CACNA1A Mutations Causing Early Onset Ataxia: Profiling Clinical, Dysmorphic and Structural-Functional Findings

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1. Introduction

The CACNA1A gene, located on chromosome 19p13, encodes the pore-forming α1A subunit of the voltage-gated Cav2.1 (P/Q-type) Ca\(^{2+}\) channel, which mediates the intracellular entry of Ca\(^{2+}\) ions [1]. CACNA1A plays a major role in neurotransmitter release...
throughout the nervous system, especially in cerebellar Purkinje cells and in all brain areas involved in the pathogenesis of migraine [1].

The Cav2.1 (P/Q-type) Ca\textsuperscript{2+} channel is a high-voltage-activated channel consisting of a principal pore-forming subunit (\(\alpha_{1A}\) or CACNA1A), associated with auxiliary subunits [2,3]. \(\alpha_{1A}\) consists of four repeated homologous domains (DI–DIV), each with six transmembrane regions (S1–S6) that constitute two functional modules: a voltage sensor (S1–S4) and a \(\text{Ca}^{2+}\)-selective pore (S5-P loop-S6) [2]. The movement of 5–6 positively charged residues located at the S4 helices (Arg\textsuperscript{+} or Lys\textsuperscript{+}, every 3rd-4th amino acid (defined as R0-R5 gating charges)) induces the conformational changes required for pore opening in response to membrane depolarization [2].

Mutations in the CACNA1A gene result in great clinical heterogeneity, and present with chronic progressive symptoms or paroxysmal events (including sporadic and familial hemiplegic migraine (HM), epilepsy and migraine), or both [4]. Initially, mutations in the CACNA1A gene were identified in patients with familial hemiplegic migraine (FHM) (MIM#141500) or episodic ataxia type 2 (MIM#108500) [4], in that phenotypes acetazolamide may prevent recurrence and improve symptoms [5]. Triplet repeat mutations in the CACNA1A gene were associated with late onset-spinocerebellar ataxia type 6 (MIM#183086) characterized by late onset, slowly progressive cerebellar ataxia, due to toxic accumulation of an expanded polyQ [6]. Other mutations of CACNA1A were identified in patients with early onset epileptic encephalopathy (MIM#617106) [7]. Recently, single case reports and short case series have described patients with congenital (non-episodic) ataxia, with or without cerebellar atrophy, carrying missense CACNA1A mutations [8,9], located around pore regions, voltage-sensing regions and S4-S5 linkers connecting these two functional modules of Cav2.1 [8]. Therefore, CACNA1A mutations should be included in the differential diagnosis of both congenital ataxia and early onset epileptic encephalopathy [7,8]. Unfortunately, clinical findings may be unspecific at early stages, radiology may show normal findings and dysmorphic features that could be of help have not yet been reported. To complete the phenotype, cognitive dysfunction, including learning difficulties and autism, have been reported among the chronic neurological manifestations associated with CACNA1A point-mutations [4,10,11].

We present the neurological and radiological features of three patients with congenital ataxia, two of them with a previously reported CACNA1A mutation and one carrying a new CACNA1A variant. We propose a pattern of dysmorphic facial features to facilitate diagnosis. Clinical and neuroradiological evolution, pharmacological management, structural localization of variants and their expected functional consequences are also depicted.

2. Results
2.1. Clinical Description Of Patients

Patients’ molecular, clinical and radiological data are presented in Table 1 and Figure 1.
Table 1. GA: Gestational age; HELLP syndrome: hemolysis, elevated liver enzymes, low platelet count, related with pregnancy; SD: standard deviation; OFC: occipito-frontal circumference; WIPPSI: Wechsler Preschool and Primary Scale of Intelligence; ADHD: attention deficit and hyperactivity disorder; MPD: Methylenidate; MVRD: Midsaggital vermis relative diameter. * Subscores: Language 77, socialization 80, coordination 75, postural 34.

| Patient/Gender | 1 Female | 2 Male | 3 Female |
|----------------|----------|--------|---------|
| Age            | 8 years  | 20 years | 7 years |
| Molecular findings/ inheritance | c.4182C>A p.Phe1394Leu/De novo | c.4991G>A p.Arg1664Gln/De novo | c.5006G>A p.Arg1669Gln/De novo |
| Pregnancy & delivery/GA | Twin pregnancy/HELP syndrome/C-section/ 35 weeks | Normal/eutocic/41 weeks | Normal pregnancy/forceps/40 + 6 weeks |
| Somatometry at birth (SD) | Weight 2150 g (+1.0SD) Height 43.5cm (+0.7 SD) OFC 32 cm (+0.5 SD) | Weight 3520 g (+0.1 SD) Height 51 cm (+0.1 SD) OFC 36 cm (+0.4 SD) | Weight 3400 g (+0.3 SD) Height 51 cm (+0.6 SD) OFC 35 cm (+0.1 SD) |
| Somatometry at last evaluation (SD) | 19.5 kg (−1.5 SD) 1.16 m (−0.5 SD) OFC 52.5 cm (+0.5 SD) | 77.2 kg (+0.2 SD) 176 m (−0.2 SD) OFC 59 cm (+1.5 SD) | 22.6 kg (−0.89 9 SD) 1.23 m (−0.69 7 SD) OFC 53.5 cm (+1.2 SD) |
| Initial neurological symptoms | Hypotonia Severe developmental delay | Hypotonia Developmental delay | Hypotonia Developmental delay |
| Cerebellar syndrome | Truncal ataxia and stereotypes Strabismus, terminal nystagmus | Mild ataxia/Dysarthria Oculomotor apraxia Nystagmus | Mild ataxia |
| Neurodevelopment | Sitting position: 27 months Walk only with stroller: 5 years No speech (guttural sounds) Special schooling | Sitting position: 8 months Independent walking: 30 months Language delay Occupational school | Sitting position: 9 months Independent walking: 30 months Language Delay Ordinary school with support |
| Other neurological symptoms | Intellectual disability Autistic traits Attention deficit (treated with guanfacine) | Mild intellectual disability Uncontrolled lateral head movements without consciousness abnormalities. | Mild intellectual disability Brunet – Lézine (30 months) 67 * WIPPSI IV (5.5 years): verbal IQ 70, performance IQ 68. ADHD (MPD) |
| Cranial magnetic resonance | 6 months: ventricular enlargement and increased extra – axial spaces. Normal posterior fossa structures. 16 months: cerebellar atrophy: increased interfolia spaces mainly in vermis. MVRD = 0.66 (mean for controls 0.77). 4 years 10 months: progression of generalized cerebellar atrophy: MVRD = 0.51 (mean for controls 0.80) | 6 years 10 months: cerebellar atrophy, with a MVRD = 0.76 (mean for controls 0.82). 13 years 2 months: progressive generalized cerebellar atrophy. MVRD = 0.64 (mean for controls 0.87) | 15 months: no signs of cerebellar atrophy. 24 months: cerebellar atrophy: increase of interfolia spaces in the superior vermis |
| ICARS assessment | No collaboration, no comprehension | 17 years: 14/100 19 years: 12/100 | 4 years: 28/100 6 years: 19/100 |
| Acetazolamide therapy(at least during 8 months) | 12 mg/kg/day in two doses Improvement in muscle tone and communication intention. No objective positive response in the long term. | 250 mg /12 h Improvement in motor symptoms. Abolished stereotyped episodes. Withdrawn due to lithiasis | 12 mg/kg/day in two doses. No objective positive response |
and a high palate. They all present joint laxity, but also long fingers, with clinodactyly of fifth fingers combined with marked camptodactyly of fifth fingers in the oldest patient.

Figure 1. Clinical and radiological features of patients. Above, the magnetic resonance sagittal and coronal images show a progression in the cerebellar atrophy in Patients 1 and 2, despite clinical stabilization. Immediately below the images, the midsagittal vermis relative diameter (MVRD) has been calculated in the sagittal sequences for patients 1 and 2. MVRDs are detailed and compared to controls’ values. In the middle, pictures from the patients are shown. In the bottom, Human Phenotype Ontology (HPO) codes are included. y: years; mo: months; MVRD: midsagittal vermis relative diameter.

Concerning neuroimaging, cranial MRI characteristics are detailed in Table 1, and images are included in Figure 1. In sequential MRI studies a progression in the cerebellar atrophy is marked, with unspecific or no findings in the supratentorial structures. Their MVRD was compared with two sex- and age-matched controls.

2.2. Molecular Characterization of Patients

Regarding molecular findings, patient 1 had a likely pathogenic heterozygous missense mutation c.4182C>A (p.Phe1394Leu, F1394L) in the CACNA1A gene encoding α1A isoform 2, NM_023035.2. Patient 2 and 3 had a similar likely pathogenic heterozygous missense mutation with different nomenclature due to isolation from two different

Pregnancies were uneventful; all the patients showed developmental delay, with particularly marked speech delay in Patient 1. They all presented persistent cerebellar symptoms in different degrees of severity: global hypotonia, truncal and limb ataxia, dysarthria or slurred speech and oculomotor symptoms such as nystagmus, oculomotor apraxia and strabismus. Patients 2 and 3 walk independently and are able to maintain a conversation at 20 years and 7 years of age, respectively. Patient 1, the most severely affected, is able to walk only with a walking frame and has severe communication impairment at 8 years of age. They have not shown neurological regression at any time.

Acetazolamide was initiated in all three patients, applying a compassionate use formula, and maintained for at least 8 months. Responses are detailed in Table 1. In the case of Patient 2, at the age of 17 years he came into the emergency room because of subtle uncontrolled lateral head movements without consciousness abnormalities. A video EEG register during the episode ruled out epileptic activity, and the initiation of acetazolamide limited the episodes and improved the motor symptoms (three points in the ICARS). However, after 12 months of therapy he presented kidney lithiasis and the treatment was
stopped. Subtle worsening in motor abilities was evident but no new abnormal movements appeared after the withdrawal.

The two younger patients showed abnormal executive functions and fulfilled attention deficit with hyperactivity disorder (ADHD), with positive response to guanfacine and methylphenidate, respectively.

The three patients show common dysmorphic traits (Figure 1) such as a mildly asymmetric and oval face with large and prominent forehead, mild bilateral ptosis, strabismus, hypertelorism, telecanthus, downsloping palpebral fissures and narrow nasal bridge. Patients 1 and 2 also show low-set ears. In the midface, patients 1 and 3 show a long philtrum and a high palate. They all present joint laxity, but also long fingers, with clinodactyly of fifth fingers combined with marked camptodactyly of fifth fingers in the oldest patient.

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By using the structure of the rabbit Cav1.1 complex [12], the localizations of Cav2.1 mutations can be predicted given their high level of homology. Thus, F1394L is located at the S5 segment of α1A domain III (DIII), a few amino acids downstream from the beginning of the pore-loop between S5 and S6 helices. R1664Q and R1669Q are the same mutation in α1A isoforms 3 and 2, respectively, and they affect the R2 gating charge at the voltage sensor S4 segment of domain IV (DIV) (Figure 2). The two affected residues, F1394 and R1664/R1669, are fully conserved throughout evolution (Figure 3a for comparison of orthologous Cav2.1 channels) and among all the human Cav channel family [4] (Figure 3b). Supplementary Table S1 encompasses previously CACNA1A variants linked to congenital ataxia.
Figure 2. Location of the mutations in CaV2.1 α1A channel subunit. (a) Location of the variant residues of α1A channel subunit isoforms 2 and 3 in the secondary structure of the pore forming α1S and several regulatory subunits [12] (see also panel B). N- and C-termini and the intracellular loops are shown in gray, voltage-sensor modules (S1–S4) from the four domains are in red—with the S4 α-helices colored in dark red—and Ca2+-selective pore modules (S5-P loop-S6) are in blue. (b) Sequence alignment of the regions affected by variants between the human α1A channel subunit isoforms 2 and 3 and rabbit CaV1.1 α1S subunit. The α1A subunit segment of each alignment is indicated at the top. Mutations are highlighted with yellow circles on the human CaV1.1 (hCaV1.1) sequences. The gating charged residues of S4 segments (labeled R0-R5) are shaded in red. Amino acids are colored depending on their physicochemical properties: small and hydrophobic are in red, acidic in blue, basic in magenta and G and amino acids containing hydroxyl, sulfhydryl or amine groups in green. Below, a consensus code indicates fully conserved residues (*), conservation between residues with strongly similar properties (:), or with weakly similar properties (.). The Uniport IDs of the sequences aligned are for hCaV2 (α1A): O00555-2 (isoform 2) and O00555-3 (isoform 3) and for rabbit CaV1.1 (α1S): P07293. The sequence alignments were made using Multiple Sequence Alignment Clustal Omega. (c) Three-dimensional location of the amino acid variants on a CaV channel model. The structure of the α1S subunit of the CaV1.1 channel (PDB 5GJV) [12] was used as a model considering their high level of homology (panel B). N- and C-termini and the intracellular loops are shown in gray, voltage-sensor modules (S1–S4) are in gray and Ca2+-selective pore modules (S5-P loop-S6) are in dark gray. The two regions where mutations are located are highlighted in yellow for S5 helix of DIII and green for S4 helix of DIV. The residues of CaV1.1 (α1S) equivalent to those mutated in CaV2.1 (α1A) and identified in the patients, according to the sequence alignment on panel B, have been highlighted in magenta.
3. Discussion

We present three patients with non-progressive congenital ataxia without HM or epilepsy and common dysmorphic traits. Facial dysmorphic features of all patients include: strabismus, hypertelorism, telecanthus, downslanting palpebral fissures and narrow nasal bridge. We have identified only one previous article describing dysmorphic traits in a patient harboring a pathogenic variant in CACNA1A [13]. She had congenital ataxia with
severe HM and dysmorphic facial features, including round face with high forehead and an OFC in the 90–97th centile, and carried a ΔF1502 gain-of-function variant (located at the inner pore vestibule of the CaV2.1 channel) [13]. Genetic diseases presenting congenital ataxia are frequently difficult to diagnose as cerebellar atrophy on the MRI may not be present at early stages of development and muscular hypotonia, weak deep tendon reflexes, and delayed motor milestones are unspecific neurological signs. However, a particular gestalt may help to identify genetic diseases, and, at the present time, new technologies of facial pattern recognition have demonstrated their usefulness in a wide number of genetic conditions [14].

The cerebellum expresses a variety of different functions in addition to motor action, including affective skills, working memory, response timing and attentional control, and many of the patients reported on in the literature show intellectual disability [15]. Moreover, the cerebellum is strongly associated with autism spectrum disorder (ASD); pathological studies have found consistent abnormalities in Purkinje cells in ASD patients; in addition, the cerebellum is involved in associative learning, cognition, and emotional function, which are altered in ASD [16]. Two of our patients show intellectual disability, and the most severely affected patient (patient 1) also shows autistics traits. The younger patients have ADHD with remarkable good response to methylphenidate and guanfacine, which might be explained by the impairment in cognitive executive functions and working memory described in cerebellar disorders [15].

The three patients showed an improvement in all neurodevelopmental areas, despite two of them showing a progressive cerebellar atrophy in sequential MRI. The most severe patient shows the greatest cerebellar atrophy, which suggests that there might be a correlation between the neurological impairment (including motor, speech and cognitive skills) and the degree of cerebellar atrophy (Figure 1).

Acetazolamide therapy showed clear benefit in patient 2, but not in patient 3 carrying the same CaV2.1 amino acid substitution; however, the development of renal calculi prompted us to stop the therapy. In patient 1, parents perceived a benefit in muscle tone and communication intention after acetazolamide treatment, but the lack of specific scales to evaluate cerebellar syndrome before and after the treatment limits our conclusions.

Knowledge of the mutation’s effect on protein function is important to improve our understanding of the phenotype of patients with CACNA1A-related disease and to contribute to the development of new treatment approaches. In this respect, no functional studies are available for the previously unreported F1394L variant. Still, the affected residue is located in a region of the S5 segment at DIII contributing to the channel pore. This region includes invariant amino acids conserved in the rabbit CaV1.1 (Figure 2), in CaV2.1 through evolution (Figure 3a, left) and in all human CaV channels (Figure 3b, left), which suggests high functional relevance. In this sense, two other amino acid substitutions in the same S5 pore segment where F1394L is located, Y1385C (corresponding to Y1387C in α1A isoform 2 and Y1384C in isoform 3) and V1396M (corresponding to V1399M in α1A isoform 2), have been reported in association with congenital ataxia with cerebellar atrophy, psychomotor delay, intellectual disability, epilepsy (including different kinds of refractory generalized seizures), HM and acute coma (Figure 3, left) [17–19]. For mutation V1396M/V1399M (five residues upstream of F1394L in α1A isoform 2), the functional analysis of the heterologously expressed murine CaV2.1 ortholog carrying the equivalent variant (V1347M) (Figure 3a, left) shows gain-of-function effects due to higher current density and lower voltage threshold for channel activation, when compared to the wild-type (WT) channel [18]. Such increase in channel activity is consistent with the close proximity of the affected residue to the initial pore-loop region that contributes to both the entrance of Ca2+ into the selectivity filter vestibule and into the docking site for the regulatory δ subunit [12], which is essential for the proper functional expression of the pore-forming subunit at the plasma membrane [3]. Residues V1396/V1399 and F1394 are in fact fully conserved in the pore-forming α1S subunit of the rabbit CaV1.1 channel for which structural data are available [12], where they correspond to residues V947 and
F942, respectively (Figure 2b). Therefore, we can evaluate whether the changes of these amino acids to methionine and leucine, respectively, and linked to cerebellar dysfunction may modify structural features of the channel subunit. According to Yasara calculation of amino acid interactions on the rabbit Cav1.1 structure, V947 mainly establish hydrophobic contacts with three residues at the P-loop of domain III (F997, V1000 and A1003) and with the amino acid E452 at the extracellular loop between S1 and S2 of domain II (Figure 4 and Table 2 top). All these residues are conserved or semiconserved in human Cav1.2, except E452 (the equivalent amino acid is a valine) (see Table 2 Legend). By using the Amino Acid Interactions (INTAA) web server, we also estimated pairwise interaction energy (kJ/mol) between the side-chain of V947 and its neighbor residues and found that mutation V947M worsen all hydrophobic interactions, in particular with E452 (even when this amino acid is substituted by a valine as found in human Cav1.2). This may alter the correct conformation of the voltage sensor, thus explaining the increase in voltage sensitivity reported for the VM mutant channel [18].

Figure 4. Internal Cav1.1 channel amino acid interactions affected by mutations F942L and V947M. Structure of the rabbit Cav1.1 channel showing the side-chains of F942 and V947 and Yasara-identified interacting residues from side (a) and top (b) views. Residues L938 and M941 are hidden in panel B for visualization purposes. DII S1-S2 loop and DIII S5, P-loop and S6 are colored in red, blue, green and yellow, respectively.

In the case of F942, Yasara identifies the interactions with other residues located at regions delineating the channel pore in domain III: L938, M941 and I945 at the S5 segment, L1007 at the P-loop, and F1044 and Y1048 at the distal half of S6 segment that faces the cytosolic side and line the inner pore vestibule of the channel (Figure 4). Again, all these interactions, specially between F942 and Y1048 (corresponding to F1394 and Y1497 in Cav1.2), are expected to be energetically worse by mutation F942L (Table 2), in this case caused by the loss of the aromatic ring and consequently the interaction. This may prevent the proper 3D arrangement of the channel inner pore. Although further research, including electrophysiological analysis, is required to confirm this hypothesis, interestingly, a gain-of-function Cav1.2 genetic variant in that precise S6 pore area (AF1502) associated to congenital ataxia and hemiplegic migraine, has been reported to improve voltage-dependent gating of Cav1.2 by strongly decreasing the voltage threshold for channel opening, fastening activation kinetics, and slowing down both the deactivation and the inactivation of the channel [13,20].
Table 2. Calculation of pairwise interaction energy (kJ/mol) between the side-chains of residues V947 (top) or F942 (bottom) (and their corresponding methionine and leucine mutants, respectively) and Yasara-calculated interacting residues. The values have been obtained with the Amino Acid Interactions (INTAA) web server using the WT or mutant rabbit CaV1.1 models, as indicated (PDB id: 5gjv). All residues interacting with V947 and F942 are conserved or semiconserved in human CaV2.1, except E452. Thus, V947 corresponds to V1396, F997 to Y1443, V1000 to V1446, A1003 to A1449 and E452 to V507 (the numbering of these CaV2.1 residues are referred to isoform 3). In the same way, F942 corresponds to F1394, L938 to F1390, M941 to I1393, I945 to V1397, L1007 to L1456, F1044 to F1493 and Y1048 to Y1497 (in this case, the numbering of the CaV2.1 amino acids are referred to isoform 2). As residue E452 is not conserved in human CaV2.1 (where the corresponding residue is a valine), we also evaluated how V947M mutation could affect the energy interaction with a valine at the same position (V452) by exchanging the sequence of the DII loop S1–S2 of rabbit CaV1.1 for that of human CaV2.1.

|      | E452 | V452 | F997 | V1000 | A1003 |
|------|------|------|------|-------|-------|
| V947 | −3.98| −3.96| −2.02| −3.06 | −2.08 |
| M947 | 47.27| 30.3 | −1.09| −1.93 | −0.67 |
| Δ    | +51.25| +34.26| +0.93| +1.13 | +1.41 |
| L938 | 5.54 | −8.22| −4.72| −2.96 | −2.34 | −9.41 |
| M941 | −3.74| −3.30| −3.71| −2.94 | 7.26  | 37.47 |
| Δ    | +1.8 | +4.92| +1.01| +0.02 | +9.6  | +46.88 |

The functional consequences of a third mutation at the S5 segment of CaV2.1 domain III, Y1385C, has been recently reported and both gain- and loss-of-function effects have been observed [21]. As found for V1396M, Y1385C also favors channel opening by voltage (gain-of-function) [21]. However, Y1385C has the contrary effect on CaV2.1 current density, which is strongly reduced by the mutation without alteration of channel trafficking to the cell membrane (loss-of-function) [21]. Besides, Y1385C also reduces the voltage threshold for channel inactivation (loss-of-function). Nevertheless, the combination of all these alterations results in a global gain-of-function due to higher persistent activity when compared to WT channels, which can lead to a greater Ca\(^{2+}\) influx into cells over a physiologically relevant window of membrane potentials near the resting potential [21]. This is consistent with the association of Y1385C to HM, which is produced by CACNA1A gain-of-function mutations [1].

In the same F1394 position, a single nucleotide deletion (c.4182delC) that resulted in a putative truncated protein due to premature termination, was found in several members of an EA2 family showing variation of clinical symptoms among the carriers, ranging from severe early-onset (at the age of 1-2) weekly episodes to less severe phenotype with first attacks occurring in childhood and prolonged attack-free periods [22,23]. As all CaV2.1 truncations, it is expected to produce channel loss-of-function that is the cause of the majority of EA2 cases [1].

Several mutations affecting residues located at other CaV2.1 S5 segments have also been linked to neurological disorders, including episodic ataxia type 2 (EA2) and HM (Figure 3c, left). Two mutations affecting the Y248 residue at the S5 helix of domain I (DI) (Y248C and Y248N) and mutation L621R at the S5 helix of domain II (DII) were found in association with EA2 [24,25], a disease mostly due to CaV2.1 null or reduced activity [1]. Only mutation L621R has been studied at the functional level after heterologous expression. Unfortunately, no significant effect on CaV2.1 channel function was found to corroborate the expected loss-of-function effect. Thus, L621R did not alter CaV2.1 current density, nor channel rate of activation or inactivation [26]. Among the mutations linked to HM (mainly produced by CaV2.1 gain-of-function) are G230V at S5-DI [27], V1695I (referred to α1A isoform 3 and corresponding to V1700I in α1A isoform 2) [28] and...
I1710T (also referred to as I1709T and corresponding to I1714T in α1A isoform 2) [29], both at S5-DIV. Functional study of the heterologously expressed murine CaV2.1 ortholog carrying the equivalent G230V mutation (G232V) suggests a loss-of-function effect due to reduced channel expression at the cell surface [18]. Whether this effect occurs only after heterologous expression in mammalian cells but not in patients’ neurons remains controversial. Indeed, most HM-linked CACNA1A mutations show decreased density of functional CaV2.1 channels in the plasma membrane depending on the cell expression system [1], making it difficult to evaluate both the consequences of this effect in vivo and its relevance for the associated clinical phenotype, if any. In contrast, functional analysis of mutation V1695I/V1700I reveals increased channel activity due to reduced voltage threshold for CaV2.1 activation (by ~4 mV), slowed channel inactivation, and lessened direct G protein-mediated inhibition [30,31].

Regarding the R1664Q/R1669Q mutation affecting the second gating charge (R2) at DIV S4 voltage-sensing helix, it has been previously found to be linked to different ataxic phenotypes, such as EA2 and congenital ataxia [32–34]. In some cases, carriers present cerebellar atrophy and other symptoms, such as psychomotor delay, intellectual disability, and migraine without focal deficits. Studies performed in transgenic Drosophila flies show that the introduction of the R1664Q/R1669Q variant in the equivalent CaV2.1 (cacophony calcium-channel) does not allow rescue of synaptic transmission in a CaV2.1-deficient background, as happens with the WT channel [33]. This suggests that R1664Q/R1669Q is a loss-of-function variant, which is consistent with its linkage to EA2, mainly produced by CACNA1A loss-of-function mutations.

Other mutations of clinical relevance also modify R2 residues, as occurs with the R1664Q/R1669Q variant. This is the case with R198Q, linked to EA2 and affecting the R2 gating charge at the DI S4 segment, (Figure 3c, right), and a gain-of-function mutation linked to congenital ataxia, psychomotor delay, intellectual disability, febrile seizures, and HM (R1350Q, named as R1349Q by some authors and corresponding to R1352Q in α1A isoform 2) that locates at the equivalent R2 gating charge of DIII S4 helix (Figure 3c, right) [4,35–38].

4. Materials and Methods

Patients with a molecular confirmation of de novo variants in CACNA1A, attended at the Neurology Department of Hospital Sant Joan de Déu, and presenting with early onset ataxia were eligible. Evaluations from November 2017 to November 2020 were included. Patients with CACNA1A variants showing early epileptic encephalopathy or those with familial antecedents of HM were excluded.

For measuring cerebellar syndrome, neurological exam and assessment through ICARS, recently been validated in children with cerebellar syndrome, were performed [39]. Regarding dysmorphic evaluation, patients’ measures were compared with age and gender-related reference values from the Hall’s Handbook of Normal Physical Measurements [40].

Brain MRI exams included T1- and T2-weighted, diffusion-weighted and FLAIR sequences. To evaluate MRI cerebellar images, 2D analysis was performed using the midsagittal vermis relative diameter (MVRD) already validated in children with cerebellar atrophy [41]. This is calculated using a midsagittal section and measuring total posterior cranial fossa diameter in a linear segment from the posterior commissure to the opisthion and the largest sagittal diameter of the cerebellum parallel to the previous linear segment. The MVRD was compared to two sex- and age-matched controls from a historical cohort [41]. Lower indices denote greater cerebellar atrophy.

For molecular studies genomic DNA was isolated from venous whole blood. Mutational analysis was performed by genomic DNA analysis both in patient’ and parent samples. In the three patients a targeted gene panel of ataxia-causing genes was run. The identified likely pathogenic heterozygous variants were confirmed by Sanger sequencing in the proband and the parents, ratifying a de novo condition.
For analysis of interaction energies, calculations were performed using the rabbit CaV1.1 model (PDB id: 5gjv) and the F942L and V947M mutants, which were generated using Chimera’s rotamers tool [42]. We used Yasara’s algorithm [43] to minimize energy of models and identify interactions between residues of interest. The INTAA web server [44], which uses Lennard-Jones potential and point charges electrostatics, was used to calculate pairwise side chain interaction energies.

5. Conclusions

In conclusion, our results suggest that among the broad spectrum of CACNA1A-related phenotypes, non-progressive congenital ataxia is associated with cognitive impairment and dysmorphic features, constituting a recognizable syndromic neurodevelopmental disorder. Further studies in larger series of patients are needed to establish whether the recognition of this pattern in patients with nonspecific motor and/or cognitive impairment and cerebellar atrophy might aid in early diagnosis of CACNA1A-related disease, thereby allowing a targeted management and care of the patients and their families. Deepening of our knowledge of the effect of each mutation on protein function, to distinguish between both gain- and loss-of-function, is required to advance in the development of new personalized therapies.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/ijms22105180/s1, Table S1: Previously reported CACNA1A variants linked to congenital ataxia (CA) including functional consequences and molecular localization (protein domain).

Author Contributions: Conception and design of study: A.F.M.-M., D.C.-A., A.E., M.I.-S., J.M.F.-F. and M.S. Acquisition and analysis of data: all authors. Critical revision of manuscript: all authors. Drafting manuscript and figures: all authors. All authors have read and agreed to the published version of the manuscript.

Funding: This work was funded by the Spanish Ministry of Health, Consumer Affairs and Social Welfare, the Spanish Ministry of Science and Innovation, the State Research Agency (AEI, Agencia Estatal de Investigación), and FEDER Funds (Fondo Europeo de Desarrollo Regional): Grants RTI2018-094809-B-100 to J.M.F.F. and CEX2018-000792-M through the “María de Maeztu” Programme for Units of Excellence in R&D to “Departament de Ciències Experimentals i de la Salut”. M.S. is supported by the Generalitat de Catalunya (PERIS SLT008/18/00194) and National Grant PI17/00101 from the National R&D&I Plan, cofinanced by the Instituto de Salud Carlos III (Subdirectorate-General for Evaluation and Promotion of Health Research) and FEDER (European Regional Development Fund).

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki of 1964, as revised in October 2013 in Fortaleza, Brazil.

Informed Consent Statement: Parents gave their written informed consent and the adolescent gave his assent for publication.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to protection data laws protecting personal medical information.

Acknowledgments: We thank the patients and their families for their collaboration.

Conflicts of Interest: The authors declare no conflict of interest.

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