Infantile and childhood onset PLA2G6-associated neurodegeneration in a large North African cohort

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Background and purpose: Mutations in the PLA2G6 gene are causative of PLA2G6-associated neurodegeneration (PLAN), a spectrum of neurodegenerative conditions including infantile, childhood and adult onset forms.

Methods: Seventeen North African patients with a clinical suspicion of infantile-onset PLAN underwent clinical, neurophysiological and neuroimaging examinations, and PLA2G6 sequencing. Haplotype analysis was performed to date the identified founder mutation.

Results: All patients carried biallelic mutations in PLA2G6. Sixteen children had the commonest form of infantile-onset PLAN, with early onset of psychomotor regression, hypotonia, pyramidal and cerebellar signs, and abnormal ocular movements. The phenotype was highly homogeneous, with rapid development of severe spastic tetraparesis, cognitive impairment and optic atrophy. Neuroimaging showed cerebellar atrophy and claval hypertrophy to be the commonest and earliest signs, whilst cerebellar cortex hyperintensity and pallidal iron deposition were later findings. Motor or sensory-motor neuropathy and electroencephalogram fast rhythms were also frequent. Nine patients from six families shared the same founder mutation (p.V691del) which probably arose by the late seventeenth century. Only one patient fitted the diagnosis of the much rarer childhood-onset PLAN. Despite the early onset (18 months), clinical progression was slower, with behavioral disturbances and dystonia. Typical features of infantile-onset PLAN such as hypotonia, nystagmus/strabismus, optic atrophy, electroencephalogram fast rhythms and motor neuropathy were absent. Cerebellar atrophy, claval hypertrophy and pallidal hypointensity were evident at brain magnetic resonance imaging. This patient carried a missense variant predicted to be less deleterious.

Conclusions: The PLAN-associated phenotypes and the challenges of diagnosing the childhood-onset form are delineated, and a common North African founder mutation is identified.

Introduction

Neurodegeneration with brain iron accumulation (NBIA) defines an expanding group of disorders that have recently been classified based on the causative gene’s name. Amongst these, recessively inherited mutations of the PLA2G6 gene are causative of...
PLA2G6-associated neurodegeneration (PLAN), which includes conditions previously known as infantile neuroaxonal dystrophy (INAD), atypical later-onset NAD, Karak syndrome and early-onset dystonia-parkinsonism with cognitive impairment [1].

Infantile-onset PLAN (previously defined as classic INAD, MIM256600) is a severe disorder usually presenting in the first 2 years of life with psychomotor regression, axial hypotonia and/or ataxia. Nystagmus and strabismus are frequent, as well as early optic atrophy. The disease is rapidly progressive, with spastic tetraparesis and cognitive decline. Extra-pyramidal features may be present at a later stage, and death often occurs by the end of the first decade. The clinical suspicion is supported by specific findings that include abnormal visual evoked potentials or evidence of optic disk pallor at ophthalmological examination, high-voltage fast rhythms at electroencephalogram (EEG), electromyographic (EMG) signs of chronic denervation, and accumulation of spheroid bodies at skin, rectal or nerve biopsy. Brain magnetic resonance imaging (MRI) shows cerebellar atrophy as an early and constant finding that is variably associated with T2-weighted hyperintensity of the cerebellar cortex due to progressive gliosis, as well as with hypointensity of the globi pallidi (without ‘eye of the tiger’ sign) and sometimes of the substantia nigra due to iron accumulation [2].

The PLA2G6 gene encodes a calcium-independent group VI phospholipase A2 (iPLA2b). Phospholipase A2 enzymes catalyze the hydrolysis of glycerophospholipids with release of free fatty acid, which is critical in cell membrane homeostasis and repair [3]. Besides the several point mutations, rare genomic rearrangements have been described [4–6]. Here, detailed findings of a large cohort of North African patients with genetically confirmed infantile- or childhood-onset PLAN, followed for up to 7 years after onset, are reported.

Materials and methods

Patients

Seventeen patients were admitted at the Department of Child and Adolescent Neurology (National Institute Mongi Ben Hmida of Neurology, Tunis) between 2005 and 2012 with a clinical suspicion of infantile-onset PLAN. Most patients underwent routine blood tests, EEG, EMG with electroneurography and brain MRI. Written informed consent was obtained from all families, and the study was approved by the local ethics committee.

Mutation analysis of the PLA2G6 gene

All coding exons and splice sites of PLA2G6 (NM_003560) were bidirectionally Sanger sequenced (primers and polymerase chain reaction conditions available upon request). Novel variants were searched in public databases dbSNP and Exome Variant Server. The potential pathogenicity of novel missense variants was predicted using Polyphen-2, Sift and Provean software. ClustalW2 was employed to assess evolutionary conservation of mutated residues by multiple sequence alignment of human PLA2G6 protein (NP_003551.2) and its orthologues.

Haplotype analysis and dating of p.V691del mutation

Eight microsatellite markers (of which one was intragenic) and three intragenic single nucleotide polymorphisms that spanned approximately 12 Mb around the PLA2G6 locus were genotyped. The age of the mutation was assessed as described in Data S1 and Table S1.

Results

Clinical findings

Seventeen patients (10 girls and seven boys) from 13 unrelated families are described. Several patients were examined more than once, and age at last examination ranged from 1.6 to 9 years. Clinical features and laboratory, neurophysiological and neuroimaging data are summarized in Tables 1 and 2, respectively.

Eleven families originated from Tunisia, one was Algerian and one Libyan. Consanguinity was present in eight families, and family history was positive in seven. Pregnancy and delivery were uneventful in all cases. Early psychomotor development was normal in all children, but only four eventually acquired walking.

Fifteen patients were referred for psychomotor regression that started at a mean age of 18 months (range 11–24 months), whilst two children were first examined at age 18 months for either psychomotor delay or gait disturbance. In four patients, strabismus was reported to be the first manifestation of the disease, appearing at 8–16 months of age (about 4–8 months before the onset of psychomotor regression). One patient exhibited focal seizures at age 10 months that were well controlled under valproate; therapy was discontinued at age 3 years without recurrence.

The latest neurological examination occurred at different stages of disease. In the two patients aged
<table>
<thead>
<tr>
<th>Patients</th>
<th>1</th>
<th>2</th>
<th>3A/3B</th>
<th>4A/4B</th>
<th>5</th>
<th>6&lt;sup&gt;a&lt;/sup&gt;</th>
<th>7A/7B</th>
<th>8&lt;sup&gt;b&lt;/sup&gt;</th>
<th>9&lt;sup&gt;a&lt;/sup&gt;</th>
<th>10&lt;sup&gt;a&lt;/sup&gt;</th>
<th>11A/11B</th>
<th>12</th>
<th>13</th>
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<td>F</td>
<td>M/F</td>
<td>M/M</td>
<td>M</td>
<td>F</td>
<td>F/F</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>F/F</td>
<td>F</td>
<td>M</td>
</tr>
<tr>
<td>Origin</td>
<td>Tun</td>
<td>Tun</td>
<td>Tun</td>
<td>Tun</td>
<td>Tun</td>
<td>Lib</td>
<td>Tun</td>
<td>Tun</td>
<td>Tun</td>
<td>Alg</td>
<td>Tun</td>
<td>Tun</td>
<td>Tun</td>
</tr>
<tr>
<td>Consanguinity</td>
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<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Protein change</td>
<td>V691del hom</td>
<td>E786Sfs*29 hom</td>
<td>H124_A126dup V691del hom</td>
<td>+ R635X hom</td>
<td>V691del hom</td>
<td>L481Q + V691del hom</td>
<td>E547G hom</td>
<td>R745P hom</td>
<td>R741W V691del hom</td>
<td>R745P hom</td>
<td>F568V hom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AAO</td>
<td>1.8</td>
<td>0.11</td>
<td>0.11/1.6</td>
<td>2/1.8</td>
<td>2</td>
<td>1.4</td>
<td>1.2/0.11</td>
<td>1.6</td>
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<td>1.6</td>
<td>2/1.6</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
<td>First symptoms</td>
<td>PMR</td>
<td>PMR</td>
<td>PMR/PMR</td>
<td>str&lt;sup&gt;c&lt;/sup&gt;, PMR /str&lt;sup&gt;c&lt;/sup&gt;, PMR</td>
<td>PMR</td>
<td>PMR</td>
<td>PMR/ PMR</td>
<td>PMR, GD</td>
<td>PMR</td>
<td>str&lt;sup&gt;c&lt;/sup&gt;, PMR</td>
<td>PMR/ PMR</td>
<td>PMR</td>
<td></td>
</tr>
<tr>
<td>Last follow-up</td>
<td>5</td>
<td>4</td>
<td>4.8/2.7</td>
<td>3/3</td>
<td>9</td>
<td>2</td>
<td>5/2.4</td>
<td>8</td>
<td>8</td>
<td>1.6</td>
<td>5/2.9</td>
<td>4</td>
<td>3.8</td>
</tr>
<tr>
<td>Hypotonia</td>
<td>+</td>
<td>+</td>
<td>+/+</td>
<td>+/+</td>
<td>+</td>
<td>+</td>
<td>+/+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+/+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pyramidal signs</td>
<td>+++</td>
<td>++</td>
<td>+++/++</td>
<td>+++/++</td>
<td>+++</td>
<td>+++</td>
<td>+++/++</td>
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<td>+++</td>
<td>+++</td>
<td>+++/++</td>
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<td></td>
</tr>
<tr>
<td>Cognitive decline</td>
<td>+++</td>
<td>++</td>
<td>+++/++</td>
<td>+++/++</td>
<td>+++</td>
<td>+++</td>
<td>+++/++</td>
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<td>+++</td>
<td>+++</td>
<td>+++/++</td>
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<td></td>
</tr>
<tr>
<td>Nystagmus/strabismus</td>
<td>ny str, ny</td>
<td>str, ny/ny</td>
<td>str, ny/str, ny</td>
<td>str, ny/str</td>
<td>ny</td>
<td>str, ny</td>
<td>str, ny</td>
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<tr>
<td>Optic atrophy</td>
<td>+</td>
<td>+</td>
<td>+/+</td>
<td>+/+</td>
<td>+</td>
<td>+</td>
<td>+/–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>+/–</td>
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<tr>
<td>Cerebellar signs</td>
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<td>+</td>
<td>–/–</td>
<td>–/–</td>
<td>–</td>
<td>–</td>
<td>–/–</td>
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<td>–</td>
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<td>–/–</td>
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<td></td>
</tr>
<tr>
<td>Achillan areflexia</td>
<td>–</td>
<td>+</td>
<td>+/+</td>
<td>+/+</td>
<td>+</td>
<td>+</td>
<td>+/+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–/–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Other signs</td>
<td>Epilepsy</td>
<td>amyotrophy</td>
<td>amyotrophy</td>
<td>amyotrophy</td>
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<td>amyotrophy</td>
<td>amyotrophy</td>
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</tr>
</tbody>
</table>

AAO, age at onset; Alg, Algerian; amyotrophy, amyotrophy; contract, severe contractures; extrapyr, extrapyramidal signs; F, female; GD, gait disturbance; gen, generalized; hom, homozygous; Lib, Libyan; M, male; ny, nystagmus; PMD/PMR, psychomotor delay/regression; str, strabismus; Tun, Tunisian. Cognitive decline: +, mild; ++, moderate; ++++, severe. Pyramidal signs: +, pyramidal signs with no over spasticity; ++, mild to moderate spasticity; ++++, marked spastic tetraparesis. The symbol / separates data from two affected siblings.  
<sup>a</sup>Affected relative(s) followed in another center; <sup>b</sup>diagnosed as childhood-onset PLAN; <sup>c</sup>strabismus was the very first symptom, appearing at age 1.4 (family 4), 0.10 (family 10) and 0.8 (family 13); <sup>d</sup>deceased.
Table 2  Laboratory, neurophysiological and neuroimaging findings

<table>
<thead>
<tr>
<th>Patients</th>
<th>1</th>
<th>2</th>
<th>3A/3B</th>
<th>4A/4B</th>
<th>5</th>
<th>6</th>
<th>a</th>
<th>7A/7B</th>
<th>8</th>
<th>b</th>
<th>9a</th>
<th>10a</th>
<th>11A/11B</th>
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<th>13</th>
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</thead>
<tbody>
<tr>
<td>Elevated AST</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Elevated LDH</td>
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<td>+</td>
<td>+</td>
<td>n.a.</td>
<td>+</td>
<td>+</td>
<td>n.a.</td>
<td>+</td>
<td>n.a.</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>EMG MN</td>
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<td>2.9</td>
<td>norm (1.6)</td>
<td>MN</td>
<td>&gt;</td>
<td>MN</td>
<td>&gt;</td>
<td>MN</td>
<td>&gt;</td>
<td>MN</td>
<td>&gt;</td>
<td>MN</td>
<td>&gt;</td>
<td>MN</td>
<td>&gt;</td>
</tr>
<tr>
<td>EEG</td>
<td>n.a.</td>
<td>gen sp</td>
<td>9</td>
<td>n.a.</td>
<td>FR</td>
<td>2.6</td>
<td>/</td>
<td>n.a.</td>
<td>FR</td>
<td>4.7</td>
<td>FR</td>
<td>1.6</td>
<td>FR</td>
<td>4.7</td>
<td>FR</td>
</tr>
<tr>
<td>MRI CA, ClH</td>
<td>2</td>
<td>CA, ClH</td>
<td>norm (2.9)</td>
<td>CA, ClH</td>
<td>2</td>
<td>CA, ClH</td>
<td>2</td>
<td>CA, ClH</td>
<td>2</td>
<td>CA, ClH</td>
<td>2</td>
<td>CA, ClH</td>
<td>2</td>
<td>CA, ClH</td>
<td>2</td>
</tr>
</tbody>
</table>

AST, aspartate aminotransferase; BrA, brain atrophy; CA, cerebellar atrophy; CCH, cerebellar cortex hyperintensity; ClH, claval hypertrophy; EEG, electroencephalogram; EMG, electromyography; GPH, globi pallidi hypointensity; LDH, lactate dehydrogenase; MRI, magnetic resonance imaging; n.a., not available; norm, normal; MN, motor neuropathy; ONA, optic nerve atrophy; SMN, sensory-motor neuropathy; sp, spikes; SW, slow waves.

The symbol > indicates two subsequent examinations of the same patient (age in parentheses). The symbol / separates data from two affected siblings.

2 years or younger (mean 20 months, range 18–24 months), the phenotype was characterized by axial hypotonia, abnormal ocular movements, mild cognitive impairment and pyramidal tract signs but no overt spasticity. One child, aged 2 years, had already developed optic atrophy. Eight patients were between 2 and 4 years at last clinical follow-up (mean 3.2 years, range 2.4–4 years). They showed mild to moderate cognitive regression, generalized hypotonia and spasticity. Five children had developed optic atrophy, and three had signs of amyotrophy.

Finally, seven patients were last examined after the age of 4 years (mean 6.4 years, range 4.8–9 years): the clinical presentation was severe in all of them with irritability, severe cognitive decline, marked spastic tetraparesis, feeding and swallowing difficulties. Optic atrophy was present in all but one patient, and four showed generalized amyotrophy. Strabismus and/or nystagmus appeared early in the disease course and were overall present in all but one patient from all age groups. Conversely, overt cerebellar signs were difficult to appreciate especially in older children due to the marked worsening of spasticity. In our series, extrapyramidal signs were rare, being observed only in two children aged 4 and 8 years, respectively. All patients received symptomatic treatment associating vitamin therapy and physiotherapy. Baclofen was prescribed in three patients to control marked spasticity. By the time of the study and after a mean follow-up period of 2.9 years (range 0 months to 7 years), only one patient had died at age 5 years.

**Laboratory, neurophysiological and neuroimaging findings**

Fifteen patients underwent routine biochemical testing. Creatine phosphokinase and alanine aminotransferase levels, renal and thyroid function, amino acid and organic acid chromatography, cerebrospinal fluid and blood lactate levels were normal. All patients showed mildly increased levels of aspartate aminotransferase (range 33–131 U/l, normal values <30) and/or lactate dehydrogenase (range 455–1189 U/l, normal values 230–430) even at early stages of the disease.

Electromyography showed involvement of the lower motor neuron in 14 out of 16 patients tested, with associated sensory neuropathy in four (Table S2). In most cases, EMG abnormalities became manifest after 2 years of age.

Electroencephalogram was abnormal in eight of 14 patients, being characterized by fast rhythms variably associated with few slow waves and spikes. Only one
child had generalized spike waves, predominantly in the center-temporal regions.

Brain MRI was performed in 15 patients. At latest examination, cerebellar atrophy was detected in 13 patients, with additional T2-hyperintensity of the cerebellar cortex in eight and pallidal/nigral T2-hypointensity indicative of iron accumulation in four. Optic nerve atrophy was also evident in four patients. Claval hypertrophy, a recently described neuroradiological sign of INAD [7], was observed in all but one child.

Representative MRI images of two patients are presented in Fig. 1, whilst Fig. 2 summarizes the prevalence of clinical features and laboratory findings in the three age groups.

PLA2G6 mutation screening

Molecular screening of the PLA2G6 gene identified bi-allelic mutations in all patients that were homozygous in 11 families and compound heterozygous in two (Tables 1 and S3). Five missense changes, one nonsense, one frameshift, one three-amino-acid duplication and one one-amino-acid deletion were encountered. This last mutation recurred in six apparently unrelated families, of which five were homozygous. Segregation with the disease was confirmed in all families.

Five variants (p.E547G, p.F568V, p.R745P, p.H124_A126dup and p.E786Sfs*29) were novel, whereas four (p.L481Q, p.R635X, p.R741W and p.V691del) had been reported previously [8–10]. None of the newly identified variants was reported in public databases. Two novel missense changes affected residues that were fully conserved amongst different species and were homogeneously predicted as deleterious; the p.E547G variant affected a partially conserved residue, for which in silico prediction tools gave contradictory results regarding potential pathogenicity (Fig. S1).

Dating of the p.V691del founder mutation

Five Tunisian and one Libyan family were found to share the same p.V691del mutation (five in homozygosity and one in compound heterozygosity with p.L481Q). Haplotype analysis of the PLA2G6 locus identified only one informative microsatellite marker for which the same allele was shared by all individuals carrying the mutation, defining a maximum common region of about 1 Mb between flanking markers D22S346 and Chr22_38917 (Fig. 3).

Based on the number of subjects sharing the haplotype and on its greatest length, p.V691del was estimated to have arisen at least 12 generations back. Assuming an average of 25–30 years per generation, the onset of this mutation could be dated approximately in the late seventeenth century or even before.

Discussion

The phenotypic spectrum of PLAN has recently been reclassified to include three distinct conditions that present at variable ages: infantile-onset PLAN (corresponding to classic INAD), childhood-onset PLAN (including atypical NAD and Karak syndrome) and adult-onset PLAN (comprising early onset parkinsonism, variably associated to dystonia and cognitive decline) [1,11]. Moreover, single heterozygous muta-
tions in the PLA2G6 gene have been reported in patients with typical late-onset Parkinson’s disease, possibly contributing to the risk of developing this common neurodegenerative disorder [12–15].

Here a large cohort of North African patients with genetically confirmed PLAN is presented. All 17 children were initially referred with a clinical diagnostic suspicion of infantile-onset PLAN; however, upon detailed investigation one of them (patient 8) was reclassified as having childhood-onset PLAN. This phenotype is much rarer than the infantile-onset form, with only 13 patients reported to

Figure 2 Prevalence of major clinical features and laboratory findings in patients with infantile-onset PLAN. For clinical assessments and neurophysiological/neuroimaging tests performed more than once in the same patient, all the available examinations have been included, each being counted within the corresponding age group. The severity of cognitive impairment and spasticity significantly worsened in all patients with advancing age. Patient 8, diagnosed with childhood-onset PLAN (Discussion), was removed from the graph.

Figure 3 Haplotype analysis of microsatellite markers in the genomic region encompassing the PLA2G6 gene. Different gray shades identify distinct haplotypes shared amongst families. Family F7 is heterozygous for the p.V691del mutation.
date [8,10,16,17]. Age at onset ranges from 1.6 to 6 years, with gait abnormalities, speech delay or regression and diminished social interaction, this last feature possibly leading to a misdiagnosis of autism before the occurrence of other neurological signs. Optic atrophy, nystagmus and seizures present with comparable frequency to infantile-onset PLAN, but other common features of this form such as truncal hypotonia, strabismus, EEG abnormalities and EMG signs of denervation are much rarer. The disease course is usually characterized by progressive dystonia, dysarthria, speech regression and neurobehavioral disturbances (impulsivity, hyperactivity and emotional lability) [1,18]. Besides cerebellar atrophy, iron deposition in the basal ganglia seems to universally develop in the cases so far described, whilst this is infrequently observed in the infantile-onset form, at least in the early stages of the disease [10,17,19].

Patient 8 was first examined at age 5 years. The first signs of psychomotor regression and gait ataxia had occurred at 18 months, which initially led to a diagnostic suspicion of infantile-onset PLAN. However, this girl showed a slower disease progression that was mainly characterized by spastic-ataxic gait (loss of ambulation was at 7 years), dysarthria and dystonia. There was progressive language regression, attention deficit and hyperactivity. Even at the latest follow-up at 8 years, hypotonia, abnormal ocular movements, optic atrophy and amyotrophy were absent, and cognitive decline was mild. EMG and EEG examinations performed at 6 years were normal. Brain MRI showed cerebellar atrophy and claval hypertrophy, which were accompanied, at age 8 years, with bilateral hypointensity of the pallidi and the substantia nigra due to iron accumulation. Interestingly, this patient was found to be homozygous for a novel PLA2G6 missense change (p.E547G) that was the only variant in our series not fully conserved amongst species and predicted as damaging only by a subset of tools. Whilst the pathogenetic impact of this variant cannot be established in the absence of specific functional studies, it is tempting to speculate that this missense change may exert only a mild effect on the protein function, suggesting a possible correlation with the less severe phenotype of childhood-onset PLAN.

Genotype–phenotype correlates have already been proposed in PLAN: the coexistence of two null mutations was invariably associated with the severe infantile-onset presentation, whilst patients with the childhood- and adult-onset phenotypes always carried at least one missense change with potential residual protein function and not unequivocally predicted as pathogenic [8,10,11,16,17]. These correlates find at least a partial explanation in functional studies of the enzymatic activity of PLA2G6 mutants. Indeed it has been shown that some PLA2G6 mutations associated with classic INAD result in a nearly complete loss of enzymatic activity, whilst mutations causative of later-onset dystonia-parkinsonism do not directly impair the protein catalytic function [3]. However, further studies are needed to better explain the clinical variability associated with PLA2G6 variants, especially regarding the ‘intermediate severity’ phenotype of childhood-onset PLAN.

All other 16 patients in our cohort fit the diagnosis of infantile-onset PLAN, as widely described in the literature. The onset was between 11 and 24 months with psychomotor regression and axial hypotonia, followed by the early appearance of pyramidal signs, frequent nystagmus and/or strabismus. Spastic tetraparesis progressively increased in severity, often leading to limb contractures and masking underlying cerebellar signs. After 2 years of age, all children progressively developed optic atrophy and many of them showed achillean areflexia and amyotrophy. In our series, dystonia and epilepsy were very rare, being each present in one patient only, and literature data on their prevalence in infantile-onset PLAN are conflicting. A report on 14 patients showed a high frequency of dystonia, ranging from 75% in children younger than 3 years to nearly 100% when reaching 7 years of age [20]. However, other studies did not mention dystonia as a major feature of classic INAD [9,10,19,21]. The occurrence of seizures is also variable amongst different studies, being either absent [20] or reported in up to 15%–30% of patients [10,11,19].

Electroencephalogram and EMG abnormalities were common features that usually became manifest after 2 years of age, whilst cerebellar atrophy was an early sign, being already present in several patients who underwent brain MRI at or before 2 years. Interestingly, in our series, hypertrophy of the clava (also known as gracile tubercle), recently reported in patients with PLAN [7,17], was present in 13 out of 14 patients with infantile-onset PLAN (as well as in the child with childhood-onset), being the only appreciable abnormality in two children aged 1.6 years. This observation confirms the proposed value of this neuroradiological sign as a supportive diagnostic marker of PLAN even in very young children. Three patients with infantile-onset PLAN showed signs of iron deposition, which appeared late in the disease course. In fact, none of the MRI examinations performed in children younger than 5 years showed pallidal and/or nigral hypointensity.
An unexpected finding was the detection in all patients of mildly elevated values of aspartate aminotransferase and/or lactate dehydrogenase enzymes that were detectable from the early stages of the disease and progressively increased with time. These biological abnormalities have never been reported in PLAN, and their significance remains to be assessed. Six unrelated families from Tunisia and Libya were found to share the same p.V691del mutation on a common 1 Mb haplotype, suggesting a founder effect dating back at least 12 generations. This change affects a highly conserved amino acid and it was found to nearly abolish the enzymatic activity of PLA2G6 [3]. Prior to our study, p.V691del was identified in a Jordanian patient with Karak syndrome, and in a sporadic case and several individuals with classic INAD from two consanguineous Israeli Bedouin kindred [8,9]. It is tempting to speculate that all these patients, originating from countries abutting the Mediterranean Sea, have inherited the same mutation from a common ancestor living in the late seventeenth century.

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Disclosure of conflicts of interest

The authors declare no financial or other conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Electropherograms of the novel missense mutations identified in this study and conservation of the affected residues in PLA2G6 orthologues.

Table S1. Microsatellite markers spanning the PLA2G6 locus.

Table S2. Electroneurography data.

Table S3. Nucleotide and protein changes identified in the study.

Data S1. Methods.

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