
Vps	vacuolar protein sorting
AP	autophagosome
LC3	Microtubule-associated protein 1A/1B-light chain 3
mTOR	mammalian target of rapamycin
CTSD	Cathepsin D
v-ATPase	vacuolar ATPase
UPR	unfolded protein response
DYRK1A	Dual specificity tyrosine phosphorylation regulated kinase 1A
RCAN1	Regulator of calcineurin 1
SOD1	Superoxide dismutase 1
CSTB	Cystatin B
MAPT	Microtubule-associated protein tau
GSK-3β	Glycogen synthase kinase 3 β
cdk5	cyclin-dependent kinase 5
LCLs	lymphoblastoid cell lines
iPSCs	induced pluripotent stem cells
MMU16	mouse chromosome 16

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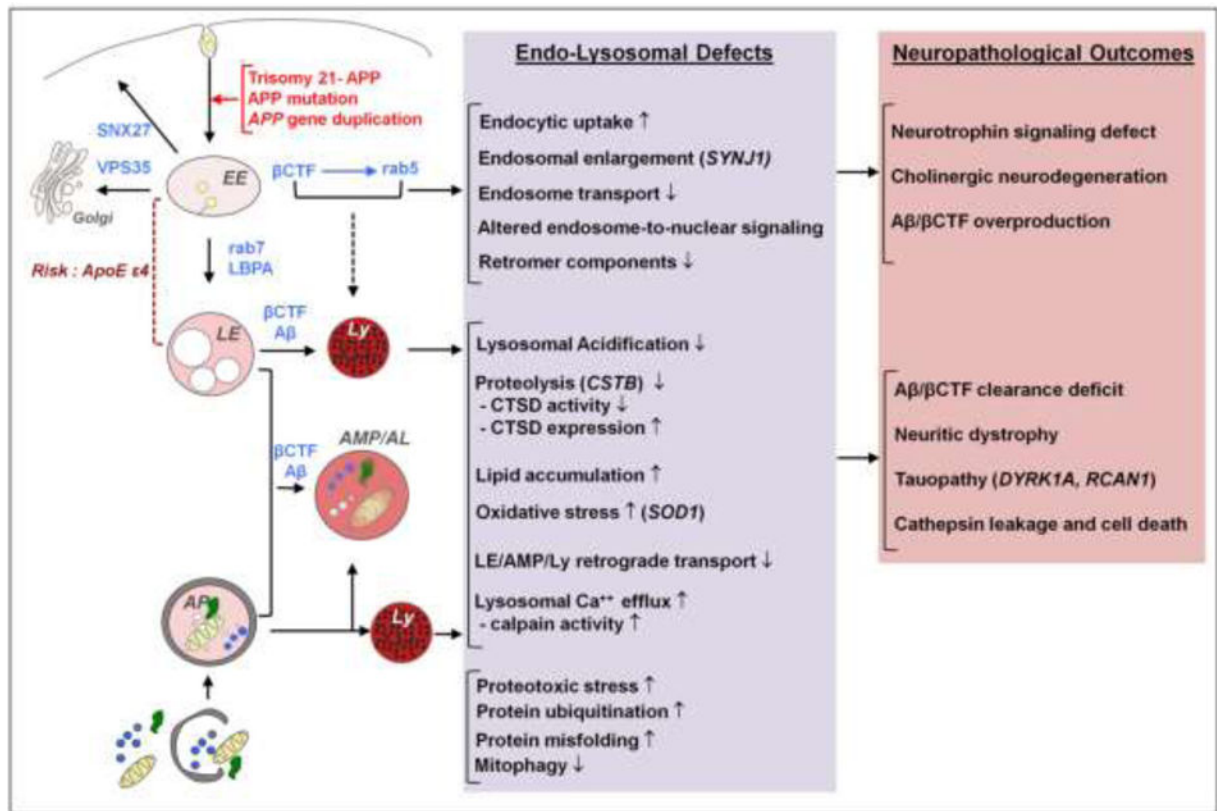


Figure 1.

Overview of the shared pathways and pathological mechanisms driven by the APP gene and its cleavage products (βCTF/Aβ) in DS and AD: known and predicted endocytic and lysosomal factors contributing to neurodegeneration. Genetic contributors to neurodegeneration in DS other than APP are shown in parentheses. ApoE ε4, a known genetic risk factor for AD, contributes to endolysosomal dysfunction in AD and potentially in DS. EE, early endosome; LE, late endosome; AP, autophagosome; Ly, Lysosome; AMP/AL, amphisome/autolysosome.

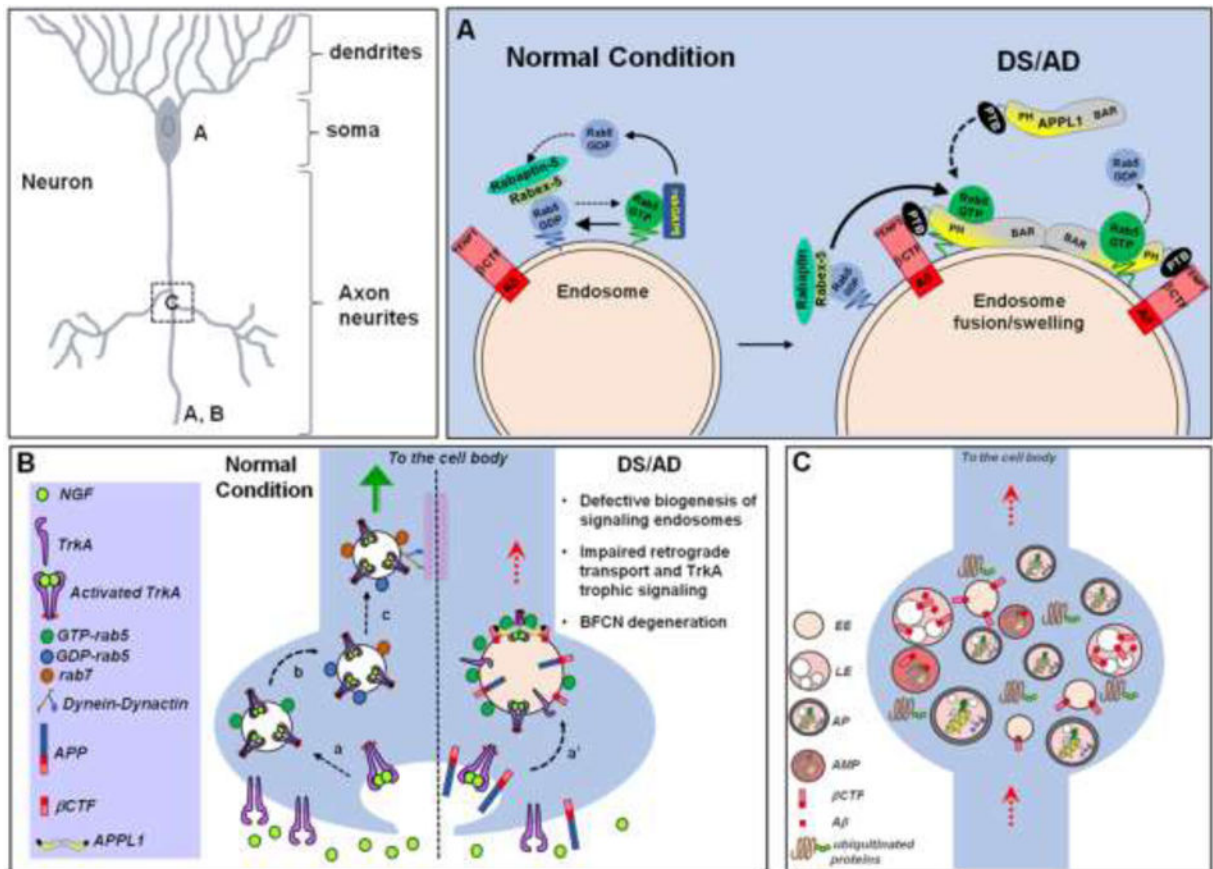


Figure 2.

Endosomal pathology and altered endosome trafficking in DS and AD contribute to aberrant neurotrophin transport and signaling and neuritic dystrophy. A) Under normal conditions, the conversion of rab5 from an active to an inactive state occurs rapidly and it is highly regulated by the coordinated action of rab5 effectors rabex-5/rabaptin-5 and rabGAP5, respectively. In DS and AD, APP-βCTF mediates pathological activation of rab5 by recruiting APPL1 to endosomes. APPL1 binds to the “YENPT” motif of βCTF through its phosphotyrosine binding (PTB) domain. Upon dimerization via its BAR domains to facilitate vesicle curvature, APPL1 binds to GTP-rab5 via its PH domain, which stabilizes rab5 in its activated state on endosomes. Over-activation of rab5 causes acceleration of endocytosis and endosome fusion leading to characteristic swelling of endosomes. B) Under normal conditions, biogenesis of long-lived signaling endosomes involves endocytosis of activated TrkA receptors (NGF-bound TrkA) into rab5-positive endosomes (a), followed by deactivation of rab5 and acquisition of rab7 (b), which binds to dynein/dynactin motor complexes regulating long-range retrograde axonal transport (c, green arrow). In DS and AD, prolonged rab5 hyperactivation by APP/APP-βCTF and APPL1 causes enlargement of NGF/TrkA endosomes (a') and slows retrograde endosome transport (red dotted arrow), thus diminishing TrkA signaling which ultimately leads to the loss of trophic support for basal forebrain cholinergic neurons (BFCNs). C) DS and AD are characterized by the pathological accumulation of ubiquitinated misfolded/aggregated proteins and of enlarged early and late endosomal structures (EE and LE, respectively) containing βCTF/Aβ fragments.

Autophagosomes (AP), amphisomes (AMP), and endolysosomal compartments containing incompletely degraded material accumulate within neuritic swellings (“dystrophic neurites”) as a result of the β CTF/A β -mediated impairment of retrograde transport and lysosomal function.

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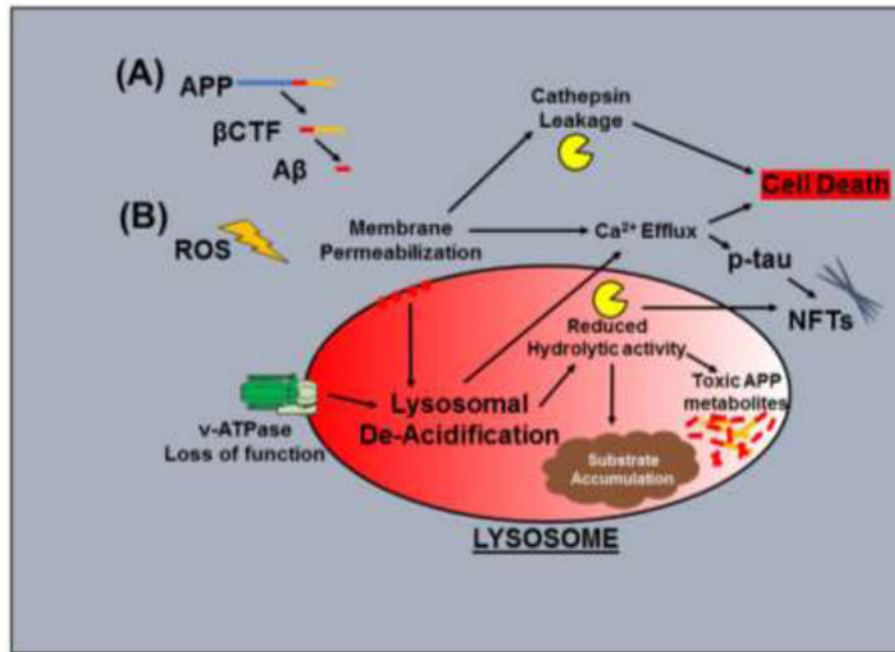


Figure 3. Common mechanisms of lysosomal dysfunction in neurons during AD and DS: (A) APP and its cleavage products, β CTF and $A\beta$, contribute to lysosomal de-acidification and lysosomal membrane permeabilization, leading to a decline in lysosomal function. (B) Reactive oxygen species (ROS), which are increased in DS and in AD, also contribute to impaired lysosomal function. Congruent elements of lysosomal dysfunction; including lysosomal de-acidification, reduced proteolysis, lysosomal membrane permeabilization leading to loss of membrane integrity, and the release of Ca^{2+} and cathepsins represent common pathological outcomes in AD and DS, leading to accumulation of intralysosomal waste, toxic APP and tau metabolite accumulation, neurofibrillary tangle (NFT) formation, and cell death.