Published in final edited form as:

J Clin Neuromuscul Dis. 2017 March ; 18(3): 147–151. doi:10.1097/CND.0000000000000132.

A Novel Missense Variant in the *AGRN* Gene; Congenital Myasthenic Syndrome Presenting With Head Drop

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Abstract

Congenital myasthenic syndromes (CMS) are a heterogeneous group of diseases of the neuromuscular junction caused by compromised synaptic transmission. Clinical features include early-onset weakness of limbs and oculobulbar muscles resulting in hypotonia, bulbar paresis, ptosis, and hypoventilation. The first dropped head syndrome in children were detected in 2 patients with *LMNA* and *SEPN1* mutations. We report a 17-month-old boy with dropped head and limb-girdle weakness, who had no ptosis or ophthalmoplegia at presentation. We performed whole exome sequencing, which revealed a homozygous missense variant in the *AGRN* gene c.5023G>A, p.Gly1675Ser in the LG2 domain, which is predicted to be likely disease causing by in silico tools. Agrin is known to play a critical role in the development and maintenance of the neuromuscular junction. Agrin-related CMS is one of the rarest subtypes. Of note, our patient is the first described patient with agrin-related CMS with dropped head phenotype.

Keywords

congenital myasthenic syndrome; *AGRN*; dropped head; whole exome sequencing

INTRODUCTION

Congenital myasthenic syndromes (CMS) are a group of inherited diseases of the neuromuscular junction (NMJ), where the transmission is compromised at the motor endplate.¹ Typical presentations of CMS include early-onset weakness and/or fatigability of limbs and oculobulbar muscles resulting in hypotonia, bulbar paresis, ptosis, hypoventilation, and apneic episodes.² The first dropped head syndrome defined in children

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CONSENT

Written informed consent was obtained from the patient for publication of this case report and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

The authors report no conflicts of interest.

were in 2 patients with *LMNA* and *SEPNI* mutations.³ In the literature, no CMS patient with dropped head phenotype has been reported.

CASE REPORT

Informed consent was obtained from the parents for genetic investigations, recording, and publishing the disease-related information. The proband of Turkish origin, from a consanguineous marriage between first cousins, was born with normal weight and APGAR scores. There was no described hypoventilation or apneic episodes during the early postnatal period. He was able to hold his head, sit unsupported, stand and walk unsupported a few steps by 1 year of age, but beginning at age 1, his parents noticed frequent head droppings and neck weakness throughout the day. First visit to pediatric neurology clinic at 17 months of age revealed prominent neck extensor muscle weakness along with mild limb-girdle weakness. There was no ptosis, ophthalmoplegia, or distal muscle weakness at presentation. Creatine kinase level was 84 U/L. His language and social skills were consistent with his age. Over the following year, he was followed with a provisional clinical diagnosis of nonprogressive congenital myopathy with neck weakness (Fig. 1).

At 30 months of age, he presented with bilateral ptosis and restrictive eye movements deteriorating during the day. His weakness of extensor neck muscles and lordotic gait were still present. At this point, the clinical picture was more consistent with myasthenia, thus screens for acetylcholine receptor antibodies and muscle-specific receptor tyrosine kinase (MuSK) were done, with negative results. Electromyographic study showed 10%–30% electrodecremental response during repetitive stimulation at 3.5 Hz stimulation of the right ulnar nerve, whereas no decline was observed at 20 Hz stimulation. His initial presentation with prominent neck weakness sparing the eyes was not considered as a typical presentation of CMS. Thus, we considered *LMNA* -or *SEPNI*-related myopathies in the differential diagnosis. In the interim, pyridostigmine and salbutamol treatment was started based on the progression of symptoms consistent with CMS. During his most recent visit at 5 years of age, he still had difficulty in holding head in an upright position. However, it was observed that the patient's symptoms typically improved after salbutamol and the first few hours of pyridostigmine administration.

As the yield achieved from all diagnostic tests was unsatisfactory, whole exome sequencing (WES) was performed. WES of the proband's DNA isolated from peripheral blood revealed a homozygous missense variant in the *AGRN* gene in exon 29, g.chr1:985853G>A, c.5023G>A, p.Gly1675Ser (NM_198576), which encodes agrin. This variant was not present in the Exome Variant Server databases but was detected in the heterozygous state in 3/8548 East Asian and 1/64,402 European chromosomes by the Exome Aggregation Consortium. Glycine at 1675 is conserved in vertebrates, and analysis of the serine variant at this position is predicted to be disease causing by Mutation Taster. Review of the WES data did not reveal any potential pathogenic mutation in *SEPNI*, *LMNA*, or any other known muscle disease-related genes. The *AGRN*Gly1675Ser variant was confirmed by Sanger sequencing in the proband and his parents. As predicted from the WES results, the proband appeared homozygous for the chr1:985853A variant. The sequence chromatograms for DNA from both parents clearly contained both the normal (control) peak representing a G at this

position and an overlapping second A peak, demonstrating that both are heterozygotes for the same mutation (Fig. 1).

DISCUSSION

The patient described in this report harbor a homozygous Gly1675Ser mutation in the laminin globular domain (LG2) of the agrin protein. Agrin is a heparan sulfate proteoglycan known to play a critical role in the development and maintenance of the NMJ.⁴ Together with the low-density lipoprotein receptor-related protein 4 (LRP4) and MuSK, agrin forms agrin–MuSK–LRP4 signaling system, which is crucial for the development and maintenance of NMJ.⁵ Secreted by the nerve terminal into the synaptic space, agrin plays the trigger role in activation of MuSK and clustering of MuSK and LRP4.⁵

Agrin-related CMS is very uncommon. A recent study from the United Kingdom assessing the prevalence of genetic defects in childhood myasthenia revealed *CHRNE*, *RAPSN*, and *DOK7* as the most common genes identified in the patients, and no *AGRN* mutation was detected in the study.⁶ However, we acknowledge that the prevalence of subtypes in the United Kingdom may not represent the prevalence of the subtypes in Turkey.

Nicole et al described 5 patients from 3 different kinship in different range of age groups. Homozygous and heteroallelic mutations dispersed through the entire gene resulted in a distinct phenotype associated with slowly progressive distal muscle weakness and muscle atrophy with fat replacement sparing axial and ocular muscles (Table 1).⁸ Maselli et al⁴ reported a different and more severe phenotype in a 39-year-old man, including respiratory failure, ophthalmoplegia, and facial weakness, which were caused by 2 heteroallelic mutations (p.Gln353* and p.Val1727Phe). The mutations were shown to be located in follistatin-like domain and laminin globular domain (LG2) of the protein (Table 1). Huze et al⁷ reported a milder phenotype in 42- and 36-year-old siblings resulting from a missense homozygous mutation (Gly1709Arg), in which bilateral ptosis, mild external ophthalmoplegia, and thin thorax were prominent features. The mutation was located in the LG2 domain, and in vitro expression studies revealed that the mutation perturbs endplate maintenance without interfering postsynaptic differentiation.⁷ The mutation identified in our patient is predicted to be located in LG2 domain based on the known structure of the mouse agrin.⁴ These data suggest features like ophthalmoplegia and limb–girdle weakness that can be common findings resulting from the mutations in LG2 domain, which interacts with alpha-dystroglycan and integrins.^{4,7} Our case with typical head drop shows a distinctive presentation among other reported *AGRN*-related CMS cases.

Salbutamol treatment is recommended to patients with mutations in the agrin–LRP4–MUSK–DOK7 pathway,⁹ thus we included salbutamol in the treatment protocol. Of note, we observed no deterioration in the symptoms after pyridostigmine was added to the protocol.

Although there is evidence suggesting pathogenicity of the homozygous Gly1675Ser variant in our patient, additional studies are needed to clarify its clinical significance. Additional

data demonstrating a damaging effect on agrin function would be needed to clarify the significance of this variant.

CONCLUSIONS

Our case demonstrated a clinical picture of CMS with dropped head phenotype. WES identified a homozygous missense variant of the *AGRN* gene, strongly suggesting the molecular defect behind this phenotype. Sequence analysis of additional similar cases, and functional studies on protein levels and function, will be important to confirm this variant as a disease causing mutation and to further define the clinical spectrum of findings associated with *AGRN* mutations.

Acknowledgments

The authors thank Michelle Graff for assistance with Sanger sequencing. The authors would like to thank the patients and families for their enrollment.

Supported by the National Institutes of Health (USA) Grants R01 AR044345 and R01 HD075802. DNA sequencing in the Boston Children's Hospital IDDRC Molecular Genetics Core was supported by the NIH Grant P30 HD18655. O. Ceyhan-Birsoy was supported by the Dubai-Harvard Foundation for Medical Research postdoctoral fellowship and is a Schlumberger Foundation Faculty for the Future grant.

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FIGURE 1.

Phenotype and genetic analysis. A–C, Patient at 4 years of age. Prominent neck weakness, ophthalmoplegia, and bilateral ptosis were noted. Increased lumbar lordosis is notably seen. Head dropping despite adequate ambulation is noted. D, Sanger confirmation of the variant in the family. The patient was homozygote, and parents were heterozygotes for the Gly1675Ser variant. The variant was present only in heterozygous state and at extremely low frequencies in control populations. E, The conservation of glycine at position 1675 within

various species shown by homologue-generated amino acid alignment (<http://www.ncbi.nlm.nih.gov/homologene>).

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TABLE 1

Clinical and Genetic Overview of the Patients Reported in the Literature

Article	Clinical Phenotype	Age at Presentation, yrs	Mutation	Exon	cDNA Change	Protein Alteration	Altered Domain
Huze et al ⁷	Prosis, facial weakness, ophthalmoplegia, thin thorax, and pelvis	Patient 1: 42 Patient 2: 36	Missense homozygous	29	c.5125G>C	p.Gly1709Arg	LG2
Maselli et al ⁴	Prosis, ophthalmoplegia, respiratory insufficiency	39	Compound heterozygous	6	N/A	Gln353*	FS
Nicole et al ⁸	Distal muscle weakness, distal muscle atrophy	Patient 1: 45 Patient 2: 43 Patient 3: 32 Patient 4: 23 Patient 5: 25	Compound heterozygous	30 2	c.226G>A	Val11727Phe Gly76Ser	LG2 Nia
This study	Dropped head phenotype, proximal muscle weakness	1.5	Missense homozygous	29	c.5023G>A	Gly1675Ser	LG2

All reported disease-causing *AGRN* mutations are shown to be recessive inheritance.^{4,7,8}

cDNA, complementary DNA; FS, follistatin-like domain; LG2, laminin G-like domain 2; LG3, laminin G-like domain 3; Nia, N-terminal domain.