Clinical Observations

Twinkle-Associated Mitochondrial DNA Depletion

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ABSTRACT

BACKGROUND: Autosomal recessive mutations in the nuclear Twinkle (C10orf2) gene cause a mitochondrial DNA depletion syndrome (MDS) characterized by early onset hepatoencephalopathy.
METHODS: We report a severe, early onset encephalopathy and multisystem failure case caused by novel recessive Twinkle gene mutations. Patient clinical, laboratory, and pathological features are reported and Twinkle-associated MDS literature reviewed.
RESULTS: Typical presentation includes symptom onset before age six months, failure to thrive, psychomotor regression, epileptic encephalopathy, sensory axonal neuropathy, cholestatic liver dysfunction, and occasionally, renal tubulopathy, movement disorders, and ophthalmoplegia. Death is typical before age four years.
CONCLUSIONS: In the differential diagnosis of early onset encephalopathy and multisystem failure, MDS should be considered.

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Introduction

Mitochondrial depletion syndromes (MDS) are comprised of autosomal recessive disorders caused by mutations in nuclear genes encoding for key enzymes involved in mitochondrial DNA (mtDNA) replication and/or maintenance. These mutations result in decreased mtDNA copy number, leading to impaired energy production in various tissues.

The *C10orf2* gene, also called *PEO1* or Twinkle, encodes the mtDNA replicative Twinkle helicase—important for mtDNA replication. Dominant mutations in the Twinkle gene can cause multiple mtDNA deletions and result in chronic progressive external ophthalmoplegia or mitochondrial myopathy. Recessive Twinkle mutations cause mtDNA depletion.[1]

MDS are phenotypically heterogeneous and manifest clinically as early onset encephalopathy, hepatoencephalopathy, encephalomyopathy, infantile onset spinocerebellar ataxia, or Perrault syndrome.[2,3] The hepatocerebral form of MDS is associated with mutations in *Twinkle, POLG1, DGUOK*, and *MPV17*. MDS associated with *POLG1* is known as Alpers-Huttenlocher (Alpers) syndrome.

We describe a child with severe, early onset encephalopathy and multisystem failure caused by recessive Twinkle gene mutations that have never been documented before. We also review the literature on *Twinkle*-associated MDS.

Patient description

This girl was born vaginally at 38+5 weeks gestation to a 32-year-old G4P3 mother, on venlafaxine and bupropion throughout pregnancy, and a healthy 33-year-old father. The parents are Caucasian and non-consanguineous.

Declarations of interest: None.
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There were four healthy siblings. Intrauterine growth restriction was noted at 32 weeks gestation. Birth weight was 2.63 kg (5th percentile). She had poor oromotor coordination from birth, requiring gastrostomy tube feeding by age four months.

At two months of age, weight, length, and head circumference were all less than the third percentile. Examination of extraocular movements was suggestive of internuclear ophthalmoplegia. Visual fixation was inconsistent. Severe hypotonia with significant head lag was present. Deep tendon reflexes were absent. Episodes of nonepileptic choreiform tongue movements were observed. A prolonged generalized seizure occurred in the context of hyponatremia (Na 117 mmol/L).

Initial laboratory investigations at age two months revealed a mild raised anion gap metabolic acidosis. By age three months, serum lactate was mildly elevated to 5 mmol/L (normal < 2.8). Urine amino acids revealed a generalized aminoaciduria. Plasma amino acids, ammonia, urine organic acids, plasma acylcarnitine profile, transferrin isoelectric focusing, and very long chain fatty acids were normal. Creatine kinase was normal. Initial liver function testing revealed mild elevations in alanine aminotransferase (ALT) 46 U/L (normal ≤ 33), aspartate aminotransferase (AST) 61 U/L (normal ≤ 32), gamma-glutamyltransferase (GGT) 467 U/L (normal ≤ 204), and lactate dehydrogenase 535 U/L (normal < 300). Serum alpha-fetoprotein level was elevated at age 3.5 months (488 ug/L; normal ≤ 5).

The presence of proteinuria, elevated urine/microalbumin/creatinine ratio (167 mg/mmol creatinine; normal 0 to 2.8), elevated urine pH (>9.0), and ongoing need for oral sodium and bicarbonate supplementation were suggestive of a tubular nephropathy.

Abdominal ultrasound revealed mild splenomegaly, and chest x-ray showed thymic hypoplasia. Echocardiogram, electrocardiogram, brain magnetic resonance imaging (MRI) were normal initially. Nerve conduction studies (motor: tibial and peroneal, sensory; median, radial, ulnar, superficial peroneal, and sural) showed absent sensory responses and normal motor responses. Repetitive nerve stimulation was normal. Needle electromyography showed myopathic units. Electroencephalogram revealed bilateral, independent temporal interictal epileptiform discharges.

By age four months, she developed stridor requiring supraglottoplasty for laryngomalacia; hypertension, treated with amlodipine; bilateral asymmetric ptosis, not responsive to pyridostigmine trial; and refractory status epilepticus requiring pentobarbital infusion. She developed severe lactic acidosis, refractory to sodium bicarbonate boluses. On the last day of life, serum lactate was persistently elevated above 15 mmol/L. Cerebrospinal fluid (CSF) revealed elevated lactate 45 mmol/L (normal 0.62-2.10), pyruvate 0.25 mmol/L (normal 0.06-0.22), and lactate/pyruvate ratio 182 (normal 10-25). A mild increase in concentration of most amino acids was noted in the CSF, which can be secondarily increased in all conditions with lactic acidosis and hyperammonemia. Liver function testing revealed marked elevations in ALT 209 U/L, AST 306 U/L, ALP 605 U/L (normal ≤ 449), GGT 874 U/L, ammonia 90 umol/L (normal 11-48), and INR 3.4 (normal 0.9-1.1), as well as low Klaus Fibrinogen 0.85 g/L (normal 2-4) and albumin 19 g/L (normal 38-54). Repeat

brain MRI at four months demonstrated interval development of mild cerebral atrophy. MR spectroscopy was normal. Following discussion with family, care was withdrawn and the patient passed away at age four months.

Autopsy revealed severe microvesicular steatosis of the liver tissue with ascites and bilateral pulmonary edema. Muscle histology demonstrated type 1 fiber predominance, with significant size variability in the muscle fibers and increases in sarcoplasmic lipid droplets and internal nuclei. Electron microscopy showed structurally abnormal mitochondria, with marked vacuolation, disarray of the cristae, and a close association between abnormal mitochondria and the lipid droplets. There was slight evidence of fiber necrosis and inflammatory infiltrate. Ragged red and blue fibers were not evident through Gomori trichrome staining and NADH-TR, respectively. There were a few scattered cox-negative fibers. Numerous fibers indicated an increase in oil red O staining. Respiratory chain enzyme studies revealed decreased activity of all mitochondrial respiratory chain complexes. Central nervous system neuropathological examination revealed widespread "metabolic" encephalopathic changes, characterized by neuronal loss, secondary gliosis, focal mineralization of thalamic neurons, and astrocytic changes reminiscent of Alzheimer type II cells.

Chromosomal microarray was performed initially with normal results. Subsequently, a comprehensive next generation sequencing panel of 183 genes associated with muscular dystrophy and myopathy revealed two heterozygous novel variants in the *C10orf2* gene. The first variant, c.853C>T, was reportedly the type expected to be pathogenic and results in change of an arginine residue to a stop codon at amino acid position 285 (p.Arg285Ter), predicted to cause premature truncation of the protein. The second variant, c1592+4A>G is an intronic splice site variant, resulting in change from an adenine to a guanine residue located at c.1592+4 and was predicted to alter the exon 3 donor site with potential to alter splicing. The data were insufficient for definitive classification and the variant classified as of unknown significance. Sanger sequencing confirmed the results. Parental testing revealed the p.R285* variant was paternally and the c.1592+4A>G variant maternally inherited.

Discussion

We describe the clinical, laboratory, pathologic and molecular features of a patient with two novel heterozygous mutations in the *Twinkle* gene, presenting with early onset encephalopathy, status epilepticus, and liver failure, resulting in death at age four months.

Only ten patients have been previously reported with MDS caused by autosomal recessive *Twinkle* mutations, with survival ranging from three months to 4.5 years (Table).[4–8]

Commonly reported clinical features of failure to thrive, severe hypotonia, seizures, and abnormal eye movements were represented in our patient. Dyskinesias[5,8] and ataxia[5] have been reported in a subset of patients. These features presented after a variable period of normal psychomotor development, with symptom onset before age six months in all reported patients. Until

Table.
Comparison of Clinical Features in Patient with Twinkle-Associated Mitochondrial DNA Depletion Syndrome

Case	Symptom Onset	Survival	Neurological Features	Non-Neurological Features	MRI Brain	Electrophysiology	Mutation(s)	Ref
1	Neonatal	3 years	Neonatal: Axial hypotonia/ peripheral hypertonia, developmental delay, muscle atrophy (with elevated CK) 10 months: seizures, areflexia	Neonatal: mild hepatic dysfunction, lactic acidosis.	6 months: subdural collection, mild ventricular dilatation	N/A	Homozygous c.1370C>T (p.Thr457Ile) (first cousin of cases 2 and 3)	[3]
2	Not reported	2 years	Axial hypotonia, seizures, peripheral sensory neuropathy, abnormal eye movements.	Failure to thrive, mild liver insufficiency, elevated lactate.	N/A	N/A	Homozygous c.1370C>T (p.Thr457Ile) (sibling of case 3)	[3]
3	Not reported	2 years	Axial hypotonia, seizures, peripheral sensory neuropathy, abnormal eye movements.	Failure to thrive, mild liver insufficiency, elevated lactate.	N/A	N/A	Homozygous c.1370C>T (p.Thr457Ile) (sibling of case 2)	[3]
4	6 months	4.5 years	6 months: Abnormal eye movements, 8 months: Hypotonia, psychomotor regression 13 months: Poor visual fixation, ophthalmoparesis, areflexia 18 months: Hearing impairment 3.5 years: Ataxia, distal amyotrophy, extensor plantar responses 4.5 years: Epilepsia partialis continua progressing to status epilepticus	8 months: Feeding intolerance 12 months: Hepatic dysfunction (transaminitis, elevated AFP and LD), elevated lactate.	3.5 years: T2-hyperintensities around 4th ventricle, superior cerebellar peduncle, and dentate nuclei; cerebellar atrophy	EMG (30 months): Absent sensory responses Auditory evoked potentials (18 months): Abnormal	Compound heterozygous c.1523A>G (p. Tyr508Cys) and c.952G>A (p. Ala318Thr) (sibling of case 5)	[4]
5	5 months	Age 4 years at time of report (2012)	5 months: Abnormal eye movements. 6 months: Hypotonia, poor visual fixation, ataxia (suspected), areflexia, athetosis 11 months: Dysphagia 25 months: Myoclonus and epilepsia partialis continua progressing to refractory generalized convulsive status epilepticus 4 years: Ophthalmoparesis	11 months: Feeding intolerance, failure to thrive. 8.5 months: Hepatic dysfunction (transaminitis).	8.5 months: Normal	EEG (8.5 months): Normal EEG (25 months): Focal irritation with generalization EMG (8.5 months): Absent sensory responses	Compound heterozygous c.1523A>G (p. Tyr508Cys) and c.952G>A (p. Ala318Thr) (sibling of case 4)	[4]
6	Neonatal	6 months	10 weeks: Dysconjugate gaze, seizures	Neonatal: Hypoglycemia, hyperammonemia. 10 weeks: Renal tubulopathy, hepatomegaly and liver failure	4 days/10 weeks (with MRS): Both normal	Visual and auditory evoked potentials (10 weeks): Abnormal	Compound heterozygous C85C>T (p.Arg29Ter) and c1523A>G (p. Tyr508Cys)	[5]
7	Unknown	3 months	Limited information available	Limited information available. 3 months: Renal tubulopathy, lactic acidosis.	N/A	N/A	Homozygous c.1183T>C (p.Phe395Leu) (sibling of patient 8 and 9)	[6]

Table (*Continued*)

Case	Symptom Onset	Survival	Neurological Features	Non-Neurological Features	MRI Brain	Electrophysiology	Mutation(s)	Ref
8	Neonatal	4 months	6 weeks: Irritability	Neonatal: Jaundice, vomiting, feeding intolerance, elevated lactate. 6 weeks: Hypoglycemia, renal tubulopathy, hepatic dysfunction (mild transaminitis), elevated tyrosine (urinary succinylacetone normal), lactic acidosis.	N/A	N/A	Homozygous c.1183T>C (p.Phe395Leu) (sibling of patient 7 and 9)	[6]
9	Neonatal	6 months	0-3 months: Irritability, lethargy hypotonia, poor suck/swallow, abnormal visual behaviors	Neonatal: Hypoglycemia, hepatic dysfunction (hepatomegaly, mild transaminitis, conjugated hyperbilirubinemia) newborn screen positive for tyrosinemia (urinary succinylacetone negative), renal tubulopathy, failure to thrive, feeding intolerance.	3 months: Normal	EEG (3 months): Diffusely slow, poorly organized background	Homozygous c.1183T>C (p.Phe395Leu) (sibling of patient 7 and 8)	[6]
10	Neonatal	21 months	5 months: Hypotonia, psychomotor regression 7 months: Abnormal eye movements, lingual dyskinesias 11 months: Chorea (upper limbs, face), areflexia 12-21 months: Loss of visual fixation	0-5 months: Recurrent vomiting 7 months: Failure to thrive 1 year: Elevated AFP, abnormal glycosylation profile suggestive of type I CDG	1 year (brain and spine): Normal	EEG (1 year): Poorly organized background Auditory evoked potentials (1 year): Bilateral sensorineural hearing loss. EMG (1 year): Sensorimotor neuropathy.	Compound heterozygous c.316G>A (p.Lys106-Glu) and c.1181G>A (p. Arg394His)	[7]
11	Neonatal	4 months	2 months: Irritability, severe hypotonia, psychomotor delay, dysconjugate gaze, poor visual fixation, lingual dyskinesias, areflexia 4 months: Bilateral ptosis, refractory status epilepticus	Neonatal: Hypoglycemia, failure to thrive. Hyponatremia 2 months: Renal tubulopathy, lactic acidosis, hepatic dysfunction (mild transaminitis). 3 months: laryngomalacia, hypertension. 4 months: Refractory lactic acidosis, liver failure.	2 months: Normal 4 months (with MRS): Mild cerebral atrophy	EEG (2 months): Bilateral independent temporal discharges. EEG (4 months): Multiple independent spike foci EMG (2 months): Absent sensory responses; mild myopathic features EMG (4 months): Unchanged	Compound heterozygous c.853C>T (p. Arg285Ter) and c1592 +4A>G	Our patient

Abbreviations: AFP, alpha-fetoprotein; CDG, congenital disorder of glycosylation; LD, lactate dehydrogenase; MRS, magnetic resonance spectroscopy.

now, ptosis has been reported in other mitochondrial disorders, but not Twinkle-associated MDS.

Common laboratory features include neonatal hypoglycemia and, later, lactic acidosis and cholestatic liver dysfunction, as seen in our patient. In one patient reported by Bouchereau et al., plasma and CSF lactate remained normal and the patient had an abnormal glycosylation profile suggestive of congenital disorder of glycosylation type I.[8] Renal tubulopathy has been reported in four patients, including ours.[6,7]

In previously reported patients, MRI findings ranged from normal to cerebellar atrophy. In our patient, mild cortical atrophy was present by four months. Electromyography (EMG) findings were consistent, with sensory neuropathy in the majority of reported patients.

Muscle biopsy findings were described in the patients reported by Sarzi et al. and Hakonen et al.[4,5] Pathology included lipid accumulation and either decreased cox staining or cox-negative fibers, also seen in our patient. Muscle pathology was normal at autopsy in the patient reported by Goh et al. and one by Prasad et al., both presenting with predominant hepatic and renal failure.[6,7] Liver pathology has demonstrated micro- or macrovesicular steatosis and cirrhosis in most patients. Mitochondrial DNA depletion was demonstrated in the patient tissues reported by Sarzi et al. and Goh et al.[4,6]

Unfortunately, there is limited evidence for effectiveness of supplements that have been used in other mitochondrial disorders. However, mitochondrial transplantation has been demonstrated to rescue mitochondrial respiratory function in animal models of myocardial and liver ischemia, as well as in cells harboring the mitochondrial DNA mutation MERRF A8344G, and may present a therapeutic option in patients with mitochondrial diseases in future.[9]

Conclusions

The typical presentation of autosomal recessive MDS is characterized by early onset hepatoencephalopathy, failure to thrive, psychomotor delay and/or regression, epileptic encephalopathy, sensory axonal neuropathy, progressive liver dysfunction, and in some patients, renal tubulopathy, movement disorders, and ophthalmoplegia. As such, MDS should be considered on the differential of early onset encephalopathy and multisystem failure. The description of this patient and the documentation of new mutations in the Twinkle gene is an important contribution to the understanding of this complex and severe neurodegenerative condition.

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