

## Review Article

# Dysregulation of the Autophagy-Endolysosomal System in Amyotrophic Lateral Sclerosis and Related Motor Neuron Diseases

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Amyotrophic lateral sclerosis (ALS) is a heterogeneous group of incurable motor neuron diseases (MNDs) characterized by a selective loss of upper and lower motor neurons in the brain and spinal cord. Most cases of ALS are sporadic, while approximately 5–10% cases are familial. More than 16 causative genes for ALS/MNDs have been identified and their underlying pathogenesis, including oxidative stress, endoplasmic reticulum stress, excitotoxicity, mitochondrial dysfunction, neural inflammation, protein misfolding and accumulation, dysfunctional intracellular trafficking, abnormal RNA processing, and noncell-autonomous damage, has begun to emerge. It is currently believed that a complex interplay of multiple toxicity pathways is implicated in disease onset and progression. Among such mechanisms, ones that are associated with disturbances of protein homeostasis, the ubiquitin-proteasome system and autophagy, have recently been highlighted. Although it remains to be determined whether disease-associated protein aggregates have a toxic or protective role in the pathogenesis, the formation of them results from the imbalance between generation and degradation of misfolded proteins within neuronal cells. In this paper, we focus on the autophagy-lysosomal and endocytic degradation systems and implication of their dysfunction to the pathogenesis of ALS/MNDs. The autophagy-endolysosomal pathway could be a major target for the development of therapeutic agents for ALS/MNDs.

## 1. Introduction

Amyotrophic lateral sclerosis (ALS) is a heterogeneous group of inexorable neurodegenerative disorders characterized by a selective loss of upper and lower motor neurons in the brain and spinal cord [1, 2]. Most patients die of respiratory failure within 3–5 years. Although ALS is one of the best studied and a well-known form of motor neuron diseases (MNDs), the molecular pathogenesis of ALS is still unclear [1, 2]. To date, no effective therapeutic interventions to cure or even relieve symptoms are available [3].

Most cases of ALS are sporadic, while approximately 5–10% cases are familial. Recent advances in human genetics and genomics greatly facilitate the chromosomal mapping of disease loci, and the identification of causative genes

and mutations predisposing to many familial forms of ALS/MNDs [1]. Thus far, more than 16 ALS causative genes including *SOD1*, *ALS2*, *SETX*, *SPG11*, *FUS*, *VAPB*, *ANG*, *TARDBP*, *FIG4*, *OPTN*, *ATXN2*, *VCP*, *C9orf72*, *UBQLN2*, *SIGMAR1*, and *CHMP2B* have been identified [1, 4] (<http://neuromuscular.wustl.edu/index.html>) (Table 1). The following characterizations of the disease-causing and -related gene products, in conjunction with the creation of animal models, have successfully unveiled the molecular basis underlying the pathogenesis of ALS/MNDs, such as oxidative stress, endoplasmic reticulum (ER) stress, excitotoxicity, mitochondrial dysfunction, neural inflammation, protein misfolding and accumulation, dysfunctional intracellular trafficking, abnormal RNA processing, and noncell-autonomous damage [4–11]. It is currently believed that

TABLE 1: Genes associated with ALS and other neurodegenerative diseases.

Disease type	Locus	Gene	Protein	Inheritance*	Onset	Function	Mutation linked to other diseases
ALS1	21q22.11	<i>SOD1</i>	SOD1	D	Adult	Oxidative and ER stress response	
ALS2	2q33.1	<i>ALS2</i>	ALS2/alsin	R	Juvenile	Trafficking and protein degradation	PLSJ, IAHSP
ALS3	18q21	—	—	D	Adult	—	
ALS4	9q34.13	<i>SETX</i>	Senataxin	D	Juvenile	DNA damage response	AOA2
ALS5	15q21.1	<i>SPG11</i>	Spatacsin	R	Juvenile	—	SPG11
ALS6	16p11.2	<i>FUS</i>	FUS	D	Adult	DNA and RNA metabolism	ALS-FTD
ALS7	20p13	—	—	D	Adult	—	
ALS8	20q13.32	<i>VAPB</i>	VAPB	D	Adult	ER and Golgi membrane trafficking	SMA4
ALS9	14q11.2	<i>ANG</i>	Angiogenin	D	Adult	Neuroprotection	PD or ALS-PD
ALS10	1p36.22	<i>TARDBP</i>	TDP-43	D, R, or S	Adult	DNA and RNA metabolism	ALS-FTD, FTD
ALS11	6q21	<i>FIG4</i>	FIG4	D or S	Adult	PI (3,5) P2 regulation	CMT4J
ALS12	10p13	<i>OPTN</i>	Optineurin	D or R	Adult	NFkB regulation	GLC1E
ALS13	12q24.12	<i>ATXN2</i>	Ataxin-2	D	Adult	Gene regulation	SCA2
ALS14	9p13.3-p12	<i>VCP</i>	VCP or p97	D	Adult	Protein degradation	IBMPFD
ALS15	Xp11.21	<i>UNQLN2</i>	Ubiquilin-2	D	Adult	Protein degradation	ALS-FTD
ALS16	9p13.3	<i>SIGMAR1</i>	SIGMAR1	R	Juvenile	ER chaperon	
ALS-FTD1	9q21-q22	—	—	D or S	Adult	—	
ALS-FTD2	9p21.2	<i>C9orf72</i>	C9ORF72	D or S	Adult	—	FTD
ALS-FTD3	3p11.2	<i>CHMP2B</i>	CHMP2B	D	Adult	Trafficking and protein degradation	
DHN-7B	2p13.1	<i>DCTN1</i>	Dynactin-1	D	Adult	Trafficking	Perry syndrome
CMT2B	3q21.3	<i>RAB7</i>	Rab7	D	Adult	Trafficking and protein degradation	
CMT2O	14q32.31	<i>DYNC1H1</i>	Dynein	D	Adult	Trafficking	SMA-LED and MRD13
ALS**	5q35.3	<i>SQSTM1</i>	Sequestosome or p62	?	Adult	Protein degradation	PDB

\*Inheritance (D: dominant, R: recessive, and S: sporadic). FTD: Frontotemporal dementia, DHN: distal hereditary motor neuronopathy, CMT: Charcot-Marie-Tooth disease, PDB: Paget disease of bone, PLSJ: primary lateral sclerosis juvenile, IAHSP: infantile-onset ascending hereditary spastic paralysis, AOA: ataxia-ocular apraxia-2, SPG: spastic paraplegia, SMA: spinal muscular atrophy, PD: Parkinson's disease, GLC1E: glaucoma 1, open angle, E, SCA2: spinocerebellar ataxia-2, IBMPFD: inclusion body myopathy with dementia and Paget disease of bone, SMA-LED: spinal muscular atrophy with lower limb predominance, and MRD13: mental retardation, autosomal dominant 13. \*\*ALS: Fecoto et al. reported several novel SQSTM1 mutations in patients with ALS and predicted 8 of 9 missense variants behave like a pathogenic mutant by in silico analysis [64].

a complex interplay of such multiple toxicity pathways, rather than a single independent mechanism, is implicated in the ALS/MND's pathogenesis [4–6].

Among these pathogenic mechanisms, ones that are associated with disturbances of protein homeostasis have been highlighted, as the accumulation of insoluble protein aggregates is the cardinal pathological feature for ALS and other neurodegenerative diseases [12]. Although it remains to be determined as to whether such protein aggregates have a toxic or protective role in the pathogenesis of ALS/MNDs, it is conceivable that the formation of them results from the imbalance between generation and degradation of misfolded proteins within neuronal cells. In eukaryotes, there are two main degradation systems for cytoplasmic proteins, that is, the ubiquitin-proteasome system (UPS) and autophagy. The UPS is mainly involved in selective clearance for short-lived proteins [13], while autophagy is the mechanism by which the long-lived as well as misfolded proteins can be removed by the endolysosomal system [14, 15]. It is also noted that

the involvement of endocytosis and vesicle trafficking in the regulation of protein homeostasis and degradation have recently emerged [10, 16–18].

In this paper, we aim to give a comprehensive view on the autophagy-endolysosomal system and implication of its dysfunction to the pathogenesis of ALS/MNDs. Excellent review articles specialized on the role of the UPS in ALS/MNDs can be found elsewhere [13, 19].

## 2. The Autophagy-Endolysosomal System

**2.1. Autophagic Pathways.** Autophagy is an evolutionally conserved lysosomal degradation system that is tightly linked to a wide variety of physiological processes such as protein homeostasis, removal of pathogens, and antigen presentation. There are at least three forms of autophagic pathways; macroautophagy, microautophagy, and chaperon-mediated autophagy, among which macroautophagy, hereafter referred to as “autophagy”, plays a crucial role in the removal

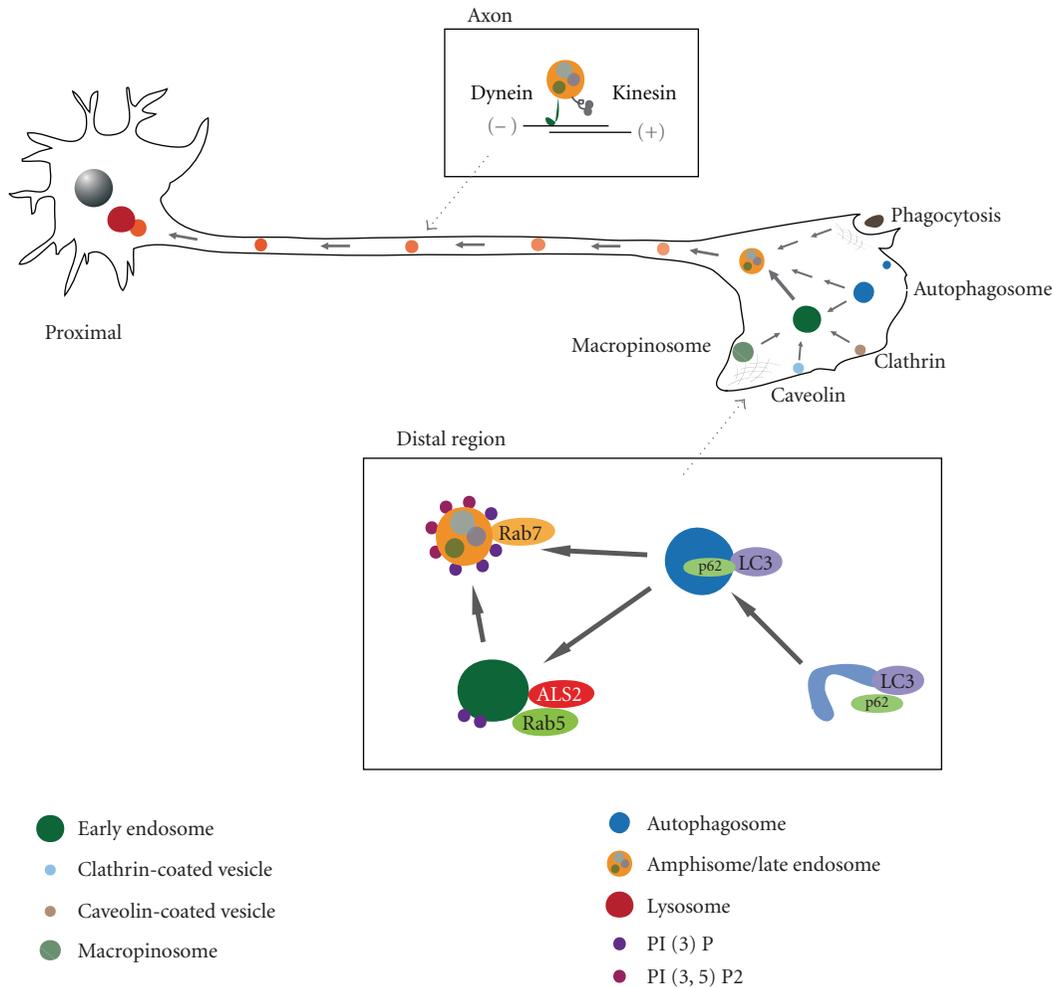


FIGURE 1: Endocytic trafficking and the autophagy-endolysosomal system in neurons.

of cytoplasmic long-lived as well as misfolded proteins [14, 20–23]. Autophagy comprises three sequential steps; autophagosome formation, maturation, and degradation within lysosomes, through which entrapped cargo molecules within autophagosomes can be degraded and reutilized for the synthesis of newer cellular components.

These multiple steps of autophagy are highly orchestrated by a common group of proteins called ATG (autophagy-related), such as Atg5 and Atg7 [14], and Rab GTPases, a large family of small G proteins [24]. While most autophagic pathways are Atg5/Atg7-dependent, an Atg5/Atg7-independent but Rab9-dependent alternative autophagic pathway has recently been found in mammals [25]. Although autophagy is highly upregulated under stress conditions such as nutritional starvation [14] and exercise [26] (BCL2/Beclin-1-dependent inducible autophagy), several lines of evidence support the existence of basal or constitutive autophagy (BCL2/Beclin-1-independent basal autophagy) in most cell types including neuronal and muscle cells [26–30]. In fact, despite that starvation does not induce autophagy in the brain [31], either Atg5 or Atg7 deficiency in neurons results

in the accumulation of misfolded proteins and neurodegeneration [29, 30], indicating that basal autophagy has a crucial role in the central nervous system (CNS).

Whichever autophagic mechanisms except Atg5/Atg7-independent alternative [25] are involved, their activation can be monitored by the level and distribution of two autophagy-associated proteins. One is microtubule-associated protein 1 light chain 3 (LC3), a yeast Atg8 homolog, whose lipidated forms (LC3-II) are highly enriched onto autophagosomal membranes [32] (Figure 1). The other is p62 (a.k.a. sequestosome 1/SQSTM1), an adaptor molecule for selective autophagosomal degradation of ubiquitinated targets, which directly binds to LC3, thereby promoting the recruitment and engulfment of cargos to autophagosomes [33–35] (Figure 1).

**2.2. Endocytic Pathways.** Endocytosis is an evolutionally conserved cellular process involving the internalization of a wide variety of molecules from the surface of cells. There are at least four distinct well-recognized endocytic pathways in eukaryotes: phagocytosis, macropinocytosis,

clathrin-mediated endocytosis (CME), and caveola-mediated endocytosis [36–38] (Figure 1). In addition, several other uncharacterized clathrin- and caveolin-independent pathways exist [38]. Each endocytic pathway mediates the transport of specific cargo molecules and delivers them to the correct destinations within cells. It is highly appreciated that most internalized vesicles and/or vacuoles containing specific cargos mature to or fuse with early endosomes before the cargos are delivered to their end destinations [38], and that a variety of the distinctive Rab GTPases, such as Rab5 and Rab7, control endocytosis and vesicle trafficking as well as cargo transportation [39]. Interestingly, a recent study has demonstrated that certain types of endocytosis (macropinocytosis) and autophagy are oppositely regulated by a phospholipid binding protein Annexin A5, suggesting a coordinated cross-talk between endocytic and autophagic pathways [40].

**2.3. Maturation of Autophagosomes, Macropinosomes, and Endosomes.** Nascent autophagosomes undergo a stepwise maturation, resulting in the creation of amphisomes and autolysosomes by fusion with multiple endocytic compartments, such as early endosomes, multivesicular bodies (MVBs), late endosomes, and lysosomes [41, 42]. Amphisomes, an intermediate hybrid vesicular compartment, contain both autophagosomal and endosomal contents, while autolysosomes are formed either from amphisomes or directly from autophagosomes by fusion with lysosomes [41] (Figure 1). Notably, not only the internalized vacuoles such as macropinosomes that are generated through macropinocytosis but also early endosomes themselves sequentially mature into MVBs, late endosomes, and lysosomes [15] (Figure 1). Further, it has recently been shown that fusion of autophagosomes with early endosomes is required for autophagy [43]. These findings strongly support an intimate crosstalk between autophagic and endocytic pathways particularly in their maturation steps.

Elaborate molecular mechanisms regulating the maturation of intracellular vesicular/vacuolar compartments have recently begun to emerge. Those include Rab-switching, phosphatidylinositol (PI) conversion, endosomal sorting complex required for transport (ESCRT) machinery, as well as luminal acidification, as reviewed in detail elsewhere [15, 41, 42, 44]. Among the systems, the sequential action of the small GTPases Rab5 and Rab7, that is, the Rab5-Rab7 switching, plays a central role in the early step of the endosome maturation; early to late endosomes [15, 45, 46]. Further, it has been reported that the sequential reaction of Rab5-Rab21-Rab7 plays a pivotal role in macropinocytosis and the macropinosome maturation [47]. PI conversions mediated by VPS34 and PIKfyve also tightly link to the maturation from Rab5- to Rab7-positive endosomes [15]. Of particular, the Class III phosphatidylinositol-3 kinase (PI3K) complexes containing p150, Beclin-1, VPS34, and UVRAG positively regulate the maturation of both autophagosomes and endosomes [15, 42, 48]. On the other hand, the ESCRT complexes play a major role in the later step of the autophagosome, amphisome, and endosome maturation [49]. Moreover, histone deacetylase-6 (HDAC6), a

ubiquitin-binding deacetylase acting as a central component of basal autophagy, selectively targets the ubiquitinated proteins to autophagosomes [35], and controls the autophagosome maturation rather than the autophagosome formation [50]. Despite of such recent progress, molecular mechanisms underlying coordinated regulation of multiple maturation steps by these factors are still incompletely understood.

Since autophagosomes as well as endosomes are motile within cells [51, 52], it is reasonable that their movements are linked to their maturation stages, particularly in differentiated neuronal cells. Indeed, Rab5 and Rab7 act in a coordinated manner in controlling the early stage of maturation and the axonal retrograde transport of vesicles in motor neurons [52]. Further, Snapin, a neuronal SNARE-binding protein acting as an adaptor linking late endosomes to the dynein complex, plays key roles not only in dynein-mediated retrograde transport but also in late endosomal-lysosomal maturation in neurons [53]. Most recently, it has also been shown that autophagosomes are formed and fuse with late endosomes and/or immature lysosomes distally, and their maturation progresses during transport along the axons in primary dorsal root ganglion (DRG) neurons [54].

**2.4. Lysosomal Degradation.** The final step of the autophagy-endolysosomal system is degradation of cargo molecules within lysosomes. Two classes of proteins; lysosomal acid hydrolases and lysosomal membrane proteins (LMPs), play essential roles in degradation of cargos in lysosomes. Lysosomal acid hydrolases such as cathepsins are involved not only in bulk degradation of substrates (cargos) but also in other physiological processes such as antigen processing. On the other hand, LMPs have a wide variety of functions including luminal acidification, import of cytosolic proteins, and transport of degraded materials to cytosol. Excellent review article specialized on lysosome biogenesis is available elsewhere [55].

Lysosomal positioning is dynamically regulated by nutritional conditions, in which a starvation induces preferential relocalization of lysosomes from cell peripheries to the juxtannuclear regions close to the microtubule-organizing center (MTOC), thereby regulating the autophagic flux in cells [56]. In neurons, bidirectional movements of lysosomes within axons are observed [57, 58]. While autophagosomes and endosomes are also bidirectionally moved in the distal axons [54], they are rather exclusively transported in a retrograde direction upon fusion with lysosomal-associated membrane protein 1 (LAMP-1)-positive late endosomes and/or immature lysosomes [51, 52, 54, 57]. Further, fully matured lysosomes containing active lysosomal hydrolases are confined to the proximal region of axons or the cell body [54, 57]. Thus, autophagosomes and endosomes formed in axons must be transported to the cell body for a complete digestion of their cargos [27] (Figure 1). Recent evidence showing that defective Snapin-dynein-mediated retrograde transport in neurons results in the aberrant accumulation of immature lysosomes and impaired lysosomal degradation [53] supports this notion. Taken together, lysosomal degradation of either engulfed or internalized cargos in neurons

might be strictly dependent on retrograde transport and late endosomal-lysosomal trafficking [51, 53, 58].

### 3. Dysfunction of the Autophagy-Endolysosomal System in Motor Neuron Diseases

Growing evidence supports a role of the autophagy-endolysosomal pathway in the pathogenesis of ALS/MNDs. Indeed, the accumulation of autophagosomes were observed in the spinal cord of sporadic ALS patients [59], indicating autophagic dysfunction in ALS. Autophagic dysfunction includes defects in the initiation (formation of autophagosomes) and/or maturation stages of autophagic processes, as well as imbalance between them, resulting in aberrant accumulation of misfolded and/or aggregated proteins within cells. Such pathological conditions disturb neuronal homeostasis, leading to neurodegeneration. In this section, we focus on causative and/or associated genes for ALS/MNDs, whose gene products functionally link to the autophagy-endolysosomal system; including *SOD1* [60], *FIG4* [61], *VCP* [62], *CHMP2B* [63], *SQSTM1* [64], *DCTN1* [65], *DYNC1H1* [66], and *RAB7A* [67] (Table 1). We describe *ALS2* [68, 69] and its product ALS2/alsin, an emerging regulator for autophagy-endolysosomal system [70, 71], in a separate section (see Section 4). Other ALS/MND causative genes, such as *TARDBP* [72], *OPTN* [73], and *UBQLN2* [74], which are also associated with protein degradation, are described in detail elsewhere [4, 75].

**3.1. Superoxide Dismutase 1 (*SOD1*): *ALS1*.** Mutations in *SOD1* that encodes superoxide dismutase 1 (*SOD1*) account for an approximately 20% of familial ALS cases [1]. It is currently believed that the *SOD1*-mediated dismutase enzymatic activity is not a major determinant for the phenotypic modification in ALS, since there is no correlation between disease severities and the *SOD1* dismutase activities [76, 77]. Rather, the propensity for the aggregate formation associated with mutant *SOD1* proteins, that is, gain of toxic function, might be related to the phenotypic expression of disease [78]. Recently, it has been reported that the normal as well as mutant *SOD1* proteins are degraded both by the UPS and the autophagy-endolysosomal system [70, 79]. *SOD1* mutants can be recognized by p62 in an ubiquitin-independent manner and targeted for degradation through the autophagy-endolysosomal pathway [80, 81]. Importantly, progressive enhancement of autophagy and/or decrease of autophagic flux are detected in a mutant *SOD1* (*SOD1*<sup>G93A</sup>)-expressing ALS mouse model [82–84]. Most recently, heat-shock protein 70 (*Hsp70*) and *Bcl2*-associated athanogene 3 (*BAG3*) mediate the ubiquitination-independent autophagic degradation of misfolded proteins including *SOD1* mutants [85]. It is also noted that *SOD1* mutants directly bind to the retrograde motor protein complex, thereby disturbing axonal transport [86–88] (see Section 3.6). Taken together, it is conceivable that increased accumulation of *SOD1* mutants as disease progresses disturb the autophagy-endolysosomal system.

**3.2. Phospholipid Phosphatase *Fig4*: *ALS11*.** Mutations in *FIG4* account for a form of autosomal recessive Charcot-Marie-Tooth type 4J (*CMT4J*) [89]. Interestingly, an approximately 2% of patients with ALS and primary lateral sclerosis (PLS) carry heterozygous deleterious mutations (nonsynonymous variants) in *FIG4* [61], indicating that *FIG4* is implicated in the pathogenesis of both peripheral neuropathy and ALS/MNDs. *FIG4* encodes a phosphoinositide 5-phosphatase, *Fig4*, that regulates the intracellular level of phosphatidylinositol-3,5-bisphosphate (*PI(3,5)P<sub>2</sub>*). It has been shown that mutation in *FIG4* results in a significant reduction of the *PI(3,5)P<sub>2</sub>* level in cultured cells [88]. Further, mice lacking *Fig4*, exhibit the accumulation of LC3-II, p62, and *LAMP-2* in neurons and astrocytes, and die earlier than wild-type litters [90]. Thus, deregulation of the autophagy-endolysosomal system, namely the later stage of autophagosome and/or endosome maturation, might be associated with the pathogenesis of *FIG4*-linked ALS/MNDs (Figure 2).

**3.3. Valosin-Containing Protein (*VCP/p97*): *ALS14*.** Mutations in *VCP* have previously been identified in patients with inclusion body myopathy associated with Paget disease of bone and frontotemporal dementia (*IBMPFD*) [91]. Recently, exome sequencing reveals *VCP* mutations as a cause of familial ALS, accounting for 1-2% of familial ALS [62]. *VCP* encodes valosin-containing protein (*VCP/p97*) that belongs to the AAA+ (ATPases associated with various activities) protein family, being implicated in multiple cellular processes including the UPS [92, 93]. A recent study has shown that *VCP/p97* regulates endolysosomal sorting of endocytosed ubiquitinated cargos such as caveolin-1 [94]. Further, loss of *VCP/p97* accelerates the accumulation of autophagosomes [95], and expression of *IBMPFD*-linked mutants results in the impaired maturation of autolysosomes with accompanying the cytoplasmic accumulation of TAR DNA-binding protein (*TDP-43*), a causative gene product for *ALS10* and a major constituent of ALS-linked cytoplasmic inclusions [72, 95–97]. Thus, *VCP/p97* might play essential roles not only in the maturation of autophagosomes and endolysosomes, but also in the regulation of intracellular dynamics of *TDP-43*.

**3.4. Charged Multivesicular Body Protein 2B (*CHMP2B*): *ALS-FTD3*.** Mutations in *CHMP2B* have been identified in patients with FTD and ALS-FTD [63, 98]. *CHMP2B* encodes charged multivesicular body protein 2B (*CHMP2B*), a component of the ESCRT-III complex. The ESCRT complexes are known to play important roles in MVB biogenesis and autophagosomal-endolysosomal maturation [99]. Either functional loss of ESCRT-III or ectopic expression of disease-linked *CHMP2B* mutants causes the accumulation of LC3-positive autophagosomes accompanying protein aggregates containing ubiquitinated proteins and p62 [49], and results in dendritic retraction prior to neurodegeneration [100]. Interestingly, ESCRT-depleted cells also exhibit the accumulation of *TDP-43* positive cytoplasmic inclusions [49]. These results indicate that deregulation of MVB biogenesis and

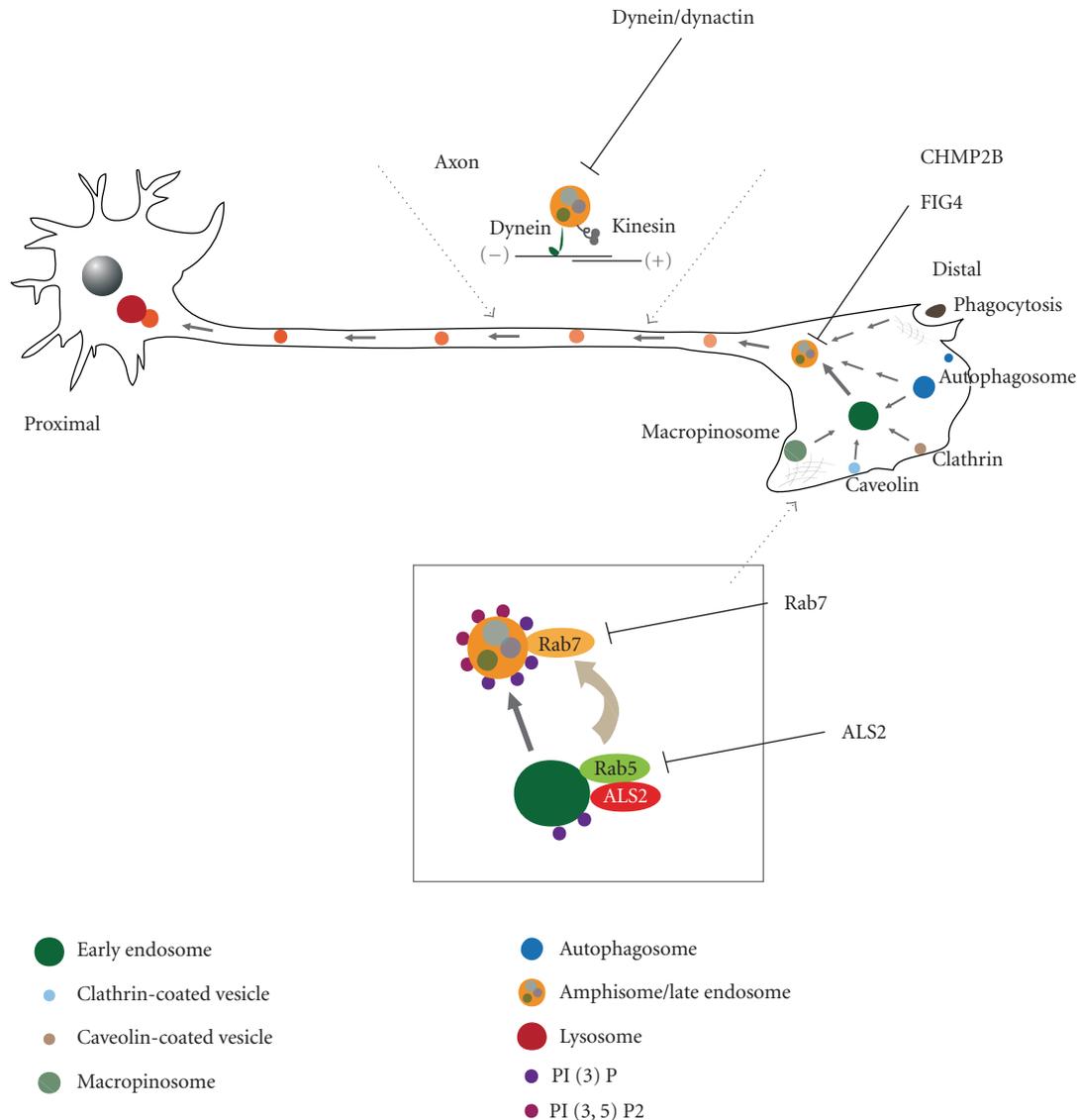


FIGURE 2: ALS-linked mutations in the genes, whose protein products are associated with autophagy-endolysosomal system and/or endocytic trafficking, underlie the pathogenesis of ALS and related motor neuron diseases.

autophagy is implicated in the pathogenesis of *CHMP2B*-linked FTD and ALS-FTD (Figure 2).

3.5. *Sequestosome 1 (SQSTM1/p62)*. *SQSTM1* encodes SQSTM1/p62 that was originally isolated as an interacting protein for the atypical protein kinases (aPKCs) [101]. It has been shown that p62 acts as an adaptor and/or scaffold protein that regulates not only the NF- $\kappa$ B activation through the binding with aPKCs but also the selective-autophagy via association with ubiquitinated misfolded proteins [102–104]. Further, accumulation of p62 by defective autophagy causes competitive inhibition of the oxidative-stress responsive transcription factor Nrf2-Keap1 interaction, resulting in activation of Nrf2 and its target antioxidative stress genes [105]. Conversely, genetic inactivation of *Sqstm1* in mice results in the accumulation of hyperphosphorylated tau and neurodegeneration [106]. Although mutations in

*SQSTM1* have originally been identified in patients with Paget disease of bone (PDB) [107], a recent study has revealed several missense variants in *SQSTM1* in familial as well as sporadic ALS [64]. It is notable that abundant p62-positive inclusions in the brain are a typical pathological feature of ALS or ALS-FTD associated with hexanucleotide repeat expansion in *C9orf72* [108, 109]. Considering the facts that two independent genes linking to forms of Paget disease of bone; *VCP* for IBMPFD and *SQSTM1* for PDB, are also associated with ALS and/or ALS-FTD, and that both *VCP* and p62 are key regulators for the autophagy-endolysosomal system, dysregulation of such *VCP/p62*-associated common pathological pathways might account for these seemingly different diseases.

3.6. *Dynein/Dynactin Complex*. Mutation in *DCTN1* encoding the p150 subunit of the transporter protein dynactin

has been identified in autosomal dominant form of lower MNDs [65]. Dynactin functions as an adaptor between dynein and various cargos, thereby regulating the efficiency of dynein motor [11]. It has also been shown that expansion of polyglutamine-tract in androgen receptor, which causes a form of motor neuron disease; spinal and bulbar muscular atrophy (SBMA), results in polyglutamine-dependent transcriptional dysregulation of dynactin [110]. Moreover, overexpression of dynamitin (p50) subunit of dynactin, which causes a dissociation of the dynactin complex, thereby interfering the dynein/dynactin-dependent retrograde transport, causes MND in mice [111]. On the other hand, several studies identify mutations in the component of dynein motor complex itself. Exome sequencing reveals the mutation in *DYNC1H1* encoding cytoplasmic dynein heavy chain in patients with dominant form of axonal CMT [66]. Mutations in a mouse homolog *Dync1h1* have also been identified, resulting in progressive motor neuron degeneration in mice [86]. It has been demonstrated that a mutant SOD1-expressing ALS mouse model carrying dynein mutation shows a defective axonal transport [86, 87]. Further, SOD1 mutants preferentially interact with the dynein complex, disturbing their functions [88]. Interestingly, decreased dynein function impairs the autophagy-dependent clearance of misfolded protein aggregates in parallel with the increased level of LC3-II-positive autophagosomes [112]. Collectively, defects in dynein/dynactin-mediated retrograde axonal transport are involved in etiology of ALS/MNDs [11] (Figures 1 and 2).

**3.7. Small GTPase Rab7.** Charcot-Marie-Tooth type 2B (CMT2B) is an autosomal-dominant peripheral neuropathy caused by the missense mutations in *RAB7A* [67]. These mutations cause the constitutive activation of its encoding protein Rab7 [113], a regulator of maturation of autophagosomes, amphisomes, and late endosomes in cells [114–116] (Figures 1 and 2). Although the molecular mechanism by which dysfunction in a ubiquitously expressed Rab7 affects only sensory and/or motor neurons remains unclear, recent studies have demonstrated that these CMT2B-associated Rab7 mutants exhibit a persistent elevation of endosome-mediated nerve growth factor (NGF) signaling [117], and inhibit neurite outgrowth in cultured neuronal cells [118].

#### **4. ALS2/alsin:**

##### **A Regulator of Autophagy-Endolysosomal Protein Degradation**

Loss of function mutations in the *ALS2* gene accounts for juvenile recessive amyotrophic lateral sclerosis (ALS2), juvenile primary lateral sclerosis (JPLS), and infantile-onset ascending hereditary spastic paralysis (IAHSP) [68, 69, 119, 120]. The *ALS2* gene encodes a 184 kDa protein of 1657 amino acids, ALS2 or alsin, comprising three predicted guanine nucleotide exchange factor (GEF) domains: the N-terminal RCC1-like domain (RLD), the central Dbl homology and pleckstrin homology (DH/PH) domain, and the C-terminal vacuolar protein sorting 9 (VPS9) domain

[68]. Indeed, it has been shown that ALS2 acts as a GEF for Rab5 [120–122], and regulates endosome fusion and trafficking by activating Rab5 [120, 121, 123] (Figure 1). ALS2 is also involved in Rac1-activated macropinocytosis and the following macropinosome trafficking and fusion [124, 125]. In particular, fusion between early endosomes and macropinosomes is, at least in part, regulated by ALS2 in an ALS2-associated Rab5 GEF activity-dependent manner [124]. Further, ALS2 plays some modulatory roles in axonal outgrowth in neuronal cells [125, 126], and in cytoprotection from oxidative stress-induced insults [127–130].

Recently, we have demonstrated that activated Rac1 interacts with ALS2 and induces the relocalization of ALS2 from cytoplasm to membranous compartments; for example, membrane ruffle, macropinosome, and endosome [124]. This Rac1-mediated relocalization of ALS2 is required for the ALS2-mediated Rab5 activation on the membranous compartments [71, 124]. It is noted that ALS2 is also colocalized with LC3/p62-positive autophagosomes and/or amphisomes [70, 71]. Conversely, pathogenic missense ALS2 mutants fail to be localized to such vesicular compartments, and lose the competence to enhance the formation of amphisomes [71], indicating that the Rac1-induced relocalization of ALS2 might be crucial to exert the ALS2-associated function linking to the autophagy-endolysosomal degradative pathway. Indeed, loss of ALS2 results in a slower degradation of endocytosed epidermal growth factor (EGF) in mouse embryonic fibroblasts [131]. Further, an ALS2-deficient SOD1<sup>H46R</sup>-expressing ALS mouse model exhibits the aberrant accumulation of autophagosomes and vesicular compartments in axons, delayed protein degradation by the autophagy-endolysosomal system, accelerated neurodegeneration, and earlier death [70]. Although the exact physiological function of ALS2 remains to be clarified, it is currently believed that ALS2 plays an important role in trafficking and maturation of several distinct vesicular compartments, including macropinosome, endosome, and autophagosome, and is implicated in the autophagy-endolysosomal degradative pathways (Figure 2).

#### **5. Conclusions and Perspectives**

Thus far, a large number of successful therapeutic interventions in preclinical animal studies have failed to translate into human clinical applications in ALS/MNDs. Even in such a discouraging situation, enormous efforts have continuously been made towards defining the molecular pathogenesis of these devastating diseases. The autophagy-endolysosomal system is among the underlying mechanisms, whose dysfunction is tightly associated with a variety of neurodegenerative diseases. It plays a pivotal role not only in ALS/MNDs as discussed in this paper, but also in other neurodegenerative diseases including Alzheimer's disease [57], Parkinson's disease [132], and Huntington's disease [133, 134]. Thus, the autophagy-endolysosomal pathway could be a major target for the development of novel therapeutic agents for neurodegenerative diseases [135]. Indeed, the induction of autophagy by lithium administration results in a reduced level of aggregated proteins and extends

lifespan in a SOD1<sup>G93A</sup>-expressing ALS mouse model [136]. However, a recent preclinical animal study has demonstrated that the treatment with rapamycin, an another inducer of autophagy, rather causes the accumulation of p62, more severe mitochondrial impairment, higher Bax levels, and greater caspase-3 activation, thereby augmenting motor neuron degeneration in a same ALS mouse model [137]. These conflicting results imply that the simple pharmacological induction of autophagy cannot be always beneficial *in vivo*. As such, our understanding of the intricate autophagy-endolysosomal system and its functional linking to other physiological systems in the CNS is still incomplete. Future studies, which could uncover the molecular mechanisms of a selective neurodegeneration in greater detail, will be required for the development of proper and effective therapeutic agents for the treatment of ALS/MNDs and other neurodegenerative diseases.

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