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Late-onset leukoencephalopathy in a patient with recessive *EARS2* mutations

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Mitochondrial aminoacyl-transfer RNA (tRNA) synthetases catalyze the attachment of specific amino acids to their cognate tRNA, enabling intramitochondrial protein synthesis. Recessive mutations in their coding nuclear genes are associated with heterogeneous clinical presentations, often displaying leukoencephalopathy.[1]

Biallelic mutations in *EARS2*, encoding the mitochondrial glutamyl-tRNA synthetase, result in an infantile-onset neurologic disorder hallmarked by extensive symmetrical white matter abnormalities sparing the periventricular zone, symmetrical signal abnormalities of the thalami and brainstem and thin corpus callosum (leukoencephalopathy with thalamus and brainstem involvement and high lactate [LTBL], OMIM#614924). High blood lactate and mitochondrial dysfunction in muscle and fibroblasts can be observed, especially in more severe cases.[2]

Clinical spectrum of patients with LTBL ranges from severe neonatal or early infantile disease with delayed psychomotor development, seizures, hypotonia, and persistent lactate increase to a more favorable form, characterized by a transient psychomotor regression in the first year of life followed by stabilization and, in some cases, partial recovery of lost skills by age 2 years. Of interest, some cases display an even milder phenotype characterized only by minor clinical regression and abnormalities on brain MRI, suggesting that some mutation carriers can escape the genetic diagnosis.[2] Long-term clinical follow-up of *EARS2*-mutated patients has never been reported.[3]

Here, we describe a male patient with late-onset multisystemic neurodegenerative disorder presenting with behavioral abnormalities, pyramidal, and extrapyramidal signs and progressive cognitive decline. Whole-exome sequencing (WES) analysis in the proband allowed the identification of 2 novel *EARS2* mutations. The Ethics Committee of the IRCCS Foundation Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy) approved the study. Written informed consent was obtained from the patient.

The patient was born at term after an uneventful pregnancy. Reportedly, developmental psychomotor milestones were reached with some delay. He completed high school and attended military service. He got married and had a son. He worked as an office worker until retirement. At age 63 years, he presented mood deflection for which he was examined by a psychiatrist who diagnosed an atypical form of depression. The following year, the patient developed postural and intention tremor of the right upper limb, which initially responded favorably to beta-blocking therapy. At age 68 years, the tremor got significantly worse involving the left limb and presenting at rest. Neurologic examination revealed the presence of upper limb dysmetria, plastic rigidity of the 4 limbs, and diffuse hyperreflexia. The Mini-Mental State Examination score was 25/30. The serum lactate level was within normal limits. Brain MRI showed extensive confluent almost symmetrical white matter abnormalities and callosal atrophy, without thalamus and brainstem involvement (figure, A). Head CT did not show pathologic calcifications. Brain ^{18}F-fluorodeoxyglucose PET displayed

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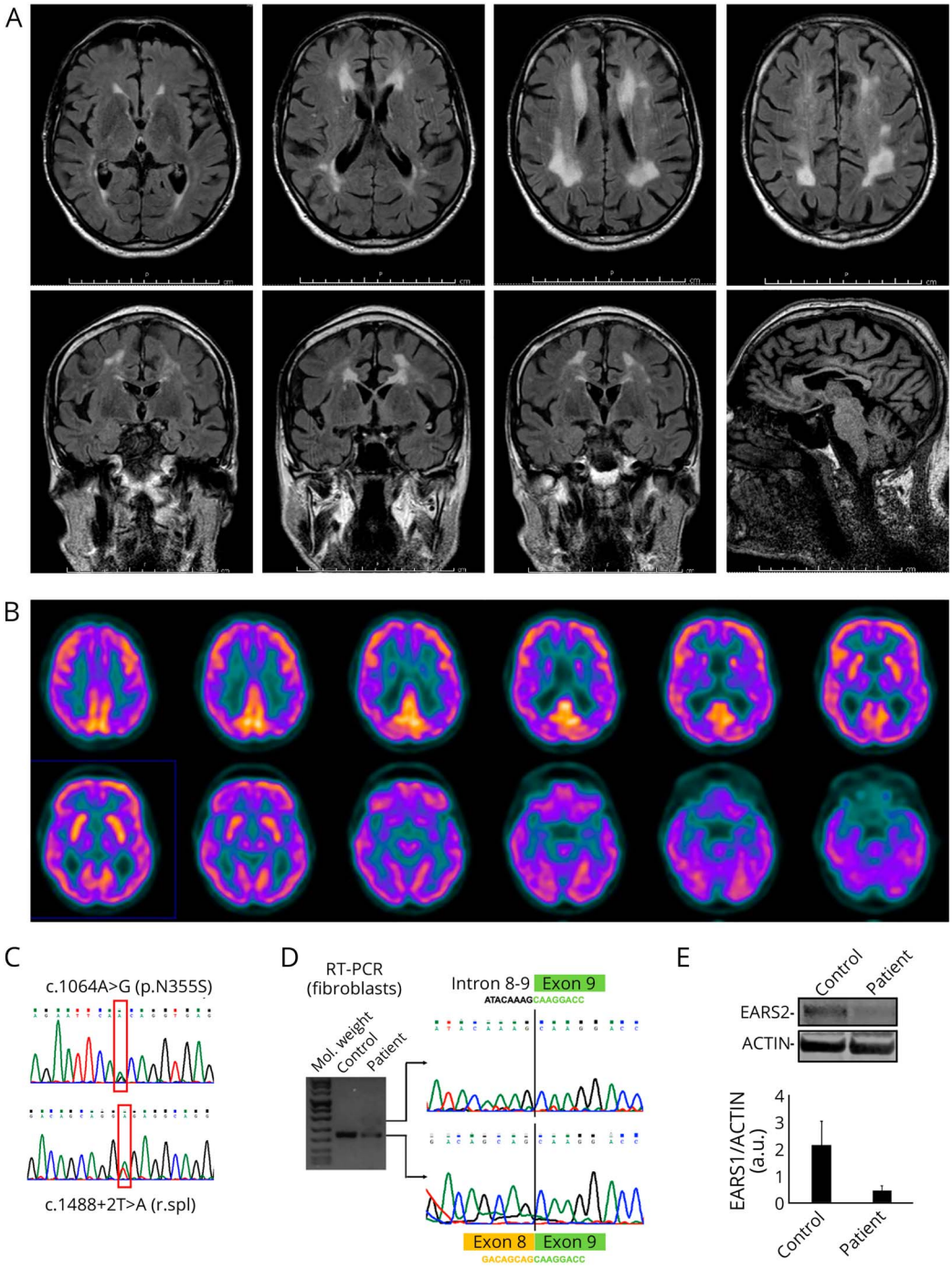
Figure Neuroradiologic findings in the patient and molecular and biochemical analyses of the identified EARS2 mutations



(A) Brain MRI of the proband showing extensive supratentorial almost symmetrical white matter abnormalities and callosal atrophy, without thalamus and brainstem involvement. (B) Brain ^{18}F-fluorodeoxyglucose PE revealing marked symmetrical hypometabolism of the thalamus and temporoparietal cortex. (C) Sequence electropherograms showing the nucleotide substitutions c.1046A>G and c.1488+2T>A detected in the patient. (D) Gel electrophoresis of RT-PCR amplicons encompassing exons 4 and 9 in *EARS2* transcript (fibroblasts) showed an additional band in the patient caused by partial retention of intron 8 in patient cDNA. cDNA allele-specific sequence electropherograms showing the intron retention effect of c.1488+2T>A mutation on the *EARS2* transcripts at exon 8–9 boundary. (E) SDS-PAGE/Western blot analysis of EARS2 (Sigma-Aldrich, SAB2100641, 1:1000) in protein lysates of fibroblasts from the patient and healthy control. Densitometry, after normalization to ACTIN, showed a marked reduction of EARS2 signal in patient cells compared with controls. cDNA = complementary DNA; RT = reverse transcriptase.

symmetrical hypometabolism of the thalamus and the medial temporal and parietal cortices (figure, B). He started levodopa therapy with some benefit on rest tremor. Starting from age 70 years, the patient displayed a progressive cognitive deterioration and reduced autonomy in daily activities and is now completely dependent on others. In the late stages of the disease, he developed a peculiar movement disorder characterized by repetitive motor and vocal stereotypies.

WES was performed using the Nextera Exome Library protocol (Illumina) and an Illumina NextSeq500 sequencing platform. Variant calling and annotation were performed by GATK and ANNOVAR (annovar.openbioinformatics.org/en/latest/) tools. No variants in late-onset leukoencephalopathy-related genes were found, including the *CSF1R* gene, which appeared a good a priori candidate gene in this patient.[4] Conversely, variant prioritization disclosed 2 heterozygous variants in *EARS2* (NM_001083614): c.1064A>G and c.1488+2T>A, which were confirmed by Sanger sequencing (figure, C) and resulted absent in public databases (1000G and gnomAD). The son of the proband carried only the c.1064A>G, indicating that the identified *EARS2* mutations are associated in trans. The c.1064A>G substitution leads to the amino acid change p.Asn355Ser. The affected asparagine is conserved across mammalian orthologues, and its change is predicted deleterious by in silico tools (MutationTaster: score 0.999—disease causing; PolyPhen-2: score 0.756—possibly damaging; CADD-PHRED: score 22.0—highly deleterious). The c.1488+2T>A substitution abrogates a physiologic splicing consensus sequence (PhyloP score = 4.312; PhastCons score = 1), likely activating a cryptic donor site 102 nucleotides downstream exon 8. Transcript analysis in patient fibroblasts showed the partial retention of intron 8 (figure, D), preserving *EARS2* reading frame and introducing 34 additional amino acids in the C-terminal anticodon binding domain. *EARS2* transcript levels were maintained (not shown), whereas residual protein levels were found reduced in the patient compared with control cells (figure, E), suggesting an increased degradation rate of the mutated protein. The stability and activity of respiratory chain complexes, evaluated by SDS-PAGE (sodium dodecyl sulphate - polyacrylamide gel electrophoresis) and spectrophotometric analysis respectively, did not differ between patient and control fibroblasts (not shown).

The clinical phenotype of the proband is different from that shown by leukoencephalopathy with thalamus and brainstem involvement and high lactate (LTBL), particularly in terms of age at onset, clinical course, and brain MRI. The finding of novel *EARS2* variants in a patient with leukoencephalopathy but without LTBL phenotype represents a diagnostic dilemma and requires future confirmations. Considering the unbiased diagnostic approach of WES, the risk of finding functionally deleterious mutations that are not causative of the patient's phenotype is a concrete possibility; therefore, this chance cannot be completely excluded in the case presented here. In addition, in a recent report, McNeill et al. found 2 functionally deleterious *EARS2* variants in a patient affected by leukoencephalopathy with calcifications and cysts, a phenotype completely explained by the presence of biallelic *SNORD118* mutations, raising some doubts on the pathogenicity of *EARS2* variants in cases without a classic LTBL phenotype.[5,6] However, it should be pointed out that the patient described there, carrying both *EARS2* and *SNORD118* mutations, died prematurely (age 16 years), keeping open the possibility of the subsequent development of *EARS2*-related late-onset disease, as in the case reported here.

Of interest, the clinical presentation of the proband presented here overlaps with clinical findings in patients harboring mutations in *AARS2*, encoding the mitochondrial alanyl-transfer RNA synthetase, in which psychiatric symptoms usually precede a rapid neurologic deterioration with pyramidal signs and progressive cognitive decline in adult patients in association with leukoencephalopathy.[7]

Overall, the findings presented here may suggest a possible pathogenic role of the identified mutations in a late-onset form of genetic leukoencephalopathy. We speculate that the combination of a missense change and a splice site mutation preserving reading frame does not severely affect EARS2 function, permitting a later disease onset and milder neuroradiologic phenotype. Our study prompts the screening of this gene in other cases with late-onset leukoencephalopathy to confirm or reject this possible etiologic link.

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Appendix Authors

Name	Location	Contribution
Edoardo Monfrini, MD	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Italy	Analyzed NGS data, performed clinical assessment, and drafted the manuscript
Dario Ronchi, PhD	University of Milan, Italy	Performed molecular studies and critical revision of the manuscript
Giulia Franco, MD	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Italy	Performed clinical assessment
Manuela Garbellini, BSc	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Italy	Performed biochemical studies
Letizia Straniero, PhD	Humanitas University, Milan, Italy	Performed wet phase of NGS
Elisa Scola, MD	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy	Performed brain imaging studies

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Name	Location	Contribution
Federica Arienti, MD	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Italy	Performed clinical assessment
Stefano Duga, PhD	Humanitas University, Humanitas Clinical and Research Center IRCCS, Milan, Italy	Supervised the study and critical revision of the manuscript
Giacomo Pietro Comi, MD	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Italy	Supervised the study and critical revision of the manuscript
Nereo Bresolin, MD	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Italy	Supervised the study and critical revision of the manuscript

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Alessio Di Fonzo, MD, PhD	Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan, Italy	Study design, supervised the study, and critical revision of the manuscript

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