THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Congenital Myasthenic Syndromes

Congenital myasthenic syndromes (CMS) are heterogeneous inherited disorders of neuromuscular transmission characterized by fatigable weakness of the skeletle muscle with onset at or shortly after birth or in early childhood (1). In CMS, the safety margin of neuromuscular transmission is compromised, and clinical evaluation should involve detalied electromyographic (EMG) studies to demonstrate a defect in neuromuscular transmission (2). Severity and progression can vary. Major findings in the neonatal onset subtype include feeding difficulties, poor suck and cry, choking spells, ptosis, facial, bulbar and generalized weakness (1). Later childhood onset subtypes show abnormal muscle fatiability, motor milestones may be delayed, ptosis, and fixed or fluctuating extraocular muscle weakness (1).

Our Congenital Myasthenic Syndromes Sequencing Panel includes analysis of the 20 genes listed below.

Congenital Myasthenic Syndrome Panel				
AGRN	*CHRNA1*	*COL13A1*	*GFPT1*	*RAPSN*
ALG14	*CHRNB1*	*COLQ*	*LRP4*	*SCN4A*
ALG2	*CHRND*	*DOK7*	*MUSK*	*SNAP25*
CHAT	*CHRNE*	*DPAGT1*	*PREPL*	*SYT2*

Genes and Associated Disorder	Inheritance	Clinical Features/Molecular Pathology
ALG14 [OMIM# 607905] Myasthenic syndrome, congenital, 15, without tubular aggregates [OMIM# 616227]	AR	Compound heterozygous mutations in *ALG14* were described in a family with two siblings with congenital myasthenic syndrome characterized by childhood onset progressive fatigable proximal weakness (3). *ALG14* is concentrated at the muscle motor endplates and small interfering RNA silencing of ALG14 results in reduced cell-surface expression of muscle acetylcholine receptor (3).
ALG2 [OMIM#612866] Myasthenic syndrome, congenital, 14, with tubular aggregates [OMIM# 616228]		The same mutation in *ALG2* has been described in two different Saudi Arabian consanguineous families, one with congenital myasthenic syndrome-14, which is characterized by slowly progressive limb girdle muscle weakness with onset in early childhood (3, 4). A different mutation was also identified in an Italian patient in the homozygous state (3).
AGRN [OMIM#103320] Myasthenia, limb-girdle, familial [OMIM#254300]	AR	Huze *et al,* 2009 identified a homozygous mutation in *AGRN* in two siblings with CMS (5). Agrin induces the aggregation of acetylcholine receptors and other postsynaptic proteins on muscle fibers and plays a critical role in the formation of the neuromuscular junction.
CHAT [OMIM#118490] Myasthenic syndrome, congenital, associated with episodic apnea [OMIM#254210]	AR	Mutations in *CHAT* account for approximately 4-5% of patients with CMS (1). Choline acetyltransferase catalyzes the biosynthesis of acetylcholine.
CHRNA1 [OMIM#100690] *CHRNB1* [OMIM#100710] *CHRND* [OMIM#100720] *CHRNE* [OMIM#100725] Myasthenic syndrome, fast-channel congenital [OMIM#608930] Myasthenic syndrome, slow-channel congenital [OMIM#601462] Myasthenic syndrome, congenital, associated with acetylcholine receptor deficiency [OMIM#608931]	AR/AD	Gain of function mutations in the genes encoding the acetylcholine receptor (*CHRNA1, CHRNB1, CHRND, CHRNE*) results in autosomal dominant slow channel CMS, and loss of function mutations in these genes result in autosomal recessive CMS (1). Two particular founder mutations in *CHRNE* (c.1327delG and c.1353dup) account for approximately 50% and 20% of affected individuals from European Roma and Maghreb ancestry respectively.

COL13A1 [OMIM#120350] Myasthenic syndrome, congenital-19 [OMIM#6161720]	AR	Homozygous loss-of function mutations in COL13A1 were identified in three patients from two unrelated families with congenital myasthenic syndrome. All patients presented with respiratory insufficiency, hypotonia and feeding difficulties of varying severity. The *COL13A1* gene encodes a nonfibrillar transmembrane collagen protein, which plays an important role in in the formation and maintenance of the neuromuscular junction (6).
COLQ [OMIM#603033] Endplate acetylcholinesterase deficiency [OMIM#603034]	AR	Mutations in *COLQ* account for approximately 10-15% of patients with CMS (1). Mutations (missense, frameshift, stop and splice site mutations) causing AR CMS have been identified in more than 100 CMS families (7). The *COLQ* gene encodes a collagen-like strand that associates into a triple helix to form a tail that anchors catalytic subunits of acetylcholinesterase.
DOK7 [OMIM#610285] Myasthenia, limb girdle, familial [OMIM#254300]	AR	*DOK7* mutations have been reported in CMS patients primarily affected the proximal limb muscles. Selcen *et al*, 2009 identified 17 different truncating mutations in the DOK7 gene amongst 16 patients with limb-girdle congenital myasthenic syndrome (8).
GFPT1 [OMIM#614828] Myasthenia, congenital, with tubular aggregates 1 [OMIM#610542]	AR	Mutations in *GFPT1* account for approximately 2% of patients with CMS (1). Senderek *et al*, 2011 identified 18 homozygous and compound heterozygous mutations (nonsense, frameshift and missense) in 13 families with limb-girdle myasthenia with tubular aggregates-1 (9). *GFPT1* is a homodimeric cytoplasmic enzyme and is the first and rate-limiting enzyme of the hexosamine biosynthetic pathway.
LRP4 [OMIM#604270] Myasthenic syndrome, congenital, 17	AR	Compound heterozygous missense mutations in *LRP4* have been reported in one 17 year old female with congenital myasthenic syndrome who presented in infancy with respiratory and feeding difficulties (10). LRP4 is expressed on the surface of the postsynaptic membrane of the neuromuscular junction, is a receptor for neurally secreted agrin, and LRP4 bound by agrin activates MuSK, which is also associated with CMS. The identified mutations in LRP4 are located at the edge of its 3rd beta-propeller domain and decrease binding affinity of LRP4 for both MuSK and agrin (10).
MUSK [OMIM#601296] Myasthenic syndrome, congenital, associated with acetylcholine receptor deficiency [OMIM#608931]	AR	Mutations in *MUSK* have been reported in eight individuals from three families with CMS and are a rare cause of CMS (1). MUSK encodes the postsynaptic muscle-specific receptor tyrosine kinase.
PREPL [OMIM# 609557]	AR	One patient with biallelic mutations involving the *PREPL* gene has been described, this patient had congenital myasthenic syndrome with pre- and post-synaptic features, in addition to growth hormone deficiency (11).
RAPSN [OMIM#601592] Myasthenic syndrome, congenital, associated with acetylcholine receptor deficiency [OMIM#608931]	AR	Mutations in *RAPSN* account for approximately 15-20% of patients with CMS (1). Nearly 200 mutations in *RAPSN* have been identified to date (7) and multi-exon deletions may account for up to 15% of mutations in patients (12). *RAPSN* plays an essential role in the clustering of acetylcholinesterase at the endplate.
SCN4A [OMIM#603967] Myasthenic syndrome, acetazolamide-responsive [OMIM#608390]	AR	Tsujino *et al*, 2003 identified compound heterozygous missense mutations in a patient with CMS (13). Mutations in *SCN4A* have also been identified in patients with hyperkalemic periodic paralysis, paramyotonia congenital and hypokalemic periodic paralysis type 2. The SCN4A protein mediates the voltage-dependent sodium ion permeability of the postsynaptic membrane.
DPAGT1 [OMIM#191350] Myasthenia, congenital, with tubular aggregates 2 [OMIM#614750]	AR	Belaya *et al,* 2012 identified 7 compound heterozygous mutations in *DPAGT1* in 5 patients from 4 families with limb-girdle congenital myasthenic syndrome with tubular aggregates-2 (14). DPAGT1 catalyzes the first step in the dolichol cycle.
SNAP25 [OMIM#600322] Myasthenic syndrome, congenital-18 [OMIM#616330]	AD	Shen *et al*. identified a de novo heterozygous sequence change in the *SNAP25* gene in a patient who presented at birth with stiffness, respiratory insufficiency and joint contractures. In vitro studies showed a dominant-negative effect of the missense mutation on liposome fusion and inhibition of exocysosis of catecholamine-containing vesicles in chromaffin cells (15)

SYT2 [OMIM# 600104] Myasthenic syndrome, congenital, 17, presynaptic [OMIM# 616040]	AD	Hermann et al, 2014, identified two different heterozygous missense mutations in the SYT2 gene in two different families with autosomal dominant congenital myasthenic syndrome (16).

Test methods:
Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interests are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All novel and/or potentially pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp. Our CNV detection algorithm was developed and its performance determined for the sole purpose of identifying deletions and duplications within the coding region of the gene(s) tested. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Regions of high homology and repetitive regions may not be analyzed. This methodology will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of our deletion/duplication assay may be reduced when DNA extracted by an outside laboratory is provided.

Congenital Myasthenic Syndrome Panel (20 genes)

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	$2,000
CPT codes:	81406 81407
Turn-around time:	8 weeks

Note: We cannot bill insurance for the above test.

Results:
Results, along with an interpretive report, will be faxed to the referring physician. Additional reports will be provided as requested. All abnormal results will be reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

References:

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