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Original article

Missense mutations of *CACNA1A* are a frequent cause of autosomal dominant nonprogressive congenital ataxia

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ABSTRACT

Background: Mutations in the *CACNA1A* gene, encoding the pore-forming CaV2.1 (P/Q-type) channel α1A subunit, localized at presynaptic terminals of brain and cerebellar neurons, result in clinically variable neurological disorders including hemiplegic migraine (HM) and episodic or progressive adult-onset ataxia (EA2, SCA6). Most recently, *CACNA1A* mutations have been identified in patients with nonprogressive congenital ataxia (NPCA).

Methods: We performed targeted resequencing of known genes involved in cerebellar dysfunction, in 48 patients with congenital or early onset ataxia associated with cerebellar and/or vermis atrophy.

Results: De novo missense mutations of *CACNA1A* were found in four patients (4/48, ~8.3%). Three of them developed migraine before or after the onset of ataxia. Seizures were present in half of the cases.

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Alpha-1a subunit (CACNA1A)
Targeted resequencing

Conclusion: Our results expand the clinical and mutational spectrum of *CACNA1A*-related phenotype in childhood and suggest that *CACNA1A* screening should be implemented in this subgroup of ataxias.

1. Introduction

CACNA1A encodes the α1 subunit of the Voltage-dependent Ca^{2+} channels, which mediate the intracellular entry of Ca^{2+} ions and are involved in a variety of Ca^{2+}-dependent processes, such as neurotransmitter release, and gene expression.[1] CACNA1A spans approximately 300 kb and contains 50 exons. It encodes CaV2.1 (P/Q-type) Ca^{2+} channel, located in the plasma membrane, composed of a main transmembrane pore-forming subunit (α1A) which consists of four repeated domains (I–IV) each containing six transmembrane regions (S1–S6) with a voltage sensor (S1–S4) and a pore region (S5, P-loop and S6). The N and C-terminal regions and the large intracellular loops between α1A domains serve as platforms for channel gating regulation processes, which includes the functional interaction with proteins of the synaptic vesicle docking/fusion machinery and with calmodulin.[2,3] At the postsynaptic side, it contributes to neuronal excitability[4] and Ca^{2+} signaling microdomains.[5] CACNA1A is expressed in the cerebellum and in all brain areas involved in the pathogenesis of migraine, including the cerebral cortex and nociceptive pathways.[6] Loss of function mutations of CACNA1A cause spinocerebellar ataxia type 6 (SCA6; MIM 183086), characterized by late onset, slowly progressive cerebellar ataxia due to the toxic accumulation of the expanded polyQ and subsequent selective degeneration of cerebellar Purkinje cells. Episodic Ataxia type 2 (EA2; MIM 108500) is also associated with CACNA1A loss of function mutations.[7] Gain of function mutations of CACNA1A in the α1A channel subunit located around the pore or the voltage sensor regions, responsible for channel activation at lower voltages and increased channel opening probability[8,9] have been linked to 50% of cases of familial and sporadic hemiplegic migraine (FHM/SHM) type 1 (MIM 141500).[10] FHM phenotypes have also been associated with loss of function mutations and recently biallelic mutations of *CACNA1A* (one truncating and one missense) were identified in a patient with early onset epilepsy, ataxia and optic nerve atrophy.[11,12] Although the clinical phenotypes of the three allelic disorders are highly overlapping and data from the literature suggests that in 20% of cases of FHM the disease is associated with a mild permanent cerebellar ataxia, there are few reports of patients with congenital (non episodic) ataxia carrying missense *CACNA1A* mutations.[13–15] We used a targeted next generation sequencing approach to screen known ataxia genes in a series of patients with congenital or early onset ataxia associated with cerebellar and/or vermis atrophy without any cause found in previous screening.

2. Patients and methods

2.1. Patients

48 patients from unrelated families were enrolled in this study as part of a whole cohort of patients affected by non syndromic congenital or early onset cerebellar ataxia, cerebellar atrophy with or without intellectual disability, recruited at the Bambino Gesù Children's Hospital from January 2000 to December 2015. Following the identification of the mutations, all clinical and neuroimaging data sets were retrospectively analyzed by expert pediatric neurologists. Informed consent was obtained from all participating subjects according to the Declaration of Helsinki.

2.2. Targeted next generation sequencing

We used standard methods (Qiagen DNA extraction kit) to isolate genomic DNA from peripheral blood of the patients and their family members. The exonic sequences of 5 genes known to be associated with pediatric ataxia (*CACNA1A*, *ITPR1*, *KCNC3*, *ATP2B3 and GRM1*) were analyzed using a Truseq Custom Amplicon (TSCA) (Illumina Inc., San Diego, CA) targeted capture and paired end library kit. Targeted resequencing was performed using TSCA enrichment sequenced on an Illumina MiSEQ desktop sequencer (Illumina Inc.). Selected variants were analyzed using standard pathogenicity prediction programs including PolyPhen-2,[16] SIFT,[17] and the mutation interpretation software Alamut (http://www.interactive-biosoftware.com/).

2.3. Sanger sequencing

Sanger sequencing was used to confirm *CACNA1A* (Genbank accession NM_000068.3; ENST00000614285) variants and segregation analysis.

2.4. Protein sequence alignment and molecular modeling

Homology modeling of CACNA1A protein across residues 1244–1360 (encompassing the S1–S4 transmembrane segments of the repeat III) was made with MODELLER (9v13) https://salilab.org/modeller.[18] Comparative protein modeling by satisfaction of spatial restraints, using the structure of a voltage-gated sodium channel as template (Protein Data Bank entry 4BGN) according to the sequence alignment is shown in Fig. 2. The two proteins exhibit 27% amino acid identity along this amino acid interval. Homology modeling of the voltage-dependent P/Q-type calcium channel subunit alpha-1A