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Novel pathogenic *ALG2* mutation causing congenital myasthenic syndrome: A case report

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Abstract

ALG2 mutations are extremely rare causes of congenital myasthenic syndromes (CMS). The clinical phenotype and treatment response is therefore not well described. We present the case of a baby who immediately after birth presented with pronounced truncal hypotonia, proximal muscle weakness and feeding difficulties. Single fibre electromyography showed neuromuscular transmission failure and salbutamol and ephedrine treatment improved both muscle weakness and neuromuscular transmission. Genetic analysis revealed a likely pathogenic variant c.1040del, p.(Gly347Valfs*27) in exon 2 and a variant of uncertain significance, c.239G>A, p.(Gly80Asp) in exon 1 of the *ALG2* gene. Western blot in whole cell lysates of HEK293 cells transfected with p.Gly80Asp, or p.Gly347Valfs*27 expression constructs indicated that p.Gly347Valfs*27 is likely a null allele and p.Gly80Asp is pathogenic through marked reduction of ALG2 expression. This case highlights the utility of functional studies in clarifying variants of unknown significance, in suspected cases of CMS.

Keywords: Congenital myasthenic syndrome; CMS; ALG2; Salbutamol; Case report.

1. Introduction

Congenital myasthenic syndromes (CMS) constitute a heterogeneous group of disorders caused by mutations in genes encoding the pre-, intra-, or postsynaptic proteins of the neuromuscular junction (NMJ). The pattern of inheritance in CMS is usually autosomal recessive and the characteristic phenotype is exercise induced weakness and fatigability. The diagnosis is particularly challenging in the limb-girdle CMS (LG-CMS), where ptosis may be absent[1]. Instead, in LG-CMS, the diagnosis is clinically supported by limb-girdle weakness accompanied by disturbed neuromuscular transmission on repetitive nerve stimulation (RNS) or single fibre electromyography (SFEMG)[1]. Genetic aetiology plays a pivotal role for which treatment strategy to choose[2]. Different subgroups of CMS respond to different treatments, such as acetylcholinesterase inhibitors (AChEIs), selective $\beta 2$ adrenergic agonists (salbutamol), α- and β adrenergic agonists (ephedrine), 3,4-diaminopyridine and fluoxetine. LG-CMS is less commonly associated with mutations in the *ALG2* gene [1,3], which encodes an α-1,3-mannosyltransferase that catalyses early steps in the asparagine-linked glycosylation pathway[4], important for glycosylation of NMJ proteins. Since *ALG2* mutations are an extremely rare cause of CMS, the clinical phenotype and treatment response is less well defined.

2. Case report

We present a nonconsanguineous Swedish family with one female child confirmed to have two variants in the *ALG2* gene. She was compound heterozygous for a likely pathogenic c.1040del, p.(Gly347Valfs*27) variant and a c.239G>A p.(Gly80Asp) variant of unknown significance (VUS).

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The child was born full term after an uneventful pregnancy and delivery. Birth weight was normal (3,450 grams). The family history was unremarkable; her parents and a 20-month older male full sibling were all healthy. She was hypotonic at birth and pregnancy was only notable for prenatally identified bilateral club foot deformity. At day 1 after birth, a paediatric neurological examination revealed pronounced truncal hypotonia and proximal muscle weakness, absence of Moro reflex (most likely due to muscle weakness) and tendon reflexes as well as weak grip reflexes in both hands and feet. Her facial expression and pupillary light reflexes were judged normal, but the suction reflex was absent. There was a suspicion of slight dysmorphic features, such as low-seated ears. Due to her hypotonia and feeding difficulties, she immediately received a nasogastric tube, which could be removed at 8 weeks of age. She was treated in the neonatal intensive care unit due to nutritional and respiratory difficulties, including inspiratory stridor and weak cry; however, ventilatory support was never needed. A rhinolaryngoscopy performed by an ear, nose and throat consultant demonstrated laryngomalacia. Echocardiography performed by a paediatric cardiology consultant was normal. Genetic testing for spinal muscular atrophy was negative (normal *SMN1* gene analysis) as was microarray analysis.

Due to suspected neuromuscular disease, further medical examinations were performed at two weeks of age at the department of clinical neurophysiology, Uppsala University Hospital. Within the differential diagnosis work-up, motor and sensory nerve conduction studies revealed normal amplitudes and conduction velocities for her age and electromyography (EMG) of the left lateral vastus muscle showed absence of abnormal spontaneous activity and normal motor unit potentials (Fig. 1A). Low frequency RNS at 3 Hz was performed with recordings of the abductor digiti quinti (ADQ) and trapezius muscles, although due to technical reasons it was not possible to conclude a pathological decrement. Especially in the ADQ, RNS was technically difficult to perform due to the short distances and discomfort [5]. SFEMG during voluntary contraction in the left orbicularis oculi muscle showed abnormal jitter in 15 out of 19 examined fibre pairs, with blockings in several pairs, with a mean consecutive difference (MCD) jitter value of 120 μs, markedly elevated compared to the suggested reference values below 45 μs in infants[6].

Due to the abnormal SFEMG findings of disturbed neuromuscular transmission and coherent clinical findings, the most comprehensive gene panel available at that time for CMS-diagnostics was ordered (Invitae 16-gene panel; *AGRN, ALG2, CHAT, CHRNA1, CHRNB1, CHRND, CHRNE, COLQ, DOK7, DPATG1, GFPT1, GMPPB, MUSK, PREPL, RAPSN* and *SLC5A7*). A likely pathogenic variant c.1040del, p.(Gly347Valfs*27) in exon 2 and a VUS, c.239G>A, p.(Gly80Asp) in exon 1 were identified in the *ALG2* gene. Through parental testing, the variants were confirmed to be on opposite alleles. The likely pathogenic variant in exon 2 results in a premature translational stop signal in the *ALG2* gene (p.Gly347Valfs*27), which is expected to disrupt the last 70 amino acids of the ALG2 protein and has been reported to affect ALG2 protein function. The VUS in exon 2 results in a replacement of glycine in codon 80 to aspartic acid in the ALG2 protein. The glycine residue is weakly conserved and there is a moderate physiochemical difference between glycine and aspartic acid. In addition, the variant had not been reported in the literature on individuals with *ALG2*-related conditions. Hence, the available evidence was insufficient to determine the role of the variant in disease. Serum acetylcholine receptor (AChR) and muscle specific tyrosine kinase (MuSK) antibodies were absent.

Based on the SFEMG and clinical findings, a treatment trial with AChEI, pyridostigmine, was initiated at 3 weeks of age with weekly incremental dosing until a maximum dosage of 6.7 mg/kg/day. Neither the parents nor the physiotherapist noted any obvious clinical improvement upon AChEI treatment. Therefore, a follow-up SFEMG examination was performed 1.5 hours after intake of pyridostigmine, one month later. Quantitative analysis of 9 muscle fibre pairs showed increased jitter in 5 pairs (blockings in 4 pairs) with a mean MCD of 115 μs, basically unchanged compared to the first SFEMG examination. Based on this, AChEIs were judged not to have any beneficial effect on the proximal muscle weakness nor neuromuscular transmission. Instead, β2-receptor agonist treatment (salbutamol 0.26 mg/kg/day divided into three doses) was initiated at 8 weeks of age with subsequent and continuous improvement of muscle weakness mainly in her arms, shoulders and trunk. A follow-up electrophysiological examination at 14 months of age showed no decrement in the ADQ muscle on RNS. SFEMG of the orbicularis oculi muscle showed only 4 out of 13 fibre pairs with increased jitter and the rest with normal jitter, mean MCD was clearly improved at 40 μs. Despite a delayed acquisition of gross motor skills throughout her first 2 years, her muscle strength has continued to improve. Major motor milestones, like rolling over from front to back and vice versa, getting into sitting position and crawling were achieved by 11-, 17- and 18 months, respectively. At 27 months of age, ephedrine was introduced as an add-on treatment (slowly titrated to 1.4 mg/kg/day divided into three doses) with a clear parentally reported improvement in functional outcomes and less fatigability, in addition to improved neuromuscular transmission on SFEMG. At the last clinical follow-up (at 34 months of age), she was able to get up from prone to standing position, sit without support, and move around by crawling and using a walker.

To verify a functional effect on *ALG2*, analysis of serum transferrin proteoforms was done at Skåne University Hospital, Sweden, but no abnormalities were found. Further analysis with Western blot in whole cell lysates of HEK293 cells transfected with pcDNA3.1-*ALG2* wild type, p.Gly80Asp, or p.Gly347Valfs*27 expression constructs (Fig. 2) was performed at Oxford University, UK (as previously described in [3]). A protein band at approximately 45 kDa is seen for wild type *ALG2*, but expression is greatly reduced for *ALG2* harbouring the missense mutation p.Gly80Asp, and for the frameshift p.Gly347Valfs*27 a corresponding protein band is not clearly visible indicating

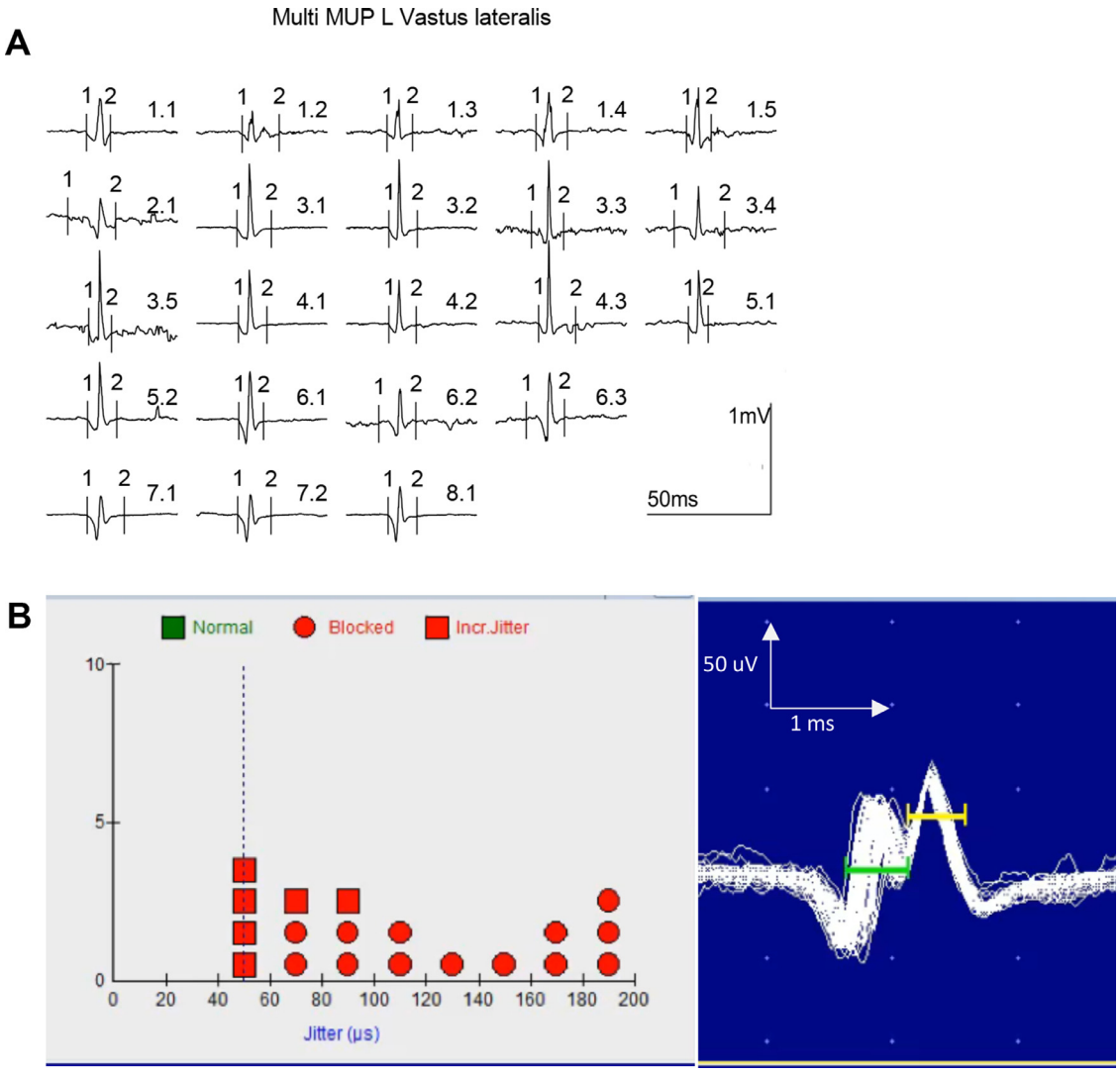

Fig. 1. Electrophysiological evaluation at age 2 weeks. A) Electromyography (EMG) of the left lateral vastus muscle revealed normal motor unit potentials of normal duration and amplitude. B) Single-fibre EMG of the left orbicularis oculi muscle showed abnormal jitter with a mean MCD of 120 μs (left panel) and several pairs with blockings, an example of jitter in one muscle fibre pair (right).

either this truncated protein is unstable or nonsense-mediated decay of the mRNA transcript. Thus, p.Gly347Valfs*27 is likely a null allele and p.Gly80Asp is pathogenic through a marked reduction of expression.

3. Discussion

The diagnosis of CMS is made based on clinical phenotype and ≥1 of the following criteria: neuromuscular transmission failure on RNS and/or SFEMG, molecular genetic confirmation, response to pyridostigmine in addition to absence of serum antibodies against AChR and MuSK. Confirmation of the genetic diagnosis is critical, since incorrect treatment in CMS may have detrimental effects or even be dangerous [7]. For example, patients harbouring mutations in AChR subunits giving rise to slow channel syndrome or in the LRP4-MUSK-DOK7 pathway may be worsened by AChEIs. In our case, the diagnosis of CMS was strongly suggested by the finding of neuromuscular transmission failure along with two different variants in *ALG2*. However, since bi-allelic pathogenic or likely pathogenic variants would strengthen our preliminary diagnosis, further variant classification was performed. Through Western blot analysis and finding of a significantly reduced *ALG2* protein expression the diagnosis could finally be confirmed.

The first case of *ALG2* related CMS was described in 2003 as a multisystemic disorder with intellectual disability, defective myelinization, seizures, coloboma of the iris, hepatomegaly and coagulation abnormalities [8]. Since then, only a handful of cases have been reported, predominantly with LG-CMS [1,3]. Our presented case is in line with previously published cases with predominantly disturbed neuromuscular transmission; manifested as delayed motor milestones, hypotonia and primarily proximal muscle weakness [1,3]. Nevertheless, our case did not

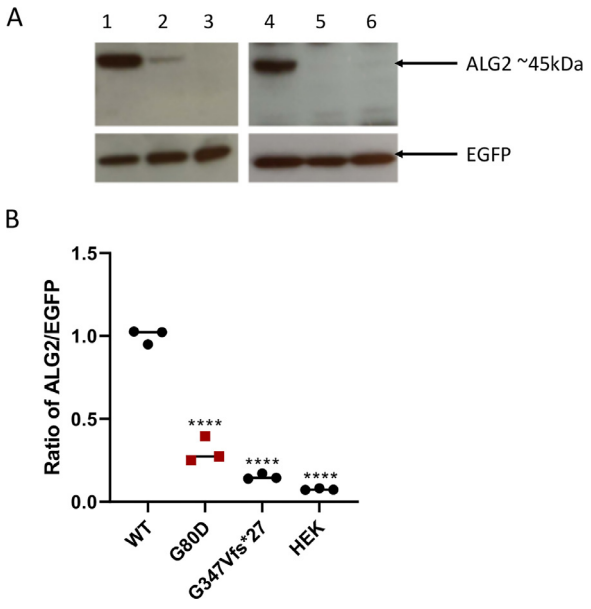

Fig. 2. **Expression of *ALG2* variants in HEK293 cells**. HEK 293 cells were seeded at 3.5×10^5 cells per well on a 6-well plate and transfected with 3µg pcDNA3.1-*ALG2* wild type or mutants. 48 hours after transfection, expression levels were analysed by western blotting. **A)** Representative western blots from whole cell lysates transfected with pcDNA3.1-*ALG2* wild type, -p.Gly80Asp (G80D), or -p.Gly347Valfs*27 (G347Vfs*27) constructs. *ALG2* was detected using rabbit polyclonal anti-*ALG2* antibody (Aviva, ARP48974_P050, 1:1000). *EGFP* was detected using rabbit polyclonal anti-GFP antibody (Abcam, Ab6556, 1:2000). Lane 1: *ALG2* WT transfected lysate. Lane 2: *ALG2* G80D transfected lysate. Lane 3: *EGFP* transfected lysate. Lane 4: *ALG2* WT transfected lysate. Lane 5: *ALG2* G347Vfs*27 transfected lysate. Lane 6: *EGFP* transfected lysate. B) Quantitation of the levels of *ALG2* expression. Experiments were carried out three times. *ALG2* protein expression was quantified as ratio of *ALG2/EGFP*. HEK293 transfection with *EGFP*-alone was used as a negative control. **** $p<0.0001$, one-way ANOVA with multiple comparisons.

improve clinically on AChEIs, which have previously been suggested to improve the clinical picture [3]. Instead, our case significantly improved both clinically and regarding neuromuscular transmission failure on SFEMG with $\beta 2$ adrenergic agonists (salbutamol) and ephedrine. These observations are supported by a recent study in a zebrafish model of CMS caused by defects in two other postsynaptic proteins MUSK and DOK7. The study found that $\beta 2$ adrenergic agonist (salbutamol) caused morphological improvement of neurogenesis, with improvement of postsynaptic AChR clustering and size of synaptic contacts most likely through the cAMP dependent pathway [9]. Thus, it is important to consider alternative treatment strategies (i.e., $\beta 2$ adrenergic agonists and ephedrine) when AChEIs fail. A beneficial response to these agents could be equally suggestive of a CMS diagnosis.

The strengths of this case report include functional assessment of the found VUS in *ALG2* on protein expression and how this impacts the clinical and electrophysiological picture as well as treatment response; verifying the CMS diagnosis. Limitations include, as always with case reports, limited ability to generalize the specific findings to other cases.

In summary, availability of genetic testing for CMS has increased the ability to identify causative genes. Additional functional studies can be helpful in expanding our knowledge of variants of unknown significance. Sharing information on clinical and electrophysiological phenotype and treatment responses are important for optimal management of the patients with rare CMS types.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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