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Autophagy in Polyglutamine Disease: Imposing Order on Disorder or Contributing to the Chaos?

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Abstract

Autophagy is an essential, fundamentally important catabolic pathway in which double membrane-bound vesicles form in the cytosol and encircle macromolecules and organelles to permit their degradation after fusion with lysosomes. More than a decade of research has revealed that autophagy is required for normal central nervous system (CNS) function and plays a central role in maintaining protein and organelle quality controls in neurons. Neurodegenerative diseases occur when misfolded proteins accumulate and disrupt normal cellular processes, and autophagy has emerged as a key arbiter of the cell's homeostatic response to this threat. One class of inherited neurodegenerative disease is known as the CAG / polyglutamine repeat disorders, and these diseases all result from the expansion of a CAG repeat tract in the coding regions of distinct genes. Polyglutamine (polyQ) repeat diseases result in the production polyQ-expanded proteins that misfold to form inclusions or aggregates that challenge the main cellular proteostasis system of the cell, the ubiquitin proteasome system (UPS). The UPS cannot efficiently degrade polyQ-expanded disease proteins, and components of the UPS are enriched in polyQ disease aggregate bodies found in degenerating neurons. In addition to components of the UPS, polyQ protein cytosolic aggregates co-localize with key autophagy proteins, even in autophagy deficient cells, suggesting that they probably do not reflect the formation of autophagosomes but rather the sequestration of key autophagy components. Furthermore, recent evidence now implicates polyQ

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proteins in the regulation of the autophagy pathway itself. Thus, a complex model emerges where polyQ proteins play a dual role as both autophagy substrates and autophagy offenders. In this review, we consider the role of autophagy in polyQ disorders and the therapeutic potential for autophagy modulation in these diseases.

Graphical abstract

OVERVIEW OF THE CAG / POLYGLUTAMINE REPEAT EXPANSION DISORDERS

Polyglutamine disorders are adult-onset progressive neurodegenerative diseases caused by an expansion of a CAG triplet repeat within the coding region of affected genes. The respective resulting proteins thus carry abnormally long polyglutamine (polyQ) tracts, and disease severity, measured as the age of onset and extent of pathology, directly correlates with the length of the polyQ tract. Although disease usually presents late in life, polyQ disorders display a phenomenon known as ‘anticipation’, as the expanded CAG repeat is inherently unstable, resulting in earlier ages of onset and more severe disease course in successive generations [1]. There are nine described polyQ disorders, including Huntington’s disease (HD), X-linked spinobulbar muscular atrophy (SBMA), dentatorubral-pallidoluysian atrophy (DRPLA) and six spinocerebellar ataxias (SCA1, 2, 3, 6, 7 & 17). The causative mutant proteins, although evolutionarily and functionally unrelated, all have wide patterns of expression and are readily detected in many cell types, both within the CNS and outside of it. Despite this widespread expression, all polyQ disorders exhibit selective neurotoxicity, targeting specific neuronal populations and presenting with varied clinical manifestations.

PolyQ-expanded disease proteins misfold and accumulate as proteinaceous aggregates that cannot be efficiently degraded [1]. They thus belong to a superfamily of human neurodegenerative disorders, including Alzheimer’s disease, Parkinson’s disease, amyotrophic lateral sclerosis, frontotemporal dementia, and prion diseases. These ‘proteinopathies’ are all characterized by the accumulation of mutant misfolded proteins in inclusions or aggregates. In polyQ disease, these aggregates – known as intraneuronal nuclear inclusions – are highly enriched in peptide fragments of the aggregation-prone

polyQ-expanded disease protein, as well as components of the proteasome, protein chaperones and basal transcription factors [1].

The mechanisms underlying neurodegeneration in polyQ disorders are still controversial [1]. Seminal discoveries have demonstrated that a toxic gain of function upon polyQ-expansion of affected proteins is key in polyQ disease pathogenesis, but evidence also suggests that loss of native protein function contributes to polyQ disease protein toxicity [2, 3]. Most likely, for any given polyQ disease, more than one mechanism determines the pattern of observed neuron dysfunction and death, but alterations of certain key cellular pathways appear to be shared among the different polyQ disorders. Repeatedly targeted pathways include transcription regulation, mitochondrial function and cellular proteostasis. Maintenance of cellular protein homeostasis is achieved via a delicate balance between protein synthesis and protein degradation. Neurons in particular depend heavily upon maintaining protein quality control through highly efficient degradation mechanisms. Macroautophagy (hereafter called simply autophagy), an evolutionarily conserved lysosomal degradation pathway, fulfills a principal proteostasis function in neurons, where it is highly active and functions to eliminate toxic macromolecules and dysfunctional organelles, such as protein aggregates and damaged mitochondria.

AUTOPHAGY DYSREGULATION IN NEURODEGENERATIVE DISEASE

The importance of basal neuronal autophagy was demonstrated by the conditional CNS knock-out of key autophagy genes Atg5 and Atg7, which, in the absence of any additional stress, resulted in neurodegeneration similar to that observed in many neurodegenerative disorders [4, 5]. Neuronal autophagy also positively regulates synapse development and remodeling of neuronal terminals required for neuronal plasticity [6]. This implies that neurons require efficient autophagy not just for survival, but also to maintain proper function. Indeed, neurons are particularly sensitive to autophagy/lysosomal perturbations, as reflected by the high frequency of neurological disorders caused by mutations targeting the endo-lysosomal network [6, 7].

One of the most common types of insults faced by neurons is the accumulation of aberrantly folded/aggregated proteins and dysfunctional organelles. Autophagy plays a key role in degrading these toxic components, which are hallmarks of many neurodegenerative diseases of the CNS, such as Alzheimer's, Parkinson's and Huntington's diseases [7]. This suggests autophagy has powerful neuroprotective potential, degrading the causative misfolded, mutant proteins. However, the autophagy pathway itself might be a target of disease, as some causative disease gene products in neurodegenerative diseases (such as presenilin-1, huntingtin, α -synuclein, parkin, Leucine-rich repeat kinase 2 (LRRK2) and dynein) directly impact proper autophagy progression at multiple steps [7]. Indeed, recent evidence indicates that the accumulation of autophagic vesicles (AVs) observed in neurodegenerative diseases is due not only to increased autophagy, but in some cases to decreased autophagic flux [6, 7]. This suggests that deficits in autophagy-mediated degradation could be involved in the pathology of these diseases, because disease proteins are normally involved in the regulation of the autophagy pathway in the CNS [1, 7].

Autophagy impairment in polyglutamine disease

Degradation of misfolded proteins typically occurs through two distinct pathways: the ubiquitin proteasome system (UPS) or autophagy. Importantly, eukaryotic proteasomes may not efficiently degrade long polyQ tracts, and recent work indicates that aberrant sequestration of key UPS components can prevent efficient delivery of misfolded proteins to the nuclear proteasome in polyQ disorders [8]. Indeed, nuclear inclusions are enriched in UPS components and heat-shock protein chaperones, suggesting a generalized deficiency in the UPS' ability to degrade these proteins, resulting in their abnormal accumulation. On the other hand, polyQ-expanded proteins, both aggregated and soluble forms, are amenable substrates for autophagy degradation [9]. Thus, failure of the UPS may lead to up-regulation of autophagy via cross-talk between degradation pathways in the cell's attempt to maintain normal proteostasis. However, while the UPS controls clearance of both cytosolic and nuclear proteins, autophagy degradation appears limited to the cytosol. Thus, cellular distribution of protein aggregates is a critical determinant when considering autophagy-mediated turnover of pathogenic entities. This is particularly important in polyQ disorders, where the localization of aggregates varies between disorders. While in SCA1, SCA7, SCA17 and SBMA, aggregates predominate in the nucleus, they are mainly cytoplasmic in SCA2 and SCA6, and are present in both subcellular locations in HD, SCA3, and DRPLA. This suggests that up-regulation of autophagy would result in degradation of only cytosolic polyQ protein complexes, preventing their import into the nucleus. As we discuss later, this is highly relevant to polyQ disease, as the nucleus has arisen as a central player in polyglutamine disease pathogenesis.

Huntington's Disease

Huntington's disease (HD), the most common of the polyQ diseases, is an autosomal dominant neurodegenerative disorder characterized by involuntary motor movement, cognitive decline, and psychiatric illness. HD is caused by a CAG repeat expansion (36) located in the amino-terminal region of the huntingtin (Htt) protein.

Gross accumulation of AVs, as well as alterations in the endo-lysosomal network, were early discoveries in analysis of HD patient brains [10]. Disruptions of membranous organelles such as Golgi apparatus and ER, and Htt positive tubule-vesicular structures were also found in abundance in HD brains, supporting polyQ-Htt dependent disruption of endocytic compartments [10, 11]. Analysis of the brain of HD transgenic mice shows striatum-specific increases in autophagy markers p62 (a cargo-adaptor) and LC3-II (an autophagosome membrane marker) [12], suggesting autophagy alterations are indeed relevant for HD pathogenesis (TABLE 1). In agreement with impairment of autophagy pathway function in HD, the mammalian Target of Rapamycin (mTOR), a serine/threonine protein kinase and master regulator of autophagy, is sequestered by polyQ-Htt aggregates, inhibiting its kinase activity and inducing autophagy [13]. Beclin-1, a key autophagy initiation protein, is also recruited to polyQ-Htt aggregates, impairing its ability to initiate autophagosome formation [14]. Since lysosomal clearance and autophagy activity also decline in the aging brain [6, 14], polyQ-Htt sequestration of autophagy proteins could be exacerbating HD disease progression in an age-dependent fashion.

Cargo recognition, which may be defined as the specific targeting of cytosolic components for autophagic degradation, is a key step in the autophagy pathway. Following substrate ubiquitination, cargo is delivered to autophagosomes via protein adaptors like p62/SQSTM1 and its relative NBR1, which bind both to ubiquitin and to the autophagosome membrane marker Atg8/LC3. Analysis of HD patient fibroblasts and transgenic mouse brains has revealed that AVs form at normal rates in HD cells and are adequately cleared by lysosomes, but they fail to efficiently trap cytosolic cargo in their lumen [15]. PolyQ-Htt is present at various organelle membranes [16, 17] and can bind directly to polyubiquitinated protein aggregates [9] and to p62 [18], suggesting that it could be directly interfering with organelle or aggregate recognition by AVs. Interestingly, a recently characterized family of adaptors, known as CUET proteins (CUE-domain targeting adaptors; Cue5-Tollip proteins), are also crucial for autophagic removal of polyQ-Htt aggregates [19]. Although the relevance of CUET proteins to HD pathogenesis is unknown, it is particularly intriguing that Tollip seems to bind to ubiquitinated substrates with higher affinity than p62 [19], suggesting a cooperative effort between p62 and other adaptors for targeting polyQ proteins for autophagy degradation.

Lysosomal clearance of autophagy substrates, the final step in the autophagy pathway, is also altered in HD. Striking accumulation of lipofuscin, a non-degradable intra-lysosomal polymer, is a prominent feature of post-mortem HD brains [10]. Intralysosomal accumulation of lipofuscin impairs lysosomal clearance of autophagic cargo, and enhancing neuronal autophagy results in normalization of lipofuscin levels in brains of *Hdh* 140Q knock-in mice [20]. Accumulation of intralysosomal lipofuscin may thus reflect defects in autolysosomal degradative capacities in HD.

In agreement with this hypothesis, recent data suggests that Htt itself is a key regulator of the autophagy pathway. Htt normally resides in the cytosol, localizing to cytoplasmic vacuoles and other membranous compartments, but upon polyQ tract expansion, Htt localization is significantly altered [16, 17]. Expression of either normal or mutant Htt induces endolysosomal network remodeling, stimulating endosome tubulation and autophagy [17]. Recently, Ochaba et al. postulated that Htt could be acting as a protein scaffold, bringing the autophagic core machinery into contact with targets for degradation [21]. The C-terminal domain of Htt is structurally similar to yeast Atg11 protein, and can interact with the Atg1/ULK1 complex and with Atg8-related proteins such as LC3B and GABARAP, potentially functionally mimicking the role of Atg11 in yeast [21]. Additionally, other domains in Htt share sequence homology with Atg23 and vacuolar protein 8 (Vac8), components of the yeast cytoplasm-to-vacuole-targeting pathway [21]. This suggests that Htt could be performing a variety of autophagy-related functions, but the relevance of these findings in vivo or to HD pathogenesis remains unclear. In support of a key role for Htt in neuronal autophagy, conditional CNS knock-out of Htt in mice results in autophagy defects characterized by p62 accumulation and pronounced lipofuscin and ubiquitin deposits [21]. Furthermore, the glutamine stretch in normal Htt appears to play a central role in this putative autophagy-regulator function of Htt, as deletion of the glutamine stretch in normal murine Htt (to create a Q-Htt isoform) activated autophagy in an mTOR-independent manner in mice [22]. Q-Htt autophagy induction was physiologically relevant,

as heterozygous expression of the Q-Htt allele in trans to the polyQ-Htt allele in Hdh140Q/Q knock-in mice significantly reduced Htt aggregate load, ameliorated motor phenotypes and improved survival in this HD mouse model [22]. It is particularly intriguing that the Androgen Receptor, another polyQ-expanded disease causing protein, has also been recently described as a co-regulator of autophagy activity [2, 23], suggesting a novel role for polyQ-tract containing proteins in maintaining cellular proteostasis.

Finally, polyQ-Htt can also interfere with Rhes-mediated regulation of autophagy [24]. Rhes (Ras homolog enriched in striatum) is a small guanine nucleotide-binding protein that appears to be selectively localized to the striatum, and binds Beclin-1 to release it from its inhibitory interaction with Bcl-2, thus inducing autophagy. Rhes also binds to both normal and polyQ-expanded Htt [25], and co-expression of polyQ-Htt blocks Rhes-induced autophagy activation [24]. Expression levels of Rhes are decreased in human HD brains [26] and in HD N171Q-82Q mouse striatum [26, 27]. Furthermore, striatum lentiviral overexpression of Rhes rescues behavioral and biochemical phenotypes of HD mice, although activation of autophagy under these conditions was not explored [27]. Rhes is a key regulator of mTORC1 in striatum [27], suggesting a dual loss of Rhes-on-Beclin-1 and Rhes-on-mTOR regulation, both leading to autophagy dysfunction, could underlie HD striatal neurodegeneration. Even more intriguingly, since Rhes expression is restricted to the striatum, aberrant polyQ-Htt-Rhes interactions could explain the striatal selectivity of HD pathology despite ubiquitous Htt expression [25].

Rhes (and its brain homologue, Rheb), are both key activators of mTORC1 in vivo [28]. Furthermore, recent evidence also suggests that Htt directly promotes mTORC1 signaling by forming a transient complex with mTOR and Rheb, and this effect is enhanced by polyQ-Htt [29]. In contrast, impaired mTORC1 signaling has been documented in HD transgenic mice [13, 27], and mTORC1 inhibition by Rapalogues is protective in both fly and the N171-82Q mouse model of HD [13]. Thus, rescue of HD phenotypes through Rheb-mediated activation of mTOR in HD striatum appears somewhat contradictory in the face of previously published data. However, recent evidence suggests the benefits observed after systemic delivery of mTOR inhibitors could be due to the beneficial effects of rapalogues on skeletal muscle and not the CNS [30]. Indeed, mTORC1 activity is enhanced in skeletal muscle of R6/2 HD mice, suggesting tissue-specific and opposite effects of polyQ-Htt on mTOR activity. Differential effects of polyQ-protein expression between affected tissues have now also been reported for SBMA [2], suggesting that tissue-specific polyQ protein interactions can indeed result in opposing physiological effects. Furthermore, such aberrant protein interactions between polyQ protein Ataxin-1 and its interactors are also thought to underlie pathogenesis in SCA1 [3], highlighting the importance of native polyQ protein function in polyQ disease. What is clear is that the relationship between qualitycontrol autophagy and mTOR activity in the context of HD is highly complex, and that much care should be taken before systemic therapies of mTOR inhibition are developed for this disease.

Cleavage of Htt by calpains and caspases, releasing an N-terminal fragment containing the polyglutamine tract, is a key event in the pathogenesis of HD. Evidence suggests that overexpression of Htt also leads to activation of key lysosomal hydrolases cathepsin D and cathepsin L [31]. Indeed, affected areas in HD brains are enriched in cathepsin D staining

and activity [17, 32]. Interestingly, polyQ-Htt appears more resistant to cathepsin proteolysis [33], suggesting that this compensatory mechanism is unable to aid HD neurons in coping with polyQ-Htt proteotoxic stress, perhaps in part due to dysfunctional autolysosomal degradative capacity. However, the potential to correct such deficits and thus facilitate cathepsin degradation of polyQ-Htt as a therapeutic target in HD deserves further exploration.

HD, like all polyQ disorders, is an inherited disease transmitted in an autosomal dominant manner. However, environmental and genetic factors outside of Htt repeat size can alter onset, progression, and severity of disease. A proposed genetic modifier of HD onset is peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC-1 α), a master transcriptional regulator of mitochondrial biogenesis and metabolism. PGC-1 α transcription interference contributes to the mitochondrial dysfunction and metabolic abnormalities in HD [34, 35]. Furthermore, recent evidence suggests a co-induction regulatory loop between PGC1 α and transcription factor E-B (TFEB), a master regulator of the autophagy-lysosome pathway [36, 37]. In agreement with a key role of the PGC1 α -TFEB axis in HD pathogenesis, impaired TFEB expression and activity was reported in HD transgenic mice [37]. Additionally, TFEB also exerts a global transcriptional control on lipid catabolism through its interaction with PGC-1 α [36]. One of the key clinical features of HD patients is bioenergetics dysregulation, which results in significant weight loss and muscle atrophy. This raises the possibility that polyQ-Htt-PGC1 α transcription interference on TFEB activity could also account for such observed metabolic manifestations of HD, but the basis for this phenomenon remains unresolved.

Atg7, a key protein in autophagy initiation, has also been proposed as another autophagy-related genetic modifier of HD. A valine-to-alanine substitution at residue 471 in Atg7 was associated with earlier age of HD onset [38], but the mechanism for this effect is unclear. However, two independent regulators of autophagy as genetic factors that accelerate HD age of onset strongly suggest that autophagy is indeed neuroprotective in HD, and position autophagy as an important target for therapy development in this disorder.

Spinal and Bulbar Muscular Atrophy

Spinal and bulbar muscular atrophy (SBMA), also known as Kennedy's disease, is an X-linked inherited neuromuscular disorder characterized by lower motor neuron degeneration leading to weakness and atrophy of bulbar, facial, and limb muscles. SBMA patients display signs of androgen insensitivity, with full disease penetrance restricted to adult males [18]. The causative mutation in SBMA is a CAG trinucleotide repeat expansion in the first exon of the androgen receptor (AR) gene [39]. AR is a transcription factor that controls the expression of androgen-responsive genes upon ligand-mediated nuclear translocation [40], and disease pathogenesis is likely to involve two distinct pathways: gain-of-function toxicity due to production of misfolded polyQ-AR protein and loss or alteration of AR normal function. Transcriptional dysregulation thus represents an important finding in models of SBMA [2, 41–43]. Indeed, decreased expression levels of important growth factors required for neuron survival, as well as sequestration of transcriptional co-activators into nuclear inclusions have been reported in patient tissues and animal models of SBMA [44].

Similar to polyQ-Htt, over-expression of polyQ-AR leads to the accumulation of LC3 puncta [45] and the formation of electron dense AVs [46]. In a fly model of SBMA, expressing polyQ-AR in the eye leads to a classic degenerative phenotype, accompanied with AV and multivesicular-body accumulation [47]. Additionally, motor neurons of a transgenic mouse model expressing 100 CAG repeats (AR100) have increased numbers of AVs at postsymptomatic stages [2], suggesting alterations in the autophagy pathway are indeed features of SBMA.

Recent evidence by our lab suggests that transcriptional inhibition of autophagy signaling, rather than activation of autophagy, underlies the observed accumulation of AVs in SBMA [2]. By undertaking an exhaustive analysis of stable cell lines, transgenic mice and patient-derived iPSCs neuronal progenitor cells (NPCs), we uncovered a profound transcriptional inhibition of TFEB signaling in models of SBMA. We determined that while SBMA cells and motor neurons were competent for autophagy initiation and autophagosome formation, they failed to successfully complete autophagic degradation (TABLE 1). Dynamic measurements of autophagy markers revealed impairments in autophagic flux in SBMA cells, which correlated with marked deficits in TFEB target gene expression [2]. We identified a novel interaction between TFEB and polyQ-AR, suggesting that TFEB dysregulation might account for autophagic flux impairments present in SBMA models.

Importantly, we restored autophagy flux by over-expressing TFEB in patient-derived NPCs [2], as a proof-of-concept that modulation of TFEB activity could be an important target for therapy development for SBMA and other disorders characterized by inhibition of autophagic flux. Indeed, treatment of SBMA transgenic mice expressing 97Q (AR97Q) with paneoflorin, a plant extract, partly exerted therapeutic effects on behavioral and pathological phenotypes by strongly upregulating TFEB expression [48].

Importantly, we also found evidence of an interaction between normal Q-length AR and TFEB, and we detected enhanced TFEB signaling and increased autophagy pathway activity when normal AR is overexpressed. [2]. Our data suggest that AR can normally interact with TFEB to promote its function, functionally and spatially regulating TFEB activity in response to testosterone. Thus, we have identified a previously unknown AR function on autophagy through interaction with TFEB, and documented a change-of-function upon expansion of the polyQ tract in AR, resulting in TFEB inhibition [23]. As AR normally interacts with many transcription co-regulators and studies of a SBMA fly model indicate that polyQ-expanded AR may promote neurotoxicity by reducing the function of the coregulators with which it interacts [49], reduced availability of a co-activator protein shared by AR and TFEB may result in decreased TFEB transactivation function in SBMA.

Similar to HD, evidence suggests that autophagy is also playing a neuroprotective role in SBMA. Genetic ablation of autophagy in *Drosophila* exacerbates polyQ-AR eye degeneration phenotypes [47] and depletion of p62 in AR97Q transgenic mice significantly worsens motor and neurological phenotypes [50]. Conversely, pharmacological activation of autophagy through Rapamycin treatment suppresses polyQ-AR eye degeneration, and this effect is dependent on functional autophagy and HDAC6 [47]. Treatment of AR97Q mice with 17-allylamino-17-demethoxygeldanamycin (17-AAG) also markedly ameliorated

motor impairments by reducing amounts of monomeric and aggregated mutant AR [51]. 17-AAG is a potent Hsp90 inhibitor, and experiments have shown that it can also potentially enhance autophagic degradation of polyQ-AR [52], although the dependence on autophagy function for SBMA disease rescue in vivo remains unknown.

Like other aggregate prone proteins, cytosolic polyQ-AR can be a substrate of autophagy [53]. Although the nucleus has emerged as the principal site of polyQ disease pathogenesis, degradation of cytoplasmic polyQ-AR oligomers would reduce nuclear polyQ-AR available for aggregation and toxicity. In agreement with this, transgenic mice expressing polyQ-AR with a deleted nuclear localization signal (AR dNLS112Q), which results in cytoplasmic retention of AR, show substantially improved motor phenotypes compared to full-length polyQ-AR models [53]. Autophagy induction was detected in AR dNLS112Q cultured motor neurons, and inhibition of autophagy augmented testosterone-mediated toxicity in this model [53], further suggesting that targeting of cytosolic polyQ-AR for autophagy degradation could have important beneficial effects in SBMA. Indeed, 17-AAG inhibition of Hsp90 also prevents conformational changes in AR necessary to bind androgens, preventing its nuclear translocation and thus facilitating autophagic clearance of polyQ-AR [52]. Furthermore, depletion of p62 induces the accumulation of insoluble, aggregated AR in AR97Q mice, strongly suggesting a population of polyQ-AR is indeed degraded through autophagy in vivo [50]. Similar to data reported for Htt [20], p62 was also shown to interact with both normal and polyQ-AR [50]. Although autophagy cargo recognition was not analyzed in this particular study, it is interesting to postulate a common mechanism of polyQ disease proteins affecting p62 function. Intriguingly, in vivo overexpression of p62 resulted in amelioration of behavioral and motor phenotypes in AR97Q mice, enhancing the formation of neutral polyQ-AR inclusion bodies [50]. Further studies addressing the role of p62 in polyQ disorders are needed to clarify the basis for these effects.

SBMA is a neuromuscular disorder, characterized by adult onset proximal muscle weakness due to lower motor neuron degeneration. Recently, our lab has also demonstrated that skeletal muscle plays a primary role in SBMA pathogenesis, superseding motor neurons as the key site of polyQ-AR toxicity [54, 55]. Interestingly, while TFEB activity in SBMA motor neurons and patient-derived NPCs was significantly reduced, analysis of quadriceps muscle samples from symptomatic 14 month-old AR100 transgenic mice yielded an opposite and dramatic up-regulation of TFEB target genes [2], consistent with studies in SBMA knock-in AR113Q mice [56]. This suggests a muscle-specific process of supraphysiological induction of TFEB in diseased SBMA muscle cells. Since uncontrolled autophagy is thought to underlie muscle wasting in models of muscular dystrophy [57], excessive activation of autophagy could also be responsible for SBMA skeletal muscle phenotypes. In agreement with this hypothesis, global reduction of autophagic activity by Beclin-1 haploinsufficiency in SBMA knock-in AR113Q mice increased skeletal muscle fiber size and significantly extended lifespan in this model [58].

The mechanisms responsible for the different responses in TFEB dysregulation by polyQAR expression between tissue types remain unknown, but strongly suggest that systemic delivery of autophagy therapies could actually have deleterious effects in SBMA. Understanding the cross-talk between SBMA skeletal muscle and motor neurons, and

identifying key players that regulate TFEB activity in a tissue-specific manner will be essential for SBMA research. Importantly, however, the non-cell autonomous nature of motor neuron toxicity in SBMA and the accessibility of skeletal muscle will facilitate therapeutic delivery of drugs to the affected neuronal populations.

Spinocerebellar ataxia type 3

Spinocerebellar ataxia type 3 (SCA3, also known as Machado–Joseph disease or MJD), is among the most common of the autosomal dominant spinocerebellar ataxias worldwide [1]. It is a devastating neurodegenerative disorder resulting from the expansion of a polyQ repeat region of the ataxin-3 (ATXN3) protein. The main clinical features of SCA3 are progressive ataxia, peripheral amyotrophy, muscle atrophy, parkinsonian features, dystonia, and spasticity. Although the exact function of ATXN3 remains unknown, it appears to be involved in the ubiquitin signaling system [59], although it has also been implicated in the regulation of histone acetylation [1]. The expansion of the polyQ tract in ATXN3 does not alter its ubiquitin protease activity [59], but as for other polyQ disorders, polyQ-ATXN3 toxicity seems to stem predominantly from gain-of-function effects.

Like other polyQ disease proteins, ATXN3 is also an autophagy substrate [9, 60]. Indeed, autophagy seems to primarily clear soluble polyQ-ATXN3, rather than the large aggregates [61]. Moreover, genetic ablation of autophagy in a *Drosophila* eye degeneration model increased retinal toxicity of polyQ-ATXN3, suggesting autophagy is neuroprotective in SCA3 [62]. Interestingly, genetic modifiers of polyQ-ATXN3 toxicity identified in this eye degeneration model, including several members of the heat-shock protein (Hsp) chaperone family, were found to be dependent on intact autophagy activity [62].

Analysis of SCA3 patients' putamen, one affected brain region in SCA3, revealed abnormal expression of endogenous autophagy markers p62 and Atg16L, as well as punctate immunostaining patterns for LC3 [63]. These results were confirmed in a transgenic mouse model of SCA3 expressing ATXN3 with 71Q. Additionally, in vivo lentiviral transduction of polyQ-ATXN3 in rat brain, a conditional model of SCA3, led to an accumulation of autophagosomes at late stages of disease progression. Analysis of Beclin-1 protein levels in striatum of SCA3 transgenic mice and MJD patient-derived fibroblasts found significant decreases in transgenic SCA3 mice compared to non-transgenic controls [63]. Similar to what has been reported in HD [14], Beclin-1 co-localizes with polyQ-ATXN3 inclusions [63], suggesting that sequestration of key autophagy proteins is an early feature of SCA3 pathology, and could also underlie autophagy dysfunction in this disorder (TABLE 1).

Importantly, overexpression of Beclin-1 in both SCA3 transgenic mice and lentiviral-ataxin3 models results in enhanced polyQ-ATXN3 clearance, improved neuronal marker staining and reduced pro-inflammatory signaling [63]. Indeed, these biochemical effects of Beclin-1 overexpression translated into improved motor phenotypes in SCA3 transgenic mice, even when administered well after onset, at a stage of pathology resembling a late, severe cerebellar ataxia [64]. SCA3 models thus appear amenable to autophagy induction therapy, resulting in improvements in disease pathology when autophagy is upregulated [64]. In agreement with this, systemic treatment of a transgenic mouse model of SCA3 expressing

ATXN3 carrying 70Q with Rapamycin analog Temsirolimus rescued motor performance and decreased aggregate number in the brain [61]. This effect correlated with inhibition of mTOR signaling and increased autophagy levels in CNS [61], similar to what has been reported for HD [13]. Activation of autophagy through co-injection of autophagy regulator microRNA let-7 into the lentiviral polyQ-ATXN3 rat model of SCA3 also reduced ATXN3 aggregates in the brain [65], suggesting that polyQ-ATXN3 can indeed be cleared through autophagy activation in the CNS.

Treatment of a novel ATXN3-135Q transgenic mouse model with 17-DMAG (another Hsp90 inhibitor) dramatically reduced polyQ-ATXN3 aggregates in the brainstem, concomitant with an increase in Beclin-1 and LC3-II protein levels [66]. Although treatment resulted in improved motor performance, similar to what was reported for SBMA mice [51, 67], the beneficial effects of 17-DMAG treatment were not maintained throughout SCA3 disease progression, suggesting either a higher dose of treatment is required or refractory mechanisms in affected SCA3 cell populations are impeding long-term effects. Similarly, while lithium chloride treatment of a *Drosophila* model of SCA3 rescued eye degeneration, improved motor phenotypes, and extended lifespan of SCA3 transgenic flies [68], no such benefits were noted when treatment was tested in SCA3 transgenic mice [69], despite positive induction of autophagy in both systems. Thus, it is clear that more research into the mechanisms of polyQ-ATXN3 pathogenesis, and a better understanding of the potential disruption in the autophagy pathway in SCA3 are required before successful long-term autophagy therapies can be developed.

Spinocerebellar ataxia type 7

Spinocerebellar ataxia type 7 (SCA7) is a dominantly inherited disorder characterized by cerebellum, brainstem, and retinal degeneration. SCA7 is caused by a polyQ repeat expansion in the ataxin-7 gene. The ataxin-7 (ATXN7) protein is ubiquitously expressed, and is a component of the SPT-TAF-ADA-GCN5 acetyltransferase (STAGA) transcriptional co-activator complex that remodels chromatin and possesses histone acetyltransferase activity.

Until recently, very few studies had addressed the role of autophagy dysregulation in SCA7. However, common findings with other polyQ disorders strongly suggested that autophagy could also be a target of polyQ-ATXN7 toxicity. Nuclear inclusions in SCA7 patient brains are positive for proteasome subunits, protein chaperones and ubiquitin [70]. Importantly, overexpression of polyQ-ATXN7 in cells leads to an increased number of AVs, [70, 71], and there are increases in LC3-II in the cerebellum of SCA7 transgenic mice [71].

Like other polyQ disease proteins, proteolytic processing of ataxin-7 by caspases generates a toxic amino-terminal fragment, containing the polyQ expansion tract, and this N-terminal polyQ-ATXN7 peptide is enriched in nuclear inclusions that accumulate over time. N-terminal fragments of polyQ proteins have long been reported to be bona fide autophagy substrates [72], and indeed macroautophagy preferentially degrades proteolytically cleaved ATXN7 fragments [73]. Similar to what has been reported for Htt, the selectivity of autophagy for polyQ vs. normal fragment degradation is mediated by post-translational

modification. In particular, deacetylation of lysine 257 in ATXN7 facilitates autophagic degradation of polyQ-ATXN7 [71]. Interestingly, this is in direct opposition to the effect of this post-translational modification on polyQ-Htt, wherein acetylation of the polyQ-Htt fragment targets it for autophagy-mediated turnover [74]. This suggests a complicated relationship between post-translational modification and polyQ disease protein clearance. Further studies will be necessary to identify additional regulatory post-translational modifications and analyze their effects on the stability and degradation of these mutant proteins.

More recently, Alves et al. demonstrated alterations in autophagy in models of SCA7. Using a knock-in SCA7 mouse model expressing 266 glutamines, they documented accumulation of the autophagy-related proteins mTOR, Beclin-1, and p62 in polyQ-ATXN7 inclusions [75], consistent with observations from several other polyQ disorders [13, 15]. Additionally, analysis of the cerebellum of SCA7 transgenic mice revealed increased number of AVs, elevated levels of LC3, and numerous lysosomal markers associated with autophagic degradation (TABLE 1). These increases in LAMP-1 and LC3 were also found in an affected patient cerebellum, but were not detected in the striatum, an area generally spared in SCA7 pathology [75]. Further work documented decreased expression levels of the key autophagy initiation proteins Atg7, the Atg12-Atg5 complex, and Atg16L2 in the cerebellum of SCA7 knock-in mice. This finding, along with accumulations of ubiquitin and p62-positive aggregates, suggests a likely impairment in autophagic flux [75]. This situation is reminiscent of HD, where polyQ-Htt interaction with p62 is thought to impair cargo recognition and autophagic flux [15]. Further studies are needed to confirm impaired autophagy function in SCA7, and if noted, at which step of the autophagy pathway this inhibition occurs. However, these results further indicate that inhibition of autophagy pathway flux is a pervasive theme in the polyQ disease field.

Not surprisingly, a second mechanism of autophagy dysfunction could be at play in SCA7. p53, a major intracellular regulator of autophagy and apoptosis, has been shown to both induce autophagy via nuclear transcription activation of the mTOR signaling pathway, and to inhibit autophagy by directly interacting with cytosolic FIP200, a member of the ULK1-FIP200-ATF13-ATG101 autophagy initiator complex [6]. p53 is recruited to polyQ-ATXN7 inclusions [70], and recent evidence indicates that mutant polyQ-ATXN7 expression results in an increased interaction between p53 and FIP200, co-aggregating with mutant ATXN7 [76]. This aberrant interaction yields decreased soluble FIP200 levels and subsequent destabilization of ULK1, directly impacting autophagy initiation [76]. Treatment with a polyQ-ATXN7 aggregation blocker rescued soluble levels of FIP200 and ULK1, and restored autophagy activity [76]. Interestingly, neuronal specific deletion of FIP200 in mice results in progressive loss of cerebellar granule and Purkinje cells, as well as ataxia – a constellation of phenotypes that overlap with SCA7 [77]. Taken together, these findings suggest that p53-mediated disruption of autophagy via FIP200 inhibition may contribute to the selective vulnerability of Purkinje cell and other cerebellar neurons in SCA7 (TABLE 1).

Other spinocerebellar ataxias

Our knowledge of the role of autophagy in other spinocerebellar ataxias is still incomplete. Nonetheless, a number of reports provide evidence for autophagy dysfunction in other polyQ SCAs. Among these are SCA1, a late-onset neurodegenerative disease characterized by pathology of the cerebellum, brainstem and spinocerebellar tracts, that is due to a polyQ repeat expansion in the ataxin-1 (ATXN1) protein. ATXN1 is both nuclear and cytosolic, but has been found to accumulate in the nuclei of Purkinje cell neurons in SCA1 transgenic mice. Importantly, cytoplasmic polyQ-ATXN1 can be degraded by autophagy [72]. SCA1 transgenic mice display cytoplasmic vacuoles in affected Purkinje cells, and present with increased brain LC3-II with no changes in p62 levels (TABLE 1), suggesting possible autophagic flux impairment [78].

SCA6 is characterized by a loss of motor coordination and balance, with selective degeneration of Purkinje cell neurons. The polyQ expansion mutation occurs in the cytoplasmic tail domain of the Cav2.1 channel, a pore-forming subunit of the P/Q type voltage-gated calcium channels [79]. Interestingly, whereas polyQ-inclusions in other polyQ disorders are heavily ubiquitinated, mutant Cav2.1 channels form inclusions in the cytoplasm of SCA6 Purkinje cells that typically lack ubiquitin immunoreactivity [79]. Recently, a novel knock-in mouse model of SCA6 was generated by introducing a splice site mutation in the CACNA1A locus that favors splicing of the polyQ-expanded isoform (MPI 118Q/118Q) of Cav2.1 [80]. These mice display motor impairments and gait ataxia, and they suffer an age-dependent specific loss of Purkinje cells [80]. As previously reported for SCA6 patient samples, MPI 118Q/118Q mice form inclusions, which are negative for ubiquitin, but were heavily stained for lysosomal markers cathepsin B and LAMP2 (TABLE 1). Ultrastructural immunogold analysis revealed that cytoplasmic nuclear inclusions of mutant Cav2.1 channels are also associated with LAMP1, and this finding was confirmed in SCA6 patient cerebellar tissue [80]. Interestingly, MPI 118Q/118Q mice had no detectable autophagic changes, suggesting that while there is lysosomal involvement in SCA6 aggregate turnover, the pathway may be independent of canonical macroautophagy. Although most SCA6 work has focused on the effect of the polyQ tract expansion on the Cav2.1 alpha subunit, recent work indicates that this gene also encodes a bona fide transcription factor [81]; thus, future studies need to consider if transcription dysregulation in SCA6 could alter autophagy pathway function, as has been shown for many of the other polyQ disease proteins.

SCA17 involves the expression of polyQ-expanded TATA-binding protein (TBP), a general transcription initiation factor. TBP interacts with other protein factors, including high mobility group box 1 (HMGB1), to regulate gene expression. HMGB1 was found to be sequestered into polyQ-TBP aggregates in vitro, leading to impaired starvation-induced autophagy [82]. This is consistent with previously reported reductions in soluble HMGB1 in polyQ-Htt and polyQ-ATXN1 expressing neurons [83]. HMGB1 is an important autophagy regulator [84], raising the prospect of a general mechanism for autophagy dysfunction in all polyQ diseases via HMGB1 inhibition. In support of this hypothesis, HMGB1 overexpression rescued polyQ disease pathology in HD primary neurons and a *Drosophila*

HD model [83], and HMGB1 reduced TBP aggregation in SCA17 cell culture models [82], highlighting HMGB1 modulation as a plausible therapy target in polyQ disease.

Dentatorubral pallidoluysian atrophy

DRPLA is a rare autosomal dominant neurodegenerative disorder caused by an expansion of a polyQ tract in Atrophin-1. Clinical manifestations include ataxia, choreoathetosis, and dementia, and age-of-onset ranges from infancy to late adulthood. The most pathologically affected tissues in DRPLA are the brainstem and the cerebellum, and there is also characteristic, generalized atrophy of the brain and spinal cord. Although its exact function is unknown, atrophin-1 has been shown to act as a transcriptional co-regulator.

Drosophila eye models of DRPLA have revealed accumulation of AVs in degenerating retinas and glial cells, where truncated forms of Atrophin-1-75Q are being expressed [85]. Increased numbers of AVs in affected photoreceptor cells were attributed to decreased lysosomal degradative function (TABLE 1), since they were accompanied by intra-luminal lysosomal lipofuscin and cytosolic p62 puncta accumulation [85]. Thus, autophagy dysfunction in DRPLA flies appears to be much more reminiscent of abnormalities in lysosomal storage disorders, where inhibition of lysosomal digestion underlies autophagy impairment [86]. Indeed, polyQ-Atrophin-1 expression can directly block lysosomal degradation, but not lysosomal acidification or fusion of lysosomes with autophagosomes [85]. Although the mechanism by which Atrophin-1 impinges lysosomal clearance is unknown, one possible scenario is that the autophagy pathway stalls due to the accumulation of partially digested autophagosomes. Indeed, unlike what has been reported for other models of polyQ toxicity, genetic or pharmacological induction of autophagy actually increases neurotoxicity in DRPLA flies, with enhanced formation of giant autolysosomes with intact, undigested contents [85]. If these results are further validated in mammalian DRPLA models or DRPLA patients, then therapeutic strategies to boost autophagy pathway function may be contraindicated in this particular polyQ disorder.

CONCLUDING REMARKS

The importance of neuronal autophagy and its dysregulation in neurodegenerative disease is undeniable. Nonetheless, the precise events leading to autophagy dysfunction, and the key steps affected in each disorder remain unclear. Polyglutamine repeat diseases comprise a family of neurodegenerative diseases caused by the expansion of encoded CAG repeat tracts at distinct gene loci, and are all characterized by the predominant accumulation of protein aggregates in neurons and non-neural CNS cells. Impairment of autophagy has been repeatedly observed in HD, and recent discoveries based upon work done on models of SBMA, SCA3, and SCA7 indicate that the autophagy pathway is a common target in polyQ disease pathogenesis and progression.

Autophagy plays an essential role in the adaptive response of cells to stress and is crucial for the maintenance of protein homeostasis. With rare exception, up-regulation of autophagy *prior to disease onset* consistently yields therapeutic benefits in models of polyQ disease by enhancing degradation of polyQ proteins. However, recent evidence also suggests that

global up-regulation of autophagy can sometimes be detrimental as a means of disease therapy. Indeed, in the context of polyQ protein toxicity, tissue-specific autophagy inhibition and autophagy over-activation can occur together in degenerating neurons in polyQ repeat disease models, yielding a scenario of excessive energy consumption in support of a failed proteostasis effort, which burdens the neuron with an enormous number of autophagosomes and autolysosomes that block axonal and cytoskeletal transport pathways. Understanding the nature of autophagy dysfunction for each polyQ disorder, and then identifying drugs that can appropriately target autophagy in a tissue-specific and stage-specific manner will be necessary, if autophagy therapy development efforts are to be successful.

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Highlights

- Autophagy dysfunction is a feature in the pathogenesis of neurodegenerative disorders
- Polyglutamine diseases are caused by the expansion of a CAG triplet in affected genes
- Singular affected steps in the autophagy pathway vary in different polyQ disorders
- Targeting autophagy for polyQ disease therapeutics should be approached with care

Fig. 1.

Table 1

Polyglutamine Disease	Causative protein	Normal Function of Protein	CAG repeat size range (in patients)	Autophagy Phenotypes
	Huntingtin	N-terminal fragment is transcription factor; scaffold for signaling; autophagy factor	~36–121	Autophagic vacuole accumulation Inhibition of autophagy signaling Defective cargo recognition
Spinobulbar Muscular Atrophy (SBMA)	Androgen Receptor	Transcription factor of androgen responsive genes	~37–70	Autophagic vacuole accumulation TFEB dysregulation, Impaired autophagy flux
Spinocerebellar ataxia type 1 (SCA1)	Ataxin-1	Transcriptional co-repressor	~39–91	Cytoplasmic vacuoles accumulation Increased levels of LC3II
Spinocerebellar ataxia type 2 (SCA2)	Ataxin-2	Involved in RNA processing and regulation of protein translation	~32–200	None reported
Spinocerebellar ataxia type 3 (SCA3)	Ataxin-3	Deubiquitinating enzyme	~52–86	Impaired autophagy inductionAutophagy flux defects
Spinocerebellar ataxia type 6 (SCA6)	CACNA1A	Voltage gated calcium channel subunit	~18–30	Lysosomal proteins in aggregates Impaired induction of autophagy
Spinocerebellar ataxia type 7 (SCA7)	Ataxin-7	Component of histone acetyltransferase complex (STAGA), regulation of protein transcription	~34–300	Autophagic vacuole accumulation Impaired p53 signaling
Spinocerebellar ataxia type 17 (SCA17)	TATA-binding protein (TBP)	Universal basal transcription factor	~43–66	Decreased autophagy activation via HMGB1 sequestration
Denatorubral-pallidoluysian atrophy (DRPLA)	Atrophin-1	Possible transcriptional co-repressor	~49–88	Impaired lysosomal degradation