Encephalopathy due to mutation in mitochondrial fission factor gene

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ABSTRACT

Mitochondrial fission factor (MFF) is a part of the protein complex that promotes mitochondrial and peroxisome fission. Mitochondria and peroxisome fission are complex processes of vital importance for cell growth and survival. Here, we are presenting a case of 5-year-old boy with spastic diplegic cerebral palsy, global developmental delay, and intellectual disability with a history of perinatal asphyxia and encephalopathy. MRI brain was suggestive of symmetrical hyper-intensities in bilateral thalami. Whole-genome sequencing indicates homozygous MFF gene mutation at exon 3 of c.19_20delAGinsTT; p.Ser7Phe variant.

Key words: *Mitochondrial fission factor, Spastic diplegic cerebral palsy, Perinatal asphyxia, Encephalopathy, Mitochondria, Peroxisomes*

Mitochondria are dynamic organelles that continually divide and fuse. Mitochondrial fission facilitates the redistribution of mitochondria in response to local changes in the demand for ATP, whereas mitochondrial fusion is needed to exchange mitochondrial DNA and other components that may become damaged over time [1,2]. The rates of fission and fusion are finely balanced and mutations in some of the key players of this system have been associated with human disease. Dynamin- related protein 1 (DRP1) encoded by the DNM1L gene is the main effector of mitochondrial fission and division of peroxisomes [3]. DRP1 is mainly present in the cytosol and requires proper signals to be recruited to the mitochondria.

Mitochondrial fission factor (MFF) is a nuclear gene encoding a protein that promotes recruitment and association of DRP1 to the mitochondrial surface. Mutations in genes encoding some of these proteins result in the impaired mitochondrial dynamics leading to mitochondrial disorders. The outer membrane protein MFF is presumably the major recruitment factor for DRP1. The knockdown of MFF results in mitochondrial elongation and formation of tubular peroxisomes. Its biallelic mutation has been reported as an extremely rare, autosomal disorder characterized by delayed psychomotor development, severe hypotonia with the inability to walk, microcephaly with variable features, including early-onset seizures, optic atrophy, and peripheral neuropathy.

CASE REPORT

A 5-year-old male child, born to non-consanguineous parents, presented to our outpatient department with delayed development, intellectual disability, balance, and problems in walking with stiffness in both the legs which was more than the arms. Further history from the parents revealed insignificant antenatal events. The patient was born at term with a birth weight of 3.1 kg through vaginal delivery at home attended by a dai (traditional birth attendant). There was no history of difficult labor, meconium stained liquor or failed progress at the time of delivery of the baby. As per mother, the child did not cry immediately after birth and was referred by attending birth attendant to the hospital.

At the hospital, child was diagnosed with perinatal asphyxia and admitted to the NICU. The baby was put on mechanical ventilation for 3 days then weaned off to CPAP for 3 days, and subsequently was given oxygen by nasal prongs for 5–7 days. Oxygen was gradually weaned off, and feeding was started with nasogastric tube along with other supportive care. Cranial sonography showed normal ventricles, brain parenchyma, basal ganglia, and brain stem, without hypoechoic or hyperechoic lesion. The child started to accept breastfeed at 13th day of life and was discharged from the hospital on 14th day with advice for breastfeed, calcium, and supportive care.

As per history given by the patient's mother, she also had growth and developmental delay with intellectual disability in her childhood. No similar complaints were noted in the patient's father and other siblings. The rest of the family history was insignificant for known neurological or genetic disorders. The immunization status of the patient was as per the national immunization schedule.

On general examination, microcephaly was present with no facial dysmorphism or neurocutaneous stigmata. The patient developed a social smile at the age of 6 months, and started recognizing his mother at the age of 12 months. At present,

patient can sit with support, has immature pincer grasp, can feed himself with a spoon with some spilling, has monosyllable speech but does not speak any definitive words, and indicates the desire and toilet needs. According to the Gross Motor Function Classification System (GMFCS), level IV was observed.

Neurological examination revealed patient to be alert, able to track, fix, and follow the object. On cranial nerve examination, divergent squint was present and the rest cranial nerves were normal. On motor examination, he did not have wasting or atrophy of any of the muscle groups, and the tone was increased in all four extremities (more in legs than arms). Deep tendon reflexes were brisk in biceps, triceps, knee, and ankle with bilaterally extensor plantars. Nystagmus, signs of autonomic dysfunction, and meningeal irritation were absent. Dystonic movements of the affected limbs without any prior history of seizures were present. The review of other systems was within normal limits. The ophthalmological and ear examination were also normal.

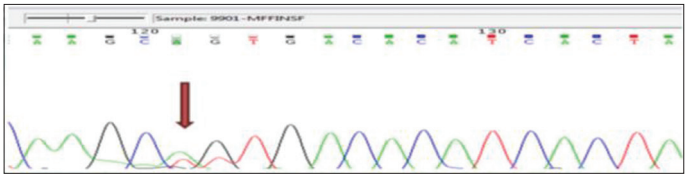

Figure 1: Sanger sequencing evaluation of patient's father

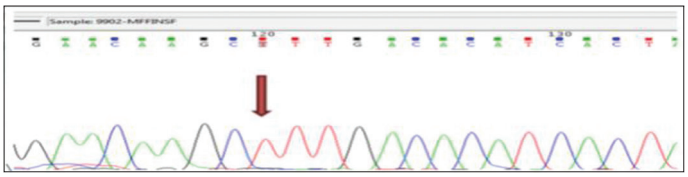

Figure 2: Sanger sequencing evaluation of patient's mother

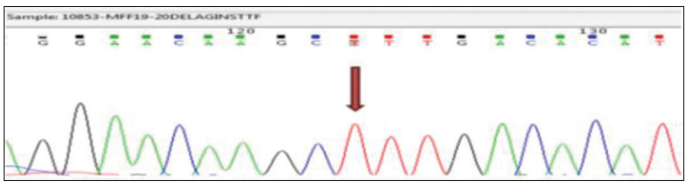

Figure 3: Sanger sequencing evaluation of fetus of patient's mother

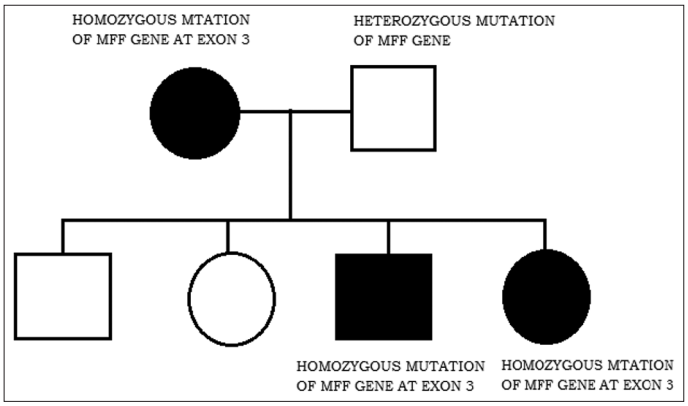

Figure 4: Pedigree of the family

Table 1: Whole-genome sequencing of the patient and Sanger sequencing evaluation of patient's father, mother, and fetus

Sample	Gene	Location	Variant	Zygosity
Patient	MFF	EXON 3	c.19_20delAGinsTT; p.Ser7Phe	Homozygous
Father				Heterozygous
Mother				Homozygous
Fetus				Homozygous

Laboratory investigations such as complete blood count, blood urea, serum creatinine, SGPT, random blood sugar, serum T3, T4, and TSH, serum vitamin D3, and serum vitamin B12 levels were normal except for the increased levels of serum lactate. Very long chain fatty acids (VLCFA), phytanic, and pristanic acids were normal. MRI brain findings were suggestive of symmetrical hyperintensity in bilateral thalami. Cerebellum, brain stem, and pituitary gland were normal. Lateral, 3rd, and 4th ventricles were normal in size, shape, and position.

Due to the neurological involvement, growth and developmental delay with intellectual disability and raised serum lactate levels, the possibility of mitochondrial disorder was considered. To confirm the possibility of a genetic disorder, we advised whole-genome sequencing for the patient. Whole-genome sequencing was performed and homozygous mutation for MFF gene on exon 3 with variant c.19_20delAGinsTT; p.Ser7Phe; and transcript NM_020194.5 was observed. A deletion "AG" and insertion "TT" were detected between nucleotide position "19" and "20" leading to change in amino acid from serine to phenylalanine at codon 7 [4-6].

Sanger sequencing was performed on both the parents. Sanger sequencing evaluation of the patient's father revealed the heterozygous status for the MFF gene on exon 3 with a variant c.19_20delAGinsTT; p.Ser7Phe. Sanger sequencing evaluation of the patient's mother revealed the homozygous mutation for the MFF gene on exon 3 with variant c.19_20delAGinsTT; p.Ser7Phe. The variation was confirmed by sequencing with both forward and reverse primers. MFF gene-targeted mutation analysis was performed by the polymerase chain reaction, followed by automated DNA sequencing. The data obtained were subsequently analyzed for the presence or absence of mutation in the hot spot region.

We started treating the patient with intensive physiotherapy, occupational therapy, speech therapy, and supportive care. The patient was called for regular follow-up and neuro-ophthalmological evaluation. The patient's mother was 28 weeks pregnant with the next child at the time of the presentation of their 5-year-old to us. We advised her for Sanger sequencing evaluation of the fetus for MFF mutation which revealed homozygous mutation for the MFF gene on exon 3 with variant c.19_20delAGinsTT; p.Ser7Phe. After consultation with neurologist, obstetrician, and genetic team, we advised her to continue the pregnancy as there was a variable expression of MFF gene mutation in the patient and his mother, thus the clinical spectrum of the fetus with homozygous mutation of the similar gene cannot be ascertained. Furthermore, the mother was 28 weeks pregnant and medical termination of pregnancy at this stage was not possible.

Table 2: Genetic findings, biochemical, and phenotypic features of MFF mutation

Patient	P1 (Shamseldin *et al.*, 2012)	P1 (Koch *et al.*, 2016)	P2 (Koch *et al.*, 2016)	P3 (Koch *et al.*, 2016)	P4 (Nasca *et al.*, 2018)	Present report
Consanguinity	+	–	+	+	+	–
Origin	Saudi Arabian	Austrian	Turkish	Turkish	Italian	Indian
MFF variants	c.(190C>T);(190C>T) p.(Gln64*);(Gln64*)	c.(184dup);(892C>T) p.(Leu62Profs*13);(Arg298*)	c.(453_454del); (453_454del) p.(Glu153Alafs*5); (Glu153Alafs*5)	c.(453_454del); (453_454del) p.(Glu153Alafs*5); (Glu153Alafs*5)	c.(892C>T);(892C>T) p.(Arg298*);(Arg298*)	c.19_20delAGinsTT; p.Ser7Phe
Phenotypic features						
Sex	**Male**	**Male**	**Male**	**Male**	**Male**	**Male**
Age at onset	First year	4 months	4 months	11 months	9 months	5 months
Microcephaly	+	+	+	+	+	+
Regression/loss of skills	n.c.	+	+	+	+	+
Severe developmental delay/ intellectual disability	+	+	+	+	+	+
Spasticity	+	+	+	+	+	+
Growth retardation	n.c.	–	–	+	+	+
West syndrome	–	+	+	–	-	_
Other epileptic and developmental encephalopathies	+	–	–	–	+	+
External ophthalmoparesis	–	+	+	+	+	_
Optic atrophy	+	+	–(9 months)	+	+	_
Peripheral neuropathy	n.c.	+	n.d.	+	–	_
Movement disorder	–	–	–	–	Upper limb dystonia	Dystonia
Brain MRI lesions	Pallidum	Putamen, pallidum, caudate, mesencephalon, dentate nucleus optic radiation, cerebellar atrophy	Pallidum, mesencephalon, thalamus	Putamen, pallidum, caudate, thalamus, mesencephalon, cerebellar atrophy	Putamen, caudate, mesencephalon, cerebellar atrophy	Symmetrical hyper-intensity in bilateral thalamic
Increased brain lactate (liquor/MRS)	n.c.	–	n.d.	n.d.	+	n.d.
Laboratory findings						
Increased plasma lactate	–	+	+	+	+	+
MRC in muscle	n.d.	Normal	n.d.	Normal	Slight increased SDH	n.d.
MRC fibroblasts	Normal	n.d.	n.d.	n.d.	Normal	N.d.
VLCFA increased	–	–	–	n.d.	–	_
Plasmalogens abnormal	n.d.	–	n.d.	n.d.	–	_
Pristanic acid abnormal	n.d.	–	n.d.	n.d.	–	_
Phytanic acid abnormal	n.d.	–	n.d.	n.d.	–	_
Bile acid metabolites abnormal	n.d.	–	n.d.	n.d.	–	_
Bone abnormalities	n.d.	–	n.d.	n.d.	Osteoporosis	_
Kidney cysts	n.d.	–	n.d.	n.d.	–	_

+: Present, –: absent, n. c.: not commented, n. d.: not determined

DISCUSSION

The MFF gene contains nine coding exons. Exon 1, 5, 6, and 7 are subject to alternative splicing and exon 2 contains an alternative translational initiation codon. Northern blot analysis detected high MFF expression in the heart, kidney, liver, brain, muscles, and stomach. Knockdown and overexpression studies revealed that MFF affected mitochondrial morphology in a way indistinguishable from that of DNM1L, a MFF [7].

MFF mutations are extremely rare. Only five patients have been described until now. The first cases were two brothers reported by Shamseldin *et al*. [8]. These two patients carried a homozygous nonsense mutation (p.Gln64*); the marked difference in the mitochondrial and peroxisomal morphology was observed in patient fibroblasts, indicating that MFF defect was the cause of the disease (details in Table 2). A singleton case and two additional siblings with MFF mutations were later described by Koch *et al*. [9]. The main features of these patients were early onset epileptic encephalopathy, acquired microcephaly, optic atrophy, external ophthalmoparesis, and neuroradiological findings evocative of Leigh syndrome, all together suggesting mitochondrial dysfunction.

Another case report by Nasca *et al*. [10] described a patient with neurological phenotype dominated by spasticity and dystonic postures of the upper limbs. Our patient's phenotypic features were quite similar, suggesting mitochondrial and peroxisomal involvement but laboratory analysis is less informative as serum lactate level was found to be elevated with other biochemical values in the control range. However, neither mitochondrial respiratory chain nor biomarkers of peroxisomal dysfunction was observed.

Similar results were obtained in the other MFF patients, with plasma lactate only occasionally increased and other biochemical values in the control range. The absence of overt mitochondrial and peroxisomal biochemical impairment is in line with what is reported in other disorders affecting the dynamics of intracellular organelles (e.g., DNM1L mutations).

The mitochondrial disease mainly affects neurons because they are the most energy-consuming cell types. However, in addition to bioenergetics defects, evidences are indicating a key role of impaired mitochondrial dynamics in neurodegenerative diseases, in particularly mitochondrial transport along axons. Impaired mitochondrial fission causes the formation of larger organelles, deleterious effect on mitochondrial mobility, synaptic homeostasis, and mitophagy [11-14].

CONCLUSION

Our findings establish MFF loss of function as a cause of disturbed mitochondrial and peroxisomal dynamics associated with early-onset encephalopathy. Since most cases of perinatal asphyxia are due to genetic and metabolic cause we suggest that such cases with clinical features suggestive of mitochondrial disorder warrant genetic testing for MFF mutations even if laboratory findings are not indicative of mitochondrial or peroxisomal dysfunction.

REFERENCES

1. Okamoto K, Shaw JM. Mitochondrial morphology and dynamics in yeast and multicellular eukaryotes. Annu Rev Genet 2005;39:503-36.
2. Chan DC. Mitochondrial fusion and fission in mammals. Annu Rev Cell Dev Biol 2006;22:79-99.
3. Li X, Gould SJ. The dynamin-like GTPase DLP1 is essential for peroxisome division and is recruited to peroxisomes in part by PEX11. J Biol Chem 2003;278:17012-20.
4. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, *et al*. 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 2015;17:405-24.
5. Landrum MJ, Lee JM, Benson M, Brown G, Chao C, Chitipiralla S, *et al*. ClinVar: Public archive of interpretations of clinically relevant variants. Nucleic Acids Res 2016;44:D862-8.
6. Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H, *et al*. The NHGRI GWAS catalog, a curated resource of SNP-trait associations. Nucleic Acids Res 2014;42:D1001-6.
7. Gandre-Babbe S, van der Bliek AM. The novel tail-anchored membrane protein MFF controls mitochondrial and peroxisomal fission in mammalian cells. Mol Biol Cell 2008;19:2402-12.
8. Shamseldin HE, Alshammari M, Al-Sheddi T, Salih MA, Alkhalidi H, Kentab A, *et al*. Genomic analysis of mitochondrial diseases in a consanguineous
9. Koch J, Feichtinger RG, Freisinger P, Pies M, Schrödl F, IusoA. Disturbed mitochondrial and peroxisomal dynamics due to loss of MFF causes Leigh-like encephalopathy, optic atrophy and peripheral neuropathy. J Med Genet 2016;53:270-8.
10. Nasca A, Nardecchia F, Commone A, Semeraro M, Legati A, Garavaglia B, *et al*. Clinical and biochemical features in a patient with *mitochondrial fission factor* gene alteration. Front Genet 2018;9:625.
11. Sheng ZH, Cai Q. Mitochondrial transport in neurons: Impact on synaptic homeostasis and neurodegeneration. Nat Rev Neurosci 2012;13:77-93.
12. Itoh K, Nakamura K, Iijima M, Sesaki H. Mitochondrial dynamics in neurodegeneration. Trends Cell Biol 2013;23:64-71.
13. Burté F, Carelli V, Chinnery PF, Yu-Wai-Man P. Disturbed mitochondrial dynamics and neurodegenerative disorders. Nat Rev Neurol 2015;11:11-24.
14. Pellegrino MW, Haynes CM. Mitophagy and the mitochondrial unfolded protein response in neurodegeneration and bacterial infection. BMC Biol 2015;13:22.

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