



Genetic Testing for Centronuclear & Myotubular Myopathy

Clinical Features:

Centronuclear myopathy (CNM) is a rare muscle disease associated with non-progressive or slowly progressive muscle weakness that can develop from infancy to adulthood (1, 2). On muscle histopathology, patients with CNM have increased frequency of central nuclei, as well as type 1 fiber predominance and hypotrophy, in the absence of other significant abnormalities. Other neuromuscular conditions can have similar findings on muscle biopsy, so these features are not always diagnostic for CNM (1, 2).

Patients with [X-linked myotubular myopathy \(XLMTM\)](#) [OMIM#310400] generally present with hypotonia, feeding difficulties, respiratory distress, and delayed motor milestones. Death in infancy is common in males with the classic form of this condition. Milder forms of XLMTM have been identified and are characterized by fewer respiratory complications and longer life expectancy than observed in the severe cases (3). Intelligence is usually normal (1). Muscle of patients with XLMTM appears similar to fetal myotubes, with small rounded muscle fibers and no surrounding contractile elements. In the presence of a family history consistent with X-linked inheritance, these findings are suggestive of XLMTM. Female carriers generally do not have significant muscle weakness or notable features of XLMTM, although there have been several cases of symptomatic carriers with skewed X-inactivation (4). *MTM1* testing can be considered in females with a biopsy consistent with CNM. Muscle biopsies are generally not used to identify XLMTM carrier females, as only 50-70% of carriers will have an abnormal biopsy (3).

The majority of patients with [autosomal dominant or later onset CNM](#) [OMIM#160150], including *DNM2*-associated CNM, are ambulatory into adulthood (1, 2). Some patients with *DNM2*-associated CNM have a more severe infantile onset and may have early feeding and respiratory issues, as well as delayed milestones (5). Intelligence is usually normal (1), but at least one family with a *DNM2* mutation has been reported to have mild cognitive impairment, as well as mild axonal peripheral nerve involvement (6). NADH staining of patients with *DNM2* mutations often reveals radial arrangement of sarcoplasmic strands, which is highly characteristic, but not diagnostic, of *DNM2*-associated CNM (6).

Patients with [autosomal recessive *BIN1*-associated CNM](#) [OMIM#255200] appear to have an intermediate presentation that is typically less severe than classic XLMTM but more severe than most cases of dominant or *DNM2*-associated CNM (7). The small number of patients reported thus far have presented at birth or in childhood with slowly progressive proximal or diffuse muscle weakness and/or delayed motor milestones (7, 8). Respiratory insufficiency appears to be less common and less severe than in XLMTM, although it has been reported in at least two patients (7-9). Several affected individuals from one family had decreased fetal movements, joint contractures, and other congenital abnormalities (7). Scapular winging, mental retardation, and rare radial arrangement of sarcoplasmic strands on NADH stains of muscle tissue were also reported in one individual (8).

Patients with [*RYR1*-associated CNM](#) typically present at birth with profound weakness and hypotonia (10, 11). Reduced fetal movement and polyhydramnios may also be noted in the prenatal period (10, 11). Frequent respiratory tract infections and feeding difficulties associated with bulbar involvement are common, but often exhibit substantial improvement over time (10). Slow improvement in muscle strength is typically observed, and there is no known association with cognitive involvement (10). Patients share similar phenotypic features including myopathic facies with inverted V-shaped mouth, and external ophthalmoplegia (10, 11). Key histopathological findings include a lower total number of central nuclei than is seen with other genetic forms of CNM, fibers with internal nuclei (often multiple), a marked increase in fat and connective tissue, and a notable absence of necrosis and regeneration (10).

Inheritance:

[XLMTM](#) is an X-linked condition that occurs in 1 in 50,000 male live births (12). Less than 20% of these cases are due to *de novo* mutations of the *MTM1* gene (12). Recurrence risk for a carrier female is 50% (1 in 2). All daughters of affected males are obligate carriers and at risk for having affected sons. Germline mosaicism has been observed (3).

***DNM2*-associated CNM** is a rare condition and is generally believed to be less common than XLMTM. The majority of cases appear to be autosomal dominant, but *de novo* mutations are not uncommon and several recurrent *de novo* mutations have been identified (13). Recurrence risk for an affected parent is 50% (1 in 2). Germline mosaicism has not been reported in *DNM2*-associated CNM but remains a possibility, meaning that the recurrence risk for a *de novo* mutation does exist.

***BIN1*-associated CNM** is a rare condition and is generally believed to be less common than XLMTM and *DNM2*-associated CNM. All cases reported thus far have been homozygous for a recessive mutation (7, 8). Recurrence risk is 25% (1 in 4) for the same parents.

RYR1 mutations have also been identified in patients with CNM, with one study finding compound heterozygous mutations in 14 out of 24 affected individuals, and one heterozygous mutation inherited from an asymptomatic parent in three additional individuals, suggesting an autosomal recessive pattern of inheritance (10). Recurrent *RYR1* mutations associated with common haplotypes have been found in South African patients, suggesting the presence of founder effects in this population (10). A *de novo* heterozygous dominant missense *RYR1* mutation has also been identified in one patient with CNM (11). Recurrence risk is 25% (1 in 4) for the same parents in cases of autosomal recessive inheritance.

Molecular Genetics:

CNM can be caused by mutations in at least four genes, including *MTM1*, *DNM2*, *BIN1* and *RYR1*. **XLMTM** is caused by mutations in the *MTM1* [OMIM#300415] gene located at Xq28 (14, 15). *MTM1* codes for the myotubular protein, a highly conserved phosphatase thought to be involved in cellular transport and trafficking (3). Over 190 disease-associated mutations have been identified to date in the *MTM1* gene. Truncating and splice site mutations are more likely to be associated with the severe neonatal form, whereas the milder phenotypes are often caused by missense mutations outside of the functional domains (3). Missense mutations may result in a mild or severe phenotype based on their position in the *MTM1* gene (16). Approximately 80% of males with a diagnosis of myotubular myopathy by muscle biopsy will have a mutation in *MTM1* identifiable by sequence analysis. About 7% of mutations in *MTM1* are deletions (15).

DNM2 [OMIM#602378] is the only gene currently known to be associated with **autosomal dominant CNM**. *DNM2* mutations account for most, but not all, cases of CNM with autosomal dominant inheritance or later onset. The *DNM2* gene, located at 19p13.2, encodes the dynamin 2 protein, a ubiquitously expressed GTPase primarily involved in endocytosis and membrane trafficking (13). The protein is composed of 5 different domains, including the middle and PH domain. To date, fewer than 10 disease-associated mutations have been identified in the *DNM2* gene. Mutations in the PH domain of the protein are more likely to be associated with severe neonatal onset, whereas the milder phenotypes with later onset are often caused by mutations in the middle domain (5). The majority of mutations identified thus far are missense mutations, but at least one small deletion has been reported (5, 13). Mutations in *DNM2* have also been associated with dominant intermediate Charcot-Marie-Tooth disease, type B [OMIM#606482].

BIN1 [OMIM#601248] is associated with **autosomal recessive CNM**. *BIN1* mutations appear to be relatively rare, accounting for approximately 25% of cases of CNM with apparent recessive inheritance (7) but only a small percentage of all CNM cases combined. Located at 2q14, the *BIN1* gene codes for Bridging Integrator 1, also known as amphiphysin II, and has a muscle specific isoform. Amphiphysin II is regulated by phosphoinositides and is believed to be involved in membrane remodeling and T tubule organization. To date, only a small number of mutations have been described in the *BIN1* gene, including several missense changes and one nonsense mutation (7, 8).

RYR1 [OMIM#180901] is typically associated with **autosomal recessive CNM**, although a *de novo* autosomal dominant mutation in this gene has also been reported (11). The *RYR1* gene, located at 19q13.2, encodes the skeletal muscle ryanodine receptor, which is the principal sarcoplasmic reticulum calcium release channel with a crucial role in excitation-contraction coupling (10). CNM-associated mutations identified in *RYR1* have included missense, frameshift, and intronic mutations (10, 11). Mutations in *RYR1* have also been associated with malignant hyperthermia [OMIM#145600], central core disease [OMIM#117000] and multi-minicore disease [OMIM#255320]. In contrast to Central Core Disease due to heterozygous dominant *RYR1* mutations, no mutational hotspots have been observed in *RYR1*-associated CNM.

Additional Resources:

**The Information Point for Centronuclear
and Myotubular Myopathy**
<http://centronuclear.org.uk/>

Congenital Myopathy Research Program
Beggs Laboratory, Childrens Hospital Boston
Phone: (617) 919-2169
Email: lswanson@enders.tch.harvard.edu

Joshua Frase Foundation

Phone: (617) 715-1155

<http://www.joshuafrase.org/>

Muscular Dystrophy Association (MDA)

Phone: (800)572-1717

www.mda.org

Test methods:

We offer sequence analysis of all coding exons and intron/exon boundaries of *MTM1*, *DNM2*, *BIN1*, and *RYR1*. Targets of interests are enriched and prepared for sequencing using the Agilent SureSelect system. The constructed genomic DNA library is sequenced 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 pathogenic and likely 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 20bp.

We also offer deletion/duplication analysis of these genes by oligonucleotide array-CGH. Deletion/duplication analysis of the *MTM1*, *DNM2*, *BIN1* and *RYR1* genes by oligonucleotide array-CGH identifies copy number changes involving one or more exons. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Array-CGH will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of this assay may be reduced when DNA is extracted by an outside laboratory. Deletion/duplication analysis of the *MTM1* gene may also be performed by MLPA.

MTM1 sequencing analysis

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	\$1000
CPT codes:	81406
Turn-around time:	4 weeks

MTM1 deletion/duplication analysis

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	\$1000
CPT codes:	81405
Turn-around time:	4 weeks

Note: The sensitivity of our assay may be reduced when DNA is extracted by an outside laboratory.

DNM2 sequencing analysis

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	\$1000
CPT codes:	81406
Turn-around time:	4 weeks

DNM2 deletion/duplication analysis

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	\$1000
CPT codes:	81405
Turn-around time:	4 weeks

Note: The sensitivity of our assay may be reduced when DNA is extracted by an outside laboratory.

BIN1 sequencing analysis

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	\$1000
CPT codes:	81406
Turn-around time:	4 weeks

BIN1 deletion/duplication analysis

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	\$1000
CPT codes:	81405
Turn-around time:	4 weeks

Note: The sensitivity of our assay may be reduced when DNA is extracted by an outside laboratory.

RYR1 sequencing analysis

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	\$1000
CPT codes:	81408
Turn-around time:	4 weeks

RYR1 deletion/duplication analysis

Sample specifications:	3 to10 cc of blood in a purple top (EDTA) tube
Cost:	\$1000
CPT codes:	81407
Turn-around time:	4 weeks

Note: The sensitivity of our assay may be reduced when DNA is extracted by an outside laboratory.

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:

1. Pierson CR, Tomczak K, Agrawal P et al. X-linked myotubular and centronuclear myopathies. *J Neuropathol Exp Neurol* 2005; 64: 555-564.
2. Fardeau M, Tome F. Congenital Myopathies. In: Engel A, Franzini-Armstrong C, eds. *Myology*. New York, NY: McGraw-Hill, 1994: 1500-1505.
3. Das S, Dowling J, Pierson C. X-Linked Myotubular Myopathy. In: Pagon R, Bird T, Dolan C, eds. *GeneReviews* [Internet]. Seattle: University of Washington, 2002.
4. Kristiansen M, Knudsen GP, Tanner SM et al. X-inactivation patterns in carriers of X-linked myotubular myopathy. *Neuromuscul Disord* 2003; 13: 468-471.
5. Bitoun M, Bevilacqua JA, Prudhon B et al. Dynamin 2 mutations cause sporadic centronuclear myopathy with neonatal onset. *Ann Neurol* 2007; 62: 666-670.
6. Echaniz-Laguna A, Nicot AS, Carré S et al. Subtle central and peripheral nervous system abnormalities in a family with centronuclear myopathy and a novel dynamin 2 gene mutation. *Neuromuscul Disord* 2007; 17: 955-959.
7. Nicot AS, Toussaint A, Tosch V et al. Mutations in amphiphysin 2 (BIN1) disrupt interaction with dynamin 2 and cause autosomal recessive centronuclear myopathy. *Nat Genet* 2007; 39: 1134-1139.
8. Claeys KG, Maisonobe T, Böhm J et al. Phenotype of a patient with recessive centronuclear myopathy and a novel BIN1 mutation. *Neurology* 2010; 74: 519-521.
9. Mejaddam AY, Nennesmo I, Sejersen T. Severe phenotype of a patient with autosomal recessive centronuclear myopathy due to a BIN1 mutation. *Acta Myol* 2009; 28: 91-93.
10. Wilmshurst JM, Lillis S, Zhou H et al. RYR1 mutations are a common cause of congenital myopathies with central nuclei. *Ann Neurol* 2010; 68: 717-726.
11. Jungbluth H, Zhou H, Sewry CA et al. Centronuclear myopathy due to a de novo dominant mutation in the skeletal muscle ryanodine receptor (RYR1) gene. *Neuromuscul Disord* 2007; 17: 338-345.
12. Laporte J, Biancalana V, Tanner SM et al. MTM1 mutations in X-linked myotubular myopathy. *Hum Mutat* 2000; 15: 393-409.
13. Bitoun M, Maugeen S, Jeannet PY et al. Mutations in dynamin 2 cause dominant centronuclear myopathy. *Nat Genet* 2005; 37: 1207-1209.
14. de Gouyon BM, Zhao W, Laporte J et al. Characterization of mutations in the myotubularin gene in twenty six patients with X-linked myotubular myopathy. *Hum Mol Genet* 1997; 6: 1499-1504.
15. Laporte J, Guiraud-Chaumeil C, Vincent MC et al. Mutations in the MTM1 gene implicated in X-linked myotubular myopathy. ENMC International Consortium on Myotubular Myopathy. European Neuro-Muscular Center. *Hum Mol Genet* 1997; 6: 1505-1511.
16. McEntagart M, Parsons G, Buj-Bello A et al. Genotype-phenotype correlations in X-linked myotubular myopathy. *Neuromuscul Disord* 2002; 12: 939-946.

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