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Clinical, genetic, molecular, and pathophysiological insights into spinocerebellar ataxia type 1

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Abstract
Spinocerebellar ataxia type 1 (SCA1) is a late onset neurodegenerative disease characterized by cerebellar ataxia with variable degrees of ophthalmoplegia, pyramidal and extrapyramidal signs, and peripheral neuropathy. SCA1 is caused by the toxic effects triggered by an expanded polyglutamine (polyQ) within the protein ataxin 1 (Atxn1) resulting in variable degrees of neurodegeneration in the cerebellum, brainstem, and spinocerebellar tracts. The toxic gain-of-function mechanisms by which the polyQ expansion induces neuronal cell death are not fully understood and no effective therapies are yet available. Alterations in transcriptional regulation, calcium homeostasis, glutamate signaling/excitotoxicity, and impaired protein degradation are few recurrent events in the pathogenesis in SCA1. However, elucidating the molecular routes regulated by ataxin 1 is leading to the discovery of new pathways that are implicated in SCA1. This suggests that dominant-negative effects exerted by the mutant protein, rather than just gain-of-function mechanisms, might be also implicated in SCA1 pathogenesis. The challenge now is to determine how these responses account for the clinical manifestation of the disease symptoms and, ultimately, how this knowledge can be translated into the development of therapeutic strategies. Herein, we review the phenotype and most recent advances in our understanding of the physiopathological mechanisms of neurodegeneration in SCA1.

Key words: *Spinocerebellar ataxia, SCA1, cerebellum, ataxin 1, polyglutamine, neurodegeneration, therapy*

Clinical, electrophysiological, and neuropathological features of SCA1

Spinocerebellar ataxia type 1 (SCA1, OMIM #164400) is a late onset autosomal dominant neurodegenerative disorder characterized by cerebellar ataxia associated with variable degrees of oculomotor abnormalities, pyramidal, extrapyramidal features, peripheral neuropathy, and cognitive impairment (1). SCA1 is one of the spinocerebellar ataxias that can be classified on clinical grounds as ADCA type I, according to Harding's classification (2,3). The age at onset of the disease is usually around the third decade of life, but can occur as early as 4 and as late as 74 years of age. The disease duration is usually 10–20 years from the age of onset. The majority of patients show a multisystemic involvement at early stages of the disease, 1–2 years of the disease duration. At the disease onset, the clinical signs consist of cerebellar ataxia syndrome, pyramidal signs, which sometimes even precede ataxia, and in most patients, ophthalmoplegia (4,5). As the disease progresses, other symptoms occur in variable degrees like dysphagia, dysphonia, tongue atrophy, deep sensory loss, peripheral sensory-motor axonal neuropathy, pes-cavus, amyotrophy, and fasciculations. Dystonia is the most common extrapyramidal symptom. Rarely, rigidity, tremor, and chorea have also been reported (5,6). Usually, the latter symptoms are present in the late stages of the disease. The clinical phenotype is more homogeneous than previously thought when disease duration and age at onset are taking into account (4). At the onset of the disease, ocular movement abnormalities include gaze-evoked nystagmus, impairment of vestibular ocular reflex, and increased amplitude of saccades with normal velocity (7,8). In half of the patients, the external ocular movements are full and in the other half there is upgaze or lateral gaze limitation. In later stages of the disease there is severe ophthalmoplegia in all directions with absent nystagmus and slow velocity in saccades. Neuropsychological features in SCA1 include impairment of executive dysfunction (5,9).

Efforts have been made to identify distinctive electrophysiological characteristics in genetically defined SCA subtypes (10). In SCA1, motor evoked

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potentials (MEP) recordings have frequently proved subclinical affection of the corticospinal tracts. Almost all SCA1 patients present with prolonged central motor conduction times (CMCT) and peripheral motor conduction times (PMCT) in legs and upper extremities (11). Since MEP from leg muscles are lost in several cases and no longer enable determination of CMCT, recordings from hand muscles are predestined to indicate characteristic changes of SCA1. Using magnetic stimulation of the motor cortex and cervical radices, complete differentiation of SCA1 from other genotypes was demonstrated in a large cohort of SCA patients with CMCT to the first dorsal interosseus >10 ms and PMCT >18 ms characteristic of the SCA1 group (10). Nerve conduction studies have revealed slowing of sensory and motor nerves in most patients with SCA1.

Structural neuroimaging in SCA1 shows olivopontocerebellar atrophy similar to SCA2 and SCA3 (Figure 1). No abnormalities are identified in T2-weighted images. Functional magnetic resonance (MR) imaging identifies reduction in volume of the cerebellum and the levels of N-Acetylaspartate (NAA) in the cerebellum in SCA1 as well as SCA2. A recent MR functional study reported correlation between the brainstem changes and the neurological deficits in SCA1 (12). Position emission tomography (PET) has been used to identify functional impairment in the brains of spinocerebellar patients, in particular in SCAs 2 and 3, and to correlate this with the severity of ataxia. Interestingly, the cerebellar metabolism did not correlate with the severity of ataxia in SCA patients (13). Significant differences were detected when healthy controls were included. In another PET study focusing on speech with different SCA subtypes, including SCA1, significant reductions in mean regional blood flow were correlated in the cerebellum, but not in supratentorial regions in ataxic subjects (14).

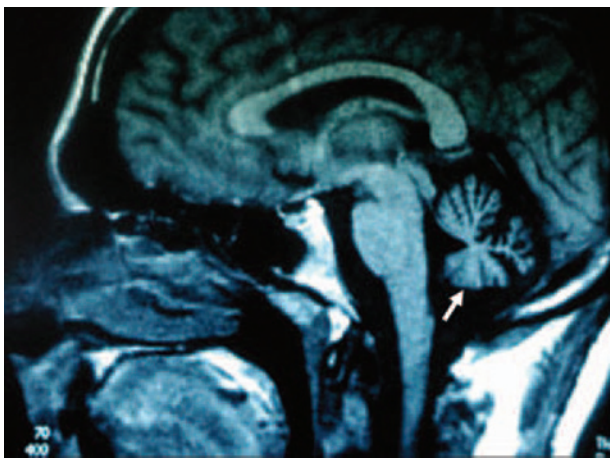

Figure 1. Characteristic T1-weighted mid-sagittal neuroimage of a SCA1 patient with disease symptoms existing for 10 years showing marked atrophy of the cerebellar vermis and brainstem.

The sites of neurodegeneration in SCA1 in the cerebellum include neurons in the dentate nuclei and Purkinje cells (PC), with several torpedo-like formations in their axons, Bergman's gliosis, variable loss of granule cells, and some atrophy of the middle cerebellar peduncles (4,5,15). Basal ganglia nuclei are found relatively spared. The substantia nigra has been found mildly depigmented with variable degrees of neuronal loss and gliosis. In the mesencephalon, nuclei of the 3rd and 4th cranial nerves and the dorsal nucleus of the raphe show severe neuronal loss. Atrophy of the ventral part of the pons is remarkable, with reduced number of neurons and increased number of astrocytes in the pontine nuclei. In bulbar sections, the inferior olivary nuclei are severely affected. Involvement of the reticular formation, nucleus ambiguus, dorsal nucleus of the vagus nerve, and neuronal loss of hypoglossal nuclei vary from one patient to another. In the spinal cord, there is diffused demyelination of posterior columns and of the spinocerebellar tracts. A mild loss of motor neurons is found at the cervical and lumbar enlargement. Examination of the posterior and anterior roots revealed a mild loss of fibers, more evident posteriorly. Golgi and calbindin immunocytochemistry studies show loss of cerebellar Purkinje cell dendrites, reduced dendritic trees, decreased formation of proximal spines, abnormal variable accumulation of neurofilaments, and early formation of axonal spheroids (15).

Inheritance of the SCA1 mutation

The basic genetic defect in SCA1 consists of the expansion of a translated trinucleotide CAG repeat located within exon 8 of the *SCA1* gene (16). The CAG repeat is highly polymorphic in the normal population and the number varies between 4 and 39 repeats (17). In SCA1, the CAG repeat is unstable and expands to about 40–83 repeats. Longer expansions result in earlier age of onset and more severe clinical manifestations of the disease (17–19). Strikingly, most paternal transmissions of the mutant *SCA1* allele are highly unstable and show an increase in repeat number, whereas most maternal transmissions show no change or a decrease in repeat number. The instability of the CAG repeat, predominantly through paternal transmission, is the molecular basis of a phenomenon called anticipation in SCA1. Unexpanded *SCA1* alleles have an interrupted repeat configuration, whereas a contiguous uninterrupted repeat $(CAG)_n$ is found in expanded alleles (20). While expanded alleles have been identified in control chromosomes, they contain CAT interruptions (21). This indicates that the repeat instability in SCA1 is more complex than a simple variation in repeat number and that the loss of CAT interruptions predisposes the mutant alleles containing $\geqslant 40$ CAG repeats to expansion. It also

indicates that in addition to the repeat number, the configuration in expanded alleles is also important to determine pathogenesis. The CAG repeat encodes for a polyglutamine (polyQ) tract, and the disease symptoms are triggered by the expanded polyglutamine located within the N-terminus region of a novel protein denoted ataxin 1 (Atxn1) (16). Thus, together with SCA1, at least eight additional inherited neurodegenerative diseases, including Huntington's disease (HD), Spinobulbar muscular atrophy, dentatorubral pallidoluysian atrophy (DRPLA), and SCA subtypes 2, 3, 6, 7, and 17 are currently caused by expansions of glutamine-encoding repeats in genes whose sequences are otherwise unrelated (22). Compelling evidence indicates that common molecular pathways and biological mechanisms might underlie neurodegeneration in these diseases.

What are the mechanisms of neurodegeneration in SCA1?

The mechanisms mediating SCA1 pathogenesis are still not completely understood, but some general principles have emerged. Genetic studies in mice and flies support a toxic gain-of-function mechanism since mice lacking Atxn1 do not develop ataxia or cerebellar Purkinje cell pathology (23). This argues against a loss-of-function or haploinsufficiency of ataxin 1 as the underlying pathogenic mechanisms in SCA1. Lines of transgenic mice expressing a mutant human *SCA1* allele have shown that eventual development of ataxia is not attributable to cell death *per se*, but to neuronal dysfunction and morphological alterations that occur long before ataxia and cell loss (24,25). The earliest time examined at which cerebellar Purkinje cells from SCA1 mice show morphological differences when compared to those of control animals is at P25 (25). At this time, PC somata contain cytoplasmic vacuoles in the form of distended cisternal structures that probably originate from invagination of the outer cell membrane since they contain proteins from the somatodendritic membrane, including mGluR1, GluRΔ1/Δ2, GluR2/3, and PKCγ (26). At 4 weeks of age, single large intranuclear aggregates containing mutant Atxn1 are detected in a subset of PC. By 5 weeks of age, PC show decreased proximal branches, reduced dendritic arbors, and smaller cell bodies when compared to control counterparts. At 8 weeks of age, mild gliosis is detected in the cerebellar molecular layer. Mild cerebellar cell loss is first observed at 12 weeks of age. Shortly thereafter, at 15 weeks of age, there is obvious shrinkage of the cerebellar molecular layer and the presence of heterotopic PC. Albeit subtle motor deficits are first detected in mutant mice on the rotating-rod at 5 weeks of age in the absence of deficits in balance and motor coordination, ataxia is not first apparent until 12 weeks of age. Thus, significant neuropathology develops in PC of *SCA1* transgenic mice before the onset of ataxia. Overall, these findings are consistently similar to those observed in knockin mice with a targeted expansion of 154 CAG repeats within the murine *Sca1* gene ($Sca1^{2Q/154Q}$) (27).

The basal electrophysiological properties of PC in SCA1 transgenic mice are similar to WT neurons (28), in spite of their morphological and molecular differences such as the presence of intranuclear inclusions in mutant PC, and the fact that expression of some important Ca^{2+}/Glu-regulating molecules differ in the two cell types (29,30). Electrophysiological studies performed in knockin $Sca1^{2Q/154Q}$ mice revealed age-dependent derangement of hippocampal synaptic plasticity, in agreement with the learning and memory cognitive deficits observed (27). Strikingly, no synaptic alterations (at 5 weeks of age) in climbing fiber-mediated (CF) and parallel fiber-mediated (PF) cerebellar excitatory post-synaptic currents (EPSC) were detected. Since this study did not address cerebellar synaptic function in mutant mice at later stages of the disease, it is not possible to evaluate the effects, if any, of the intranuclear inclusions in the properties of Purkinje cell electrophysiology.

It is generally assumed that the expanded polyglutamine causes the mutant ataxin 1 to misfold, adopt aberrant conformations leading to exposure of hydrophobic residues, and form insoluble aggregates in the nucleus, which lead to neuronal dysfunction and eventually cell death in SCA1 (22). Intranuclear insoluble inclusions are neuropathological hallmarks identified in SCA1 post-mortem human and mouse brain tissues. Studies with animal models and patient tissue indicate that in addition to expanded ataxin 1, the nuclear protein accumulations contain chaperones and components of the ubiquitin-proteasome system, such as ubiquitin and proteasome subunits (31–33). This suggests that the misfolded protein triggers a stress response to diminish the amount of mutant ataxin 1 thus playing a key role in toxicity. A possible mechanism for aggregate formation by the mutant protein would be by loss of native state stability by the expanded polyglutamine and, thus, leading to the formation and accumulation of a partially unfolded, aggregation-prone protein, resulting in fibrillization. This might account for the earlier age of onset and increased severity of disease symptoms observed when mutant ataxin 1 contains a longer stretch of glutamines. A number of suppressors of neurodegeneration in animal models have coupled reduced protein accumulation or modulation of ability to handle misfolded mutant Atxn1, to mitigate degeneration (34,35). Alterations in protein conformation due to the expanded polyglutamine may also enable mutant ataxin 1 to recruit cellular proteins through a series of aberrant interactions

(Table I). This could explain the restricted cytotoxicity observed in a few neuronal subtypes of the CNS in SCA1 despite the fact that ataxin 1 is expressed systemically, most likely through alterations of cell-type-restricted functions. In this regard, Anp32a/Lanp stands out as an appealing candidate to mediate selective SCA1 neurodegeneration for, among a few reasons, its ability to interact with mutant ataxin 1 and the fact that Anp32a/Lanp expression is confined to the primary sites of SCA1 pathology including cerebellar Purkinje cells and pontine nuclei neurons (36, Matilla-Dueñas, unpublished data). This might explain the early onset selective cerebellar and brainstem deficits identified in early stages of the disease in SCA1 patients. Evidence from transgenic mice has shown that the polyglutamine expansion stabilizes mutant ataxin leading to its accumulation (34). This might underlie some of the toxic gain-of-function effects exerted by mutant ataxin 1 in SCA1. Since protein-protein interactions are sensitive to the conformational state of proteins and because the conformation of the polyglutamine expanded ataxin 1 differs from that of the wild-type protein, disruption of interactions or creation of new ones involving the participation of ataxin 1 are probably an occurring mechanism during the pathogenic process.

While the role of the polyglutamine expansion in mediating SCA1 pathogenesis is well established, it is also becoming increasingly clear the contribution of the protein framework, in addition to the expanded polyglutamine, to the pathogenic mechanisms of neurodegeneration. Regions outside the polyglutamine sequence dramatically alter SCA1 pathogenesis. Both the AXH (Ataxin 1/HMG-box protein 1) domain (SMART Database accession number SM00536), a highly conserved globular module near the C-terminal region of ataxin 1 exhibiting significant sequence similarity to a region in the High-mobility group box 1 (HBP1) (37,38) and boat (35) transcription factors, and phosphorylation of Atxn1 in Ser776 (34,39), appear to determine neuronal toxicity in flies and mice (35,40). Transcriptional dysregulation mediated by the soluble non-aggregated form of the mutant protein of genes encoding for calcium homeostasis and glutamate signaling at early stages of Purkinje cell dysfunction, is an important feature of the pathogenic mechanisms (29,30). Importantly, this seems to precede the onset of disease symptoms in SCA1, which might result from alterations of protein-protein interactions with at least 12 transcriptional co-regulators (Table I), including the cerebellar leucine-rich acidic nuclear protein Anp32a/Lanp (36), polyQ-binding protein (PQBP1) (41), silencing mediator of retinoid/thyroid hormone receptors (SMRT) (42), Boat (35), Gfi-1/Senseless (40), and Sp1 (Goold and Matilla-Dueñas, unpublished data), which have all been shown to bind ataxin 1 *in vitro* and/or *in vivo* studies. These observations support the view that the expanded polyglutamine is likely to interfere with the cellular function of ataxin 1 and highlight the possible contribution of the biological function of the protein in SCA1 pathogenesis.

Potential biological functions of ataxin 1

Ataxin 1 is a 98-kDa soluble protein of about 816 amino acids depending on the number of glutamines included. It is widely expressed in humans and mice, with a nuclear localization in neurons and a cytoplasmic localization in non-neuronal cells (43). In cerebellar Purkinje cells, the primary neuropathological target in SCA1, Atxn1 has both a nuclear and cytoplasmic distribution. This widespread localization of Atxn1 has not helped to understand its function. Within the nucleus, Atxn1 is found co-localizing with the nuclear matrix associated promyelocytic leukemia protein (PML) in PML-oncogenic domains (POD) (36,44). Several structural domains have been identified in ataxin 1 (Figure 2A). In addition to the amino terminal polyQ region, which aggregates through a beta rich conformation in agreement with the polar zipper structure proposed by Max Perutz, ataxin 1 has four low complexity regions (aa 47–64, 88–99, 154–169, 366–377), and an AXH domain (aa 570–689), a highly conserved globular module near the C-terminus exhibiting significant sequence similarity to a region in the high-mobility group box 1 (HBP1) and boat transcription factors (35,37,38). Determination of the structure of the AXH domain in Atxn1 (Figure 2B) has shown that it forms stable homodimers and contains an OB-fold (45,46), a structural motif found in many oligonucleotide-binding proteins, supporting the proposed role of Atxn1 in RNA binding (47). Furthermore, the AXH module has a cluster of charged surface residues that provide a surface for protein-protein interactions. Ataxin 1 also contains a functionally active carboxy(C)-terminal nuclear localization signal (NLS), which targets the protein from the cytoplasm to the nucleus (48), and altering the NLS sequence causes Atxn1 to accumulate in the cytoplasm. Atxn1 is subject to a variety of post-translational modifications including phosphorylation at Ser776 (39), sumoylation at multiple residues (49), and ubiquitination (50). Several proteins have been shown to interact with ataxin 1, and most of the interactions occur through the C-terminus region containing the AXH module (Table I). Among the known Atxn1 binding partners, at least 16 are implicated in regulation of transcription and 9 in RNA binding/metabolism. Besides its role in transcription, the fact that ataxin 1 is capable of binding to RNA (amino acids 541–767) points to a possible role for Atxn1 in nuclear and cytoplamisc functions typical of RNA

Table I. Proteins interacting with ataxin 1.

UniProt	Protein	Interaction shown by	Interaction regions within Atxn1	Does PolyQ modulate interaction?	Biological significance of interaction	Reference
P54253	ITSELF	2HS	SAR	No	Unknown	(60)
Q9NRR5	A1UP/UBQLN4	2HS, IF	SAR	No	Degradation by UPS, targets proteins to the ER	(61,62)
Q9NWB1	A2BP1	2HS, AP	Not shown	Unknown	RNA binding, translation regulation	(62)
P39687	ANP32A/LANP	2HS, IF, IP	PolyQ, C-terminus	Yes	Phosphorylation, transcription regulation	(36, Matilla, unpublished)
Q99700	ATXN2	2HS, AP	Not shown	Unknown	Unknown	(62)
P48634	BAT2	2HS, AP	Not shown	Unknown	pre-mRNA splicing	(62)
Unassigned	BOAT	2HS, IF, IP	SAR	No	Transcription regulation	(35)
Q6P1W5	C1ORF94	2HS, AP	Not shown	Unknown	Unknown	(62)
O755909	CCNK	2HS, AP	Not shown	Unknown	Transcription regulation	(62)
P23528	CFL1	2HS, AP	Not shown	Unknown	Cytoskeleton	(62)
Q96RK0	CIC	2HS, AP	Not shown	Unknown	Transcription regulation	(62)
P38432	COIL/P80	2HS, AP, IP	C-terminus	No	snRNP maturation, pre-RNA splicing	(62,63)
P46108	CRK	2HS, AP	Not shown	Unknown	Signal transduction	(62)
Q15038	DAZAP2	2HS, AP	Not shown	Unknown	Transcription regulation	(62)
Q8TE02	DERP6	2HS, AP	Not shown	Unknown	Transcription regulation	(62)
Q96EY1	DNAJA3	2HS, AP	Not shown	Unknown	Modulation of apoptosis, transcription regulation	(62)
Q8N684	FLJ12529	2HS, AP	Not shown	Unknown	Pre-mRNA 3' processing	(62)
P04406	GAPDH	2HS, IP	N-terminus	No	Unknown	(64)
Q99684	GFI-1/ SENSELESS	2HS, IP	AXH	No	Transcription regulation	(40)
O15379	HDAC3	IF, IP, TAP	Not shown	Yes	Transcription regulation	(42, Goold and Matilla, unpublished)
P15822	HIVEP1	2HS, AP	Not shown	Unknown	Transcription regulation	(62)
P53990	KIAA0174	2HS, AP	Not shown	Unknown	Unknown	(62)
Q53G59	KLHL12	2HS, AP	Not shown	Unknown	Unknown	(62)
Q9Y618	NCOR2/SMRT	2HS, IF, IP	C-terminus (most likely AXH)	No	Transcription regulation	(42)
O43809	NUDT21	2HS, AP	Not shown	Unknown	Pre-mRNA processing	(62)
Q9HAU0	PLEKHA5	2HS, AP	Not shown	Unknown	Unknown	(62)
O60828	PQBP1	IF, IP	Not shown	Yes	Transcription regulation	(41)
Q14671	PUM1	2HS, AP	Not shown	Unknown	RNA binding, translation regulation	(62)
O43251	RBM9	2HS, AP	Not shown	Unknown	RNA binding, translation regulation	(62)
Q93062	RBPMS	2HS, AP	N-terminus	Unknown	RNA binding, translation regulation	(62)
Q8N196	SIX5	2HS, AP	Not shown	Unknown	Transcription regulation	(62)
Q96T58	SPEN	2HS, AP	Not shown	Unknown	Transcription regulation	(62)
P080473	SP1	IP, TAP	AXH	Yes	Transcription regulation	(53)
Q9UNE7	STUB1/CHIP	IF, IP	Not shown	No	Ubiquitination. modulation of chaperon activity	(50)
Q92609	TBC1D5	2HS, AP	Not shown	Unknown	Signal transduction	(62)
Q12933	TRAF2	2HS, AP	Not shown	Unknown	Signal transduction	(62)
Q13049	TRIM32	2HS, AP	Not shown	Unknown	Protein ubiquitination	(62)
P26368	U2AF2	2HS, AP	Not shown	Unknown	Pre-mRNA splicing	(62)
Q6P1N6	USP54	2HS, AP	Not shown	Unknown	Deubiquitination	(62)
Q93009	USP7	2HS, IF, IP	C-terminus	Yes	Deubiquitination, stabilization	(65)
P62258/ P63104	YWHAE/YWHAZ	2HS, IP	C-terminus	Yes	Stabilization, signal transduction	(34)
Q9H869	YY1AP1	2HS, AP	Not shown	Unknown	Transcription regulation	(62)
Q9UKY1	ZXH1	2HS, AP	Not shown	Unknown	Transcription regulation	(62)

2HS, two-hybrid system; AP, affinity purification; IF, immunofluorescence; IP, immunoprecipitation; SAR, self-association region; TAP, tandem affinity purification.

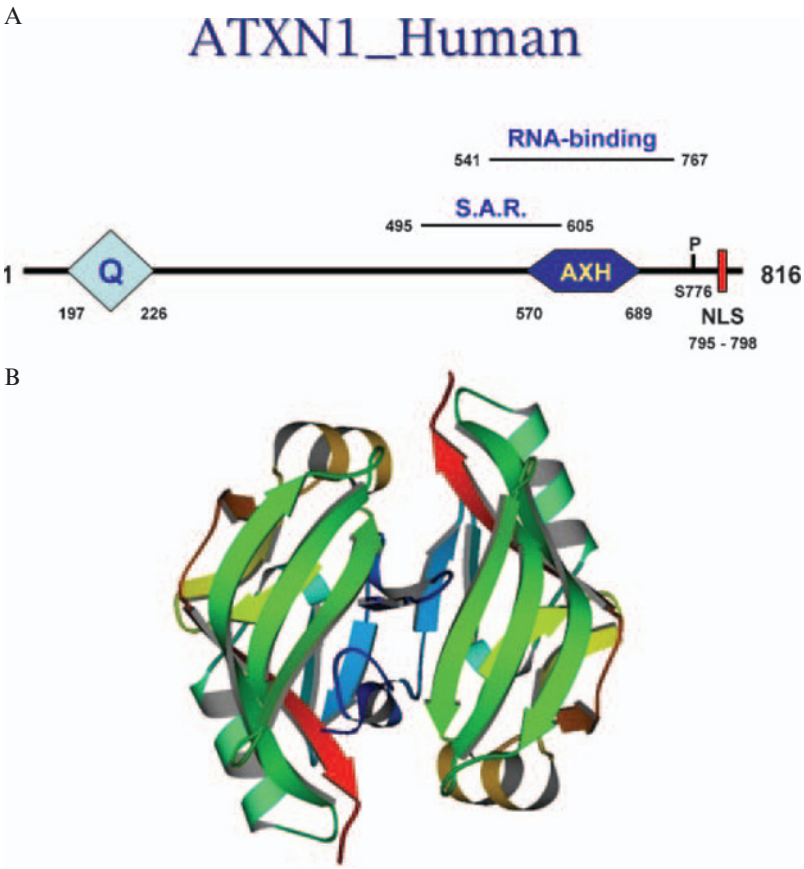

Figure 2. Functional regions within ataxin 1. (A) The localization of the polyglutamine (polyQ) tract, the AXH module, the nuclear localization signal (NLS), the self-associating region (S.A.R.), and the known regions participating in RNA binding and protein-protein interactions (adapted from 38). (B) Structure of the AXH domain in Atxn1. The AXH domain is dimeric, contains an OB-fold motif, and a cluster of charged surface residues (from 45).

binding proteins, including regulation of RNA splicing, mRNA stability, mRNA transport and nuclear export, or mRNA translation (47,51).

While much effort has been dedicated to elucidate the molecular pathogenic mechanisms underlying neurodegeneration in SCA1, only a few studies have addressed the biological function of ataxin 1. During cerebellar development there is a transient burst of mRNA expression of Atxn1 at postnatal day 14, when the murine cerebellar cortex becomes physiologically functional (52). This indicates a possible functional role for Atxn1 at specific stages of cerebellar development. Loss of ataxin 1 function in Atxn1-null (*Sca1*$^{-/-}$) mice leads to hippocampal alterations in short-term synaptic plasticity and several neurobehavioral abnormalities in the absence of apparent neuroanatomical abnormalities (23). They include exploratory behavior in a novel environment, spatial learning impairment, and deficits in motor and locomotor activities. This evidence led us to propose a role for ataxin 1 in cerebellar motor functions and some forms of learning and memory. Recent evidence has shown that the motor and locomotion deficits observed in Atxn1-null mice are most likely caused by transcriptional dysregulation of genetic programs implicated in motor functions including the dopaminergic-signaling pathway (Matilla-Dueñas and Goold, unpublished).

Ataxin 1 regulates genetic programs involved in motor functions in mice

Microarray analysis has recently revealed that loss of ataxin 1 function leads to dysregulation of expression of genes implicated in translational initiation, Wnt-receptor signaling, RA/Thyroid hormone signaling, nucleic acid binding, and intracellular signaling cascades (53). These findings and the fact that HBP1 transcription factor also regulates genes of the Wnt pathway putatively through its AXH domain (54), provide new evidence linking Atxn1 and its AXH module in Wnt-signaling. Wnt-signaling interacts with RA-signaling to regulate neurogenesis, neuronal differentiation, and proper specification (55,56). Alterations in Wnt receptor- and RA-signaling in Atxn1-null mice suggest that Atxn1 might regulate genetic programs required for proper neuronal specification during development. Strikingly, expression levels of the dopamine receptor D2 (Drd2) are significantly reduced in cerebellum of null mice lacking Atxn1 and in young 5-weeks-old SCA1 transgenic mice expressing human ataxin 1 with an expanded polyglutamine in

cerebellar Purkinje cells (53). This suggests that signaling pathways that are regulated by ataxin 1 are also dysregulated in SCA1, and supports the view that the biological function of the protein contributes to SCA1 pathogenesis. Recent evidence has shown that Atxn1 interacts and functions synergistically with zinc-finger transcription factor Sp1 to co-regulate Drd2 expression (53). The interaction, transcriptional effects, and occupancy of the Drd2 promoter by ataxin 1 are mediated by the AXH module within Atxn1 and are abrogated by the expanded polyglutamine in the mutant protein. Drd2 receptors modulate ion channels, phospholipases, receptor tyrosine kinases, glutamatergic and GABAergic neurotransmission, and stimulate MAP kinase pathways (57). Remarkably, ablation of Drd2 function in mice results in reduced motor and locomotor activities, associative deficits, altered drug-responses, and modification of the electrophysiological characteristics of Drd2-expressing neurons (58). These observations highlight the role of the Drd2-dependent dopaminergic-signaling pathway in regulating motor behaviors and some forms of learning and memory, and point to possible alterations of Drd2-dependent functions underlying the neurobehavioral deficits in Atxn1-null mice and the early motor deficits in SCA1.

Towards therapeutic strategies in SCA1

Although the *SCA1* gene has been known for 14 years (16), its identification has not helped to create effective medicines or prevention strategies yet. It is therefore important to give priority to pharmacogenetic research with potentially high clinical relevance. Genome- and proteome-wide approaches, such as oligonucleotide microarrays and proteomics respectively, have provided one direction for supplementing our knowledge of cellular pathways and molecular routes that might be relevant to drug response, for instance calcium homeostasis, glutamate excitotoxicity, and dopaminergic signaling. These studies are enabling to increase the number of potential targets identified for treatment. We have learnt that transcriptional dysregulation occurs at early stages during SCA1 pathogenesis (29,30). Therefore, targeting transcriptional regulation by modulating histone acetylation/deacetylation is currently being exploited as a possible strategy for therapy. Unfortunately, disappointing results in a few studies have discouraged the use of modulators of histone acetylation to treat neurodegeneration due to lack of target specificity and low effectivity. In contrast, silencing the *SCA1* mutant allele using RNA interference is a strategy that has proved useful to prevent initiation of a cascade of neurotoxic events and, thus, it has decreased intracellular aggregates and improved the behavioral phenotype in SCA1 mice (59). Making mutant Atxn1 less toxic by modifying a phosphorylation site has proved capable of preventing ataxia and neurodegeneration (39). PI3K/Akt signaling, which activates pro-survival pathways, is found commonly dysregulated in neurodegenerative diseases, including SCA1 (34). Therefore, manipulating this downstream signaling pathway may provide a rational strategy for the therapeutic intervention of SCA1 and other neurodegenerative conditions. Increasing number of studies are evidencing the contribution of the biological function of ataxin 1 in mediating pathogenesis, and are pointing to previously unidentified targets that could be used in therapeutic strategies. In this regard, molecular pathways regulated by ataxin 1 recently identified, such as the retinoid/thyroid-, Wnt-, and Drd2-dopaminergic-signaling pathways, which all appear to modulate brain motor functions, are most likely implicated in SCA1 (Matilla-Dueñas, unpublished). This suggests that dominant-negative effects, rather than just gain-of-function mechanisms might be implicated in pathogenesis, predominantly occurring at the initial stages of the disease. Therefore, identifying the cellular pathways regulated by ataxin 1 is leading to comprehend the molecular mechanisms mediating neurodegeneration and offers the opportunity to identify potential therapeutic targets that could be used to prevent neuronal dysfunction and motor deficits at presymptomatic stages of the disease in SCA1 patients.

Concluding remarks

Intensive ongoing research is providing a better understanding of the mechanisms of neurodegeneration implicated in spinocerebellar ataxia type 1, which is leading to the identification of potential targets for developing therapeutic strategies. Since most human neurodegenerative conditions are caused by disruption of common molecular pathways, this offers the unique opportunity to design therapeutic strategies to halt or prevent neuronal dysfunction and death in patients with SCA1 and other neurodegenerative diseases.

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