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Supporting Data

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Biallelic Variants of *MRPS36* Cause a New Form of Leigh Syndrome

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ABSTRACT: Background: The *MRPS36* gene encodes a recently identified component of the 2-oxoglutarate dehydrogenase complex (OGDHC), a key enzyme of the Krebs cycle catalyzing the oxidative decarboxylation of 2-oxoglutarate to succinyl-CoA. Defective OGDHC activity causes a clinically variable metabolic disorder characterized by global developmental delay, severe neurological impairment, liver failure, and early-onset lactic acidosis.

Methods: We investigated the molecular cause underlying Leigh syndrome with bilateral striatal necrosis in two siblings through exome sequencing. Functional studies included measurement of the OGDHC enzymatic activity and *MRPS36* mRNA levels in fibroblasts, assessment of protein stability in transfected cells, and structural analysis. A literature review was performed to define the etiological and phenotypic spectrum of OGDHC deficiency.

Results: In the two affected brothers, exome sequencing identified a homozygous nonsense variant (c.283G>T, p.Glu95*) of *MRPS36*. The variant did not affect transcript processing and stability, nor protein levels, but resulted in a shorter protein lacking nine residues that contribute to the structural and functional organization of the OGDHC complex. OGDHC enzymatic activity was significantly reduced.

The review of previously reported cases of OGDHC deficiency supports the association of this enzymatic defect with Leigh phenotypic spectrum and early-onset movement disorder. Slightly elevated plasma levels of glutamate and glutamine were observed in our and literature patients with OGDHC defect.

Conclusions: Our findings point to *MRPS36* as a new disease gene implicated in Leigh syndrome. The slight elevation of plasma levels of glutamate and glutamine observed in patients with OGDHC deficiency represents a candidate metabolic signature of this neurometabolic disorder. © 2024 International Parkinson and Movement Disorder Society.

Key Words: 2-oxoglutaratedehydrogenase complex; chorea; dystonia; Leigh syndrome; mitochondrial disorders

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α-Ketoglutarate dehydrogenase or 2-oxoglutarate dehydrogenase complex (α-KGDH or OGDHC) is a key multienzyme complex of the Krebs cycle that catalyzes the oxidative decarboxylation of α-ketoglutarate/2-oxoglutarate to succinyl-CoA,[1] generating the reducing equivalents NADH and $FADH_2$ for the electron transport chain. This multicomponent enzyme includes three catalytic subunits (2-oxoglutarate dehydrogenase/E1, dihydrolipoyl succinyl transferase/E2, and dihydrolipoamide dehydrogenase/E3), each encoded by different nuclear genes. The E2 component forms a large multimeric core that binds to the peripheral E1 and E3 subunits. The E3 subunit, which is encoded by the *DLD* gene,

is shared across OGDHC, pyruvate dehydrogenase (PDH), and branched-chain α-keto acid dehydrogenase (BCKDC) enzymatic complexes.[1] The defect of this subunit has been associated with a rare disorder characterized by clinical and biochemical features of combined PDH and BCKDC deficiency, including global developmental delay (GDD), severe neurological impairment, liver failure, lactic acidosis, accumulation of pyruvate and branched-chain amino acids in plasma, and branched chain α keto acids in urine.[2]

A fourth subunit necessary for the stable recruitment of E3 to the E1–E2 core of OGDHC, called KGD4, has recently been characterized in mice.[3] Murine Kgd4, previously annotated as Mrps36, was originally considered a putative component of the mitochondrial ribosome, is a highly conserved protein whose function appears analogous to that of PDX1 in the PDH complex.[3,4] When absent, the activity of the whole OGDHC is significantly reduced, although the activities of the single catalytic sites seem unaffected.[3] More recently, the structural organization of the eukaryotic OGDHC has been resolved by means of complexome profiling and cross-linking mass spectrometry studies,[4] confirming that MRPS36/KGD4 (E4) is an adaptor protein linking the E3 subunit to the E1–E2 core.

Over the years, multiple cases with isolated OGDH deficiency (Mendelian Inheritance in Man [MIM]: 203740) without a molecularly confirmed diagnosis sharing a phenotype ranging from fatal neonatal lactic acidosis to a variable association of slowly progressive movement disorder (MD), GDD, epilepsy, and Leigh syndrome have been reported, suggesting the existence of mutations affecting one or multiple genes encoding the individual subunits of OGDHC.[5-9]

Leigh syndrome, or subacute necrotizing encephalomyelopathy, is a clinically heterogeneous disorder caused by a broad spectrum of defects related to energetic metabolism failure. To this date, more than 75 genes, including nuclear and mitochondrial DNA genes, have been associated with the disorder. They are all either directly involved in the oxidative phosphorylation reaction or encode proteins that are essential for mitochondrial structural integrity, mitochondrial DNA maintenance and translation, pyruvate metabolism and vitamin and cofactor transport and metabolism.[10]

Recently, Yap et al[11] reported two siblings with OGDH deficiency and a homozygous loss-of-function variant in the *OGDH* gene, encoding the E1 subunit of the enzyme. These individuals presented with progressive GDD, elevated lactate, ataxia, seizure, and severe MD. Magnetic resonance imaging (MRI) scan showed symmetric atrophy of the frontotemporal lobes and symmetric basal ganglia cystic lesions.

Here, we report two siblings with a phenotype consistent with Leigh syndrome, and markedly reduced OGDHC activity, who were identified to share a homozygous truncating variant in *MRPS36* (Online MIM *611996), encoding the MRPS36/KGD4 (E4) subunit of OGDHC. The clinical features of the previously reported patients with isolated OGDH deficiency, with and without a molecular diagnosis, were reviewed to more accurately profile the clinical phenotype associated with this rare neurometabolic disorder.

Methods and Materials

Exome sequencing (ES) and ES data analysis was performed as previously reported.[12-15] Extended methods and experimental workflow related to clinical data collection, genomic analysis, biochemical analyses, structural modeling, in vitro functional characterization, and literature review are available as Supporting Data (Supplementary Methods, Table S1).

Results

Patient 1 was born at term, after an uneventful pregnancy from consanguineous (first cousins) Italian parents. The pedigree included one healthy older sister and a previous pregnancy that resulted in a spontaneous miscarriage. Perinatal cyanosis and jaundice at birth were reported. He presented in the first months with failure to thrive (FTT), irritability, severe motor delay, and hypotonia. The only developmental milestones achieved in the first year of life were visual tracking, social smiling, and responding to sounds. Spastic-dystonic tetraparesis with acute worsening during fever emerged at that time and slowly progressed throughout his life. He had always been wheelchair-bound and never developed verbal language, whereas social interaction and simple sentence comprehension were relatively preserved. A first electroencephalography (EEG) at the age of 1 year was unremarkable. At the age of 2 years, he presented with febrile seizures associated with EEG showing a diffuse slowing of background activity. At that time a diagnosis of primary measles encephalitis was advanced. From the age of 2 to 8 years, he suffered from reflex epilepsy with photo-dependent myoclonic seizures (eyelid myoclonia and upper limb jerks).

At the age of 20 years, when he was first seen in our clinic, he was seizure free, and EEG did not show any epileptic abnormalities, but an altered background activity.

Neurological examination at that time showed severe generalized dystonia, with prominent cranial and axial involvement leading to severe scoliosis, choreic movements, and lower limb spasticity. Voluntary movements and emotional stimuli triggered dramatic opisthotonos

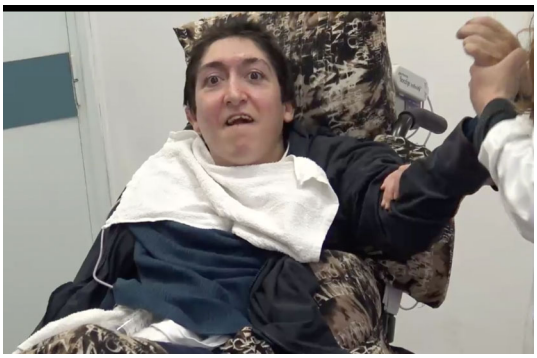
with apneas. Baclofen and lorazepam were ineffective, whereas tetrabenazine had some efficacy on chorea.

At the age of 21 years, brain MRI disclosed bilateral T2 weighted hyperintensities in the putamina compatible with striatal necrosis and diffuse cerebral atrophy. Brainstem auditory evoked potentials (BAEPS), sensory evoked potentials (SEPs), electroneurography/electromiography (ENG/EMG), and visual evoked potentials (VEPs) were unremarkable. Motor evoked potentials (MEPs) showed absent motor responses to cortical stimulation bilaterally. Echocardiography revealed mild hypertrophy of the left ventricle, dilatation of the aortic bulb and ascending aorta, and redundant mitral flaps. The patient died of respiratory failure at the age of 28. Mild and persistent lactic acidosis (2–10 mmol/L; reference range (r.r.) 0.5–1.2) and occasional increase of glutamic acid (80.4 μmol/L; r.r. 7–65) and glutamine (958.7 μmol/L; r.r. 581–709) were detected over time. Urine organic acid assessment did not detect tricarboxylic (TCA) aciduria. Morphological and biochemical (respiratory chain and PDH activity) study of muscle biopsy did not detect any alteration. No pathogenetic variants were detected on mitochondrial DNA.

Patient 2, the 33-year-old younger brother of patient 1, was born at term after an uneventful pregnancy. There was no explicit history of perinatal asphyxia, but he had meconium-tainted amniotic fluid. As his brother, he presented with FTT and severe GDD with relative sparing of social interaction and language comprehension. A severe progressive hyperkinetic MD became evident during the first years. At the age of 13 years, he was a wheelchair-bound boy with severe intellectual disability, generalized dystonia with prominent cranial and axial involvement and paroxysmal bouts of choreic, ballistic and dystonic movements, triggered by emotions and other nonspecific stressors (Video 1). When re-examined at the age of 25 years, the clinical status was relatively stable with a prevalent akinetic status associated with dystonia and superimposed dyskinetic bouts with apnea, which was triggered by excitement/emotion or other stressors (Video 1). At the age of 26 years, he underwent a tracheostomy during an episode of acute respiratory failure because of pneumonia. A percutaneous endoscopic gastrostomy tube was placed the same year because of worsening dysphagia.

Several treatments were tried: risperidone (1 mg/day) attenuated chorea and supplementation with thiamin (400 mg/day), riboflavin (150 mg/day), and carnitine (2 g/day) improved strength and environment participation.

At the age of 15 years, MRI documented caudate atrophy, bilateral T2 weighted hyperintensities in the putamina and diffuse cerebral atrophy (Fig. 1). Echocardiography from the age of 13 years showed hypertrophic cardiomyopathy, which remained stable during adulthood (intraventricular septum thickness 13 mm at ages 13 and 25) associated with sclerosis and dilation of the aortic bulb (27 mm at age 13, 35 mm at 25) and the ascending aorta and mild mitral and tricuspidal regurgitation. Persistent lactic acidosis (2.7–5.5 mmol/L) and a slight increase of glutamic acid (90 μmol/L) and glutamine (865.7 μmol/L) were detected over time. As for his brother morphological and biochemical studies on muscle biopsy were normal.



Video 1. Patient 2 at the age of 29 years with a complex neurological phenotype characterized by severe generalized dystonia with superimposed abrupt choreo-ballistic and dystonic movements of the four limbs and axis, so severe that he needs to be tied to his wheelchair to avoid falling. Severe oromandibular dystonia and limb rigidity can be seen during the examination. The patient's interaction and situational comprehension abilities are relatively spared.
Video content can be viewed at https://onlinelibrary.wiley.com/doi/10.1002/mds.29795

Based on the documented parental consanguinity, a recessive inheritance model of the disorder was postulated. ES performed on the proband (patient 1) excluded the occurrence of functionally relevant variants compatible with known Mendelian disorders based on the expected inheritance model and clinical presentation as well as the involvement of variants affecting the *OGDH* gene. However, an extended analysis directed to explore occurrence of variants in functionally relevant genes allowed us to detect a homozygous nonsense variant in *MRPS36* (c.283G>T, NM_033281.6; p.Glu95*, NP_150597.1) as the best candidate underlying the condition (Supplementary Table S1). The variant had not previously been reported in public (gnomeAD, https://gnomad.broadinstitute.org/) and in-house (>3000 population-matched exomes/genomes) databases. It was predicted as damaging by multiple in silico prediction tools (Combined Annotation Dependent Depletion phred: 43). The gene is widely expressed, and particularly highly expressed in brain and heart (Human Protein Atlas, https://www.proteinatlas.org/ENSG00000134056-MRPS36/tissue). Sanger sequencing confirmed the homozygous status of the variant in his sibling, its heterozygous state in their parents, and homozygosity for the wild-type allele in the sister. Functional validation was performed by assessing the enzymatic OGDHC activity,

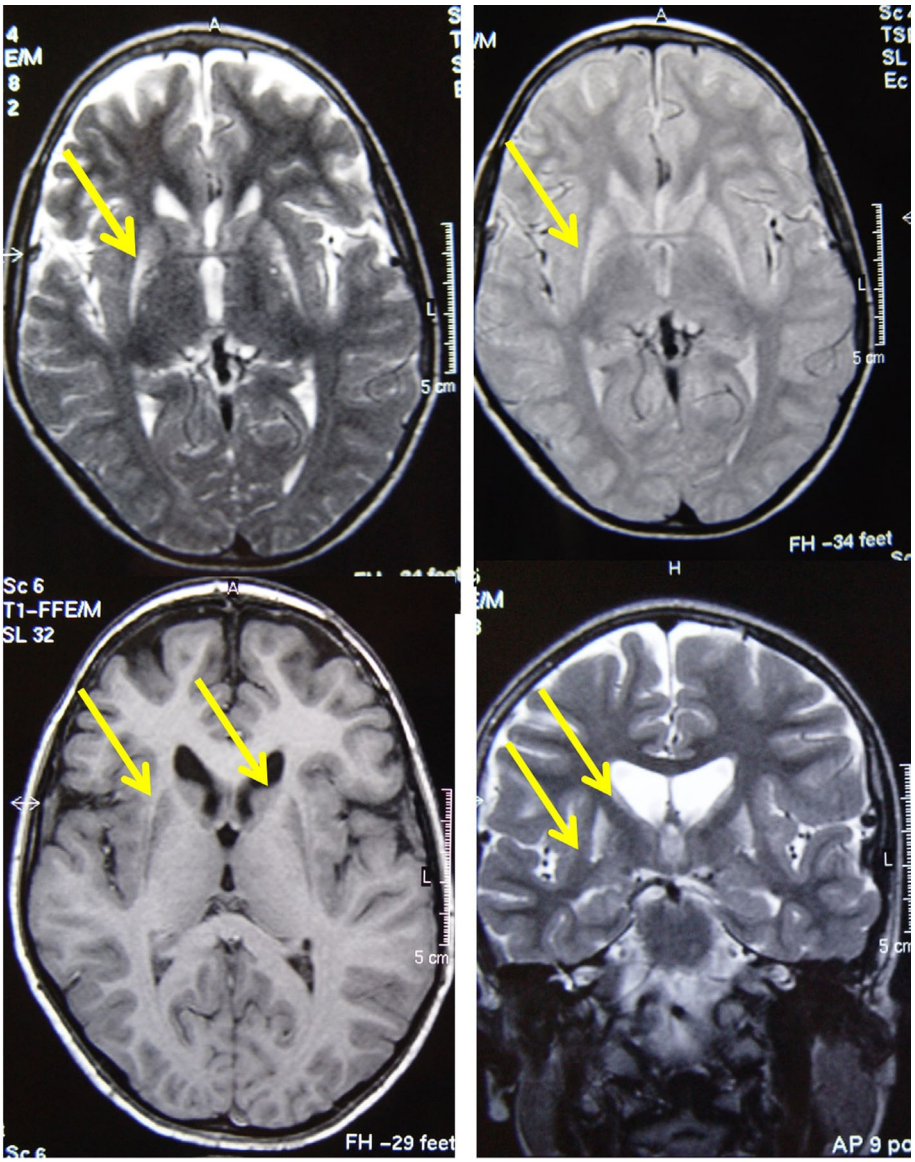


FIG. 1. Patient 2 brain magnetic resonance imaging at the age of 15 showing diffuse cerebral atrophy and striatal necrosis and atrophy (bilateral and symmetric T2 weighted hyperintensities of the putamina [hypointense in T1], volume loss of the head of caudate). [Color figure can be viewed at wileyonlinelibrary.com]

which disclosed a significantly reduced catalytic activity in primary fibroblasts obtained from patient 2 (20.1 mU/U citrate synthase, r.r. 48–120 based on 10 controls). Based on the American College of Medical Genomics (ACMG) criteria, taking into account the annotation and functional data, the variant was classified as likely pathogenic (PP1, PS3, PM2, and PM3). Notably, this substantially reduction in activity was not associated with either a reduced *MRPS36* mRNA level in patient 2's fibroblasts nor with a reduced level of the mutated protein in transfected COS1 cells (Supplementary Fig. S1.).

To further investigate the molecular mechanism underlying defective OGDHC function, the available three dimensional model describing the structural organization of the multimeric complex was considered (Fig. 2A). MRPS36 interacts via its C- and N-terminal regions with the E2 and E3 subunits, respectively.[4] Therefore, the nonsense variant, which results in the truncation of the C-terminal sequence of the protein (residues 95–103), directly affects the interaction of MRPS36 with the E2 subunit.[4] Specifically, the C terminus of MRPS36 directly interacts with the E2 subunit through an extended network of electrostatic interactions (Fig. 2B). Among these, the carboxylic groups of Glu^{95} and Glu^{103} are found at distances below 6 Å from charged groups in the E2 subunit (Arg^{289}, Arg^{197}, Lys^{201}, and Lys^{211}), therefore, indicating rather stable intermolecular salt bridges (Fig. 2C). This electrostatic network also encompasses Arg^{99}, which stabilizes the local secondary structure of MRPS36 by forming ion-pair interactions with the aforementioned anionic

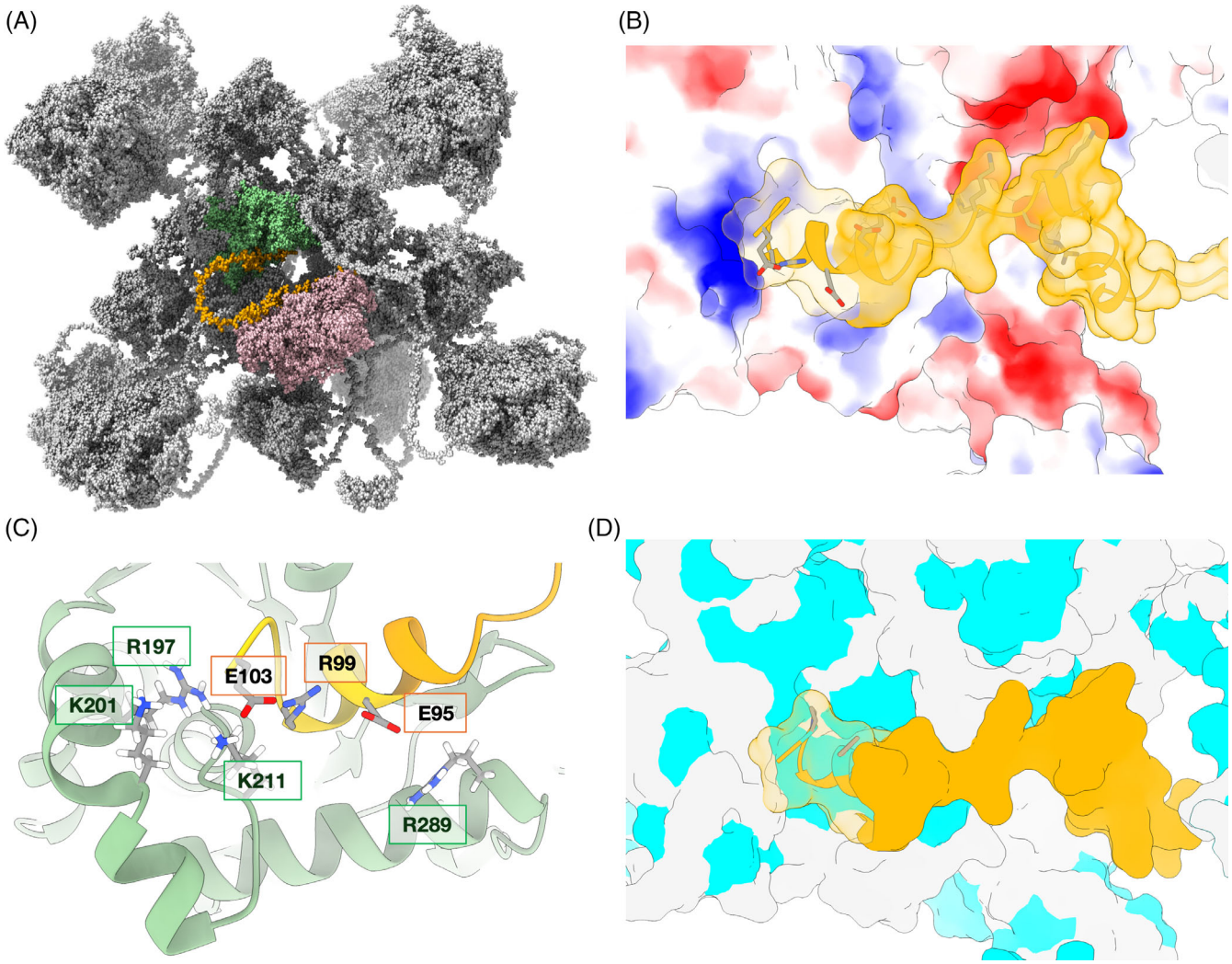


FIG. 2. Structural organization of the 2-oxoglutarate dehydrogenase complex (OGDHC) complex, and structural determinants of the interactions between MRPS36 (residues 95–103) and the E2 subunit. (**A**) OGDHC counts three catalytic subunits (2-oxoglutarate dehydrogenase-E1; dihydrolipoyl succinyl transferase-E2; dihydrolipoamide dehydrogenase-E3) (stechiometry: 16:24:12). The E3 subunits are connected to the OGDHC "core" by six MRPS36 subunits. Representative E2, E3, and MRPS36 subunits are shown in green, pink, and orange, respectively. (**B**) The molecular surface of the E2 subunit is color-coded based on the electrostatic potential. A cluster of positively charged residues (depicted in blue) is close to the MRPS36 C terminus (colored in orange). (**C**) Structural details of the ion pair interactions involving the charged residues in the C terminus of MRPS36 (E103, R99, and E95, colored in orange) with the E2 subunit (R197, K201, K211, and R289, colored in green). (**D**) The molecular surface of the E2 subunit is shown with the hydrophobic residues colored in cyan. The MRPS36 C terminus (orange) fills a large hydrophobic pocket present in the E2 subunit. [Color figure can be viewed at wileyonlinelibrary.com]

residues (Glu^{103} and Glu^{95}). Interestingly, most of intermolecular electrostatic interactions stabilizing E2-MRPS36 binding involve residues located at the C terminus. Notably, this region also fills the largest hydrophobic pocket at the E2-MRPS36 interface with its hydrophobic residues Phe^{96}, Ile^{97}, and Pro^{102} (Fig. 2D). These structural organization points to a defective ability of the mutant MRPS36 protein to bind to the E2 subunit as the molecular mechanism underlying defective OGCHC function.

Discussion

Defects in enzymes involved in the Krebs cycle are extremely rare, presenting with a combination of severe neurological features, cardiomyopathy and liver dysfunction.[16] Within this group, PDH and pyruvate carboxylase deficiency are well-established causes of Leigh syndrome.[17] Furthermore, E1 and E3 deficiency because of *OGDH* and *DLD* gene mutations, respectively, the only two disorders previously associated with isolated OGDHC deficiency, have been reported as the molecular basis of disorders in the spectrum of Leigh syndromes in four patients.[11,18-20]

Overall, isolated OGDH deficiency has been described in 12 patients from seven unrelated families presenting with a neurodegenerative disorder associated with prominent hyperkinetic MD. The clinical, biochemical, and genetic features of previously reported patients have been summarized in Supplementary Table S2. Two main phenotypes might be outlined, including a condition characterized by neonatal/early-

onset lactic acidosis and severe encephalopathy (six patients),[5,6,7] and a later-onset presentation with FTT, GDD, neurological deterioration, milder and fluctuating metabolic alterations, and chronic relapsing–remitting course (six patients).[8,9,11,21] MD is prominent in both phenotypes and characterized by choreoathetosis and dystonia in the majority of patients. Severe trunk dystonia with opisthotonos has been described in two patients,[6,11] and later emergence of pyramidal signs have been reported in seven patients.[6-9] Epilepsy was reported in three cases.[6,8,11] Four patients died in early infancy.[6,7] All had moderate to severe GDD, with prominent motor involvement and relatively spared social interaction and language comprehension. The most frequently observed MRI finding was cortical atrophy, whereas bilateral striatal necrosis was reported in two patients.[8,11] A metabolic signature consisting in a variable association of lactic acidosis, increased TCA cycle intermediates, and increased glutamate plus glutamine appears to characterize the disease (Supplementary Table S2).

Five of 12 patients received a genetic diagnosis, namely an atypical form of E3 deficiency (without biochemical evidence of PDH and BCKDC deficiency) because of biallelic *DLD* variants in three patients,[6,20] and E1 deficiency because of biallelic missense variants in the *OGDH* gene in two patients.[11] In the remaining seven cases, a reduction of the OGDH enzymatic activity without metabolic sign of E3 deficiency was demonstrated.[5,7-9,21] The present cases showed a phenotype that overlaps with that of patients reported over time, shifted toward the milder spectrum of the disease. They have never experienced major metabolic decompensations, nor showed TCA intermediates in urine samples.[8] The slight elevation of plasma glutamate and glutamine observed in these and previously reported patients with OGDHC deficiency (three later diagnosed with E3 deficiency and one without a molecular diagnosis) could be considered a metabolic signature.[6,8]

The multifaceted function of the OGDHC and its complex structural organization is in line with the pleiotropic and variable clinical spectrum associated with the defective function of the enzyme. Genetic variability in terms of affected genes and differential impact of pathogenic variants likely account for the observed variation in terms of clinical severity and disease course. It is reasonable to speculate that pathogenic variants affecting the catalytic sites, as shown by the three cases reported with mutated *DLD*, result in severe/rapidly fatal forms, whereas those involving *MRPS36*, are associated with a relatively milder phenotype, because of residual catalytic activity of the enzymatic complex.

In summary, we described two siblings affected by a severe, early-onset, and slowly progressive neurometabolic disorder with prominent MD and bilateral striatal necrosis, which is consistent with Leigh syndrome. The biochemical evidence of OGDHC deficiency and the role of MRPS36 in the enzymatic complex suggest that biallelic loss-of-function variants of *MRPS36* can affect the enzymatic function of the complex, pointing to *MRPS36* as a new disease gene for this rare neurometabolic disorder. ■

Data Availability Statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary data. The ES data are available upon request from one of the senior authors (M.T.). The data are not publicly available due to due to privacy/ethical restrictions.

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

De novo *FRMD5* Missense Variants in Patients with Childhood-Onset Ataxia, Prominent Nystagmus, and Seizures

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ABSTRACT: Background: *FRMD5* variants were recently identified in patients with developmental delay, ataxia, and eye movement abnormalities.
Objectives: We describe 2 patients presenting with childhood-onset ataxia, nystagmus, and seizures carrying pathogenic de novo *FRMD5* variants. Weighted gene co-expression network analysis (WGCNA) was performed to gain insights into the function of *FRMD5* in the brain.
Methods: Trio-based whole-exome sequencing was performed in both patients, and CoExp web tool was used to conduct WGCNA.
Results: Both patients presented with developmental delay, childhood-onset ataxia, nystagmus, and seizures. Previously unreported findings were diffuse choreoathetosis and dystonia of the hands (patient 1) and areas of abnormal magnetic resonance imaging signal in the white matter (patient 2). WGCNA showed that *FRMD5* belongs to gene networks involved in neurodevelopment and oligodendrocyte function.
Conclusions: We expanded the phenotype of *FRMD5*-related disease and shed light on its role in brain function and development. We recommend including *FRMD5* in the genetic workup of childhood-onset ataxia and nystagmus. © 2024 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: ataxia; *FRMD5*; nystagmus; genetics; weighted gene co-expression network analysis

FERM domain-containing proteins (FDCP) are a group of proteins that play a crucial role in anchoring the cytoskeleton to the plasma membrane, thereby regulating cell motility and interaction with the extracellular environment.[1]