Report of the Third Family with Multiple Mitochondrial Dysfunctions Syndrome 5 Caused by the Founder Variant p.(Glu87Lys) in *ISCA1*

Anju Shukla[1] Parneet Kaur[1] Katta M. Girisha[1]

[1] Department of Medical Genetics, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, Karnataka, India

J Pediatr Genet 2018;7:130–133.

Address for correspondence K. M. Girisha, MD, Department of Medical Genetics, Kasturba Medical College, Manipal Academy of Higher Education, Manipal, Karnataka, India (e-mail: girish.katta@manipal.edu).

Abstract

Iron-sulfur cluster assembly 1 (ISCA1) is one of the essential proteins operating in the mitochondrial iron-sulfur (Fe-S) cluster biogenesis pathway. We reported the variant c.259G > A [p.(Glu87Lys)] in homozygous state in exon 4 of the *ISCA1* gene as the likely cause of multiple mitochondrial dysfunction syndrome 5 in a previous publication. We now report the third patient with the same phenotype and variant, further supporting the possibility of a founder event. Our observation confirms the clinical presentation associated with a probable founder variant in this condition.

Keywords

- multiple mitochondrial dysfunctions syndrome 5
- ISCA1
- founder effect

Introduction

Iron-sulfur cluster assembly 1 (ISCA1) is a mitochondrial protein involved in the biogenesis of iron-sulfur clusters (ISCs) essential for electron transport reaction.[1–3] Recently, we reported four patients from two unrelated families with a homozygous missense variant in *ISCA1* leading to multiple mitochondrial dysfunctions syndrome 5 (MMDS5; MIM #617613).[4] Here, we report the same variant in another family with similar clinical and neuroimaging findings. Our findings further confirm this nosological entity.

Materials and Methods

Clinical Report

A 6-month-old male child was the first born to a nonconsanguineous couple by lower segment cesarean section (►**Fig. 1A**). At birth, weight was 2.6 kg (–1 standard deviation [SD]), length was 47 cm (normal), and head circumference was 33 cm (normal). He had no feeding difficulty and the perinatal history was unremarkable. He presented with myoclonic jerks starting at the age of 3 months and developmental delay. On examination at 6 months of age, the head circumference was noted to be 40.5 cm (–2 SD), total length was 66 cm (normal), and weight was 6.5 kg (normal). He had mild spasticity in all four limbs. He had normal deep tendon reflexes. The rest of the systemic examination was unremarkable. Testing for serum amino acids, acylcarnitine profile, and thyroid function showed normal results. Magnetic resonance imaging of the brain at age 6 months showed pachygyria, dilated ventricles, and diffuse white matter abnormalities in the cerebrum, cerebellum, as well as brain stem (►**Fig. 1B**, **C**). Magnetic resonance spectroscopy showed an elevated lactate peak. The patient was recruited for further investigations after obtaining informed consents. This work has been approved by the local Institutional Ethics Committee.

Molecular Analysis

Targeted Sanger sequencing of exon 4 and flanking intronic regions of *ISCA1* was done for the proband and his parents as the clinical and radiographic features suggested possibility of MMDS5 as the likely diagnosis. Further, whole-exome sequencing was done to rule out other pathogenic variants and to analyze the founder effect as described earlier.[4] Variant prioritization and filtering strategy is outlined in ►**Supplementary Table S1** (online only). Autozygosity mapping was done using FILTUS v.1.0.4 (Oslo University Hospital and University of Oslo, Oslo, Norway).[5]

received
December 30, 2017
accepted after revision
February 28, 2018
published online
April 5, 2018

DOI https://doi.org/10.1055/s-0038-1641177.
ISSN 2146-4596.

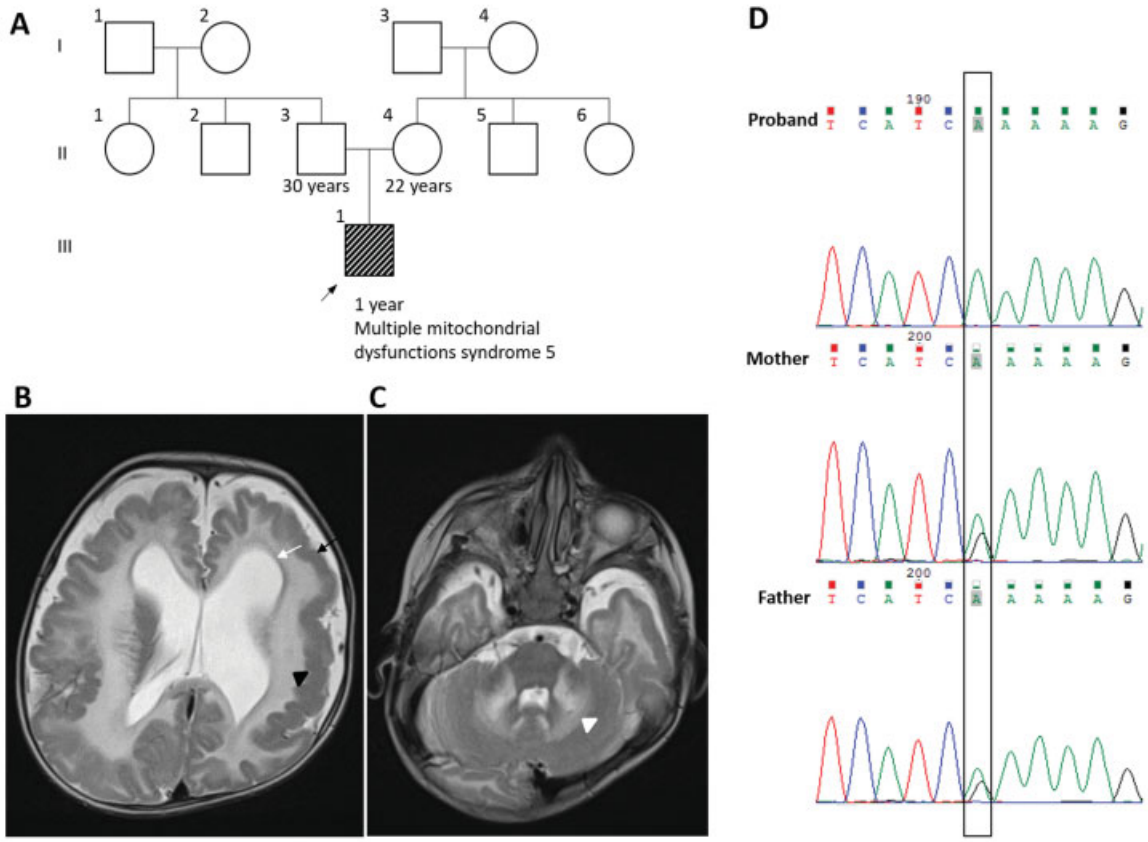

Fig. 1 (**A**) Pedigree of the family. (**B**) T2-weighted magnetic resonance imaging of brain shows pachygyria (black arrow), dilated ventricles (white arrow), and extensive hyperintensities in cerebral deep white matter (black arrowhead). (**C**) Extensive hyperintensities were also seen in deep cerebellar nuclei (white arrowhead) on T2-weighted magnetic resonance imaging of brain. (**D**) Homozygous variant c.259G > A [p.(Glu87Lys)] was observed on targeted Sanger sequencing in patient index, parents were heterozygous carriers.

Results

Targeted Sanger sequencing confirmed homozygous missense variant, c.259G > A [p.(Glu87Lys)] in exon 4 of *ISCA1* (NM_030940.3). Biallelic segregation of the variant was validated in the parents of the proband (►**Fig. 1D**). The variant was observed on integrated genomic viewer with read frequency of 100% from exome data.[6] No other clinically relevant variants were observed in any other gene in the proband's exome. A total of 587 regions of homozygosity were observed from exome sequencing data spanning 311 Mb. *ISCA1* was seen in one of the large regions of homozygosity of 8.25 Mb (Chr9:82006605–90258248). This block was then manually checked for the haplotype reported earlier in patients with the same variant in *ISCA1*.[4] All three families were found to carry a common haplotype around the variant (►**Supplementary Table S2**, online only).

Discussion

Biogenesis of ISCs operate in the mitochondria, cytosol, and nucleus of eukaryotic cells and is imperative for several cellular functions such as respiration, translation, DNA repair, and gene expression regulation.[7,8] In vivo functional studies in mice demonstrate critical roles of *ISCA1* in maturation and maintenance of mitochondrial ISCs of neuronal cells.[9] Recently, we reported a homozygous missense variant c.259G > A [p.(Glu87Lys)] in *ISCA1* associated with MMDS5.[4] We hereby report the third family with the same phenotype and the genotype confirming the association. Clinical features in the proband and the affected individuals reported earlier include early-onset seizures, developmental delay, spasticity, and early demise. Characteristic brain imaging findings of pachygyria, ventriculomegaly, and extensive bilateral white matter abnormalities in cerebral and cerebellar hemispheres and brain stem were noted to be identical in all affected individuals. The detailed clinical characteristics of the previously reported and the present proband are provided in ►**Table 1**.

The marked similarity between the clinical presentation of the proband described here when compared with our previously reported subjects with MMDS5 led us to perform targeted testing for the same genetic variant, c.259G > A [p.(Glu87Lys)] in *ISCA1*. The in silico prediction tools, PolyPhen-2, Sorting Intolerant from Tolerant (SIFT), and MutationTaster, are consistent in predicting the pathogenicity of the said genetic variant.[10–12] The variant is not present in homozygous state in the 1000 Genomes Project, the Exome Aggregation Consortium (ExAC), gnomAD, and in our in-house exome data of 377 unrelated individuals.

Table 1 Clinical findings observed in the proband and families reported earlier

Clinical findings	Present study	Shukla et al, 2017			
		Family 1		Family 2	
	III.1	II.3	II.4	II.1	II.2
Sex	Male	Female	Male	Female	Male
Age at assessment	6 mo	9 mo	8 mo	7 mo	1 y 11 mo
Age at demise	NA	1 y 7 mo	5 y	11 mo	2 y 3 mo
Birth weight (kg)	2.6	3	3.6	2.5	2.7
Weight (kg/SD)	6.5 (normal)	7/–1	NA	6.4/–2	5.8/–6
Length (cm/SD)	66 (normal)	63/–1	NA	NA	76/–5
OFC (cm/SD)	40.5/–2	41/–2	42/–2	42.5/–1	43.5/–6
Seizures	+	+	+	+	+
Onset of seizures	3 mo	NA	4 mo	3 mo	2 mo
Developmental delay	+	+	+	+	+
Milestones achieved	Social smile sometimes		Partial head control		No milestones achieved
Feeding difficulty	–	+	+	+	+
Tone	Spasticity	Spasticity	Spasticity	Spasticity	Spasticity
Deep tendon reflexes	Normal	Exaggerated	Exaggerated	Exaggerated	Exaggerated
Strabismus	–	+	–	–	+
Other clinical findings	–	History of incessant cry, nystagmus	History of incessant cry, tremors in hands	–	–
Pachygyria	+	+	+	NA	–
Cerebral white matter abnormalities	+	+	+	+	+
Cerebellar white matter abnormalities	+	+	+	+	+
Cerebral ventriculomegaly	+	+	+	+[a]	+
MRS findings	Elevated lactate peak	Elevated lactate peak	Elevated lipid-lactate peak	NA	Elevated lipid-lactate peak
EEG findings	NA	Normal	NA	Abnormal	Normal
Ophthalmological findings	NA	Normal VEP and fundus	Stippled pigmentation of fundus	NA	NA
Hearing evaluation	Normal	NA	NA	Impaired	Normal
Blood lactate (mg/dL)	NA	20.7	36	65.8	40.5
CPK (normal: 22–198 U/L)	NA	132	568	NA	42

Abbreviations: +, present; −, absent; CPK, creatine phosphokinase; EEG, electroencephalogram; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; NA, not available; OFC, occipitofrontal circumference; SD, standard deviation; VEP, visual evoked potential.
[a]Observed on computed tomography.

All the patients and families with the reported pathogenic variant are from the same geographical region. Exome sequencing was done to validate the founder region haplotype as observed in the previously reported patients who demonstrated an identical haplotype (G-A-T-G-C-G-A-A-T-T-G-T-T-T-C-G) in all the three families, thus confirming the founder effect.

This report further validates *ISCA1* as a causative gene for MMDS5. It also illustrates that MMDS5 has very characteristic and recognizable clinical and radiological findings. Also, the confirmation of a founder effect is likely to aid in diagnosis of MMDS5 by targeted variant testing for individuals from this geographic region.

Conflict of Interest
None.

Acknowledgments
We thank the patient and his family for participating in the study. We thank the Department of Health Research, Ministry of Health and Family Welfare, Government of India, for funding the project entitled "Clinical and molecular characterization of leukodystrophies in Indian children" (V.25011/379/2015-GIA/HR).

References

1 Cózar-Castellano I, del Valle Machargo M, Trujillo E, et al. hIscA: a protein implicated in the biogenesis of iron-sulfur clusters. Biochim Biophys Acta 2004;1700(02):179–188
2 Song D, Tu Z, Lee FS. Human ISCA1 interacts with IOP1/NARFL and functions in both cytosolic and mitochondrial iron-sulfur protein biogenesis. J Biol Chem 2009;284(51):35297–35307
3 Sheftel AD, Wilbrecht C, Stehling O, et al. The human mitochondrial ISCA1, ISCA2, and IBA57 proteins are required for [4Fe-4S] protein maturation. Mol Biol Cell 2012;23(07):1157–1166
4 Shukla A, Hebbar M, Srivastava A, et al. Homozygous p.(Glu87Lys) variant in ISCA1 is associated with a multiple mitochondrial dysfunctions syndrome. J Hum Genet 2017;62(07):723–727
5 Vigeland MD, Gjøtterud KS, Selmer KK. FILTUS: a desktop GUI for fast and efficient detection of disease-causing variants, including a novel autozygosity detector. Bioinformatics 2016;32(10):1592–1594
6 Thorvaldsdóttir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Brief Bioinform 2013;14(02):178–192
7 Brancaccio D, Gallo A, Mikolajczyk M, et al. Formation of [4Fe-4S] clusters in the mitochondrial iron-sulfur cluster assembly machinery. J Am Chem Soc 2014;136(46):16240–16250
8 Netz DJ, Mascarenhas J, Stehling O, Pierik AJ, Lill R. Maturation of cytosolic and nuclear iron-sulfur proteins. Trends Cell Biol 2014;24(05):303–312
9 Beilschmidt LK, Ollagnier de Choudens S, Fournier M, et al. ISCA1 is essential for mitochondrial Fe_4S_4biogenesis in vivo. Nat Commun 2017;8:15124
10 Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nat Methods 2010;7(08):575–576
11 Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. Nat Methods 2010;7(04):248–249
12 Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 2009;4(07):1073–1081