REVIEW

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Ataxin-2 gene: a powerful modulator of neurological disorders

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Purpose of review
To provide an update on the role of Ataxin-2 gene (*ATXN2*) in health and neurological diseases.

Recent findings
There is a growing complexity emerging on the role of *ATXN2* and its variants in association with SCA2 and several other neurological diseases. Polymorphisms and intermediate alleles in *ATXN2* establish this gene as a powerful modulator of neurological diseases including lethal neurodegenerative conditions such as motor neuron disease, spinocerebellar ataxia 3 (SCA3), and peripheral nerve disease such as familial amyloidosis polyneuropathy. This role is in fact far wider than the previously described for polymorphism in the prion protein (*PRNP*) gene. Positive data from antisense oligo therapy in a murine model of SCA2 suggest that similar approaches may be feasible in humans SCA2 patients.

Summary
ATXN2 is one of the few genes where a single gene causes several diseases and/or modifies several and disparate neurological disorders. Hence, understanding mutagenesis, genetic variants, and biological functions will help managing SCA2, and several human diseases connected with dysfunctional pathways in the brain, innate immunity, autophagy, cellular, lipid, and RNA metabolism.

Keywords
Ataxin-2 gene, C9ORF72, cytosine adenine guanine-repeats, spinocerebellar ataxia 2, TDP43

INTRODUCTION

Spinocerebellar ataxia 2 (SCA2) is an autosomal dominant lethal disease caused by ≥32 cytosine adenine guanine (CAG) repeats in Ataxin-2 gene (*ATXN2*) [1–3]. In SCA2, the main affected tissues are the cerebellum, pons, olive, brainstem, frontal lobe, medulla oblongata, cranial and peripheral nerves. The pattern of neurodegeneration extends into the substantia nigra, and affects motoneurons as well [4]. The clinical manifestations of SCA2 include progressive gait ataxia, dysarthria, dysphagia, cognitive decline, slow eye movements, ophthalmoplegia, Parkinsonism, pyramidal features, and/or neuropathy.

The pathological CAG expansion is unstable in both germinal and somatic tissues, hence offspring may inherit a shorter or larger version of the pathogenic repeats. ATXN2 CAG somatic mosaicism, i.e., the presence of different CAG, cytosine uracil guanine (CUG), and/or polyQ length stretches, exists in different body tissues. Intermediate CAG expansions in *ATXN2* (≥29CAG/CAA repeats) increase the risk for many other neurological diseases. The genetic alteration locates in the first exon of *ATXN2* with locus at 12:111,452,214–111,599,676 (GRCh38) harboring 25 exons and encoding a very ubiquitous RNA binding protein. In the same *ATXN2* locus a novel gene, called *ATXN2-AS* encoding a natural antisense transcript (NATs) with a CUG tract, has been described. For this review, we cover relevant articles on *ATXN2* published during the last 18 months.

Spinocerebellar ataxia 2 MUTAGENESIS AND FOUNDER EFFECTS

Mutagenesis in SCA2 is thought to occur in predisposed haplotypes, particularly in those alleles with loss of 5′ CAA located within the CAG sequence [5,6]. This haplotype is universal for SCA2 families

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KEY POINTS

- Advances in the understanding of *ATXN2* CAG repeat expansions mutagenesis and the impact of founder effect help to understand not only SCA2 but also to explain the origin of intermediate alleles and its role in other neurodegenerative diseases (e.g., ALS, FTD, ALS/FTD, and Parkinson's Disease).
- ATXN2 isoforms are prominently expressed in brain and its role in RNA, lipid, and amino acids metabolism influence human brain functioning.
- Novel *ATXN2* variants may play a role as modifiers factor but their occurrence needs to be evaluated in diverse populations.
- Advances in deep phenotyping of prodromal SCA2 stage improve our understanding of the pathophysiology and may provide a therapeutical window.
- Progress in therapeutical targeting of *ATXN2* in animal models seems promising not only for treating SCA2 but also for other more common neurological diseases.
- Future investigations may combine SCA2 haplotype information to address allele-specific targeting and its impact on wet biomarkers for both prodromal and manifest SCA2.

and is shared with a lower range of CAG repeats (22–31CAG), suggesting a common mutagenic mechanism [7] (Fig. 1A,B).

The second most frequent CAG/CAA sequence pattern is (CAG)13CAA(CAG)8 and had an opposite distribution in a Chinese SCA2 cohort compared to a control population [8]. This is interesting since the allele 13CAG-CAA-8CAG shares the SCA2 risk haplotype in other populations [5] where the loss of 5′CAA was originally proposed to be the initial step for SCA2 mutagenesis. Taken together, the CAG expansions have emerged in different populations independently of each other.

SCA2 is highly prevalent in Holguin, Eastern Cuba. The original founder haplotype was introduced in this region ~375 years ago according to predictions with Monte-Carlo simulation [9] using microsatellites markers in 13 families (Fig. 1C). This point in time corresponds to 1615, 70 years after the foundation of Holguin village. Thus, the Spanish *conquista* introduced either intermediate alleles and/or a founder SCA2 mutation. The genetic profiles of SCA2 Cuban families are very similar to Spanish ones (Laffita-Mesa *et al.* manuscript in preparation). African slaves are an unlikely source for SCA2 founder effect as they were brought into this part of Cuba several years later since sugar plantations were not so prominent. Most likely, a full penetrant mutation was not the original founder event as individuals carrying such mutations would be negatively selected. Instead, a premutation or low penetrance alleles (clinically irrelevant) within predisposing haplotype seems to have been a more reasonable event. Social conditions, cultural replicators, endogamous and closed-cycle marriages, religion, wars, syndemics resulted in a bottleneck effect increasing the number of premutation carriers among the Spanish settlers.

Ataxin-2 gene expression

Expression of *ATXN2* is ubiquitous (https://gtexportal.org/home/gene/ENSG00000204842.14). At least 27 splice variants are predicted in Gtex but only few have been experimentally confirmed [10] (Fig. 1D).

Ataxin-2 gene and brain functioning, health and neurodegeneration

ATXN2 is essential for neurodevelopment in *Drosophila* [11]. Both *ATXN2* levels and its subcellular location determine the spacing of dendritic branches for the optimal dendritic receptive fields in sensory neurons in *Drosophila* [12]. This may also have implications for neuronal synaptic plasticity, where FMR1 and ATX2 function together in a microRNA-dependent process mediating long-term olfactory habituation [13].

Massive Atxn2-CAG expansion in mice cause early disruption of excitability and communication in cerebellar neuronal layers [14].

In humans, an antagonistic pleiotropic role in cognition is emerging. ATXN2 and its substitute ATXN2L positively influence several cognitive domains such as verbal–numerical reasoning, reaction-time, educational attainment, and cognitive resilience [15,16]. Nevertheless, *ATXN2* CAG repeat length polymorphisms, are associated with smaller volume in both the putamen and thalamus, but also with atrophy in amygdala and globus pallidus conferring cognitive decline in old age [17].

On the other hand, many lines of evidence suggest that *ATXN2* genetic alterations may to lead disruption of innate immunity, autophagy, Krebs cycle, amino acid homeostasis, lipid, and RNA metabolism [18,19]. This altogether with the intracellular dynamics [20▪▪] explain the global effect in brain functioning caused by *ATXN2* gain and/or loss of function.

Beyond CAG repeats, duplication in Ataxin-2 gene lowers age of onset in spinocerebellar ataxia 3 and C9-ALS

Recently, we identified a 9bp-duplication c.109_117 delinsCGGAGCGGG/GCCTCGCCC in the *ATXN2-S/AS* region as modifier in familial neurodegeneration.

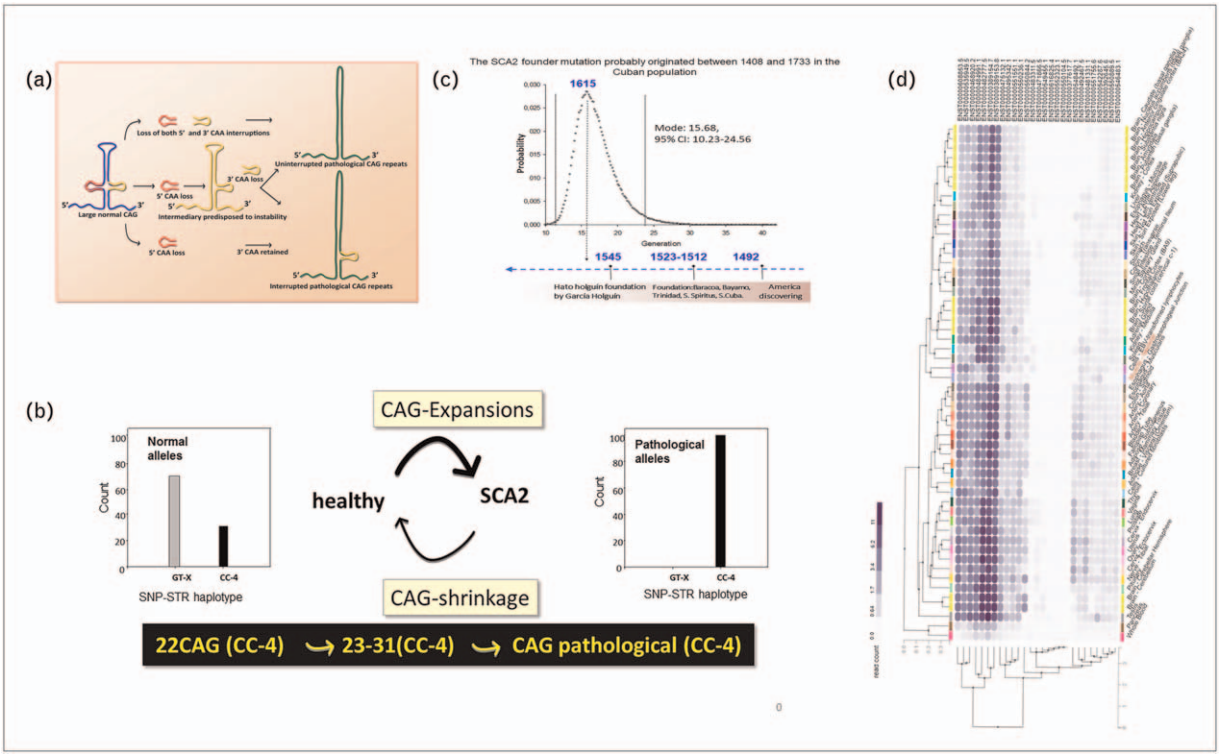

FIGURE 1. Mutagenesis, founder effect and *ATXN2* isoforms. (A) Mutagenesis in SCA2 is thought to occurs under predisposed haplotypes, where 5′ CAA interruption loss occurs with more propensity [5,6]. (B) Low range CAG repeats interrupted by CAA (33–34repeats) shared the same STR-SNP haplotype, loci: D12S1333, D12S1672, rs695871 and rs695872, with uninterrupted full expansions [7]. (C) Prediction of the age for the Cuban SCA2 founder effect with DMLE [9] using six STR markers (D12S1328-D12S1332-D12S1672-D12S1333- D12S1329-88-5) in the vicinity of SCA2 mutation in 13 affected families. The curve represents the probability density of the mutation age for growth rate 0.45 within the period 1774–2007. The dashed lines correspond to the 95% credible set of values. The origin was predicted to have occurred 15 generations ago. According to this, the founder effect occurred likely 375 years ago, at year ~1615 which is 70 years after the Holguin Villa foundation. (D) Isoform Expression of ATXN2 in different tissues (https://gtexportal.org/home/gene/ENSG00000204842.14#gene-transcript-browser-block). ATXN2, Ataxin-2 gene; CAG, cytosine adenine guanine; STR-SNP, short tandem repeat-single nucleotide polymorhphism.

Furthermore, a mosaic with a different CAG/CAA-repeat sequence pattern within the 22CAG allele associated with the 9-bp duplication was also found [21▪▪]. Other structural alterations in this region are a 17 bp deletion CCCGCCCGCTCCGCCGC, a 9 bp insertion, CCCGCCCGCT dbSNP (rs1249427887), and 18 bases insertion CCCGCCCGCTCCGCCGCG all of them located at the same loci 12–111599394-C-CCCGCCC GCTCCGCCCGCT and at frequency, 6.6e-5, 3e-3, 6.6e-5 with CADD values of 12.2. 12.6 and 22.7 (gnomAD v3.1). The 17 bp deletion encodes a polypeptide 39.85% homologous to ATXN2, when aligned with exon-1 (Fig. 2A,B).

The 9bp-duplication [21▪▪] significantly decreased the age at onset for both spinocerebellar ataxia 3 (SCA3) and C9ORF72-amyotrophic lateral sclerosis (ALS). Droplet digital PCR experiments demonstrated its inclusion in the main transcript. In addition, in *ATXN2-AS*, the splice variant profile in 9bp-dup carriers differed as compared with controls. As shown in Fig. 2C,D, the controls had the predicted v1_v2 peaks at ~800 bp and ~440 bp [22] but mutants showed a shifted v2 profile, with no exon-2 variant and less 2+3 exon for v2 and v1 respectively suggestive for aberrant processing of the *ATXN2-AS* transcripts in patients with the 9bp-dup (Fig. 2C,D). Furthermore, this genetic change duplicates the phosphorylation and methylation sites on Serine and Arginine residues, Ser_{38}-Gly-Arg_{40}-Ser_{41}-Gly-Arg_{43} located in the N-terminal intrinsically disordered region (IDRs). Indeed, this region is targeted by multiple kinases, such as PKC, GSK3, and Cdk5 [23].

Ataxin-2 gene CAG length variation in neurological diseases

The presence of at least one *ATXN2* allele >22CAG decreased age at onset by 6 years in Portuguese patients with transthyretin familial amyloid polyneuropathy associated with the Val30Met variant

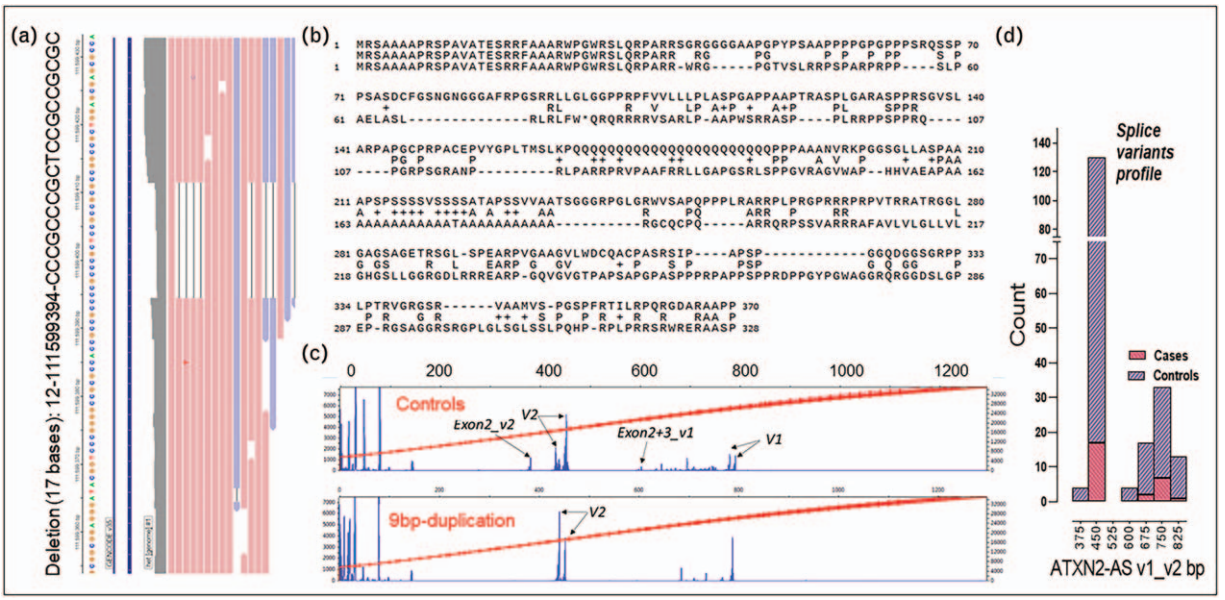

FIGURE 2. Novel ATXN2 variants and aberrant Splice variant profiles. (A) Deletion (17 bases) at 12-111599394 in ATXN2 (p.Ala35GlyfsTer39). (B) Local alignment for the first exon of wild-type ATXN2 and the predicted polypeptide resulting from the 17 bp deletion. (C) Typical fragment analysis traces for the ATXN2-AS gene expression profiling in healthy controls (top) and cases with ATXN2 duplication mutation of 9 bp duplication (bottom). Two ATXN2-AS splice variants were analyzed v1 (~800 bp) and v2 (~450 bp), arrows indicate the predicted length. In addition to the expected bands, other bands can be seen at 360, 480, 600–760 bp. Strand Specific-RT-PCR was performed in cDNA using primers and conditions previously published [22] with small modifications. Two rounds of PCR were done, and samples were ten-fold replicated. To obtain a pool of amplicons, 5ul of cDNA was amplified with R2/LK-F6 or LK-F7 primers and a second PCR amplification of 1/100 of the first PCR was done with LK/R3–6FAM primers. Five microliters of reaction amplicons were cleaned with 2ul ExoSAP-IT Express PCR and were mixed with GeneScan 1200 LIZ dye Size Standard (Applied Biosystems) and were ran during 4hrs with 50 cm capillary by Capillary Electrophoresis in a 3730 genetic Analyzer. Peak Scanner software was used to obtain splice variants sizes and height peaks. The data table was exported directly to be analyzed using height peaks≥150 and size ≥300. (D) Histogram with ATXN2-AS splice variant profile. Peaks showing differences of 40 bp sizes were binned together for data presentation purposes.

[24]. *ATXN2* intermediate alleles lowers AO in frontotemporal dementia (FTD) [25], in addition, those patients had parkinsonism and psychotic symptoms at the time of disease onset [25]. Intermediate alleles are overrepresented also in Alzheimer's disease and behavioral FTD suggesting a potential link between *ATXN2* with tauopathies [26].

An autopsy performed in an individual with corticobasal syndrome recently confirmed *ATXN2* genotype with 27/39CAG. The full expanded allele, which otherwise cause SCA2 at midlife, was interrupted by four CAA motifs (CAG8–CAA–CAG4–CAA–CAG4–CAA–CAG9–CAA–CAG10) and accompanied by a 27CAG intermediate allele (CAG8–CAA–CAG4–CAA–CAG4–CAA–CAG8) [27]. The patient had not cerebellar ataxia, neuroimaging abnormalities or neuropathological hallmarks for SCA2. This aggressive phenotype is in contrast with the late levodopa-responsive parkinsonism associated with interrupted expansions. It is claimed that CAA interruptions may influence variable phenotypes (Fig. 3A,B). Likewise, allelic interaction may determine a phenotype different than the expected for *ATXN2* intermediate alleles in ALS and full CAG expansions in SCA2. This notion, of allelic interaction, is reinforced by appearance of late onset SCA2 resulting from homozygous 31/31CAG, and a case ALS with cognitive decline and 28/28CAG in homozygous state [28,29].

The phenotype compatible with ALS/SCA2 in a woman with *ATXN2* 32CAG repeats suggests that the length of the CAG determines the timing of ALS and ataxia phenotypes in a disease continuum [30]. This continuum may also include Parkinsonism, pure cerebellar SCA2, and infantile-onset SCA2, with genetic and neuropathological overlap with TDP43 pathology.

Ataxin-2 gene as a genetic risk factor, spinocerebellar ataxia 2 mutations, variable phenotype and massive expansions in different populations and whole-genome sequencing projects

Increasingly, *ATXN2* CAG repeat expansions have a prominent role in different populations either as

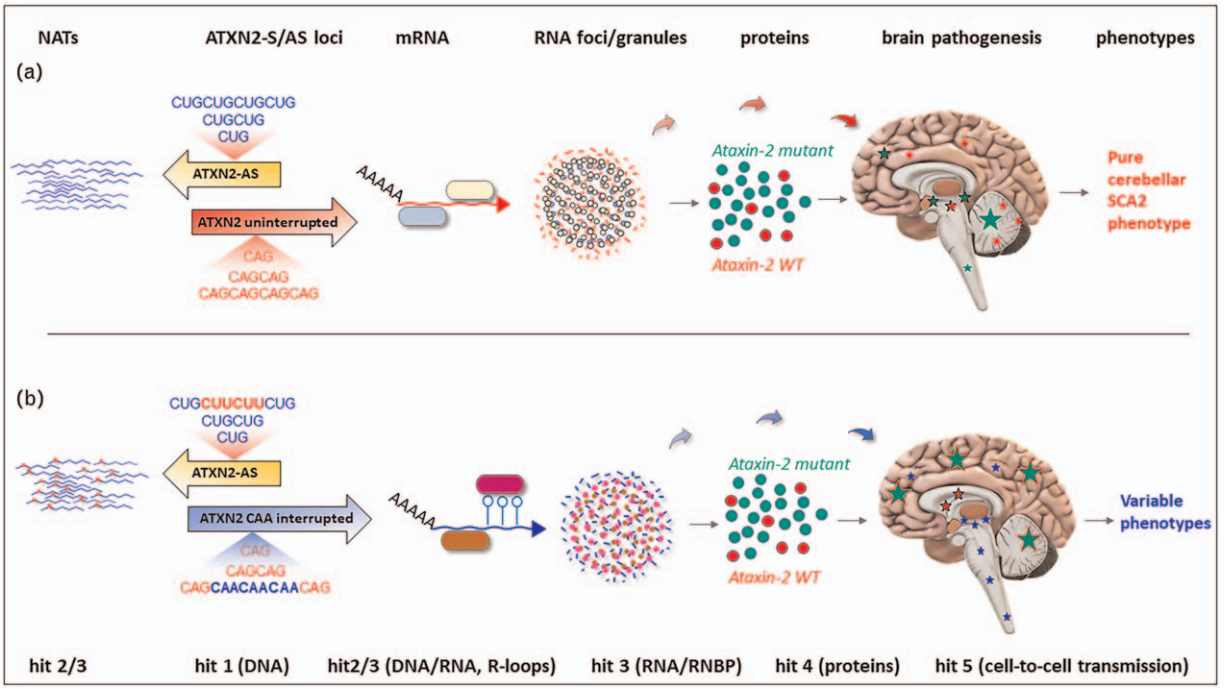

FIGURE 3. Variable phenotype may involve the CAA interruptions within the *ATXN2* CAG repeats. This may entail different interactors at different pathological hits, but the most likely is the level involving RNA/RNA-binding proteins. CAG, cytosine adenine guanine.

genetic risk factor, SCA2 *de novo* cases or massive repetitions causing SCA2 in the childhood. Gardiner *et al.*, 2019 analyzed 14 196 individuals with a wide age range (18–99 years) from The Netherlands, Scotland, and Ireland, and found that ~11% carried intermediate alleles in the major nine genes causing polyQ diseases [31▪]. For *ATXN2*, they found both intermediate alleles and pathological expansions (30–36 units). Furthermore, Akçimen *et al.*, 2020 used ExpansionHunter to estimate the trinucleotide repeats in whole-genome sequencing (WGS) data of unrelated healthy individuals from different geographic regions. The frequency of abnormal *ATXN2* CAG repeats was 3.32% with a CAG number ranging between 27–36 units. Pathological SCA2 alleles were of low penetrance ≥34CAG repeats [32]. Likewise, Ibanez *et al.* identified patients with variable phenotypes, ALS (22/33CAG), levodopa-responsive early-onset Parkinson's disease (31/41CAG), progressive cerebellar ataxia (22/40CAG), and SCA2 (22/42CAG) [33].

One girl with intellectual disability had 99CAG repeats with apparently healthy parents [33]. Two little sisters with more than 180 CAG repeats presented with systemic features including global developmental delay, infantile spasms, hypotonia, seizures, dysautonomia, hearing and visual impairment, dysphagia, sleep, and mood disorders. The germinal instability started in the grandfather with 22/37CAG who transmitted an unstable *ATXN2* mutation (43CAG) to one of his dizygotic sons with further massive expansions of up to ≥180CAG [8]. Another child was reported in Mexico. The maternal SCA2 mutation, 49CAG, expanded to ~884 in her son whom at age 5yrs presented with ataxia, gaze-evoked nystagmus, and spasticity [34].

Prodromal spinocerebellar ataxia 2 and phenotype insights

Jacobi *et al.* described the prodromal features for European SCA1, 2, 3, and 6 cohorts and evaluated the sensibility of SARA and other clinical tools to detect changes in SCAs prodromal phase [35▪▪]. The longitudinal observation was performed between 2008–2015, with assessments at baseline, 2, 4, and 6 years. Twenty-two (59%) of SCA2 mutation carriers phenoconverted into SCA2, with a median age of onset around ~36 years. They had an increase in the SARA from the time of inclusion, which was influenced by the age at first visit and the CAG expansion. There was a progressive cerebellar functioning deterioration determined by clinical scales and supported by volume loss in the cerebellum and pons. This was paralleled by annual decline in non-ataxia signs and sleep quality. On the other hand, Nigri *et al.* found isolated volume loss in the pons, and cortical thinning in specific frontal and parietal areas in preclinical SCA2 [36]. No other clinical or cognitive changes were evidenced in this one-year

longitudinal study. In the prodromal phase, mutation carriers had larger variability in both gait and postural sway control [37], suggesting deterioration of the vestibulocerebellar network. Furthermore, there is a gradual worsening of speech and swallowing [38]. These preclinical abnormalities seem to be dampened with physical rehabilitation [39]. All this knowledge provides insights that will help design future disease modifying therapies (Fig. 5B,C). One challenge will be to make a fair prediction of the 'estimated years to symptom onset' (EYO) based on the different expanded CAG alleles and to identify gene modifiers of this stage.

Antisense therapy proved to be efficient in ALS and spinocerebellar ataxia 2 preclinical models

ATXN2 and TDP43 interact through RNA molecules (Fig. 4) [40,41,42▪,43]. In spinal cord neurons of ALS

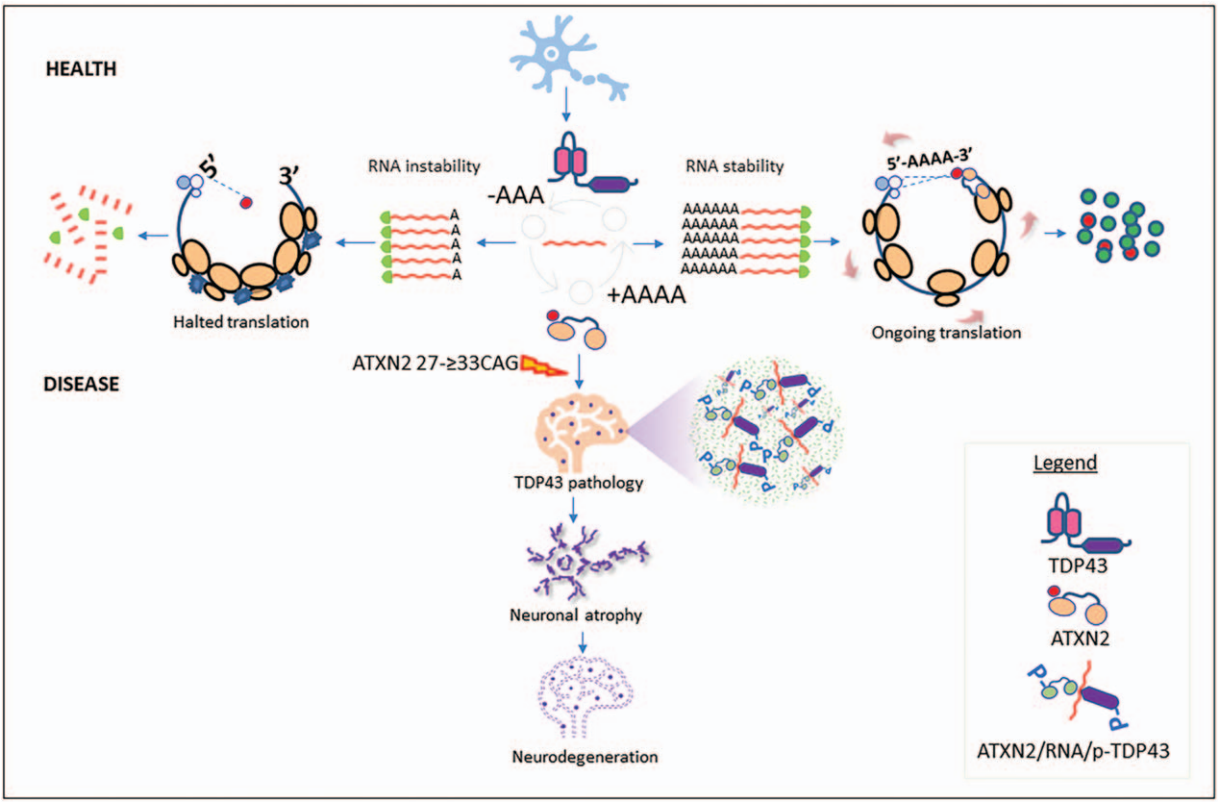

FIGURE 4. *ATXN2* function and RNA metabolism in health and disease. For efficient protein synthesis, *ATXN2* C-terminal PAM2 motif mediates interaction with the poly(A)-binding protein PABPC1 tethered to poly-(A) 3′tail mRNAs [40]. This complex interacts with eIF4GI at the 5′ tail, creating the required circularization mRNA linking 5′-3′ ends. Yokoshi *et al.* found that ATXN2 binds, stabilizes, and regulates the translation of more than 4,000 mRNA molecules [41]. For this, ataxin-2 predominantly binds to AU rich *cis*-regulatory elements at the 3′ tail of target mRNAs. The breadth of cellular processes targeted by this ATXN2 functions includes RNA splicing, mRNA polyadenylation, 3′tail processing and cellular metabolism. The pathological polyQ expansion perturbs this ataxin-2 physiological role, as Q31 (ALS) and Q39 (SCA2) downregulates the abovementioned processes in a dependent polyQ length manner [41]. It was not clear how ATXN2 stabilizes its target transcriptome, but Inagaki *et al.* showed polyadenylation enzymatic activity for ATXN2 [55]. The mechanism implies that ATXN2 binds to both *cis*-regulatory elements in the target mRNA and to PABP and others RNP trough the PAM2 motif. This complex may activate and stabilize their target transcriptome, for instance TDP43 and Cyclin-D1, through catalytic polyadenylation. In turn, TDP43 protein accelerates deadenylation of target mRNAs, which is a critical step in the RNA degradation. Therefore, regulating the length of the of the poly(A) tail plays a key role in the control of the mRNA stability [42▪]. This provides evidence for a common cellular process where both proteins cooperate with antagonistic functions regulating common substrates. Likewise, could suggest that an immediate pathological consequence of the abnormal interaction driven by polyQ in ATXN2, would be the loss of TDP43 function when work on stabilizing and activating their common transcriptomes. This may also affect the regulation of circadian genes as *Drosophila* ATX2 complex may switch distinct modes of posttranscriptional regulation through its associating factors to control circadian clocks and ATX2-related physiology [43]. It is not clear whether ATXN2 undergone any other posttranslational modification like the pathological TDP43 phosphorylation. ALS, amyotrophic lateral sclerosis.

patients, ataxin-2 and TDP43 are abnormally localized. Similarly, TDP-43 mislocalized in SCA2 and there is neuropathological and phenotype overlapping with Frontotemporal Lobar Degeneration (FTLD) cases manifest as pure FTLD-ALS without ataxia [44,45]. Pathological phosphorylation of TDP43 is seen in ALS, and its levels are increased in cases with ALS with *C9ORF72* or *ATXN2* expansions [46].

Lowering *ATXN2* expression with antisense oligo in TDP-43 ALS mice prolongs their survival which is therapeutically relevant for both ALS and SCA2 [47]. However, recent studies have shown reduced ATXN2 expression suggesting either loss of function as part of the disease process or neuroprotective role in FTLD-TDP and other TDP-43 proteinopathies [20▪▪]. The last notion is well supported as proper ataxin-2 amounts are needed it to ensure optimal dendritic arborization in *Drosophila* neurons. In contrast, upregulated ATXN2 levels affects the distribution of FMRP and other RNA binding proteins inducing aberrant dendritic morphology [12].

The same antisense oligo therapy reduced the levels of both wild-type and mutant ataxin-2 in SCA2 bacterial artificial chromosome mice (BAC-Q72). Moreover, ALS-related proteins Eaat2, Pcp4, TBK1 and p62/Sqstm1 were significantly dysregulated in spinal cord and cerebellum. This therapy corrected the expression levels of all but two ALS proteins and also improved motor coordination [19].

These promising preclinical results raise hope for future trials in humans. However, since *ATXN2* function is pleiotropic and the long-term effect of reducing both the normal and mutated ataxin-2 alleles in humans remains unclear, an allele-specific therapy is the most desirable approach.

One future direction would be combining haplotype information with oligo antisense silencing. All SCA2 families share the universal SNPs haplotype C–C for rs695871 and rs695872 markers located in *ATXN2* exon-1. Furthermore, about 45% of ALS cases with intermediate alleles have the same SCA2 haplotype [5,48]. Therefore, this information would be relevant for developing allele-specific therapies. In SCA3, another polyQ disease, Prudencio *et al.*, 2020 demonstrated the effectiveness of this approach in silencing the pathological *ATXN3* allele [49].

Of note, the novel compound naphthyridine-azaquinolone (NA) was found to specifically binds

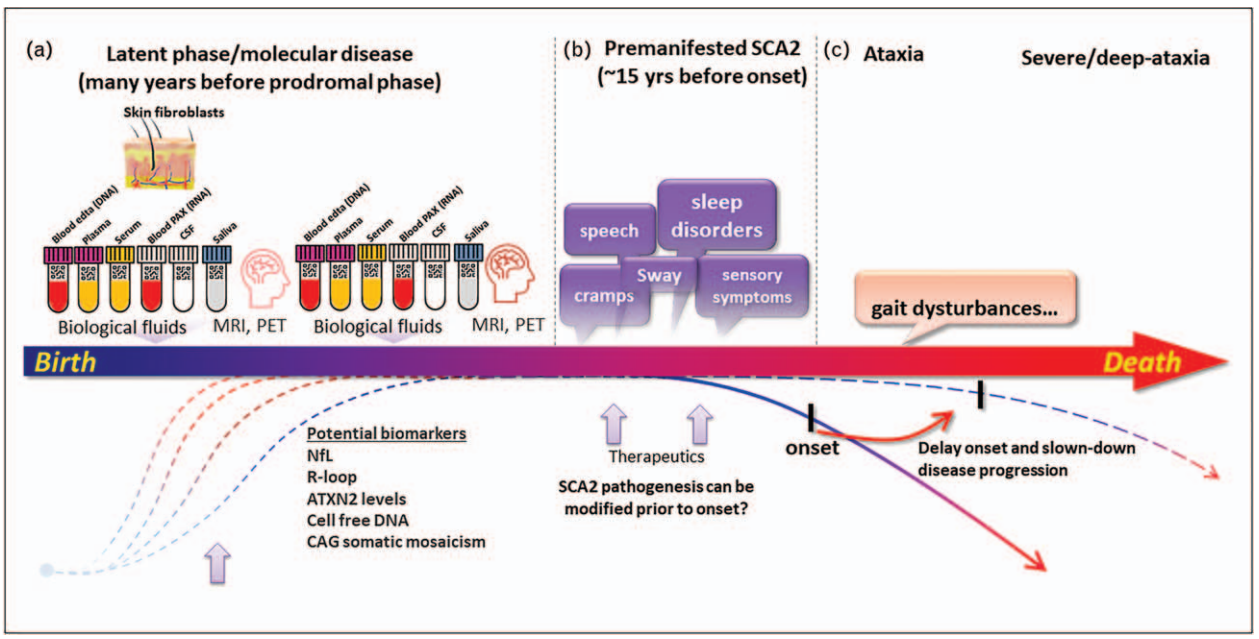

FIGURE 5. Prodromal SCA2 and biomarkers. (A) Identification of preclinical biomarkers in biological fluids and fibroblasts, paralleled with MRI and standardized clinical evaluation in asymptomatic individuals at risk [57]. Importantly, individuals are not aware of their genetic statuses. This approach, will be of great help in local or global SCA projects aimed at uncovering wet biomarkers like free circulating DNA, expanded ataxin-2 in CSF, somatic mosaicism, neurofilaments, DNA/RNA-derivatives improving the read outs for future trials. BC) SCA2 is featured by two major stages, early on it is featured by the nonmotor phase and later by a predominant and invaliding motor stage. Genetic factors or therapies acting in early stages have the potential to slowdown the neurodegeneration and postpone the onset of ataxic symptoms. Arrows in the bottom suggest potential time points for therapies targeting modifiers or disease causative factors. SCA2, spinocerebellar ataxia 2.

Table 1. Potential biomarkers, approaches and sample types

Biomarker	Commentary	SCA2 phases[a]	ATXN2 mutation specific?	Techniques	Sample type
ATXN2 mRNA levels	New alternative splicing variants were found lacking exon 12 and exon 24, and this could be relevant for disease progression [10]. Furthermore, genotyping of both DNA and RNA levels may shed evidence about mutant allele expression, as was recently shown in SCA3 and C9ORF72 ALS [21▪▪].	A^{+++}, B^{++}, C^{+}	Y^{+++}	QPCR, DDPCR	Blood-edta, blood pax, fibroblasts.
ATXN2 CAG somatic mosaicism	Mosaicism of unstable CAG repeats was confirmed in SCA2 brain and lymphoblasts cell lines. The mosaicism varied within the brain and spinal cord and was smaller in the cerebellum than in other brain tissues [58]. Furthermore, CAG somatic mosaicism in DNA and mRNA are correlated in ATXN3 gene [59].	A^{+++}, B^{++}, C^{+}	Y^{+++}	Fluorescent PCR, fragment analysis	Blood-edta, blood pax, fibroblasts.
ATXN2-AS profile	ATXN2-AS transcripts with a CUG repeat expansion are toxic in an SCA2 cell model. This was associated with aberrant splicing of amyloid beta precursor protein and N-methyl-D-aspartate receptor 1 in SCA2 brains [22].	?	Y^{+++}	Fluorescent PCR, fragment analysis	Blood-edta, blood pax, fibroblasts.
ATXN2 AUUUA binding function	Yokoshi *et al.* found that ATXN2 binds, stabilizes, and regulates the translation of more than 4,000 mRNA molecules [41].	?	Y	*In vitro* RNA binding	Blood-edta, blood pax
R-loop	Human ATXN2 deficiency leads to genome-destabilizing R-loops accumulation [60]. TDP-43 is also crucial in maintaining genomic stability through a co-transcriptional process that prevents aberrant R-loop accumulation [61] and controlling replication stress.	?	Y	Qpcr, DDPCR	blood-edta, blood pax
Cell free DNA	Cell-free circulating DNA in plasma is significantly increased in Friedreich's ataxia, SCA2 and in SCA12 patients. It was possible to distinguish between ataxia patients and healthy controls using plasma DNA [62].	?	Y/N	WGS, QPCR, DDPCR, PicoGreen fluorescent assay	CSF, Plasma, serum
NFL	Levels of serum NfL were elevated in SCA3 individuals and correlated with disease severity. The was also high concentrations starting from early disease stage and correlated with disease severity [63]. Likewise, blood NfL levels were increased with proximity to the predicted onset of ataxia in SCA3 individuals [64].	A^{+++}, B^{++}, C^{+}	N	Different platforms	CSF, Plasma, serum
Micro-RNA, and small RNAs	Bañez- *et al.*, 2012 provide evidence involving HTT CAG repeats interfering with cell viability at the RNA level. Pathological CAG repeats $\geq$40 units induced neuronal cell death and increased levels of small CAG-repeated RNAs (sCAGs) of $\approx$21 nucleotides in a Dicer-dependent manner [65]. Furthermore, the severity of the toxic effect of HTT mRNA and sCAG generation correlated with CAG expansion length. Likewise, Creus–Muncunill *et al.*, 2021 demonstrated that sRNA produced in the putamen of HD patients are sufficient to recapitulate HD pathophysiology *in vivo* [66].	?	Y/N	WGS, QPCR, DDPCR	Blood-pax

Table 1 (*Continued*)

Biomarker	Commentary	SCA2 phases[a]	ATXN2 mutation specific?	Techniques	Sample type
Lipid metabolism, cholesterol metabolites	ATXN2 CAG repeat variations may contribute to the missing heritability of obesity by affecting body mass index in the general population [67]. Loss of ATXN2 function by therapeutics silencing with ASO7 evidenced liver X receptors and farnesoid X receptor activation (LXR/RXR, FXR/RXR) which are nuclear receptors regulating lipid and glucose metabolism. Ligands for these receptors include fatty acids, oxysterols and bile acids as well as rexinoids, pointing to their relevance in the regulation of metabolic pathways [68]. This metabolic change agrees with the observation of ATXN2 CAG repeats as determinant of obesity-related risk parameters in Mexican adolescents whenever CAG stretch deviated from the most common 22CAG repeat [69]. Serum testosterone levels are also decreased in SCA2 male individuals and associates with disease duration [70]. Therefore, the ATXN2 loss of functions affects metabolic processes connected with underlying causes for metabolic syndrome-related diseases.	A,B,C^{+}	Y/N	Different platforms	Plasma, serum
Stau-1, Pcp2, Calb1 and RGS8 mRNA levels	Stau-1 physically interact with ATXN2 in an RNA dependent manner. Both are relocated to Stress granules and increased Stau-1 levels were found in cerebellum of SCA2 mouse model. Furthermore, Stau-1 and ATXN2 had similar effects reducing levels of Pcp2 and Calb1 [71].	A^{++}	?	QPCR, DDPCR	Blood-edta, blood pax

ALS, amyotrophic lateral sclerosis; ATXN2, Ataxin-2 gene; CAG, cytosine adenine guanine; CUG, cytosine uracil guanine; SCA2, spinocerebellar ataxia 2.
[a]Referred to SCA2 stages in Fig. 5A,C.

slipped-CAG DNA intermediates of Huntington's disease (HD) mutations. The therapeutic potential is highlighted as NA reduces both somatic mosaicism for the HD expanded allele and mutant HTT protein aggregates in striatum [50▪].

Genome editing technologies, such as CRISPR-Cas9, may harbor great potential for future therapies in SCA2 and other polyQ disorders. These techniques include the use of guided RNAs to either alter, excise, or insert a specific DNA sequence in a precise or predetermined manner. In one patent the use of rare cutting endonucleases and transposases for altering *ATXN2* expression is claimed [51]. Another genome editing application (WO/2018/154462) comprises *ex* and *in vivo* methods for correcting abnormal *ATXN2 CAG* repeats [52]. Significant improvement are expected of genome editing applied for correcting the pathological *ATXN2* CAG repeats and other polyQ diseases -SCA1, SCA3 and HD- [53–55]. However, finding biomarkers that may be precede the prodromal clinical changes and predictors of phenoconversion remain as other unmet needs in the field [56,57] (Fig. 5A, see Table 1).

CONCLUDING REMARKS

Almost three decades ago, the goal in the field was to provide accurate SCA2 presymptomatic diagnostic, prenatal diagnosis, and symptomatic treatment [72]. At present, preimplantation genetic testing by karyomapping enabled the first birth of three healthy babies without pathogenic CAG expansions for HD, SCA2 or SCA12 [73]. Currently, drug screening, experimental disease modelling, and deep-phenotyping studies in patients with SCA2 prepare the ground for future trials. Many of the SCA2 features overlap with different diseases. Hence, if we accurately dissect all SCA2 phenotypes it may uncover features of other disorders, or human traits

influenced whenever *ATXN2* homeostasis and/or sequence is altered. The increasing knowledge of *ATXN2* in several disparate neurological diseases resembles the major impact polymorphisms at different codons in the prion protein gene (*PRNP*) have over both sporadic and familial prion diseases. Research on *ATXN2* is intense, productive, therapeutically driven and aimed to understand gain and loss of *ATXN2* functions. Understanding the SCA2 mutagenesis and novel *ATXN2* genetic alterations will help in to improve genetic counseling and to design future therapeutical trials for SCA2.

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Conflicts of interest

There are no conflicts of interest.
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