

HGGA, Volume 5

Supplemental information

**The severity of *MUSK* pathogenic variants
is predicted by the protein domain they disrupt**







Benjamin T. Cocanougher, Samuel W. Liu, Ludmila Francescato, Alexander Behura, Mariele Anneling, David G. Jackson, Kristen L. Deak, Chi D. Hornik, Mai K. ElMallah, Carolyn E. Pizoli, Edward C. Smith, Khoon Ghee Queenie Tan, and Marie T. McDonald

Heterozygous variants observed in patients involving the Ig1 domain

Variant effect	Loss of protein	Loss of Ig1 domain	Loss of Ig1 domain	Missense in Ig1	Frameshift	Missense in Ig1	Frameshift	Missense in Ig1	Loss of protein	Missense in Ig1
DNA changes	out of frame deletion exons 7-11	in frame deletion exons 2-3	in frame deletion exons 2-3	c.114T>A	c.421delC	c.220C>T	c.790C>T	c.296G>T	c.496C>T	c.308A>G
Protein changes				p.D38E	p.141fs	p.R74W	p.R264*	p.C99F	p.166*	p.N103S
Phenotype	CMS		CMS		FADS		FADS		CMS	
Onset	Neonatal		Infantile		fetal		Fetal		Neonatal	
Reference	this paper		Gallenmüller et al., 2019		Li et al., 2019		Ding et al., 2020		Giarrana et al., 2015	

Supplemental Figure 1: Compound heterozygous allele combinations with missense variants in the Ig1 domain. Five allele combinations in compound heterozygosity involve the Ig1 domain. They all result in fetal or neonatal presentation, with the exception of the p.N103S which has a variable presentation (observed as neonatal, infantile, adolescent, and adult). Two cases have been reported with the in-frame deletion of Ig1. The p.R74W and p.C99F allele, when in combination with a null allele, result in FADS. The p.N103s allele is the most variable and most common pathogenic allele observed in the cohort.

Compound heterozygous variants observed in patients with biallelic missense variants in the kinase domain

	Allele 1	Allele 2				
Variant effect	Missense in kinase domain	Missense in kinase domain	Missense in kinase domain	Missense in kinase domain	Missense in kinase domain	Missense in kinase domain
						
DNA changes	c.1815G>A	c.2180C>T	c.1948C>A	c.2384T>G	c.2365G>A	c.2180C>T
Protein changes	p.M605I	p.A727V	p.P650T	p.I795S	p.V790M	p.A727V
Phenotype	CMS		CMS		CMS	
Onset	Neonatal		Childhood		Adolescence	
Reference	Maselli et al., 2010		Luan, Tian, and Cao 2016		Owen et al., 2018	

Supplemental Figure 2: Compound heterozygous allele combinations with missense variants in the kinase domain. Three combinations of variants were observed with two separate missense variants in the kinase domain. The p.A727V appeared in both a neonatal case in combination with p.M605I and an adolescent case with p.V790M, suggesting that either (1) the p.M605I is a more severe variant or (2) additional genetic or environmental modifiers were present in the patients reported. The p.V790M, when paired with a null allele, leads to neonatal onset CMS (Supplemental Figure 3).

Heterozygous variants observed in patients with one loss of MuSK and one missense variant in the kinase domain

	Allele 1	Allele 2		Allele 1	Allele 2		Allele 1	Allele 2		Allele 1	Allele 2
Variant effect	Frameshift	Missense in kinase domain		Frameshift	Missense in kinase domain		Splice site disruption	Missense in kinase domain		Truncating before kinase	Missense in kinase domain
DNA changes	c.497delA	c.2365G>A		c.220insC	c.2365G>A		c.79+2T>G	c.2158A>G		c.2062C>T	c.2324T>C
Protein changes	p.K156Rfs*20	p.V790M		p.R75fs*	p.V790M		p.?	p.K720E		p.Q688X	p.F775S
Phenotype	CMS			CMS			CMS			CMS	
Onset	Neonatal			Neonatal			Neonatal			Neonatal	
Reference	Maggi et al., 2013			Chevessier et al., 2004			Al-Shahoumi et al., 2015			Shen et al., 2020	
Variant effect	Frameshift	Missense in kinase domain		Truncating after kinase	Missense in kinase domain		Splice site disruption	Missense in kinase domain			
DNA changes	c.496C>T	c.1634T>C		c.2446C>T	c.2887G>A		c.79+2T>G	c.2165T>C			
Protein changes	p.R166*	p.L545P		p.R816*	p.A763T		p.?	p.V722A			
Phenotype	CMS			CMS			CMS				
Onset	Adult			CVCP			CVCP, adult onset limb-girdle weakness				
Reference	Owen et al., 2018			Murali et al., 2019			Pinto et al., 2019				

Supplemental Figure 3: Compound heterozygous allele combinations with one null and one

missense variant. The most common allele class observed in compound heterozygous patients is one null and one missense allele (11/15 observations). Patients with one null allele and one kinase missense variant had variable phenotypes from neonatal CMS to isolated CVCP. Even residues that are very close in space (p.V722A and p.K720E) had disparate clinical presentations.

Gene	DNA change	Protein change	Zygosity	dbSNP rsID	Variant interpretation	Related Disease (inheritance)
MUSK	Deletion exons 7-11	Premature stop codon	Heterozygous	N/A	Pathogenic (maternally inherited)	Congenital Myasthenic Syndrome (AR)
MUSK	Deletion exons 2-3	In frame	Heterozygous	N/A	Pathogenic (paternally inherited)	Congenital Myasthenic Syndrome (AR)
MUSK	c.667G>A	p.Val223Ile	Heterozygous	rs774463260	Uncertain significance (paternally inherited)	Congenital Myasthenic Syndrome (AR)
CHRND	c.68A>T	p.Asn23Ile	Heterozygous	Not reported	Uncertain significance	Congenital Myasthenic Syndrome (AR)
HNRNPDL	c.273A>G	Silent mutation	Heterozygous	rs372961029	Uncertain significance	Limb girdle muscular dystrophy (AD)
SPEG	c.3017G>A	p.Arg1006His	Heterozygous	rs571127512	Uncertain significance	Centronuclear myopathy (AR)
GAA	c.2065G>A	p.Glu689Lys	Heterozygous	rs1800309	Benign (Pseudodeficiency allele)	Pompe Disease (AR)

Supplemental Table 1: Results from a comprehensive neuromuscular panel revealed two pathogenic variants in *MUSK*, four variants of uncertain significance, and a benign pseudodeficiency allele in *GAA* were detected. This result is consistent with a diagnosis of *MUSK*-related CMS.