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Late-onset episodic ataxia type 2 associated with a novel loss-of-function mutation in the *CACNA1A* gene

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ABSTRACT

We report a patient with typical features of episodic ataxia type 2 (EA2) but with onset in the sixth decade and associated interictal hand dystonia. He was found to bear the novel heterozygous missense mutation p.Gly638Asp (c.1913G>A) in the *CACNA1A* gene. Functional analysis of the mutation on P/Q channels expressed in HEK 293 cells revealed a reduction of Ca^{2+} current densities, a left-shift in the apparent reversal potential, the slowing of inactivation kinetics and the increase in the rate of current recovery from inactivation. These results are consistent with a decrease in Ca^{2+} permeability through mutant P/Q channels. To our knowledge, this is just the second patient with late onset EA2 linked to a *CACNA1A* mutation and the first to carry a loss-of-function missense mutation.

1. Introduction

Episodic ataxia type 2 is a neurological disorder characterized by recurrent attacks of ataxia and vertigo of minutes to a few days of duration. These episodes are often brought about by emotional stress, physical exercise, alcohol or caffeine. In most patients onset occurs before age 20, although late onset, after age 50, has also been reported [1,2]. Some patients may exhibit mild interictal cerebellar ataxia or isolated nystagmus or, more rarely, progressive ataxia [3]. More than 50% of the cases show a dramatic response when treated with the carbonic anhydrase inhibitor acetazolamide.

EA2 is inherited as an autosomal dominant trait with incomplete penetrance, and since 1996 the condition is known to be linked to mutations in the *CACNA1A* gene, encoding the α_{1A} subunit of the P/Q-type voltage-gated calcium channel $Ca_V2.1$ [4]. Around 60% of the familial cases show linkage to the *CACNA1A* locus on chromosome 19 [5]. More than 50 mutations in *CACNA1A* have been described in EA2 patients [6]: sixty percent of these are truncating nonsense mutations or insertions or deletions of a few nucleotides inducing a premature stop codon; 15% involve splice sites and the remaining 25% are missense changes that cluster around highly conserved and important functional regions of the α_1 channel subunit, mainly including the line pore (S5-P-S6 segments) and the S2 and S4 segments of the voltage sensors [6,7]. Exceptionally, small expansions of the unstable CAG repeat, usually leading to the spinocerebellar ataxia type 6, may also produce EA2 [8].

The functional consequences of EA2-related *CACNA1A* mutations have been analyzed. The results imply a loss of function of the mutated channels, associated with a diminished current density due to a decrease in channel conductance, deficiencies in channel trafficking to the plasma membrane, or both [9].

Here we report a patient with onset of EA in his late sixth decade, bearing a novel mutation in the pore-forming subunit of the $Ca_V2.1$ channel. The change resulted in a loss of function of the channel probably due to a decreased Ca^{2+} permeability, which supports mutation pathogenicity.

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2. Patients and methods

2.1. Case report

A 60-year old man presented with a 2-year-long history of episodes of instability with associated dizziness and paleness, during which he was unable to walk and showed dysarthric speech. An occasional episode would provoke the patient to fall to the ground. These paroxysms would occur on an almost daily basis and lasted 30 to 60 min. He also complained of frequent bouts of hand clumsiness and posturing that, on retrospect, were diagnosed as dystonic hand movements. No family history of a similar disorder was retrieved, although the proband daughter, who was not available for examination or genotyping, allegedly developed motor tics with onset in adulthood. The patient's personal history revealed ischemic cardiopathy since age 50 and intermittent claudication episodes that the patient was able to differentiate from the ataxic attacks. His interictal examination was unremarkable; of note, there was no evidence of ataxia, nystagmus or movement disorder.

Two brain MRI studies, including angio-MR, were performed at age 60 and at age 64, with normal results. EEG showed spike activity over the right temporal region, more prominent during both hyperpnea and photic stimulation. The patient was treated for several months with antiepileptic drugs, including phenytoin, gabapentin and vigabatrin. Although vigabatrin reduced attacks to 1–2 per month, it was only with EA2 diagnosis and the introduction of acetazolamide that the episodes receded completely. EA2 attacks were well controlled until age 70, when the patient suffered a rapid neurological deterioration and died of glioblastoma multiforme.

2.2. DNA isolation and mutation analysis

Genomic DNA was extracted using the QIAamp DNA Blood Maxi Kit (QIAGEN, Hilden, GE) after obtaining informed consent from the

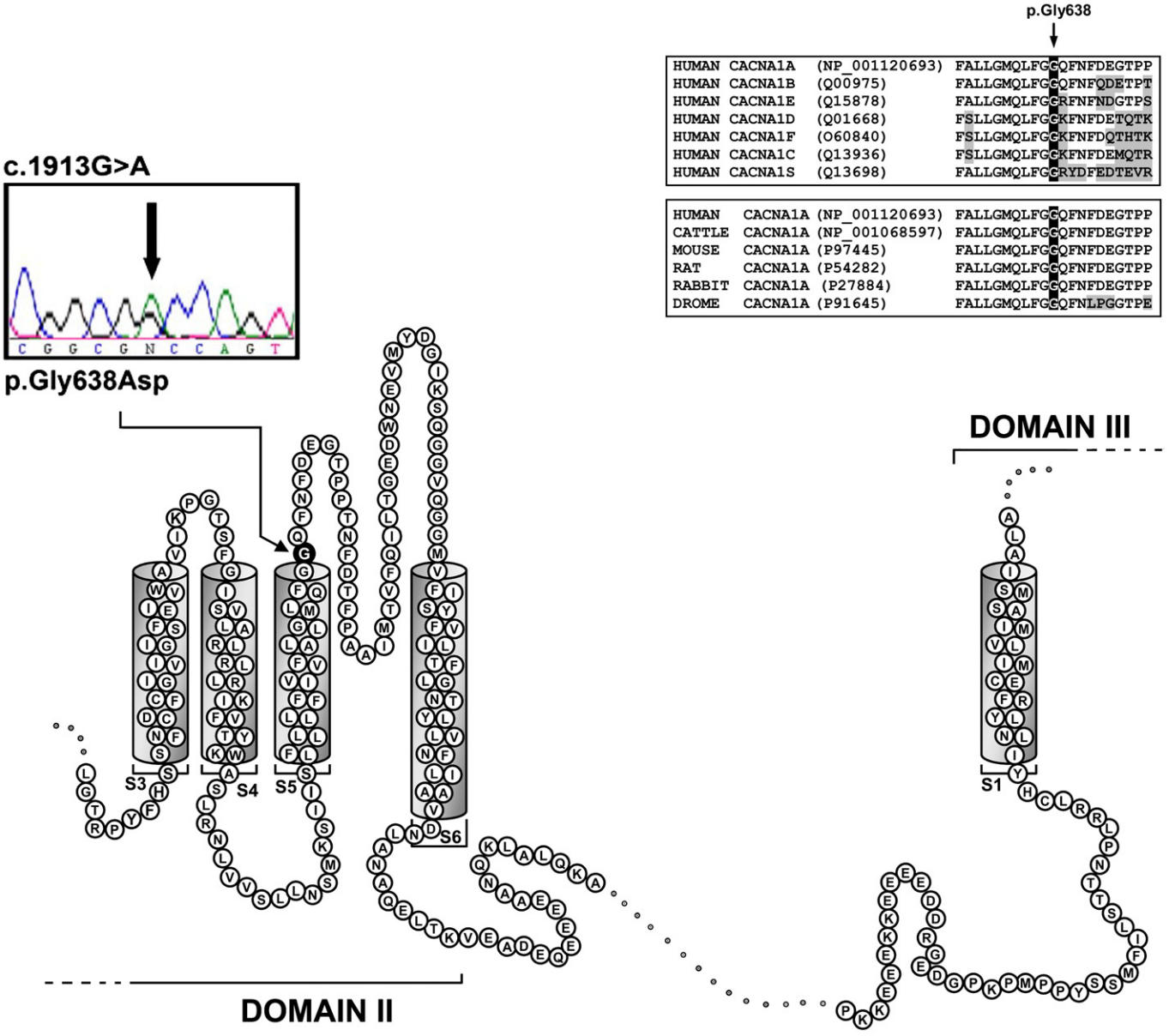

Fig. 1. Schematic representation of a fragment of the human CACNA1A protein (bottom) showing the mutation identified in this study, the corresponding sequence electropherogram of the proband (upper left) and the protein alignment (upper right) performed with ClustalW (http://www.ebi.ac.uk/Tools/clustalw2/index.html). The Gly^{638} residue is conserved in all the human calcium channel α_1 subunits studied (CACNA1A, B, E, D, F, C and S) as well as in the orthologous CACNA1A proteins of cattle, mouse, rat, rabbit and fruit fly (DROME). Non-conserved amino acids are emphasized in grey. In brackets, the reference sequence code of each protein.

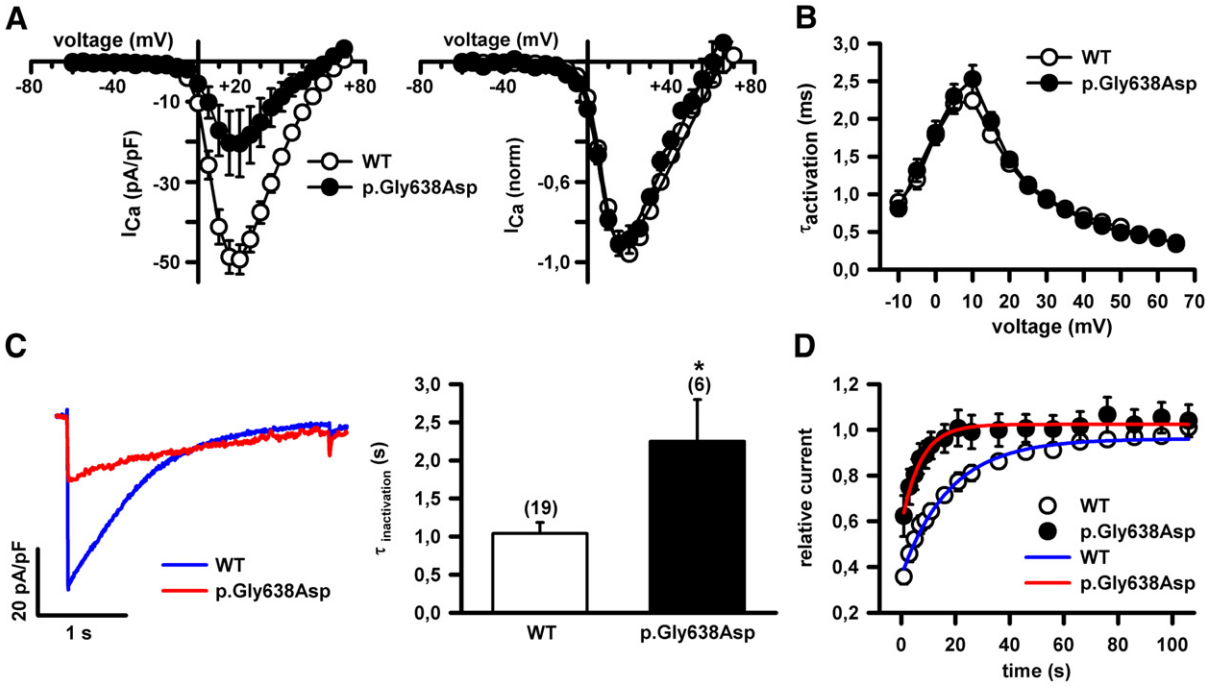

Fig. 2. p.Gly638Asp reduces current densities and alters inactivation properties of heterologously expressed P/Q channels. A, Current density–voltage relationships (left panels) and normalized I–V curves (right panels) for WT (○, $n=62$) and p.Gly638Asp P/Q (●, $n=8$) channels expressed in HEK 293 cells. $V_{1/2,\ act}$, k_{act} and V_{rev} values were (in mV): WT (○, $n=62$) 7.55 ± 0.43, 3.93 ± 0.33 and 64.47 ± 0.81; p.Gly638Asp (●, $n=8$) 6.66 ± 0.44, 4.18 ± 0.32 and 58.87 ± 0.69, respectively. B, Average τ_{act} for WT (○) and p.Gly638Asp channels (●) at the indicated voltages. C, Current traces in response to 3 s depolarizing pulses to +20 mV, illustrating differences in current density and inactivation kinetics (left panels) and average τ_{inact} (right panels) for WT (1.04 ± 0.14 s, $n=19$) and p.Gly638Asp (2.25 ± 0.54 s, $n=6$) channels (*$P<0.01$). D, Time course of I_{Ca} recovery from inactivation for WT and p.Gly638Asp channels. Average τ of current recovery from inactivation obtained after fitting the data to a single exponential, were (in s): WT (○, $n=19$) 19.24 ± 2.79; p.Gly638Asp (●, $n=5$) 6.91 ± 1.26 ($P<0.01$).

proband. A project for *CACNA1A* mutation analysis in a cohort of EA2/hemiplegic migraine patients was previously approved by the Ethics Committee of Vall d'Hebron University Hospital.

The repeat expansion detection (RED) method [10] was used to quantify trinucleotide expansions in *SCA1,2,3,6,7,8,12* and *DRPLA* genes.

The 47 exons of the *CACNA1A* gene and their corresponding exon/intron junctions including splice sites and branch points, were PCR-amplified and sequenced in 42 independent PCR products as previously reported [11]. Two hundred control chromosomes were screened by means of *Ita*I digestion of PCR products for the presence of the change found in the proband.

The mutation identified in the *CACNA1A* gene was named according to Human Genome Variation Society guidelines (www.hgvs.org), using RefSeq accession number NM_001127221 as a cDNA reference sequence, with nucleotide 237, the A of the ATG initiation codon, corresponding to +1, and NP_001120693 as the protein reference sequence. Both sequences correspond to the *CACNA1A* transcript variant 3.

2.3. DNA constructs and site-directed mutagenesis

cDNAs of the α_{1A} and $\alpha_2\delta$ subunits of the $Ca_V2.1$ neuronal calcium channel were obtained from rabbit, and the $Ca_V\beta_{2a}$ was from rat (all cDNAs provided by Dr. Lutz Birnbaumer, NIH, USA). The mutation was introduced using the QuickChange™ Site-Directed Mutagenesis XL kit (Stratagene, La Jolla CA). All cDNA clones were sequenced in full to confirm the integrity and transferred to the pcDNA3 vector for subsequent transfection in HEK 293 cells.

2.4. Heterologous expression and electrophysiology

HEK 293 cells were transfected using a linear polyethylenimine (PEI) derivative, the polycation ExGen500 (Fermentas Inc., Hanover, Maryland, USA) as previously reported (8 equivalents PEI/3.3 μg DNA/dish) [12]. Transfection was performed using the ratio for α_{1A} (wild-type (WT) or p.Gly638Asp), $Ca_V\beta_{2a}$, $\alpha_2\delta$, and EGFP (transfection marker) of 1:1:1:0.3. Recordings were done 24–72 h after transfection at room temperature (22–24 °C).

Calcium currents (I_{Ca}) through WT or p.Gly638Asp P/Q channels were measured using the whole-cell configuration of the patch-clamp technique [13]. Pipettes had a resistance of 2–3 MΩ when filled with a solution containing (in mM): 140 CsCl, 1 EGTA, 4 Na_2ATP, 0,1 Na_3GTP and 10 Hepes (pH 7.2–7.3 and 295–300 mosmol/l). The external solution contained (in mM): 140 tetraethylammonium-Cl, 3 CsCl, 2.5 $CaCl_2$, 1.2 $MgCl_2$, 10 Hepes and 10 glucose (pH 7.3–7.4 and 300–305 mosmol/l). Recordings were obtained with a D-6100 Darmstadt amplifier (List Medical, Germany), filtered at 1 kHz (only for short pulses) and corrected for leak and capacitive currents. Currents were acquired at 33 kHz (for short pulses) or 5 kHz (for long pulses). The pClamp8 software (Axon Instruments, Foster City, CA, USA) was used for pulse generation, data acquisition and subsequent analysis.

Peak inward Ca^{2+} currents were measured from cells clamped at −80 mV and pulsed for 20 ms from −60 mV to +70 mV in 5 mV steps. A modified Boltzmann equation (Eq. (1)) was fitted to normalized current–voltage (I–V) to obtain the voltage dependence of activation

$$I = G_{max}(V - V_{rev})/\left(1 + \exp\left(-\left(V - V_{1/2,act}\right)/k_{act}\right)\right) \quad (1)$$

where I is the peak current, G_{max} is the maximum conductance of the cell, V is the membrane potential, V_{rev} is the extrapolated reversal potential of I_{Ca}, $V_{1/2,\ act}$ is the voltage for half-maximal current activation, and k_{act} is the slope factor of the Boltzmann term.

Time constant for activation (τ_{act}) was obtained from single exponential fits of I_{Ca} activation phase.

Voltage-dependent inactivation kinetic during depolarization was estimated during 3-s pulses from a holding potential of −80 mV to a test potential of +20 mV, by fitting the inactivation phase of I_{Ca} to a single exponential.

Time course of I_{Ca} recovery from inactivation ($\tau_{recinact}$) was tested applying a second pulse of 50 ms to +20 mV at increasing time intervals (1–106 s) after the inactivating prepulse. Normalized peak I_{Ca} at different times was fitted to a single exponential.

2.5. Statistics

Data are presented as the means ± S.E.M. Statistical tests included Student's *t* test or Mann–Whitney test, as appropriate. Differences were considered significant if $P<0.05$.

3. Results

3.1. Mutation analysis

The CAG/CTG expansions in *SCA* and *DRPLA* genes were within the normal range.

A new gene variant was identified in the proband after extensive sequencing of *CACNA1A*. A change from G to A at cDNA nucleotide 1913 in exon 14 was detected, prompting the p.Gly638Asp change located at the P-loop of domain II of the protein, which is part of the ion selective pore (Fig. 1). It was not possible to determine if this was a *de novo* mutation or if it was inherited, since DNA from the patient first-degree relatives was not available. Restriction analysis of the corresponding PCR product allowed confirmation of the mutation in the proband and exclusion in 100 unrelated control subjects.

Several facts pointed to a causative role of the variant identified, and prompted us to perform functional analyses: First, the residue involved is located within the ion selective pore; second, the amino acid substitution from the small, hydrophobic glycine to the acidic aspartate is likely to imply a change in the electrophysiological properties of the pore-forming subunit; and third, the glycine at position 638, as well as the neighboring amino acids, are highly conserved in paralogous human α_1 subunits of Ca_V2 and Ca_V1 channels (CACNA1A, B, C, D, E, F and S) as well as in orthologous CACNA1A subunits of cattle (*Bos taurus*), mouse (*Mus musculus*), rat (*Rattus norvegicus*), rabbit (*Oryctolagus cuniculus*) and fruit fly (*Drosophila melanogaster*) (Fig. 1, upper right pannel).

3.2. Current density and activation/inactivation properties of heterologously expressed WT and p.Gly638Asp P/Q channels

WT and p.Gly638Asp mutant channels were expressed and analyzed using the whole-cell patch clamp technique in HEK 293 cells. We observed a significant ($P<0.01$) 58% decrease in maximum current density in the mutant p.Gly638Asp compared to the WT P/Q channel (from −49.34 ± 3.67 pA/pF ($n=62$) to −20.42 ± 8.34 pA/pF ($n=8$)) (Fig. 2A, left panel and 2C). No differences between WT and p.Gly638Asp channels were observed regarding their voltage for half-maximal Ca^{2+} current (I_{Ca}) activation ($V_{1/2,\ act}$) ($P>0.16$) or the steepness of the activation curve (k_{act}) ($P>0.59$). However, the apparent reversal potential (V_{rev}) was significantly ($P<0.0001$) shifted to hyperpolarized potentials for p.Gly638Asp channels (by ~5 mV), suggesting a decrease in Ca^{2+} permeability (Fig. 2A, right panel). Activation kinetics were similar for both P/Q channel types (Fig. 2B). To investigate whether the p.Gly638Asp mutation affects the time course of channel inactivation we analyzed I_{Ca} decay during a 3-s test pulse elicited from a holding potential of −80 mV to +20 mV. p.Gly638Asp channels inactivated significantly slower than WT channels (Fig. 2C) and consequently, the rate of recovery from inactivation in mutant channels was accelerated (Fig. 2D).

4. Discussion

We reported a patient where the prototypic signs of EA2 arose in the sixth decade of life. The diagnosis was confirmed after the identification of the underlying molecular defect, a novel missense mutation in the *CACNA1A* gene, resulting in a glycine-to-aspartate change at position 638 of the protein. In order to properly establish the causative role of the p.Gly638Asp mutation in the disease, we studied its functional consequences on P/Q channels expressed in HEK 293 cells. Our data show that p.Gly638Asp reduces P/Q current densities, shifts the apparent reversal potential to lower voltages (~5 mV), slows inactivation kinetics and increases the rate of I_{Ca} recovery from inactivation.

The current EA classification embraces, at least, seven clinical variants [14]. Among these, only EA4 [15] and EA5 [16] are known to present after age 20 and are thus considered "late-onset" disorders; in both conditions vertigo is a common ictal feature and oculomotor disturbances are frequent interictal findings. EA5 was described after screening index individuals from 71 EA kindreds for mutations in the epilepsy-related *CACNB4* gene [16]. The only mutant belonged to a pedigree with EA2-like features, including acetazolamide responsiveness, where *CACNA1A* mutations had been ruled out [14]. The gene defect underlying EA4 is unknown.

Classical EA2 presents in childhood and onset before age 20 is the rule [17]. To our knowledge, late onset in *CACNA1A*-linked EA2 has been reported only once [2]. The authors reported sudden-onset dysarthria and gait ataxia with onset at age 61 in the index case, who carried a 9-base pairs (bp) insertion in the *CACNA1A* gene. The mutation duplicates the previous 9 bp and predicts an insertion of three amino acids within the intracellular linker connecting domains II and III of the protein. Mutant cDNA produced a marked reduction in current amplitudes when injected into oocytes [2]. However, in a previous report [18], one out of four unrelated, sporadic, acetazolamide-unresponsive, late-onset EA patients that were screened for mutations in *CACNA1A* was found to bear a missense change in the C-terminus region of the protein. Since clinical findings were considered atypical for EA2 and the mutation was non-truncating, the authors proposed that their cases represented a new clinical entity. More recent studies have proved that missense mutations do underlie EA2 in some cases [19]. The patient reported herein, therefore, confirms that acetazolamide-responsive EA2 may present in the late sixth decade.

This patient was also unusual in that he displayed some degree of dystonia involving both upper limbs. Interictal dystonia, either segmental or focal, occurred in two EA2 pedigrees [20]. The authors postulated that the dyskinetic disorder may have followed progression of the cerebellar degenerative process, although defective $Ca_V2.1$ function in the basal ganglia in their or our patient cannot be ruled out.

Although the most common causative mutations of the EA2 phenotype are nonsense, some missense mutations, typically involving the pore loop region of the protein and, rarely, CAG repeat expansions, have been also reported [6]. Our patient was found to carry the missense mutation p.Gly638Asp, that replaces a highly conserved tiny and neutral glycine by a negatively charged aspartate. The mutation is located in the pore loop of the channel between segments S5 and S6 of the second domain of the α_{1A} subunit, the same location of p.Thr666Met, the most common CACNA1A mutation, usually associated with familial hemiplegic migraine. Missense mutations have been described also in the pore loops of all other domains of the α_{1A} subunit [6].

Data available from functional studies in the described EA2 missense mutations strongly support the hypothesis that episodic ataxia is a consequence of a partial or complete loss of $Ca_V2.1$ channel function due to reduced conductance and/or deficiencies in protein misfolding and channel membrane targeting [9]. Our functional analysis of the p.Gly638Asp P/Q channel is in agreement with this idea and support mutation pathogenicity. The observed reduction in current density in the mutant channel is probably due to a reduction in Ca^{2+} permeability, as suggested by the left-shift in the

apparent reversal potential of the measured Ca^{2+} currents. Furthermore, although P/Q Ca^{2+} currents inactivate during prolonged depolarization mainly due to a voltage-dependent process, a certain fraction of the inactivation is due to the Ca^{2+} influx generated by the opening of these channels (Ca^{2+}-dependent inactivation) [21]. Therefore, a reduced Ca^{2+} permeability of p.Gly638Asp P/Q channels may account for the slowing in current inactivation and the consequent acceleration in the rate of recovery from inactivation observed in mutant channels.

In summary, these results confirm and extend previous observations of a late onset of the classical EA2 phenotype. Just as in early-onset EA2, the molecular defect underlying these unusual variants may involve loss-of-function mutations in the *CACNA1A* gene.

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References

[1] Baloh RW, Yue Q, Furman JM, Nelson SF. Familial episodic ataxia: clinical heterogeneity in four families linked to chromosome 19p. Ann Neurol 1997;41:8–16.
[2] Imbrici P, Eunson LH, Graves TD, Bhatia KP, Wadia NH, Kullmann DM, et al. Late-onset episodic ataxia type 2 due to an in-frame insertion in CACNA1A. Neurology 2005;65:944–6.
[3] Vahedi K, Denier C, Ducros A, Bousson V, Levy C, Chabriat H, et al. CACNA1A gene de novo mutation causing hemiplegic migraine, coma, and cerebellar atrophy. Neurology 2000;55:1040–2.
[4] Ophoff RA, Terwindt GM, Vergouwe MN, van Eijk R, Oefner PJ, Hoffman SM, et al. Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca2+ channel gene CACNL1A4. Cell 1996;87:543–52.
[5] Jen J, Kim GW, Baloh RW. Clinical spectrum of episodic ataxia type 2. Neurology 2004;62:17–22.
[6] Jen JC, Graves TD, Hess EJ, Hanna MG, Griggs RC, Baloh RW. Primary episodic ataxias: diagnosis, pathogenesis and treatment. Brain 2007;130:2484–93.
[7] Mantuano E, Veneziano L, Spadaro M, Giunti P, Guida S, Leggio MG, et al. Clusters of non-truncating mutations of P/Q type Ca2+ channel subunit Ca(v)2.1 causing episodic ataxia 2. J Med Genet 2004;41:e82.
[8] Jodice C, Mantuano E, Veneziano L, Trettel F, Sabbadini G, Calandriello L, et al. Episodic ataxia type 2 (EA2) and spinocerebellar ataxia type 6 (SCA6) due to CAG repeat expansion in the CACNA1A gene on chromosome 19p. Hum Mol Genet 1997;6:1973–8.
[9] Guida S, Trettel F, Pagnutti S, Mantuano E, Tottene A, Veneziano L, et al. Complete loss of P/Q calcium channel activity caused by a CACNA1A missense mutation carried by patients with episodic ataxia type 2. Am J Hum Genet 2001;68:759–64.
[10] Pujana MA, Volpini V, Gratacos M, Corral J, Banchs I, Sanchez A, et al. Uncloned expanded CAG/CTG repeat sequences in autosomal dominant cerebellar ataxia (ADCA) detected by the repeat expansion detection (RED) method. J Med Genet 1998;35:99–102.
[11] Cuenca-Leon E, Corominas R, Fernandez-Castillo N, Volpini V, Del Toro M, Roig M, et al. Genetic analysis of 27 Spanish patients with hemiplegic migraine, basilar-type migraine and childhood periodic syndromes. Cephalalgia 2008;28:1039–47.
[12] Fernandez-Fernandez JM, Tomas M, Vazquez E, Orio P, Latorre R, Senti M, et al. Gain-of-function mutation in the KCNMB1 potassium channel subunit is associated with low prevalence of diastolic hypertension. J Clin Invest 2004;113:1032–9.
[13] Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflugers Arch 1981;391:85–100.
[14] Kerber KA, Jen JC, Lee H, Nelson SF, Baloh RW. A new episodic ataxia syndrome with linkage to chromosome 19q13. Arch Neurol 2007;64:749–52.
[15] Damji KF, Allingham RR, Pollock SC, Small K, Lewis KE, Stajich JM, et al. Periodic vestibulocerebellar ataxia, an autosomal dominant ataxia with defective smooth pursuit, is genetically distinct from other autosomal dominant ataxias. Arch Neurol 1996;53:338–44.
[16] Escayg A, De Waard M, Lee DD, Bichet D, Wolf P, Mayer T, et al. Coding and noncoding variation of the human calcium-channel beta4-subunit gene CACNB4 in patients with idiopathic generalized epilepsy and episodic ataxia. Am J Hum Genet 2000;66:1531–9.
[17] Mantuano E, Veneziano L, Jodice C, Frontali M. Spinocerebellar ataxia type 6 and episodic ataxia type 2: differences and similarities between two allelic disorders. Cytogenet Genome Res 2003;100:147–53.
[18] Julien J, Denier C, Ferrer X, Ducros A, Saintarailles J, Lagueny A, et al. Sporadic late onset paroxysmal cerebellar ataxia in four unrelated patients: a new disease? J Neurol 2001;248:209–14.
[19] Wan J, Khanna R, Sandusky M, Papazian DM, Jen JC, Baloh RW. CACNA1A mutations causing episodic and progressive ataxia alter channel trafficking and kinetics. Neurology 2005;64:2090–7.
[20] Spacey SD, Materek LA, Szczygielski BI, Bird TD. Two novel CACNA1A gene mutations associated with episodic ataxia type 2 and interictal dystonia. Arch Neurol 2005;62:314–6.
[21] Cens T, Rousset M, Leyris JP, Fesquet P, Charnet P. Voltage- and calcium-dependent inactivation in high voltage-gated Ca(2+) channels. Prog Biophys Mol Biol 2006;90:104–17.