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Genetic testing in a cohort of patients with potential epilepsy with myoclonic-atonic seizures

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ABSTRACT

Epilepsy with myoclonic-atonic seizures (EMAS) accounts for 1–2% of all childhood-onset epilepsies. EMAS has been shown to have an underlying genetic component, however the genetics of this disorder is not yet well understood. The purpose of this study was to review genetic testing results for a cohort of EMAS patients. A retrospective chart review was conducted for 77 patients evaluated at Children's Hospital Colorado with a potential diagnosis of EMAS. Genetic testing and biochemical testing was reviewed. Family history data was also collected. Seventy-seven percent of the cohort had at least one genetic test performed, and a molecular diagnosis was reached for six patients. Thirty-seven patients had a microarray, six of which identified a copy number variant. Only one was felt to contribute to the phenotype (2p16.3 deletion including *NRXN1*). Fifty-one patients had an epilepsy panel, two of which were positive (likely pathogenic variant in *SCN1A*, pathogenic variant in *GABRG2*). Of the six patients who had whole exome sequencing, two were negative, three were positive or likely positive, and one had multiple variants not felt to explain the phenotype. While EMAS is widely accepted to have a strong genetic component, the diagnostic yield of genetic testing remains low. This may be because several genes now thought to be associated with EMAS are not included on the more commonly ordered epilepsy panels, or have only recently been added to them.

1. Introduction

Epilepsy with myoclonic-atonic seizures (EMAS), also known as Doose syndrome, is an epilepsy syndrome accounting for one to two percent of all childhood-onset epilepsies (Kelley and Kossoff, 2010). EMAS is characterized by multiple seizure types with onset typically between one and five years of life (Kelley and Kossoff, 2010). Development is usually normal prior to seizure onset, with a subsequent plateau or regression in most patients.

Since first described by Dr. Hermann Doose in 1970, the disorder has been felt to have an underlying genetic component given the high prevalence of epilepsy and EEG abnormalities among family members of affected individuals. In Doose's original cohort of 117 patients with EMAS, 32% had a positive family history of seizures, while 68–80% had a family history of abnormal EEG findings (Kelley and Kossoff, 2010; Doose et al., 1970). However, the prevalence of myoclonic or atonic seizures among family members was much lower (about two percent), suggesting significant phenotypic variability within affected families (Kelley and Kossoff, 2010; Doose et al., 1970).

The variability of presentation among individuals with EMAS and their family members suggests that inheritance may be multifactorial or polygenic in some cases Kelley and Kossoff, 2010; Doose et al., 1970; Tang and Pal, 2012. This has complicated the identification of clear genetic etiologies. While several genes have been associated with EMAS, (including *SCN1A* (Ebach et al., 2005; Escayg et al., 2001), *SCN1B* (Kelley and Kossoff, 2010; Scheffer et al., 2001; Wallace et al., 1998), *SCN2A* Scheffer et al., 2001; Sugawara et al., 2001; Wolff et al., 2017), *GABRG2* (Kelley and Kossoff, 2010; Scheffer et al., 2001; Kang and Macdonald, 2016), *CHD2* (Meller et al., 2017), *STX1B* (Vlaskamp et al., 2016), *SLC2A1* (Larsen et al., 2015; Mullen et al., 2011), *SLC6A1* (Carvill et al., 2015; Johannesen et al., 2018), and *GABRB3* (Meller et al., 2017)), analysis of these genes in previously reported EMAS cohorts has yielded positive results in a minority of patients, suggesting that the genetics of EMAS remains poorly understood. The purpose of this study is to assess the testing practices and diagnostic yield of clinical genetic evaluations in patients with a potential diagnosis of EMAS at a tertiary epilepsy center, as well as to review the existing literature regarding genetic associations with EMAS.

2. Material and methods

A retrospective chart review was conducted at Children's Hospital

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Colorado. Patients were identified from a clinical database of patients with a potential diagnosis of EMAS. The database included children with epilepsy onset between May 2004 and April 2017. Eligibility criteria included consideration of a diagnosis of EMAS by a child neurology faculty member at any point in the patient's course. A definitive diagnosis of EMAS was not required. Patients were excluded if on detailed review they never had any clinical or electrographic evidence of drop seizures. Drop seizures were defined as a brief, sudden event that could cause the patient to fall. Additionally, patients were excluded if they had a clear structural etiology on imaging. The cohort was defined by these clinically relevant parameters as evolution of individual patient phenotype over time can result in diagnosis switching and delay in final diagnosis (Eschbach et al., 2018), yet genetic testing may already be completed prior to this time. The full electroclinical spectrum of this cohort and diagnosis switching is described in detail in the initial publication on this cohort (Eschbach et al., 2018). Genetic testing was reviewed, including chromosomal microarray, mitochondrial DNA analysis, single gene testing, epilepsy panels, and whole exome sequencing (WES). Overall percentage of the cohort tested for key genes previously associated with EMAS (*SCN1A, SCN1B, SCN2A, GABRG2, CHD2, STX1B, SLC2A1, SLC6A1,* and *GABRB3)* through any form of testing was noted. For variants of uncertain significance (VUS), results of parental testing were also noted. Results of panel testing were considered "normal" if completely negative, or if all variants were identified in a heterozygous state in an autosomal recessive gene, in which case they would not be expected to be clinically relevant.

Pertinent biochemical testing was also reviewed to identify any metabolic abnormalities and to assess the utility of this testing in an EMAS cohort. This included 3-methylglutaconic acid, acylcarnitine, serum amino acids, ammonia, glucose, lactate, pyruvic acid, very long chain fatty acids, urine organic acids, and cerebrospinal fluid studies (amino acids, glucose, BH4/neopterin, folate/5MTHF, neurotransmitters, protein, and pyridoxal-5-phosphate).

Family history data was collected to determine the prevalence of epilepsy family history in the cohort and to assess whether familial variants had been inherited from symptomatic or asymptomatic individuals. Family history was specifically reviewed for childhood onset epilepsy, febrile seizures, intellectual disability, and autism, and any other relevant neurodevelopmental history was also noted. All data analyses were descriptive in nature using frequencies and percentages. This study was reviewed and approved by the University of Colorado Multiple Institutional Review Board (COMIRB) with waiver of consent.

3. Results

The cohort consisted of 77 patients with a suspected diagnosis of EMAS, including 19 females (25%) and 58 males (75%). As described in the earlier publication regarding this cohort, 50 patients (65%) had a change in epilepsy diagnosis over time and 57 patients (74%) had a final diagnosis of EMAS (Eschbach et al., 2018). Overall, 77% of patients (59/77) had at least one genetic test performed, and a definitive molecular diagnosis was reached for six (10%) of the patients who underwent testing. The breakdown of genetic and biochemical testing for this cohort is shown in Fig. 1.

Thirty-seven patients (48%) had a chromosomal microarray. Thirty of these were normal, five had copy number variants not felt to be clinically relevant, and one had multiple regions of homozygosity, which was expected given reported consanguinity. One patient had an abnormal finding that is potentially clinically significant; a 461 kb deletion at 2p16.3, including the *NRXN1* gene (Al Shehhi et al., 2018a). The details of this patient's phenotype are shown in Table 1A. Overall yield of microarray in this cohort was therefore 2.7%.

Sixteen patients (31%) had single gene testing, most commonly for *SCN1A, SLC2A1*, and *POLG1*, none of which yielded abnormal results. Thirteen of those patients subsequently had an epilepsy panel. There were four different epilepsy panels utilized with number of genes analyzed ranging from 38 – 89. This variability depended on the type of epilepsy panel ordered and date of testing. Some of these panels have been updated over time as new genes have been discovered (including several genes with a proposed association with EMAS), so the presence or absence of these genes was noted for each patient's panel. Overall, 51 patients (66%) had an epilepsy panel. Thirty-nine of these (76.5%) were normal, including 24 negative panels and 15 with one or more heterozygous variants in autosomal recessive genes. Ten patients (19.6%) had variants of uncertain significance in autosomal dominant genes, including three in the *GABRG2* gene, which has previously been associated with EMAS. Of the *GABRG2* variants identified, two were inherited from asymptomatic fathers and the inheritance is unknown for the other. Specifics of these patients' clinical presentations are shown in Table 1B. The remaining seven patients had variants which were inherited from an asymptomatic parent and /or were inconsistent with the patient's clinical presentation (Supplemental Table 1). Two patients (4%) were found to have pathogenic variants, one in the *SCN1A* gene and one in the *GABRG2* gene, both of which have previously been associated with EMAS. The overall yield of genetic testing in this cohort for the genes most commonly associated with EMAS is shown in Fig. 2.

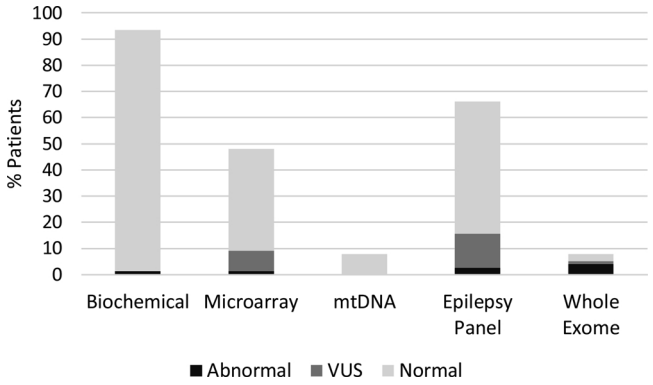

Fig. 1. Results of biochemical and genetic testing for patients with suspected EMAS. Black represents the percentage of patients with positive (abnormal) test results, dark gray represents patients with variants of uncertain significance, and light gray represents patients with normal testing.

Of the six patients who had whole exome sequencing, two were negative and one had variants of uncertain significance in *NIPA1, SZT2,* and *FAH*. Three patients had abnormal findings which were felt to at least partially explain their symptoms, including a *de novo* pathogenic variant in *CHD2*, a *de novo* likely pathogenic variant in *CSNK2A1,* and compound heterozygous variants in *PIGN*.

Of the 72 patients (93.5%) who had biochemical testing, only one (1.4%) had abnormal results significant enough to prompt additional studies, which was an elevation of 3-methylglutaconic acid. A methylglutaconic aciduria genetic testing panel was subsequently sent which was negative. Six patients (8%) had mitochondrial DNA testing, all of which was unremarkable.

Forty-two percent of patients had a family history of childhood-onset epilepsy (Fig. 3). An additional 19 percent had any family history of seizures, including febrile seizures, and adult-onset epilepsy. Four patients (5%) had no known family history of seizures, but did have relatives with other neurodevelopmental issues, including developmental delays, learning disabilities, and autism. Overall, 66% of patients had a positive family history for neurologic disease.

4. Discussion

While several genes have been described as associated with EMAS, no single gene has been implicated in the majority of patients with this diagnosis (Kelley and Kossoff, 2010). This retrospective review of genetic testing suggests that pathogenic variants in previously associated

Table 1A
Summary of pathogenic/ likely pathogenic variants identified in genes previously associated with EMAS, patients' clinical presentation, and final epilepsy diagnosis.

A. Pathogenic/ Likely Pathogenic Variants in Genes Previously Described in EMAS

Gene	Coding DNA	Variant	Classification	Mode of Inheritance	Parental Testing	Family History	Clinical Summary	Final Epilepsy Diagnosis
SCN1A	c.5104G > T	p.D1702Y	Likely Pathogenic	AD	Not Completed	Paternal history of seizures in grandfather, great aunt, and first cousin once removed.	History of febrile seizures and epilepsy onset within the first year of life with multiple seizures types, including generalized tonic-clonic, atonic, and absence seizures. Seizures are drug resistant and now s/p corpus callosotomy. Initial developmental delay / regression and now intellectual disability. EEG with diffuse background slowing and multifocal spikes. Normal brain MRI.	Dravet Syndrome
CHD2	c.3067-2A > G	IVS24-2A > G	Pathogenic	AD	*De Novo*	Father with history of generalized tonic-clonic seizures with onset at 18 years, now seizure free for 10 years.	Absence seizures beginning at 7 months, myoclonic-atonic, myoclonic, and myoclonic-absence seizures beginning at 2 years with ongoing drug resistant epilepsy. He had a single generalized tonic-clonic seizure at 10 years. Developmental delay after seizure onset, intellectual disability, and autism. EEG with diffuse slowing, generalized and multifocal spikes, and one with PFA. Normal brain MRI.	EMAS
GABRG2	c.770-1G > A	IVS6-1G > A	Pathogenic	AD	Not Completed	History of seizures in father which resolved by five years w/o meds, single seizure in brother.	Febrile seizure at 20 months, afebrile myoclonic, absence, and generalized tonic-clonic seizures beginning at 3 years. Now 11 years old and seizure free for 2 years on dual therapy. Normal development. EEG with generalized spikes, no PFA or SSW. MRI with non-specific scattered T2 hyperintensities.	EMAS

Table 1B
Summary of variants of uncertain significance identified in genes previously associated with EMAS, patients' clinical presentation, and final epilepsy diagnosis.

B. Variants of Uncertain Significance in Genes Previously Described in EMAS

Gene	Coding DNA	Variant	Classification	Mode of Inheritance	Parental Testing	Family History	Clinical Summary	Final Epilepsy Diagnosis
GABRG2	c.1112A > C	p.K371T	VUS	AD	Not Completed	Negative	Generalized tonic-clonic, myoclonic-atonic, and absence seizures beginning at 3 years, now seizure free for 3 years. Normal development. EEG with no PFA or SSW. Normal brain MRI.	EMAS
GABRG2	c.1088G > A	p.R363Q	VUS[a]	AD	Paternally Inherited (Father is asymptomatic)	History of seizures in mother and brother, both resolved by 5 years.	Epilepsy onset at 3 years old with multiple seizure types, including generalized tonic-clonic, myoclonic-atonic, atypical absence, and tonic. 10 years later with drug resistant epilepsy s/p VNS and corpus callosotomy. Developmental regression, intellectual disability, and autism. EEG with diffuse slowing, multifocal and generalized spikes, PFA, and SSW. Normal MRI brain.	Lennox-Gastaut Syndrome
GABRG2	c.1113_1115delAAA	p.K374del	VUS[b]	AD	Paternally Inherited (Father is asymptomatic)	Negative.	Generalized tonic-clonic and atonic seizures beginning at 2 years. Now at 6 years, has infrequent absence seizures s/p VNS and corpus callosotomy. Developmental and cognitive delay. EEG with generalized discharges and mild diffuse slowing. Normal brain MRI.	EMAS

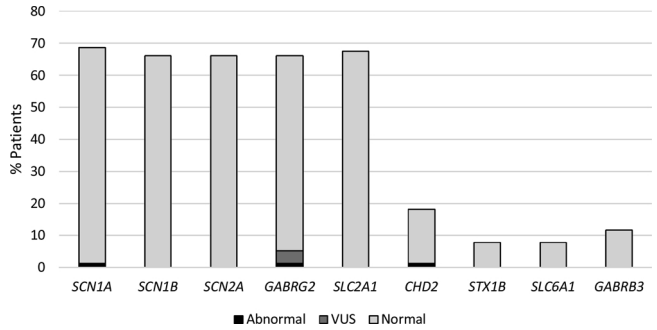

Fig. 2. Results of genetic testing for genes previously associated with EMAS. Black represents patients with pathogenic/ likely pathogenic variants, dark gray represents patients with variants of uncertain significance, and light gray represents patient with negative testing of the gene.

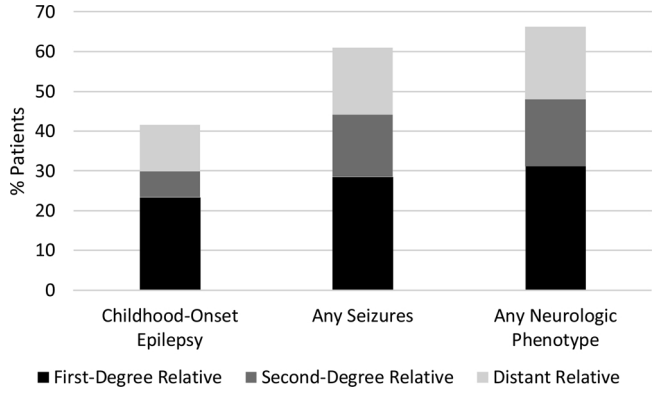

Fig. 3. Family history of childhood-onset epilepsy, of any history of seizures (including febrile and adult-onset), and of any neurologic phenotype (including epilepsy, febrile seizures, developmental delays, learning disabilities, and autism).

genes (*SCN1A, SCN1B, SCN2A, GABRG2, SLC2A1,* and *CHD2*) are relatively low yield, and the pathogenicity of variants can be uncertain in EMAS patients. Furthermore, only patients with mutations with *GABRG2* and *CHD2* had final diagnoses of EMAS, and the *CHD2* patient would be considered atypical for EMAS but did not fit neatly into another epilepsy syndrome. More recently described genes, including *SLC6A1, STX1B,* and *GABRB3* have not been included in clinical testing long enough to assess their yield. Three of the nine patients with a positive genetic testing result had significant family histories for epilepsy that did not segregate with the discovered gene. It is unclear to what degree these variants contribute to disease, if at all. The prevalence of each potential genetic etiology should be assessed in a large multicenter cohort that may help clarify which variants are sufficient to cause disease regardless of genomic background.

4.1. Sodium channelopathies

The *SCN1A* gene encodes a subunit of a sodium channel expressed primarily in the brain and involved in neuronal signaling (National Library of Medicine (US), 2018). *SCN1A* was reported in association with EMAS in a 2001 study in which an *SCN1A* mutation was identified in a family with a generalized epilepsy with febrile seizures plus (GEFS +) phenotype, including a single individual with EMAS (Escayg et al., 2001). Since then, several independent studies have identified additional *SCN1A* pathogenic variants in EMAS patients within GEFS + families, but rarely in sporadic cases, suggesting that *SCN1A* is not the primary genetic cause of EMAS (Ebach et al., 2005; Nabbout et al., 2003; Sun et al., 2008). Fifty-three of our 77 patients had evaluation of the *SCN1A* gene, including 51 epilepsy panels and two single gene tests. A single likely pathogenic missense variant was detected in the *SCN1A* gene (yield of 1.9%) and that patient was ultimately felt to have a phenotype more consistent with Dravet Syndrome (Table 1A). This variant was reported as a strong candidate for a disease-causing mutation, given its absence from a database of 6500 healthy individuals and the fact that the altered amino acid is in a highly conserved position within a crucial domain of the SCN1 A protein. While the patient was ultimately felt to have Dravet syndrome, there can be overlap between EMAS and Dravet syndrome which may make *SCN1A* testing reasonable, particularly early in a patient's course. Distinguishing between Dravet Syndrome and EMAS may improve with the consensus clinical criteria recently developed for Dravet syndrome, but this does not exclude some atypical Dravet syndrome presentations (Wirrell et al., 2017).

The *SCN1B* gene provides instructions for making a different sodium channel subunit expressed in the brain, skeletal muscle, and heart (National Library of Medicine (US), 2018). A study in 1998 identified a *SCN1B* pathogenic variant which segregated with disease in a large GEFS + family, including one individual with EMAS (Wallace et al., 1998). An additional study in 2003 involved testing of *SCN1A, SCN1B,* and *GABRG2* in 22 patients with sporadic EMAS; no causal mutations were identified (Nabbout et al., 2003). Fifty-one patients in our cohort were tested for *SCN1B* (all through epilepsy panels) and no pathogenic variants were identified.

The *SCN2A* gene encodes yet another sodium channel subunit involved in generation and propagation of action potentials in neurons and muscle (National Library of Medicine (US), 2018). Pathogenic variants in this gene can cause a variable phenotype ranging from an early infantile epileptic encephalopathy to self-limited familial infantile seizures (National Library of Medicine (US), 2018). An *SCN2A* mutation was described in a single Japanese patient with an EMAS phenotype in 2001 (Sugawara et al., 2001). The variant was inherited from his father, who had a history of two febrile seizures in childhood, but no afebrile events. An additional study of 71 patients with pathogenic *SCN2A* variants included *de novo* missense mutations in two patients with EMAS (Wolff et al., 2017). Fifty-one patients in our cohort had testing of this gene through an epilepsy panel, however no variants were identified.

Our results appear consistent with what has been previously reported in the literature – that sodium channelopathies are rare in patients with classic EMAS, and more common when there is a clear family history of epilepsy (particularly GEFS+). Given the phenotypic overlap between EMAS and Dravet syndrome, it is worth considering a diagnosis of Dravet syndrome in any EMAS patients found to have a pathogenic variant in the *SCN1A* gene.

4.2. GABRG2

The *GABRG2* gene encodes the gamma2 subunit of the gamma-aminobutyric acid (GABA) receptor type A protein (National Library of Medicine (US), 2018). While *GABRG2* pathogenic variants have been associated with an EMAS-like phenotype within GEFS + families, *GABRG2* mutations in patients with classic EMAS are rarely identified (Kelley and Kossoff, 2010; Scheffer et al., 2001; Sun et al., 2008). There is report of a single child with EMAS who was found to have a pathogenic variant in *GABRG2* as part of a targeted resequencing study of 500 epilepsy patients (including 81 with EMAS) in 2014 (Carvill et al., 2013). In the previously mentioned 2003 study, no mutations in the *GABRG2* gene were identified (Nabbout et al., 2003). Fifty-one of our patients underwent analysis of the *GABRG2* gene via an epilepsy panel. Only one patient was found to have a clear pathogenic variant (yield of 2%) (Table 1A). Three additional patients were found to have variants of uncertain significance in the gene (Table 1B). Two of these variants were inherited from asymptomatic fathers and did not provide strong evidence of pathogenicity. They are therefore likely benign, or contributing only through a polygenic mechanism. Parental studies have

not been completed for the third patient, who has classic EMAS and no pertinent family history. Interestingly, the patient with the pathogenic variant and the patient with the potentially pathogenic VUS (given that parental testing has not been completed) both had a more mild presentation with normal development, although they did still have multiple seizures types. This raises the question of whether these patients more closely resemble early-onset absence epilepsy, given the known association between *GABRG2* mutations and childhood absence epilepsy (Kang and Macdonald, 2016).

4.3. SLC2A1

SLC2A1 provides instructions for producing the glucose transporter type 1 (GLUT1) protein, which is the major transporter of glucose across the blood-brain barrier and within the brain itself (National Library of Medicine (US), 2018). *SLC2A1* was initially explored as a genetic cause of EMAS given the similar presentation to patients with GLUT1 deficiency syndrome (early onset absence and myoclonic seizures) and shared responsiveness to the ketogenic diet (Mullen et al., 2011). A 2011 study involving *SLC2A1* analysis of 84 patients with EMAS identified four pathogenic variants (yield of 5%), although three of these patients had additional features not typically associated with EMAS and the fourth had a family history of absence epilepsy and movement disorders (Mullen et al., 2011). Three of these four patients had confirmatory CSF studies demonstrating hypoglycchorachia (Mullen et al., 2011). A similar study in 2015 of 120 patients with EMAS did not identify any pathogenic variants in the *SLC2A1* gene (Larsen et al., 2015). These two studies suggest that *SLC2A1* mutations may not be a genetic cause of classic EMAS, and are more likely to be found in patients with an "EMAS+" phenotype; however, given the potential treatment implications, this is still an important gene to consider in the genetic workup of patients with a potential diagnosis of EMAS. Fifty-two patients in our cohort had testing of the *SLC2A1* gene, including 51 panels and one single gene test. All testing was negative for variants within *SLC2A1*.

4.4. CHD2

CHD2, which codes for a chromodomain helicase DNA-binding protein, is important for many essential cellular processes and has been associated with a variety of epilepsy and neurodevelopmental disorders (National Library of Medicine (US), 2018). A targeted resequencing study in 2014 identified *CHD2* mutations in two of 81 patients with EMAS, and a second study in 2015 identified a mutation in one of 20 EMAS patients (Trivisano et al., 2015). While pathogenic variants in the *CHD2* gene have been reported in several patients with an EMAS-like phenotype, the patients described in the literature do not appear to have a classic EMAS presentation (specifically, the majority had developmental delay prior to seizure onset) (Trivisano et al., 2015). Only thirteen of our patients had analysis of the *CHD2* gene (including seven through panel testing and six through WES), as it was not included on epilepsy panels until early 2016. There was a single patient found to have a *de novo* pathogenic variant in *CHD2*, detected through whole exome sequencing (yield of 7.7%). Interestingly, this patient is now ten years old with a classic EMAS phenotype, including typical development prior to seizure onset. However, he continues to have ongoing drug-resistant epilepsy.

4.5. More recently-described EMAS genes

The *STX1B* gene encodes for the syntaxin-1B protein, which interacts with the protein encoded by the previously described *STXBP1* gene to regulate synaptic transmission of neurotransmitters (National Library of Medicine (US), 2018). *STX1B* was first described in 2012 in a cohort of patients with fever-associated epilepsy syndromes, including two patients with EMAS (Schubert et al., 2014), and an additional EMAS patient with a full gene deletion of *STX1B* was reported in a 2016 case report (Vlaskamp et al., 2016).

The *SLC6A1* gene encodes a neuronal GABA reuptake transporter (National Library of Medicine (US), 2018). *SLC6A1* was identified as a cause for neurodevelopmental disorders through two independent whole exome sequencing studies, which showed *de novo* mutations in two patients with intellectual disability and autism (Carvill et al., 2015). A 2015 publication provided further evidence for *SLC6A1* as a genetic cause for EMAS (Carvill et al., 2015). This included discussion of an EMAS patient with a full gene deletion of *SLC6A1,* and identification of likely pathogenic *SLC6A1* variants in four of 85 EMAS patients in an additional cohort of patients with epileptic encephalopathies. A subsequent validation cohort of 75 EMAS patients identified *SLC6A1* mutations in two additional patients, including one inherited from a mother also diagnosed with EMAS. A recently published study with the aim of defining the spectrum of *SLC6A1* mutations found that while many patients with pathogenic variants in this gene do have a presentation consistent with EMAS (16/34 in this study), there are other phenotypes that can be seen, including childhood absence epilepsy, early-onset absence epilepsy, and other unclassified generalized epilepsy disorders, as well as a few patients with intellectual disability, but no seizure history (Johnannesen et al., 2018).

The *GABRB3* gene encodes the beta3 subunit of the gamma-aminobutyric acid (GABA) receptor type A protein (Meller et al., 2017). Mutations in *GABRB3* have been reported in large-scale studies of patients with severe epilepsy disorders (Meller et al., 2017). A 2017 study involving *GABRB3* sequencing for 416 patients with childhood-onset epilepsy identified mutations in 22 patients, including five with a diagnosis of EMAS, leading the authors to propose *GABRB3* as an EMAS-associated gene (Meller et al., 2017). Only three patients in this cohort had an epilepsy panel including the *GABRB3* gene, which was added to most panels in early 2017, and no mutations in *GABRB3* were identified.

Because the majority of the patients in our cohort had much of their genetic workup prior to 2016, none of the epilepsy panels in this group included the *STX1B* or *SLC6A1* genes. None of the six patients who had whole exome sequencing were found to have mutations in *STX1B, SLC6A1,* or *GABRB3*. While WES would have identified any sequence variants present, and chromosomal microarray would have detected larger deletions or duplications, exon-level copy number variants may not have been detected through either of these methods. Therefore, even patients who have had an extensive genetic workup may not have been sufficiently tested for any of these more recently described genes.

4.6. Other positive results

There were three additional patients in our cohort found to have pathogenic or likely pathogenic variants in genes that have not previously been associated with EMAS (Table 1C). The first is a 2p16.3 deletion including the first five exons of the *NRXN1* gene, identified in an eight-year-old male by chromosomal microarray. This patient has an epilepsy phenotype which could be consistent with an atypical EMAS presentation, with generalized tonic-clonic, myoclonic, and frequent absence seizures with onset at five years of age, and mild language and fine motor delays. *NRXN1* encodes for a neurexin protein, a cell-surface receptor protein which is required for efficient neurotransmission and involved in the formation of synaptic contacts (National Library of Medicine (US), 2018). Pathogenic variants in the *NRXN1* gene have been previously associated with intellectual disability, severe language delay, autism spectrum disorder, seizures, and hypotonia, with variable expressivity (National Library of Medicine (US), 2018)· *NRXN1* gene mutations demonstrate an estimated penetrance of 46% (Al Shehhi et al., 2018b) with higher rates of penetrance noted in patients with exonic deletions including one or more of exons 1–4, as in our patient (Lowther et al., 2017). This suggests that this deletion may indeed be pathogenic, although the variability in phenotype of *NRXN1* gene

Table 1C
Summary of pathogenic/ likely pathogenic variants identified in genes not previously associated with EMAS, patients' clinical presentation, and final epilepsy diagnosis.

C. Pathogenic/ Likely Pathogenic Variants in Genes Not Previously Described in EMAS

Gene	Coding DNA	Variant	Classification	Mode of Inheritance	Parental Testing	Family History	Clinical Summary	Final Epilepsy Diagnosis
NRXN1	Exon 1 – 5 deletion		Pathogenic	AD	Not Completed	No history of seizures. Brother with developmental delay and autism.	Myoclonic, generalized tonic-clonic, and absence seizures beginning at 5 years. Frequent atypical absence vs. myoclonic absence seizures at 8 years. Fine motor, language, and cognitive delay. EEG with multifocal and generalized spikes and occasionally SSW. Normal brain MRI	Genetic generalized epilepsy vs. atypical EMAS
PIGN	c. 1940delA; c.932T > G	p.K647Rfs*12; p.L311W	Pathogenic; Likely Pathogenic	AR	Paternally Inherited; Maternally Inherited	Mother with seizures in infancy, resolved by 2 years. Paternal great uncle with seizures in childhood.	Myoclonic seizures with neonatal onset and now ongoing atypical absence, myoclonic, and myoclonic-atonic seizures. Seizures intractable at 5 years, s/p corpus callosotomy. Developmental and cognitive delay, nonverbal. EEG with diffuse slowing, generalized and multifocal spikes, and SSW. Normal brain MRI.	Lennox-Gastaut Syndrome
CSNK2A1	c.426+1G > T	IVS6+1G > T	Likely Pathogenic	AD	*De Novo*	Maternal great uncle and two maternal second cousins with seizures.	Seizure onset in the first year of life with drug resistant myoclonic and tonic seizures, later development of absence and myoclonic-tonic seizures. Mild developmental delay. Language and cognitive delay. EEG with diffuse slowing and generalized spikes, rare slow spike-and-wave. Normal MRI brain.	Genetic generalized epilepsy

Abbreviations: PFA = paroxysmal fast activity, SSW = slow spike-and-wave. a = clinical interpretation likely benign given inheritance from asymptomatic father in patient with maternal family history of seizures b = clinical interpretation likely benign given inheritance from asymptomatic father.

mutations, as with other recently identify epilepsy genes, makes it difficult to determine if the mutation is truly pathogenic or acts as a susceptibility factor for neurodevelopmental disorders, including epilepsy (Demarest and Brooks-Kayal, 2018).

Additionally, whole exome sequencing identified compound heterozygous variants (one pathogenic, one likely pathogenic) of the *PIGN* gene in one patient. This patient is a five-year-old male who was at one point diagnosed with EMAS, however his course is more consistent with an early onset epileptic encephalopathy that evolved into Lennox-Gastaut syndrome. He presented with myoclonic seizures in the neonatal period, and later developed myoclonic-atonic, myoclonic-absence, atypical absence, and generalized tonic-clonic seizures. His seizures have been refractory to numerous anti-seizure medications. He also has moderate to severe developmental delay, vision impairment, hypotonia, and dysmorphic features. The *PIGN* gene encodes a protein involved in anchoring proteins to the cell surface (National Library of Medicine (US), 2018). Pathogenic variants in this gene are known to cause autosomal recessive multiple congenital anomalies-hypotonia-seizures syndrome 1 (MCAHS1), characterized by neonatal hypotonia, severe developmental delays, seizures, dysmorphic features, and variable congenital anomalies, consistent with our patient's presentation (National Library of Medicine (US), 2018).

A third genetic change not previously reported in EMAS patients was identified in an eight-year-old female. Whole exome sequencing revealed a *de novo*, likely pathogenic, intronic variant (predicted to cause abnormal gene splicing) in *CSNK2A1*. She has myoclonic and tonic seizures with onset in infancy, which responded well to felbamate. She has mild cognitive and language delays, but has caught up significantly since her seizures have improved. While initially felt to have EMAS, she now has a less specific diagnosis of genetic generalized epilepsy. The *CSNK2A1* gene encodes a serine/threonine protein kinase which is highly expressed in the brain and important for proper brain development and function (National Library of Medicine (US), 2018). Heterozygous pathogenic variants in this gene have been reported in patients with developmental delay, intellectual disability, behavioral problems, hypotonia, microcephaly, and pachygyria (National Library of Medicine (US), 2018). While our patient has epilepsy and developmental delay but lacks the other features, very few patients with *CSNK2A1* mutations have been reported thus far, therefore it is possible that the full spectrum of this disorder is yet to be defined.

Biochemical testing did not yield any positive results in this cohort. For the single patient with an elevated 3-methylglutaconic acid level, further testing with a methylglutaconic aciduria gene panel was negative. Mitochondrial DNA analysis also did not result in any diagnoses, although only six patients in the cohort had this testing. Single gene testing was performed for 16 patients with no positive results. Thirteen of these patients later had a full epilepsy panel, leading to a diagnosis in one patient. While single gene testing was common during the first several years of this analysis, panel testing is now more common, as it is more efficient and cost effective. This approach is also typically more practical in disorders such as epilepsy where there is a lot of phenotypic overlap, making it more difficult to pinpoint one specific gene.

The primary limitation of this study is the retrospective design. Due to the retrospective review, testing was performed over several years and sometimes sent by institutions other than our own. Additionally, the evaluation for each patient was not standardized. As testing has become more comprehensive, accessible, and affordable, many of the more newly diagnosed patients have had a more thorough genetic workup than those who presented five to ten years ago. Even the more recently diagnosed patients in our cohort had testing prior to *SLC6A1, STX1B,* and (in some cases) *CHD2,* being added to commercial epilepsy panels, giving us little ability to determine the true yield of testing for these genes in EMAS patients. An additional limitation of this study is the accuracy of phenotyping. While all patients in the cohort were given a clinical diagnosis of EMAS at some point in their course, this was not always the final diagnosis. This is a persistent problem in the literature as there is a lack of consensus regarding the features that define EMAS, as well as significant variability in clinical practice (Nickels et al., 2018).

5. Conclusions

EMAS is widely accepted as having a strong genetic component which is supported by the frequent family history of epilepsy in these patients, although complex family histories that do not segregate with pathogenic mutations raise the possibility of polygenic inheritance patterns. *SCN1A* mutations may play a role in EMAS but may better represent the phenotypic variation seen in Dravet Syndrome. *SCN2A* and *SCN1B* have an unclear role in EMAS and likely explain very few patients. *GABRG2* patients seem to have significant overlap with early onset absence epilepsy patients with normal development. This study supports an overlap between the *CHD2* spectrum of disease and EMAS and adds *NRXN1* as a potential etiology for these patients. *SLC6A1*, *STX1B,* and *GABRB3* are potentially important etiologies in EMAS and require ongoing study, however they have only recently been added to the more commonly ordered epilepsy panels. This highlights the importance of selecting a laboratory and panel that is updated on a regular basis to include analysis of newly classified genes. Given the clinical heterogeneity of many of the genes associated with EMAS and potential for evolution of phenotypes over time (Eschbach et al., 2018), it is worth considering whole exome/genome sequencing platforms early in the diagnostic algorithm for early onset generalized epilepsies. This will be more practical as the cost of whole exome/genome sequencing platforms become more economical and may reduce the delay in updating panels with rapid knowledge regarding gene associations. Further research involving whole exome/genome sequencing, as well as targeted testing of genes previously implicated in EMAS, will help to further elucidate the yield of known genes and identify new candidate genes for this disorder.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.eplepsyres.2019.01.008.

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