Variants of *SLC18A3* leading to congenital myasthenic syndrome in two children with varying presentations

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SUMMARY

This report describes the variation in presentation of two unrelated patients found to have a rare form of presynaptic congenital myasthenic syndrome. Both patients presented with hypotonia, ptosis, poor weight gain and apneic episodes. Through whole exome sequencing, our patients were found to have the same likely pathogenic biallelic variants in W315X and I200N of *SLC18A3*, encoding vesicular acetylcholine transporter (VAChT). These specific variants in *SLC18A3* have not been previously described in the literature. We illustrate the variety in clinical presentation and course of children with mutations in *SLC18A3*, leading to presynaptic congenital myasthenic syndrome through VAChT deficiency.

BACKGROUND

Congenital myasthenic syndromes (CMS) are a group of genetic disorders caused by dysfunction of neuromuscular transmission at the level of the neuromuscular junction. Patients typically present with fatigable muscle weakness and characteristic electromyography (EMG) findings, either decremental EMG or abnormal single-fibre EMG response.[1] CMS are rare disorders with a cited prevalence of 9.2 per million children in the UK,[2] with North American data not in published literature. CMS are typically caused by defects in postsynaptic transmission but can be caused by defects in presynaptic transmission, synaptic transmission and glycosylation; clinical presentation and treatment options vary based on type of defect.[1–3] Currently, more than 30 genes are associated with CMS, with this number likely to increase with advances in next-generation sequencing.[2 4]

SLC18A3 (solute carrier family 18 (vesicular acetylcholine), member 3) encodes the vesicular acetylcholine transporter (VAChT), an exon within the first intron of CHAT gene on chromosome 10q11.23. VAChT is responsible for transporting acetylcholine (ACh) into vesicles in the presynaptic terminal, allowing for eventual release into the synaptic space.[5] This report describes two unrelated patients with varied phenotypes, found to have CMS due to mutations in *SLC18A3*.

CASE PRESENTATION

Patient 1

Patient 1 was the first-born child of nonconsanguineous Caucasian parents. He was born at 38+1 weeks by caesarian section secondary to non-reassuring fetal heart rate. Fetal movements were normal. Apgar Scores were 4 at 1 min, 6 at 5 min and 6 at 10 min. His weight was 4 kg (85th percentile), head circumference 34.5 cm (50th percentile) and length 46.5 cm (third percentile). He required positive pressure ventilation at delivery for poor respiratory effort and a heart rate of 80. He was transitioned to continuous positive airway pressure (CPAP) at 43 min of life, which was weaned on the first day of life. He remained in the neonatal intensive care unit (NICU) for 9 days, with stay prolonged by two unexplained desaturations, excessive secretions and jaundice.

At 3 months of age, his parents sought medical attention due to concerns with weight gain, felt to be secondary to gastro-oesophageal reflux. He was also noted to be slow with his development, particularly with head control. He was found to have complete head lag and was unable to lift his head from prone. His weight at that time had dropped below the third percentile (4.915 kg), and he was started on omeprazole for presumed gastro-oesophageal reflux disorder (GERD). By 6 months of age, his parents noticed brief episodes of drooping eyelids, usually when tired. His weight had improved (above 15th percentile). He continued to require head support and had persistent truncal hypotonia. Head MRI showed no abnormalities to account for significant truncal hypotonia.

At 12 months of age, he was started on pyridostigmine after EMG findings were consistent with CMS. He continued to have mild ptosis, even with treatment. He was unable to sit up before falling over with no saving reaction. At 18 months of age, he was unable to get to a sitting position by himself but was able to sit once placed into sitting position. He was started on 3,4-diaminopyridine (3,4-DAP) at 2 years of age, with further improvements noted.

Patient 2

Patient 2 was the first-born child to nonconsanguineous Caucasian parents. He was born at 34+1 weeks by caesarian section due to non-reassuring fetal heart rate. Fetal movements were normal. Apgar Scores were 7 at 1 min and 8 at 5 min. His weight was 2.098 kg (30th percentile), head circumference 32 cm (70th percentile) and length 44 cm (35th percentile). He required CPAP at birth for respiratory distress, with apneas noted on the first day of life. He was weaned off respiratory support at 36 hours of age. He had a 3 ½-week NICU admission, prolonged secondary to feeding

difficulties and jaundice requiring phototherapy. While in the NICU, nursing staff noted he was slow to feed by bottle but was able to finish entire feeds.

Three weeks after discharge, he was admitted to hospital with apneic episodes during feeding. These episodes persisted during feeding and with nasal suctioning, each lasting between 20 and 50 s and resolving with stimulation. Invasive ventilation was not required. He was started on omeprazole for presumed GERD along with nasogastric feeds. Multiple investigations were performed including upper gastrointestinal (GI) series, upper GI endoscopy, bronchoscopy and echocardiogram, which were all normal. Swallowing assessment showed incoordination. Due to poor weight gain along with persistent reflux symptoms, a nasojejunal tube was placed.

At 1-month corrected age, he was noted to have poor suck, weak cry and copious oral secretions. Deep tendon reflexes were present, and no appreciable muscular weakness was noted. Ophthalmology reported variable nystagmus (both vertical upbeat nystagmus and horizontal nystagmus), however no evidence of ptosis. Head ultrasound, head MRI and electroencephalogram (EEG) were normal. At 2-month corrected age, he was treated with pyridostigmine after whole exome sequencing results. Within 1 week, he had improvements in his cry, suck and amount of oral secretions.

INVESTIGATIONS

Genetic testing for patient 1 was initially targeted towards hypotonia. A microarray test was requested to rule out copy number variants, in addition to DNA methylation studies for Prader-Willi syndrome and molecular testing for spinal muscular atrophy and myotonic dystrophy. Initial investigations were normal. A comprehensive muscular dystrophy/myopathy panel was completed, which was also normal. EMG studies were completed. Repetitive nerve stimulation showed a decrement of more than 20% of the amplitude on several occasions over the abductor digiti minimi muscle, highly suggestive of a neuromuscular junction disorder. Edrophonium (Tensilon) is currently not approved for use in Canada[6]; therefore, this test was not performed. Based on EMG results, a CMS panel was completed and found to be normal; however, this panel did not include the *SLC18A3* gene. Finally, whole exome sequencing in a trio was completed at GeneDx.

Patient 2 had a normal microarray. Whole exome sequencing in a trio was requested through GeneDx, due to worsening apneic episodes and failure to thrive.

MOLECULAR ANALYSIS

Whole exome sequencing in a trio was performed through GeneDx for both patient 1 and patient 2. For patient 1, whole exome sequencing found likely pathogenic biallelic variants in the SLC18A3 gene. The c.945G>A, p.Trp315Ter (W315X) variant was paternally inherited. This variant is predicted to cause loss of normal protein function through protein truncation and has not previously been reported.[7] The c.599T>A, p.Ile200Asn (I200N) variant was maternally inherited. This variant results in a non-conservative amino acid substitution, which is likely to impact secondary protein structure and has not previously been reported.

Whole exome sequencing for patient 2 showed the same likely pathogenic biallelic variants as patient 1. For patient 2, the c.599T>A, p.Ile200Asn (I200N) variant in *SLC18A3* was paternally inherited, and the c.945G>A, p.Trp315Ter (W315X) variant in *SLC18A3* was maternally inherited.

OUTCOME AND FOLLOW-UP

Patient 1 is 4 years old. He is unable to stand up without support and ambulates with use of a walker. He has a speech delay. He does not drool and is able to swallow and chew his food. He continues to have ptosis, more significant on the right eye than left.

Patient 2 is currently 6 months of age corrected. He can maintain head control when sitting with support. His muscle strength appears to be improving, but he is still generally weak as demonstrated by inability to lift his head from prone and truncal hypotonia. He required a gastrostomy tube for poor weight gain. He continues to have nystagmus, which has remained stable.

DISCUSSION

Both of our patients had clinical features consistent with CMS along with genetic confirmation of *SLC18A3* mutation. With initiation of pyridostigmine for both patients and 3,4-DAP for patient 1, there has been some clinical improvement without full resolve of symptoms, as expected with most cases of CMS. Interestingly, both patients have the same variants of *SLC18A3* with no known relation between these families. However, our geographical population does have significant founder effects; therefore, it is possible that these families share common ancestors.

Within the presynaptic neuron, the role of VAChT is to package ACh synthesised by cholineacetyltransferase (ChAT) into vesicles, through exchange of cytoplasmic ACh with vesicular protons.[8] Release of ACh from vesicles at the neuromuscular junction is important in both central and peripheral nervous systems. Presynaptic CMS caused by dysfunctional VAChT activity leads to impairment of both muscarinic and nicotinic ACh receptors, which can lead to more severe phenotypes.[5]

Cognitive defects may occur in VAChT defects due to altered cholinergic synaptic transmission mediated by neuronal nicotinic ACh receptors in the brain.[5] Studies in VAChT knockout mice have shown impairments in social memory, which the authors attributed to decreased cholinergic tone, which is known to be important for memory and learning.[9 10] While cognitive impairment is reported to be rare in CMS, mutations in *SLC18A3* have been shown to manifest as neurodevelopmental delay.[4]

It is important to note that use of 3,4-DAP could deplete supply of vesicles filled with ACh in the nerve terminals if VAChT is not functional and unable to refill vesicles; this could therefore lead to crisis due to insufficient ACh release.[11] Seizures and liver dysfunction are known side effects of this medication. In our patient with presynaptic CMS, 3,4-DAP can be helpful but should be still used with caution.

Five patients have been described in the literature with mutations in *SLC18A3* (see table 1).[5 12 13] The first study describes two patients from separate families.[5] The first patient was found to have a 10q11.21–q11.23 deletion associated with a hemizygous mutation of *SLC18A3* mutation, c.557G>C, p.Gly186A. He presented with exercise intolerance and ptosis at age six and later with continued ptosis, ophthalmoplegia and facial weakness at age 14. He was also noted to have cognitive deficits and mild reduction of left ventricular systolic function, which resolved with initiation of pyridostigmine. Clinical improvement was noted at age 16, 2 years after treatment was initiated. The second patient in this study was found to have a homozygous mutation in *SLC18A3*, c.1192G>C, p.Asp398His. She presented earlier in life, with meconium ileus at 3 days of life, apneas at 1 month of age and hypotonia and feeding difficulties at 3 months of age. She was also found to have fluctuating

Table 1 Summary of patients described with *SLC18A3* mutation in published literature

	Patients (n)	Mutation in *SLC18A3*	Age at publication (years)	Current function	Pharmacological treatments
O'Grady *et al*[5]	2	c.557G>C, p.Gly186Ala c.1192G>C, p.Asp398His	16 6	Some clinical improvement Loss of ambulation at age 5 Gastrostomy feeds	Pyridostigmine Pyridostigmine 3,4-Diaminopyridine ephedrine
Aran *et al*[12]	2	c.1078G>A, p.Gly360Arg	Death at 5 days of life 4.5	Death at age 5 days Mechanical ventilation Profound hypotonia GDD Progressive microcephaly Nystagmus	No pharmacological treatment for either patient
Schwartz *et al*[13]	1	c.154G>T, p.Val52Phe	13	Loss of ambulation Ptosis ophthalmoplegia Facial weakness	Pyridostigmine

GDD, global developmental delay; SLC18A3, solute carrier family 18 (vesicular acetylcholine), member 3.

nystagmus, ophthalmoplegia, ptosis and hypotonia on presentation. Pyridostigmine, 3,4-DAP and ephedrine are used for symptomatology; however, she experienced loss of independent ambulation at 5 years old.

Another study described two siblings of nonconsanguineous parents with more severe presentations of *SLC18A3* mutations.[12] Both siblings were found to be homozygous for *SLC18A3* c.1078G>A, p.Gly360Arg. These siblings were found to have the same clinical features including retrognathia, severe hypotonia and distal arthrogryposis in all extremities, dislocated hips and genital abnormalities including undescended testes and micropenis. The first child died at 5 days of life from respiratory failure. The second child was reported to be 4.5 years old and remained on mechanical ventilation since birth. No pharmacological treatments were started at family's request. He has profound hypotonia, global developmental delay, progressive microcephaly and nystagmus.

Finally, a fifth patient has been described with a 10q11.22–q11.23 deletion associated with a hemizygous *SLC18A3* mutation, c.154G>T, p.Val52Phe.[13] She was initially admitted to an intensive care unit at 30 hours of life after cardiorespiratory arrest. She was prescribed pyridostigmine for poor suck, ptosis and poor facial weakness. She received a trial off medication at 4 months of age; however, it was restarted after a second cardiorespiratory arrest. At 13 years old, she continued to have ptosis, ophthalmoplegia and facial weakness. She uses a wheelchair for ambulation.

We describe two patients with the same likely pathogenic biallelic mutations in *SLC18A3* from unrelated families. While both patients have the same genotype, their presentations, response to treatment and progress have varied. They both showed feeding issues and failure to thrive with generalised weakness. The second child had life-threatening apneas, which only stopped with the addition of pyridostigmine. With only five described clinical cases in the literature, we add two further clinical cases confirmed through genetic diagnosis.

Learning points

- Congenital myasthenic syndromes are a rare group of disorders caused by dysfunction of neuromuscular transmission at the level of the synapse.
- Our patients showed feeding issues, failure to thrive and hypotonia with delayed motor development. The second patient also had life-threating apneic events.
- Treatment with pyridostigmine was helpful for both patients, with life-threatening apneas stopping once pyridostigmine started.
- Use 3,4-diaminopyridine with caution due to potential of insufficient acetylcholine release, but it can be considered in children older than 2 years for symptom improvement.

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REFERENCES

1 Engel AG, Shen X-M, Selcen D, *et al*. Congenital myasthenic syndromes: pathogenesis, diagnosis, and treatment. *Lancet Neurol* 2015;14:420–34.
2 Rodríguez Cruz PM, Palace J, Beeson D. The neuromuscular junction and wide heterogeneity of congenital myasthenic syndromes. *Int J Mol Sci* 2018;19:1677–23.
3 Engel AG, Shen X-M, Selcen D, *et al*. What have we learned from the congenital myasthenic syndromes. *J Mol Neurosci* 2010;40:143–53.
4 Finsterer J. Congenital myasthenic syndromes. *Orphanet J Rare Dis* 2019;14:57.
5 O'Grady GL, Verschuuren C, Yuen M, *et al*. Variants in SLC18A3, vesicular acetylcholine transporter, cause congenital myasthenic syndrome. *Neurology* 2016;87:1442–8.
6 Canada H. Product information tensilon, 2019. Available: https://health-products.canada.ca/dpd-bdpp/dispatch-repartition.do [Accessed 20 Jan 2020].
7 GeneDx. *Genetic testing report*. Gaithersburg, MD, 2019.
8 Nguyen ML, Cox GD, Parsons SM. Kinetic parameters for the vesicular acetylcholine transporter: two protons are exchanged for one acetylcholine. *Biochemistry* 1998;37:13400–10.
9 Prado VF, Martins-Silva C, de Castro BM, *et al*. Mice deficient for the vesicular acetylcholine transporter are myasthenic and have deficits in object and social recognition. *Neuron* 2006;51:601–12.
10 de Castro BM, De Jaeger X, Martins-Silva C, *et al*. The vesicular acetylcholine transporter is required for neuromuscular development and function. *Mol Cell Biol* 2009;29:5238–50.
11 Lee M, Beeson D, Palace J. Therapeutic strategies for congenital myasthenic syndromes. *Ann N Y Acad Sci* 2018;1412:129–36.
12 Aran A, Segel R, Kaneshige K, *et al*. Vesicular acetylcholine transporter defect underlies devastating congenital myasthenia syndrome. *Neurology* 2017;88:1021–8.
13 Schwartz M, Sternberg D, Whalen S, *et al*. How chromosomal deletions can unmask recessive mutations? deletions in 10q11.2 associated with ChAT or SLC18A3 mutations lead to congenital myasthenic syndrome. *Am J Med Genet A* 2018;176:151–5.