Clinical Reasoning: Siblings with progressive weakness, hypotonia, nystagmus, and hearing loss

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Neurology® 2018;90:e625-e631. doi:10.1212/WNL.0000000000004973

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Section 1
Case 1

A Yemeni boy was born healthy at full term after an uncomplicated pregnancy and delivery. Head circumference (HC) at birth is unknown. At 3 months of age, he developed vomiting and failure to thrive. He was diagnosed with renal tubular acidosis (RTA). Targeted DNA analysis revealed *ATP6V0A4* mutations associated with autosomal recessive distal RTA. Around 12 months of life, the patient developed horizontal nystagmus. At that time, his HC and height were approximately 10th percentile for age (45 cm and 74.9 cm, respectively). His development, the remainder of his neurologic examination, and brain MRI were normal. There were no birthmarks. He was lost to follow-up until 30 months of age, when he developed leg pain, progressive weakness of the lower, then upper extremities, dysarthria, decline in language skills, and sensorineural hearing loss following a mild viral illness. Examination at 30 months revealed normal muscle bulk, hypotonia, hyporeflexia, weakness (4/5 muscle strength throughout), and a wide-based gait. Bilateral wrist drop, finger contractures, decreased muscle bulk, and microcephaly (HC 2nd percentile, 46 cm) were noted at 53 months of age.

Case 2

Case 1's younger sister was born at full term after an uncomplicated pregnancy and delivery. Her HC at birth is unknown, but at 1.5 months of age was less than 2nd percentile (32.50 cm), and her length was less than the 3rd percentile (47 cm). She presented with RTA around 3 months of age, and was likewise found to have homozygous *ATP6V0A4* gene mutations. At 12 months of age, gastroesophageal reflux disease and failure to thrive manifested. Around 15 months of age, she developed horizontal and rotary nystagmus, weakness (4/5 proximally and 3/5 distally), and regression of motor milestones, including the gradual loss of her ability to stand, then sit. By 30 months of age, she developed sensorineural hearing loss and torticollis. Her examination was notable for microcephaly, nystagmus, esotropia, pale optic nerve heads, diminished muscle bulk, wrist drop, hypotonia, weakness, and hyporeflexia.

The parents were first cousins and had 4 unaffected older children. The mother had 6 first-trimester miscarriages. The maternal grandmother also had multiple miscarriages. The eldest sister of the sibling pair had a child with microcephaly of undetermined cause. The family history was otherwise unremarkable with respect to the chief complaint (figure 1).

Questions for consideration

1. What is the differential diagnosis?
2. What testing would help narrow the differential diagnosis?

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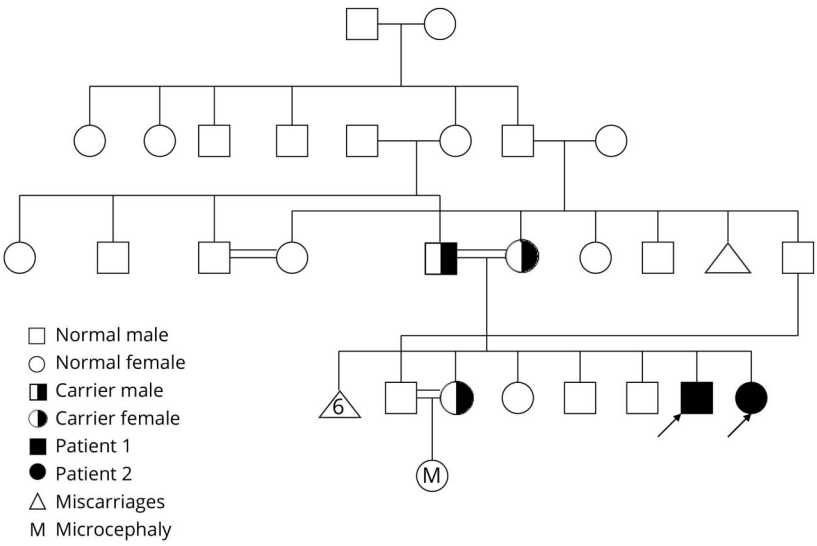
Figure 1 Three-generation pedigree analysis



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Section 2

These siblings presented with childhood-onset progressive distal and proximal muscle weakness, hyporeflexia, nystagmus, optic atrophy, hearing loss, gastroesophageal reflux, failure to thrive, and developmental regression, suggestive of combined peripheral nervous system and CNS involvement. The differential diagnosis is shown in table 1 and includes metabolic disorders, riboflavin transporter deficiency, infantile neuroaxonal dystrophy, or spinal muscular atrophy. Other investigations and results for both patients are listed in table 2.

The boy underwent extensive evaluations (tables 1 and 2). The newborn screen revealed risk for short chain acyl-CoA dehydrogenase deficiency and isobutyryl Co-A dehydrogenase deficiency. Urine organic acid analysis revealed ethylmalonic aciduria (SCAD deficiency/mitochondrial dysfunction). The acylcarnitine profile showed mild elevation of propionylcarnitine (C3), butyryl/isobutyryl carnitine (C4) (SCAD or isobutyryl CoA dehydrogenase deficiency), and octanoyl carnitine (C8) (MCAD deficiency). These findings can also occur in riboflavin transporter deficiency. EMG/nerve conduction studies of both lower extremities revealed a motor axonal polyneuropathy without acute denervation (tables 1 and 2). Muscle biopsy revealed widespread myofiber atrophy with mild fiber-type grouping and a marked increase in esterase staining, suggestive of a neurogenic pathology (figure 2). Staining for succinate dehydrogenase and cytochrome C oxidase showed occasional small foci of subsarcolemmal staining in scattered fibers, which is nonspecific, or suggestive of mitochondrial dysfunction. His brain MRI and magnetic resonance spectroscopy revealed mild nonspecific changes (tables 1 and 2).

Due to the similarities between the siblings' clinical manifestations, the sister's evaluation was limited (table 2).

Questions for consideration

1. Based on these findings, what is the differential diagnosis?
2. What testing could clarify the diagnosis?

Table 1 Differential diagnosis and relevant extensive evaluations

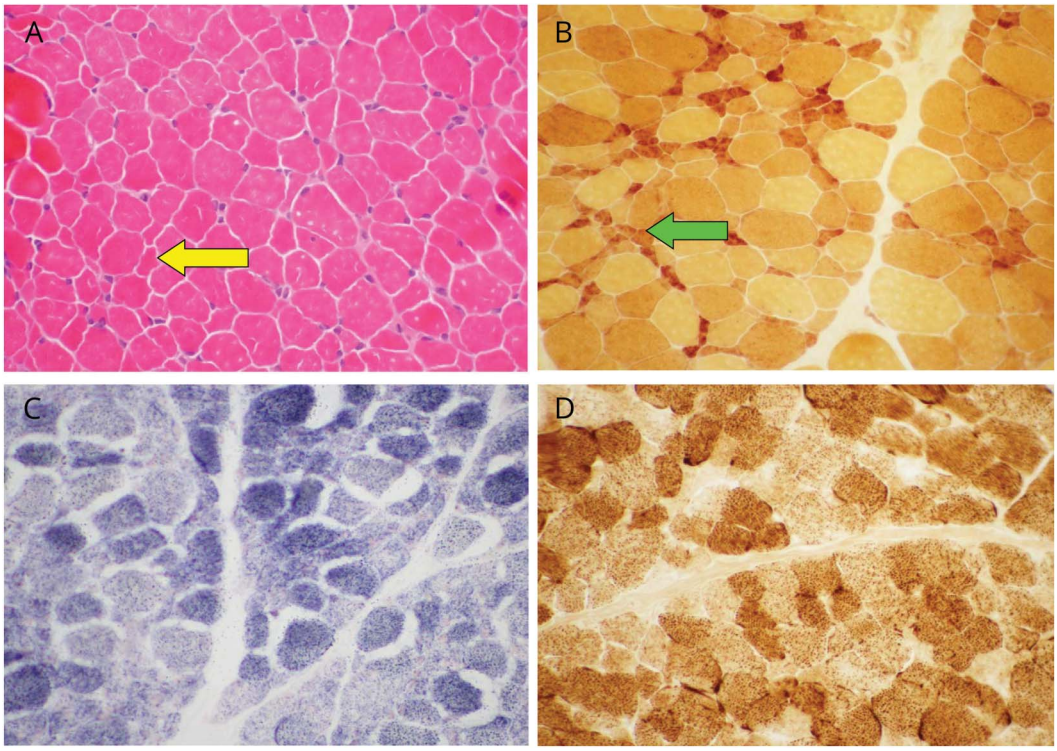
Differential diagnosis	Investigations	Case 1 results
Small molecular disorder		
Amino acid disorder	Serum amino acid	Nonspecific elevations
Organic acid disorder	Urine organic acid	Ethylmalonic aciduria (suggests SCAD deficiency and mitochondrial respiratory chain defects)
Congenital lactic acidosis	Serum lactate	Normal
	Serum pyruvate	Normal
Fatty acid oxidation defect and riboflavin transporter deficiency	Acyl carnitine profile	Mild elevation of propionylcarnitine C3 butyryl/isobutyryl carnitine C4 (suggesting SCAD deficiency or isobutyryl CoA dehydrogenase deficiency) and octanoyl carnitine C8 (MCAD deficiency)
Peroxisomal disorder	Very long chain fatty acids	Normal
Lysosomal disorder	Lysosomal enzyme assay	Normal
Genetic disease		
SCAD deficiency	*ACADS* gene sequencing (for SCAD)	Homozygous G209S variant (nonspecific)
IBD deficiency	*ACAD8* gene sequencing (for IBD)	Normal
Spinal muscular atrophy	*SMN1* gene	Normal
Chromosomal disorder	Chromosomal microarray	18p 11.31 Duplication of unknown significance
X-linked mental retardation	XLMR panel	Normal
Mitochondrial disease	Mitochondrial respiratory chain enzyme analysis	No deficiency of respiratory chain activity; citrate synthase elevated suggestive of mitochondrial proliferation
	Muscle biopsy	Neurogenic atrophy (figure 2); succinate dehydrogenase and cytochrome C oxidase showed nonspecific occasional small foci of subsarcolemmal staining

Table 2 Comparison between published cases of Brown-Vialetto-Van-Laere syndrome type 2 and our cases

Features	37 Reported cases of RFVT2 deficiency with molecular diagnosis[6]	Patient 1 (male)	Patient 2 (female)
Age at presentation	5.6 y (0.25–27 y)	12 mo	24 mo
Clinical features	(1) Cranial nerve deficit VII-XII (73%); (2) hearing loss (89%); (3) weakness: upper > lower extremity shoulder girdle/distal hand muscle weakness (92%); (4) hyporeflexia/areflexia; (5) sensory symptoms, ataxic gait with sensory-motor axonal neuropathy (51%); (6) feeding difficulty, PEG/G-tube (24%); (7) respiratory symptoms (57%); (8) optic atrophy (70%)	Microcephaly, nystagmus, sluggish pupillary response, FTT, GERD, developmental regression, optic nerve atrophy, corneal clouding, SNHL/auditory neuropathy, polyneuropathy, nephrocalcinosis, hypotonia, areflexia, distal renal tubular acidosis type 1	Same as first patient, esotropia
Biochemical laboratory	Abnormal acylcarnitine profile (61%)	Positive newborn screen for suspected short chain acyl-CoA dehydrogenase deficiency and isobutyl Co-A dehydrogenase deficiency	Same as first patient
Brain MRI	All normal	Optic chiasm atrophy and shortening of the anteroposterior length of corpus callosum	Shortened anteroposterior length of corpus callosum
EMG	Sensory-motor axonal neuropathy	Motor axonal neuropathy	Motor axonal neuropathy
Molecular diagnostics	Reported mutation in *SLC52A2*, c.368T>C, missense p.Leu123Pro, c.419C>T2, missense p.Pro140Leu, c.916 G>A2, missense p. Gly306Arg, c.1016T>C, nonsense p.Leu339Pro	Mutation in *SLC52A2* c.1327T>C missense mutation at the cDNA level or p.Cys 443 Arg (C443R) at protein level associated with (1) c.1185delC mutation in the *ATP6V0A4* gene; (2) *TOP1MT* (a mitochondrial DNA toposiomerase, c.1030C>T); (3) *PLEC1* (a gene associated with epidermolysis bullosa ± limb-girdle muscular dystrophy type 2Q ± pyloric atresia, c.5843 G>A)	Same as first patient

Abbreviations: FTT = failure to thrive; GERD = gastroesophageal reflux disease; PEG = percutaneous endoscopic gastrostomy tube; SNHL = sensorineural hearing loss.

Figure 2 Muscle biopsy showing atrophic fibers



(A) Hematoxylin & eosin stain (magnification ×40) shows diffuse myofiber atrophy, including both type 1 and type 2 fibers, which vary in diameter, with moderately to severely atrophic fibers intermixed with fibers of normal or slightly increased diameter. This pattern suggests neurogenic atrophy. (B) Esterase staining (magnification ×40) shows increased staining in many of the small diameter, severely atrophic fibers scattered throughout the fascicles. (C) Succinate dehydrogenase and (D) cytochrome C oxidase stain (magnification ×40) show occasional small foci of subsarcolemmal staining in scattered fibers without large accumulations, potentially suggestive of mitochondrial disease.

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Section 3

The differential diagnosis for these patients can be narrowed to infantile neuroaxonal dystrophy, a mitochondrial disorder, or riboflavin transporter deficiency.

PLA2G6 gene testing for infantile neuroaxonal dystrophy was negative. Mitochondrial respiratory chain enzyme analysis was unremarkable. A peripheral nerve biopsy of the boy showed severe axonal neuropathy with variable degeneration. Visual evoked potentials of both were abnormal without reproducible P100 peaks. Auditory evoked potential testing showed absent responses, suggestive of an auditory neuropathy. Given the evidence for the motor, sensory, and cranial neuropathies, both children received occupational and physical therapies, and were treated empirically for possible riboflavin deficiency and mitochondrial disorder, with 30 mg/kg/d of riboflavin, 4 mg/kg/d of coenzyme Q10, and 330 mg TID of carnitor. Whole exome sequencing was initially deferred due to cost.

By 5 years of age, both children developed mild scoliosis, diffuse muscle atrophy, and extensor posturing of the upper extremities with bilateral hand contractures. They were referred to the NIH undiagnosed disease program when patient 1 was 5.5 years old; they were initially evaluated at NIH when patient 1 was 6 years of age. In addition to their known *ATP6V0A4* gene mutations (RTA), whole exome sequencing also revealed homozygous gene variants in *TOP1MT* (a mitochondrial DNA topoisomerase, c.1030C>T), *PLEC1* (a gene associated with epidermolysis bullosa, limb-girdle muscular dystrophy type 2Q, and pyloric atresia, c.5843 G>A), and homozygous c.1327T>C (p, C443R) mutations in the *SLC52A2* gene (a riboflavin/vitamin B_2 transporter gene). The mutations in the *SLC52A2* gene have not been previously described, but are believed to be causative of the rare diagnosis of Brown-Vialetto-Van-Laere syndrome (BVVLS) type 2.

The healthy eldest sister, who has the infant daughter with microcephaly, was sequenced and subsequently found to be a carrier of the *ATP6V0A4*, *SLC52A2*, and *PLEC1* genes. She is also homozygous for the *TOP1MT* gene mutation. She is married to her maternal first cousin.

Discussion

BVVLS is a rare neurologic disorder with motor (limb, neck, and respiratory muscle weakness), sensory (gait ataxia), and cranial neuropathies (bulbar palsy, hearing loss, facial weakness, optic atrophy, and nystagmus) due to riboflavin transporter deficiency. BVVLS1 is caused by mutations in *C20orf54/SLC52A3* gene on chromosome 20p13, which encodes RFVT3, the predominantly intestinal riboflavin transporter.[1] In contrast, BVVLS2 is caused by mutations in *SLC52A2* gene on chromosome 8q24, which encodes RFVT2, the main riboflavin transporter in brain and spinal cord.[2,3] RFVT2 is highly expressed in brain and has been associated with neurodegenerative disorders. Maternal riboflavin deficiency due to mutations in *SLC52A1/GPR172B* gene (encoding RFVT1) is reported to cause a glutaric aciduria type 2–like condition in newborns.[4]

The age at presentation of BVVLS2 varies from infancy to young adulthood. In infancy, initial symptoms often include neuropathy-mediated respiratory weakness. In children or young adults, sensorineural hearing loss may occur first, followed by other cranial neuropathies in combination with lower motor neuron signs and ataxia. Some cases may resemble amyotrophic lateral sclerosis.[5] In 2016, Jaeger and Bosch[6] published a review of 37 molecularly confirmed patients with BVVLS2. Typical biochemical abnormalities may include an abnormal acylcarnitine profile. Nerve conduction studies typically show axonal sensory motor neuropathies, and most patients reported thus far have had a normal brain MRI.[3] BVVLS2 can be differentiated from Fazio-Londe syndrome and amyotrophic lateral sclerosis, which do not involve sensorineural hearing loss. BVVLS2 can also be differentiated from Nathalie syndrome, which does not involve lower cranial nerve symptoms, and Bolthauser syndrome, which does not affect the lower motor neurons. Similarly, Madras motor neuron disease can be distinguished by the relatively benign course and most common occurrence in Southern India. The differential diagnosis also includes multiple acyl-CoA dehydrogenase deficiency, which lacks motor and sensory neuronopathies, and spinal muscle atrophy, which does not involve sensory nerves.

While the c.1327T>C genetic change in *SLC52A2* seen in both of our patients has not been reported in patients with BVVLS2, their symptoms can be explained by this disorder (table 2). Treatment with riboflavin has been shown to improve the clinical course in some individuals with this condition.[3] Both of the patients reported seem to have stabilized with riboflavin supplementation.

The mitochondrial c.1030C>T mutation in the *TOP1MT* gene (TOP1MT, mitochondrial DNA topoisomerase) found in both siblings could potentially explain some of the muscle biopsy features, including the small foci of subsarcolemmal staining in scattered fibers. This is less likely to be pathogenic, however, because the eldest asymptomatic sister is also homozygous for TOP1MT 1030C>T mutation. The *PLEC1* gene mutations remain of unknown clinical significance in these cases.

The parents received extensive genetic counseling regarding the 25% recurrence risk of RTA type 1 and BVVLS2 among future children. The roles of preimplantation genetic diagnosis or invasive prenatal testing were discussed.

Conclusion

Our cases illustrate the pitfalls of single gene evaluations and support the necessity of more extensive testing in complex cases to identify multiple mutations that may potentially affect the clinical phenotype, and ultimately direct clinical management. In our cases, earlier exome sequencing would have obviated the need for invasive muscle biopsy. This study also

highlights the importance of proper genetic counseling, both to explain complex variants of unknown significance, as well as to provide the family tools with which to guide future planning.

Author contributions

K.K.S. and A.M.H. conceived the study and analyzed and interpreted the data. K.K.S. and A.R.B.W. wrote the manuscript. A.R.B.W. performed critical revision of the manuscript for intellectual content. F.J.S. helped analyze data and revise the manuscript. All authors read and approved the manuscript.

Acknowledgment

The authors thank the NIH Undiagnosed Disease Program for whole exome sequencing; Dr. W. Kupsky and Department of Neuropathology, Wayne State University, Detroit, Michigan, for providing the muscle biopsy slides; and Dr. Pinki Chowdhury, University of Michigan, for genetic analysis and formatting the manuscript.

Study funding

No targeted funding reported.

Disclosure

The authors report no disclosures relevant to the manuscript. Go to Neurology.org/N for full disclosures.

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