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ACO2 mutations: A novel phenotype associating severe optic atrophy and spastic paraplegia

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Aconitase 2 (*ACO2*) encodes the mitochondrial aconitase (ACO2), an enzyme catalyzing interconversion of citrate into isocitrate in the Krebs cycle. *ACO2* mutations have been initially associated with infantile cerebellar-retinal degeneration combining optic atrophy, retinal degeneration, severe encephalopathy, epilepsy, and cerebellar ataxia[1–3]; subsequently, *ACO2* mutations have also been associated with milder presentations including isolated optic atrophy[2] or cerebellar ataxia without optic atrophy.[4] We report here a patient presenting with a novel *ACO2* phenotype associating optic atrophy with spastic paraplegia.

Methods

Mini-exome sequencing by exon capture (TruSight One Sequencing Panel kit—Montpellier NGS platform) was performed in a patient with familial syndromic optic atrophy. Written informed consent was obtained. Aconitase enzymatic activity was measured as previously described.[5]

Case report

A now 56-year-old white lady issued from a nonconsanguineous union presented because of severe optic atrophy and spastic paraplegia. Her symptoms had been slowly progressive since infancy and associated with delayed motor (walking at about 3 years) and mental development. An ophthalmologist (C.H.) first examined her at the age of 38 years because of her visual problems. She came to the neurologic clinic at the age of 49 years because of aggravation of the spastic paraplegia, requiring a walking aid. She currently presents with mild cognitive involvement allowing a relatively autonomous life with a sheltered social work. Her sister (not directly examined) has a similar but more severe clinical presentation with visual problems, spasticity, and mental retardation. Cerebral MRI showed mild vermian cerebellar atrophy and nonspecific T2 and fluid-attenuated inversion recovery hyperintensities in cerebellar dentate nuclei and supratentorial white matter (figure, A–D). Neurologic examination revealed severe visual impairment, mild upper limb ataxia, diffuse hyperreflexia, and severe spastic paraplegia with bilateral extensor plantar reflex. Ophthalmological evaluation confirmed the presence of bilateral optic atrophy without retinal involvement, globally stable since first examination at the age of 38 years. Visual acuity was severely reduced (right eye: counts the fingers at 30 cm; left eye: counts the fingers at 1 m). The patient did not present any signs of peripheral neuropathy, and the EMG was normal. Mini-exome analysis identified compound heterozygous mutations in the *ACO2* gene (c.2135C>T [p.(Pro712Leu)][4]; c.940+5G>C) in the patient and her

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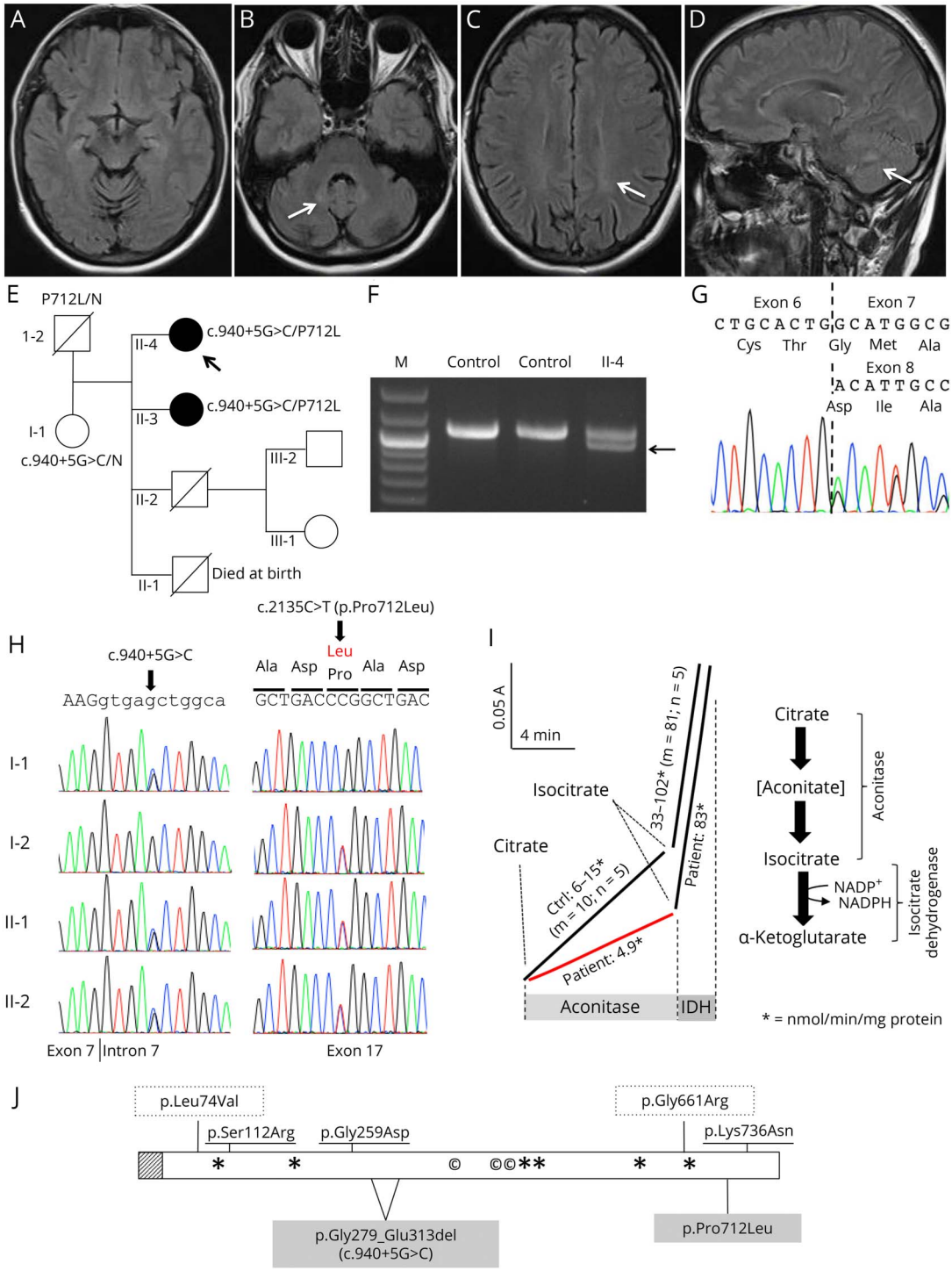
Figure Cerebral MRI and molecular and enzymatic analysis



Mild vermian cerebellar atrophy (A, axial FLAIR w.i.); bilateral dentate hyperintensities (white arrow in B, axial FLAIR w.i., and D, sagittal FLAIR w.i); and supratentorial white matter hyperintensities (white arrow in C, axial FLAIR w.i). (E) Pedigree showing segregation of the disease with mutation c.940+5G>C causing the in-frame skipping of exon 7 and with mutation c.2135C>T causing the p.(Pro712Leu) missense change (P712L). Analysis of reverse transcription PCR (RT-PCR) products of the patient skin fibroblasts (F): the c.940+5G>C mutation (SplicePort score reduction from 1.17 to 0.21) affects exon 7 splicing and results in an abnormal RT-PCR fragment of 980 bp (arrow), whereas only the normal product of 1,085 bp is seen for control fibroblasts. Sequence of RT-PCR products (G): skipping of exon 7 leading to an in-frame deletion of 35 amino acids (p.[Gly279_Glu313del]). (H) Sanger sequencing results of parents and patients: Both patients are compound heterozygous for the mutations c.940+5G>C and p.(Pro712Leu), the mother (I.1) is a heterozygous carrier for the c.940+5G>C mutation, and the father (I.2) is a heterozygous carrier for the p.(Pro712Leu) mutation. (I) Mitochondrial aconitase activity is measured for 10 minutes (citrate injection), followed by isocitrate dehydrogenase activity for 2 minutes (isocitrate injection) through NADPH production in fibroblasts. Patient II.4 aconitase activity (red line) is reduced to about 50% of controls (black line), whereas isocitrate dehydrogenase activity, used for normalization, is similar to controls. (J): Predicted location of mutations on the ACO2 enzyme and substrate and cofactor binding sites. Stripe box: mitochondrial targeting signal; *Substrate binding sites; ©Iron-sulfur binding sites; highlighted mutations: present report; boxed (dotted) mutations: optic atrophy 9 (OPA9) phenotype; underlined mutations: infantile cerebellar-retinal degeneration phenotype (ICRD). FLAIR = fluid-attenuated inversion recovery.

affected sister (figure, E–H); the second mutation involves exon 7 donor splice site and causes the in-frame skipping of exon 7, as confirmed by reverse transcription PCR (RT-PCR) analysis on patient's skin fibroblasts (figure, F and G). No relevant variants were found in other genes causing isolated or syndromic optic atrophy. Total aconitase enzymatic activity on the patient's cultured skin fibroblasts showed a 50% decrease in activity in the presence of citrate and a 40% decrease in activity in the presence of cis-aconitate (compared with isocitrate dehydrogenase activity as a reference) (figure, I), suggesting, in the presence of the two *ACO2* mutations, ACO2 impairment. We did not find mt-DNA depletion in the patient's fibroblasts and leukocytes (not shown).

Discussion

In this report, we expand the phenotype of *ACO2* mutations describing a patient with severe spastic paraplegia and symptomatic optic atrophy; the patient also had a relatively mild cognitive involvement, while cerebellar involvement, usually associated with *ACO2* mutations,[1–4] was clinically very mild, although present at the imaging level.

The pathogenicity of the mutations was confirmed by the segregation in the family and by the quantification of the enzymatic activity, consistent with deficiencies observed in previously reported milder *ACO2* cases.[2] Moreover, the pathogenicity of the intronic mutation was confirmed by RT-PCR studies.

The presently described phenotype might evoke a spastic paraplegia, optic atrophy, and peripheral neuropathy–like phenotype[6] but differs because of absent peripheral neuropathy.

Mutations in genes coding for enzymes of the Krebs cycle are globally rare and are often responsible for a large spectrum of disease ranging from severe early-onset encephalopathy to various types of tumors.[7] The pathophysiologic mechanism could be disruption of energy metabolism and oxidative phosphorylation, but defects in mitochondrial DNA maintenance have also been identified, notably for *ACO2*[4] and *SUCLA2*.[8]

Ocular phenotypes have already been reported in ACO2 deficiency, with optic atrophy and retinal dystrophy,[1] but also in isocitrate dehydrogenase deficiency, with pigmentary retinopathy, succinyl-CoA synthetase deficiency, with ophthalmoplegia, and succinate dehydrogenase deficiency (SDH), with optic atrophy and pigmentary retinopathy.

Slowly progressive spastic paraplegia has never been reported in ACO2 or in other Krebs cycle defects. Moreover, patients with Krebs cycle defect and isolated organ-specific involvement or milder survival into adulthood are rare and were described for SDH, succinyl-CoA synthetase deficiency (*SUCLA2*), and fumarase deficiency. The presently reported patient underscores striking variable clinical presentations resulting from *ACO2* mutations, ranging from early-onset severe epileptic encephalopathy with cerebello-ocular syndrome (ICRD)[1] to isolated optic atrophy (OPA9),[2] complex ataxia without optic atrophy,[4] and now spastic paraplegia and optic atrophy with survival into adulthood (figure, J). The detection of these milder and new phenotypes is possible because of next-generation sequencing techniques. Milder phenotypes are generally associated, as this is the case of our patient, with moderate residual ACO2 enzymatic activity[2]; however, a case of a relatively mild phenotype despite marked ACO2 enzymatic activity reduction has already been reported, suggesting complex genotype-phenotype correlations.[4]

In addition to severe encephalopathic and cerebello-ocular phenotypes, *ACO2* mutations should be investigated in the presence of milder phenotype with slowly progressive spastic paraplegia and optic atrophy.

Author contributions

Cecilia Marelli: writing of the manuscript, design and conceptualization of the study, analysis and interpretation of the data, study supervision, and critical revision of the manuscript for intellectual concept. Christian Hamel: design and conceptualization of the study, analysis and interpretation of the data, and study supervision. Melanie Quiles, Bertrand Carlander, Lise Larrieu, Cecile Delettre, Emmanuelle Sarzi, Dominique Chretien, and Pierre Rustin: analysis and interpretation of the data and critical revision of the manuscript for intellectual concept. Claire Guissart and Michel Koenig: analysis and interpretation of the data, critical revision of the manuscript for intellectual concept, and study supervision.

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