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Review

Efficient Prevention of Neurodegenerative Diseases by Depletion of Starvation Response Factor Ataxin-2

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Ataxin-2 (ATXN2) homologs exist in all eukaryotic organisms and may have contributed to their origin. Apart from a role in endocytosis, they are known for global effects on mRNA repair and ribosomal translation. Cell size, protein synthesis, and fat and glycogen storage are repressed by ATXN2 via mTORC1 signaling. However, specific liver mitochondrial matrix enzymes and the mitochondrial repair factor PINK1 require ATXN2 abundance. During periods of starvation, ATXN2 is transcriptionally induced and localized to cytosolic stress granules, where nuclear factors dock to compensate RNA pathology. These physiological actions were now revealed to be crucial for human neurodegenerative diseases, given that ATXN2 depletion is surprisingly efficient in preventing motor neuron and cerebellar atrophy, as demonstrated in mouse models, flies, and yeast.

Introduction

Age-associated human diseases are a major burden for developed society. The underlying factors include environmental stressors and hereditary vulnerabilities. Whether toxic life events repeatedly overwhelm the defense capacity of an organism will depend on specific genes and their variation as well as on nutrient availability and stored energy. In particular, with the domestication of endosymbionts of bacterial origin, namely, mitochondria and chloroplasts, it must have become important for eukaryotic cells to expand their genetic machinery to compensate the deleterious consequences of chronic oxidative stress and carbohydrate/lipid depletion. The repair of fragile single-stranded RNA had to be ensured, and the recruitment of alternative fuels from storage needed to be managed. Besides, cell growth had to be repressed during periods of excessive bioenergetic demands or of nutrient starvation. The chronic dysregulation of these processes would prominently affect cells with high rates of mitochondrial respiration such as neurons, which cannot undergo cell division and will insidiously accumulate the deleterious consequences during the organism's life span. Ataxin-2 (human gene symbol *ATXN2*) is now emerging as a key factor for human neurodegenerative diseases, and it appears to act within this machinery of nutrient disposal and damage repair.

Ataxin-2 Homologs in Phylogenesis

During phylogenetic evolution, a protein with a combination of domains similar to ATXN2 was never found among prokaryotes, but it appears in every eukaryotic organism, irrespective of whether it contains mitochondria or chloroplasts. Land plants have several copies of the gene

Trends

Ataxin 2 (ATXN2) orthologs containing Lsm, PAM2, and PRD motifs are now identified from man to yeast and plants.

Global growth repression in stress periods by ATXN2 via inhibition of mTORC1 phosphorylation signals was recently shown in yeast, worms, and man.

Compensatory breakdown of lipids via ATXN2 was observed in worms, flies, and mammals.

Both effects are modulated by specific mitochondrial matrix enzymes (e.g., IVD, ACADS) under the control of ATXN2. Factors responsible for elimination of dysfunctional mitochondria and bacteria (e.g., PINK1) are also governed by ATXN2, so it may have contributed to eukaryotic origin.

Depletion of ATXN2 levels is neuroprotective in ALS and in SCA2 mouse models; this confirms previous observations in yeast and flies.

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family (CID3/CID4 in *Arabidopsis thaliana*), with each plant species having at least one copy. Yeast strains contain one copy (PBP1 in *Saccharomyces cerevisiae*), while vertebrate animals have two copies, except birds [1]. The complete gene family targets RNAs to adapt cells to rapid environmental changes [2].

The conserved protein structure of ATXN2 homologs is composed of three elements: First, it contains a PAM2 motif toward the C terminus, which mediates direct protein interaction with the cytosolic poly(A)-binding protein PABPC1, and thus the association with the 3′-untranslated region of all mRNAs during ribosomal translation in periods of cell growth, or its association with these mRNAs in stress granules in periods of stalled translation and RNA quality control [3,4]. Second, toward the N terminus it contains an Lsm (like Sm) domain and an Lsm-associated domain (LsmAD), implicated in the cytosolic refolding and maturation of a wide spectrum of RNAs and in their degradation. Apparently, this triggers a preferential interaction of Ataxin-2 with uridine-rich *cis*-regulatory elements in the 3′-untranslated region of specific mRNAs, such as the growth-associated AU-rich elements [5]. Third, it commonly contains proline-rich domains (PRD), which are known to mediate the association with the receptor endocytosis apparatus and influence the trophic state of cells [6,7]. In this way, mammalian ATXN2 interacts with GRB2 and endophilin-A, presumably influencing both the clathrin-independent fast-acting endocytosis and the dendritic spine morphogenesis of neurons [8–10].

It is interesting to note that a polyglutamine (polyQ) domain (encoded by a CAG repeat) is present only among primates and among insects [1]. The unstable expansion of this repeat in humans causes an age-associated multisystem neural atrophy with preferential affection of motor neurons, cerebellar Purkinje neurons, and basal ganglia neurons including the thalamus [11,12]. This condition was termed as ‘spinocerebellar ataxia type 2 (SCA2)’, and thus provided the name for Ataxin-2.

Influence of Ataxin-2 on mRNA Translation

Apparently, ATXN2 is needed to counteract starvation, given that it is transcriptionally induced in human and mouse cells that are deprived of glucose, amino acids, and lipid-containing serum [13]. During periods of low glucose levels, oxidative stress, overheating, or viral infection, yeast PBP1 and the other members of the ATXN2 protein family will relocalize together with its interactor poly(A)-binding protein to cytosolic stress granules, where the repair of RNAs occurs and their decay is decided [13,14]. This cellular damage compensation effort occurs in interaction with several other RNA-binding proteins that are relocalized from the nucleus to stress granules, such as TDP-43, FUS, or TIA-1, and this seems essential for the long-term health of neurons [15–21]. Gene redundancy in mammals is ensured by another homolog, which was termed Ataxin-2-like (*ATXN2L*) or Ataxin-2-related protein (A2RP), but it lacks the polyQ domain. It has a stronger association with the nucleus than ATXN2 in periods of health, also relocalizes to stress granules during arsenite or heat treatment, and is able to heterodimerize with ATXN2 [22–25].

Effects of ATXN2 on mRNA translation were first established as the cause of conspicuous phenotypes in several species. Ambivalent evidence emerged that it acts as a global translational repressor in stress periods, as would be expected from a component of stress granules, and also as a translational enhancer of specific mRNAs in growth periods, as expected in view of its association with polysomes. The absence of its ortholog ATX-2 in the nematode *Caenorhabditis elegans* leads to abnormal masculinization of the germ line via insufficient translation of specific factors, for example, the *rme-2* mRNA that is controlled by MEX-3 and GLD-1, an ortholog of the myelination factor QKI [26]. In *Drosophila melanogaster* flies, its ortholog dATX2 causes actin-dependent phenotypes together with female sterility [27]. Similarly, its suppression in flies causes an altered circadian rhythm via insufficient translation of

the clock-component PERIOD via LSM12–TYF interaction [28–30], with some circadian phenotypes also detectable in *Atxn2*-knockout mice [31], but at the same time it mediates an opposite silencing effect on NOT1 via DDX6/ME31B interaction [30]. Initial findings showed the global translation rate of mouse cells with ATXN2 deficiency to be reduced and suggested ATXN2 to act as global translation activator. However, these experiments involved a starvation period followed by maximal growth stimuli, so unresolved RNA damage may have reduced translation rates [13,32]. It is important to consider that the deletion of yeast PBP1 suppresses the lethality due to deletion of the global translation activator PAB1, the poly(A)-binding protein [33]. This indicates the importance of PBP1/ATXN2 and suggests that it acts as an inhibitor of global mRNA translation. Indeed, the global effect of ATXN2 on growth seems to be repressive, given that body size, cell size, and nutrient storage increase after ATXN2 depletion [34,35]. A role of ATXN2 as translation suppressor for specific targets was also reported, since the expression of a Ca^{2+}/calmodulin-dependent protein kinase II translational reporter was increased by the knockdown of dATX2 [36]. In worms, ATX-2 depletion impedes microtubule growth and nuclear cytokinesis, via direct protein interaction with the prion-like protein SZY-20, so centrosome size is inversely correlated with ATX-2 abundance [37,38]. These data may explain why ATXN2-depleted cells with excess nutrients increase in size instead of entering proliferation.

Other ATXN2 protein interactors also contain RNA-binding domains, and thus underscore its importance for RNA processing and brain diseases: (i) The Ataxin-2 binding protein 1 (A2BP1 or RBFOX1) is a nuclear splicing factor that modifies neuronal excitability and was implicated in autism [39–41]. (ii) The DEAD/H-box RNA helicase DDX6 as a component of stress granules and P-bodies associates with ATXN2 and with ATXN2L to modulate mRNA storage in periods of stalled translation as well as the degradation of mRNAs [3,22,42]. (iii) The FMRP protein, whose mutations are responsible for the fragile X mental retardation syndrome, associates with polysomes and interacts with ATXN2 for olfactory habituation in a process that involves the microRNA pathway and synaptic plasticity [36,43] (Figure 1).

Modulation of Growth in Stress Periods by Ataxin-2 Occurs via mTORC1 Signaling

It was now shown that ATXN2 acts as a repressor of mechanistic target of rapamycin (mTOR) signaling in mouse fibroblasts and human neural cells, thus blocking protein biosynthesis and cell growth in times of bioenergetics deficit [13]. It is still a matter of controversy how this is achieved precisely. In yeast, a transient sequestration of the mTORC1 ortholog TOR1 to stress granules in periods of excessive heat is observed, which is prevented by PBP1 deletion and driven by PBP1 overexpression. This was confirmed by a coimmunoprecipitation experiment, indicating a direct interaction between an overexpressed N-terminal PBP1 fragment and TOR1 [44]. This TOR1 sequestration occurs in response to a PBP1 phosphorylation by the PAS domain containing serine/threonine kinase PSK1, itself regulated via phosphorylation by the AMP kinase ortholog SNF1 as a response to low energy levels [45]. A slightly different scenario has been proposed in *C. elegans*, where ATX-2 also suppressed mTOR signals, but in an indirect manner via association with a component of the tuberous sclerosis complex [34]. These data from two model organisms agree with findings in mammalian cells that the functions of ATXN2 and ATXN2L are governed by phosphorylation events [46]. In addition, recent data suggest that ATXN2 affects cell growth via several downstream phosphorylation cascades, as repressors of mTOR and phosphoinositide 3-kinase signals and as enhancers of PINK1 kinase [13,47]. Thus, ATXN2 is centrally placed within phosphorylation cascades that control the trophic state of cells, particularly mRNA translation, lipid signals, and autophagy.

Many lines of evidence suggest that Ataxin-2 family members play a role in starvation-induced autophagy, in view of their interaction with growth factor receptor endocytosis and signaling

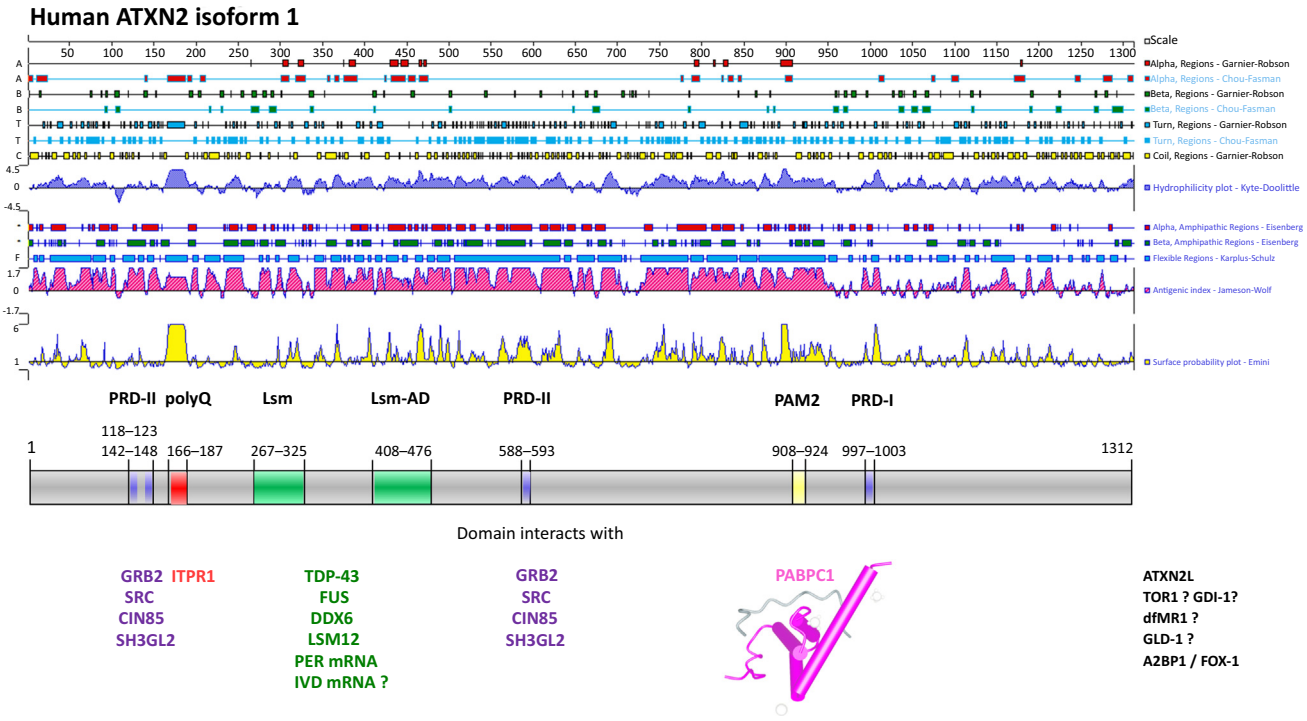


Figure 1. ATXN2 Protein Features with Interaction Targets. Direct association of ATXN2 with proteins and mRNA targets under physiological conditions at the ribosomal translation apparatus and at the endocytosis machinery differs from interactions with stress granule components in periods of starvation and oxidative stress, and during accumulation and aggregation. The polyQ domain has strong hydrophilicity and surface probability, so its expansion probably alters the conformation, with consequences of decreased solubility and aggregation of the entire protein, in parallel to aberrant interaction with ITPR1. Among the four PRDs, the N-terminal and central domains show best experimental interaction. A superstructure of alpha, beta, and coil regions is not apparent.

[6,7,48], with the AMP kinase ortholog SNF1 [45], with mTOR signaling [13], and with PINK1 [47], all of them being known coordinators of the macroautophagic pathway. These global repressive effects on growth may explain several indirect effects of ATXN2 on diverse pathways, simply as consequences of general transcription and translation rates. For example, PBP1 is a modifier of the average poly(A)-tail length of mRNAs, which is controlled by deadenylases and depends on translational activity [5,49]. Similarly, deficiency in PBP1 or ATXN2 leads to accumulation of nuclear RNA–DNA hybrids, a process that is repressible by Mg^{2+}, dependent on RNaseH1, and can be counteracted by calorie restriction [50]. RNA–DNA hybrids appear as by-products of normal transcriptional activity, so an increase in their number may simply reflect the increased size of ATXN2-depleted cells. Consequently, dATX2 enhances another PAS domain containing factor, the circadian rhythm factor PER [28,29], which triggers the general repression of metabolism and protein synthesis during nighttime (Figure 2).

Ataxin-2 Effects on Nutrients and Life Span Are Mediated by Which mRNA Targets?

In patients with SCA2, a depletion of subcutaneous fat stores is evident before the first subjective complaints and objective signs of motor deficits appear. Conversely, in mice with ATXN2 deficiency, the overall phenotypes are due to an accumulation of hepatic/gonadal/intestinal/subcutaneous fat and of glycogen in liver granules, with the consequence of obesity, dyslipidemia, insulin resistance, and infertility [48]. In *D. melanogaster*, the knockdown of dATX2 exclusively in the larval fat body leads to reduced growth and elevated death of the flies during development, presumably due to inadequate lipid breakdown [51]. Similarly, in

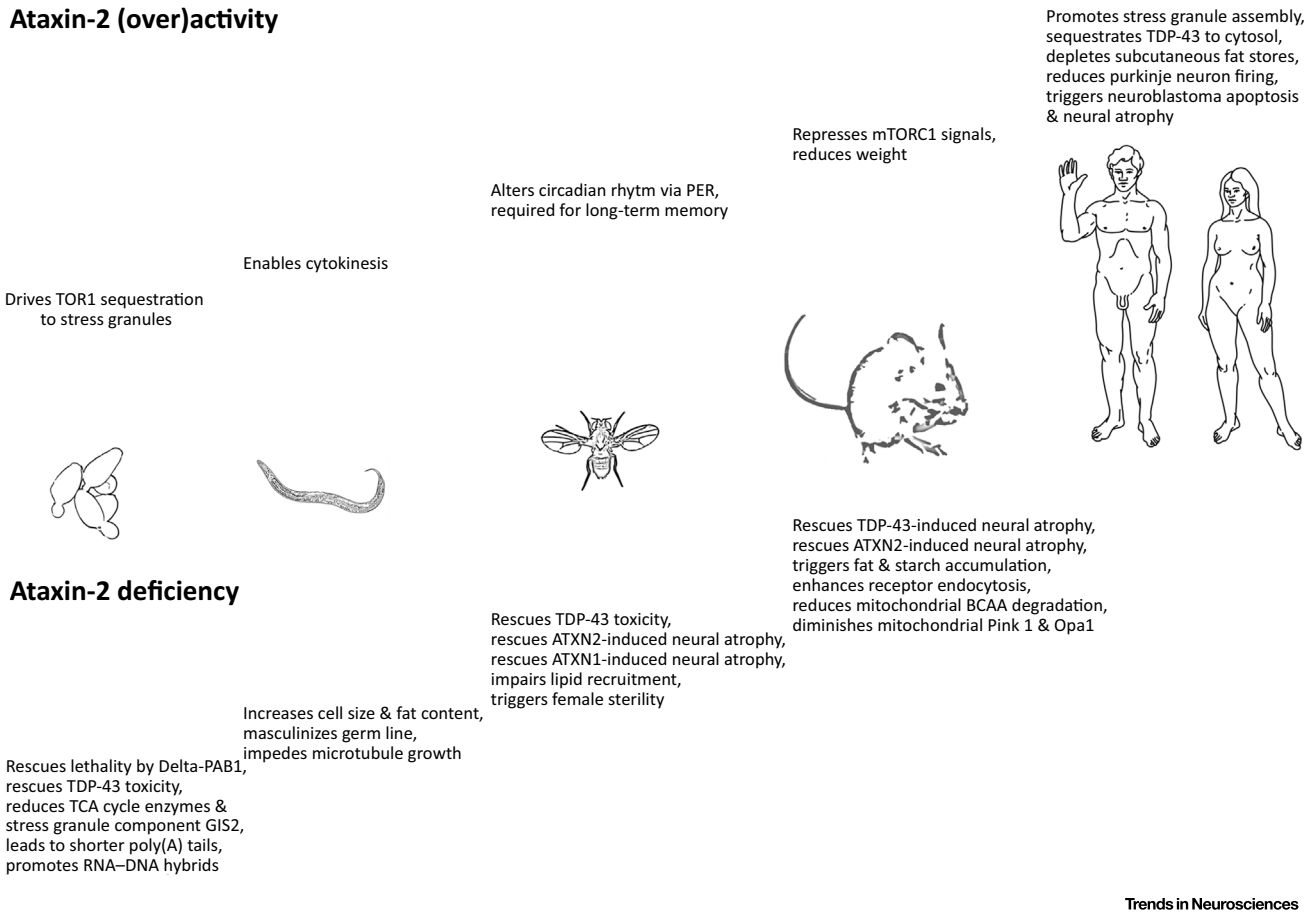


Figure 2. Evolution of ATXN2 Functions. Yeast, worm, fly, and mammalian evidence supports the roles of ATXN2 for RNA translation at ribosomes and RNA repair at stress granules, for the suppression of global protein synthesis via mTORC1, for the storage of fat/glycogen, and for fuel breakdown at mitochondria in periods of excessive bioenergetic demands. TCA, tricarboxylic acid.

C. elegans it was recently shown that fat content of dietary-restricted worms increases by ATX-2 deficiency [34]. Consistently, these results underline the key role of Ataxin-2 in the recruitment of stored reserve fuels.

The identity of some mRNAs that are controlled by ATXN2 to mediate all these effects was revealed in several publications over the past two years, and interestingly a large part of them encode mitochondrial precursor proteins or control the cytosolic breakdown of stored fuels. The investigation of the global transcriptome of mouse brain with ATXN2 deficiency revealed a marked reduction in the mRNA levels of isovaleryl dehydrogenase as a mitochondrial matrix enzyme crucial for the breakdown of the amino acid leucine, and of several factors associated with the endoplasmic reticulum [52], in parallel to an increase in the mRNA levels of several components of the translation machinery [32]. Leucine levels are a determinant of mTORC1 signaling and protein synthesis [53]. Profiling efforts of the global proteome and metabolome of mouse brain and liver with ATXN2 deficiency confirmed the substantial decrease in the levels of isovaleryl dehydrogenase (to 7% upon quantitative immunoblots in liver) and of other mitochondrial matrix enzymes that degrade short fatty acids and branched chain amino acids, such as ACADS, ALDH6A1, ALDH7A1, MCCC2, PCCA, and OTC. In addition, decreased levels were also demonstrated for components of the mitochondrial citric acid cycle [54]. Another study defined the global proteome profile of yeast with PBP1 deletion without or with

stress from NaN_3 or heat administration, again detecting significant downregulations of bioenergetics key enzymes, such as KGD2 (a component of the mitochondrial alpha-ketoglutarate dehydrogenase complex) and CIT1 (the rate-limiting enzyme of the mitochondrial citric acid cycle) in the absence of stress, together with reduced levels of the stress granule component GIS2 during heat or NaN_3 stress [55]. Interestingly, it was also shown that a reduction of several eisosome proteins, involved in fast endocytosis, occurs as a consequence of PBP1 deletion during stress [55]. Thus, in parallel to the repression of global protein synthesis during stress periods, it requires ATXN2 to maintain normal levels of specific mitochondrial precursors.

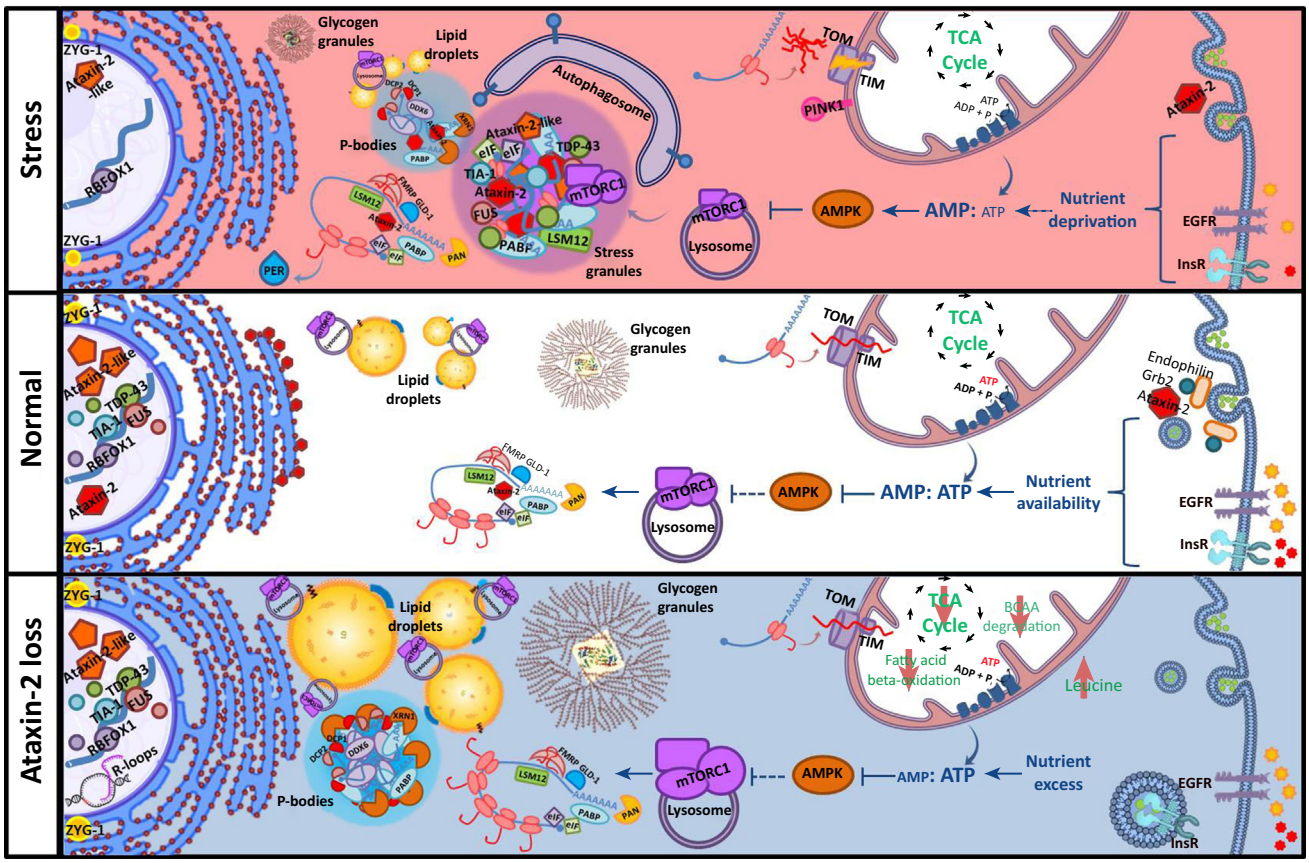
Moreover, yeast PBP1 is crucial to rescue mitochondrial dysfunction. A recent genome modifier survey studied cells with a mitochondrial dysfunction that diminishes the proton gradient and impairs the import of nuclear-encoded mitochondrial precursor proteins. The cell death of these cells was shown to occur by cytosolic overaccumulation of the precursors; this toxicity could be rescued by overexpression of (i) the stress granule components GIS2 and PBP1, (ii) components of the cytosolic ribosomal translation apparatus, (iii) modifiers of mTOR growth signals, or (iv) the RNA processing machinery [35]. Therefore, an ATXN2-mediated repression of mTOR and global protein synthesis would seem to be the plausible mechanism to prevent mitochondrial precursor overaccumulation stress. Previously, it had been shown that the viability of yeast cells lacking mitochondrial DNA depends on altered mitochondrial import and can be modified by mutations of cytosolic PBP1 [56]. The effects of ATXN2 on stressed mitochondria were also confirmed in patient blood, mouse tissue, and human neural cells, where it was shown to enhance the mRNA levels of PINK1 [47], a mitochondrial quality control factor whose absence triggers precursor import deficits [57]. ATXN2 also enhances the levels of OPA1 [47], a crucial factor for mitochondrial repair by fusion, whose mutation triggers an autosomal dominantly inherited neurodegenerative process of the optic nerve [58]. It is relevant to note that ATXN2 dysfunction leads to dysregulated calcium homeostasis in neurons; this might also be due to mitochondrial dysfunction, not only to the proposed pathology at the endoplasmic reticulum by interaction of accumulated expanded ATXN2 with ITPR1 [52,59]. Curiously, within the first 100 N-terminal amino acids of the reference sequence of human ATXN2, a mitochondrial targeting motif is predicted by some algorithms and indeed this recombinant fragment localizes to mitochondria (unpublished observation), but current research suggests the start methionine codon of ATXN2 to lie beyond this sequence [60], and indeed the subcellular localization, fractionation, and cosedimentation studies of full-length ATXN2 have never detected a mitochondrial association even under stress conditions [4,13]. Overall, ATXN2 emerges as a key cytosolic factor that modifies mitochondrial energy production and health. Given that it governs the mitochondrial usage of fuels stored in the cytosol and that it controls the repair and elimination of dysfunctional mitochondria via PINK1, the ATXN2 protein family throughout eukaryotic evolution may have contributed to the domestication of endosymbionts.

Gain-of-Function versus Loss-of-Function, Neural Atrophy versus Metabolic Excess and Cancer

The gain-of-function of ATXN2 triggered by unstable expansion of the polyQ domain with subsequent protein aggregation always results in neural atrophy, affecting different neuronal projections in dependence on severity. The normal size of the polyQ domain contains 22–23 successive glutamines, encoded by the nucleotide sequence $(CAG)_8CAA(CAG)_4CAA(CAG)_{8–9}$. Intermediate expansions of ATXN2 (Q27–33) trigger a degeneration of motor neurons, with the clinical picture of amyotrophic lateral sclerosis (ALS) and appearance of cytosolic TDP-43 inclusion bodies [18,61,62]. Depending on either the presence of CAA interruptions or the presence of modifier genes from Asian background, intermediate expansions of ATXN2 were repeatedly observed in Chinese individuals affected by Parkinson's disease with preferential

degeneration of dopaminergic midbrain neurons [63,64]. Large polyglutamine expansions in ATXN2 (≥Q34) result in the multi-system-atrophy of neural tissue, which became known as 'SCA2' and shortens life span to about 15 years from clinical manifestation to death [65,66]. Clinical and neurophysiological evidence in individuals at the prodromal stage of SCA2 suggests that demyelination is a relatively early feature in this neurodegenerative disorder [67,68].

Conversely, depletion of ATXN2 in mouse triggers obesity, dyslipidemia, and insulin resistance, in a combination of findings that is typical of the human metabolic excess syndrome [48]. It is important to note that genetic variants at the chromosomal locus of human ATXN2 are associated with abnormal metabolism of lipids and carbohydrates and also carry a risk for obesity, hypertension, and diabetes mellitus, thus acting as relevant genetic modifiers for the life span of centenarians [69,70]. Children with extreme early onset obesity were found to display an association with expansions and single-nucleotide polymorphism alleles within ATXN2 [25]. In an Egyptian family with ATXN2 polyQ expansion (several patients with Q39–52), it was noted that the affected individuals at midstages of disease exhibited increased consumption of all types of food with concomitant obesity and presented skin fat deposits in the absence of altered blood lipid profiles; at prefinal stages they manifested reduced swallowing and weight loss [71]. To interpret this time course, it is important to know that mTORC1 in the brain



Trends in Neurosciences

Figure 3. ATXN2 Effects on Diverse Subcellular Compartments. A coordinate stress response recruits nuclear factors for RNA repair and stored cytosolic fuels, triggering modifications of mRNA translation, mitochondrial function, lysosome-associated mechanistic target of rapamycin (mTOR) signals, actin-dependent vesicular trafficking, and nuclear cytokinesis, to compensate damage and to adapt cell growth. EGFR, epidermal growth factor receptor; eIF, Eukaryotic initiation factor; TCA, tricarboxylic acid.

hypothalamus acts as the central regulator of food intake and body weight [72]. The clinical course thus suggests mutant ATXN2 initially to be deficient in mTORC1 repression, while the prefinal progressive accumulation of ATXN2 aggregates leads to excessive mTORC1 repression (Figure 3).

Therapeutic Suitability of Ataxin-2 Regulation

Ataxin-2 was proposed as a general modifier gene for neurodegenerative processes, when a first compendium of modifiers on the basis of experimental evidence in the organisms *D. melanogaster*, *C. elegans*, and *S. cerevisiae* was assembled [73]. The findings so far show that ATXN2 overexpression potentiates the severity of atrophy in models of TDP-43-triggered ALS, SCA1, SCA3, and tauopathies [18,74–76], while the deficiency of ATXN2 postpones the pathology and reduces progression rate at least in the first two disorders [18,74,77]. In the case of an ALS mouse model with a life span below 30 days, it was recently revealed that the genetic ablation of ATXN2 may extend life span to over 300 days. This was accompanied by reduced confluence of stress granules, and by reduced insolubility of the nuclear RNA-binding protein TDP-43, whose abnormal deposition in cytosolic aggregates is a pathological hallmark of motor neurons in ALS. Intriguingly, a significant rescue of survival was also achieved through *ATXN2* knockdown via antisense oligonucleotides [77]. This technical approach was also successful in reducing the toxic overactivity of ATXN2 in a mouse model of SCA2 [78], providing proof-of-principle that preventive therapy for patients is now within reach. It is presently unclear to what degree this neuroprotective effect of ATXN2 depletion can also be applied to Parkinson's disease and Alzheimer's disease.

Conversely, the modulation of Ataxin-2 abundance also seems to be a potent modifier of tumor growth, since it was shown that the spontaneous regression in about 10% of childhood neuroblastoma tumors coincides with the upregulation of Ataxin-2 expression. Therefore, recombinant overexpression could be successfully applied to drive neuroblastoma cells into apoptosis [79].

It will be compelling to explore if the regulation of ATXN2 function via phosphorylation can be indeed targeted by drugs in man, which would be widely applicable in the treatment of neural atrophy, metabolic excess syndrome, and cancer.

Concluding Remarks

Depletion of ATXN2 is an unexpectedly efficient way to prevent and mitigate the atrophy of motor neurons and cerebellar Purkinje cells in animal models of ALS and SCA2, making experimental therapy of patients imminent. The physiological roles of ATXN2 in all eukaryotic organisms, which may have initially contributed to the domestication of endosymbionts, are diverse, but recent advances have defined its crucial function among stress responses. It is critical to recruit stored lipids and glycogen in periods of low glucose availability and of high energy demands, maintaining their breakdown in mitochondria. Furthermore, it promotes stress granule formation to repair damaged RNAs, while repressing ribosomal mRNA translation and restricting cell growth. It serves as a central hub within phosphorylation cascades, responding to activation signals from AMP kinase, inhibiting mTOR kinase, and promoting PINK1 kinase. Probably, it further influences the initiation of autophagy. Future investigations will focus on ATXN2 activity regulations and the redundancy with its homolog ATXN2L, to not only advance neuroprotective treatments, but also to modulate the metabolic excess syndrome and cancer (see Outstanding Questions).

Author Contribution

G.A. and N-E.S. conceived, designed, and wrote the manuscript. D.M., A-N.B., and A.D.G. analyzed the data and revised the manuscript accordingly.

Outstanding Questions

By which enzymes does ATXN2 regulate glycogen breakdown in periods of glucose depletion?

How does ATXN2 recruit lipids from subcutaneous adipocytes and from lipid droplets during stress?

What cargoes are endocytosed and which growth factor receptors are controlled by ATXN2?

Which protein domains and what specific adaptor molecules mediate the effects of ATXN2 on the actin cytoskeleton?

Do selective mRNA and microRNA targets of ATXN2 exist, beyond the general quality control?

Can we identify a specific domain within ATXN2 that is responsible for mTORC1 repression?

Given that many mammalian orthologs of yeast SNF1 and PASK exist, what are the key factors upstream from ATXN2 and how are they responding to Ataxin-2 mutations?

Is the role of ATXN2 in autophagy obvious?

Does ATXN2 also influence nuclear constitutents, further confirming its role in eukaryotic origin?

Will components of specific pathways, which are sequestrated into the cytoplasmic aggregates of polyQ-expanded ATXN2, provide clues to preventive therapy of SCA2 and ALS?

Are the neuroprotective effects of Ataxin-2 deficiency selective for motor neurons and cerebellar Purkinje neurons, or does ATXN2 also influence pathology in dopaminergic midbrain neurons and in neurons of the cerebral cortex, which are primarily responsible for Parkinson's disease and Alzheimer's dementia?

Do circadian rhythms modulate ATXN2 production?

The role of ATXN2 in obesity and insulin resistance – is it a relevant therapeutic target?

Disclaimer Statement

The authors declare no conflict of interest.

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References

1. Jimenez-Lopez, D. and Guzman, P. (2014) Insights into the evolution and domain structure of Ataxin-2 proteins across eukaryotes. *BMC Res. Notes* 7, 453
2. Jimenez-Lopez, D. *et al.* (2015) Evolutionary history exposes radical diversification among classes of interaction partners of the MLLE domain of plant poly(A)-binding proteins. *BMC Evol. Biol.* 15, 195
3. Nonhoff, U. *et al.* (2007) Ataxin-2 interacts with the DEAD/H-box RNA helicase DDX6 and interferes with P-bodies and stress granules. *Mol. Biol. Cell* 18, 1385–1396
4. van de Loo, S. *et al.* (2009) Ataxin-2 associates with rough endoplasmic reticulum. *Exp. Neurol.* 215, 110–118
5. Yokoshi, M. *et al.* (2014) Direct binding of Ataxin-2 to distinct elements in 3′ UTRs promotes mRNA stability and protein expression. *Mol. Cell* 55, 186–198
6. Nonis, D. *et al.* (2008) Ataxin-2 associates with the endocytosis complex and affects EGF receptor trafficking. *Cell. Signal.* 20, 1725–1739
7. Drost, J. *et al.* (2013) Ataxin-2 modulates the levels of Grb2 and SRC but not ras signaling. *J. Mol. Neurosci.* 51, 68–81
8. Boucrot, E. *et al.* (2015) Endophilin marks and controls a clathrin-independent endocytic pathway. *Nature* 517, 460–465
9. Renard, H.F. *et al.* (2015) Endophilin-A2 functions in membrane scission in clathrin-independent endocytosis. *Nature* 517, 493–496
10. Yang, Y. *et al.* (2015) Endophilin A1 regulates dendritic spine morphogenesis and stability through interaction with p140Cap. *Cell Res.* 25, 496–516
11. Rub, U. *et al.* (2003) Thalamic involvement in a spinocerebellar ataxia type 2 (SCA2) and a spinocerebellar ataxia type 3 (SCA3) patient, and its clinical relevance. *Brain* 126, 2257–2272
12. Gierga, K. *et al.* (2005) Involvement of the cranial nerves and their nuclei in spinocerebellar ataxia type 2 (SCA2). *Acta Neuropathol.* 109, 617–631
13. Lastres-Becker, I. *et al.* (2016) Mammalian ataxin-2 modulates translation control at the pre-initiation complex via PI3K/mTOR and is induced by starvation. *Biochim. Biophys. Acta* 1862, 1558–1569
14. Swisher, K.D. and Parker, R. (2010) Localization to, and effects of Pbp1, Pbp4, Lsm12, Dhh1, and Pab1 on stress granules in *Saccharomyces cerevisiae*. *PLoS One* 5, e10006
15. Farg, M.A. *et al.* (2013) Ataxin-2 interacts with FUS and intermediate-length polyglutamine expansions enhance FUS-related pathology in amyotrophic lateral sclerosis. *Hum. Mol. Genet.* 22, 717–728
16. Kim, H.J. *et al.* (2014) Therapeutic modulation of eIF2alpha phosphorylation rescues TDP-43 toxicity in amyotrophic lateral sclerosis disease models. *Nat. Genet.* 46, 152–160
17. Figley, M.D. *et al.* (2014) Profilin 1 associates with stress granules and ALS-linked mutations alter stress granule dynamics. *J. Neurosci.* 34, 8083–8097
18. Elden, A.C. *et al.* (2010) Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. *Nature* 466, 1069–1075
19. Heck, M.V. *et al.* (2014) Dysregulated expression of lipid storage and membrane dynamics factors in Tia1 knockout mouse nervous tissue. *Neurogenetics* 15, 135–144
20. Damrath, E. *et al.* (2012) ATXN2-CAG42 sequesters PABPC1 into insolubility and induces FBXW8 in cerebellum of old ataxic knock-in mice. *PLoS Genet.* 8, e1002920
21. Li, Y.R. *et al.* (2013) Stress granules as crucibles of ALS pathogenesis. *J. Cell Biol.* 201, 361–372
22. Kaehler, C. *et al.* (2012) Ataxin-2-like is a regulator of stress granules and processing bodies. *PLoS One* 7, e50134
23. Kaehler, C. *et al.* (2015) PRMT1-mediated arginine methylation controls ATXN2L localization. *Exp. Cell Res.* 334, 114–125
24. Shibata, H. *et al.* (2000) A novel protein with RNA-binding motifs interacts with ataxin-2. *Hum. Mol. Genet.* 9, 1303–1313
25. Figueroa, K.P. and Pulst, S.M. (2003) Identification and expression of the gene for human ataxin-2-related protein on chromosome 16. *Exp. Neurol.* 184, 669–678
26. Ciosk, R. *et al.* (2004) ATX-2, the *C. elegans* ortholog of ataxin 2, functions in translational regulation in the germline. *Development* 131, 4831–4841
27. Satterfield, T.F. *et al.* (2002) A *Drosophila* homolog of the polyglutamine disease gene SCA2 is a dosage-sensitive regulator of actin filament formation. *Genetics* 162, 1687–1702
28. Zhang, Y. *et al.* (2013) A role for *Drosophila* ATX2 in activation of PER translation and circadian behavior. *Science* 340, 879–882
29. Lim, C. and Allada, R. (2013) ATAXIN-2 activates PERIOD translation to sustain circadian rhythms in *Drosophila*. *Science* 340, 875–879
30. Lee, J. *et al.* (2017) LSM12 and ME31B/DDX6 define distinct modes of posttranscriptional regulation by ATAXIN-2 protein complex in *Drosophila* circadian pacemaker neurons. *Mol. Cell.* 66, 129–140 e7
31. Pfeffer, M. *et al.* (2017) Impact of Ataxin-2 knock out on circadian locomotor behavior and PER immunoreaction in the SCN of mice. *Chronobiol. Int.* 34, 129–137
32. Fittschen, M. *et al.* (2015) Genetic ablation of ataxin-2 increases several global translation factors in their transcript abundance but decreases translation rate. *Neurogenetics* 16, 181–192
33. Mangus, D.A. *et al.* (1998) Pbp1p, a factor interacting with *Saccharomyces cerevisiae* poly(A)-binding protein, regulates polyadenylation. *Mol. Cell. Biol.* 18, 7383–7396
34. Bar, D.Z. *et al.* (2016) Cell size and fat content of dietary-restricted *Caenorhabditis elegans* are regulated by ATX-2, an mTOR repressor. *Proc. Natl. Acad. Sci. U. S. A.* 113, E4620–E4629
35. Wang, X. and Chen, X.J. (2015) A cytosolic network suppressing mitochondria-mediated proteostatic stress and cell death. *Nature* 524, 481–484
36. Sudhakaran, I.P. *et al.* (2014) FMRP and Ataxin-2 function together in long-term olfactory habituation and neuronal translational control. *Proc. Natl. Acad. Sci. U. S. A.* 111, E99–E108
37. Stubenvoll, M.D. *et al.* (2016) ATX-2, the *C. elegans* ortholog of human Ataxin-2, regulates centrosome size and microtubule dynamics. *PLoS Genet.* 12, e1006370
38. Gnazzo, M.M. *et al.* (2016) The RNA-binding protein ATX-2 regulates cytokinesis through PAR-5 and ZEN-4. *Mol. Biol. Cell* 27, 3052–3064
39. Kiehl, T.R. *et al.* (2001) Identification and expression of a mouse ortholog of A2BP1. *Mamm. Genome* 12, 595–601

Could tumor suppression be achieved by ATXN2 gain of function in any type of cancer?

Is it possible to define phosphorylation sites that control localization, interactions, and activity of ATXN2 and that may become useful drug targets?

To what degree does ATXN2L compensate for ATXN2, and which distinct targets does it have?

Would the manipulation of ATXN2 orthologs in plants and other food-related microorganisms increase carbohydrate and lipid content, growth, cell size, fertility, and stress resistance?

40. Gehman, L.T. *et al.* (2011) The splicing regulator Rbfox1 (A2BP1) controls neuronal excitation in the mammalian brain. *Nat. Genet.* 43, 706–711
41. Voineagu, I. *et al.* (2011) Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature* 474, 380–384
42. Ayache, J. *et al.* (2015) P-body assembly requires DDX6 repression complexes rather than decay or Ataxin2/2L complexes. *Mol. Biol. Cell* 26, 2579–2595
43. McCann, C. *et al.* (2011) The Ataxin-2 protein is required for microRNA function and synapse-specific long-term olfactory habituation. *Proc. Natl. Acad. Sci. U. S. A.* 108, E655–E662
44. Takahara, T. and Maeda, T. (2012) Transient sequestration of TORC1 into stress granules during heat stress. *Mol. Cell* 47, 242–252
45. DeMille, D. *et al.* (2015) PAS kinase is activated by direct SNF1-dependent phosphorylation and mediates inhibition of TORC1 through the phosphorylation and activation of Pbp1. *Mol. Biol. Cell* 26, 569–582
46. Asada, A. *et al.* (2014) Cyclin-dependent kinase 5 phosphorylates and induces the degradation of ataxin-2. *Neurosci. Lett.* 563, 112–117
47. Sen, N.E. *et al.* (2016) Search for SCA2 blood RNA biomarkers highlights Ataxin-2 as strong modifier of the mitochondrial factor PINK1 levels. *Neurobiol. Dis.* 96, 115–126
48. Lastres-Becker, I. *et al.* (2008) Insulin receptor and lipid metabolism pathology in ataxin-2 knock-out mice. *Hum. Mol. Genet.* 17, 1465–1481
49. Kimura, Y. and Irie, K. (2013) Pbp1 is involved in Ccr4- and Khd1-mediated regulation of cell growth through association with ribosomal proteins Rpl12a and Rpl12b. *Eukaryot. Cell* 12, 864–874
50. Abraham, K.J. *et al.* (2016) Intersection of calorie restriction and magnesium in the suppression of genome-destabilizing RNA–DNA hybrids. *Nucleic Acids Res.* 44, 8870–8884
51. Vianna, M.C. *et al.* (2016) *Drosophila* ataxin-2 gene encodes two differentially expressed isoforms and its function in larval fat body is crucial for development of peripheral tissues. *FEBS Open Bio.* 6, 1040–1053
52. Halbach, M.V. *et al.* (2017) Atxn2 knockout and CAG42-knock-in cerebellum shows similarly dysregulated expression in calcium homeostasis pathway. *Cerebellum* 16, 68–81
53. Zhang, S. *et al.* (2017) Novel metabolic and physiological functions of branched chain amino acids: a review. *J. Anim. Sci. Biotechnol* 8, 10
54. Meierhofer, D. *et al.* (2016) Ataxin-2 (Atxn2)-knock-out mice show branched chain amino acids and fatty acids pathway alterations. *Mol. Cell. Proteom.* 15, 1728–1739
55. Seidel, G. *et al.* (2017) Quantitative global proteomics of yeast PBP1 deletion mutants and their stress responses identifies glucose metabolism, mitochondrial, and stress granule changes. *J. Proteome Res.* 16, 504–515
56. Dunn, C.D. and Jensen, R.E. (2003) Suppression of a defect in mitochondrial protein import identifies cytosolic proteins required for viability of yeast cells lacking mitochondrial DNA. *Genetics* 165, 35–45
57. Gispert, S. *et al.* (2009) Parkinson phenotype in aged PINK1-deficient mice is accompanied by progressive mitochondrial dysfunction in absence of neurodegeneration. *PLoS One* 4, e5777
58. Fuhrmann, N. *et al.* (2010) Solving a 50 year mystery of a missing OPA1 mutation: more insights from the first family diagnosed with autosomal dominant optic atrophy. *Mol. Neurodegener.* 5, 25
59. Kasumu, A.W. *et al.* (2012) Chronic suppression of inositol 1,4,5-triphosphate receptor-mediated calcium signaling in cerebellar Purkinje cells alleviates pathological phenotype in spinocerebellar ataxia 2 mice. *J. Neurosci.* 32, 12786–12796
60. Scoles, D.R. *et al.* (2012) ETS1 regulates the expression of ATXN2. *Hum. Mol. Genet.* 21, 5048–5065
61. Gispert, S. *et al.* (2012) The modulation of amyotrophic lateral sclerosis risk by ataxin-2 intermediate polyglutamine expansions is a specific effect. *Neurobiol. Dis.* 45, 356–361
62. Lahut, S. *et al.* (2012) ATXN2 and its neighbouring gene SH2B3 are associated with increased ALS risk in the Turkish population. *PLoS One* 7, e42956
63. Schols, L. *et al.* (2015) No parkinsonism in SCA2 and SCA3 despite severe neurodegeneration of the dopaminergic substantia nigra. *Brain* 138, 3316–3326
64. Yamashita, C. *et al.* (2014) Evaluation of polyglutamine repeats in autosomal dominant Parkinson's disease. *Neurobiol. Aging* 35, 1779. e17–e21
65. Auburger, G.W. (2012) Spinocerebellar ataxia type 2. *Handb. Clin. Neurol.* 103, 423–436
66. Almaguer-Mederos, L.E. *et al.* (2013) Estimation of survival in spinocerebellar ataxia type 2 Cuban patients. *Clin. Genet.* 83, 293–294
67. Velázquez-Pérez, L. *et al.* (2017) Corticomuscular coherence: a novel tool to assess the pyramidal tract dysfunction in spinocerebellar ataxia type 2. *Cerebellum* 16, 602–606
68. Velázquez-Pérez, L. *et al.* (2016) Abnormal corticospinal tract function and motor cortex excitability in non-ataxic SCA2 mutation carriers: a TMS study. *Clin. Neurophysiol.* 127, 2713–2719
69. Auburger, G. *et al.* (2014) 12q24 locus association with type 1 diabetes: SH2B3 or ATXN2? *World J. Diabetes* 5, 316–327
70. Sebastiani, P. *et al.* (2012) Genetic signatures of exceptional longevity in humans. *PLoS One* 7, e29848
71. Abdel-Aleem, A. and Zaki, M.S. (2008) Spinocerebellar ataxia type 2 (SCA2) in an Egyptian family presenting with polyphagia and marked CAG expansion in infancy. *J. Neurol.* 255, 413–419
72. Hu, F. *et al.* (2016) Hypothalamic roles of mTOR complex I: integration of nutrient and hormone signals to regulate energy homeostasis. *Am. J. Physiol. Endocrinol. Metab.* 310, E994–E1002
73. Na, D. *et al.* (2013) NeuroGeM, a knowledgebase of genetic modifiers in neurodegenerative diseases. *BMC Med. Genom.* 6, 52
74. Al-Ramahi, I. *et al.* (2007) dAtaxin-2 mediates expanded Ataxin-1-induced neurodegeneration in a *Drosophila* model of SCA1. *PLoS Genet.* 3, e234
75. Lessing, D. and Bonini, N.M. (2008) Polyglutamine genes interact to modulate the severity and progression of neurodegeneration in *Drosophila*. *PLoS Biol.* 6, e29
76. Shulman, J.M. and Feany, M.B. (2003) Genetic modifiers of tauopathy in *Drosophila*. *Genetics* 165, 1233–1242
77. Becker, L.A. *et al.* (2017) Therapeutic reduction of ataxin 2 extends lifespan and reduces pathology in TDP-43 mice. *Nature* 544, 367–371
78. Scoles, D.R. *et al.* (2017) Antisense oligonucleotide therapy for spinocerebellar ataxia type 2. *Nature* 544, 362–366
79. Wiedemeyer, R. *et al.* (2003) Ataxin-2 promotes apoptosis of human neuroblastoma cells. *Oncogene* 22, 401–411