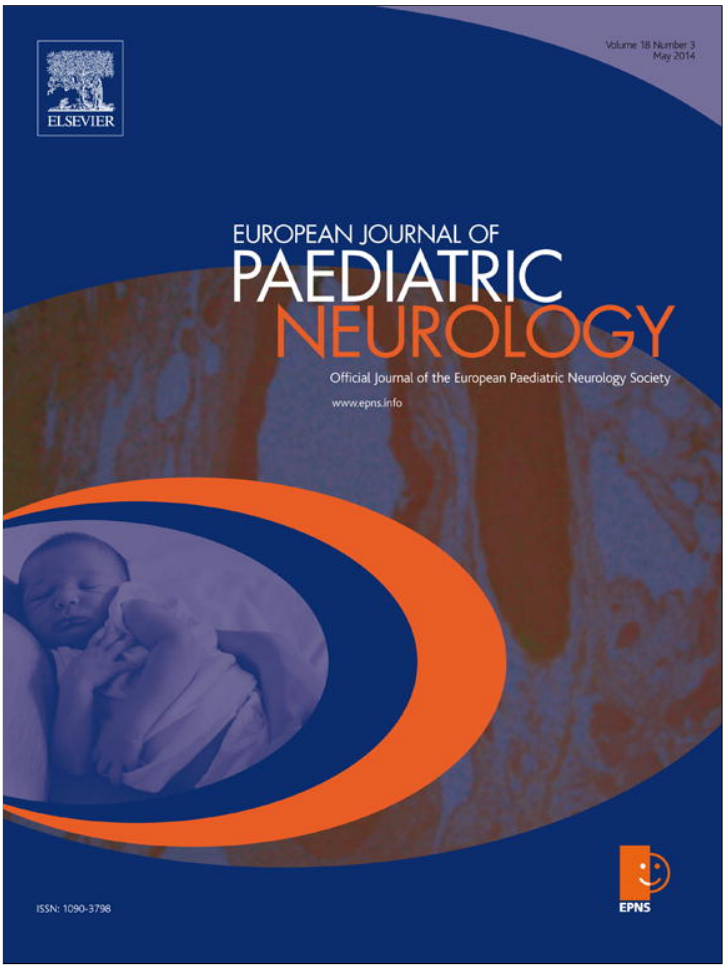

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Case study

A loss-of-function *CACNA1A* mutation causing benign paroxysmal torticollis of infancy

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ABSTRACT

Benign paroxysmal torticollis of infancy (BPTI) is a rare paroxysmal disorder characterized by recurrent episodes of head tilt and accompanying general symptoms which remit spontaneously. The rare association with gain-of-function *CACNA1A* mutations, similar to hemiplegic migraine, has been reported. We report here two new BPTI patients from the same family carrying a heterozygous mutation in the *CACNA1A* gene leading to the change p.Glu533Lys. Functional analysis revealed that this mutation induces a loss of channel function due to impaired gating by voltage and much lower current density. Our data suggest that BPTI, a periodic syndrome commonly considered a migraine precursor, constitutes an age-specific manifestation of defective neuronal calcium channel activity.

1. Introduction

Benign paroxysmal torticollis of infancy (BPTI) is a rare paroxysmal disorder characterized by recurrent episodes of head tilt to one side and variable behavioral and autonomic changes. Attacks start during infancy, last minutes to days and usually recur monthly. They are accompanied by one or more of the following signs: pallor, irritability, malaise, vomiting and ataxia. Neurological examination between

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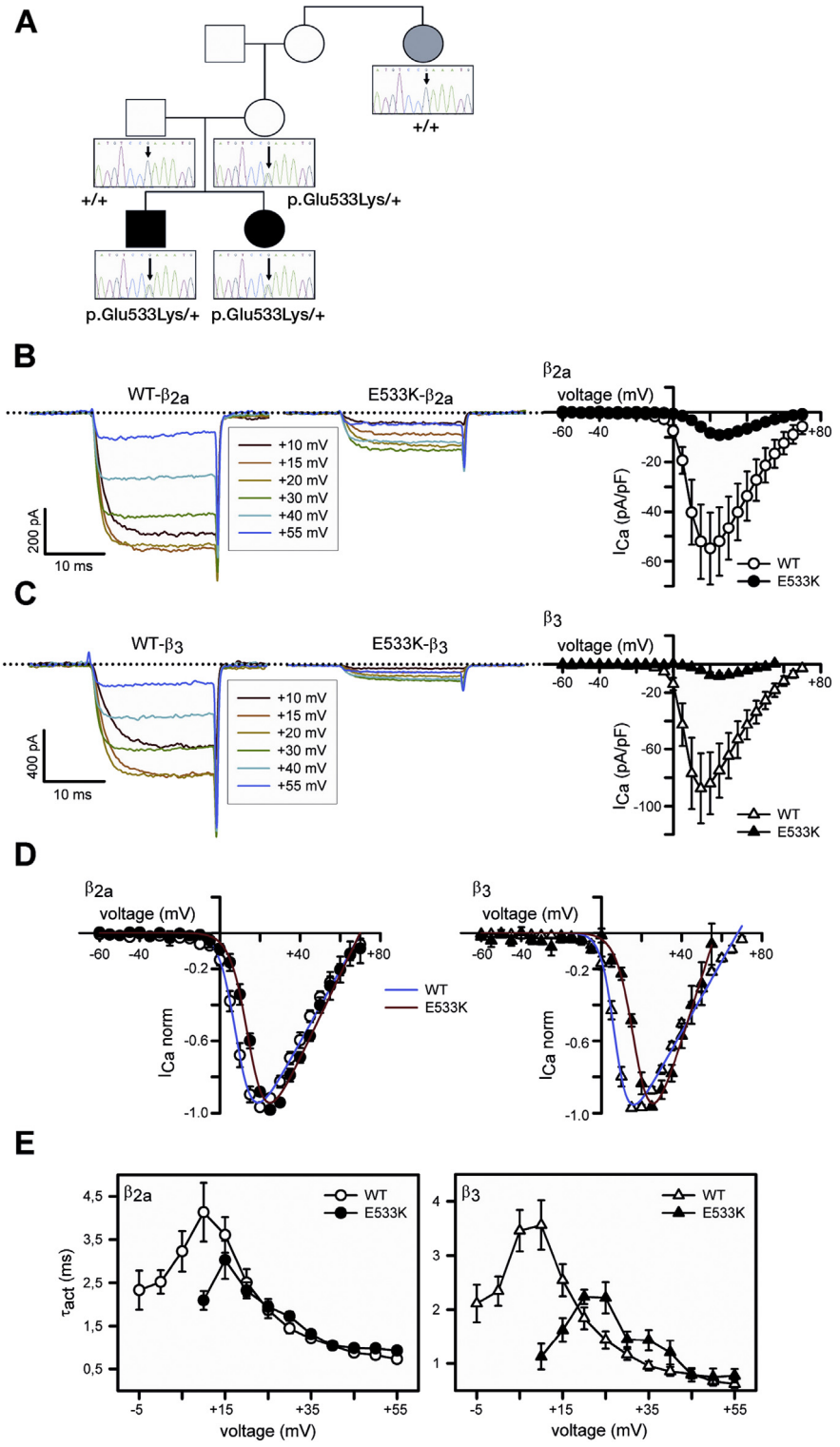

Fig. 1 – A) Pedigree of the family with the electropherograms showing the mutated or the wild-type residue in *CACNA1A*. White symbols indicate healthy individuals, black BPTI and grey epilepsy. B-C) Current traces illustrating voltage-dependence and activation kinetics (τ_{act}) of WT (left) and p.Glu533Lys (center) $Ca_V2.1$ channels, in response to 20 ms voltage pulses. Dotted lines in the current traces indicate the zero current level. Current density–voltage relationships are shown in the right panels (B: for channels containing the β_{2a} subunit; C: for channels containing the β_3 subunit). D) Normalized *I*–*V* curves for WT (open symbols) and p.Glu533Lys (closed symbols) $Ca_V2.1$ channels expressed in HEK293 cells. A modified Boltzmann equation was fitted to normalized current–voltage (*I*–*V*) to obtain the voltage dependence of activation for each $Ca_V2.1$ channel (indicated by $V_{1/2,\ act}$ values): $I = G_{max}(V - V_{rev})/(1 + \exp(-(V - V_{1/2,\ act})/k_{act}))$. $V_{1/2,\ act}$ values were (in mV): WTβ_{2a} (○, $n = 10$) 8.8 ± 1.1; p.Glu533Lysβ_{2a} (●, $n = 11$) 14.5 ± 0.7; WTβ_{3a} (△, $n = 9$) 7.1 ± 0.8; p.Glu533Lysβ_3 (▲, $n = 8$) 17.3 ± 0.9. E) Average τ_{act} of WT (open symbols) and p.Glu533Lys channels (closed symbols) at the indicated voltages. The presence of either the regulatory β_{2a} subunit or the β_3 subunit is indicated at each panel. 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 $P < 0.05$.

episodes is normal. It usually improves by age 2 years and tends to resolve by age 3–5, often evolving into benign paroxysmal vertigo (BPV)[1] or migraine with aura (MA).

There is some clinical and genetic evidence pointing to this childhood periodic syndrome as one of the infantile migraine precursors. Clinically, it is a paroxysmal disorder with accompanying signs similar to some of the non-headache features of migraine. Genetically, it has been occasionally associated with mutations in *CACNA1A*, a gene that encodes the ion-conducting pore and voltage-sensing α subunit of the neuronal $Ca_V2.1$ (P/Q-type) calcium channel and that has been linked to familial hemiplegic migraine (FHM).

We report a new case of this clinical-genetic association and provide functional evidence that the mutation is indeed disease-causing.

2. Case study

A 3year-old boy was referred with a history of recurrent episodes of torticollis starting at the age of 9 months and occurring twice per month ever since. He experienced acute episodes of lateral flexion of neck, with ipsilateral flexion of the trunk and ataxia, and no useful ipsilateral hand movement. During the episodes, which lasted no longer than 5 min and were relieved by sleep, the patient became irritable, unsteady, aphasic and held onto his mother. No nystagmus or loss of consciousness were recorded. After age 2 years the patient appeared drowsy and apathetic during the episodes. His psychomotor development and interictal examination are normal. No pharmacological treatment was attempted due to the low frequency of the attacks at the age of referral. Her 10-year-old sister had experienced similar attacks between ages 13 months and 3 years. They occurred monthly and lasted from 30 min to 24 h and some reportedly associated upgaze deviation and severe global hypotonia. Treatment with carbamazepine worsened them. Her EEG and RM were normal. No overt migraine attacks have developed.

Peripheral blood samples were collected from both patients, parents and an epileptic aunt. Genomic DNA was isolated using a standard salting-out method. Promoter, all exons and flanking intronic regions of *CACNA1A* were amplified by PCR, purified and sequenced using BigDye Terminator cycle sequencing kit v3.1 and the automated sequencer ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Copy Number Variant (CNV) studies for *CACNA1A* were performed by Multiplex Ligation-Dependent Probe Amplification (MLPA) using the SALSA MLPA kit P279-A2 (MRC-Holland, Amsterdam, the Netherlands) and Quantitative Multiplex PCR of Short Fluorescent Fragments (QMPSF) using four sets of primer pairs that covered 16 additional exons (details available upon request).

A heterozygous G to A transition at cDNA position 1597 (c.1597G > A, reference sequence GeneBank NM_023035) was found in both patients and their asymptomatic mother, but not in their father nor their aunt (Fig. 1A). This mutation results in a substitution of glutamate for lysine at residue 533 (p.Glu533Lys). Such residue is located at the second voltage sensor domain (VSD) of the $Ca_V2.1$ channel and it has been suggested to necessarily contribute to the energy pathway required for the movement of the voltage sensor to gate the channel. No CNV was found.

$Ca_V2.1$ wild-type (WT) and p.Glu533Lys (E533K) mutant human channels were heterologously expressed in HEK293 cells and analyzed using the whole-cell patch clamp technique as previously described.[2] We observed a significant decrease in maximum current density in the mutant p.Glu533Lys compared to WT P/Q channels containing functionally different regulatory β subunits (in channels containing the β_{2a} subunit: from -54.9 ± 14.6 pA/pF ($n = 10$) to -9.2 ± 1.6 pA/pF ($n = 11$), $P < 0.01$ (Fig. 1B); in channels containing the β_3 subunit: from -87.6 ± 24.5 pA/pF ($n = 9$) to -8.3 ± 1.9 pA/pF ($n = 8$), $P < 0.001$ (Fig. 1C)). The potential for half-maximal activation ($V_{1/2,\ act}$) was also significantly shifted to depolarized potentials for p.Glu533Lys channels (by ~ 6 mV for channels containing the β_{2a} subunit ($P < 0.001$) and by ~10 mV for channels containing the β_3 subunit ($P < 0.0001$)). Consistently, the maximum current amplitude was elicited by depolarizing pulses to +20/+15 mV or +25 mV for WT or p.Glu533Lys channels, respectively (Fig. 1D). Furthermore, activation kinetics of p.Glu533Lys mutant channels were also right shifted (by ~5 mV for channels containing the β_{2a} subunit and by ~10 mV for channels containing the β_3 subunit when compared to WT channels) (Fig. 1E).

3. Discussion

We have described the unusual instance of two sibs with BPTI carrying a loss-of-function mutation in *CACNA1A*. This observation adds to previous evidence suggesting that BPTI can be an early manifestation of a calcium neuronal channelopathy.

Because of the accompanying general symptoms, complete recovery between attacks, development of migraine or other related syndromes at follow-up, and the presence of family history of migraine, BPTI is often considered a childhood migraine precursor or equivalent. In fact, three previous reports have identified mutations in *CACNA1A* in patients[2,3] or in relatives of patients[4] with BPTI. Here we report the *CACNA1A* p.Glu533Lys mutation in two siblings affected with BPTI and their presumably asymptomatic mother. The latter might have displayed reduced penetrance or have suffered the same symptoms in infancy, but without being cause of concern.

This is the fourth mutation that links this syndrome to the α subunit of the neuronal $Ca_V2.1$ channel, encoded by the *CACNA1A* gene. The first mutation (p.Tyr1854*) was found in a BPTI patient from a kindred with FHM and episodic ataxia type-2 (EA-2).[4] The patient was described as having attacks from six weeks of age, starting with tilting of the head, head turning to the left for 15–30 min, followed by vomiting and unsteadiness of gait, and lasting 1–3 days. The second mutation (p.Tyr1245Cys) was found and functionally characterized by our group in the clinical context of neonatal BPTI that evolved with aging, first into BPV and then into HM.[2] The third one (p.Gln736*) was found in a BPTI patient from a kindred with paroxysmal tonic upgaze and EA.[3] The patient was described as having attacks from 16 months of age, starting with head tilting, accompanied by unsteadiness, moaning,

sweating and sometimes vomiting, and lasting for one to several hours.

The results of our functional studies of the p.Glu533Lys mutation on $Ca_V2.1$ channels expressed in HEK293 cells show that this mutation induces a loss of channel function due to an impaired gating by voltage and much lower current density. The later effect might be due to a reduction in the traffic of P/Q channels to the plasma membrane, as suggested for other EA-2 mutation (p.Glu147Lys) affecting a glutamate residue placed at a similar position but on the first VSD of the $Ca_V2.1$ channel. The p.Tyr1854* was analyzed *in vitro* elsewhere showing also a loss-of-function of the mutant channel. Moreover, the premature stop codon can lead to nonsense-mediated RNA decay (NMD) of the resulting transcripts, a process that, if triggered, would induce a loss-of-function due to haploinsufficiency. The p.Gln736* mutation has not been functionally studied, but the early position of the resulting premature stop codon made the authors suppose it would lead to NMD of the mutated transcripts and therefore to haploinsufficiency of the CACNA1A protein. These mutations all lead to a loss-of-function of the channel. However, our functional analysis of the p.Tyr1245Cys mutation showed a clear gain of function due to improved channel activation by voltage and decreased direct channel inhibition by G-proteins.[2]

Thus, the p.Glu533Lys mutation identified in our family and the two previously described mutations with premature stop codon (p.Tyr1854* and p.Gln736*), all induce a loss-of-function of the calcium channel, the most common functional correlate of mutations causing EA-2. Indeed, the p.Glu533Lys mutation has been previously found in a EA-2 patient and her affected relatives.[5] Her spells started at age 12, lasted about 20 min and were sometimes precipitated by stress and preceded by headache.

Mutations in the *CACNA1A* gene have been now associated with a wide variety of paroxystic syndromes, including EA-2, FHM, BPTI, other childhood periodic syndromes and a variant of alternating hemiplegia of childhood (AHC).

On the basis of its association with loss-of-function *CACNA1A* mutations, as in the cases reported here, or with *CACNA1A* haploinsufficiency, we hypothesize that BPTI may represent an age-specific, early manifestation of defective neuronal calcium channel activity.

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