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To Be or No B2: A Rare Cause of Stridor and Weakness in a Toddler

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Abstract

We present a case of a young child with a rare metabolic disorder whose clinical presentation resembled that of autoimmune myasthenia gravis. The differential diagnosis was expanded when autoantibody testing was negative and the patient did not respond to standard immunomodulatory therapies. Rapid whole genome sequencing identified 2 rare variants of uncertain significance in the *SLC52A3* gene shown to be in compound heterozygous state after parental testing. Biallelic mutations in *SLC52A3* are associated with Riboflavin Transporter Deficiency, which in its untreated form, results in progressive neurodegeneration and death. Supplementation with oral riboflavin has been shown to limit disease progression and improve symptoms in some patients. When the diagnosis is suspected, patients should be started on supplementation immediately while awaiting results from genetic studies.

Keywords

muscle weakness, gene mutation, riboflavin, whole genome sequencing, *SLC52A3*

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Case

A 13-month-old boy presented with 3 months of intermittent activity-induced stridor. The patient was born term by normal vaginal birth with no perinatal complications. He had normal acquisition of developmental milestones and did not have any significant prior medical history or contributory family history. One week prior to presentation, he developed upper respiratory tract symptoms, persistent stridor, respiratory distress, easy fatigability with short bursts of activity, dysphagia, and weak cry. He was initially evaluated in an ambulatory clinic for stridor, and subsequently admitted to the pediatric intensive care unit for further evaluation and management. His neurological examination was notable for normal cognition, bilateral eyelid ptosis worse on the left, mild facial weakness, axial hypotonia and mild proximal weakness of the lower extremities with intact deep tendon reflexes.

The clinical presentation was highly suspicious for a neuromuscular junction disorder, specifically autoimmune myasthenia gravis with rapid symptom onset was highly considered, although a congenital myasthenic syndrome or infantile botulism were also on the differential. A neurogenic or myopathic

process could not be excluded. Initial diagnostic evaluation noted mildly elevated inflammatory markers with otherwise normal complete blood count, chemistry panel, thyroid studies, and creatine kinase. A nasopharyngeal viral respiratory PCR was positive for coronavirus (non SARS-CoV2). Direct laryngoscopy and bronchoscopy showed bilateral vocal cord immobility with cords in the paramedian position. Magnetic Resonance Imaging (MRI) of the brain and chest were normal.

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Due to the concern for a myasthenic crisis with worsening respiratory symptoms, he was empirically treated with 5 days of intravenous immunoglobulin (IVIG) followed by prednisone and azathioprine, a steroid-sparing immunomodulating agent. He had mild improvement in symptoms initially, but this was short lived. A second course of IVIG with a trial of pyridostigmine did not result in any clear clinical improvement. Repeat flexible laryngoscopy 2 weeks into treatment demonstrated persistent immobile vocal folds. During this time, the patient developed worsening stridor and respiratory distress requiring tracheostomy.

Given the presentation and minimal improvement with immunomodulatory therapies and acetylcholinesterase inhibitors, additional evaluations were pursued. His newborn screen was confirmed normal. Serum acetylcholine receptor (AChR) and Muscle-Specific Kinase (MuSK) antibodies sent on admission ultimately resulted negative 1 week into the hospital course. A congenital myasthenic syndrome gene panel ordered 2 weeks into the hospital course (Fulgent) resulted negative after 36 days from the time of sample collection. While awaiting these results, rapid whole genome sequencing (WGS) through the Rady Children's Institute for Genomic Medicine was performed 4 weeks into the hospital course. A preliminary report was released 24 hours after sample collection. The WGS identified 2 rare heterozygous missense variants in *SLC52A3* (NM_033409.4), which encodes for a riboflavin transporter protein, a c.374C>A (p.Thr125Asn) and a c.721C>T (p.Leu241Phe) that, through parental studies, were subsequently shown to be on opposite chromosomes (in trans configuration). The maternally-inherited heterozygous missense variant c.374C>A (p.Thr125Asn) has been previously reported as single heterozygous variant in a 10-year-old patient with Brown-Vialetto-Van Laere syndrome¹ and as a compound heterozygous change with a nonsense variant in a 11-month-old patient with weakness, mild axonal neuropathy and sensorineural hearing loss.² This variant is extremely rare in the general population with an allele frequency of 0.0028%,³ and affects a highly conserved amino acid that is predicted to have a deleterious effect on protein function based on SIFT (v5.1.1), PolyPhen-2 and MutationTaster in-silico tools. The second variant is a novel, paternally-inherited heterozygous missense variant at c.721C>T (p.Leu241Phe) that is absent from population databases.³ The c.721C>T affects a conserved amino acid and is predicted to result in a deleterious effect on protein function based on SIFT (v5.1.1), PolyPhen-2 and MutationTaster in-silico tools. Both variants were orthogonally confirmed with Sanger sequencing.

Biallelic pathogenic variants in *SLC52A3* are associated with autosomal recessive riboflavin transporter deficiency (RTD) neuropathy, a rare progressive neurometabolic condition that can be fatal if left untreated. Since the clinical presentation in our patient showed substantial similarities to patients with RTD, the variants were interpreted as the likely etiology of the patients' symptoms.^{4,5} Based on these genetic results, high dose riboflavin supplementation was initiated at 45 mg/kg/day divided 4 times daily on the same day the

variants were reported to prevent further disease progression. Ubiquinol was added to support mitochondrial function. His immunosuppressant medications were weaned off over the next 2 weeks, and a gastrostomy tube was placed for nutritional support.

Additional metabolic investigations including serum ammonia, pyruvate and lactate were unremarkable. Urine amino acids were normal and urine organic acids showed marginal elevations in 3-hydroxybutyric and acetoacetic. The serum acylcarnitine profile was normal. Serum vitamin B2 level was 26 nmol/L (reference range 5-50 nmol/L) when tested 2 hours after the patient received 100 mg oral riboflavin.

Motor nerve conduction studies of the bilateral facial nerves showed significantly decreased compound muscle action potentials with normal latencies which are findings consistent with an axonal neuropathy. Needle electromyography (EMG) of selected muscles of the face and extremities showed increased spontaneous activity with decreased recruitment and rare large amplitude motor unit action potentials with fast firing rates. Taken together, these findings were consistent with a neurogenic process further supporting the diagnosis of RTD. The EMG abnormalities were more severe in the facial muscles compared to the muscles of the extremities which were clinically less affected. His ophthalmologic examination was normal. Brainstem auditory evoked response testing showed bilateral sensorineural hearing loss consistent with auditory neuropathy spectrum disorder.

At 6-month follow-up, the patient remained tracheostomy dependent with supplemental oxygen requirement at night. His dysphagia improved, and he was able to take some feedings by mouth. Hearing aids were prescribed for his sensorineural hearing loss, and he had improvements in his strength, endurance and motor skills as well as other developmental domains.

Discussion

Riboflavin, or vitamin B2, is a water soluble vitamin that serves as a precursor for 2 important redox cofactors, flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN), making it vital for cells and mitochondrial energy production.⁶ Humans cannot synthesize riboflavin endogenously, therefore it must be acquired from the diet. Riboflavin is found in many foods including milk, eggs, meats and green vegetables. It is absorbed in the small intestine and renal urinary ducts and subsequently distributed throughout the body including crossing the blood brain barrier where it can be taken up by cells for use in the central nervous system.⁷

The *SLC52A3* gene encodes for the riboflavin transporter protein RFVT3 which is involved with gut absorption of riboflavin. Deficiency in RFVT3 results in a neurodegenerative disorder formerly known as Brown-Vialetto-Van Laere (BVVL) syndrome type 1 (OMIM # 211530), although a spectrum of clinical phenotypes are now recognized and the disorder is broadly referred to as RTD.^{4,8} RTD is an autosomal recessive disease that typically presents in childhood, although there are reports of patients diagnosed in adulthood.^{6,9}

The clinical presentation can be highly variable but typically includes cranial neuropathies, sensorineural hearing loss, weakness, feeding difficulties, balance and gait problems and respiratory complaints such as dyspnea or stridor while cognition typically remains intact.⁸ Patients are often asymptomatic for the first weeks to months of life before developing symptoms, most commonly failure to thrive secondary to feeding difficulties from cranial neuropathy and respiratory symptoms. Sensorineural deafness is the most common presenting feature of patients presenting after infancy.^{4,8} The diagnosis of RTD is made by genetic analysis.¹⁰

MRI of the brain is typically normal, but electrophysiologic evaluation with nerve conduction and EMG usually demonstrates a pattern consistent with sensorimotor axonal neuropathy.⁸ Metabolic studies are typically normal, however urine organic acids can show abnormalities in some patients and the acylcarnitine profile can show abnormal concentrations of short and medium-chain plasma acylcarnitines in 50-60% of patients with a confirmed molecular diagnosis of RTD.^{4,8,11} The pattern of abnormality is similar to those findings seen in multiple acyl-CoA dehydrogenation defect (MADD), an inherited defect of mitochondrial fatty acid oxidation and branched-chain amino acid catabolism.¹¹ In the electron transport chain, FAD, the important metabolite of riboflavin, is an electron acceptor of several acyl-CoA dehydrogenation reactions including 1,5-dihydro-FAD (FADH₂), important for both fatty acid oxidation and the catabolism of branched-chain amino acids. Therefore, a shortage of FAD due to riboflavin deficiency can lead to an abnormal accumulation of organic acids and acylcarnitines which can be measured in the urine or serum, respectively.¹¹ Additionally, abnormalities in plasma flavin levels have been reported in RTD, typically with low levels of FAD and FMN with or without abnormal riboflavin levels, suggesting defects in riboflavin uptake.^{4,11} Any of these biochemical abnormalities, if present, can normalize after treatment with riboflavin.^{4,8,11} As mentioned, our patient had mild elevations in 3-hydroxybutyric and acetoacetic which are reflective of ketosis likely secondary to feeding difficulties and inadequate caloric supply and do not reflect any specific pattern suggestive of an inborn error of metabolism. It should be noted that the newborn screen is not a reliable diagnostic tool to evaluate newborns for RTD since the acylcarnitine profile can be normal secondary to maternal compensation.^{10,11}

This case highlights the important role that rapid genome-wide sequencing can play for patients experiencing clinical deterioration without a clearly established diagnosis to guide management, undergoing an extensive diagnostic odyssey, or atypical or failed response to initial therapies. In this case, rapid sequencing revealed a rare, treatable neurometabolic disorder that was not previously considered during the early diagnostic evaluation. While the initial testing took several weeks to result, the turnaround time from sample collection to rapid WGS results were less than 24 hours. More recently, rapid WGS has been shown to report variants in a time-sensitive manner that meaningfully impacts the care of critically ill patients.¹²

As next generation sequencing is increasingly incorporated into clinical practice, it is important to note that a variant is reported to have unknown significance either because 1) the variant is in a gene that matches the phenotype but there is insufficient evidence to determine its pathogenicity, 2) the gene in question is not proven to be pathogenic but its molecular characteristics suggest that it may be pathogenic, or 3) the variant is in a gene that does not fit the reported phenotype.¹³ In this case, our patient fits the phenotype of patients with variants in *SLC52A3* but the variants themselves have not been proven to be pathogenic. However, the combination of the molecular characteristics and genotype-phenotype correlation highly suggest that these variants are pathogenic. Additional electrophysiologic phenotyping with EMG and nerve conduction as well as the results of the brainstem auditory evoked response in addition to the proband's arrest of deterioration and improvement on high dose riboflavin provided further support for the diagnosis.

Given that riboflavin supplementation is potentially life-saving, it should be started if RTD is in the differential and continued until genetic studies result. When RTD is left untreated, infants with RTD rarely survive beyond 1 year.^{8,10} Current treatment for RTD is enteric high dose riboflavin supplementation with a wide dosing range reported in the literature (7-60 mg/kg/day). Overall, it is seemingly well tolerated, though gastrointestinal upset is the most common complaint.⁸ This therapy appears to halt progression of the disease and in some patients has resulted in dramatic improvement of symptoms including weaning off ventilatory support.^{4,8,9} Some cases only show disease stabilization, highlighting the importance of immediate therapy to avoid further disease progression.

Conclusions

We present a case of a young child with a rare metabolic disorder whose clinical presentation resembled that of an autoimmune neuromuscular disorder. Rapid whole genome sequencing was able to identify 2 compound heterozygous variants in *SLC52A3*, consistent with the clinical diagnosis of riboflavin transporter deficiency. When the diagnosis is suspected, patients should be started on riboflavin supplementation immediately while awaiting results from genetic studies.

Authors' Note

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

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Author Contributions

ALF and JHY contributed to analysis and interpretation and drafted the manuscript. SS, AQ, and MC contributed to interpretation. LG contributed to acquisition and analysis. KW contributed to analysis and interpretation. All authors critically revised the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

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