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# Role of UCHL1 in the pathogenesis of neurodegenerative diseases and brain injury

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## Abstract

UCHL1 is a multifunctional protein expressed at high concentrations in neurons in the brain and spinal cord. UCHL1 plays important roles in regulating the level of cellular free ubiquitin and redox state as well as the degradation of select proteins. This review focuses on the potential role of UCHL1 in the pathogenesis of neurodegenerative diseases and brain injury and recovery. Subjects addressed in the review include 1) Normal physiological functions of UCHL1. 2) Posttranslational modification sites and splice variants that alter the function of UCHL1 and mouse models with mutations and deletions of UCHL1. 3) The hypothesized role and pathogenic mechanisms of UCHL1 in neurodegenerative diseases and brain injury. 4) Potential therapeutic strategies targeting UCHL1 in these disorders.

### Keywords

Ubiquitin C-terminal hydrolase 1; ubiquitin-proteasome pathway; protein degradation; neurodegeneration; brain injury

## 1. Introduction

Ubiquitin C-terminal hydrolase 1 (UCHL1), also known as neuron-specific protein PGP9.5 and Parkin 5, is one of the most abundant proteins in the brain (1–5% of total soluble protein) (Day and Thompson, 2010; Wang et al., 2017). It is also expressed at high levels in testicular tissue and its expression may be induced in other cell types often associated with oncogenesis and metastasis (Jara et al., 2013; Nakao et al., 2018). Immunochemical experiments demonstrate that UCHL1 is localized predominantly in neurons and axons in the central and peripheral nervous system (Day and Thompson, 2010; Wilson et al., 1988).

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At a molecular level, UCHL1 is a relatively small protein (27kD) composed of 223-amino-acids encoded by 9 exons (Setsuie and Wada, 2007). Although the role of UCHL1 *in vivo* remains unclear, its great abundance in neurons suggests a significant role in neuronal cell function. UCHL1 variants and modifications have been linked with neurodegenerative disorders such as Parkinson's disease (PD), Alzheimer's disease (AD) and brain injury (Leroy et al., 1998; Liu et al., 2019; Mi et al., 2021a; Nakamura et al., 2021). These modifications diminish UCHL1's functions, alter its solubility, and interfere with its normal interactions with other proteins, resulting in increased production and impaired degradation of misfolded and aggregated proteins, common features shared by many neurodegenerative disorders and brain injury.

This review focuses on the molecular mechanisms of UCHL1 that may be important in its role in the pathogenesis of neurodegenerative disorders and brain injury. First, we provide an overview of ubiquitin and deubiquitinating enzymes (DUBs) in protein degradation systems. Then we summarize known posttranslational modification sites and splice variants that alter the function of UCHL1 and mouse models that bear spontaneous mutations, deletions, or genetic manipulations in UCHL1. Afterwards, we examine the molecular mechanisms by which UCHL1 may contribute to protein metabolism and other physiological functions in neurons. Based upon this background, we then discuss the potential role and mechanisms of UCHL1 in neurological diseases. Finally, we address potential strategies targeting restoration or preservation of UCHL1 activity that may be useful in treatment of these disorders.

## 2. Ubiquitin and deubiquitinating enzymes in protein degradation

Ubiquitin plays a key role in the removal of unwanted, misfolded or aggregated proteins that may contribute to the pathogenesis of neuronal dysfunction and recovery in variety of etiologies of acute brain injury and degenerative processes (Ciechanover and Kwon, 2015; Edwards et al., 2020; Liu et al., 2021; Soto and Estrada, 2008; Sweeney et al., 2017). In eukaryotic cells, damaged and misfolded proteins are degraded by two key mechanisms: the ubiquitin-proteasome pathway (UPP), and the autophagy-lysosome pathway (ALP) which is composed of a series of catabolic processes that involves delivery of cellular components to the lysosome for degradation (Nedelsky et al., 2008). The ALP is primarily responsible for degrading long-lived proteins, whereas the UPP is regarded as one of the key degradation routes for small short-lived proteins (Collins and Goldberg, 2017; Nedelsky et al., 2008). A common feature of the UPP and ALP is the attachment of ubiquitin conjugates to specific cargo proteins that initiate these degradative processes (Kwon and Ciechanover, 2017). When misfolded and unfolded proteins are tagged with ubiquitin, these proteins will be degraded in the proteasome or lysosome (Ding and Yin, 2008; Reiss et al., 2020).

Ubiquitin is an 8.5 kDa protein composed of 76 amino acids. Post translational modification of proteins by ubiquitin has diverse cellular roles: proteins are modified either by covalent binding of a single ubiquitin molecule (monoubiquitination), or with chains of multiple ubiquitin molecules (polyubiquitination) (Kwon and Ciechanover, 2017; Livneh et al., 2017). Ubiquitin is conjugated to its protein substrates through the sequential actions of a ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzymes (E2s), and ubiquitin

ligases (E3s) (Glickman and Ciechanover, 2002). Polyubiquitin chains are formed by linking ubiquitin to the first methionine or to one of its seven lysine residues on the ubiquitin molecule: M1, K6, K11, K27, K29, K33, K48 and K63, yielding eight potential types of homogeneous polyubiquitin chains, with K48- and K63-linked polyubiquitin chains being the two most abundant polyubiquitin chain types (Kwon and Ciechanover, 2017; Ohtake et al., 2016). Attachment of K48 polyubiquitin chains to misfolded proteins tags the proteins for transport to the proteasome for degradation and serves as an important component of the UPP, whereas K63 chains act as proteasome-independent signals for endocytosis, DNA repair, kinase activation, and autophagy (Erpapazoglou et al., 2014). In addition, K63-linked polyubiquitin chains may play a part in synaptic function: modification of proteins by K63-linked polyubiquitin chains plays a significant role in postsynaptic protein scaffolding, synaptic strength and plasticity by modifying postsynaptic density protein 95, scaffolding potentials, enhancing its synaptic targeting, and promoting synapse maturation and efficacy (Ma et al., 2017).

Polyubiquitin chains with linkages other than K48 or K63 are less prevalent and have distinct functions such as regulating nuclear factor κB (NF-kB) activation, proteasome degradation of proteins, cell cycle signaling, and post-Golgi membrane protein trafficking (Bremm and Komander, 2011; Kim et al., 2011; Komander et al., 2009b; Rahighi et al., 2009; Wickliffe et al., 2011; Yuan et al., 2014).

Ubiquitination is reversible; a family of proteasome-associated DUBs cleaves ubiquitin from substrates, thereby regulating the ubiquitination process and recycling free ubiquitin, which is necessary for protein turnover (Komander et al., 2009a; Lange et al., 2022). There are ~90 DUBs in the human genome with highly specific protein substrates which are expressed in various cell types. These DUBs fall into five subfamilies: ubiquitin carboxyl-terminal hydrolases (UCHs), ubiquitin specific proteases, ovarian tumor- like proteases, JAB1/MPN/Mov34 metalloproteases, and the Machado-Jakob disease proteases (Kemp, 2016). The UCH subfamily has four members: UCHL1, UCHL 3, UCHL 5 and BRCA1 associated protein 1 with UCHL1 being the only DUB expressed at high levels in neurons.

## 3. Enzymatic Activities of UCHL1

### 3.1. Hydrolase activity

UCHL1, like many DUBs, is a cysteine protease that contains a catalytic site. UCHL1 has a high affinity for ubiquitin and can efficiently hydrolyze the isopeptide bond between the protein substrate and the C-terminal G76 of ubiquitin in *in vitro* assays (Wilkinson et al., 1992). The hydrolase catalytic site contains a catalytic triad consisting of a cysteine (C90), a histidine (H161), and an aspartate (D176) which are essential for activity. Mutation of any of the three amino acids abolishes or significantly decreases hydrolase activity (Table 1) (Larsen et al., 1998). In addition, mutation of other sites such as phenylalanine 214 to alanine (F214K) also abolished UCHL1's hydrolase activity (Osaka et al., 2003). Missense mutations in UCHL1 have been identified in PD (I93M) and other neurological disorders (E7A, R178Q and A216D) with altered hydrolase activity (Bilguvar et al., 2013; Leroy et al., 1998; Rydning et al., 2017). *In vitro* analysis of recombinant UCHL1 I93M indicated a decline in hydrolase activity of 50% as compared to wild type (Nishikawa et al., 2003;

Setsuie and Wada, 2007). The UCHL1 E7A missense mutation exhibits a near complete loss of hydrolase activity as well as an extensive loss of ubiquitin binding ability resulting in a larger than 100-fold reduction in the efficiency of UCHL1 E7A relative to wildtype counterparts (Bilguvar et al., 2013; Lee and Hsu, 2017). Similar *in vitro* analysis indicated that while UCHL1 R178Q exhibited a 4-fold increase in hydrolase activity, UCHL1 A216D was insoluble and consequently resulting in the complete loss of function (Rydning et al., 2017).

Due to restricted access to the active site, UCHL1 is not an efficient hydrolase of ubiquitinated proteins. In *in vitro* experiments using UCH DUBs and ubiquitinated substrates, UCHL1's hydrolase specific activity is 200-fold less than that of the UCHL3 which is not selectively expressed in brain (Liu et al., 2002). This observation suggests that UCHL1's hydrolase activity may not be its sole function.

### 3.2. Ligase activity

Liu et al first reported that UCHL1 has a dimer- dependent and ATP-independent ubiquitin ligase function *in vitro*; however, an active site for this activity has not been identified (Liu et al., 2002). UCHL1's ligase activity was hypothesized to produce higher susceptibility to PD via increased accumulation of α-synuclein (Liu et al., 2002). A polymorphic variant of UCHL1 that was reportedly associated with decreased PD risk (UCHL1 S18Y) exhibited reduced ligase activity but comparable hydrolase activity to the wild-type enzyme (Carmine Belin et al., 2007; Liu et al., 2002; Maraganore et al., 1999). However, multiple subsequent studies failed to identify a correlation between the UCHL1 S18Y mutation and a reduced risk of PD (Carmine Belin et al., 2007; Maraganore et al., 1999; Mellick and Silburn, 2000; Miyake et al., 2012). UCHL1 has also been proposed to prevent microtubule formation through ubiquitination of tubulins and microtubule-associated proteins (Bheda et al., 2010). However, sedimentation equilibrium experiments failed to detect the dimer, leading the authors to conclude that UCHL1 does not exist as dimers in solution and thus may be devoid of ligase activity *in vivo* (Das et al., 2006). Another study using the same approach described by Liu et al found that UCHL1 did not exhibit ubiquitin ligase activity nor was it able to ubiquitinate α-synuclein (Bilguvar et al., 2013; Liu et al., 2002). Further investigations are needed to clarify UCHL1's role in ubiquitin ligation.

## 4. Modification sites and splice variants

### 4.1. Farnesylation

Farnesyl groups consist of several lipids that act as a membrane anchor for proteins. Farnesylation occurs by the addition of a farnesyl group to the cysteine in the CAAX motif, a reaction catalyzed by the protein farnesyl transferase (Clarke and Tamanoi, 2004). UCHL1 can be farnesylated at cysteine 220 and this farnesylated UCHL1 is reported to promote α-synuclein neurotoxicity by increasing membrane-associated UCHL1 including binding to the endoplasmic reticulum (Liu et al., 2009). However, partition of UCHL1 to the membrane does not require farnesylation at the C220 site as the farnesylation resistant C220S mutation did not reduce the proportion of UCHL1 in the membrane fraction (Bishop et al., 2014).

### 4.2. S-mercuration, S-nitrosylation and reactive lipid modification site

Methylmercury (MeHg) is an environmental electrophile that covalently modifies cellular proteins. S-mercuration refers to the binding of mercury-containing compounds to cellular proteins through their reactive thiols. UCHL1 can undergo S-mercuration by MeHg at cysteine 152. This covalent modification inhibits UCHL1, leading to the potential disruption of cellular monoubiquitin pool homeostasis (Toyama et al., 2015).

Nitrosylation (S-nitrosylation) occurs at the cysteine 152 of UCHL1 in human AD brains and animal models of AD (Nakamura et al., 2021). The nitrosylated UCHL1 transfers the nitric oxide (NO) group to cyclin dependent kinase 5 then to Dynamin-related protein 1, leading to excessive mitochondrial fragmentation and bioenergetic compromise, with consequent synapse loss and cognitive impairment (Nakamura et al., 2021).

Reactive lipids such as the cyclopentenone prostaglandin (CyPg) 15-deoxy-$\Delta^{12,14}$ – prostaglandin $J_2$ (15d-PGJ2) can also covalently bind to UCHL1 cysteine 152, resulting in aggregation and/or disruption of UCHL1 enzyme activity (Koharudin et al., 2010). Mutation of cysteine 152, but not the five other cysteine residues in UCHL1, prevents the unfolding of the protein and preserves UCHL1 hydrolase activity after incubation of UCHL1 recombinant protein with CyPgs (Koharudin et al., 2010).

### 4.3. Oxidation sites

It has been reported that the full-length UCHL1 is extensively modified by carbonyl formation, methionine oxidation and cysteine oxidation in AD and PD brains (Castegna et al., 2002; Choi et al., 2004; Sultana et al., 2006). Proteomic analyses identified five oxidatively modified methionine residues to be on M1, M6, M12, M124 and M179. In addition, the cysteine residue in the carboxyl-terminal region of UCHL1 (C220) was also oxidatively modified to cysteic acid (Choi et al., 2004).

### 4.4. N-Terminal truncated UCHL1 splice variant (NT-UCHL1)

An N-terminal 11 amino acid truncated variant of UCHL1 has been identified in mouse brain tissue as well as in NCI-H157 lung cancer and SH-SY5Y neuroblastoma cell lines. Removal of these 11 amino acids from the N-terminus is sufficient for the protein to lose affinity for ubiquitin and ultimately leads to the formation of insoluble aggregates (Kim et al., 2014). Stable expression of NT-UCHL1 decreases cellular ROS levels and protects cells from $H_2O_2$, rotenone and carbonyl cyanide m-chlorophenyl hydrazone (CCCP)-induced cell death. NT-UCHL1-expressing transgenic mice are less susceptible to degeneration of nigrostriatal dopaminergic neurons seen in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD, in comparison to control animals (Kim et al., 2014). These results suggest that NT-UCHL1 may have the potential to prevent neuronal damage in diseases like PD, although approaches to increasing NT-UCHL1 levels in neurons to protect against mitochondrial or other damage may be limited by its insolubility.

Active sites, mutations, and post-translational modifications of UCHL1 are summarized in Table 1.

## 5. UCHL1 deficient and transgenic mice

Mice with spontaneous deletions or genetically engineered alterations in UCHL1 have provided much information regarding the function of UCHL1 under normal and pathological conditions. Two spontaneous UCHL1 deficient (*gad, nm3419)*, three knockout (*Uchl1*$^{tm1Dgen}$*, UCHL1 knockout, UCHL1* $^{d/d}$) and two knockin (*UCHL1 C152A* and *UCHL1 C90A*) mice have been discovered or generated (Table 2). The spontaneous UCHL1 deficient and knockout mice all display a similar ataxic phenotype of progressive paralysis, neurodegeneration, and premature death. Additionally, a variety of neuropathological features and changes in protein ubiquitination have been characterized in these mice. *UCHL1 C152A* and *C90A* knockin mice develop normally and do not exhibit any detectable differences in motor and sensory functions from their wildtype counterparts up to 18 months of age (Mi et al., 2021b).

### 5.1. UCHL1 deficient mice

The gracile axonal dystrophy (*gad*) phenotype was first described by Yamazaki et al in a strain of laboratory mice. The affected mice exhibited ataxia beginning at about 80 days of age, followed by tremor, difficulty in moving and muscular atrophy of the hind limbs. The neurological signs became progressively more severe, and death occurred by 5 to 6 months of age. Pathological examination revealed neuroaxonal dystrophy and degeneration in the gracile nucleus of the medulla oblongata and the gracile fasciculus of the spinal cord (Yamazaki et al., 1988). A study using electron microscopy revealed dystrophic axons packed with neurofilaments, mitochondria and tubulovesicular structures, potentially reflecting abnormal axonal transport (Mukoyama et al., 1989). Increased accumulation of amyloid precursor protein (APP) and deposition of amyloid β peptide within the cytoplasm of both axons and glial cells in the gracile tract of *gad* mice were also reported (Ichihara et al., 1995). Furthermore, abnormal ubiquitination of dystrophic axons and a reduced level of monoubiquitin in the central nervous system (CNS) of *gad* mutant mice was detected (Osaka et al., 2003). The *gad* mutation was mapped to an in-frame deletion including exons 7 and 8 from the UCHL1 gene, corresponding to the loss of 42 residues including the catalytic His161. No UCHL1 protein was detectable in *gad* mice although mRNA transcripts were found to be produced in equivalent amounts to their wild-type counterparts (Saigoh et al., 1999).

A second spontaneous mutation *nm3419* arose on a BALB/cJ mouse line at the Jackson Laboratory (Walters et al., 2008). The *nm3419* mice begin to exhibit signs of motor ataxia by 1 month of age and death at ~6 months (Walters et al., 2008). Sequence and genomic analysis showed that the final 24 base pairs (bps) of exon 6 and the first 771 bp of intron 6 were deleted. This mutation inserts a premature stop codon that truncates the last 78 amino acids of UCHL1. Like the *gad* mouse, no UCHL1 protein was detected; also, similar to *gad* mice, free monomeric ubiquitin was reduced by ~30% compared with WT mice. In addition, the corticospinal motor neurons (CSMN) of the *nm3419* mouse are susceptible to endoplasmic reticulum (ER) stress and display early selective, progressive, and profound degeneration, and pathological changes analogous to ALS (Jara et al., 2015). Restoration of UCHL1 specifically in CSMN of *nm3419* mice via directed gene delivery was sufficient to

improve CSMN integrity to healthy control levels (Genc et al., 2022). These data together with the data from *gad* mice demonstrate that UCHL1 is essential for the maintenance of axonal integrity.

The *Uchl1*$^{tm1Dgen}$ mutant mouse was generated by targeted deletion of exons 6 through 8 and the first 6 bps of exon 9 of UCHL1 (Chen et al., 2010). These mutant mice develop progressive motor deficits beginning at 2 mo. of age progressing to paraplegia by 4–8 mo; no mice survive past 10 months. In addition, the *Uchl1*$^{tm1Dgen}$ mice exhibit impaired synaptic transmission at the neuromuscular junction (NMJ). Morphologic analyses of the NMJ further revealed profound structural defects such as loss of synaptic vesicles, accumulation of tubulovesicular structures at the presynaptic nerve terminals, and denervation of the muscles. These data demonstrate that UCHL1 is required for the maintenance of the structure and function of the NMJ and that the loss of UCHL1 may result in neurodegeneration in the peripheral nervous system.

Another UCHL1 knockout mouse (*UCHL1 knockout*) was generated by targeting a 16 kb region spanning exon 4 which contains the catalytically essential cysteine at position 90 of the mouse UCHL1 gene (Coulombe et al., 2014). The *UCHL1 knockout* mice manifest neurodegenerative phenotypes strongly reminiscent of the *gad* spontaneous mutant (Yamazaki et al., 1988). Mice homozygous for the null mutation can be identified as early as 6 weeks of age by the failure to spread their hindlimbs when suspended by the tail. At various ages the *UCHL1 knockout* mice were reported to have a deterioration in motor performance on a rotating rod similar to that reported for *gad* mice (Yamazaki et al., 1992). Examination of histological sections of the CNS of *UCHL1 knockout* mice revealed pathology similar to that reported for *gad* mice. In addition, tyrosine hydroxylase positive axonal swellings were also observed in the striatum, which contains processes from dopaminergic neurons in the substantia nigra. GSH analysis revealed significantly depleted GSH levels in the brains of knockout mice. Furthermore, Loss of UCHL1 promoted age-related degenerative changes in the enteric nervous system. These data along with data from *Uchl1*$^{tm1Dgen}$ mice further demonstrate that UCHL1 plays important roles in the peripheral nervous system.

*Uch-l1*$^{d/d}$ knockout mice were generated by targeted deletion of exons 1–3 of UCHL1 (Reinicke et al., 2019). These mice do not express detectable UCHL1 protein and were found to have accelerated sensorimotor development in the early postnatal period. This was accompanied by increased protein turnover mediated by enhanced mTORC1 activity, leading to ER stress, energy depletion, and proteasomal impairment with accumulation of nondegraded ubiquitinated proteins. Subsequently, *Uch-l1*$^{d/d}$ mice develop sensory and motor ataxia consistent with that described in *gad* and *nm3419* spontaneous UCHL1 mutants.

### 5.2. UCHL1 knockin mice

A UCHL1 knockin mouse was generated using the bacterial artificial chromosome technique to introduce a cysteine to alanine mutation at site 152 in exon 6 *(UCHL1 C152A)* (Liu et al., 2015). As mentioned earlier, C152 is the binding site for a variety of endogenous and exogenous chemicals and molecules on UCHL1 including CyPgs which

alter the structure and function of the molecule. Mutation of C152 prevents inactivation of UCHL1 hydrolase activity by preventing unfolding of the molecule induced by binding of CyPgs to the C152 site. These mice exhibit no apparent motor deficits.

Another UCHL1 knockin mouse bearing a cysteine to alanine mutation at site 90 in exon 4 (*UCHL1 C90A*) that is devoid of hydrolase activity has also been constructed (Mi et al., 2021b). Contrary to UCHL1 deficient mice, the *C90A* mice developed normally, with no observed sensory or motor defects, and are still viable until age 18 months (Mi et al., 2021b), although the monoubiquitin level of aged *C90A* mice is decreased compared to their aged wildtype counterparts. These results suggest that the hydrolase activity of UCHL1 alone does not account for the progressive neurodegeneration and premature death seen in mice that do not express full length UCHL1.

## 6. Molecular actions of UCHL1

### 6.1. Regulation of the monoubiquitin pool

UCHL1 is a multifunctional protein that may exert its cellular actions through several molecular mechanisms. Neurons are post mitotic, highly metabolically active cells that are vulnerable to the accumulation of defective proteins. The recycling of ubiquitin is critical in brain, which has a finite amount of ubiquitin (Tramutola et al., 2016). Because UCHL1 is highly expressed in neurons (Day and Thompson, 2010; Wang et al., 2017), one of its main functions in neurons may be the regulation of cellular monoubiquitin levels via its hydrolase activity. UCHL1 hydrolyzes bonds between ubiquitin and small adducts or unfolded polypeptides *in vitro* and increases monomeric ubiquitin. UCHL1 is also involved in converting ubiquitin from its pro molecule into its active form (Larsen et al., 1998). UCHL1 cleaves the tandemly repeated ubiquitin gene products UBB (three repeats), UBC (nine repeats) and the ribosomal ubiquitin fusion protein R27A to generate monomeric ubiquitin (Hurst-Kennedy et al., 2012; Wiborg et al., 1985). Ubiquitin's half-life is increased via binding at UCHL1's D30 site, independent of ubiquitin's interaction with UCHL1 at the hydrolase site (Osaka et al., 2003). These observations are in agreement with the role of UCHL1 in maintaining a stable pool of monoubiquitin that is a key requirement for the UPP and the ALP.

### 6.2. Regulation of degradation of specific proteins

UCHL1 may regulate the degradation of proteins through direct or indirect interactions and have effects on a number of important signal transduction systems. It has been reported that UCHL1 regulates APP processing by promoting β - secretase 1 (BACE1) degradation resulting in decreased amyloid β production (Zhang et al., 2012). There is also evidence that UCHL1 can deubiquitinate tropomyosin receptor kinase B (TrkB) directly in mouse hippocampus. Blockage of UCHL1 regulated deubiquitination of TrkB resulted in the increased degradation of surface TrkB and decreased activation of TrkB and its downstream signaling pathways (Guo et al., 2017). In addition, UCHL1 enhances the activity of hypoxia-inducible factor 1-alpha (HIF1α) by regulating its degradation, which is ubiquitinated by von Hippel–Lindau (pVHL) E3 ubiquitin ligase. UCHL1 abrogates pVHL-mediated ubiquitination of HIF1α and consequently inhibits its degradation (Goto et al., 2015). Since

ubiquitination/deubiquitination regulates degradation of multiple proteins via the UPP and ALP, UCHL1 activity may regulate multiple signal transduction pathways.

### 6.3. Interaction with molecules in the ALP

In addition to maintaining a stable pool of monoubiquitin and deubiquitinating select proteins, UCHL1 may also regulate the ALP directly. UCHL1 physically interacts with lysosome-associated membrane protein type 2A (LAMP-2A), heat shock cognate protein 70 (Hsc70), and heat shock protein 90 (Hsp90) (Kabuta et al., 2008a; Kabuta and Wada, 2008). LAMP-2A forms a complex with chaperones such as Hsc70 and Hsp90 to function as a receptor for chaperone-mediated autophagy (CMA) at the lysosomal membrane. Coimmunoprecipitation assays using a series of alanine substitutions of basic and acidic residues located on the surface of UCHL1 revealed that the R63 residue of UCHL1 participates in these interactions (Kabuta et al., 2008a). Interactions of UCHL1 with these molecules are independent of monoubiquitin binding and are abnormally enhanced by a familial mutation of UCHL1 I93M, thus inhibiting CMA-dependent degradation and causing the accumulation of CMA substrates such as $\alpha$-synuclein (Kabuta et al., 2008a).

### 6.4. Structural role

Given its high level of expression in neurons, UCHL1 may also have a structural function. UCHL1 interacts with tubulin and microtubules both in the context of cell division and cytoskeletal functions. Using transformed cell lines of different origins, Bheda et al demonstrated the close association of endogenous UCHL1 with the mitotic spindle through all stages of M phase, suggesting that UCHL1 is involved in regulation of microtubule dynamics (Bheda et al., 2010). Furthermore, UCHL1 has an inhibitory effect on microtubule formation in a fibroblastic cell line overexpressing UCHL1. The inhibitory effect is likely related to UCHL1's dimer-dependent ubiquitin ligase activity (Bheda et al., 2010). Interestingly, the aberrant interaction of mutant UCHL1 I93M or carbonyl modified UCHL1 with tubulin both promote tubulin polymerization (Kabuta et al., 2008b).

### 6.5. Regulating the redox state

UCHL1 has been proposed to regulate the cellular redox state. Redox proteomics performed on the UCHL1-deficient *gad* mouse brains identified changes in protein oxidation in molecules ranging from antioxidant, glycolytic, and cell signaling, to structural proteins such as neurofilament-L (Castegna et al., 2004). UCHL1 also enhances the activity of HIF1α through inhibiting its degradation (Goto et al., 2015). The UCHL1-HIF-1 axis induces reprograming of the glucose metabolic pathway and increase production of glutathione (GSH) (Nakashima et al., 2017). Consistent with data from *gad* mice, free GSH was found to be markedly decreased in brains of UCHL1 knockout mice as compared to wild type littermates (Coulombe et al., 2014). In neurons of another UCHL1 knockout mouse ($UCHL1^{d/d}$), up-regulation of the reactive oxygen species (ROS)-detoxifying enzyme MnSOD at the presymptomatic stage was reported (Reinicke et al., 2019). Upregulation of MnSOD is likely a compensatory mechanism for cells to cope with increased oxidative stress. Thus, it is likely that UCHL1 regulates the level of cellular GSH and redox state of the neuron through multiple mechanisms.

## 7. The role of UCHL1 in pathological conditions

Increased accumulation of aggregated, misfolded, and oxidized proteins, as well as reduced efficiency in repairing and removing abnormal proteins by the UPP and ALP are common features in a number of neuropathological conditions (Baba et al., 1998; Blokhuis et al., 2013; Doyle et al., 2011; Hu et al., 2000; Smith et al., 2003; Tabira et al., 2002). Accordingly, UCHL1 dysfunction is hypothesized to contribute to the pathogenesis of neurodegenerative diseases and is also implicated in mechanisms of injury and recovery after traumatic brain injury (TBI) and cerebral ischemia.

### 7.1. Alzheimer's disease

The histopathological hallmarks of AD are extracellular amyloid plaques and intracellular neurofibrillary tangles (NFTs). The principal component of amyloid plaques is amyloid β peptide, a 39–43 amino acid fragment proteolytically produced from APP. Amyloid β peptide is generated through the amyloidogenic pathway in which APP is cleaved by BACE1 and γ-secretases sequentially in the N- and C-terminal portions of the amyloid β region (Esler and Wolfe, 2001; Kang et al., 1987; Masters et al., 1985; Selkoe, 2001). Therefore, increased BACE activity or expression promotes the generation of amyloid β. Inhibition of UCHL1 activity significantly increased BACE1 protein expression and increased concentrations of amyloid β, whereas overexpression of UCHL1 decreased amyloid β levels and delayed AD progression (Zhang et al., 2014; Zhang et al., 2012). In addition, UCHL1 deficient *gad* mice exhibited high levels of amyloid β and exogenous UCHL1 rescued amyloid β-induced decreases in synaptic function and contextual memory in APP/presenilin 1 (PS1) mice (Gong et al., 2006; Ichihara et al., 1995; Zhang et al., 2012). These studies demonstrate that decreased UCHL1 activity or UCHL1 gene deficiency promote BACE1 degradation and increase amyloid β production. In line with these findings, decreased levels of UCHL1 have been found in both human AD cases and mouse model of AD (Guglielmotto et al., 2017).

Several studies suggest that UCHL1 may regulate the formation of NFTs. The amount of soluble UCHL1 is proportional to the number of NFTs in brain sections from sporadic AD brain patients (Choi et al., 2004). Phosphorylated tau, which is associated with the formation of NFTs, is increased after knock-down of UCHL1 in a mouse model of AD. Conversely, UCHL1 overexpression reduced the level of phosphorylated tau (Zhang et al., 2014). Recently, it has been shown that inhibition of UCHL1 suppresses tau aggresome formation, a cellular protective response to sequester abnormal protein aggregates from the cytoplasmic environment and enhance the degradation of toxic proteins by autophagy (Kopito, 2000; Taylor et al., 2003; Yu et al., 2018). These studies demonstrate that UCHL1 solubility and activity may regulate tau phosphorylation and NFT formation.

Extensive oxidation and nitrosylation of UCHL1 has been reported in both human AD brains and animal models of AD (Castegna et al., 2002; Choi et al., 2004; Nakamura et al., 2021). These abnormal modifications result in decreased solubility and increased aggregation of UCHL1 molecules. Oxidized or nitrated proteins are removed by functional UPP, which is impaired in AD brains (Bonet-Costa et al., 2016). UCHL1 is highly expressed in neurons and is an important constituent of the neuronal UPP. Oxidation or nitrosylation

of UCHL1 impairs UPP function leading to increased protein aggregation, impaired cellular metabolism, and cell death (Grimm et al., 2011).

The presence of a sustained immune response in the brain has been hypothesized to be another underlying mechanism of neurodegeneration in AD. The sustained activation of microglia and other immune cells has been demonstrated to exacerbate both amyloid and tau pathology and may contribute to the pathogenesis of the disorder (Kinney et al., 2018). UCHL1 has been shown to suppress inflammatory responses in vasculature (Ichikawa et al., 2010). Inhibition of UCHL1 with Low-Dose Naltrexone (LDN) decreases anti-inflammatory mediator triggering receptor expressed on myeloid cells 2 (TREM2) and restoration of UCHL1 activity with exogenous recombinant UCHL1 fusion protein rescued amyloid-β1–42-induced decrease of TREM2 in cortical neurons (Guglielmotto et al., 2019). Furthermore, treatment with UCHL1 fusion protein returned the production of inflammatory markers interleukin-6 and tumor necrosis factor α to control levels (Guglielmotto et al., 2019). These data indicate that UCHL1 activity may also play a role in preventing neuroinflammation in AD.

### 7.2. Parkinson's disease

UCHL1 has been implicated in the pathogenesis of both familial and sporadic PD (Nawaz et al., 2020). UCHL1 co-aggregates with α-synuclein in Lewy bodies and S-nitrosylation of UCHL1 induces structural instability and promotes α-synuclein aggregation (Kumar et al., 2017; Liu et al., 2002). In addition, a missense mutation in UCHL1, the UCHL1 I93M (also known as PARK 5), has been identified in familial PD and *in vitro* analysis of recombinant UCHL1 I93M indicated decreased hydrolase activity as compared to wild type (Leroy et al., 1998; Nishikawa et al., 2003). Analysis of UCHL1 I93M mutant mice revealed the physiological phenotypes of PD and degeneration of dopaminergic neurons (Setsuie et al., 2007). The mutant version of UCHL1 I93M also exhibits increased insolubility, and aberrant interactions with other proteins such as Hsp90, Hsp70, and tubulin (Kabuta et al., 2008a; Kabuta et al., 2008b). Expression of UCHL1 I93M in cells inhibits CMA and increases α-synuclein (Kabuta et al., 2008a). In addition, UCHL1 has been identified as the binding partner of α-synuclein and PD 2 through the STRING database (Szklarczyk et al., 2017). These data demonstrate that UCHL1 plays critical roles in PD pathogenesis.

### 7.3. Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a rare and fatal neurodegenerative disorder that affects the upper and lower motor neurons of the brain and spinal cord (Mejzini et al., 2019). UCHL1 null mice *nm3419* develop progressive upper and lower motor neuron pathology analogous to ALS (Genc et al., 2016; Jara et al., 2015). Restoration of UCHL1 specifically in CSMN of UCHL1 null mice via directed gene delivery was sufficient to improve CSMN integrity to healthy control levels (Genc et al., 2022). In addition, elevated UCHL1s level have been found in cerebrospinal fluid (CSF) and serum in patients with ALS, although its prognostic value needs to be further investigated (Li et al., 2020). These data indicate that UCHL1 may be involved in the pathogenesis and progression of ALS.

### 7.4. Cerebral ischemia

Cerebral ischemia is a consequence of a mismatch in cerebral perfusion and brain energy requirements. When prolonged, cerebral ischemia results in extensive damage to both gray and white matter structures in brain leading to significant functional impairment (Kunz and Iadecola, 2009). Recovery of function after stroke is largely dependent upon repair and remodeling of axonal and synaptic connections. Since UCHL1 plays a critical role in axonal integrity and synaptic function, it may also play a significant role in the restoration of neuronal function after stroke. CyPgs and NO are produced after cerebral ischemia and covalently modify the C152 cysteine of UCHL1, unfolding the enzyme and inhibiting UCHL1 activity. Thus, UCHL1 activity may be inhibited after ischemia and impair recovery.

A number of studies have shown that modulation of UCHL1 activity alters gray and white matter injury and recovery of function after cerebral ischemia. Down-regulation of endogenous UCHL1 in mouse N2a neuroblastoma cells increases cell death induced by oxygen-glucose deprivation (Shen et al., 2006). Pharmacological inhibition of UCHL1 activity exacerbated cell death in primary neuronal cultures subjected to transient hypoxia while treatment of primary neurons with UCHL1 recombinant protein prior to hypoxia reduced neuronal cell death (Liu et al., 2011). Primary neurons derived from knockin mice bearing a C152A mutation in UCHL1 (UCHL1 C152A KI) that are resistant to modification by reactive lipids or NO were resistant to cell death and neurite damage induced by CyPgs, hypoxia, and oxygen glucose deprivation (Liu et al., 2015; Liu et al., 2019). Transient middle cerebral artery occlusion (MCAO) in UCHL1 C152A KI mice resulted in significantly attenuated gray and white matter injury and improved recovery of sensorimotor function (Liu et al., 2019). Furthermore, the UCHL1 C152A mutation preserved excitatory synaptic drive to pyramidal neurons and their excitability in the periinfarct zone; additionally, axonal conduction velocity recovered by 21 d post MCAO in UCHL1 C152 KI mice compared to WT controls (Liu et al., 2019). These results indicate that UCHL1 plays a significant role in determining gray and white matter survival and motor recovery after cerebral ischemia.

### 7.5. Traumatic brain injury

TBI is characterized by diffuse axonal injury which may lead to devasting neurological impairment (Adams et al., 1984; Frati et al., 2017). UCHL1 is released into the CSF and blood after TBI and has been employed as a biomarker in patients with suspected TBI (Papa et al., 2016; Papa et al., 2019; Wang et al., 2021). TBI is also companied by the generation of reactive lipids and NO, which may modify UCHL1 and impair its function (Koharudin et al., 2010; Kozlov et al., 2017). Thus, one may hypothesize that UCHL1 could also play a significant role in restoration of brain function after TBI. The protein transduction domain of the human immunodeficiency virus trans-activator of transcription (HIV TAT) capsid protein allows proteins to readily transduce neurons *in vitro* and *in vivo* (Cao et al., 2002). Treatment with TAT-UCHL1 fusion protein decreased axonal injury and CA3 neuronal cell death in a mouse TBI model (Liu et al., 2017). In addition, knockin mice expressing the UCHL1 C152A mutation had significantly attenuated gray and white matter injury and significantly improved sensorimotor recovery after TBI compared to their wild type controls (Mi et al., 2021a). Conversely, transgenic mice lacking UCHL1 hydrolase activity exhibited

increased TBI-induced axonal injury and neuronal death (Mi et al., 2021b). These data demonstrate that that UCHL1 plays a key role in TBI.

## 8. Pathogenic mechanisms of UCHL1 in brain

Alteration in the structure and functional impairment of UCHL1 by genetic mutation, reactive lipid species, nitrosylation and oxidation may lead to neurodegeneration through three mechanisms: disruption of the UPP and ALP, increased insolubility, and oxidative stress (Figure 1).

### 8.1. Disruption of abnormal protein degradation

A common feature of many neurological diseases is the accumulation of damaged, misfolded or aggregated proteins that may impair cellular function and induce ER stress (Baba et al., 1998; Blokhuis et al., 2013; Doyle et al., 2011; Hu et al., 2000; Smith et al., 2003; Tabira et al., 2002). The UPP and the ALP remove these abnormal proteins and aggregates collaboratively from the cell (Nedelsky et al., 2008). UCHL1 plays a critical role in maintaining the UPP and ALP function by maintaining the stable pool of ubiquitin required for both pathways. Activated monoubiquitin is essential for tagging of abnormal proteins for transport to the proteosome via the UPP. In addition, ubiquitin is required for the initiation, execution, and termination of autophagy (Chen et al., 2019). The UPP and autophagy pathways are essential for the removal of abnormal proteins and aggregates that are accumulated within neurons in neurodegenerative diseases and after acute injury from ischemia and trauma (Doyle et al., 2011). Impairment of these pathways by mutation or inactivation of UCHL1 may exacerbate and inhibit recovery from injury and speed neurodegenerative processes (Genc et al., 2016; Mi et al., 2021b; Shen et al., 2006; Yu et al., 2018; Zhang et al., 2012).

### 8.2. UCHL1 insolubility and protein aggregation

UCHL1 can be modified by reactive lipids, NO, and protein carbonyls (Castegna et al., 2004; Choi et al., 2004; Koharudin et al., 2010; Liu et al., 2013; Nakamura et al., 2021; Tramutola et al., 2016). These modifications and structural changes, such as unfolding of the molecule, may make the molecule less soluble and prone to form aggregates, a common pathological feature of neurogenerative diseases (Kabuta et al., 2008b; Koharudin et al., 2010; Liu et al., 2013; Ross and Poirier, 2004). Consistent with these observances, decreased levels of soluble UCHL1 and elevated levels of UCHL1 in NFT have been found in AD brains (Lowe et al., 1990). UCHL1 also co-aggregates with α-synuclein in Lewy bodies, the intra-neural fibrillary aggregate that is the histological hallmark of PD (Liu et al., 2002). Transgenic mice expressing the human I93M UCHL1 mutation associated with familial PD display structural changes and increased insolubility and aberrant interactions with multiple proteins such as tubulin (Kabuta et al., 2008b). These data show that insolubility and aggregation of UCHL1 may also be an important mechanism in the pathogenesis of neurogenerative diseases.

### 8.3. Oxidative stress

Oxidative stress has been hypothesized to play a pivotal role in the pathogenesis of neurodegenerative diseases and secondary injury after brain ischemia and TBI (Chamorro et al., 2016; Chen et al., 2020; Frati et al., 2017; Singh et al., 2019). Neurons are highly dependent on mitochondrial oxidative phosphorylation for their energy demands which generates ROS (Watts et al., 2018). This may lead to lipid peroxidation, protein oxidation and DNA modification (Singh et al., 2019). These modifications further result in altered membrane permeability and fluidity, protein aggregation, impaired cellular function and cell death (Grimm et al., 2011). UCHL1 regulates the level of cellular GSH, a major antioxidant in brain through multiple mechanisms including interactions with MnSOD and HIF1 α (Goto et al., 2015; Reinicke et al., 2019). UCHL1 deficient *gad* mice exhibit increased vulnerability to lipid peroxidation, and damage is further increased in neurons cultured in media deficient in Vitamin-E (α-tocopherol), an antioxidant that protects cells from ROS damage (Kikuchi et al., 1990; Nagamine et al., 2010; Sung et al., 1980). In addition, a variety of oxidized proteins have been identified in *gad* mouse brains (Castegna et al., 2004). These data suggest that oxidation of multiple protein targets may be regulated by UCHL1 and thus play a significant role in the pathogenesis of neurodegenerative disease and brain injury.

## 9. Potential therapeutic approaches targeting UCHL1

Impaired UCHL1 function has been implicated in the pathogenesis of neurodegenerative diseases, stroke and TBI. Thus, augmenting UCHL1 activity or preventing the posttranslational modification of UCHL1 has the potential to afford new therapeutic strategies in these disorders.

### 9.1. TAT-UCHL1 recombinant proteins

As mentioned in section 7.5, the prothrombin domain of the HIV TAT capsid protein confers the ability of HIV to readily enter neurons (Ballarin and Tymianski, 2018; Cao et al., 2002; Gong et al., 2006; Hill et al., 2012). By modifying UCHL1 to include the prothrombin domain of TAT with UCHL1, the resultant TAT-UCHL1 protein readily enters neurons even when administered systemically. TAT-UCHL1 has been reported to reverse deficits in long term potentiation in hippocampal slices from a mouse model of AD, and systemic administration of TAT-UCHL1 improved memory function in mice bearing mutations in APP and PS1 (Gong et al., 2006). Because UCHL1 may play an important role in axonal integrity and axonal transport, administration of TAT-UCHL1 could also be useful in other diseases where white matter and axonal function is important in recovery such as TBI, stroke, and vascular dementia (Frati et al., 2017; Hinman, 2014; Kalaria, 2016). Treatment with TAT-UCHL1 decreased axonal injury and CA3 neuronal cell death in mice after TBI (Liu et al., 2017). Thus, TAT-UCHL1 has potential as treatment for patients with neurodegenerative diseases and brain injury.

### 9.2. Viral vector mediated UCHL1 gene delivery

Gene delivery using adeno-associated virus (AAV) or lentivirus is another potential therapeutic approach for increasing UCHL1 activity. When the UCHL1 gene was delivered

selectively to CSMN in two different mouse ALS models bearing mutations on human superoxide dismutase type 1 (SOD1, G93A) and prion protein transactive response DNA-binding protein-43 (TDP-43, A315T) through AAV-mediated retrograde transduction, the expression of disease-inducing misfolded SOD1 and mutant TDP-43 were reduced and diseased CSMN retained their neuronal integrity and cytoarchitectural stability (Genc et al., 2022). Zhang and colleagues reported that intracranial injection of UCHL1-expressing AAV reduced amyloid β production, inhibited neuritic plaque formation and improved memory deficits in AD transgenic model mice (Zhang et al., 2014). In addition, stereotactic injections of lentiviral vectors containing the sequence of a nitrosylation-resistant UCHL1 (C152S) mutant into the dentate gyrus of hAPP-J20 AD transgenic mice prevented synaptic loss as measured by the presynaptic marker synaptophysin (Nakamura et al., 2021). These data suggest that use of viral vector-mediated UCHL1 gene therapy to increase UCHL1 activity in the brain could be a promising disease-modifying strategy for ALS and AD therapy.

### 9.3. Other approaches

UCHL1 has several known motifs that are sites for posttranslational modification which could be the target for drug development. The cysteine 152 may be modified by reactive lipids or NO resulting in changes in its structure and activity (Koharudin et al., 2010; Nakamura et al., 2021). Mutating UCHL1 to prevent binding of these substrates to the C152 site has been shown to decrease accumulation of polyubiquitin proteins, ameliorate white matter damage and preserve electrophysiological function and motor performance in models of TBI and cerebral ischemia (Koharudin et al., 2010; Liu et al., 2019; Mi et al., 2021a). Synaptic loss in neurons in the hippocampi of hAAP-J20 AD mice was ameliorated by transfection with a lentiviral vector expressing UCHL1 bearing a mutation in the C152 site (Nakamura et al., 2021). UCHL1 C220 may also be modified by farnesylation and may increase membrane association and susceptibility to α-synuclein toxicity (Liu et al., 2009). Thus, the posttranslational modification of the C152 and C220 sites may exacerbate neuronal degeneration in these disorders. The development of small molecule inhibitors that prevent binding of substrates to these sites could have therapeutic utility in preventing synaptic loss, white matter injury, and functional sequalae in neurodegenerative diseases, stroke, and TBI.

## 10. Conclusions

UCHL1 is a multifunctional protein that is highly expressed in the neurons of brain and spinal cord. UCHL1 plays important roles in regulating the level of cellular free ubiquitin, redox state as well as the degradation of select proteins. UCHL1 is prone to genetic and posttranslational modifications by reactive oxygen species, reactive lipids, and NO that impair its activity and result in decreased solubility and aggregation. Loss of UCHL1 function results in neurodegeneration in the central and peripheral nervous systems and may contribute to the pathogenesis of brain injury and neurodegeneration. Restoring UCHL1 activity and preventing the detrimental modification of UCHL1 may be an effective therapeutic strategy in neurodegenerative diseases and brain injury. Although its hydrolase activity plays a key role in regulating free ubiquitin and protein degradation in the neuron, selective abrogation of hydrolase activity does not produce the motor deficits and

premature death seen in UCHL1 deficient mice. This suggests that mechanisms in addition to UCHL1's ubiquitin hydrolase activity contribute to its pathogenic role. Further studies are needed to better understand UCHL1's pathogenic mechanisms and explore the therapeutic potential of various approaches to preserve UCHL1 function in neurological diseases.

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## Abbreviations

| | |
|---|---|
| **15d-PGJ2** | 15-deoxy-$\Delta^{12,14}$ - prostaglandin $J_2$ |
| **AAV** | Adeno-associated virus |
| **AD** | Azlheimer's disease |
| **ALP** | Autophagy-lysosome pathway |
| **ALS** | Amyotrophic lateral sclerosis |
| **APP** | Amyloid precursor protein |
| **BACE1** | β secretase 1 |
| **bp** | Base pair |
| **CMA** | Chaperone-mediated autophagy |
| **CSF** | Cerebrospinal fluid |
| **CSMN** | Corticospinal motor neurons |
| **CyPg** | Cyclopentenone prostaglandin |
| **DUB** | Deubiquitinating enzyme |
| **GSH** | Glutathione |
| **Hsc70** | Heat shock cognate protein 70 |
| **Hsp90** | Heat shock protein 90 |
| **LAMP-2A** | Lysosome-associated membrane protein type 2A |
| **MCAO** | Middle cerebral artery occlusion |
| **NFT** | Neurofibrillary tangles |
| **NMJ** | Neuromuscular junction |
| **NO** | Nitric oxide |
| **PD** | Parkinson's disease |

| | |
|---|---|
| **PS1** | Presenilin 1 |
| **pVHL** | Von Hippel–Lindau |
| **ROS** | Reactive oxygen species |
| **SNCA** | Synuclein alpha (not needed) |
| **SOD1** | Superoxide dismutase type 1 |
| **TAT** | Trans-activator of transcription |
| **TBI** | Traumatic brain injury |
| **TDP-43** | Transactive response DNA-binding protein-43 |
| **TREM2** | Triggering receptor expressed on myeloid cells 2 |
| **TrkB** | Tropomyosin receptor kinase B |
| **UCHL1** | Ubiquitin C-terminal hydrolase 1 |
| **UPP** | Ubiquitin-proteasome pathway |

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## Highlights

- UCHL1 has multiple functions in the normal brain including regulation of the level of cellular free ubiquitin, the redox state and degradation of select proteins
- Posttranslational modifications of UCHL1 result in decreased solubility, increased aggregation and impaired function of UCHL1
- Loss of UCHL1 function results in neurodegeneration in the central and peripheral nervous systems and may contribute to the pathogenesis of brain injury and neurodegeneration by multiple molecular mechanisms.
- Restoring UCHL1 activity and preventing the detrimental modification of UCHL1 may be an effective therapeutic strategy in neurodegenerative diseases and brain injury.
- Further investigation is needed to determine the precise mechanisms by which UCHL1 deficiency contributes to neurodegeneration.

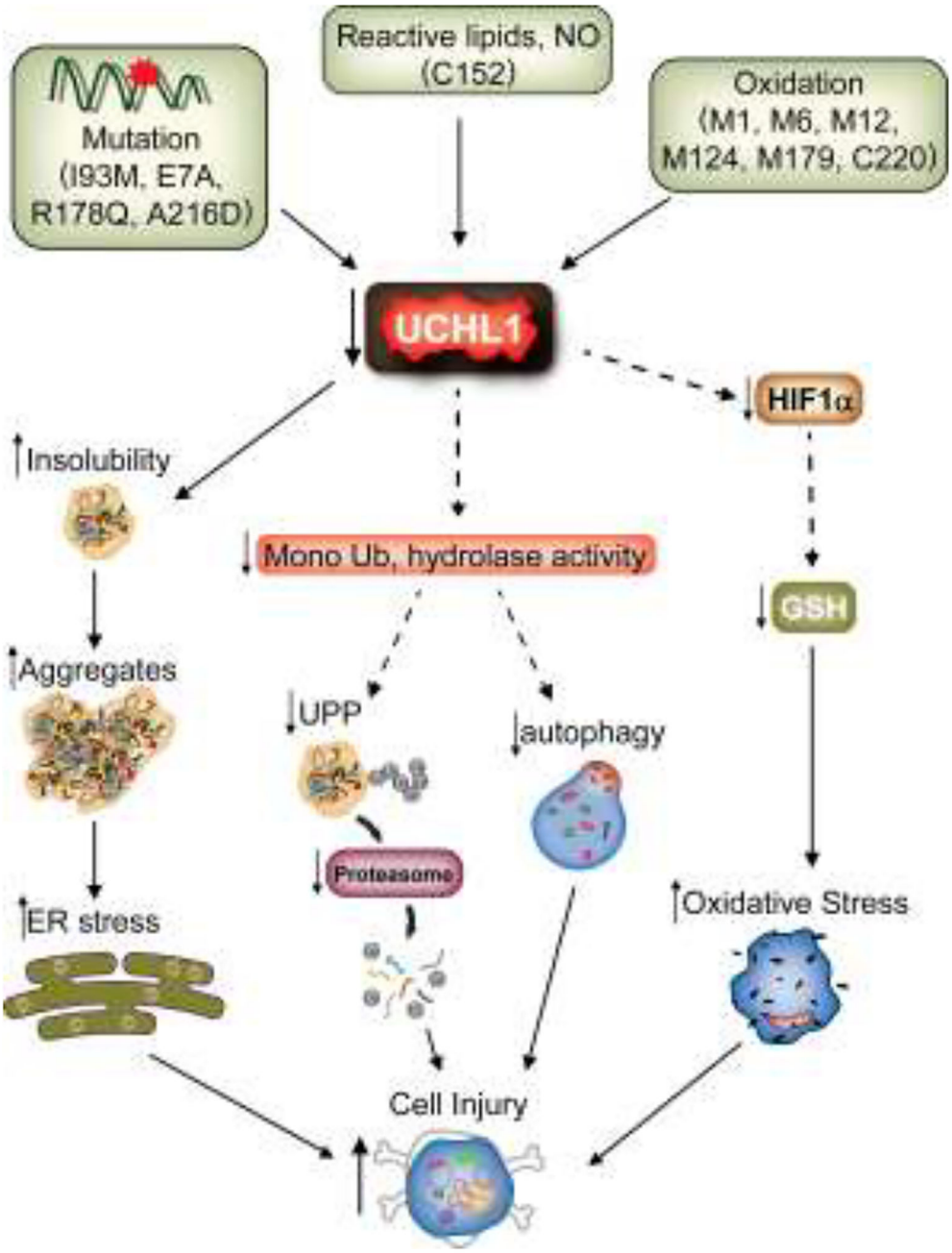


**Figure 1.**

Potential mechanisms of UCHL1's structural and functional impairment in neurodegeneration. Alterations in the structure and function of UCHL1 by genetic mutation, reactive lipid, NO and oxidation lead to neuronal cell death through disruption of UPP and ALP, increased cellular insolubility and oxidative stress.

**Table 1.**

Active sites, mutations and post-translational modifications of UCHL1

| Sites | Description | Species | Effects of modification | Publications |
|---|---|---|---|---|
| C90, H161, D176 | Active site for hydrolase activity | Human and mouse | Mutation of the sites abolishes or decreases hydrolase activity | (Bilguvar et al., 2013; Boudreaux et al., 2010; Larsen et al., 1996; Nishikawa et al., 2003) |
| D30K | Produced by mutagenesis | Human | Abolishes enzymatic activity and ubiquitin binding | (Osaka et al., 2003) |
| F214A | Produced by mutagenesis in human cell line | Human | Almost complete loss of hydrolase activity | (Boudreaux et al., 2010) |
| I93M | Spontaneous mutation associated with increased risk for PD | Human | Mutation reduces hydrolase activity | (Bilguvar et al., 2013; Liu et al., 2002; Nishikawa et al., 2003) |
| S18Y | Spontaneous mutation associated with decreased risk for PD in selected population | Human | Mutation reduces ligase activity | (Liu et al., 2002) |
| R178Q, A216D | Spontaneous mutation associated with childhood blindness, ataxia and spastic paraplegia | Human | A216D: loss of function; R178Q: increased hydrolase activity | (Rydning et al., 2017) |
| E7A | Spontaneous mutation associated with childhood blindness, ataxia and spastic paraplegia | Human | Decreased binding to ubiquitin and significantly decreased hydrolase activity | (Bilguvar et al., 2013) |
| C220 | Modification site associated with exacerbated alpha-synuclein neurotoxicity | Human cell lines, mouse primary neuronal culture, human brain tissues | Farnesylation or oxidation of the site may promote membrane association of UCHL1 and decrease activity | (Liu et al., 2009) |
| N-terminal 1–11 | Spontaneous mutation that truncates the first 11 amino acids at the N-terminal | Human cell lines, mouse brain tissues | Increases membrane association of UCHL1, decreases cellular ROS levels | (Kim et al., 2014) |
| C152 | Site of covalent modification by reactive lipids, S-nitrosylation, S-mercuriation | Mouse | Loss of hydrolase activity, structural changes, aggregation | (Koharudin et al., 2010; Nakamura et al., 2021; Toyama et al., 2015) |
| M1, M6, M12, M124, M179 | Sites of methionine oxidation | Human AD, PD brains | Hydrolase activity inhibition | (Choi et al., 2004) |

**Table 2.**

Genotypes of naturally occurring and engineered UCHL1 mutant mice

| Name | Type | Genotype | Publications |
|---|---|---|---|
| *gad* | Spontaneous | Deletion of exons 7 and 8 | (Mukoyama et al., 1989; Saigoh et al., 1999; Yamazaki et al., 1988) |
| UCHL1$^{nm3914}$ *or GAD j* | Spontaneous | Deletion of the final 24 bp of exon 6 and the first 771 bp of intron 6 | (Walters et al., 2008) |
| UCHL1$^{tm1Dgen}$ | KO | Deletion of exons 6 through 8 and the first 6 bp of exon 9 | (Chen et al., 2010) |
| UCHL1 knockout | KO | Deletion of exon 4 | (Coulombe et al., 2014) |
| UCHL1$^{d/d}$ | KO | Deletion of exons 1–3 | (Reinicke et al., 2019) |
| UCHL1 C152A | KI | Selective mutation of Cystine 152 to Alanine | (Liu et al., 2015) |
| UCHL1 C90A | KI | Selective mutation of Cystine 90 to Alanine | (Mi et al., 2021) |