Box S1 | Lysosomal storage disorders: insights into the pathogenesis and treatment of neurodegenerative disorders of ageing (NDAs)

The more than 50 recognised classes of lysosomal storage disease (LSDs)
, like Gaucher disease (GD) and Niemann-Pick disease type C, are genetically inherited disorders that arise from: 1), defects in the activity of lysosomal hydrolases that catabolise proteins and other autophagic cargo (primary storage disorders); 2), dysfunction of proteins involved in autophagic cargo sequestration and lysosomal delivery (secondary storage disorders) or 3) aberrant forms of proteins involved in cargo handling and exocytosis (tertiary storage disorders)
. Deficiencies in lysosomal hydrolases disrupt the sequential degradation of cellular substrates like proteins, sphingolipids and glycosaminoglycans
. Several LSDs are associated with neurodegeneration that commences in early childhood: for example, Tay-Sachs disease where β-hexosaminidase A deficiency causes Gb3-ganglioside accumulation. Further, since mutations are expressed body-wide, LSDs present with systemic complications like splenomegaly and cardiomypathy
.

Conceptually, NDAs might be considered, at least in part, as ‘storage’ disorders associated with the accumulation of incompletely-degraded proteins, such as α-synuclein in Parkinson disease (PD) and Aβ and tau protein in Alzheimer’s disease (AD)
. In NDAs, a reduction in lysosomal clearance of aggregated proteins and dysfunctional organelles is, then, associated with characteristic pathophysiological features (main text, Table 1).

Although the lysosome is the final site of substrate degradation, and ’classical’ LSDs are conventionally viewed as compartmentalized inborn errors of metabolism, the lysosome is part of a dynamic system integrating other cellular processes like endocytosis and autophagy (main text)
. Moreover, LSDs partly result from the sequestering of proteins and other substrates that would otherwise be recycled to serve as building blocks for meeting on-going cellular demands. A diversity of pathophysiological events including decreased lysosomal acidity, neuroinflammation and dysregulation of calcium homeostasis are associated with LSDs and provide a mechanistic link to NDAs where similar pathological changes are seen
. As for the earlier age of symptom onset in LSDs vs NDAs, this is likely explained by the more radical impact of single congenital gene defects that cause a life-long loss of lysosomal (degradative) enzyme activity and rapid accumulation of substrates
. Conversely, in NDAs the primary defect(s) are mainly extrinsic to the lysosomal compartment, the evolution of disease is slower and the rate of ‘substrate’ accumulation more protracted. Eventually, with the progressive accumulation of aggregated proteins, cellular storage surpasses a threshold (”inflection point”) that initiates a cascade of damaging events.

Parallels between LSD and NDAs are exemplified by GD and PD. In GD, heterozygote mutations in GBA1, which encodes lysosomal β-glucocerebrosidase, markedly increase the risk of PD
. Conversely, a subset of sporadic PD patients present with disrupted function of β-glucocerebrosidase and lysosomal accumulation of its substrate, glucosylceramide
. Moreover, as recently reported
, more than half of PD patients have at least one putatively deleterious mutation in genes associated with LSDs other than GBA
. Lysosomal dysfunction is also of more general relevance to the pathogenesis of familial and idiopathic AD which, amongst other processes, can be linked to disruption of Presenilin-1, a protein driving lysosomal acidification
. Further comparisons of LSDs and NDAs should yield additional insights into their underlying and common causes, and also into potential therapies for restoring lysosomal function
.

Approved disease-modifying treatments for LSDs include enzyme replacement therapies which deliver recombinant enzymes, such as β-glucocerebrosidase for GD, although this enzyme poorly enters the brain
. Substrate reduction therapy involves the inhibition of enzymes that normally synthesize the excess substrate: e.g., miglustat and eliglustat inhibit glucosylceramide synthase, although the latter has limited access to the brain
. This type of therapy with better ability to penetrate the BBB (e.g., GZ/SAR402671), together with the use of chaperones to promote β-glucocerebrosidase transfer to lysosomes, is potentially useful for PD - see main text
. Another drug undergoing clinical testing is arimoclomol which is being evaluated in Niemann-Pick disease type C1. It mimicked the ability of recombinant Heat Shock Factor 70 (a protein chaperone that promotes the binding of sphingolipid-degrading enzymes to their co-factors) to counter lysosomal defects in primary fibroblasts from this and other classes of LSD
. The first trial assessing the clinical benefit of GZ/SAR402671 has recently been launched in patients with PD bearing a GBA1 mutation (NCT02906020) (Supplementary Table 1).

Important differences between NDAs and LSDs should not be neglected: not least, a deficiency of clearance mechanisms other than the ALN is a core facet of the former diseases. Nonetheless, Research and Development programmes devoted to LSDs are proving instructive in the elucidation of pathological mechanisms underlying NDAs, and in the elaboration of novel strategies for their treatment
. The first trial assessing the clinical benefit of GZ/SAR402671 has recently been launched in patients with PD bearing a GBA1 mutation (NCT02906020) (Supplementary Table 1).

References

In addition to clearance mechanisms discussed in the main text, maintenance of proteostasis requires effective folding and assembly of newly-synthesized proteins before they exit the endoplasmic reticulum (ER). When this process is insufficient and cells begin to accumulate misfolded and harmful proteins, the unfolded protein response (UPR, see Glossary) is transiently triggered to let the cell recover. The UPR is integral to maintaining proteostasis and its modulation can affect autophagic processes.

Transient UPR activation results in increased chaperone expression for protein re-folding, and, via the kinase Protein Kinase RNA-like Endoplasmic Reticulum Kinase (PERK), a reduction in protein synthesis rates to reduce the ER burden. However, chronic over-activation of PERK signalling occurs in certain NDAs and has been implicated in neurotoxicity: hence, PERK down-regulation is a promising target for NDA treatment which can be considered as complimentary to the augmentation of clearance. In mouse models, deregulated and excess protein folding causes a sustained and marked reduction in cerebral protein synthesis leading to synaptic dysfunction and neuronal death: this is reversible by genetic and pharmacological inhibition. Similar beneficial effects were observed using trazodone, an antidepressant that inhibits PERK signalling and is being repurposed for clinical trials in human NDAs. Genetic or pharmacological modulation of the UPR also results in the activation of autophagy and has shown promising results in models of Alzheimer’s disease, Parkinson’s disease and amyotrophic lateral sclerosis.

As mentioned above and discussed in the main text, disruption of proteostasis (and accumulation of neurotoxic proteins) is toxic; so cells initiate a series of adaptive responses. These include induction of clearance by the autophagic-lysosomal network (ALN) and the ubiquitin proteasome system (UPS), and reduced generation of proteins via activation of the UPR. These adaptive responses (sometimes referred to as hormesis) can also be induced by a sub-toxic, low intensity stressor (“preconditioning”) which results in cellular protection against further toxic injury. Hormesis can be induced by many preconditioning stimuli like oxidative stress, ER stress and inflammatory stimuli. It has been proposed that preconditioning of the UPR via mild ER stress may be neuroprotective. For example, in Drosophila models of apoptosis-induced retinal degeneration and human α-synuclein overexpression, ER-preconditioning elicited either genetically or with tunicamycin promoted neuroprotection by harnessing the ALN. Similarly, in a 6-hydroxydopamine mouse model of Parkinson’s disease, preventive injection of tunicamycin reduced both dopaminergic neuron loss and locomotor dysfunction. The interconnection between the UPR and clearance is also critical for neuroprotection in models of ischemia reperfusion. Finally, modest activation of X-box binding protein 1 (XBP1), a major transcription factor triggered by the UPR, leads to autophagic activation and neuroprotection in mouse models of ALS and HD.

Collectively, the above comments indicate that two UPR-articulated strategies may provide neuroprotection in NDAs: first, abrogation of an over-persistent and extreme UPR and, second, a mild and transient recruitment of the UPR as a means to engage ALN-driven clearance of neurotoxic proteins.

In addition to the cytosolic UPR discussed above, there is also a mitochondrial UPR which is activated to protect mitochondria from stressors like accumulation of unfolded proteins in their matrix. These harmful, intra-mitochondrial proteins are digested by proteases and the resultant peptides exported to the cytosol: there, they trigger a cascade that restores proteostasis by inducing genes encoding, for example, mitochondrial chaperones. In addition, due to a decrease in the activity of mTOR, protein translation is reduced. Boosting the mitochondrial UPR and mitophagy with nicotinamide had beneficial effects on mitochondrial function in human cells expressing Aβ, as well as in mice models of AD where it helped to clear intracellular deposits of Aβ. Finally, mitochondrial stress due to failure of protein import or sorting leads to the accumulation of proteins in the cytosol and to an overburdening of the UPS. Accordingly, agents that promote UPS activity may also be of therapeutic utility in NDAs associated with mitochondrial dysfunction.

References
Box S3 | Potentially detrimental effects of (excess) autophagic–lysosomal network (ALN) activity: relevance to amyotrophic lateral sclerosis (ALS)

**Excess activity of the ALN under specific conditions and its consequences**

While modest and time-constrained ALN activation is protective, sustained and uncontrolled over-activation is potentially deleterious. Indeed, sustained and massive ALN activity in stroke or traumatic brain injury leads to tissue damage and even cell death (“autosis”) (Glossary) due to excessive catabolism and cellular stress. While there is little or no evidence for excess ALN in most classes of NDA, this may potentially occur in ALS although, as outlined below, not all observations support this - still controversial - possibility.

**Evidence for a reduction in the activity of the ALN in ALS**

In post-mortem tissues of ALS patients, protein inclusions are seen in upper and lower motoneurons, as well as in neuronal and glial cells in the midbrain, prefrontal neocortex and striatum. This argues for defective clearance of neurotoxic proteins, and one possible cause of compromised ALN activity is release by reactive astrocytes of transforming growth factor β which interferes with initiation of autophagy by stimulating mTOR. Further, a low handling capacity of the ALN - as reflected in the scarcity of beclin 1 in the ventral horn of patients with sporadic or familial ALS - may be related to development of the disorder. Indeed, by analogy to frontotemporal dementia and other classes of NDA (main text), defective autophagy has been proposed as a point of convergence of harmful events in ALS, such as endoplasmic reticulum stress, mitochondrial dysfunction, anomalous processing of mRNA/miRNAs and oxidative stress.

Mutated Fused in Sarcoma (FUS), which accounts for about 5% of familial forms of ALS, inhibits the formation of autophagosomes in neuronal cells. Negative regulation of autophagy has also been reported in cultured neurons transfected with mutated TAR DNA-binding protein-43 (TDP-43), while superoxide dismutase (SOD) 1 overexpression impairs fusion of autophagosomes and lysosomes. These results support ALN impairment, although conflicting with another paper which reported an upregulation of macroautophagy in cells transfected with TDP-43 or SOD1. Underpinning the pertinence of specific cell types, in symptomatic SOD1G93A (specific mutation) mice, expression of pro-autophagic markers is increased in the brainstem and spinal cord. These observations accord with the idea that muscle cells are more effective in clearing SOD1 by upregulating the ALN. Nonetheless, muscles of SOD1G93A mice accumulate autophagosomes when compared to wild-type mice, presumably due to inefficient ALN flux.

**Evidence for an increase in the activity of the ALN in ALS**
Despite this evidence for reduced autophagic flux in ALS patients, this may not be true in all disease stages, for all ALS patients or, as intimated above, for all cell types. Moreover, ALN status may also depend on the precise genotype as highlighted by C9orf72. A hexanucleotide repeat expansion in the C9orf72 gene is a major cause of familial ALS. Although it is not clear whether C9orf72 mutations increase the propensity for ALS due to a loss and/or gain of function, carriers of expansion repeats present with reduced expression of C9orf72. Their impact may, at least partially, be explained by a deregulation of autophagy. Indeed, C9orf72 has been proposed as a negative regulator of mTOR/macroautophagy and its silencing enhanced autophagy flux in mice. Thus, lower expression of C9orf72, or loss of function, could translate into higher autophagy flux for ALS patients. Indeed, there is clinical evidence for an up-regulation of the ALN in ALS. Expression and activity of lysosomal enzymes were higher in the spinal cord of ALS patients and animal models. Underlining complexity, genetic removal of Atg7 in motoneurons inhibited macroautophagy in SOD1G86R mice and resulted in earlier disease onset yet longer survival. The loss of macroautophagy in motoneurons was also associated with reduced glial cell reactivity. Finally, SOD1 mutations caused overactivation of autophagy and cellular degeneration in SOD1 mutant mice. This appeared to involve AMPK stimulation of Ulk1, and a reduction of AMPK activity conferred some modest neuroprotection. Accordingly, treatment of cultured motoneurons from SOD1 mice with a AMPK stimulator exacerbated cytotoxicity suggesting that autophagic activity was already elevated (and deleterious) and that further stimulation might induce autosis.

**Therapeutic control of excess ALN activity**

Collectively, the above observations are far from simple to reconcile (!), yet suggest a time, cause and cell-type dependent changes in specific components of the ALN. Mobilisation might well be beneficial for neuromuscular and neuronal integrity at disease onset, but “excess” autophagy in motoneurons may be toxic in late stages of the disease. As the main text focusses on efforts to promote ALN activity, the following comments evoke possibilities for tempering a putative hyperactivity under specific conditions in ALS.

As mentioned above, agents that dampen an overactive ALN are mainly conceived for therapeutic use in stroke, cerebral ischemia and traumatic brain injury, but data are emerging for ALS. Administration of n-butylidenephthalide, a natural compound which negatively regulates pro-autophagy proteins, extended survival of SOD1 G93A mice. Moreover, inhibition of SOD1G86R mice. Edaravone, a reactive oxygen species scavenger, reduced cerebral autophagy in vivo after ischemia and also in macrophages. This action is potentially linked to its utility for treating ALS and edaravone was approved for use in a subset of ALS patients. It would be interesting to know their ALN status compared to other subjects who were not helped by treatment. Underscoring interest in edaravone, it improved cognition and diminished Aβ levels in a rat model of Alzheimer’s disease, and also displayed neuroprotective properties in a rotenone-induced model of PD. However, its influence on Aβ appears to at least partly involve anti-aggregant properties, and it remains to be proven that ALN suppression is genuinely involved in the actions of edaravone. Furthermore, not all data support the idea of promoting ALN activity in ALS. In this light, it is interesting to consider rilmenidine, a positive modulator of mTOR-independent macroautophagy (main text). In SOD1G90A mice, rilmenidine increased ALN flux in the spinal cord but ultimately exacerbated motoneuron degeneration and the accumulation of insoluble, misfolded SOD1 protein.

**Concluding comments**

In conclusion, ALS exemplifies the complexity of ALN deregulation in NDAs. Baseline flux may be inherently low at the onset of disease, related to old age and deficient actions of proteins encoded by genes such as dynactin and progranulin (Table 1). Hence, the cell attempts to promote the ALN and this may initially be protective. However, later in the disease, under conditions of chronic cellular stress, energy imbalance and inflammation, “excess” ALN activity may become deleterious. Accordingly, there may be a phase-dependent need to therapeutically enhance or moderate ALN activity, potentially explaining contradictory findings in the literature. This remark also suggests that the duration and pattern as well as the time of drug administration would be critical. Finally, accentuating complexity, while this seems to hold for motoneurons and neuromuscular junctions, it remains to be more fully established how events unfold in spinal and cerebral neurons impacted in ALS. Much further research will be needed to clarify how to optimally harness the ALN for clearing neurotoxic proteins in ALS.

**References**


<table>
<thead>
<tr>
<th>Agent</th>
<th>Disorder</th>
<th>Clinical trial</th>
<th>Phase</th>
<th>Dose</th>
<th>Primary outcome measures</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lithium</td>
<td>FTD</td>
<td>NCT02862210</td>
<td>II</td>
<td>150-600 mg/d</td>
<td>Neuropsychiatric Inventory Scale; BDNF serum levels and changes in NPI score</td>
<td>Recruiting, negative in ALS³</td>
</tr>
<tr>
<td>Metformin</td>
<td>Ageing</td>
<td>NCT02432287</td>
<td>IV</td>
<td>1700 mg/d</td>
<td>Gene expression, insulin sensitivity</td>
<td>Ongoing⁴</td>
</tr>
<tr>
<td>Metformin</td>
<td>MCI</td>
<td>NCT00620191</td>
<td>II</td>
<td>1000 mg/2x/d</td>
<td>Memory recall, ADAS-cog, 2-deoxy-2-fluoro-D-glucose positron emission tomography</td>
<td>Completed, minor cognitive benefit; other markers negative⁵</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>AD, MCI</td>
<td>NCT00678431</td>
<td>II</td>
<td>Grape juice</td>
<td>ADAS-cog, CGIC</td>
<td>Completed, unsuccessful</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>AD</td>
<td>NCT01504854</td>
<td>II</td>
<td>500-1000 mg/2x/d</td>
<td>Aβ-amyloid 1-42 levels, brain MRI; Innate immune/inflammatory biomarkers; Cognitive and functional decline</td>
<td>Completed, no change in brain volume; positive signals in patient subset (see legend)⁴⁵</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>HD</td>
<td>NCT02336633</td>
<td>III</td>
<td>40 mg/2x/d</td>
<td>Caudate atrophy; Unified Huntington Disease Rating Scale; Total Functional Capacity; inorganic phosphate/phosphocreatine levels</td>
<td>Recruiting</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>AD</td>
<td>NCT00580931</td>
<td>I</td>
<td>1500 mg/2x/d</td>
<td>ADAS-cog</td>
<td>Completed, no report</td>
</tr>
</tbody>
</table>

Table S4 | Clinical trials undertaken in neurodegenerative disorders of aging with agents that experimentally modify the clearance of neurotoxic proteins
<table>
<thead>
<tr>
<th><strong>TRx0237 (LMTX/M)</strong></th>
<th>AD</th>
<th>NCT0162639</th>
<th>II</th>
<th>100 mg/2x/d</th>
<th>Safety and tolerability with Acetylcholinesterase Inhibitor or Memantine co-administration</th>
<th>Terminated(^1,6); Post-hoc analysis “positive” (see legend)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TRx0237 (LMTX/M)</strong></td>
<td>FTD</td>
<td>NCT01626378</td>
<td>III</td>
<td>100 mg/2x/d</td>
<td>Whole brain volume (MRI); Addenbrooke’s Cognitive Exam; Functional Activities questionnaire; Frontotemporal Dementia Rating Scale; Modified CGIC</td>
<td>Completed, unsuccessful(^7)</td>
</tr>
<tr>
<td><strong>Curcumin</strong></td>
<td>MCI</td>
<td>NCT01383161</td>
<td>II</td>
<td>465 mg/6x/d</td>
<td>Cognitive testing, inflammation markers; Aβ–amyloid 1-42 levels; FDDNP-PET</td>
<td>Ongoing</td>
</tr>
<tr>
<td><strong>Ambroxol</strong></td>
<td>PD</td>
<td>NCT02914366</td>
<td>II</td>
<td>Escalating doses 60-420 mg/d</td>
<td>Glucosylceramide and ambroxol levels in CSF; GCase activity; Montreal Cognitive Assessment; UPDRS</td>
<td>Ongoing</td>
</tr>
<tr>
<td><strong>Ambroxol</strong></td>
<td>PD</td>
<td>NCT02914366</td>
<td>III</td>
<td>525,1050 mg/d</td>
<td>ADAS-cog; CGIC; MOCS; CSF (α-syn; tau; Aβ); MRI (atrophy)</td>
<td>Recruiting</td>
</tr>
<tr>
<td><strong>Arimoclomol</strong></td>
<td>ALS</td>
<td>NCT00706147</td>
<td>II/III</td>
<td>200 mg/3x/d</td>
<td>Rate of decline on ALSFRS-R, safety and tolerability</td>
<td>Well-tolerated; low adverse effects; possible increased survival; slower ALSFRS-R decline(^8)</td>
</tr>
<tr>
<td><strong>Arimoclomol</strong></td>
<td>ALS</td>
<td>NCT00244244</td>
<td>II</td>
<td>75-300mg/3x/d</td>
<td>Safety, tolerability, pharmacokinetics; rate of decline on ALSFRS-R</td>
<td>Well-tolerated, low adverse effects; slower ALSFRS-R decline with Arimoclomol(^9)</td>
</tr>
<tr>
<td><strong>GZ/SAR402671</strong></td>
<td>PD</td>
<td>NCT02906020</td>
<td>II</td>
<td>Escalating doses TBD</td>
<td>UPDRS, Parkinson’s Disease Cognitive Rating Scale; Hoehn and Yahr score</td>
<td>Recruiting</td>
</tr>
<tr>
<td><strong>Nilotinib</strong></td>
<td>PD</td>
<td>NCT02821474</td>
<td>I</td>
<td>150, 300 mg/d</td>
<td>Safety, tolerability, pharmacokinetics and biomarkers</td>
<td>Completed, potential benefits to confirm(^10)</td>
</tr>
<tr>
<td><strong>Nilotinib</strong></td>
<td>PD</td>
<td>NCT02954978</td>
<td>II</td>
<td>150, 300 mg/d</td>
<td>Safety, tolerability, pharmacokinetics and biomarkers</td>
<td>Recruiting</td>
</tr>
<tr>
<td><strong>Nilotinib</strong></td>
<td>AD</td>
<td>NCT02947893</td>
<td>II</td>
<td>150, 300 mg/d</td>
<td>Safety, Biomarkers and Clinical Outcomes</td>
<td>Recruiting</td>
</tr>
</tbody>
</table>
This table depicts those drugs affecting autophagic-lysosomal or ubiquitin-proteasomal clearance that have been, or are being, clinically evaluated for the treatment of neurodegenerative disorders of aging. The clinical trial identifier is shown, together with the phase of testing, doses under study (oral) and primary measures/readouts used. The drugs shown were not necessarily developed as modulators of neurotoxic protein elimination per se - for example, TRx0237. However, based on experimental data, they are known to modulate clearance. In addition to the drugs and studies indicated in the Table, an open label investigation with rilmenidine was recently undertaken with a view of evaluating its efficacy in the treatment of Huntington’s disease. For mechanisms of drug action - which in several cases, like lithium, are not entirely clear - see main text and Table 2. While resveratrol did not reduce brain volume loss in the overall trial in AD and MCI, analysis of a small patient subset with CSF levels of Aβ1-42 of less than 600 ng/ml, provided evidence for a favourable influence on the blood-brain barrier (blocked leakage due to decreased levels of Matrix Metalloprotease 9), a reduction in immune-inflammatory markers, and a less marked decline in cognition and functional performance. TRx0237 (LMTX or LMTM) is a new formulation of methylene blue (methylthioninium chloride) and a successor of Trx014 (Rember™). Further analysis of the AD trial suggested that it may have beneficial effects, notably on brain atrophy, but this has been disputed and another randomized trial would be needed to verify this post-hoc interpretation. Further, the focus is now largely on the anti-aggregation properties of TRx0237, so it is unclear to what extent induction of autophagy is involved in its clinical actions. Ironically, the only drug to have received FDA authorization is edaravone yet, as discussed in Suppl Box 3, edaravone may reduce ALN activity. However, this remains controversial and edaravone has other, therapeutically-useful actions like anti-oxidant and anti-aggregant properties. Abbreviations not in main text: ADAS-Cog, Alzheimer Disease Assessment Scale; ALSFRS-R, ALS Functional Rating Scale-Revised; CGIC, Clinician’s Global Impression of Change; FDDNP-PET 2-(1-(6-[(2-[fluorine-18]fluoroethyl)(methyl)amino]-2-naphthyl)-ethylidene)malononitrile - Positron Emission Tomography; GBA, β-Glucocerebrosidase; MCI, Mild cognitive impairment; MOCS, Montreal Cognitive Score; MRI, Magnetic Resonance Imaging; NPI, Neuropsychiatric inventory; TBD, to be determined; UPDRS, Unified Parkinson’s Disease Rating Scale.

References

<table>
<thead>
<tr>
<th>Disease (age of onset)</th>
<th>Clinical and pathophysiological phenotype</th>
<th>Disruption of proteostasis</th>
<th>Autophagic–lysosomal network impairment</th>
<th>Impairment of CMA and of the UPS</th>
<th>Impairment in other modes of neurotoxic protein clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alzheimer disease</strong> (usually over 70)</td>
<td>Cognitive deficits; psychiatric symptoms; disorganized language; disrupted sleep/circadian rhythms. Neurodegeneration (entorhinal cortex, medial temporal lobe, hippocampus etc); ↓axon transport; axonal and synaptic degeneration; altered microglial phenotype.</td>
<td>Aβ oligomers disrupt neurons, synapses, aggravate tau toxicity; Aβ aggregates in extra-cellular plaques/vessels; aberrant tau cleavage, post-translational marking, folding and oligomerisation; ↑tau release and spreading; intra-cellular tau tangles (with p62 and other Ub-proteins). α-syn neuropathology in subpopulation.</td>
<td>↓Sirtuin-1; ↓Neuronal ALN flux; ↓Autophagosome maturation, transport (MAPT) and fusion with lysosomes; ↓APP loading (PICALM); APP and C-terminal fragment accumulation in endo-lysosomes; ↓Lysosomal acidity and digestion (PS-1/2, APP ApoE4); ↓GliaL ALN (TREM2, ApoE4).</td>
<td>↓CMA (disrupted by Aβ/tau aggregates); Anomalous mutant tau at LAMP2A; Slow syn and mutant forms of α-syn and LRRK2 block; Slow α-syn dissociation from LAMP2A, ↓UPS clearance (perturbed by Aβ and tau oligomers); FKBPS1 binds Hsp90 to interfere with UPS substrate loading.</td>
<td>↓Proteolytic Aβ clearance (↓IDE, Neprilysin, Plasmin), ↓BBB clearance of Aβ and, probably, tau (↓LRP1, ↓P-glycoprotein; ↑RAGE); ↓Aβ provision to BBB (ApoE4); ↓glymphatic clearance of Aβ and, probably, tau.</td>
</tr>
<tr>
<td><strong>Parkinson disease</strong> (usually over 60)</td>
<td>Motor impairment (poor gait, rigidity, bradykinesia, tremor); ↓olfaction; gastrointestinal problems; cognitive deficits; pain; depression; prodromal RBD. Neuronal loss (Dopaminergic cells in SNPC etc).</td>
<td>α-syn inclusions and Lewy Bodies (contain lipids, α-syn, Tau, other neurotoxic proteins, ubiquitin); ↑α-syn release and spreading in brain - possibly earlier, in gut. Tau neuropathology in subpopulation.</td>
<td>Many α-syn related anomalies of ALN: ATG9 mislocalisation; ↓Formation, maturation, axonal transport and lysosomal fusion of autophagosomes; ↓Lysosomal function (LRRK2, PARK9, GBA); ↓beclin 1 (LRRK2); ↓Mitophagy (PINK1, PARK2).</td>
<td>↓LAMP2A/Hsc70 levels; ↓CMA activity (aggregated α-syn and mutant forms of α-syn and LRRK2 block); Slow α-syn dissociation from LAMP2A, ↑UPS clearance (aggregates and mutant forms of α-syn block); Impaired α-syn traffic to UPS (UCH-L1).</td>
<td>↓BBB α-syn clearance; likely ↓α-syn elimination by glymphatic system.</td>
</tr>
<tr>
<td><em>ca. 5%</em></td>
<td><em>APOE4, APP, PS1, PICALM, TREM2</em></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
## Frontotemporal dementia (~40-60)

- Cognitive impairment; altered personality; mood and language deficits; cell loss prominently in inferior frontal and anterior temporal cortices, asymmetrically or bilaterally.
- Misfolded and aggregated forms of tau, TDP-43 and/or (more rarely) FUS; often found with p62 and ubiquitin in inclusions.
- Autophagosome accumulation; ↓Cargo loading into autophagosomes by p62; ↓Axonal autophagosome transport (MAPT); ↓Endosomal trafficking (CHMP28); Lysosomal dysfunction (GRN, TMEM106); ↓Gliarial ALN flux (TREM2).
- ↓CMA and UPS clearance (impeded by aggregates of tau, TDP-43 and FUS); poly-GA aggregates (caused by C9orf72 mutations) sequester and stall proteasomes; p62 dysfunction.
- Not well defined, but likely similarities to AD as regards altered BBB permeability and ↓glymphatic flow.

### Amyotrophic lateral sclerosis (~50-60)

- Motor impairment (cramps, muscle weakness, spasticity); cognitive impairment; mood disturbances (especially late-phase); ventral horn motoneuron loss; brainstem and cortical neuron degeneration.
- Misfolded and aggregated TDP-43 and (more rarely) SOD1 and FUS inclusions in brain, spinal cord and motoneurons; inclusions may contain ubiquitin and ubiquitin-ligases.
- Mainly ↓ALN, but if cellular stress severe, high ALN may actually be detrimental; ↓Autophagosome maturation (C9ORF72); ↓Cargo loading (SQSTM1, UBQLN2, OPTN, TBK1); ↓Autophagosome retrograde transport (DCTN, C9ORF72); ↓Lysosomal function (CHMP28/GRN); ↓Gliarial ALN flux (TREM2).
- Aggregated proteins block proteasome; ↓Hsp70 and Hsp40; ↓Provision of SOD1 and other proteins for UPS degradation (VCP); ↓CMA clearance of TDP-43.
- BBB disruption; ↓glymphatic flow may impede efflux of neurotoxic proteins.

### Huntington disease (~30-50)

- Inherited (ca. 8-10% de novo mutations)
- Motor dysfunction (chorea, dystonia, slurred speech); cognitive impairment; sleep disturbances; basal ganglia neuron loss, especially striatal medium spinal neurons; disruption of corticostriatal pathway; failure of axonal transport.
- Aggregates of mutant (excess CAG repeat number) Htt; mutant Htt inclusions with ubiquitin, beclin 1, mTOR1, p62 and other cargo-loading proteins; Mutant Htt and fragments of Htt are cytotoxic.
- Mutant Htt poor substrate of and disrupts ALN - and mitophagy; interference with beclin-1; ↓Autophagosome formation and cargo recognition/loading; ↓Axonal transport of autophagosomes.
- Mutant Htt poor substrate of CMA and UPS; LAMP2A and Hsc70 initially upregulated, but CMA less efficient in late stages; Possible ↓UPS (blocked by mutant forms of Htt?); ↓Hsp70.
- BBB disruption due to accumulation of Htt, but role in Htt clearance uncertain; potential ↓glymphatic clearance to establish.

### CLEARANCE MECHANISMS OF MUTANT PROTEINS

Clearance mechanisms are recruited early in disease, yet eventually become dysfunctional and/or inadequate to cope with neurotoxic burden. Not all changes can be shown, and NDAs are associated with neuroinflammation/immune deregulation, glial anomalies, disruption of cerebral bioenergetics, mitochondrial dysfunction and ER/oxidative stress. Several variants of frontotemporal dementia (FTD) include behavioural, progressive non-fluent aphasia and semantic forms. ALS shares common pathological hallmarks and risk genes with FTD like C9orf72 (Chromosome 9 Open Reading Frame 72). This and other NDA-associated risk genes linked to impaired clearance, are indicated in column one. Examples of genes/proteins incriminated in pathological processes are given in columns 3-6. APOE4 (apolipoprotein E4); PARK9 (ATPase13A2); CHMP2B (chromatin-modifying protein 2B); DCTN1 (dynactin); FUS (Fused in sarcoma); GBA1 (β-glucocerebrosidase); GRN (progranulin); HTT (huntingtin); LRRK2 (leucine-rich repeat kinase 2); MAPT (microtubule association protein, tau); OPTN (optineurin); PARK2 (parkin); PICALM (phosphatidylinositol binding clathrin assembly protein); PINK1 (PTEN-induced putative kinase 1); PS (presenilin); SNCA (α-synuclein); SOD1 (superoxide dismutase 1); SQSTM1 (sequestosome 1, p62); TBK1 (TANK-binding kinase 1); TARDBP (TAR DNA binding Protein 43); TMEM106, transmembrane Protein 106B; TREM2 (triggering receptor expressed on myeloid cells 2); UBQLN2 (ubiquitin 2); UCH-L1, Ubiquitin carboxy-terminal hydrolase L1 (a deubiquitinase) and VCP (valosin-containing protein). Aβ refers to Aβ42 and related neurotoxic fragments of APP. See text for further pathology and citations. Abbreviations not above or in text: FKBP, FK-binding protein; SNPC, substantia nigra, pars compacta and RBD, rapid eye movement sleep behavioural disorder.