Clinical and genetic analysis in alternating hemiplegia of childhood: Ten new patients from Southern Europe

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Abstract

Alternating hemiplegia of childhood (AHC) is a rare neurodevelopmental disorder featuring attacks of hemiplegia and other paroxysmal and non-paroxysmal manifestations leading to progressive neurological impairment. De novo mutations in ATP1A3 have been identified in up to 80% of patients. AHC is also associated with rare mutations in other genes involved in episodic neurological disorders. We sought to find mutations in ATP1A3, CACNA1A, ATP1A2, SCN1A and SLC2A1 in a cohort of ten unrelated patients from Spain and Greece. All patients fulfilled AHC diagnostic criteria. All five genes were amplified by PCR and Sanger sequenced. Copy number variation (CNV) analysis of SLC2A1 and CACNA1A was performed using two different approaches. We identified three previously described heterozygous missense ATP1A3 mutations (p.Asp801Asn, p.Glu815Lys and p.Gly947Arg) in five patients. No disease-causing mutations were found in the remaining genes. All mutations occurred de novo; carriers presented on average earlier than non-carriers. Intellectual disability was more severe with the p.Glu815Lys variant. A p.Gly947Arg carrier harbored a maternally-inherited CACNA1A p.Ala454Thr variant. Of note, three of our patients exhibited remarkable clinical responses to the ketogenic diet. We confirmed ATP1A3 mutations in half of our patients. Further AHC genetic studies will need to investigate large rearrangements in ATP1A3 or consider greater genetic heterogeneity than previously suspected.

1. Introduction

Alternating hemiplegia of childhood (AHC) is a complex and rare neurodevelopmental syndrome that was first described by Verret and Steele in 1971 [1]. It is characterized by (i) onset of paroxysmal events before 18 months of age, (ii) repeated periods of hemiplegia involving either side of the body lasting from a few minutes to several days caused by various factors including emotional triggers, head trauma and fatigue, (iii) episodes of bilateral hemiplegia or quadriplegia of varying intensity, (iv) other paroxysmal manifestations including tonic and dystonic episodes, ocular abnormal movements (nystagmus, strabismus) and/or autonomic disturbances occurring during hemiplegic bouts or in isolation, (v) disappearance of all abnormalities by sleep, with probable recurrence of long-lasting bouts after waking, and (vi) nonparoxysmal neurological abnormalities including developmental delay, choreoathetosis, dystonia and/or ataxia [2,3].

Analysis of whole exome sequencing in 16 proband-parent trios and whole genome sequencing in another two led to establish AHC as a genetic disorder caused by mutations in ATP1A3, encoding the neuronal α3-subunit of the Na+/K+ -ATPase pump [4–6]. Subsequent molecular analysis in 143 additional AHC patients revealed the presence of mutations in 112 of them [4–8]; the negative results in approximately 20% of patients may indicate some degree of genetic heterogeneity in AHC. In fact, some reports have linked AHC, or a very similar phenotype, to mutations in three genes encoding ionic channels or solute carriers expressed in the central nervous system: CACNA1A [9], ATP1A2 [10,11] and SLC2A1 [12].

In the present study, we sought to determine whether mutations in ATP1A3 or in any of the three genes involved in familial hemiplegic migraine (FHM), CACNA1A, ATP1A2 and SCN1A or in glucose transporter CACNA1A, ATP1A2 and SCN1A. The ketogenic diet was administered to three patients with severe AHC and it resulted in a marked clinical response.
type 1 deficiency syndrome (GLUT1DS), SLC2A1, were linked to AHC in a cohort of 10 unrelated patients from Spain and Greece.

2. Subjects

Ten sporadic AHC patients who clinically fulfilled the previously described criteria for the disorder [2,3] were recruited by neurologists at four Spanish or Greek centers. After obtaining informed consent from all patient parents or custodians, blood samples were collected and genomic DNA was extracted following standard procedures [13]. The study was approved by the local Ethics Committee at Vall d’Hebron University Hospital, Barcelona.

3. Methods

3.1. Mutation screening

All promoters, exons and flanking intronic regions of ATP1A3, CACNA1A, ATP1A2 and SLC2A1 genes and the five FHM-associated SCN1A exons (number 6, 17, 23, 24 and 26) and flanking intronic regions were amplified by PCR in all the patients (details available upon request). Purified PCR products were sequenced using the BigDye Terminator cycle sequencing kit v3.1 and the automated sequencer ABI PRISM 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). All mutations were assessed by bidirectional sequencing. Inheritance of mutations was determined after sequencing the parents of all mutation carriers.

Mutation nomenclature follows HGVS guidelines (www.hgvs.org/mutnomen) and refers to the ATP1A3 cDNA sequence NM_152296.4 (protein sequence NP_689509.1) and to the CACNA1A cDNA sequence NM_023035.2 (protein sequence NP_075461.2) with +1 corresponding to A of the ATG translation initiation codons.

3.2. Copy number variant analysis

Copy number variation (CNV) studies for SLC2A1 and CACNA1A were performed. For SLC2A1 we analyzed all exons with the Multiplex Ligation–Dependent Probe Amplification (MLPA) assay by using the SALSA MLPA kit P138 for SLC2A1 (MRC-Holland, Amsterdam, the Netherlands). For CACNA1A we used two complementary approaches to maximize gene coverage: MLPA and Quantitative Multiplex PCR of Short Fluorescent Fragments (QMPSF). For the MLPA assay we used the SALSA MLPA kit P279-A2 for CACNA1A (MRC-Holland, Amsterdam, the Netherlands) and for QMPSF we used four sets of primer pairs that covered 16 additional exons not included in the MLPA kit (experimental details and primer sequences available upon request).

4. Results

4.1. Clinical data

The main clinical features of the ten patients (5 females) are summarized in Table 1.

Briefly, all patients were from Southern Europe, seven from Spain and three from Greece. The age of onset varied from 0 to 18 months; present age is comprised between 6 and 36 years. Attacks lasted minutes to days with a daily to every two months frequency and featured typical motor signs including unilateral or bilateral paresis, hypotonia, dystonia, rigidity, ataxia, nystagmus or other abnormal eye movements, dysphagia, hand posturing and postictal drowsiness. Other frequently reported paroxysmal events included epileptic seizures, cyclic vomiting and migraine with aura. Over a 6–16 year follow-up period, 9/10 patients have developed different degrees of intellectual deterioration, mostly moderate to severe, and other signs of chronic, progressive neurological dysfunction, such as ataxia, dysarthria, spasticity, hypotonia, hypertonia, dyskinesia, tremor and pyramidal signs. Microcephaly occurred in only one patient.

All patients had MRI studies, which were all normal except for patient 6 who displayed subtle bihemispheric white matter lesions. All patients showed no abnormalities on serial EEGs.

None of the patients had familial history of AHC, however six had familial history of migraine, one of a non-classified neurodegenerative disorder, one of vertigo and one of epilepsy.

Used treatments included prophylaxis with flunarizine, which often provided some degree of improvement in severity and frequency of attacks, or topiramate. Interestingly, in three patients the number and severity of attacks were markedly reduced upon institution of the ketogenic diet (KD). Patient 1 was treated for one year with KD, at age 11. She had been on flunarizine since age 3 with no clear benefit. The frequency of her dystonic attacks while on KD decreased from one per week to one every three weeks, approximately. A more dramatic improvement was recorded in her behavioral status. Both her psychologist and caregivers described frank improvement in her school performance and resolution of mood swings and sociability problems. Because of cost-effectiveness issues the family decided to stop the treatment. Patients 4 and 5 were treated with flunarizine since the disease started with poor response. Benzodiazepines were used to take them quickly to sleep and shorten the attacks: in patient 5 rectal diazepam reduced the duration of her attacks from 2 to 3 days to 4–5 h, but frequency (1–2 a week) remained unchanged; in the case of patient 4 clonazepan in his oral mucosa reduced the attacks to 3–4 h instead of 1–2 days, but his number did also not decrease. In both of them KD brought about a cessation of the hemiplegic attacks. In addition, and according to teachers and caregivers, there were improvements in their motor clumsiness, mood, attention and, though not quantified, global cognitive functions. Of note, patient 5, a 6 year-old girl, has suffered no further attacks since she was put on the diet at age 4.

4.2. Mutation screening

The extensive sequencing of the ATP1A3 gene in ten subjects with AHC allowed the identification of three previously reported changes in five unrelated patients. We identified a G-to-A transition at cDNA position c.2401, resulting in the substitution of an aspartic acid for an asparagine at residue 801 (p.Asp801Asn), in patient 1; a G-to-A transition at cDNA position c.2443, resulting in the substitution of a glutamic acid for a lysine at residue 815 (p.Glu815Lys), in patient 2; and a G-to-A transition at cDNA position c.2839, resulting in the substitution of a glycine for an arginine at residue 947 (p.Gly947Arg), in patients 3, 4 and 5 (Fig. 1).

All mutations were heterozygous and were confirmed to be de novo, except in patient 1 whose parental DNA was not available. However, the variant found in this patient has been previously associated with AHC in the literature.

A distinctive clinical feature of ATP1A3-positive vs ATP1A3-negative patients was an earlier onset of symptoms in the former group (average age of onset: 4.8 vs 13.4 mo).

Moreover, direct sequencing of CACNA1A gene revealed a heterozygous G-to-A transition at cDNA position 13360 (c.13360G>A) in patient 5, who also bore an ATP1A3 change, and in her asymptomatic mother. This mutation results in the substitution of alanine for threonine at residue 454 (p.Ala454Thr) and is assessed as probably damaging by the prediction tool PolyPhen-2 v2.2.2 (score = 1). All mutations are listed in Table 2.

No mutations in ATP1A2, SCN1A and SLC2A1 genes nor copy number variants in SLC2A1 or CACNA1A were found in the ten patients screened.

5. Discussion

We have identified three mutations in the ATP1A3 gene in five out of ten AHC patients from Spain and Greece. ATP1A3 encodes the alpha-3 catalytic subunit of Na+/K+/ATPase pump. Na+/K+/ATPases maintain
Table 1
Clinical features of 10 patients with alternating hemiplegia of childhood. M: male; F: female; mo: months; MO: migraine without aura; MA: migraine with aura; HM: hemiplegic migraine; KD: ketogenic diet; ID: intellectual disability; -: no information. Shading indicates ATP1A3 mutation carrier.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Origin</th>
<th>Present age (years) and gender</th>
<th>Age at onset (mo)</th>
<th>Attack description</th>
<th>Body part involvement</th>
<th>Duration</th>
<th>Frequency</th>
<th>Other paroxysmal events</th>
<th>Interictal exam</th>
<th>Other</th>
<th>Family history</th>
<th>Treatment (response)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Greece</td>
<td>16 F</td>
<td>6</td>
<td>Paresis, Dystonia</td>
<td>Unilateral</td>
<td>Minutes–to–hours</td>
<td>Daily–to–weekly</td>
<td>–</td>
<td>Normal MRI, EEG, SPECT</td>
<td>Mild ID</td>
<td>No</td>
<td>Multiple antiepileptic drugs and flunarizine (no effect) KD (improved)</td>
</tr>
<tr>
<td>2</td>
<td>Greece</td>
<td>7 M</td>
<td>4</td>
<td>Flaccid paresis, Ataxia</td>
<td>Unilateral</td>
<td>1–3 days</td>
<td>1–2/week</td>
<td>Dyskinesia</td>
<td>Normal MRI, EEG</td>
<td>Severe ID and motor involvement</td>
<td>Flunarizine (improved)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Spain</td>
<td>16 F</td>
<td>4</td>
<td>Flaccid paresis, Ataxia</td>
<td>Bilateral</td>
<td>2–7 days</td>
<td>2/month</td>
<td>Rigidity episodes</td>
<td>Normal MRI, C677T mutation in MTHFR</td>
<td>Cerebellar ataxia</td>
<td>MO</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>Spain</td>
<td>37 M</td>
<td>3</td>
<td>Ataxia, Flaccid paresis, Dystonia</td>
<td>Unilateral</td>
<td>Hours/days</td>
<td>2–4/month</td>
<td>Autonomic dysfunction</td>
<td>Normal MRI, arteriography and EEG</td>
<td>Moderate ID</td>
<td>No</td>
<td>Flunarizine (no effect) KD (improved) Diazepam (shortened attacks)</td>
</tr>
<tr>
<td>5</td>
<td>Spain</td>
<td>6 F</td>
<td>0</td>
<td>Nystagmus, Chorea, Dystonia, Flaccid paresis</td>
<td>Unilateral</td>
<td>1–12 days</td>
<td>1/month</td>
<td>–</td>
<td>Normal MRI, EEG, Abnormal SPECT</td>
<td>Squint</td>
<td>MO</td>
<td>Flunarizine &amp; topiramate (no effect) KD (attacks abated)</td>
</tr>
<tr>
<td>6</td>
<td>Spain</td>
<td>13 M</td>
<td>15</td>
<td>Flaccid paresis, “drowsiness postictally”</td>
<td>Unilateral</td>
<td>1h–1 week</td>
<td>6/year</td>
<td>Epilepsy</td>
<td>White matter hyperintensities on MRI</td>
<td>Severe ID</td>
<td>No</td>
<td>Flunarizine (improved)</td>
</tr>
<tr>
<td>7</td>
<td>Spain</td>
<td>8 F</td>
<td>13</td>
<td>Hypotonia, Nystagmus, Dysphagia, Hand posturing</td>
<td>Unilateral, Bilateral</td>
<td>2–6 days</td>
<td>1/month</td>
<td>Nystagmus</td>
<td>Normal MRI, EEG, Abnormal ictal SPECT</td>
<td>Clumsiness, Normal cognition</td>
<td>MO</td>
<td>Flunarizine (improved)</td>
</tr>
<tr>
<td>8</td>
<td>Greece</td>
<td>9 M</td>
<td>18</td>
<td>Ataxia, Flaccid paresis</td>
<td>Unilateral</td>
<td>Minutes–to–days</td>
<td>2/week</td>
<td>–</td>
<td>Normal MRI, EEG, Verteigo, Paresthesia</td>
<td>Developmental delay, Tremor, Ataxia</td>
<td>Topiramate (improved)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Spain</td>
<td>16 F</td>
<td>3</td>
<td>Flaccid paresis</td>
<td>Unilateral</td>
<td>1–2 days</td>
<td>1/month</td>
<td>Epilepsy \ Cyclic vomiting</td>
<td>Normal MRI, EEG, MO</td>
<td>Severe ID</td>
<td>MO</td>
<td>Epilepsy</td>
</tr>
<tr>
<td>10</td>
<td>Spain</td>
<td>12 M</td>
<td>18</td>
<td>Nystagmus, Chorea, Dystonia</td>
<td>Unilateral</td>
<td>Minutes–to–days</td>
<td>1–2/month</td>
<td>Epilepsy, MA, Myoclonus</td>
<td>Normal MRI, EEG, MO</td>
<td>Severe ID</td>
<td>MO</td>
<td>Diazepam (shortened attacks)</td>
</tr>
</tbody>
</table>
Mutations found in several AHC patients and appeared de novo, as shown in all available trios. The mutations identified in our cohort are also the most frequent AHC-causing mutations in the five previous ATP1A3-screened case series, where p.Asp801Asn was the most frequent, followed by p.Glu815Lys and p.Gly947Arg [4–8]. In our series, p.Gly947Arg was the most frequently encountered mutation. All of these changes are G>A transitions located within hypermutable CpG dinucleotide sequences and have been only described as de novo mutations.

Recently, a Japanese study on 33 AHC cases attempted to establish genotype–phenotype correlations by grouping their case series according to the following ATP1A3 mutation types: p.Asp801Asn, p.Glu815Lys or other mutations [8]. It was suggested that the p.Glu815Lys group had a more severe clinical course, while the p.Asp801Asn group resulted in a moderate form of AHC. Our results, despite the smaller sample size, may concur with these findings, since patients with the p.Gly947Arg or the p.Asp801Asn variants all had mild to moderate intellectual disability whereas the patient bearing the p.Glu815Lys variant had a severe and global psychomotor involvement. Conversely, patients with no mutation in ATP1A3 also displayed variable degrees of neurological impairment, though they tended to present later in life.

Compared to ours, previous genetic studies in AHC showed a higher incidence of ATP1A3 mutations. Although we have not ruled out the presence of ATP1A3 CNVs in our patients, data derived from the functional analyses of ATP1A3 mutations suggest that such CNVs would be more apt to produce a DYT12 phenotype, since DYT12-causing mutations reduce protein expression, whereas AHC-causing mutations seem to modulate pump activity [5,14]. Also, the possibility of additional AHC loci remains open, particularly considering the specific geographic origin of the present cohort.

Prior to the description of ATP1A3 as the major genetic cause of AHC, genetic screenings in smaller AHC cohorts, or single case reports, identified mutations in three genes encoding ion–channels, i.e. ATP1A2 [10, 11], CACNA1A [9] or the solute carriers SLC2A1 [12] and SLC1A3 [16]. The proteins encoded by three of these genes play important roles at glutamatergic synapses: CACNA1A encodes a presynaptic neuron

The proteins encoded by three of these genes play important roles at glutamatergic synapses: CACNA1A encodes a presynaptic neuron

Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gene</th>
<th>Exon</th>
<th>cDNA level</th>
<th>Protein level</th>
<th>Inheritance</th>
<th>Frequency in AHC patients screened for this gene (see refs. [4–8])</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ATP1A3</td>
<td>17</td>
<td>c.2401G&gt;A</td>
<td>p.Asp801Asn</td>
<td>Parental DNA unavailable</td>
<td>48%</td>
</tr>
<tr>
<td>2</td>
<td>ATP1A3</td>
<td>18</td>
<td>c.2443G&gt;A</td>
<td>p.Glu815Lys</td>
<td>De novo</td>
<td>33%</td>
</tr>
<tr>
<td>3</td>
<td>ATP1A3</td>
<td>21</td>
<td>c.2839C&gt;A</td>
<td>p.Gly947Arg</td>
<td>De novo</td>
<td>8%</td>
</tr>
<tr>
<td>4</td>
<td>ATP1A3</td>
<td>21</td>
<td>c.2839C&gt;A</td>
<td>p.Gly947Arg</td>
<td>De novo</td>
<td>8%</td>
</tr>
<tr>
<td>5</td>
<td>ATP1A3</td>
<td>21</td>
<td>c.2839C&gt;A</td>
<td>p.Gly947Arg</td>
<td>De novo</td>
<td>8%</td>
</tr>
<tr>
<td>6</td>
<td>CACNA1A</td>
<td>11</td>
<td>c.1360G&gt;A</td>
<td>p.Ala454Thr</td>
<td>Inherited from asymptomatic mother</td>
<td>17%</td>
</tr>
</tbody>
</table>
calcium channel involved in glutamate neuroscience, while ATP1A2 encodes the astrocytic ATPase isomorph α2 involved in glutamate reuptake; SLC2A1, in turn, encodes the glial glutamate and aspartate transporter EAAT1. Because of these previous findings and the existing clinical overlap between severe forms of FHM [17] and atypical AHC, we performed sequence analysis of the three known FHM genes and of SLC2A1, which encodes the glucose transporter at the blood–brain barrier. Mutations in SLC2A1 are the cause of GLUT1DS, a syndrome that shows wide phenotypic variability and shares many clinical signs with AHC, such as delayed development, episodic eye movements, transient abnormal involuntary movements – including hemiparesis – and epilepsy. Mutational analysis of these four genes did not reveal the genetic cause of the disease in any of the patients that were negative in the ATP1A3 screen. This agrees with a recent Italian study investigating SLC2A1 mutations in AHC [18]. However, we identified the p.Ala454Thr variant in CACNA1A in patient 5 and her asymptomatic mother. This mutation was first considered a polymorphic variant with a frequency of 0.02 in the control population of a genetic screen performed in FHM and episodic ataxia patients [19]. It was later associated with early-onset progressive ataxia [20]. More recently, we found this mutation in two subjects displaying the milder phenotype in a family segregating both FHM and migraine with aura [21]. The functional in vitro analysis concluded that this mutation reduced the secretion efficiency of the channel, which prompted us to consider the p.Ala454Thr mutation as a negative modulator of the aura severity. Our patient 5 is also carrying an ATP1A3 mutation. A possible relationship between CACNA1A and ATP1A3 proteins has only been considered in a study where presynaptic Ca2+ buffers were shown to control the strength of a fast post-tetanic hyperpolarization mediated by the ATP1A3 pump [22]. This led us to speculate that the consequences of the ATP1A3 mutation in patient 5 could be modulated by the found CACNA1A variant and result in a milder AHC phenotype. It is conceivable that the many genes regulating membrane excitability are liable to act as each other’s modifiers in paroxysmal neurological phenotypes and that variable expression in these dominantly inherited disorders may relate to epistatic or other types of gene–gene interaction.

A unique feature of three of our patients was the clinical response to KD institution, particularly concerning the paroxysmal symptoms. This concurs with two recent observations of KD-induced amelioration of paroxysmal signs in two AHC patients carrying mutations in ATP1A3, both initially diagnosed with GLUT1DS and one of them effectively harboring a SLC2A1 rare variant [23,24]. At present there is no clear rationale for the use of KD therapy in AHC. Use in our patients was empirical and occurred before molecular diagnosis was known. Institution of the diet was decided in patients with very frequent attacks and lack of response to other treatments, and was based on previous observations of an AHC-like phenotype in GLUT1DS [12] and the finding of interictal abnormal cerebral glucose metabolism in the frontal lobes, ipsilateral putamina and cerebellum in AHC Japanese patients, as detected by means of FDG-PET studies [25]. Whatever the mechanism, all three patients where KD was tried underwent a substantial reduction of attacks and a long-lasting resolution in one (patient 5), only to recur very recently in association with intense emotional stress.

Our results confirm ATP1A3 mutations as a common cause of AHC at both ends of the Mediterranean area, but also raise the issue of the existence of genetic heterogeneity. Studies focused on AHC patients who are negative for mutations in ATP1A3, including data mining of their existing massive sequencing results will hopefully identify novel genes or deep-intronic sequence variants associated with this devastating disorder.

Note added in proof

During the processing of this article, we performed a MLPA analysis, using the SALSA MLPA P059 Dystonia probemix (MRC, Amsterdam, The Netherlands), of samples from patients 6–10. No ATP1A3 CNVs were detected.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgments

We thank the patients and their families for accepting to participate in this study. We acknowledge the valuable contribution of referring physicians, M.T. García-Silva and M.R. Domingo. M.V-P is supported by Fundación Instituto de Recerca Vall d’Hebron and C.S. by MINECO (Spain). This work is supported by Agència de Gestió d’Ajuts Universitaris i de Recerca (Catalunya, Spain), grants SGR 2009/0078 and SGR 2009/0971.

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