EUROPEAN JOURNAL OF PAEDIATRIC NEUROLOGY 23 (2019) 537–540

Official Journal of the European Paediatric Neurology Society

Case study

# Expanding phenotype of mitochondrial depletion syndrome in association with *TWNK* mutations

*Biayna Sukhudyan* [a,*], *Ani Gevorgyan* [a], *Ashot Sarkissian* [b], *Eugen Boltshauser* [c]

[a] *Department of Pediatric Neurology, Arabkir Medical Center, 30 Mamikonyants str., 0014 Yerevan, Armenia*
[b] *Department of Pediatrics, Yerevan State Medical University, 2 Koryun str., 0025 Yerevan, Armenia*
[c] *Department of Pediatric Neurology, University Children's Hospital, 75, Steinwiesstrasse, 8032 Zurich, Switzerland*

ARTICLE INFO

*Article history:*
Received 3 December 2018
Received in revised form
25 January 2019
Accepted 7 February 2019

*Keywords:*
Mitochondrial diseases
Mitochondrial depletion syndromes
*C10orf2* mutations
Renal tubulopathy
*TWNK* mutations

ABSTRACT

Mitochondrial DNA depletion syndromes (MDS) are a group of clinically and genetically heterogeneous autosomal recessive disorders characterized by a reduction of mtDNA. We report two siblings of Armenian origin with early onset neurodegenerative disease characterized by encephalopathy, severe hypotonia, facial dyskinetic movements, abnormal eye movements, severe failure to thrive, and abnormal renal and hepatic function. Sanger sequencing confirmed two variants in the *C10orf2* gene (*TWNK*) and indicated a diagnosis of MDS. Our recent observation confirms that nephrocalcinosis and proximal tubulopathy can be a part of a clinical picture of MDS associated with *TWNK* mutations and document peculiar ocular and orobuccolingual dyskinesias. Wrist myoclonia and tongue tremor were new clinical features in our patients. We suggest that the above-mentioned clinical constellation could potentially provide the basis for the diagnosis of MDS.



## 1. Introduction

MtDNA depletion syndromes (MDS) are a genetically and clinically heterogeneous group of autosomal recessive disorders characterized by low mtDNA levels in specific tissues.[1] Mitochondrial diseases are often relentlessly progressive with high morbidity and mortality. Clinical diversity and genetic heterogeneity of mitochondrial diseases make diagnosis difficult. Attempts have been made to create diagnostic algorithms considering clinical history and examination, biochemical parameters, neuroimaging and neurophysiology, and suspected mode of inheritance for determination of clinical phenotype to allow targeted testing for specific genes.[2] Hence, we suggest that expansion of the phenotype may potentially support clinically guided gene testing. Eighteen entries for mtDNA depletion syndromes are listed in OMIM. MDS can be divided into four clinical presentations: hepatocerebral, myopathic, encephalomyopathic and neurogastrointestinal. These syndromes are due to defects in

* *Corresponding author.*
E-mail addresses: biayna_sukh@yahoo.com (B. Sukhudyan), aniggr@mail.ru (A. Gevorgyan), ash_sarkissian@yahoo.com (A. Sarkissian), eugen.boltshauser@bluewin.ch (E. Boltshauser).
https://doi.org/10.1016/j.ejpn.2019.02.002
1090-3798/

mtDNA maintenance caused by mutations in nuclear genes involved in nucleotide synthesis or mtDNA replication.

*TWNK* codes the motor protein TWINKLE that acts as a helicase at the replication fork. Dominant mutations in this gene are known to cause progressive external ophthalmoplegia. *TWNK* mutations (dominant/recessive) are now being reported as the genetic basis for infantile onset sensory ataxia with or without epilepsy, as well as sensory ataxia associated with dysarthria, and hepatocerebral forms of mtDNA depletion disorders in infancy and adulthood[3] and Perrault syndrome.

We report on two siblings from non-consanguineous parents of Armenian origin with early onset symptoms of severe encephalopathy, hypotonia, facial dyskinetic movements, abnormal visual behavior, severe failure to thrive, and abnormal renal and hepatic function.

## 2. Case study

This retrospective case report did not require the approval of the local Institutional Review Board; however, the parents consented to this publication.

Patient 1, a girl, was born at 40 weeks after first uncomplicated pregnancy with normal birth weight and APGAR scores. Early development was normal until the age of 3 months, when continuous conjugated and non-conjugated multi-directional and rotatory ocular deviations of different speed, mostly down-beating movements accompanied by widening of the palpebral fissure, and sun set phenomena started (video 1). Early occurrence of tongue tremor prompted testing for SMA with negative result (video 2). Full-blown manifestation of the disease with a severe muscle weakness was noticed at 6 months. At the age of 7 months, she lost head control, developed severe muscle hypotonia, hyporeflexia and poor visual fixation. Liver function tests (LFT) were elevated (ALT - 120 U/L, AST - 70 U/L) (N < 50 U/L). CK was normal. Coagulation tests were in normal range. At the age of 12 months the girl presented with secondary microcephaly, severe failure to thrive, inability to suck and swallow, multiple seizures, abnormal eye movements in the absence of fixation, hoarse voice, tongue tremor and wrist myoclonia (video 3 and 4). The patient had severe muscle weakness, areflexia and very poor spontaneous movements. Hepatomegaly was detected at 3 cm below costal margin. Renal ultrasound (US) was compatible with nephrocalcinosis. Fundoscopy was normal. Nerve conduction velocities were below norm values for the age, and action potentials were of markedly reduced amplitude, suggesting a mixed demyelinating-axonal polyneuropathy. EEG did not show epileptiform activity during constant myoclonic jerks in wrists. Audiometry showed decreased hearing from the right. Biochemical tests showed low concentration of phosphate 0.7 mmol/L (1.2–2.1 mmol/L), ionized calcium 1.08 mmol/L (1.22–1.32 mmol/L) and bicarbonates 13,2 mmol/L (18–25 mmol/L). Renal reabsorption of calcium and sodium was 99% (N), whereas renal reabsorption of phosphate was 68% (normal value is >75%). Glucosuria was detected once. This constellation suggested a proximal tubulopathy. LFT continued to be elevated (ALT - 85 U/L, AST - 159 U/L). We did not observe cholestatic origin of liver affection. In addition, iron-deficient anemia was detected. Lactate was 4 mmol/L only once (0.5–2.2 mmol/L).

Supplementary video related to this article can be found at https://doi.org/10.1016/j.ejpn.2019.02.002.

Testing of amino acids in plasma and urine as well as urine organic acid assays showed no abnormalities. Mitochondrial DNA depletion syndrome was considered in view of this particular constellation of multisystem involvement but genetic testing was not possible for financial restraints. The child died at the age of 15 months from pneumonia. Postmortem was not performed.

Patient 2, a boy, was born after the second non-complicated term pregnancy with normal birth weight and APGAR scores. Early development was normal until 7 months. First symptom was muscle hypotonia. A month later there was an acute deterioration in the patient's neurologic state. Intercurrent illness triggered the appearance of continuous orobuccolingual dyskinesias that interfered with normal feeding of the child. During clinical examination, the child showed involuntary mouth opening accompanied by repetitive protrusion of his tongue. In addition, there was an abnormal opening of the palpebral fissure accompanied by periodic convergent strabismus and eye sunset phenomena (video 5). In a short time, the child lost head control and became extremely hypotonic with diminished tendon reflexes. Deviation of the spinal column from the central line and junctional kyphosis in the thoracolumbar area were seen on radiographs early in the course of disease. Nerve conduction velocities and amplitudes of action potentials were borderline low. Elevated LFT (up to 1000 U/L) were seen. Hepatomegaly was not observed. Coagulation tests showed normal results. With time, the child developed secondary microcephaly, and LFT decreased but never normalized. Along with constant wide eye opening and eye sunset phenomena the child was had normal visual fixation and tracking, and a normal social smile. The child became very irritable with hoarse cry and very marked breath holding spells. His CK level was normal; there was no biochemical evidence for renal tubulopathy. Audiometry and fundoscopy were unrevealing. Brain MRI showed non-specific findings in both siblings. Shortly before death, he was found to be severely hypotonic and unable to move. Severe muscle wasting, contractures and progressive scoliosis were seen. At the age of 30 months, he went into epileptic status and deceased from severe metabolic acidosis and cardiac arrest. Repeated renal US examinations did not reveal signs of nephrocalcinosis during the whole course of the disease. Noteworthy, that last kidney US shortly before death showed hyperintense parenchyma. The liver was also enlarged.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.ejpn.2019.02.002.

Next Generation Sequencing performed in patient 2 identified two variants in exon 1 of *TWNK* gene, confirmed by Sanger sequencing. A previously described heterozygous variant at genomic position chr10:102749163A>G, NM_021830.4:p.Asn399Ser[4] and a variant at genomic position chr:102748821G>A, NM_021830.4:p.Arg285Gln were identified. The pathogenicity of variants was predicted by *in*

*silico* analysis with the aid of different bioinformatics tools (Polyphen2-HDIV, Polyphen2-HVAR, MutationTaster, PROVEAN, SIFT, LRT). The first variant was classified as probably pathogenic, whereas the second variant was considered as variant of unknown significance. The mother was found to be a carrier of c.G854A:p.R285Q, and the father carries a c.A11A96G:p.N399S mutation. Homozygous and compound heterozygous mutations in *TWNK* have been proven to cause mitochondrial depletion syndrome type 7 (hepatocerebral type) (OMIM:271245) and Perrault syndrome, type 5 (OMIM:616138)[4].

## 3. Discussion

We report autosomal-recessive compound heterozygous mutations of the mitochondrial replicative helicase Twinkle in early onset encephalopathy with liver and renal involvement. Before 2007, the only known recessive phenotype was a disease of Finnish heritage, infantile onset spinocerebellar ataxia (IOSCA).[5] Hakonen et al. expanded the phenotypic spectrum of *TWNK* mutations to severe early encephalopathies with liver involvement. Signs of motor neuropathy and myopathy were discovered for the first time in IOSCA patients with *TWNK* mutations in 2013.[6] These results suggest that the clinical spectrum caused by *TWNK* mutations may be more variable than previously reported.

### 3.1. *Hepatic involvement*

Liver dysfunction in mitochondrial diseases is usually progressive, evolving from microvesicular steatosis with bile duct proliferation into cirrhosis, marked cholestasis, diffuse hemosiderosis.[6] More recently, an Alpers-like clinical phenotype has also been associated with recessive mutations in gene coding helicase Twinkle.[3] In addition, depletion of mtDNA has been reported in the liver. We are missing this information since none of our patients underwent autopsy. In our patients, we observed consistent elevation of LFTs but neither showed signs of cholestatic disease and increased level of bilirubin.

### 3.2. *Ocular and facial dyskinesia*

Although PEO is well known in dominant mutations of *TWNK*, Sarzi et al. firstly reported ophthalmoparesis in autosomal recessive mutations of *TWNK*. In none of our patients did we see ophthalmoplegia. Instead, both patients showed early signs of ocular dyskinesia. The pattern of ocular movements was very similar in both of them (see video 3 and 4). Abnormal eye movements are also mentioned in 2 of 3 patients carrying homozygous mutation in *TWNK* reported by Sarzi et al., 2007.[7] Two patients with compound heterozygosity for *TWNK* mutations also had peculiar abnormal ocular movements. In one of them jerky pursuit eye movements, momentary upward eye movements and nystagmus were described.[3] Patient 2 also developed orobuccolingual dyskinetic movements. These movements in early onset spinocerebellar ataxia were reported in homozygous missense mutations in *TWNK*.[6]

### 3.3. *New clinical findings*

Constant tongue tremor together with generalized muscle hypotonia in the first patient early in the course of the disease prompted exclusion of SMA type 1 (video 1). Continuous bilateral wrist myoclonia, that disappeared in sleep, in view of negative EEG is an additional new finding observed in patient 1 (video 2).

### 3.4. *Renal involvement*

Wide spectrum of nephropathies has been described in mitochondrial disorders. It includes proximal and distal renal tubular disorders, nephrotic syndrome, urolithiasis, nephrocalcinosis, renal cystic diseases, etc. Mitochondrial depletion syndromes are among those with a higher frequency of renal involvement independent of the genetic background. Recently, a renal phenotype (proximal tubular dysfunction and nephrocalcinosis) has been described in a series of patients with *TWNK* mutations.[2] The same findings were seen in our first patient. Remarkably similar mutations caused renal tubulopathy in the first but not in the second patient; intrafamilial variability is commonly observed in affected siblings. Precise mechanisms leading to proximal dysfunction is not completely understood. Tubular granular swollen cells seem to be a typical finding in mitochondrial diseases with renal involvement. Thus, some authors recommend renal biopsy in all these cases.

## 4. Conclusion

Mitochondrial DNA depletion syndromes remain a rare and poorly recognized clinical entity. Suspicion of MDS should be raised in neurodegenerative diseases associated with involvement of different organs and systems. Increasing availability of next generation sequencing may lead to speedy diagnosis. However, access to advanced genetic testing is still limited in resource-poor countries (as Armenia). Thus, careful clinical characterization of these syndromes is necessary to guide clinicians through the process of diagnosis.

Although there is no cure for MDS, a definite diagnosis may bring a psychological relief for the parents seeking possible treatment for their child.

## Conflict of interest

None.

## Acknowledgments

We thank the parents for the permission for publication. We are grateful to genetic laboratory team “GENOMED” in Moscow, Russia, and metabolic laboratory team in Children's University Hospital, Zurich, Switzerland.

## REFERENCES


1. Martın-Hernandez E, Garcıa-Silva MT, Quijada-Fraile P, Rodrıguez-Garcıa ME, Rivera H, Hernandez-Laın A, et al. Myopathic mtDNA depletion syndrome due to mutation in TK2 gene. *Pediatr Dev Pathol* 2017;**20**(5):416–20.
2. Gorman GS, Chinnery PF, DiMauro S, Hirano M, Koga Y, et al. Mitochondrial diseases. *Nat Rev Dis Primers* 2016;**2**:16080.
3. Hakonen AH, Isohanni P, Paetau A, Herva R, Suomalainen A, Lonnqvist T. Recessive Twinkle mutations in early onset encephalopathy with mtDNA depletion. *Brain* 2007;**130**:3032–40.
4. Demain LA, Urquhart JE, O'Sullivan J, Williams SG, Bhaskar SS, Jenkinson EM, Lourenco CM, Heiberg A, Pearce SH, Shalev SA, Yue WW, Mackinnon S, Munro KJ, Newbury-Ecob R, Becker K, Kim MJ, O' Keefe RT, Newman WG. Expanding the genotypic spectrum of Perrault syndrome. *Clin Genet* 2017;**91**(2):302–12.
5. Lonnqvist T, Paetau A, Valanne H, Pihko H. Recessive twinkle mutations cause severe epileptic encephalopathy. *Brain* 2009;**132**:1553–62.
6. Dündar H, Ozgül RK, Yalnizoglu D, Erdem S, Oguz KK, Tuncel D, et al. Identification of a novel Twinkle mutation in a family with infantile onset spinocerebellar ataxia by whole exome sequencing. *Pediatr Neurol* 2012;**46**:172–7.
7. Sarzi E, Goffart S, Serre V, Chrétien D, Slama A, Munnich A, Spelbrink JN, Rötig A. Twinkle helicase (PEO1) gene mutation causes mitochondrial DNA depletion. *Ann Neurol* 2007;**62**(6):579–87.