

# Dysfunction of the Ca<sub>v</sub>2.1 calcium channel in cerebellar ataxias

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## Abstract

Mutations in the *CACNA1A* gene are associated with episodic ataxia type 2 (EA2) and spinocerebellar ataxia type 6 (SCA6). *CACNA1A* encodes the  $\alpha$ -subunit of the P/Q-type calcium channel or Ca<sub>v</sub>2.1, which is highly enriched in the cerebellum. It is one of the main channels linked to synaptic transmission throughout the human central nervous system. Here, we compare recent advances in the understanding of the genetic changes that underlie EA2 and SCA6 and what these new findings suggest about the mechanism of the disease.

## Introduction and context

The Ca<sub>v</sub>2.1 calcium channel belongs to the superfamily of voltage-gated calcium channels. Its other designation, P/Q-type channel, refers to the cell types from which its constituent currents were originally isolated; the 'P' stands for Purkinje and 'Q' for granule cells of the cerebellum. The  $\alpha$ -subunit (Figure 1), encoded by *CACNA1A*, is associated with  $\beta$  and  $\alpha_2\delta$  auxiliary subunits that are thought to help in trafficking and anchoring the principal subunit to the cell membrane and also modulate the biophysical properties of the channel [1]. *CACNA1A* undergoes extensive alternative splicing resulting in Ca<sub>v</sub>2.1 channels with different properties [2-5]. These splice variants are differentially expressed throughout the central nervous system (CNS) and serve to adjust the biophysical parameters of the channel to its role in the various cell types.

The *CACNA1A* gene maps to chromosome 19p13 [6]. It is widely expressed throughout the CNS; however, in keeping with its original identification, this gene is expressed at a particularly high level in Purkinje and granule cells of the cerebellum. In much of the CNS, Ca<sub>v</sub>2.1 channels are highly expressed pre-synaptically [7], where they couple calcium influx to vesicular exocytosis in fast neurotransmission. However, in Purkinje cells, Ca<sub>v</sub>2.1 channels serve an additional post-synaptic role in coordinating AMPA

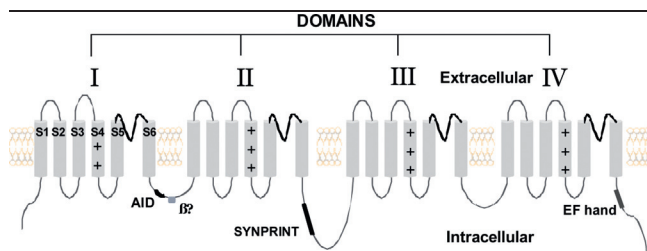
( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor activation with voltage-dependent calcium influx [8].

## Ca<sub>v</sub>2.1 and ataxia

Dominant mutations in *CACNA1A* underlie at least three allelic diseases (Table 1). A large number of different point mutations (both nonsense and missense) have been shown to cause episodic ataxia type 2 (EA2), an acetazolamide-responsive disorder characterised by paroxysmal attacks of midline cerebellar disturbance manifesting as ataxia, imbalance, vomiting, oscillopsia, and interictal nystagmus [9-15]. Attacks last from hours to days, and triggers include stress and intercurrent infection. Several other EA syndromes have been described (reviewed in [16]); mutations in *KCNA1* – the gene that encodes the K<sub>v</sub>1.1 potassium channel – underlie EA1, which is characterised by brief attacks of ataxia (lasting minutes) with interictal myokymia [17-20]. Interestingly, mutations in *CACNB4*, the gene that encodes one of the accessory  $\beta$ -subunits of Ca<sub>v</sub>2.1, also cause a form of EA [21].

EA2 mutations that result in premature stop codons are likely to generate a non-functional truncated peptide or trigger nonsense-mediated mRNA decay. Functional

**Figure 1. Two-dimensional schematic of the  $\alpha$ -subunit of  $Ca_v2.1$  showing functionally important regions of the peptide**



The subunit consists of four domains (I-IV), each containing six transmembrane segments (S1-S6). The S4 segment of each domain is lined with positively charged amino acids and acts as the voltage sensor. The S5-S6 interlinker forms the pore of the channel. The AID (alpha interaction domain) forms the binding pocket for the  $\beta$ -subunit of  $Ca_v2.1$ . The SYNPRINT (synaptic protein interaction) region interacts with SNARE (SNAP (soluble N-ethylmaleimide-sensitive factor attachment protein) receptor) proteins such as syntaxin and SNAP-25, which are involved in synaptic transmission. Sites of G-protein modulation via  $\beta\gamma$ -subunits are shown along with the 'EF' hand, which is considered to be involved in calcium-dependent facilitation.

characterisation of missense mutations in *CACNA1A* has demonstrated that mutations associated with EA2 result in a loss of  $Ca_v2.1$  channel function [22,23]. In addition, mutant subunits may disrupt the membrane trafficking of wild-type channels [24].

In contrast to EA2, spinocerebellar ataxia type 6 (SCA6) is a 'pure' progressive cerebellar syndrome that results, not from point mutations, but from an abnormal polyglutamine expansion in the channel's carboxyl-terminal domain, which is present in only certain splice isoforms of the *CACNA1A* mRNA [25]. Although changes in channel kinetics have been observed [26,27], the pathogenic mechanism of SCA6 is poorly understood.

### Recent advances

Direct sequencing of *CACNA1A* in a number of patients with clinical EA2 often fails to identify causative point mutations. However, the last year has witnessed a further step forward in understanding the genetic basis of EA2. Veneziano and colleagues [28] identified new 5' and 3' regions in the *CACNA1A* gene, including a gene promoter region and a new final exon 48, both of which harboured mutations in patients with EA. Furthermore, the mutation spectrum has expanded with the findings of large deletions and duplications in *CACNA1A* in affected individuals. Previously, nonsense and missense mutations accounted for most cases of EA2. Recently, methods such as MLPA (multiplex ligation-dependent probe amplification) and QMPSF (quantitative multiplex polymerase chain reaction of short fluorescent fragments) have demonstrated large-scale *CACNA1A* gene rearrangements in patients with EA2 [29,30]. This finding is particularly important for those patients with clinical EA2 in whom sequencing of *CACNA1A* fails to identify a point mutation.

EA2 is an autosomal dominant disease, and because large deletions in *CACNA1A* are not likely to produce functional transcripts, it is likely that reduced channel density in the cerebellar circuit (possibly in Purkinje cells, where these channels have been shown to play a central role) is sufficient to cause episodes of ataxia. Moreover, the recent observation that nonsense mutations located within a well-known alternatively spliced exon (exon 37A) [31] can cause EA2 hints at a significant role of  $Ca_v2.1$  channels containing exon 37A in the cerebellum and underpins the importance of calcium channel splicing in disease causation.

While increasing evidence points to a loss of robust  $Ca_v2.1$  expression in the cerebellum and haploinsufficiency as the underlying mechanism of EA2, calcium channel

**Table 1. Mutations in *CACNA1A* underlie three allelic disorders: EA2, FHM and SCA6**

Disease	Core clinical features	Additional features	Inheritance	Mutations	Functional consequences
Episodic ataxia type 2 (EA2)	Attacks of ataxia, vomiting, vertigo, oscillopsia lasting hours to days, and interictal nystagmus	Epilepsy, migraine, and progressive cerebellar syndrome	Autosomal dominant	Nonsense and missense mutations, small deletions and insertions, and large deletions	Loss of function
Familial hemiplegic migraine type 1 (FHM1)	Rare subtype of MA: attacks of hemiparesis and hemisensory disturbance lasting hours to days	Confusion, encephalopathy, ataxia, coma, and seizures	Autosomal dominant	Missense mutations	Gain of function
Spinocerebellar ataxia type 6 (SCA6)	Late-onset progressive cerebellar ataxia		Autosomal dominant	CAG expansion in C-terminus	Alteration of $Ca_v2.1$ channel kinetics; polyglutamine cytotoxicity?

The clinical and genetic features of each disorder are described. The effect of mutations on  $Ca_v2.1$  channel function is stated. MA, migraine with aura.

dysfunction may not be at the root of SCA6. In support of this view, the expanded CAG repeat in the SCA6 knock-in mouse does not appear to affect  $Ca_v2.1$  function [32], indicating that the polyglutamine repeat itself may have a cytotoxic effect on the cell. It has recently been suggested that cerebellar dysfunction in a related polyglutamine repeat SCA (SCA2) may arise from aberrant activation of type 1 inositol 1,4,5-trisphosphate receptors (ITPRs) in Purkinje cells by the glutamine tracts themselves [33]. If activation of the ITPRs is the mechanism of polyglutamine repeat SCAs, then SCA6 may be a result of the relative abundance of P/Q channels in Purkinje cells, rather than specific properties of the channels themselves.

### Future directions

Despite advances in the genetic basis of EA2, many questions remain unanswered. From a mechanistic point of view, the paroxysmal nature of the neurological symptoms arising from mutations in *CACNA1A* remains a mystery. In addition, the physiological basis of how attacks are precipitated by stress or indeed relieved by acetazolamide still evades explanation. Answers to some of these questions may await the development of neuronal expression systems. This will allow the study of how genetic variation in the calcium channel gene affects the biophysical properties of  $Ca_v2.1$  in its physiological environment, especially with respect to synaptic transmission and dendritic depolarisation. A recent insight into this approach came from Heeroma and colleagues [34], who demonstrated that  $K_v1.1$  mutations in EA1 exerted differential effects on excitation (rheobase) and synaptic transmission when expressed in neurons.

Although the polyglutamine tract in SCA6 is considered to confer a toxic gain of function, the exact roles of the alternative long- and short-splice variants of *CACNA1A* in Purkinje cells need to be defined. This may lead to a better understanding of how the expanded CAG repeat in SCA6 results in disease. An additional conundrum is why many spontaneous mutations in *CACNA1A* in mice result in a 3 Hz spike and wave epilepsy [35,36], whereas in humans, although a few mutations have been shown to co-segregate with EA2 and seizures [37,38], most deletions and truncations in the gene are associated with pure EA2 rather than absence epilepsy. Mutations of this gene appear to give two distinct phenotypes in humans and rodents. Understanding how these phenotypes are associated with different roles of  $Ca_v2.1$  channels in human and rodent tissues may be an important step in untangling the molecular pathogenesis of seizures and ataxia.

### Abbreviations

CNS, central nervous system; EA, episodic ataxia; EA2, episodic ataxia type 2; ITPR, inositol 1,4,5-trisphosphate

receptor; P/Q, Purkinje/granule; SCA, spinocerebellar ataxia; SCA6, spinocerebellar ataxia type 6.

### Competing interests

The authors declare that they have no competing interests.

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