The Cerebellum and its Disorders

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Spinocerebellar ataxia type 6

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Introduction

Spinocerebellar ataxia type 6 (SCA6) belongs to the group of autosomal dominant cerebellar ataxias (ADCAs) as well as to that of channelopathies (Ptacek, 1997). Its highly variable phenotype defies a clear-cut classification in any of the three major types of ADCAs, as defined by Harding (1982), though sharing the type of mutation (expansion of a CAG repeat stretch) with SCA1, SCA2, SCA3, and SCA7. The expandable sequence is embedded in a calcium channel gene, *CACNA1A*, on chromosome 19p13, coding for the α_{1A} subunit of voltage-gated calcium channels type P/Q, expressed predominantly in cerebellar Purkinje and granule cells. In many respects, SCA6 differs from ADCAs with the same type of mutation, and is unique among channelopathies for being due to the expansion of a repeat sequence.

Point mutations at the *CACNA1A* gene are known to cause episodic ataxia type 2 (EA2) and familial hemiplegic migraine (Ophoff et al., 1996). These disorders, and particularly EA2, share some features with SCA6, raising the problem of the relationship among the three allelic diseases.

Clinical features

SCA6 exhibits a highly variable clinical picture. In some studies (e.g., Zhuchenko et al., 1997 ; Geschwind et al., 1997; Filla et al., 1999) the phenotype includes a multisystem in addition to a cerebellar involvement, as observed in ADCA type I; in others (Ishikawa et al., 1997; Nagai et al., 1998; Watanabe et al., 1998; Garcia-Planells et al., 1999) the disease has been classified as a pure cerebellar ataxia (ADCA type III); and in others (Jodice et al., 1997; Jen et al., 1998; Yabe et al., 1998) it showed the features of EA2, with ataxia/vertigo episodes and interictal cerebellar deficits with variable degree of severity.

The clinical picture was first described by Zhuchenko et al. (1997) as a late-onset, slowly progressive cerebellar ataxia with nystagmus and dysarthria, brainstem involvement, vibratory and proprioceptive sensory loss, and an insidious onset characterized by 'wooziness' and momentary imbalance. The permanent and progressive character of the cerebellar deficit, the coexisting brainstem, and sensory involvement led these authors to consider SCA6 as a disorder distinct from EA2. However, a more complex picture emerged from the subsequent studies. Patients may initially experience episodes of ataxia and dysarthria, and/or of vertigo and nausea, accompanied by visual disturbance (such as dyplopia or blurred vision), and tinnitus, lasting from minutes to days and triggered by movements and physical or emotional stress. Episodes have a variable frequency (from yearly to daily) and duration (from seconds to days). Interictally, patients may be neurologically normal or show mild cerebellar signs such as gaze-evoked nystagmus and/or saccadic pursuit, mild dysarthria and dysmetria, but no overt limb or trunk ataxia (Calandriello et al., 1996; Geschwindt et al., 1997; Jodice et al., 1997) . This early phase may have a variable duration. It commonly lasts a few years before the onset of a permanent and progressive ataxia, but in some cases the disease may not progress towards a full-blown disorder (Calandriello et al., 1996; Jodice et al., 1997, Takiyama et al., 1998). Other studies, on the contrary, do not report an episodic phase (Ikeuchi et al., 1997; Matsumura et al., 1997; Stevanin et al., 1997). However, one should bear in mind that episodes may be overlooked or attributed to other causes such as low blood pressure. Patients tend to seek a neurologist's advice when ataxia becomes permanent, and the preceding episodic phase often emerges only after a prolonged and specific enquiry about it.

Unbalance and gait difficulties usually herald the transition to a permanent and slowly progressive ataxia. The neurological examination commonly shows trunk and limb ataxia, dysmetria, dysarthria, hypotonia, and a pattern of ocular movement abnormalities, similar to that described for EA2: gaze evoked nystagmus often without a downbeat component, dysmetric saccades, saccadic pursuit, and hyperactive vestibulo-ocular reflex (Gomez et al., 1997). Vertigo episodes may continue during this second phase (Gomez et al., 1997) and periodic exacerbations of the cerebellar signs can be present (Yabe et al., 1998). The clinical picture is often limited to a pure cerebellar deficit, but deep sensory deficits, ophthalmoplegia, hyperreflexia or hyporeflexia, dysphagia, and extrapyramidal signs are sometimes also reported (Geschwind et al., 1997 ; Stevanin et al., 1997; Zhuchenko et al., 1997). These latter features, however, are found more frequently in old subjects (Ikeuchi et al., 1997; Ishikawa et al., 1997; Stevanin et al., 1997) or in patients with other coexisting disorders, such as diabetes mellitus (Takiyama et al., 1998).

Neuroimaging reveals a cerebellar vermis atrophy, with or without the involvement of cerebellar hemispheres, with preservation of brainstem (Calandriello et al., 1996; Gomez et al., 1997; Jodice et al., 1997; Nagai et al., 1998; Satoh et al., 1998; Shizuka et al., 1998a; Takiyama et al., 1998). Occasionally, a size reduction of the pons has been reported (Murata et al., 1998; Arpa et al., 1999; Nakagawa et al., 1999).

When treatment with acetazolamide was tried, by analogy with EA2, patients showed a reduced frequency, duration, and severity of episodes, with little effect on permanent symptoms (Calandriello et al., 1996; Jen et al., 1998).

Neuropathology

Macroscopically, the brains of autopsied patients show a marked atrophy of the cerebellar vermis and, to a lesser extent, of the hemispheres. Microscopically, the cerebellar cortex is characterized by a remarkable loss of Purkinje cells. Granule cells are also affected, although less severely. Loss of neurons is sometimes found in the dentate and inferior olivary nuclei (Subramony et al., 1996; Gomez et al., 1997; Sasaki et al., 1998; Ishikawa et al., 1999b; Tashiro et al., 1999). Atrophy of brainstem has occasionally been described (Zhuchenko et al., 1997).

No ubiquitin immunoreactive nuclear inclusions, similar to those observed in other CAG repeat expansion disorders, have been found. However, non-ubiquitinated cytoplasmic protein aggregates, detected by anti-α_{1A} subunit antibodies, have been described in Purkinje cells (Ishikawa et al., 1999a, 1999b)

Natural history

It may be difficult to define age at onset when, as in this case, early signs are insidious and often overlooked by patients and general practitioners. In most studies, age at onset refers to the beginning of permanent gait imbalance and is, on average, around 50, with a range from 19 to 73 years (Gomez et al., 1997; Matsumura et al., 1997; Watanabe et al., 1998). As in other CAG expansion disorders, a significant inverse correlation between age at onset and size of expanded alleles has been reported in several studies (e.g., Ikeuchi et al., 1997; Ishikawa et al., 1997; Riess et al., 1997; Zhuchenko et al., 1997). Anticipation of age at onset over successive generations has been observed (e.g., Ikeuchi et al., 1997; Matsumura et al., 1997; Matsuyama et al., 1997; Watanabe et al., 1998), although in the absence of an intergenerational variation of the expanded allele size (see below). This phenomenon might be due to ascertainment biases, as pointed out by Penrose (1948). An offspring with an earlier onset than the parent is, in fact, more likely to be encountered in clinical practice, than the reverse situation. In addition, an accurate assessment of older generations might be more difficult, as a consequence of less strict diagnostic criteria in the past, or of fading memories about the exact period in which the first symptoms arose.

The disease usually progresses very slowly towards inability: autonomous walking has been observed even after 18–20 years from the onset (Geschwind et al., 1997). However, a very rapid course has also been reported (Watanabe et al., 1998). The lifespan appears to be normal, although no statistical analysis is available.

Inheritance and mutation

SCA6 is an autosomal dominant disorder, due to the expansion of a small polymorphic CAG repeat stretch at the 3′ end of the *CACNA1A* gene. Normal alleles range from 4 to 18 units (Zhuchenko et al., 1997; Shizuka et al., 1998a, 1998b). Expanded alleles range from 20 to 30 repeats (Jodice et al., 1997; Matsuyama et al., 1997 ; Shizuka et al., 1998a, 1998b), i.e., a size well within the normal range for other CAG expansion disorders. A complete sequence analysis of the gene coding region in a SCA6 patient showed that the expansion was the only detectable mutation (Jodice et al., 1997).

Homozygous patients do not differ substantially from heterozygous ones, showing a slightly earlier onset and a more rapid course (Geschwind et al., 1997; Matsumura et al., 1997; Takiyama et al., 1998).

The size of SCA6 expanded alleles is more stable than that of other repeat expansion disorders, as expected on the basis of its relatively low number of units. No variation is usually observed in families over successive generations, and no mosaicism is apparent in cells from different parts of the brain (Ishikawa et al., 1999b) or in sperm (Shizuka et al., 1998b). However, some degree of meiotic instability should be assumed, because in two families an intergenerational jump of the expanded allele size has been reported (Jodice et al., 1997; Matsuyama et al., 1997).

SCA6 expanded alleles have been found in a number of sporadic ataxia cases (Ikeuchi et al., 1997; Matsumura et al., 1997; Riess et al., 1997; Zhuchenko et al., 1997; Shizuka et al., 1998b), but a new mutation has been documented in one patient only (Shizuka et al., 1998a). Should all these cases be new mutations, the mutability of normal alleles would be very high, particularly if compared to that of SCA1, SCA2, or SCA3 alleles, for which an expansion has never (SCA1 and SCA2) or rarely (SCA3) been found among sporadic cases (Andrew et al., 1999). In addition, if a mutation/selection equilibrium is assumed, a high frequency of SCA6 de-novo mutations would be in contrast with the (presumably) small or absent selection against a disease, such as this one, with a very late onset and a long lifespan of patients. Incomplete penetrance or the presence of neglected mild episodic symptoms in relatives appears to be a more likely explanation for the high number of sporadic cases.

The gene and the protein

The *CACNA1A* gene encodes for the α_{1A} subunit of the voltage-gated calcium channel type P/Q, a pore-forming membrane protein. The gene maps on chromosome 19p13.1–p13.2 (Diriong et al., 1995) and covers about 300 kb with 47 exons (Ophoff et al., 1996).

The cDNA clones predict large peptides with molecular masses of 200 to 275 kDa with four homologous domains (I–IV), each containing six hydrophobic transmembrane segments, S1–S6 (Fig. 31.1). This primary structure gives a four-fold symmetry to the voltage-gated channels, with the pore formed at the central line of contact of four channel-forming domains. The short N-terminal and the long C-terminal tails of the protein are located in the cytoplasm.

The *CACNA1A* gene, well conserved during evolution, is expressed as a transcript of approximately 9.8 kb in the brain (Ophoff et al., 1996), more abundantly in the cerebellum than in other cerebral areas, and particularly in Purkinje cells. The transcript undergoes a considerable variety of alternative splicing, producing at least six isoforms (Mori et al., 1991; Zhuchenko et al., 1997). These differ from each other (Fig. 31.2A) according to three main variations: (1) the presence of exon 37a or of the alternative exon 37b (Trettel et al., 1999); (2) the presence or absence of exon 44; (c) the presence or absence of a five-nucleotide stretch, GGCAG, at the 3′ end of the gene between exons 46 and 47. The latter variation is critical for the expression of the CAG repeat stretch (Fig. 31.2B): when the five nucleotides are left in place, the CAG repeat is translated and expressed as a polyglutamine sequence at the protein level; when they are spliced out, a stop codon is encountered upstream of the CAG_n stretch, and the protein does not include the glutamine repeat. Isoforms both with and without the GGCAG insertion, i.e., expressing or not expressing the polyglutamine tract, have been found in the cerebellar cortex, with a predominance of the first type in SCA6 brains (Ishikawa et al., 1999a).

The distinct properties of the various isoforms are unknown, but they are thought to have regulatory and/or modulatory functions. It has been suggested, for instance, that they can modulate the binding sites for the presynaptic plasma membrane proteins, syntaxin and SNAP-25 (synaptosome-associated protein of 25 kDa), implying that a neuron could adjust the efficiency of synaptic transmission (synaptic vesicle fusion) by regulating the expression of different isoforms of the Ca^{2+} channel gene (Sheng et al., 1994; Rettig et al., 1996).

SCA6 as compared with other ADCAs

SCA6 differs from other disorders due to the expansion of a CAG repeat (SCA1–3 and SCA7, Huntington disease, dentato-rubro-pallido-luysian atrophy, and spino-bulbar muscular atrophy) in many respects. In the latter diseases, the unstable expanded CAG stretch, located within the coding regions of their genes, typically has a number of units that ranges from 35 to over 100. The gene products are nuclear or cytoplasmic rather than membrane proteins, to which the expanded polyglutamine stretch confers a gain of function, as shown, for example, by the absence of ataxia or neurodegeneration in knock-out mice (e.g., Matilla et al., 1998). Although the pathogenic role of elongated polyglutamine stretches is far from clear, intracellular ubiquitinated insoluble polyglutamine aggregates appear to be a common feature of cells specifically affected in each of these disorders (for a review, see Paulson, 1999).

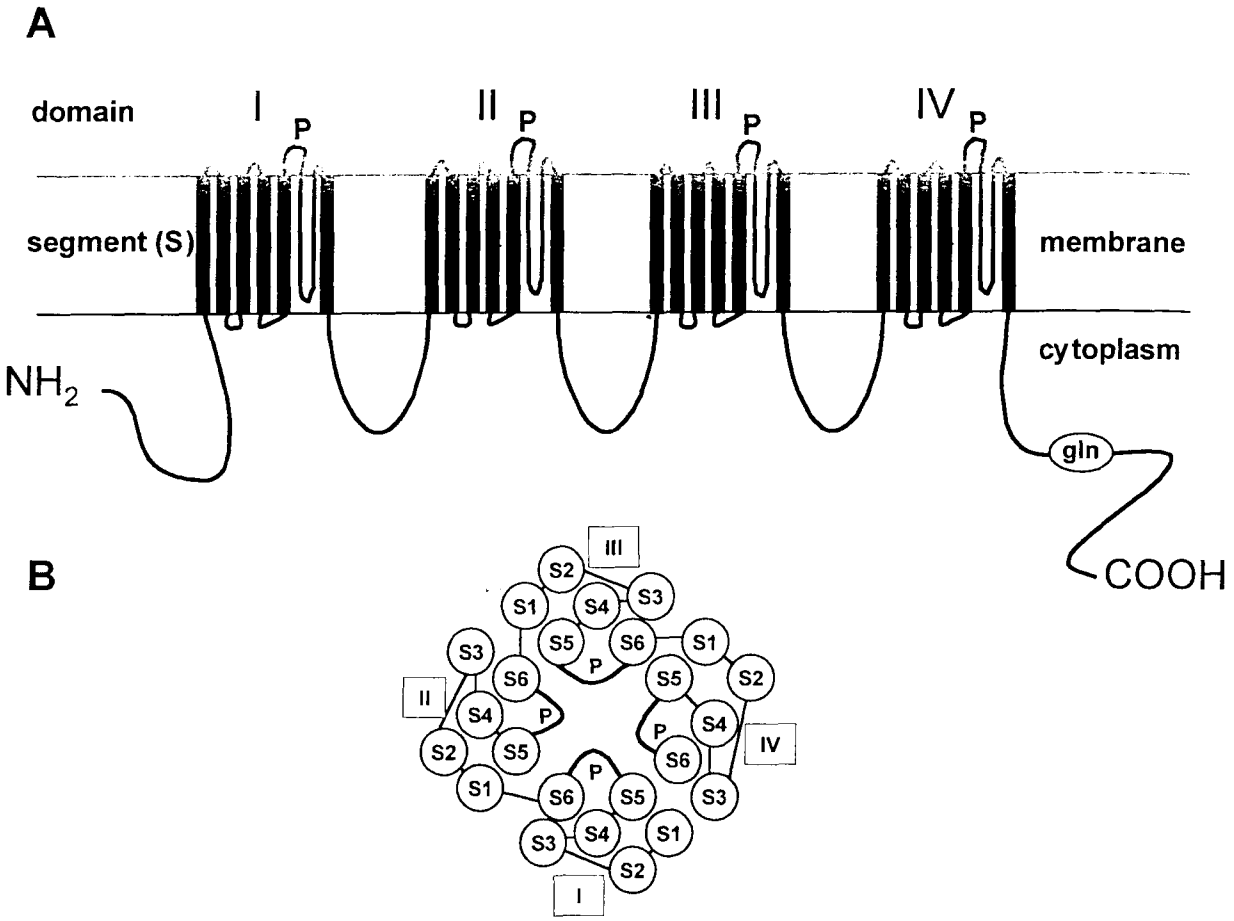

Fig. 31.1 (A) Schematic structure of the α_{1A} subunit of the voltage-gated calcium channel type P/Q. Each of the four domains (I–IV) has six α-helix transmembrane segments (S1–S6). The carboxyl terminus of the protein contains, in some of the isoforms (see text), a stretch of polyglutamines (gln). (B) Top view of the protein according to the molecular model proposed by Guy and Conti (1990), showing its four-fold symmetry and the central pore formed by the P segments.

These nuclear aggregations are found to be ubiquitinated and, as in SCA1, associated to the proteasome and nuclear matrix (Cummings et al., 1998). In addition, observations deriving from transgenic SCA1 mice and cellular models of Huntington disease suggest that the nuclear translocation of the mutant proteins has a critical pathogenic role (Klement et al., 1998; Saudou et al., 1998).

These common features of polyglutamine disorders are not shared with SCA6. The number of triplets in SCA6 expanded alleles is much shorter and stable, falling within the distribution of the normal alleles in the other diseases. The CAG repeat is not expressed in all isoforms, and recent evidence suggests that it confers to the protein a loss rather than a gain of function. Cultured cells transfected with α_{1A} subunit cDNAs, engineered to translate the poly-CAGs in 4, 24, 30, and 40 glutamine residues, showed that expansions of 30 and 40 polyglutamines induce a hyperpolarizing shift in the voltage dependence of channel inactivation (Matsuyama et al., 1999). This shift can be predicted to exert a considerable effect on channel availability, by halving the Ca^{2+} influx, which may, in turn, lead directly or indirectly to neuronal cell death (Matsuyama et al., 1999). In addition, current density of Ca^{2+} channels in transfected cells is not reduced, implying that the mutated protein is normally transported to the membrane and is not sequestered into aggregates (Matsuyama et al., 1999). Histologically, however, cytoplasmic aggregation, immunoreactive with the α_{1A} subunit antibodies, has indeed been observed in Purkinje cells of SCA6 brains (Ishikawa et al., 1999a). These aggregates, unlike those found in other polyglutamine diseases, are not ubiquitinated and are localized in the cytoplasm. Their nature and implications with the disease should be further investigated.

The available evidence shows striking analogies

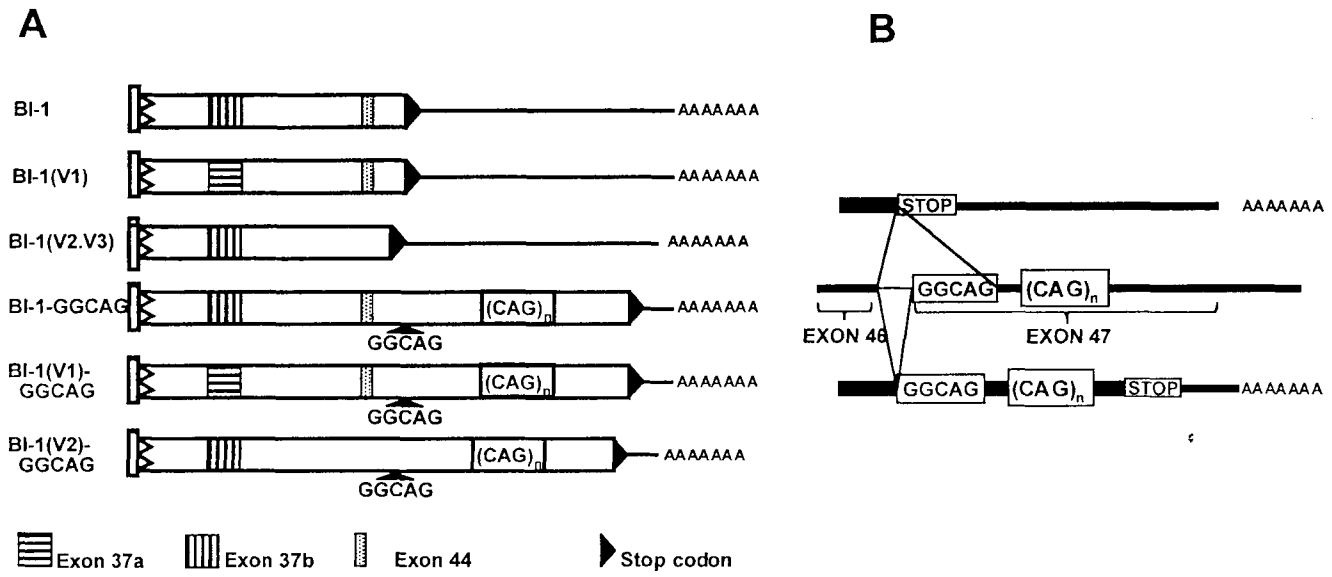

Fig. 31.2 Isoforms of the *CACNA1A* gene (modified from Zhuchenko et al., 1997). (A) The six different isoforms described by Zhuchenko et al. (1997), produced by various combinations of alternative splicing (see text). (B) Alternative splicing between exons 46 and 47. If exon 46 is joined to exon 47 downstream of the GGCAG segment, a stop codon is encountered and the poly-CAG stretch is not expressed. However, if exon 46 is joined to exon 47 upstream of the GGCAG segment, the translation continues beyond the poly-CAG, until a stop codon is encountered, thus producing a protein containing a polyglutamine stretch.

between SCA6 and EA2. The vast majority of EA2 mutations lead to a premature stop codon (Ophoff et al., 1996; Denier et al., 1999), probably producing a non-functional protein, acting either through a mechanism of haploinsufficiency or through a dominant negative effect, e.g., by interfering with the subunit assembly. Carriers of EA2 point mutations share with SCA6 patients: (a) a similar, highly variable phenotype, ranging from vertigo/ataxia episodes with interictal nystagmus and mild cerebellar signs with no overt ataxia (Denier et al., 1999) to a severe progressive pure cerebellar ataxia preceded or not by episodes (Yue et al., 1997); (b) a predominant atrophy of the cerebellar vermis (Denier et al., 1999); and (c) a sensitivity of episodes to acetazolamide treatment (Calandriello et al., 1996; Jen et al., 1998). Furthermore, a continuity between SCA6 and EA2 phenotypes has been observed in the same kindred segregating for an unstable allele with 20 or 25 CAG repeats in different family branches (Jodice et al., 1997). Patients with 25 repeats had a severe progressive ataxia similar to that described as SCA6, while those with 20 repeats had the typical features of EA2, with short vertigo episodes and interictal nystagmus.

Conclusions

SCA6 is one of the three allelic disorders due to *CACNA1A* gene mutations. The other two, familial hemiplegic migraine and EA2, display a predominantly episodic phenotype, typical of channelopathies, mainly associated with point mutations. Missense mutations are found in familial hemiplegic migraine families, suggesting that, in these cases, structural anomalies of the protein may alter the channel activity (Ophoff et al., 1996; Ducros et al., 1999), possibly by a gain of function (Hans et al., 1999). Mutations involving a premature stop codon and a truncated protein are carried by most EA2 families, indicating that a haploinsufficiency mechanism or a dominant negative effect could be responsible for this disorder (Ophoff et al.,1996; Denier et al., 1999). Expansion mutation, instead, is predominantly associated with a pure, permanent and progressive ataxia, for which a pathogenic mechanism similar to that of polyglutamine disorders could be hypothesized (Zhuchenko et al., 1997). However, several lines of evidence appear to blur this hypothetical phenotype–genotype correlation. First of all, the three allelic disorders show a considerable amount of phenotypic overlap. This holds true not only for EA2 and SCA6 (see above), but also for familial hemiplegic migraine, as shown by some families in which hemiplegic migraine is associated with a

permanent cerebellar ataxia (Ducros et al., 1999). It is interesting to note that the missense mutations in these type of families were found to confer a loss of function to the protein, as hypothesized also for EA2 (Hans et al., 1999), and for SCA6 (Matsuyama et al., 1999). In addition, a truncating mutation was reported in patients with ataxic as well as hemiplegic episodes (Jen et al., 1999). Second, different mutations can be associated with similar phenotypes: expansion mutations are also found in patients with an EA2 phenotype (Jodice et al., 1997; Jen et al., 1998), whereas a permanent and progressive cerebellar deficit, similar to that of SCA6, was reported in subjects carrying a missense mutation (Yue et al., 1997).

In this situation, more work is needed to delineate the clinical and pathological spectrum associated with the *CACNA1A* expansion mutation and the extent of its overlap with the other allelic diseases, on the one hand, and the group of disorders due to CAG_n expansions, on the other. A study of expression regulation and function of different α_{1A} subunit isoforms, particularly of those with or without the polyglutamine tract, as well as an analysis of their biophysical properties with different kind of mutations would be highly relevant to understanding the homologies and differences between SCA6 and the other allelic disorders at a cellular level. Finally, animal models could greatly contribute to the understanding of the pathogenic mechanisms involved in SCA6, namely, whether the mutation acts by altering the channel function, as in channelopathies, leading, in turn, to Purkinje cell death, or through a toxic effect of the mutated protein per se, as envisaged for other polyglutamine disorders.

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