ASOs Against ATXN2 in Preclinical and Phase 1 Trials

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Abstract Spinocerebellar ataxia type 2 (SCA2) is an autosomal dominantly inherited neurodegenerative disease caused by DNA CAG repeat expansion. The mutation is in the coding 1st exon of the *ATXN2* gene and results in an expanded polyglutamine (polyQ) domain. SCA2 is characterized by progressive ataxia and involves primarily Purkinje cells (PCs) but also other neurological systems. Some individuals with ATXN2 mutations can present as pure Parkinson or Lou Gehrig disease. Long normal ATXN2 alleles are risk alleles for amyotrophic lateral sclerosis. Comparison of mouse models expressing mutant ATXN2 (Pcp-tg-hATXN2-Q127; BAC-hATXN2-Q72) and Atxn2−/− mice clearly favors a predominant gain-of-function mechanism of repeat-expanded ATXN2 based on morphologic, transcriptomic, and slice physiology analyses. The lack of a neurodegenerative phenotype in Atxn2−/− mice led us to adopt a strategy of targeting wild-type and mutant ATXN2 with antisense oligonucleotides (ASOs). In two transgenic models, we were able to provide proof-of-principle data that targeting ATXN2 with intracerebroventricular injection of ASOs can slow progression of motor dysfunction. ASO treatment also improved expression levels of PC-specific proteins and PC firing frequencies in the acute cerebellar slice. An ASO targeting ATXN2 is currently in phase 1 human trials (BIIB105).

Keywords Ataxia · ATXN2 · Antisense oligonucleotide · Mouse

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1 SCA2 Clinical Characteristics

What is now known as SCA2 was initially described in a number of pedigrees characterized by cerebellar ataxia and slow eye movements in India (Wadia & Swami 1971). Nearly two decades later, a large population with ataxia and ophthalmoplegia was described in a founder population in the province of Holguin in eastern Cuba (Orozco Diaz et al. 1990). In 1993, the SCA2 locus was mapped to human chromosome 12q24 in several pedigrees, including Cuban individuals (Gispert et al. 1993). In the same year, we mapped an American-Italian pedigree with significant anticipation of disease onset to this region (Pulst et al. 1993).

The causative mutation was identified in 1996 as a DNA repeat expansion mutation by three groups (Pulst et al. 1996; Imbert et al. 1996; Sanpei et al. 1996). Most commonly, the *ATXN2* gene has 22 CAG repeats in controls, while ≥33 CAG repeats cause SCA2 (Fernandez et al. 2000). SCA2 is characterized by anticipation with strong inverse correlation between age of onset and CAG repeat length and meiotic instability (Figueroa et al. 2017). In the Cuban founder population, variance in age of disease onset is determined by CAG repeat size, genetic modifiers, and stochastic factors in a proportion of 50%, 25%, and 25% (Figueroa et al. 2017).

The initial clinical characterizations showed that SCA2 was a neurodegenerative disease that predominantly affected the cerebellum. While patients with SCA2 have many of the clinical characteristics that define the SCAs as a group of neurodegenerative disorders, the SCA2 phenotype, when assessed across a large number of individuals, can be clinically distinct. Gait ataxia, considered a characteristic of SCA, is the most noticeable symptom and is often the presenting symptom and sign (Pulst et al. 1993; Luo et al. 2017). A distinguishing feature of SCA2, not necessarily by its presence, but by its severity, is the slowing of saccadic eye movements (Geschwind et al. 1997; Ashizawa et al. 2013; Gwinn-Hardy et al. 2000).

Although gait ataxia is usually the first symptom, onset may also coincide with muscle cramping. Gait ataxia is followed by multiple other symptoms characteristic of cerebellar dysfunction such as appendicular ataxia with instability of stance, dysarthria, and ocular signs. In retrospect, it has become clear that some, but not all, of the families with slow eye movements described by Wadia in India had mutations in the *ATXN2* gene (Wadia and Swami 1971; Wadia et al. 1998).

Some SCA2 patients may show prominent involvement of basal ganglia or upper and lower motor neurons. This led to subsequent identification of individuals with pure outlier phenotypes. Gwinn-Hardy and colleagues described Taiwanese patients with tremor-predominant L-DOPA responsive Parkinson disease that were found to have ATXN2 mutations with later confirmation in other ethnic populations (Gwinn-Hardy et al. 2000; Payami et al. 2003). A pure ALS phenotype can also occur with *ATXN2* mutations (Tazen et al. 2013; Neuenschwander et al. 2014).

ATXN2 repeat expansions can act as recessive, dominant, or risk alleles depending on CAG repeat size (reviewed in Pulst 2018). Alleles with ≥33 repeats are dominant in causing adult-onset ataxia, and alleles of 31 and 32 repeats are recessive. Long normal repeats in *ATXN2* are risk alleles for ALS (Elden et al. 2010; Tazen et al. 2013; Neuenschwander et al. 2014).

1.1 SCA2 Models

ATXN2 is highly conserved in evolution. Its yeast ortholog is poly(A)-binding protein-1 (Pab1)-binding protein (Pbp1). In *C. elegans*, Kiehl and colleagues found that Atx2 played an essential role in patterning using RNAi (Kiehl et al. 2000). In the fly, Satterfield and Pallanck (2006) showed that dAtx2 assembled with polyribosomes and poly (A)-binding protein (PABP). Physical interaction with PABP was mediated by the N-terminal Lsm/Lsm-associated domain (LsmAD) and the PAM2 motif in dAtx2.

In a screen for modifiers of SCA3 neurodegeneration, the Bonini group pointed to an important function of fly atx2 in neurodegeneration. They showed that normal activity of Atx2 was critical for SCA3 degeneration, depending in particular on the PAM2 motif (Lessing and Bonini 2008). Similarly, dAtx2 mediates mutant Atx1 neurodegeneration in the fly (Al-Ramahi et al. 2007).

Mouse and human ATXN2 are highly homologous (Nechiporuk et al. 1998). At the nucleotide level, identity was 91% and at the amino acid level 89%. The region flanking the glutamine is significantly less conserved than the rest of the protein. Lack of conservation could potentially argue for the generation of mouse models using human cDNAs or human BACs as compared with knock-in models.

We and others have produced multiple SCA2 mouse models, including transgenic and knockout models. Recent reviews describe these mouse lines in detail (Alves-Cruzeiro et al. 2016; Scoles and Pulst 2018; Cendelin et al. 2022). In the following paragraphs, we will focus on those models that directly led to the development of ASOs for SCA2 and ALS and not discuss other transgenic models (Aguiar et al. 2006) or models using knock-in strategies of mutant ATXN2 CAG repeats (Arsović et al. 2020).

1.2 Pcp2-ATXN2 *Transgenic Mice*

We generated two transgenic lines expressing ATXN2 with mutant repeats of Q58 and Q127 under the control of the Purkinje cell protein 2 (*Pcp2*)/*L7* promoter (Huynh et al. 2000; Hansen et al. 2013). In the Pcp2-ATXN2[Q58] mice, rotarod testing demonstrated an ATXN2 dose-dependent motor phenotype for *ATXN2*-Q58 mice first observed at 6 months of age. PCs contained cytoplasmic but not nuclear inclusion bodies. The ATXN2-Q58 mouse was also used in studies demonstrating that SK positive modulators restored ATXN2 mouse motor and electrophysiological phenotypes (Liu et al. 2009; Kasumu et al. 2012; El-Sayed et al. 2022). Similar studies were performed using these mice with chlorzoxazone (Egorova et al. 2021).

To enhance the motor phenotype compared with the ATXN2-Q58 mouse, we generated a line expressing the entire human cDNA with 127 repeats under control of the Pcp2-promoter. This Pcp2-*ATXN2*-Q127 line had an earlier motor onset at 8 weeks as well as presence of cytoplasmic inclusions (Fig. 1). This line was studied

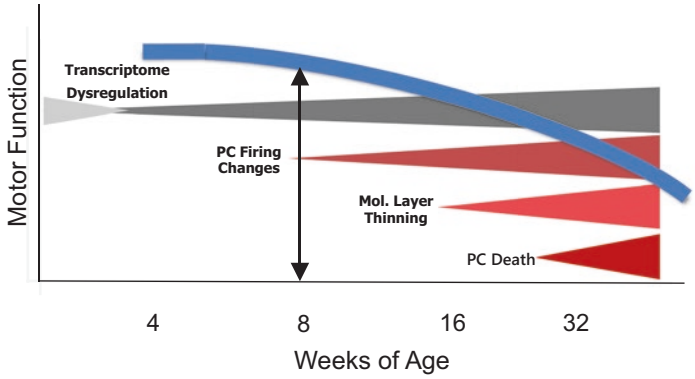

Fig. 1 Schematic of the time course of different aspects of the phenotype in Pcp2-ATXN2[Q127] mice. The blue line denotes the motor performance on the accelerating rotarod, which becomes abnormal at 8 weeks. Transcriptomes show 2 sets of differentially expressed genes (DEGs), the DEGs shown in light grey representing a developmental set, the DEGs in dark grey representing a set of progressively dysregulated mRNAs mirroring neurodegeneration

in greater detail than the Q58 line using molecular, motor, and electrophysiology in the acute cerebellar slice (Hansen et al. 2013; Pflieger et al. 2017). Although subtle mRNA expression changes were detected at 4 weeks in cerebellar mRNAs, more significant changes were seen at onset of the motor phenotype at 8 weeks. A genome-wide transcriptomic study comparing cerebellar RNAs of wild type and Q127 mice at 1 day, 3 and 6 weeks of age confirmed these results (Pflieger et al. 2017). The number of differentially expressed genes (DEGs) with stringent cutoff criteria increased from 138 at day 1 to 458 at 3 weeks and 434 at 6 weeks. Only 3 DEGs were shared across all 3 time points, whereas 87 DEGs were in common at 3 and 6 weeks (Pflieger et al. 2017). For top cerebellar DEGs, we queried the literature to identify commercially available antibodies to verify reduction in protein levels. These included RGS8, FAM107B, CEP76, HOMER3, as well as PCP2 and PCP4 (Scoles et al. 2017). On the other hand, levels of phosphorylated mTOR and p62 are increased (Paul et al. 2018, 2021).

PCs fire spontaneously at high rates, and absent synaptic inputs, firing is regular (reviewed in Meera et al. 2016). Altered PC intrinsic firing has been observed in several mouse models of ataxia including SCA2 (reviewed in Cook et al. 2021). Using the acute cerebellar slice, we examined the intrinsic spontaneous firing of PCs in wild type and Pcp2-ATXN2[Q127] mice at various time points (Hansen et al. 2013). The extracellular recordings were made by placing an electrode adjacent to a PC body, a technique, which permitted sampling of dozens of PCs in a single slice (Meera et al. 2017). We found that PCs reached their adult firing frequency of about 40 Hz at 6 weeks of age, which was maintained in wild-type mice through 40 weeks of age, but progressively decreased in mutant mice. Of note, the PC physiology in the acute cerebellar slice may differ from that observed in vivo using ATXN2-58Q mice (Egorova et al. 2021).

A significant reduction in PC spontaneous firing was first seen at 8 weeks, a time point that marked the onset of the motor phenotype on the accelerating rotarod. In summary, these results confirmed that the mouse model replicated salient feature of human SCA2, that is, an adult-onset neurodegenerative disease with only very minor developmental components.

1.3 SCA2 BAC Transgenic Mice

Testing of RNA-based therapies ideally requires mouse models that result in expression of the entire human heterogeneous nuclear RNA under control of the endogenous promoter. To address the potential problems of cDNA-based transgenes, we aimed at generating a mouse model that expressed the entire human *ATXN2* gene in as a bacterial artificial chromosome (BAC).

The human *ATXN2* gene consists of 25 exons and spans a total of 147 megabase pairs (Nechiporuk et al. 1997; Sahba et al. 1998). The largest *ATXN2* transcript is 4699 bp long including a 162 bp 5′-UTR and a 601 bp 3′-UTR. There are two in-frame start codons, the second one located just 12 bp upstream of the CAG repeat. The predicted molecular weight for ATXN2 is 144 kDa, when translated from the first start codon, and smaller by 17 kDa, when translated from the second one (Scoles et al. 2012).

We introduced a BAC containing the entire 176 kb *ATXN2* gene region including 16 kb upstream sequence and 2.5 kb downstream sequence into the mouse germline. Presently, we have two SCA2 BAC lines expressing ATXN2-Q22 or ATXN2-Q72 (Dansithong et al. 2015). Although the Q22 line has no motor, transcriptomic or neurophysiological phenotype, it has played a crucial role in the development of gene-targeted therapies as this line breeds very well and permits easy determination of target engagement of wild-type *ATXN2* mRNA.

The Q72 line has an onset of a rotarod phenotype at 8 weeks. To cross-validate our different animal models, we examined transcriptomes and found that there was a significant overlap with DEGs seen in the Q127 line as a result of BAC expression in PCs (Dansithong et al. 2015). Both models also share changes in intrinsic PC excitability (Hansen et al. 2013; Scoles et al. 2017); changes in BAC-Q72 are shown in Fig. 2.

2 ASO Development Targeting Wild-Type and mt ATXN2

2.1 Atxn2 *Knockout Mice*

A major piece of evidence needed prior to developing therapies based on targeting ATXN2 levels directly, and especially targeting wild type (wt) and mutant (mt) alleles simultaneously, is knowledge regarding the effects of ATXN2 knockout

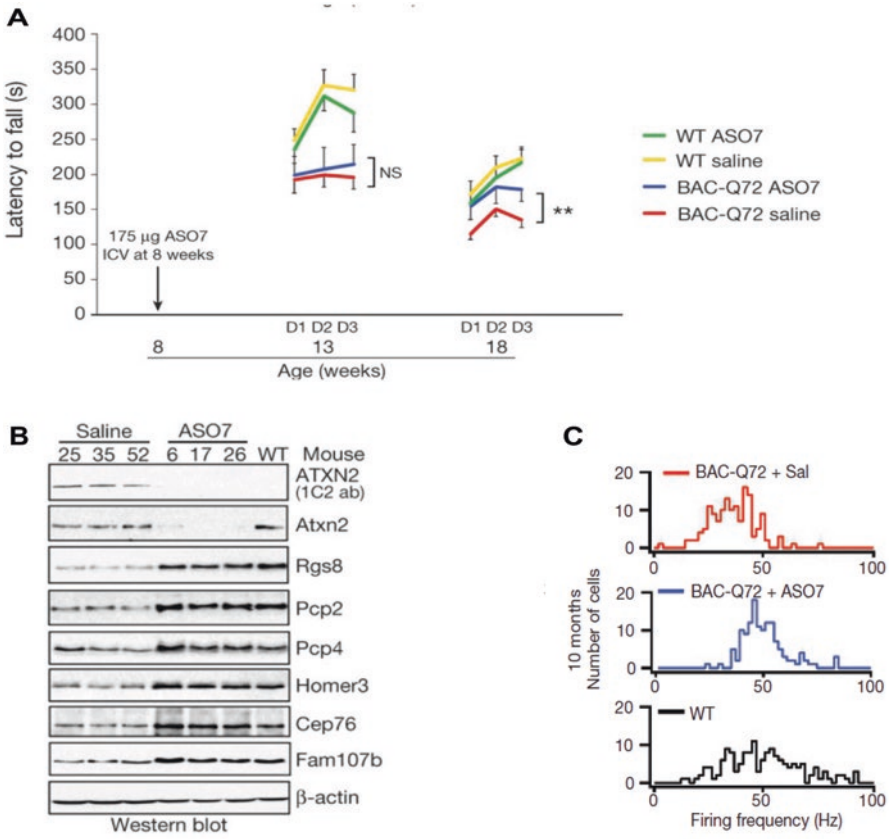

Fig. 2 Effects of ASO treatment on multiple phenotypes in the BAC-ATXN2[Q72] mice. (**a**) Improved rotarod performance of BAC-Q72 mice treated at symptom onset at 8 weeks. (**b**) Near normalization of key PC proteins by ASO treatment. Note significant reduction in mutant human ATXN2 as recognized by the 1C2 antibody, which targets expanded polyQ domains. (**c**) Analysis of intrinsic PC firing in the acute cerebellar slice. ASO7 restores normal firing frequency in BAC-Q72 mice. (Modified from Scoles et al. (2017))

in vivo. Specifically, it was not known, whether CAG repeat expansions in the *ATXN2* gene acted exclusively as a dominant gain-of-function allele or also had a loss-of-function component. To answer these questions, we generated *Atxn2* deficient mice for comparison with transgenic mice (Kiehl et al. 2006; Huynh et al. 2009).

Null mice were viable and did not have obvious morphologic central nervous system (CNS) deficits. Litters, however, showed significant sex-specific segregation distortion. Null and, to a lesser degree, heterozygote mice developed adult-onset hyperphagia and obesity. There was hyperactivity in the open cage and lack of cued and contextual fear conditioning. Long-term potentiation was impaired in the amygdala, but not in hippocampus. This was consistent with a lack of a phenotype in the Morris water maze in contrast with the observed lack of fear conditioning

(Huynh et al. 2009). Similar results were seen in an independently generated knockout mouse (Lastres-Becker et al. 2008).

In contrast to transgenic lines, we did not detect changes in intrinsic PC firing or significant transcriptomic changes (Pflieger et al. 2017). Lack of significant molecular, physiologic, or morphologic changes in the cerebellum of *Atxn2*$^{-/-}$ mice supported our notion that mutant ATXN2 acts predominantly by gain-of-function and led to our efforts targeting both wild-type and mutant *ATXN2* alleles using antisense oligonucleotides (ASOs).

2.2 Establishing Cerebellar RNA and Protein Markers for Preclinical Studies

Staining of PCs and their dendrites with antibodies to the calcium-binding protein calbindin 28K (CALB1) has been a staple for characterizing cerebellar degeneration, often with a focus on lobule VI as it can be easily identified. CALB1 is a protein that is highly expressed in PCs and involved in intracellular calcium regulation. In addition to intensity, CALB1 staining can be conveniently used to measure thickness of the molecular layer. The procedure generally involves determining a region of interest on a tissue slide and then measuring intensity with a program such as ImageJ.

With an eye on developing therapeutics, we aimed to develop methods that would sample entire cerebellar hemispheres in an operator-independent way. We began by adding quantitative reverse transcribed polymerase chain reaction (qPCR) of *Calb1* mRNA to our evaluation panel. This approach proved to be highly reproducible and allowed us to examine levels at different time points along the disease process. Levels of *Calb1* were normal at birth, significantly downregulated as early as 4 weeks of age, and exhibited a continual decline of expression after that (Hansen et al. 2013).

Our analytic methods progressing from semi-quantitative immunohistochemistry to quantitative PCR of key PC-specific genes led to the use of genome-wide transcriptional profiling. The identification of top dysregulated genes (DEGs) subsequently led to the development of protein assays that allowed us to follow progression of disease and response to therapeutic interventions (Pflieger et al. 2017; Scoles et al. 2017, 2020).

2.3 RNA-Based SCA2 Therapeutics

As transgenic models suggested a dominant (toxic) gain-of-function mechanism for pathogenesis with further support from observations in *Atxn2* null mice, we began investigating strategies to reduce ATXN2 expression. We were guided in our approach by recognition that ATXN2 disease phenotypes affect multiple neuronal

systems and that a successful treatment would have to be able to reach neurons (and potentially glia) not only in the cerebellum, but also in brainstem, cerebral hemispheres, and spinal cord. In two independent small compound screens we identified several compounds that reduced ATXN2 expression in vitro, among them cardiac glycosides, but these were predicted to be highly toxic and to have low capacity to cross the blood-brain barrier (Scoles et al. 2022).

With development of ASOs for neurologic diseases (reviewed in Scoles et al. 2019), we explored the feasibility of targeting ATXN2 in vitro and in vivo using phosphothiorated gapmer ASO in collaboration with Ionis Pharmaceuticals in 2012 (Carlsbad, USA). Gapmer ASOs have different modifications at their ends compared with the middle of the molecule. The ASOs were 20 bp in length and the backbone was phosphorothioate throughout. The terminal 5 bps at each end of the oligonucleotide had a 2′-*O*-methoxyethyl group (MOE) (Rigo et al. 2014; Scoles et al. 2017). The modifications are predicted to reduce degradation of the ASO by nucleases and at the same time to increase specificity of target mRNA interaction and degradation by RNase-H (Bennett and Swayze 2010; Crooke et al. 2021).

To identify potent ASOs, we conducted an in vitro screen with ASOs designed in silico for targeting human *ATXN2*. A total of 152 ASOs were tested in human HepG2 cells at a concentration of 4.5 μM. Delivery occurred by electroporation in two 384-well plates and *ATXN2* expression evaluated by qPCR. The 7 best ASOs were progressed to in vivo testing.

For in vivo testing, 250 μg of the 7 best ASOs were injected into the right lateral ventricle of wild type and BAC-Q72 mice and ATXN2 reduction determined after sacrifice at 7 days. To assess astroglial and microglial activation we measured cerebellar *Gfap* and *Aif1* expression by PCR. Although ASO7 reduced mouse *Atxn2* the most by 50% in wild-type mice, none of the changes of ASO7 or other ASOs were significant, which was not surprising as ASOs were directed against human *ATXN2*. One ASO significantly elevated *Aif1* expression. Three ASOs including ASO7 reduced human ATXN2 significantly in BAC-Q72 mice compared with saline injected mice. These 3 ASOs were also confirmed in their ability to reduce ATXN2 in ATXN2-Q127 mice.

When the best ASOs were analyzed at 10 weeks after injection, only ASO7 was without glial activation and we focused detailed analysis on this ASO, which targets *ATXN2* exon 11. Its uptake into PCs was confirmed by using a proprietary antibody recognizing the ASO backbone developed by Ionis Pharmaceuticals (Carlsbad, USA).

Mice were treated with ASO7 at an early symptomatic stage (8 weeks of age) or saline. Behavior was tested on the accelerating rotarod. The behavior paradigm involved testing three times per day on 3 consecutive days with the rod accelerating from 0 RPM by 1 RPM every 9 seconds. All mice including wild-type mice had fallen off the rod at 7.5 min (50 RPM). We chose this paradigm with relatively rapid acceleration and RPMs > 40 to avoid a ceiling effect that is observed in wild-type mice, which often reach the end in other rotarod paradigms without falling. This can potentially lead to a falsely reduced standard deviation in wild-type mice.

We conducted these preclinical trials following recommendations outlined by Landis and coworkers (Landis et al. 2012). This included strict randomization and

blinded evaluation with replication in two different animal models. We also opted for the use of B6/D2 hybrid mice to introduce a measure of genetic diversity. All mouse breeding followed recommendations by the Jackson laboratories. For statistical analyses of rotarod data we used Generalized Estimating Equations with the independent correlation option in Stata 12. The independent correlation option was employed, because regressions for wild-type mice frequently have more positive correlation coefficients than SCA2 mice in the 3-day rotarod paradigm.

We tested ASO7 in the Pcp2-Q127 and the BAC-Q72 models with a single intracerebroventricular (ICV) injection of 210 and 175 μg, respectively, at 8 weeks of age, when these lines are beginning to show motor deficits. Control groups were injected with saline. Our primary outcome criterion was improved motor performance, secondary outcome was reduction of ATXN2 at the end of the experiment, and tertiary outcomes were restoration of PC-specific key proteins and PC intrinsic activity.

In both models, saline-treated animals displayed the previously described deterioration of motor performance, whereas ASO-treated mice stabilized in their performance and had significantly improved performance compared with saline-treated animals. In the experiment using BAC-Q72 mice with ASO and saline treatment of wild-type and mutant mice, 10 weeks after ICV injection ASO7 had no adverse effects in wild-type mice and improved motor performance in BAC-Q72 to the level of wild-type mice (Fig. 2a).

At the endpoint (19 or 22 weeks of age), we determined the cerebellar expression of ATXN2 as a secondary endpoint. We found that ASO7 had shown long-lasting target engagement at the level of *ATXN2* mRNA and ATXN2 protein and reduced both by ≥75% in the two models.

To further demonstrate a direct effect on PC survival and function we employed quantitative PCR and western blot analyses of cerebellar extracts as well as assessment of intrinsic PC activity in the cerebellar slice. Our previously established key PC marker mRNAs and proteins (Cep76, Fam107b, Homer3, Rgs8, Pcp2, and Pcp4) showed near normalization of mRNA and protein expression. Subsets of mice were tested to determine the effect of the ASO7 on PC physiology in the acute cerebellar slice. Treatment with ASO7 treatment restored the mean PC firing frequency to that observed in age- matched wild-type mice.

3 ALS and ATXN2 ASO Phase 1 Study

In parallel with our studies in SCA2 models, the Gitler laboratory explored the role of Atxn2 in ALS with regard to TDP43 toxicity (Elden et al. 2010; Becker et al. 2017). The Gitler lab had employed yeast-2-hybrid screens to identify TDP43 as an ATXN2 interactors followed by showing that knockdown of dAtx2 improved a TDP43 induced phenotypes in the fly (Elden et al. 2010). They had also examined human genetic evidence by comparing different classes of ATXN2 repeat alleles and their association with human ALS. They found that presence of alleles with ≥27

repeats represented significant genetic risk factors for ALS (Elden et al. 2010). We and others subsequently narrowed the risk alleles to ≥30 repeats with progressively increasing risk and reaching >10-fold for individuals with ATXN2^{Q32} (reviewed in Neuenschwander et al. 2014). Frequency of the 27-repeat allele is highly variable in different populations and unrecognized population stratification can result in erroneous risk assessments. It is now well accepted that alleles from 27 to 29 repeats do not confer increased risk for ALS (Fig. 3). The molecular basis for the steep increase in ALS risk observed with the addition of a single glutamine (or CAG/CAA repeat) is currently not known.

Additional support for a role of ATXN2 in ALS has come from analysis of spinal cord transcriptomes in BAC-Q72 mice (Scoles et al. 2020). Scoles and colleagues identified DEGs in the innate immunity, the complement system, and lysosome/phagosome pathways and showed partial reversal of DEGs after ASO7 treatment. Of note, many DEGs and pathways overlapped with transcriptomes in other ALS mouse models and in those obtained by analysis of human postmortem ALS spinal cords.

TDP43 is a protein directly mutated in ALS patients and >90% of ALS patients have TDP43 aggregates in spinal neurons. Gitler and colleagues therefore analyzed whether *Atxn2* knockdown by genetic interaction or Atxn2 ASOs improved TDP43 toxicity in a mouse model overexpressing wild-type TDP43 (Becker et al. 2017). Both genetic interactions using ATXN2 knockout mice and *Atxn2*-ASO treatment by ICV injection at postnatal day 1 had a marked effect on survival in this model with some long-term survivors seen. Of note, median survival and percentage of long-term survivors increased with reducing Atxn2 abundance with the best effects seen with complete absence of Atxn2.

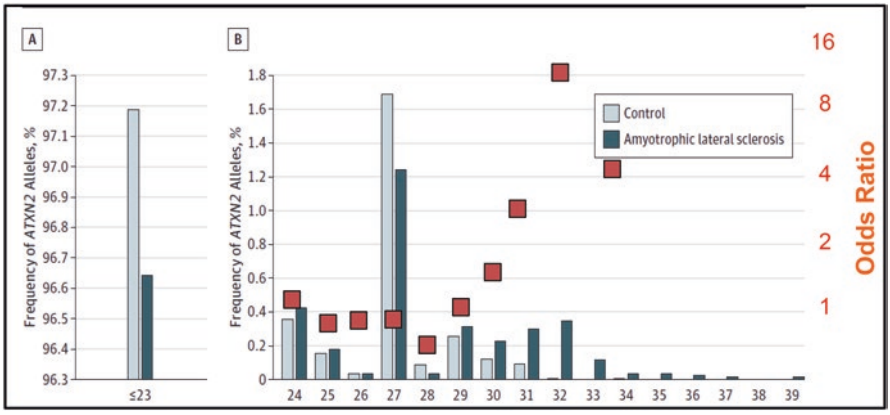

Fig. 3 Meta-analysis of the distribution of *ATXN2* repeat alleles in control and ALS populations. (**a**) Frequencies of the most common alleles with 22 and 23 repeats (rare alleles <22 are included). (**b**) Frequency of rare longer "normal" alleles. Risk of ALS does not increase until 30 repeats, but then increases steeply. Orange squares indicate odds ratio for developing ALS. (Modified from Neuenschwander et al. (2014))

3.1 Phase 1 Clinical Trial (BIIB105)

The encouraging preclinical proof-of-concept studies led to further development of improved ASOs to ATXN2 by Ionis Pharmaceuticals in collaboration with our group at the University of Utah. An IND for an ATXN2 ASO (Ionis 541) was filed on March 31, 2020 and a phase 1 dose escalation study supported by Biogen began on Sept 1, 2020, designated BIIB105. The study sponsor made the interesting decision to target wild-type ATXN2 in ALS patients, a population that does not carry mendelian deterministic *ATXN2* alleles, rather than mutant expanded *ATXN2* in SCA2 patients.

BIIB105 is in the process of recruiting two different ALS populations. The first consists of ALS patients without family history and without mutations in the *SOD1* or *FUS* genes that will receive increasing single doses of the Ionis541 ATXN2 ASO. It is interesting to note that the study sponsors did not opt to test for repeat expansions in the *C9ORF72* gene in this context, or for that matter, employed whole exome sequencing (WES) to look for other established ALS genes.

A second cohort is examining dose escalation in individuals with ALS and at least one ATXN2 allele with 30–33 CAG/CAA repeats. As of January 31, 2022, BIIB105 is ongoing and recruiting patients. It is hoped that successful dose finding in BIIB105 will lead to phase 1/2 trials in SCA2 patients.

4 Conclusions and Outlook

It took almost 50 years after the first description of SCA2 and 25 years after gene discovery to bring RNA-based therapies targeting ATXN2 into clinical trials. In 1996, only few could have imagined that ATXN2 alleles could act in a recessive and dominant fashion and also as risk alleles for other adult-onset neurodegenerative diseases. Animal models using expression of transgenes and Atxn2 knockout have been instrumental in understanding pathogenesis and were the basis for proof-of-principle studies in therapy development. ATXN2 is now being explored as valid target for the development of biologicals for SCA2 and other neurodegenerative diseases. ASO-based therapies may only represent the beginning and will likely be followed by viral and small molecule approaches.

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