Molecular Genetics and Metabolism Reports 30 (2022) 100830

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: www.elsevier.com/locate/ymgmr

A novel *MRPS34* gene mutation with combined OXPHOS deficiency in an adult patient with Leigh syndrome

L. Lenzini [a,1], M. Carecchio [b,1], E. Iori [c], A. Legati [d], E. Lamantea [d], A. Avogaro [c], N. Vitturi [c,*]

[a] University of Padova, Department of Medicine-DIMED, University Hospital, Padova, Italy
[b] Movement Disorders Unit, Department of Neurosciences, University of Padova, Padova, Italy
[c] University of Padova, Department of Medicine-DIMED, Division of Metabolic Diseases, University Hospital, Padova, Italy
[d] Unit of Medical Genetics and Neurogenetics, Fondazione IRCCS Istituto Neurologico Carlo Besta, 20126 Milan, Italy

A B S T R A C T

We report a novel pathogenic variant (c.223G > C; p.Gly75Arg) in the gene encoding the small mitoribosomal subunit protein mS34 in a long-surviving patient with Leigh Syndrome who was genetically diagnosed at age 34 years.

The patient presented with delayed motor milestones and a stepwise motor deterioration during life, along with brain MRI alterations involving the subcortical white matter, deep grey nuclei and in particular the internal globi pallidi, that appeared calcified on CT scan. The novel variant is associated with a reduction of mS34 protein levels and of the OXPHOS complex I and IV subunits in peripheral blood mononuclear cells of the case.

This study expands the number of variants that, by affecting the stability of the mitoribosome, may cause an OXPHOS deficiency in Leigh Syndrome and reports, for the first time, an unusual long survival in a patient with a homozygous *MRPS34* pathogenic variant.

1. Introduction

Leigh syndrome (LS) is a life-threatening neurodegenerative disease characterized by a heterogeneous neurological presentation, ranging from mild to severe neurological deficits and characteristic bilateral brain lesions involving the basal ganglia, thalami, and brainstem.

The syndrome is caused by mutations of more than 75 proteins encoded by either nuclear or mitochondrial genes resulting in mitochondrial dysfunction that, most often, is due to impaired assembly or function of one or more component of the oxidative phosphorylation (OXPHOS) proteins [1–3].

Mitochondria contain their own protein synthesis machinery that includes mitochondria-specific ribosomes (mitoribosome) required for the translation of mitochondrial genes. The mammalian mitoribosome (55S) contains a small subunit (28S) that decodes mRNA and mediates tRNA delivery of amino acids and a large (39S) subunit that catalyzes the formation of peptide bonds between the amino acids. Mitoribosomal components are encoded by four mitochondrial genes and 80 nuclear genes. In humans, 12S rRNA assembles with 30 mitochondrial ribosomal small subunit proteins (MRPSs) to form the small 28S subunit, while the 16S rRNA forgathers with 50 mitochondrial ribosomal large subunit proteins (MRPLs) to make the large 39S subunit.

Defects in the stability or assembly of mitoribosome impair mitochondrial protein translation, causing combined OXPHOS enzyme deficiency [4]. As the OXPHOS system generates the majority of cellular energy required by the body, disorders caused by mutations in mitoribosomal proteins are usually heterogeneous and multi-systemic, often leading to death before adulthood.

We here report on a long-surviving patient affected by LS caused by a novel biallelic mutation in the *MRPS34* gene associated with combined OXPHOS deficiency.

2. Methods

2.1. Patient description

The patient, a 34-year-old Caucasian man, was born full term after an uncomplicated pregnancy from healthy unrelated parents. He showed generalized hypotonia, poor sucking and failure to thrive soon after birth, requiring parenteral feeding via nasogastric tube. Early psychomotor delay was evident during the first year of life, including delayed motor milestones and language. Blood tests during infancy demonstrated metabolic acidosis with increased serum lactate (3.4 mmol/L; normal range: 0.1–2.2 mmol/L) and normal CPK plasma levels. During

* Corresponding author at: Department of Medicine-DIMED, Division of Metabolic Diseases, Padova University Hospital, Padova, Italy.
E-mail address: nicola.vitturi@aopd.veneto.it (N. Vitturi).
[1] These authors equally contributed to this work.

https://doi.org/10.1016/j.ymgmr.2021.100830
Received 29 October 2021; Received in revised form 2 December 2021; Accepted 2 December 2021
Available online 6 December 2021
2214-4269/© 2021 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

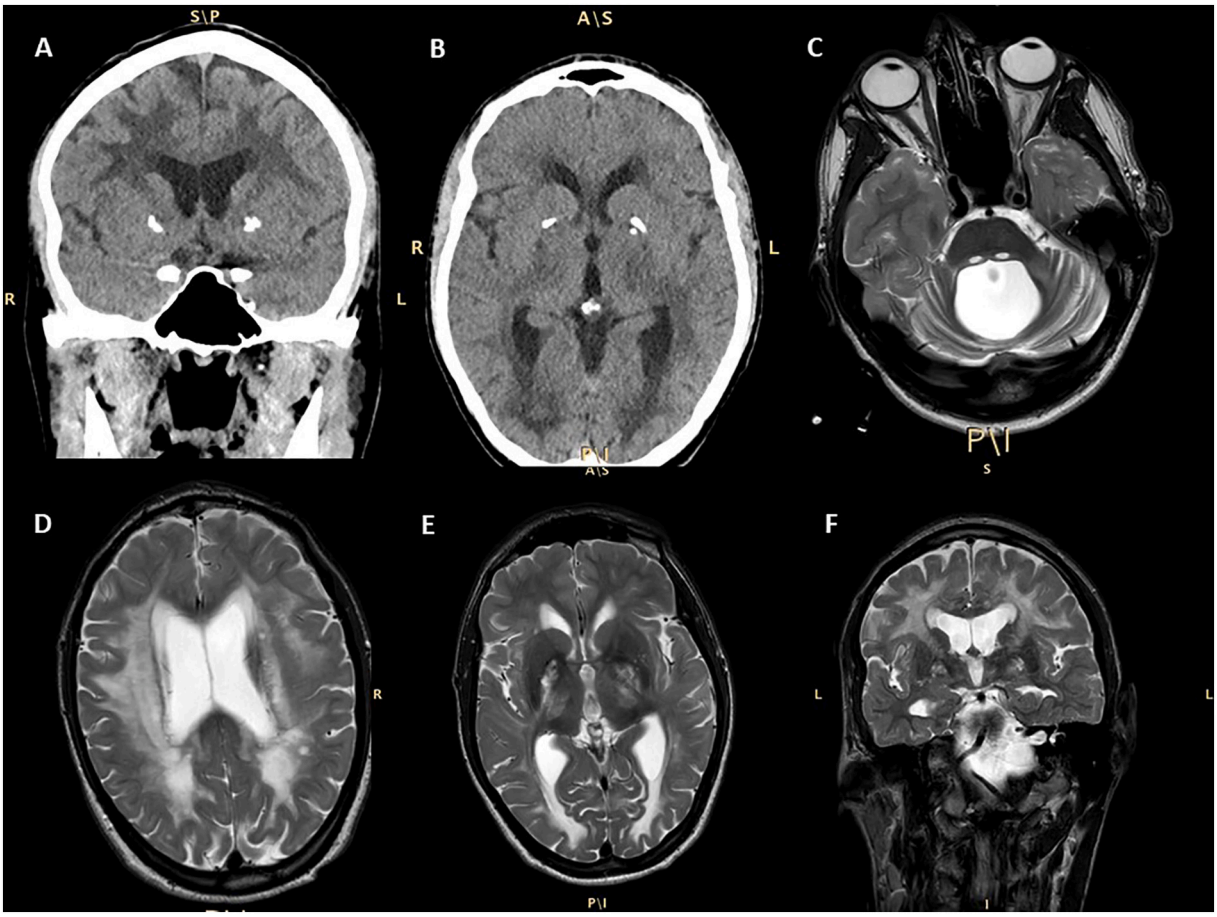

Fig. 1. Patient's brain imaging at age 33 years. A-B: coronal and axial CT scan showing bilateral internal pallidal calcification and extensive subcortical white matter hypodensity; C-F: brain MRI (T2-weighted sequences). C: abnormally dilated fourth ventricle with hyperintense dorsal brainstem nuclei; D: extensive bilateral alteration of the subcortical white matter; E: basal ganglia and thalamic nuclei signal alteration; F: coronal cut showing subcortical bilateral white matter hyperintensity and basal ganglia lesions (note the hyperintense internal pallidal signal that corresponds to calcification on CT scan).

childhood and adolescence, he underwent brain MRI showing abnormally dilated fourth ventricle, extensive subcortical white matter alterations and a bilateral, non-enhancing hyperintense signal in globi pallidi. Mild cortical atrophy was also evident. A diagnosis of LS was made at age 10 on clinical and radiological grounds. Muscular biopsy performed at age 4 showed no ragged-red fibers; combined COX/NADH-TR staining and COX/SDH staining were normal; Muscular fibers diameter varied in size between 8 and 40 μm, with rare central nuclei. No increase in endomysial or perimysial connective tissue was observed with trichromic staining, and cytoplasmatic inclusions were not detected. With basic and acid ATPase staining no tyle grouping was observed, but only mild hypotrophy of type 2 fibers. PAS (Periodic Acid Schiff), ORO (Oil Red O) and AP (Acid Phosphatase) colorations were normal. Biochemical analysis was not performed. These features suggested a hypotonia of central, rather than peripheral origin.

The patient was able to walk with support by late childhood and showed a step wise progression of neurological conditions, with major episodes of deterioration at age 5, 16 and 32 years, triggered in the last occasion by a urinary tract infection that evolved to sepsis. He was partially fed by gastrostomy by age 5 years due to severe dysphagia. When he came to our attention at age 32, the patient could take some steps with support; neurological examination showed marked spasticity in the lower limbs with spontaneous ankle clonus, inability to communicate verbally and to perform fine purposeful movements with the upper limbs. Brain MRI showed cerebral and cerebellar atrophy and an extensive signal alteration (DWI hyperintensity and T1 hypointensity) involving the subcortical white matter, globi pallidi, lateral portion of the thalami, subthalamic nuclei, midbrain and the middle cerebellar peduncles (Fig. 1). CT scan disclosed calcification involving only the GPi bilaterally, with sparing of other basal ganglia, thalami, cerebellar dentate nuclei and subcortical white matter. Despite not being specific, this radiological feature suggested a possible underlying mitochondrial disorder rather than other neurological diseases such as Primary Familial Brain Calcification (formerly known as Fahr disease).

2.2. Genetic analysis

At age 32, the patient's DNA extracted from peripheral blood mononuclear cells (PBMCs) by standard procedures (QIAquick DNA purification kit; Qiagen, Milan, Italy) was analyzed after a dedicated informed consent for genetic analyses was signed.

NGS analysis was performed through a custom-made targeted mitochondrial panel of 299 genes (Supplementary Table 1), using 50 ng of DNA as template for the construction of a paired-end library, according to the SureSelect^{QXT} Target Enrichment preparation guide (Agilent). Libraries were than sequenced on the Illumina MiSeq platform, reads produced were aligned to the NCBI human reference genome (GRCh37/hg19) using the Burrows–Wheeler Aligner (BWA) [5]. Single nucleotidevariants (SNVs) and small insertions/deletions (INDELs) calling were performed using GATK4 [6], Variant Studio software (Illumina) was used for variants annotation and filtering, focusing on rare predicted missense, frame-shift, stop-gain or stop-loss, and splicesite variants, as described in Legati A. et al. 2016 [7]. Validation of the identified candidate variant and segregation analysis in the

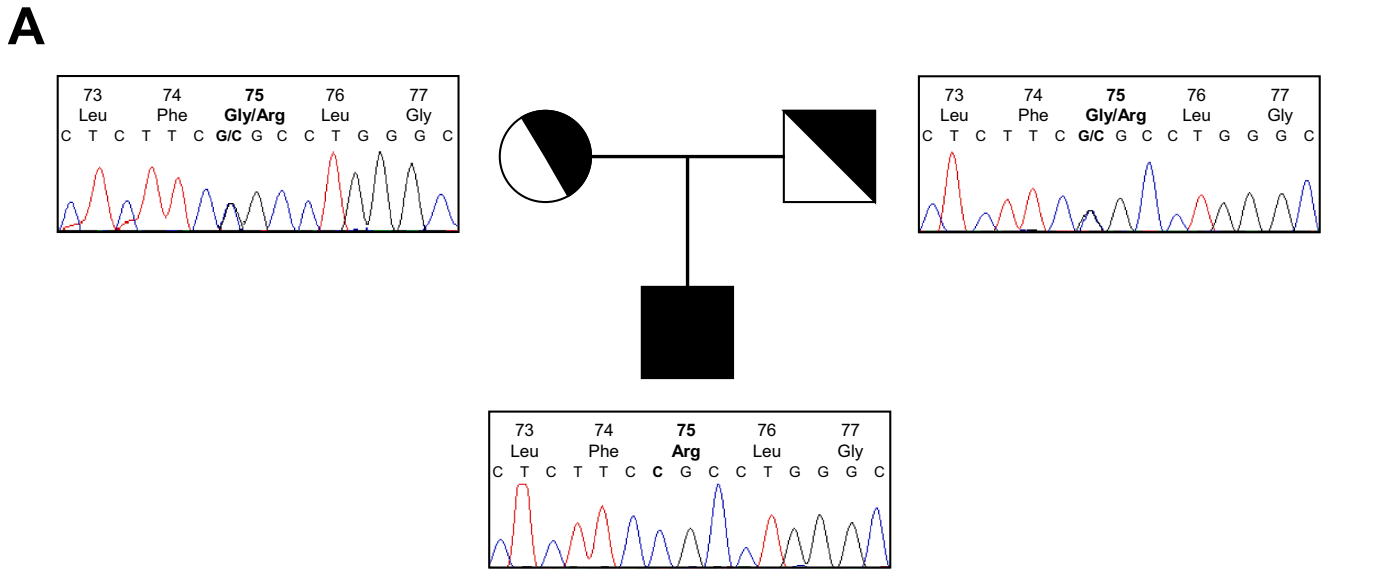

Fig. 2. A): Pedigree of the patient's family: close to each subject is reported the own Sanger sequencing of *MRPS34* exon 1 containing the c.223G > C variant, in a heterozygous state in the parents and in a homozygous state in the proband. Both nucleic and aminoacidic changes are indicated in bold. **B)**: alignment of portion of human mS34 protein sequence with its homologs in nine other vertebrate species showing the high conservation of the p.Gly75 residue.

proband's parents were performed by Sanger sequencing.

2.3. Preparation of the lymphomonocyte fraction

PBMCs were isolated from whole blood by a density gradient medium using a solution of Histopaque 1077 (Sigma-Aldrich Co. Ltd., Irvine, UK). Total proteins were extracted from PBMCs and resuspended in RIPA buffer with 10% protease inhibitor cocktail (Sigma Life Science, MI, Italy). The protein content was determined using Lowry's method.

2.4. Assessment of MRPS34 and OXPHOS expression by Western blot analysis

Proteins were separated by polyacrylamide gel electrophoresis (12%) under denaturing conditions (SDS-PAGE). Predetermined amounts of each lysate (40 μg of protein) were diluted in a loading buffer (20 mM Tris-HCl pH 6.8, glycerol 10% vol/vol, β-mercaptoethanol 1% vol/vol, SDS 1% w/vol, 0.2% bromophenol blue, deionized H_2O). Samples were loaded into gel dipped in running buffer (glycine 18.8 g/L, Tris 3 g/L, SDS 1 g/L, and deionized H_2O, pH 8.3), using the Hoefer MiniVE electrophoretic unit (Amersham Pharmacia Biotech, CA, USA) and then electro-blotted onto nitrocellulose membrane (Hybond, Amersham Pharmacia Biotech) overnight at 4 °C.

The membrane was incubated with the primary antibodies: an anti-OXPHOS cocktail which includes I-NDUFB8–18 kDa; II-SDHB-29 kDa; III-UQCRC2–48 kDa; IV-COXII-22 kDa; V-ATP5A-54 kDa (mouse, 1:500, ab110411 Abcam-Prodotti Gianni, MI, Italy), anti-mS34 (rabbit 1:500 # GTX87642 Genetex, Prodotti Gianni, MI, Italy) and anti-GAPDH (rabbit, 1: 5000, # 5174 Cell Signaling) overnight at 4 °C. The detection of protein expression was carried out by chemiluminescence reaction (Trident pico Western HPR substrate # GTX17435, Gene Tex, Prodotti Gianni) and the optical intensity of the protein bands was determined using an acquisition system chemiluminescence images (Alliance Q9 ATOM, Uvitec Cambridge). Densitometric analysis of the GAPDH expression was used as normalizer and the results were expressed as the change from the control, which was set as equal to 1.

2.5. Statistical analysis

The values obtained are reported as the mean ± SD of at least 3 technical replicates. Statistical differences of protein levels (mS34 and OXPHOS) between the case and the controls were tested by the pairwise comparison *t*-test.

3. Results

3.1. Identification of a novel mutation in MRPS34 gene

Targeted NGS analysis (mean coverage >20× in 99.6% of genes) revealed a homozygous missense variant in *MRPS34* gene: c.223G > C

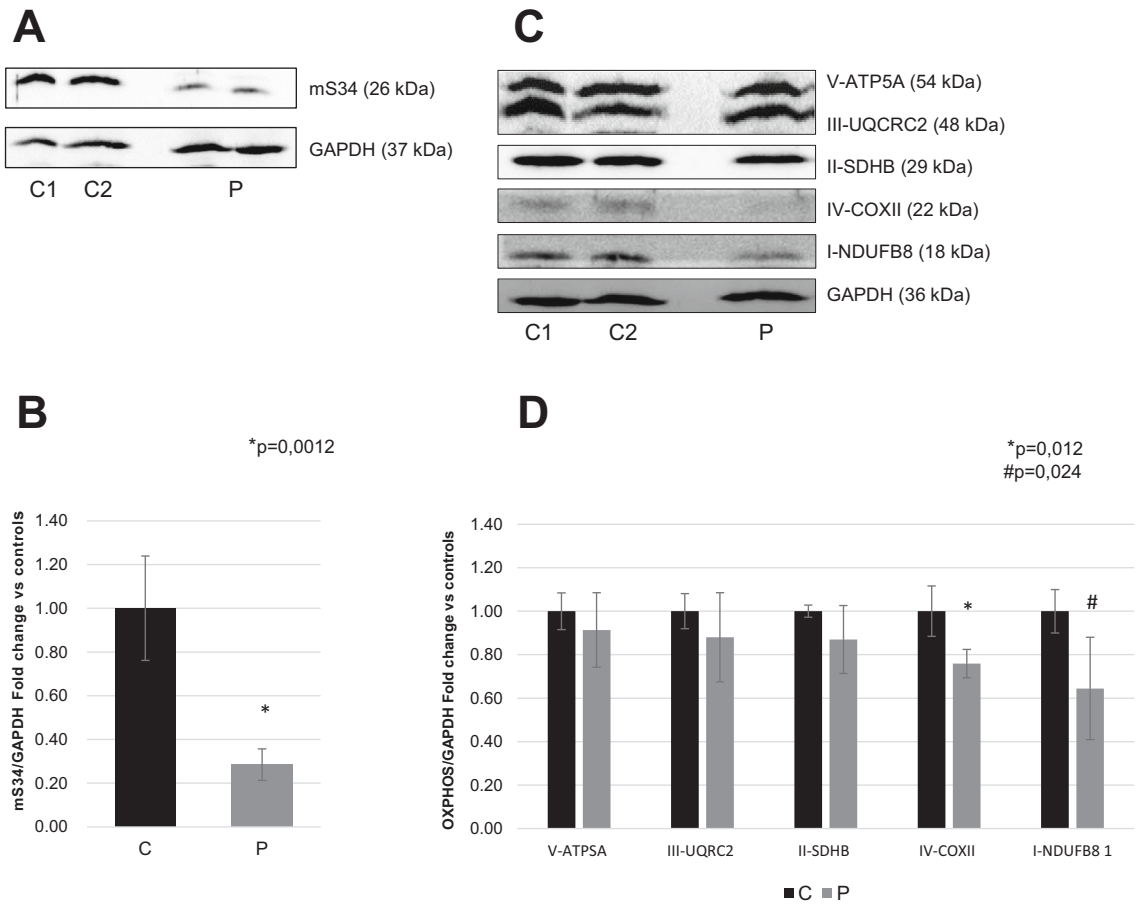

Fig. 3. Levels of mS34 and OXPHOS in the patient (P) and controls (C). **A)** SDS-PAGE Western blot analysis of mS34 and GAPDH protein expressions in PBMCs from two healthy subjects (C1 and C2) and in the patient (P); GAPDH protein expression is used as an internal control. **B)** mS34/GAPDH densitometric evaluation of blots given as mean ± SD of three different experiments. Data are expressed as fold change versus controls (set at 1). **C**: SDS-PAGE Western blot analysis of five subunits of mitochondrial OXPHOS complexes (I-NDUFB8, II-SDHB, III-UQCRC2, IV-COXII, V-ATP5A) and GAPDH protein expressions in PBMCs from two healthy subjects (C1 and C2) and the patient (P). GAPDH protein expression is used as a loading control. **D)** OXPHOS/GAPDH densitometric evaluation of blots given as mean ± SD of at least three different immunoblots. Data are expressed as fold change versus controls (set at 1).

on exon 1 (NM_001300900.2) causing the aminoacid change p. Gly75Arg in the protein. The Gly75Arg variant was never reported in public databases (GnomAD, ExAC, EVS, dbSNP) and classified as a variant of uncertain significance in accordance with the 2015 ACMG guidelines [8]. In addition, bioinformatics tools available online (SIFT, Polyphen2, MutationTaster, PROVEAN, VEST3, MutPred, MutationAssessor) [9–15] predicted a deleterious or probably deleterious effect on the functionality of the protein for this amino acid substitution.

Segregation analysis showed that the c.223G > C variant was present in a heterozygous state in both parents (Fig. 2, panel A), confirming an autosomal recessive mode of inheritance.

The alignment of human mS34 protein with its homologs in seven other vertebrate species indicated that the mutated amino acid (Gly75) is highly conserved among species (Fig. 2, **B**).

3.2. Reduction of mS34 protein levels

To investigate the effect of the p.Gly75Arg variant on mS34 protein levels, immunoblotting was performed on available cells from the subject and two healthy, age- and sex- matched individuals. Western blot analysis of PBMCs proteins showed the decrease of mS34 in the patient as compared to the controls (0.29 ± 0.07, $p = 0.0012$; Fig. 3, **A-B**).

3.3. The p.Gly75Arg MRPS34 mutation is associated with a reduction of complexes I and IV protein levels

As mS34 is a component of the mitoribosome, we sought to determine whether its low level in the affected subject is associated with a reduced expression of components of OXPHOS complex that may be due to an impairment of translation of the encoding mitochondrial DNA as already demonstrated by Lake N et al. [16]. Immunoblotting of PBMCs lysates from the case and two control individuals revealed decreased protein levels of complex I (0.65 ± 0.08, $p < 0.0001$) and IV (0.75 ± 0.18, $p = 0.012$) as shown in Fig. 3, panels C and D.

4. Discussion

We here report an adult patient who received a diagnosis of LS in childhood based on clinical and radiological features.

Genetic investigations performed during infancy assessed only the few genes known at the time causing LS, with negative results. By means of NGS techniques, we were able to identify a novel homozygous variant of the *MRPS34* gene (c.223G > C; p.Gly75Arg) during adulthood.

This nuclear gene codes for the component mS34 of the small subunit 28S of the mitoribosome and is required for the physiological functions of mitochondria [17,18]. Four pathogenic recessive mutations have been previously identified in *MRPS34* in six individuals with combined OXPHOS defects and LS or Leigh-like disease [16]. In these subjects, a

destabilization of the small mitoribosomal subunit was demonstrated along with decreased mitochondrial protein translation and OXPHOS deficiency.

Our data expands the number of variants in mitoribosomal subunits associated with LS. Moreover, this is the first patient carrying *MRPS34* mutations reported so far who reached adulthood [19–22]. In fact, among patients reported by Lake et al. [16], only one survived into late adolescence, the disorder being fatal before one year of age in two subjects; three other subjects were reported as still alive but younger. Clinical picture of our adult patient was dominated by generalized hypotonia and delayed psychomotor development during infancy and childhood and evolved to spastic quadriparesis in adulthood with a stepwise deterioration over disease course. Unlike other patients previously reported, hyperkinetic movement disorders were never observed in this subject, whereas some of his brain MRI features were strikingly similar to previously reported ones. In particular, hyperintensity of the dorsal brainstem nuclei and periaqueductal grey matter were observed at age 34 years. The bilateral and symmetric alterations of the subcortical white matter and (of) the deep grey nuclei, including the internal pallidus that showed calcification on CT scan were consistent with a mitochondrial disorder.

As the mutated Glycine in position 75 appears to be conserved in different species (Fig. 2**, B**) and the expression of mS34 protein is very low in patient's PBMCs (Fig. 3**, A-B**), we hypothesize that this variant may affect the mS34 protein structure and/or activity, but, unfortunately, due to the lack of functional experiments we were not able to demonstrate it by this study.

The hypothesis that the p.Gly75Arg variant, by destabilizing the mitoribosome assembly, could affect the mitochondrial protein synthesis was suggested by the decreased levels of subunits belonging to complex I and IV detected in patient vs controls which is concordant with what already showed for other *MRPS34* mutations [16]. Moreover, examples of how mutations in its protein component destabilize the mitoribosome assembly were already reported not only for the mS34 [16], but also for the mS28 subunit [23]. However, by this study, we cannot exclude that the observed decrease of complex I and IV levels could be due to other causes inhibiting mitochondrial translation, such as impaired transcription, mitochondrial RNA maturation or mitoribosomal function.

The partial reduction that we observed on OXPHOS complexes may be justified by the residual level of mS34 subunit. In fact, the c.223G > C variant could act as hypomorphic mutation allowing the partial expression of mS34 subunit that we detected in the PBMCs and, by consequence, a residual synthesis of mitochondrial proteins. Unfortunately, due to lack of additional biological samples, we could not determine the protein levels in other tissues, e.g. muscle or fibroblasts and, for the same reason, we could not directly compare them to the mS34 levels reported in the cases carrying other MRPS34 mutations already described; PBMCs were the only available cells from the patient as he refused to undergo muscle and/or skin biopsies.

Finally, we demonstrated that the sufficient expression of both mS34 and OXPHOS proteins in PBMCs of healthy controls further supports the use of these cells as a possible model to test the involvement of mS34 in the mitoribosome activity when the skin biopsy is not available.

However, this study has the major limitation that lacks functional data linking the observed decrease in the two OXPHOS proteins to the specific defect in *MRPS34*. In fact, the availability only of PBMCs as cell model limits the types of functional experiments that can be performed with these cells.

In conclusion, we report a novel *MPRS34* missense mutation causing LS, expanding the current knowledge on the natural disease history of patients with *MPRS34* mutations to include possible long-term survival and a step wise deterioration as frequently observed in mitochondrial diseases.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2021.100830.

Acknowledgments

L.L. is supported by the Supporting Talent in ReSearch@University of Padova (Project: "COMET, "From Phenotype to Gene Variants: a Comprehensive Approach to Gene Discovery in Adult Forms of Metabolic Rare Diseases"). E.L. and A.L. are grateful to Pierfranco e Luisa Mariani Foundation, funder of Center for the Mitochondrial Pediatric Diseases where part of this work was carried out. A.L. is supported by the Italian Ministry of Health (RF/GR-2016-02361241; "Ricerca Corrente").

Disclosure

The authors declare that they have no disclosure.

References

[1] F. Baertling, R.J. Rodenburg, J. Schaper, J.A. Smeitink, W.J.H. Koopman, E. Mayatepek, E. Morava, F. Distelmaier, A guide to diagnosis and treatment of Leigh syndrome, J. Neurol. Neurosurg. Psychiatry 85 (2014) 257–265, https://doi.org/10.1136/jnnp-2012-304426.

[2] N.J. Lake, A.G. Compton, S. Rahman, D.R. Thorburn, Leigh syndrome: one disorder, more than 75 monogenic causes, Ann. Neurol. 79 (2016) 190–203, https://doi.org/10.1002/ana.24551.

[3] A. Ardissone, C. Bruno, D. Diodato, A. Donati, D. Ghezzi, E. Lamantea, C. Lamperti, M. Mancuso, D. Martinelli, G. Primiano, E. Procopio, A. Rubegni, F. Santorelli, M. C. Schiaffino, S. Servidei, F. Tubili, E. Bertini, I. Moroni, Clinical, imaging, biochemical and molecular features in Leigh syndrome: a study from the Italian network of mitochondrial diseases, Orphanet J. Rare Dis. 16 (2021) 1–12, https://doi.org/10.1186/s13023-021-02029-3.

[4] J.A. Mayr, T.B. Haack, P. Freisinger, D. Karall, C. Makowski, J. Koch, R. G. Feichtinger, F.A. Zimmermann, B. Rolinski, U. Ahting, T. Meitinger, H. Prokisch, W. Sperl, Spectrum of combined respiratory chain defects, J. Inherit. Metab. Dis. 38 (2015) 629–640, https://doi.org/10.1007/s10545-015-9831-y.

[5] H. Li, R. Durbin, Fast and accurate short read alignment with burrows-wheeler transform, Bioinformatics 25 (2009) 1754–1760, https://doi.org/10.1093/bioinformatics/btp324.

[6] G.A. Van der Auwera, B.D. O'Connor, Genomics in the cloud: using docker, GATK, and WDL in Terra, (1st Edition)., O'Reilly Media, 2020.

[7] A. Legati, A. Reyes, A. Nasca, F. Invernizzi, E. Lamantea, V. Tiranti, B. Garavaglia, C. Lamperti, A. Ardissone, I. Moroni, A. Robinson, D. Ghezzi, M. Zeviani, New genes and pathomechanisms in mitochondrial disorders unraveled by NGS technologies, Biochim. Biophys. Acta - Bioenerg. 2016 (1857) 1326–1335, https://doi.org/10.1016/j.bbabio.2016.02.022.

[8] S. Richards, N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W.W. Grody, M. Hegde, E. Lyon, E. Spector, K. Voelkerding, H.L. Rehm, Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the american College of Medical Genetics and Genomics and the Association for Molecular Pathology, Genet. Med. 17 (2015) 405–424, https://doi.org/10.1038/gim.2015.30.

[9] S. Henikoff, SIFT: Predicting amino acid changes that affect protein function, Nucleic Acids Res 31 (2003), https://doi.org/10.1093/nar/gkg509, 3812-.

[10] I. Adzhubei, D.M. Jordan, S.R. Sunyaev, Predicting functional effect of human missense mutations using PolyPhen-2, Curr Protoc Hum Genet (2013), https://doi.org/10.1002/0471142905.hg0720s76. Chapter 7:Unit7.20.

[11] J.M. Schwarz, D.N. Cooper, M. Schuelke, D. Seelow, MutationTaster2: mutation prediction for the deep-sequencing age, Nat. Methods 4 (2014) 361–362, https://doi.org/10.1038/nmeth.2890.

[12] Y. Choi, A.P. Chan, PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels, Bioinformatics 31 (2015) 2745–2747, https://doi.org/10.1093/bioinformatics/btv195.

[13] H. Carter, C. Douville, P.D. Stenson, D.N. Cooper, R. Karchin, Identifying Mendelian disease genes with the variant effect scoring tool, BMC Genomics 14 (2013) S3, https://doi.org/10.1186/1471-2164-14-S3-S3. Suppl 3(Suppl 3).

[14] V. Pejaver, J. Urresti, J. Lugo-Martinez, K.A. Pagel, G.N. Lin, H. Nam, M. Mort, D. N. Cooper, J. Sebat, L.M. Iakoucheva, S.D. Mooney, P. Radivojac, Inferring the molecular and phenotypic impact of amino acid variants with MutPred2, Nat. Commun. 11 (2020) 5918, https://doi.org/10.1038/s41467-020-19669-x.

[15] R. Boris, A. Yevgeniy, S. Chris, Predicting the functional impact of protein mutations: application to cancer genomics, Nucleic Acids Res. 39 (2011), e118, https://doi.org/10.1093/nar/gkr407.

[16] N.J. Lake, B.D. Webb, D.A. Stroud, T.R. Richman, B. Ruzzenente, A.G. Compton, H. S. Mountford, J. Pulman, C. Zangarelli, M. Rio, N. Bodaert, Z. Assouline, M. D. Sherpa, E.E. Schadt, S.M. Houten, J. Byrnes, E.M. McCormick, Z. Zolkipli-Cunningham, K. Haude, Z. Zhang, K. Retterer, R. Bai, S.E. Calvo, V.K. Mootha, J. Christodoulou, A. Rötig, A. Filipovska, I. Cristian, M.J. Falk, M.D. Metodiev, D. R. Thorburn, Biallelic mutations in MRPS34 Lead to instability of the small mitoribosomal subunit and Leigh syndrome, Am. J. Hum. Genet. 101 (2017) 239–254, https://doi.org/10.1016/j.ajhg.2017.07.005.

[17] G. Gopisetty, R. Thangarajan, Mammalian mitochondrial ribosomal small subunit (MRPS) genes: a putative role in human disease, Gene 589 (2016) 27–35, https://doi.org/10.1016/j.gene.2016.05.008.

[18] T.R. Richman, J.A. Ermer, S.M.K. Davies, K.L. Perks, H.M. Viola, A.-M. J. Shearwood, L.C. Hool, O. Rackham, A. Filipovska, Mutation in MRPS34 compromises protein synthesis and causes mitochondrial dysfunction, PLoS Genet. 11 (2015), e1005089, https://doi.org/10.1371/journal.pgen.1005089.

[19] P. McKelvie, B. Infeld, R. Marotta, J. Chin, D. Thorburn, S. Collins, Late-adult onset Leigh syndrome, J. Clin. Neurosci. 19 (2012) 195–202, https://doi.org/10.1016/j.jocn.2011.09.009.

[20] J. Finsterer, Leigh and Leigh-like syndrome in children and adults, Pediatr. Neurol. 39 (2008) 223–235, https://doi.org/10.1016/j.pediatrneurol.2008.07.013.

[21] W. Aulbert, K. Weigt-Usinger, C. Thiels, C. Köhler, M. Vorgerd, A. Schreiner, S. Hoffjan, T. Rothoeft, S.B. Wortmann, C.M. Heyer, T. Podskarbi, T. Lücke, Long survival in Leigh syndrome: new cases and review of literature, Neuropediatrics 45 (2014) 346–353, https://doi.org/10.1055/s-0034-1383823.

[22] L. Van Maldergem, F. Trijbels, S. DiMauro, P.J. Sindelar, O. Musumeci, A. Janssen, X. Delberghe, J.J. Martin, Y. Gillerot, Coenzyme Q- responsive Leigh's encephalopathy in two sisters, Ann. Neurol. 52 (2002) 750–754, https://doi.org/10.1002/ana.10371.

[23] J. Pulman, B. Ruzzenente, L. Bianchi, M. Rio, N. Boddaert, A. Munnich, A. Rötig, M.D. Metodiev, Mutations in the MRPS28 gene encoding the small mitoribosomal subunit protein bS1m in a patient with intrauterine growth retardation, craniofacial dysmorphism and multisystemic involvement, Hum. Mol. Genet. 28 (2019) 1445–1462, https://doi.org/10.1093/hmg/ddy441.