Migrating Focal Seizures and Myoclonic Status in ARV1-Related Encephalopathy

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Abstract

Objective

To report longitudinal clinical, EEG, and MRI findings in 2 sisters carrying compound heterozygous ARV1 mutations and exhibiting a peculiar form of developmental and epileptic encephalopathy (DEE). Neuropathologic features are also described in one of the sisters.

Methods

Clinical course description, video-EEG polygraphic recordings, brain MRI, skin and muscle biopsies, whole-exome sequencing (WES), and brain neuropathology.

Results

Since their first months of life, both girls exhibited severe axial hypotonia, visual inattention, dyskinetic movements, severe developmental delay, and slow background EEG activity. Intractable nonmotor seizures started in both at the eighth month of life, exhibiting the electroclinical characteristics of epilepsy of infancy with migrating focal seizures (EIMFS). In the second year of life, continuous epileptiform EEG activity of extremely high amplitude appeared in association with myoclonic status, leading to severely impaired alertness and responsiveness. Repeated brain MRI revealed progressive atrophic changes and severe hypomyelination. WES identified a compound heterozygous in the ARV1 gene [(p.Ser122Glnfs*7) and (p.Trp163*)] in one patient and was subsequently confirmed in the other. Both sisters died prematurely during respiratory infections. Postmortem neuropathologic examination of the brain, performed in one, revealed atrophic brain changes, mainly involving the cerebellum.

Conclusions

This report confirms that biallelic ARV1 mutations cause a severe form of DEE and adds epilepsy with migrating focal seizures and myoclonic status to the spectrum of epilepsy phenotypes. Considering the potential role of human ARV1 in glycosylphosphatidylinositol (GPI) anchor biosynthesis, this severe syndrome can be assigned to the group of inherited GPI deficiency disorders, with which it shares remarkably similar clinical and neuroimaging features. ARV1 should be considered in the genetic screening of individuals with EIMFS.
The ARV1 gene encodes for Acyl-CoA cholesterol acyl transferase–related enzyme 2 required for viability 1 (ARV1), which is a highly conserved protein located in the endoplasmic reticulum membrane. Although extensive functional studies in yeasts and mice have explored the consequences of ARV1 mutations, the association of biallelic mutations of this gene with human disease is limited to a few individuals exhibiting early infantile epileptic encephalopathy, with intractable seizures and severe developmental delay.

In 1 patient, the epilepsy phenotype featured drug-resistant multifocal clustered seizures, disorganized background EEG activity, and modified hypsarrhythmia; in the remaining children, details on the epileptic encephalopathy were not provided.

We describe 2 sisters carrying compound heterozygous ARV1 mutations and exhibiting an early-onset epileptic encephalopathy with peculiar electroclinical features.

**Case Description**

**Patient 1**

Patient 1 is a first-born girl with nonconsanguineous parents who was delivered by elective C-section at 36 + 5 gestational weeks. Her birth weight was 2650 g (50th percentile), length 46 cm (25th percentile), head circumference 32.7 cm, (40th percentile), and Apgar Index 8/9.

At 4 months, hypotonia was noticed, with dyskinetic movements, opisthotonus posturing, and absence of eye tracking.
At 6 months, severe axial hypotonia, abnormal movements, and absent eye fixation persisted. EEG showed slow high-amplitude background activity (>400 μV), replaced 1 month later by subcontinuous high-amplitude multifocal spikes and waves on both hemispheres.

From age 8 months, multiple per-day focal nonmotor seizures started, with unresponsiveness, chewing automatisms, and perioral cyanosis, lasting less than a minute. Despite treatment with valproate, at age 12 months, seizures increased in frequency, appearing at times in clusters, showing multifocal origin and involving either hemisphere with a migrating pattern (figure 1), prompting repeated intensive care unit admissions. Transition from focal seizures to hemiclonic status was often seen, especially during fever (figure 2). Polygraphic EEG recordings revealed an unusual pattern of myoclonic status featuring subcontinuous rhythmic or arrhythmic spikes and slow waves of very high amplitude, associated with reduced responsiveness and erratic myoclonus predominating in the arms and face. During sleep, myoclonus disappeared, but the abnormal EEG pattern remained subcontinuous (figure 3A).

Myoclonic status and clustered multifocal seizures persisted despite several pharmacologic trials (including phenobarbital, phenytoin, ethosuximide, levetiracetam, carbamazepine, and hydrocortisone). Only the ketogenic diet reduced seizure frequency for 10 consecutive months, from several per-day to monthly clusters triggered by fever, remission of myoclonic status episodes, and improved awareness.

By age 3 years, head growth dropped to below the third percentile, and the overall clinical picture worsened because of almost continuous focal seizures and myoclonic status (figure 3B), with recurrent respiratory infections, leading to demise at 4 years 2 months due to bronchopneumonia during status epilepticus. No clinical or electrocardiographic signs of cardiomyopathy were noticed.

Extensive biological and metabolic investigations on urine, blood and CSF, karyotype, Comparative Genomic Hybridization array (CGH-array), histologic and ultrastructural examination of skin and muscle biopsy, and study of mitochondrial respiratory chain enzymes were all unrevealing. Eye fundus, sensitive evoked potential (SEP), and ERG persisted in normal limits, whereas visual evoked potentials (VEP) progressively extinguished. Brain MRI, performed at 4 months, showed a thin corpus callosum (figure 4A). A second MRI, at 24 months, showed mild brain atrophy, mainly involving the right temporal

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**Figure 2** Patient 1. Hemiclonic Seizure

On the top: during an ictal discharge of polyspikes and waves with intermixed fast activity involving the right centro-parietal region, associated with rhythmic jerks of the left arm, a similar ictal discharge independently involves the left temporo-occipital region and is associated with eye deviation to the right, lip cyanosis, and intense tachycardia. On the bottom: cyanosis and tachycardia resolve only at the end of the left temporal discharge, which lasts longer than that involving the right hemisphere.
lobe, hypomyelination, and cerebellar atrophy, more severely affecting the vermis (figure 4B–D).

Postmortem examination identified the cause of death with acute respiratory distress syndrome and a hypertrophic cardiomyopathy with subvalvular stenosis. Brain neuropathology documented cerebral atrophy, more severe in the right temporal lobe, with concomitant Ammon horn sclerosis and abnormal morphology of the parahippocampal gyrus. The corpus callosum was thin and the cerebellum atrophic, with reduced cortical thickness, especially in the vermis. The cytoarchitectonic organization of the cerebral cortex was normal, whereas severe and diffuse loss of Purkinje cells was present in the cerebellar cortex, with dystrophic dendrites in the molecular layer, cell rarefaction in the inner granular layer, and Bergmann gliosis. There was, in addition, an abnormal organization of the dentate gyri bilaterally, with mild gliosis. Subcortical gray nuclei, brainstem, and the spinal cord were normal.

Patient 2
Patient’s one younger sister was born at 37 gestational weeks, following an uneventful pregnancy; at birth, her weight was 3,210 g (75° percentile), and head circumference was 34.7 cm (75° percentile).
Severe axial hypotonia, with opisthotonos posturing and jitteriness, resulting in a hypotonic-dyskinetic quadriparesis was already apparent at 4 months (figure 5A). Visual pursuit was poor; ocular fundus examination, VEPs, and SEPs were normal. Head growth slowed down to around the 3rd percentile before age 2 years.

From age 8 months, the girl exhibited multiple seizures per day. Video-EEG recordings captured focal ictal activity arising independently from the centro-temporal-parietal regions of either hemisphere (video 1) causing psychomotor arrest, apnea, cyanosis, deviated gaze, and lasting 30–60 seconds. Prolonged focal motor seizures triggered by fever at times evolved as hemiclonic status epilepticus. Interictal EEG activity was of high amplitude (>400 μV), intermingled with multifocal discharges. Phenobarbital was ineffective; the ketogenic diet, started at 11 months, determined a temporary reduction in seizure frequency. Seizures remained uncontrolled, and at 18 months, polygraphic EEG recordings documented the same peculiar myoclonic status epilepticus observed in this patient’s sister (figure 5B). Dilated cardiomyopathy with partial hemodynamic decompensation was diagnosed. ECG documented I- and II-degree atrioventricular block, and physical examination showed hepatosplenomegaly.

During follow-up, head growth slowed down to below the 3rd percentile. The frequency of focal seizures decreased to one cluster per month, mostly during fever; myoclonic status remained unchanged (figures 5C,5D) until demise, at age 9 years during pneumonia.

Brain MRI, performed at 7 months, showed a thin corpus callosum. A new MRI, at age 3 years, showed a mildly atrophic cerebellar vermis, a small right hippocampus, and hypomyelination (figure 4E–G). At age 8 years, atrophic changes were more obvious, mainly involving the cerebellum (figure 4H).

Whole-exome sequencing, performed in patient 2 at age 8 years, identified compound heterozygous mutations in the ARV1 gene (NM_022786.3), c.363_364del (p.Ser122Glnfs*7) inherited from the mother, and c.489 G > A (p.Trp163*) inherited from the father. Sanger sequencing, performed in a DNA sample from formalin-fixed paraffin-embedded brain tissue of patient 1, confirmed both mutations. The p.Ser122Glnfs*7 mutation is present in the gnomAD control database (gnomad.broadinstitute.org/) with a frequency of 4/250,498 alleles and is not reported in the Human Gene Mutation Database (HGMD) (portal.biobaseinternational.com/hgmd/pro/start.php). The p.Trp163* mutation is not present in the gnomAD control database (gnomad.broadinstitute.org/) and in the HGMD mutation database (portal.biobaseinternational.com/hgmd/pro/start.php). Both mutations introduce a premature stop codon in the mRNA and are predicted to elicit a rapid degradation of the ARV1 mRNA through nonsense-mediated mRNA decay. The MutationTaster (mutationtaster.org/) tool predicts both mutations to be disease causing. We classified both mutations as pathogenic according to the international guidelines of the ACMG Laboratory Practice Committee Working Group.9
Data Availability
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

Discussion
These 2 sisters were compound heterozygous for the p.Ser122Glnfs*7 and the p.Trp163* truncating mutations and exhibited severe clinical features that overlap with those described in patients with ARV1-related encephalopathy, all harboring homozygous variants. Indeed, both truncating ARV1 mutations found in our patients are predicted to result in loss of function, the molecular mechanism known to be associated with ARV1 deficiency, both in humans and mice.5,8

ARV1, mapping to 1q42.2, encodes for a transmembrane protein of endoplasmatic reticulum (ER) implied in sterol homeostasis in eukaryotic cells, with a role in sterol transport between the ER and plasmatic membrane.3 As already stated,5 because of its implication in glycosylphosphatidylinositol (GPI)-anchor synthesis10,11 ER stress induced by accumulation of immature GPI-anchored proteins or aberrant lipid metabolism may be responsible for the diseases associated with ARV1 deficiency.11
Evidence of a genetic syndrome resulting from deficiency of ARV1 in humans is currently based on the description of 14 patients from 5 families, all harboring homozygous variants. The overall common reported clinical features include severe developmental delay and intractable seizures with onset in the first year of life in all and seizure onset within the 6th months in 50%. Additional clinical features included poor head control (10/14), hypotonia (8/14), visual impairment (9/14), and dystonia (6/14). Seven patients died prematurely because of respiratory infections or aspiration pneumonia, mostly during status epilepticus (1–5 years). Six remaining patients were all alive at last follow-up, between age 2 years 5 months and 18 years.5–8 Progressive deceleration of head growth is reported in 5 patients and mild-moderate dilated cardiomyopathy in 2.7

Reported MRI findings include cerebellar atrophy in 3 individuals, delayed myelination in 2, a thin corpus callosum in 1, and a hyperintense signal on T2-weighted with restricted diffusion in the cerebral central tegmental tract in one.5,7,8 No MRI abnormalities were observed in 2 individuals aged 7 months and 3 years, respectively.7,8 Overall, reported clinical features are similar to those observed in our patients, who harbored compound heterozygous variants.

In the 2 sisters we describe, severe axial hypotonia, dystonic/dyskinetic movements, poor visual contact, and head growth deceleration were already manifested a few weeks after birth and, from the third to the fourth month of life, subcontinuous myoclonic jerks intermingled to dyskinetic movements appeared. The associated electroclinical picture was quite distinctive, consisting of intractable migrating focal motor and nonmotor seizures12,13 and a peculiar myoclonic status mainly involving the face and upper limbs, resembling the electroclinical syndrome known as myoclonic status of nonprogressive epileptic encephalopathies.14,15 The overall neurologic condition progressively worsened, leaving both girls severely hypotonic, with almost continuous abnormal movements, poor eye contact, absent speech, profound intellectual disability, and recurrent respiratory infections. The older girl was affected by dilated cardiomyopathy, and her sister exhibited left ventricular hypertrophy, which was documented at autopsy not to be associated with abnormalities of the myocardial tissue.

Brain MRI findings in both patients documented malformative characteristics in association with progressive cerebellar atrophy. The association of these findings favors the hypothesis of a GPI-anchor–related disorder, which plays a key role in embryogenesis, neurogenesis, and synaptic formation.16 To additionally support this hypothesis, the core symptoms of patients with ARV1 deficiency reported previously5 and in our study, are also seen in inherited GPI deficiency conditions.17–20 Of note, migrating focal seizures, dilated cardiomyopathy, and fever sensitivity can also be found in both of these conditions.21,22

This report confirms the evidence for an autosomal recessive early-onset developmental and epileptic encephalopathy (DEE) caused by biallelic ARV1 mutations in humans and describes the first family with compound heterozygous mutations. It also suggests that ARV1 mutations should be considered a possible cause of epilepsy of infancy with migrating focal seizures.

Considering the potential role of human ARV1 in GPI-anchor biosynthesis, this genetic syndrome could be considered in the group of inherited GPI deficiency disorders.19,20,22,23

This syndrome epitomizes difficulties in sharply parting the category of the DEEs from that of the progressive neurologic disorders at the early stages of clinical diagnosis. Such dichotomous classification is obviously unsatisfactory when applied to peculiar clinical features such as those reported in this study, in which the combination of severe neurologic impairment and almost continuous epileptic activity makes it difficult to identify the main determinants of clinical presentation. The DEE category in this context is more a tentative broad category awaiting diagnostic finalization.

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**Disclosure**
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**Appendix Authors**

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### References

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