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SCA13

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Abstract Spinocerebellar ataxia 13 (SCA13), initially described in a four-generation French family, has now also been characterized in a large Filipino pedigree. Ongoing investigations continue to identify additional SCA13 families and individuals. Recently, studies have shown that mutations in the voltage-gated potassium channel KCNC3 are causative for SCA13. Sequence analysis of *KCNC3* revealed mutations 1554G→A (R420H) in the Filipino and 1639C→A (F448L) in the French pedigrees. Both mutations alter KCNC3 function in a *Xenopus laevis* oocyte expression system. $KCNC3^{R420H}$, located in the voltage sensor of the channel, has no detectable channel activity when expressed alone, and strong dominant negative effects when coexpressed with wild-type *KCNC3*. $KCNC3^{F448L}$ shifts the activation curve in the negative direction and causes an approximately sevenfold slowing of channel closure. These mutations are expected to change the output characteristics of fast-spiking cerebellar neurons, where KCNC channels confer capacity for high-frequency repetitive firing.

Keywords Neurodegeneration · Neurodevelopment · Voltage-gated potassium channel · Ataxia

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Clinical Features

French Pedigree

Spinocerebellar ataxia (SCA) 13 was originally assigned to a large French family segregating an autosomal dominant cerebellar ataxia following its description in 2000 by Herman-Bert et al. [1]. This four-generation family consisted of eight affected women (seven living at time of study) and two fourth-generation men (Fig. 1a). Of note, the female proband had affected daughters from three different partners. Unlike most dominant SCAs, a striking feature in this family was childhood onset with minimal progression of disease, other than a single individual with described age at onset (AO) of 45 years (Table 1). However, in this individual, subtle dysfunction may have been present for an extended period, and precise timing was made difficult by the presence of mild mental retardation (Stevanin and Dürr, personal communication). There was marked delay of developmental motor milestones with three affected individuals achieving independent ambulation at ages 3, 4, and 12 years. Typical of the ADCAs, patients exhibited truncal and appendicular ataxia, dysarthria, hyper-reflexia, and nystagmus. Electro-oculography testing revealed a horizontal gaze-evoked nystagmus with a vertical downbeat component in one individual. Another demonstrated absence seizures with typical 3-Hz spike and wave complexes on electroencephalogram. In addition to motor pathology, affected individuals in this family exhibited mild to moderate global cognitive impairment. Formal testing revealed intelligence quotients between 62 and 76. There was no evidence of progression in intellectual impairment.

Filipino Pedigree

In 2005, a second family with a previously unknown ADCA was described by Waters et al. [2]. This three-generation

Table 1 Clinical features in select affected French family members (+—mild, ++—moderate, +++—severe, Chd—childhood, MMS—mini-mental status exam, MR—mentally retarded)

Individual	I-1	II-2	II-3	III-1	III-8	III-9	III-10	IV-1
Age at examination	61	59	53	29	31	28	24	4
Age at onset	Chd	45	Chd	<1	<4	Chd	<3	<1
Gait ataxia	+++	+++	++	++	++	++	++	++
Limb ataxia	++	+++	++	++	+	+	+	++
Mental status (MMS)	25/30	23/30	23/30	MR	20/30 IQ 71	25/30 IQ 68	IQ 62	IQ 76
Dysarthria	+++	+++	+	++	–	++	+	++
Nystagmus	+	+	+	+	+	+	–	–
Hyper-reflexia	+	+	+	+	+	+	+	+

Filipino family segregated an adult-onset dominant ataxia with prominent cerebellar signs and symptoms as well as cerebellar atrophy on magnetic resonance imaging. The clinical and imaging phenotypes were consistent with a degenerative SCA. Nineteen individuals, including 11 clinically affected from the three generations, were examined (Fig. 1b). The 82 year-old proband was the oldest affected with disease onset at age 60 years. She has 12 living children: seven women—four affected—and five men—three affected. The seven affected offspring in generation two were between 46 and 65 years old, with AO ranging from 22 to 48 years (mean 36.4 years). The third generation has three affected individuals with a mean AO of 24.5 years. This includes a 30-year-old woman (AO 26), a 25-year-old man (AO 23), and a newly symptomatic 22-year-old man. Cerebellar signs in the 11 affected family members consist of gait ataxia, limb ataxia/dysmetria, titubation, hypotonia, dysarthria, and nystagmus (Table 2). Two had mild hyper-reflexia without a Babinski sign. The three oldest had the most severe ataxia (two wheelchair-bound) with disease duration at age of examination of 24, 43, and 34 years.

Molecular Genetics

Heterozygous mutations were detected in the affected members of each family. In the Filipino pedigree, a 1554G→A transition in exon 2 resulted in an arginine to histidine substitution (R420H) in the S4 transmembrane functional segment. S4 is the principle voltage-sensing element of the protein. Sequence analysis in affected members of the French pedigree revealed a 1639C→A transversion, also in exon 2, resulting in a phenylalanine to leucine (F448L) substitution. In contrast, this substitution occurred in the S5 transmembrane domain of the KCNC3 protein. Both mutations change amino acids that are 100% conserved among members of the human KCNC family and across phyla in the S4 and S5 domains (Fig. 2). The mutations were seen in all affected individuals but not in unaffected individuals in the respective pedigrees. Additionally, the mutations were not found in more than 400 alleles from normal individuals of Filipino or West-European descent.

Genotype/Phenotype Correlations

The French and Filipino families represent the only well-characterized genotype/phenotype correlations to date. However, initial screens of ataxia deoxyribonucleic acid repositories suggest that the R420H mutation may be recurring. An additional family with three affected members has also tested positive for the R420H mutation. These individuals had an onset in the third and fourth decades with a slowly progressive ataxia and cerebellar atrophy. Their phenotype is thus far consistent with that described in the Filipino kindred. The full spectrum of KCNC3 mutations remains to be determined.

Functional Analysis

Functional consequences of the *SCA13* mutations have been determined utilizing a *Xenopus laevis* oocyte expression system and recording the channel activity with a two-electrode voltage clamp [3]. The expression of R420H resulted in no detectable channel activity. The coexpression of wild-type *KCNC3* and $KCNC3^{R420H}$ subunits led to the suppression of current amplitude consistent with a loss-of-function-dominant negative effect. R420H did not suppress the functional expression of *Shaker*, a member of the Kv1 (KCNA1) family, indicating that this effect was not due to a general effect on channel trafficking.

The expression of F448L produced channels with altered gating. The activation of F448L was detected at −20 mV, compared to −10 mV for the wild type. Confirmation that activation was shifted ~13 mV toward the hyperpolarized direction was obtained by analysis of the probability of opening as a function of voltage. Activation kinetics of F448L and the wild type were similar at voltages where

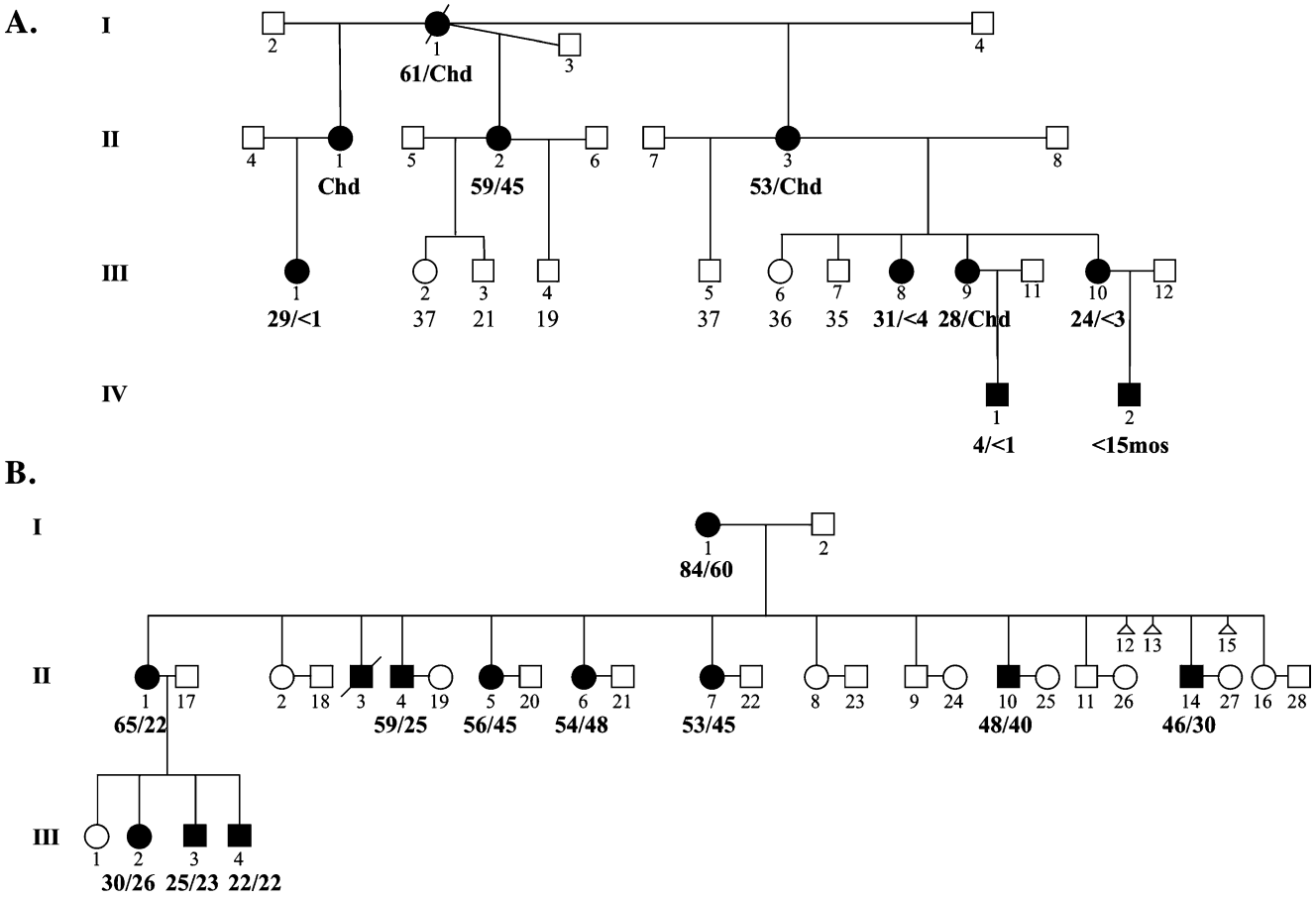

Fig. 1. Pedigrees of the original SCA13 French (**a**) and Filipino (**b**) families. Affected individuals are represented by *darkened symbols*. Age in years at examination and onset (AE/AO) are shown in *bold* below the individual designation (*Chd* childhood)

both had a maximal open probability. However, deactivation kinetics of KCNC3^{F448L} were dramatically slower. Tail currents recorded after repolarization to −90 mV revealed a approximately sevenfold slowing of channel closure in the F448L mutant. The hyperpolarized shift in the probability of opening and the slower rate of deactivation are related findings indicating that *KCNC3*^{F448L} increases the relative stability of the open state.

KCNC3 Expression Patterns

Voltage-gated potassium channels are a heterogeneous group of channels that participate in diverse ways to influence neuronal excitability [4]. The Kv superfamily is divided into 12 subfamilies [5]. The KCNC (Kv3) subfamily conveys on neurons the properties of positively shifted voltage dependencies and rapid deactivation enabling fast repolarization

Table 2 Clinical features in select affected Filipino family members (+—mild, ++—moderate, +++—severe)

Individual	I-1	II-1	II-4	II-5	II-6	II-7	II-10	II-14	III-2	III-3
Age at examination	84	65	59	56	54	53	48	46	30	25
Age at onset	60	22	25	45	48	45	40	30	26	23
Gait ataxia	+++	+++	++	++	+	+	+	+	+	+
Limb ataxia	+++	+++		+	+			+	+	+
Titubation	++			+						
Hypotonia	++	++						+	++	
Dysarthria	++	++		+				+	+	+
Nystagmus				+						
Hyper-reflexia				++				+		

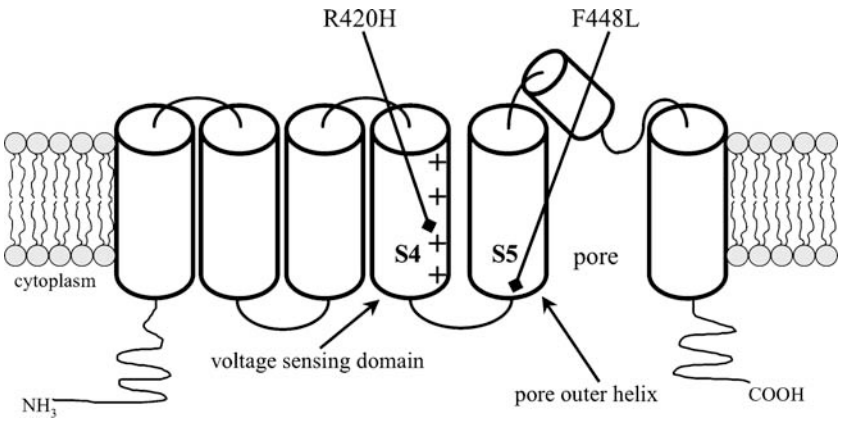

Fig. 2. Functional motif schematic of a single KCNC3 subunit illustrating the six transmembrane segments and pore re-entrant loop (6TM architecture). Segment S4 forms the main voltage sensor domain with positively charged arginine residues (*plus sign*) detecting changes in voltage. Segment S5 forms the pore outer helix and functions to couple voltage-sensor conformational changes with pore opening and closing. Locations of SCA13 mutations are designated with *arrows*

of action potentials without compromising spike initiation or height [6, 7]. This channel subfamily consists of four genes abundantly expressed in the central nervous system each with a unique expression pattern.

Initial characterization of KCNC3 expression detected the highest levels of messenger ribonucleic acid (mRNA) expression in the cerebellar cortex, inferior colliculus, hindbrain, and spinal cord [8]. Further localization studies identified particular mRNA enrichment in terminally differentiated Purkinje cells and deep cerebellar nuclei [9]. Subsequent investigations, utilizing immunohistochemical methods, revealed localization in laminae IV and V, central canal, and ventral horn of the spinal cord as well as the spinal trigeminal, cuneate, and gracilis nuclei of the medulla oblongata [10]. In situ localization has also demonstrated expression in the hippocampus and substantia nigra. Cellular localization includes both the soma and dendritic tree.

Summary

The functional properties of KCNC3 channels are distinct among voltage-gated K^+ channels. These channels activate in a more depolarized range and close more rapidly than other Kv channels facilitating high-frequency firing of action potentials with little or no adaptation. This is a characteristic of burst neuron populations found in the mammalian neocortex, hippocampus, auditory nuclei, substantia nigra, and cerebellum [7]. In common with other voltage-gated K^+ channels, KCNC3 channels are tetramers. Each subunit has six transmembrane segments and a re-entrant loop (Fig. 2) forming the voltage sensor and ion-selective pore functional domains [11]. The characteristic depolarized voltage dependence and rapid deactivation of KCNC3 are related properties conferred by specific amino acid residues in the voltage sensor and S5 [12, 13]. The R420H mutation is located in the main voltage-sensing element, S4 (Fig. 2), and changes one of the positively charged arginine residues that respond to changes in membrane potential [7, 14]. The F448L mutation is at the cytoplasmic end of S5 (Fig. 2), which is involved in coupling voltage-sensor conformational changes with the opening and closing of the pore [12].

In contrast to many other SCA genes, for which the normal function has been difficult to elucidate, the functions of KCNC3 channels have been extensively studied [6, 15–17]. KCNC3 is expressed in cerebellar granule cells, Purkinje cells, and deep cerebellar neurons, where it may form heteromultimeric channels by coassembly with KCNC1 and/or KCNC4 [18, 19]. KCNC3 channels are localized on dendrites and neuronal somata and are involved in repolarizing both somatic Na^+ spikes and dendritic Ca^{2+} spikes. These ion channels are essential for fast spiking in burst neurons that fire hundreds of action potentials per second with little or no frequency adaptation [7, 17]. The depolarized activation range of KCNC3 channels ensures channel opening only during action potentials where they contribute to fast repolarization and promote recovery of Na^+ channels from inactivation. Fast deactivation of KCNC3 channels limits the time course of the post-hyperpolarization and shortens the refractory period.

SCA13 mutations likely disrupt the firing properties of fast-spiking cerebellar neurons and may influence neuronal function in additional regions of KCNC3 expression. Pharmacological suppression of KCNC3 activity in cerebellar neurons leads to action potential broadening, spike frequency adaptation, and spike failure from accumulated Na^+ channel inactivation [17]. The R420H mutation may mimic this effect. F448L is predicted to reduce the maximal firing rate of cerebellar neurons. Due to slower closing, post-hyperpolarization would be prolonged thereby delay-

ing the return to threshold and increasing the interspike interval. The differing effects of the mutations at the cellular level may explain the contrasting phenotypes between the two pedigrees. The F448L mutation would be expected to be more severe because it alters key gating properties of KCNC3 channels. In contrast, R420H would be expected to reduce channel activity without changing the functional properties of the residual current. The childhood onset with concurrent mental retardation and seizures in members of the French pedigree and their absence in the Filipino patients is consistent with this notion.

The physiological properties of KCNC3 channels suggest potential mechanisms of neurodegeneration. First, they contribute to the repolarization of both somatic Na^+ spikes and dendritic Ca^{2+} spikes in Purkinje cells [17]. Longer duration spikes may increase Ca^{2+} influx, which in turn could contribute to neuronal death. Additionally, the functional properties of KCNC3 channels are modulated by reactive oxygen species. Mutant KCNC3 subunits may affect the ability of cerebellar neurons to cope with oxidative stress [20–22]. Last, morphological differentiation and the development of physiologic properties are tightly linked in Purkinje cells, and morphological and electrical maturation may be an interdependent phenomena [23]. Mutations that disrupt acquisition of appropriate characteristics may cause developmental defects that reduce the long-term viability of neurons. Both mutations show intrafamilial phenotypic variability implying the importance of compensatory mechanisms as well as genetic and environmental modifiers.

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