Clinical/Scientific Notes

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A SLOWLY PROGRESSIVE MITOCHONDRIAL ENCEPHALOMYOPATHY WIDENS THE SPECTRUM OF *AIFM1* DISORDERS

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To date, 3 *AIFM1* (apoptosis inducing factor mitochondrial 1, located on *Xq26.1*) mutations have been reported: 2 missense changes (c.923G>A/p.Gly308Glu; c.1478A>T/p.Glu493Val) and a 3-basepair deletion (c.601delAGA/p.Arg201del). Two mutations have been described in early-onset severe mitochondrial encephalomyopathy related to impaired oxidative phosphorylation.[1,2] A third mutation is associated with Cowchock syndrome, or Charcot-Marie-Tooth X4 (CMTX4), a slowly progressive disorder characterized by axonal neuropathy, hearing loss, and mental retardation.[3,4]

Case history. We report a 39-year-old man with an early-onset, slowly progressive mitochondrial disease associated with a novel *AIFM1* mutation, thus widening the clinical spectrum of *AIFM1*-related disorders.

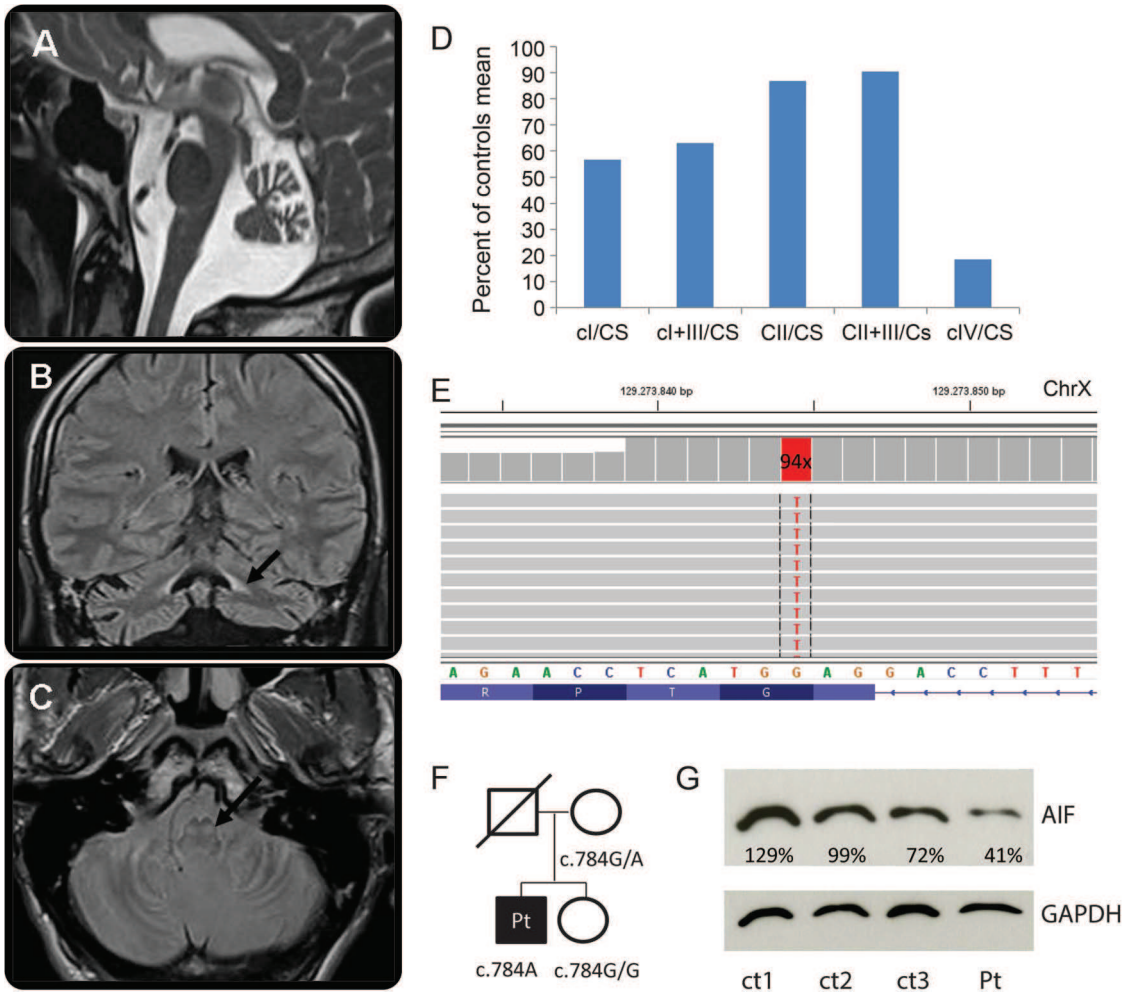
The patient's family history was unremarkable. Psychomotor development was normal until age 1 year, when he started to show walking difficulties. During childhood, he developed gait and limb ataxia (sensory and cerebellar), hearing loss, and cognitive impairment. Visual deficit and distal muscle wasting and weakness became evident at puberty, with scanned speech, head and trunk titubation, and intention tremor, progressively more marked at repeated neurologic examinations. He became chair-bound at age 20 years, needed acoustic aids at age 22 years, and developed progressive sleep apnea syndrome since age 23 years. At age 30 years, visual loss had progressed to 1-2/10 bilaterally. Only one (13 years) of several assays showed elevated lactate and pyruvate serum levels (3,051 μmol/L, normal value 580–2,100; 146 μmol/L, normal value 40–140 μmol/L, respectively). Brain CT scan and MRI were normal at age 9 years, subsequently showing cerebellar atrophy (19 years) and mild cortical and thalamic atrophy (30 years). At last MRI (35 years), further involvement of bulbar olives, dentate nuclei, and spinal cord (slight cervical atrophy with T2 hyperintensity in posterior columns) were observed (figure, A–C). Brain spectroscopy was normal. Nerve conduction study showed a moderate-to-severe axonal, more sensory than motor, neuropathy. EEG was normal. Optic atrophy and retinopathy were documented by visual evoked potentials (since his teens) and electroretinogram (25 years). F1

Results. Informed consent was obtained for biochemical and genetic analyses. Muscle biopsy (13 years) showed evidence of chronic denervation, ragged-red fibers, and fibers hyporeactive to cytochrome *c* oxidase (complex IV [cIV]) histochemical staining. Biochemical analysis revealed markedly reduced cIV activity, with partial reduction of other mitochondrial electron transfer chain (ETC) complex activities and increase of citrate synthase (figure, D). Mitochondrial DNA Southern blot and screening for *POLG1*, *SURF1*, *SCO1*, and *COX15* mutations were normal. Coenzyme Q10 and riboflavin supplementation did not modify clinical course. Recently, sequencing of a panel containing more than 100 nuclear genes associated with mitochondriopathies revealed a hemizygous c.784G>A/p.Gly262Ser mutation in *AIFM1* (NM_004208) (figure, E), the gene encoding apoptosis-inducing factor (AIF). This variant, inherited from the healthy mother (figure, F), is not reported in public databases, is predicted as probably pathogenic (table e-1 on the *Neurology*® Web site at Neurology.org), and affects a conserved amino acid residue (table e-2). Western blot analysis on patient fibroblasts showed strong reduction in AIF amount (figure, G), indicating instability of the mutant protein and confirming a deleterious outcome of the identified variant.

Discussion. AIF is a mitochondrial flavoprotein, attached by an N-terminal transmembrane domain to the inner mitochondrial membrane, with a poorly defined (FAD-dependent) NADH oxidase activity and a role in caspase-independent cell death.[5] AIF defects can result in loss of its pro-survival function related to assembly/stabilization of ETC, or in increased pro-death activity, facilitating AIF translocation from mitochondria to the nucleus and eventually leading to DNA fragmentation.[6] The few cases with *AIFM1* mutations previously reported are summarized in table e-3. Patients with a severe outcome included 2 cousins with early-onset, severe psychomotor delay, extrapyramidal signs, epilepsy,

Supplemental data at Neurology.org

Figure Brain MRI, biochemical, and genetic features



(A) Sagittal T2-weighted image. (B) Coronal fluid-attenuated inversion recovery image. (C) Axial proton density–weighted image. (A–C) Diffuse cerebellar atrophy. Hyperintensity of dentate (B, arrow on the right) and olivar (C, arrow on the right) nuclei are also present. (D) Activities of mitochondrial respiratory chain complexes (cI, cI + III, cII, cII + III, cIV) in patient muscle homogenate, reported as percentages of the controls mean. The specific activities were normalized for citrate synthase (CS) activity. (E) Snapshot from IGV software of the region containing the mutation identified in the patient. *AIFM1* is on the reverse strand, hence the highlighted nucleotide (T) corresponds to the complementary of mutant c.784A. (F) Family pedigree shows the segregation of the c.784G>A mutation. The mother, heterozygous for the mutation, was asymptomatic; none of her 6 siblings has ever presented signs of mitochondrial disorder. No neurophysiologic testing was performed. (G) Western blot analysis of fibroblasts from patient (Pt) and controls (Ct1, Ct2, Ct3) using antibodies against AIFM1 and GAPDH, the latter used as loading control. The reported percentages correspond to the values of AIFM1/GAPDH signals obtained by densitometric analysis. One hundred percent corresponds to the mean value from controls.

and axonal sensory-motor neuropathy associated with bilateral striatal necrosis[1]; and 2 siblings with prenatal ventriculomegaly.[2] Recently, *AIFM1* mutation was found in a family with CMTX4.[3,4] Mitochondrial encephalomyopathy with moderate clinical severity, slow progressive course despite early onset, and cerebellar involvement was not reported until now. Notwithstanding common clinical and instrumental features, the cases reported to date are heterogeneous. Even the biochemical profile is inconsistent, with multiple ETC deficiency reported in muscle for the most severe cases, mainly cIV deficiency found in the present patient, and normal activities described in CMTX4 individuals. These observations are partly in contrast with data obtained from mouse models suggesting that knockdown or knockout of AIF led to complex I deficiency, particularly in brain.[7] However, biochemical defects seem to reflect the clinical severity in patients, confirming the importance of AIF for proper ETC functioning and indicating that this AIF role is fundamental to reduce neuronal damage. Moreover, this case confirms the usefulness of broad genetic screening, for instance targeted sequencing, in highly heterogeneous syndromes resembling mitochondrial disorders.

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screening and analyzed exome-sequencing data. E.L. performed biochemical analyses under the supervision of B.G., D.G. monitored genetic/protein analyses. L.F. evaluated the MRIs. G.P., A.A., and D.G. wrote the manuscript. I.M. and D.P. critically revised the manuscript for important intellectual content. All authors read and approved the manuscript.

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