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The Diagnosis of Congenital Myopathies.

1. Signs and Symptoms in Congenital Myopathies.
2. Update on the Genetics of Congenital Myopathies.
3. Myoimaging in Congenital Myopathies.
4. Recently Identified Congenital Myopathies.

Molecular biology has affected the precision in the classification and nosology of congenital myopathies resulting in nomenclatorial changes. CM's were once defined as hereditary neuromuscular conditions of early childhood with characteristic myopathic features, for example central core disease, nemaline myopathy and myotubular myopathy. This definition has been diluted due to many recently defined conditions, which often lack distinct myopathic features, but are still regarded as CM's.

Gonorazky HD, Dowling JJ, Volpatti JR, et al. Signs and Symptoms in Congenital Myopathies. Semin Pediatr Neurol. 2019 Apr; 29: 3-11.

There is a wide range of clinical and histological presentations among the CM. There are no clear limits between each subgroup of CM and the clinical overlap between genes has become more evident. Next generation sequencing has produced vast amounts of genetic data that may be difficult to interpret. To interpret variants of unknown significance (VUS), the genetic diagnosis must be supported by a well characterized clinical diagnosis, making phenotype – genotype correlation a priority.

The classical presentation of CM is in the neonatal period with weakness, facial involvement, respiratory insufficiency and feeding difficulties. Despite the growing trend to classify CM based on their causative genes, traditional classification methods are still valued. These methods rely on histopathological or anatomical findings which are still highly valued due to their informative and descriptive nature. This article reviews:

1. Specific subtypes of CM.
2. Common clinical features.
3. Distinctive clinical signs.

1. SPECIFIC SUBTYPES OF CM

	Nemaline Myopathy	**Core Myopathy**	**Centronuclear Myopathy** (CNM)	**Congenital Fiber Type Disproportion** (CFTD)
Histo-pathology	Nemaline rods	Areas devoid of reactivity to oxidative stains	Presence of centrally located nuclei in >25% of muscle fibres	Type 1 fibre predominance. >40% fibre size reduction compared to Type II fibres
Classification and clinical features	5 types: 1. Severe NM 2. Intermediate 3. Typical 4. Child/Juvenile 5. Adult onset There is no correlation of the size or number of rods to the severity of the symptomatology.	CCD: central core disease. -most common CM *RYR* - floppy, ptosis, chest deform, scoliosis, contracture, hip dysplasia, absent/mild resp involvement. MmD: multiminicor e disease. *SEPN1* – paravertral mm, scoliosis, early resp involvement.	X-linked myotubular myopathy (MTM) –most common CNM. Severe phenotype – large head, long phalanges, severe lower facial mm weakness, ptosis, opthalmoparesis . High mortality – 1 year due to resp involvement	Presentation varies depending on the genetic cause (can present with dystrophic features on mm biopsy)
Genetics	13 genes described *ACTA1* – most common dominant NM (severe NM) *NEB* – most common recessive form (typical NM)	CCD – mostly due to *RYR1* mutations MmD – mostly due to *SEPN1* mutations)	7 genes (*MTM1, DNM2, BIN1, RYR1, SPEG, CCDC78, TTN*)	Pure forms associated with: *TPM3, TPM2, RYR, SEPN1, ACTA1*

2. COMMON CLINICAL FEATURES

- Prominent lower facial weakness – should lead to differential diagnosis between CM, congenital myasthenic syndromes (CMS), and myotonic dystrophies. Not a feature in muscular dystrophies.
- Generalized hypotonia and hyporeflexia.

- Brain involvement – not expected in CM. Severe presentations are at high risk of hypoxic encephalopathy. Severe NM & X-linked MTM – are associated with mild dilatation of ventricles, cortical atrophy or hemorrhagic lesions. Structural brain abnormalities should raise alarm for alternative diagnosis.

3. DISTINCTINCTIVE CLINICAL SIGNS

1. Extraocular movement restriction:
    - Most prominent clinical sign that can lead to a specific diagnosis.
    - Usually associated with ptosis. Ptosis can also be found alone.
    - Opthalmoparesis with ptosis: consistent feature of most CNM. Common genes: *MTM, BIN1, DNM2*. Only 2 forms of CNM not associated with opthlamoparesis (*TTN, CCDC78*).
    - Multiminicores in the muscle biopsy and opthalmoparesis – recessive forms of *RYR*.

2. Masticatory muscle involvement:
    - Prominent bulbar weakness with involvement of the masticatory muscles are shared features in CM.
    - Prominent lower facial muscle weakness is a distinguishing feature of NMs.
    - Other subgroups characterized by a severe compromise of the bulbar muscles are CNM.

3. Prominent Axial Weakness :
    - Most of the CM will present with generalized or proximal muscle weakness.
    - Axial pattern refers to weakness of the paravertebral muscles. It affects the cervical region and can present with dropped head syndrome. Seen in muscular dystrophies (*LMNA*), CMS, spinal muscular atrophy (SMA), inflammatory myopathies and some CM.
    - Broader paravertebral involvement can lead to early development of scoliosis and paravertebral muscle contractures causing a rigid spine syndrome. *SEPN1, RYR1* and *ACTA* related myopathies are associated with a rigid spine syndrome.
    - Early onset distal myopathies include distal nebulin myopathy, Laing DM, *TPM3* & *TPM2*, *DNM2* and *KLHL9*- related myopathies. *MYH7*-related myopathy has predominant distal muscle involvement sparing the extensor digitum brevis. Producing a sign known as the “hanging big toe” due to weakness of the extensor hallux.

4. Early Respiratory Failure:
    - Respiratory insufficiency while ambulant is not commonly seen. Seen characteristically in SEPN1-related myopathy. Also seen in mutations in *ACTA1, TPM3, NEB* and *MEGF10*.

5. Orthopaedic Abnormalities:
    - Are a common finding in early onset muscle disorders especially structural myopathies.
    - Positive symptoms (joint hyperlaxity) – can be seen in muscular dystrophies, *COL6*-related myopathies and Ehlers Danlos syndrome. This can be a

prominent subtype in some CM. *RYR1* related myopathies may have large joint hyperlaxity presenting with hip dislocation at birth.

- Negative symptoms (contractures). NMs can present with fetal akinesia associated with intrauterine growth retardation and multiple arthrogryposis. *TMP2* mutations have be a cause of distal arthrogryposis and also produce multiple pterygium syndrome (Escobar syndrome).
- King-Denborough syndrome, which is associated with dominant mutations in RYR1, is associated with a combination of orthopaedic complications. These include pectus excavatum/carinutum, kyphosis, lumbar lordosis, vertebral fusion, pes cavus and contractures.

6. Cardiac Involvement:
    - Cardiac abnormalities are rarely associated with CM. Arrhythmias along with conduction blocks are more frequently associated with CM, as compared to cardiomyopathy.
    - NM due to mutations associated with *ACTA1* can produce dilated or hypertrophic cardiomyopathy. CNM due to mutations in *TTN*, *SPEG* and rarely *BIN1* have also been associated with dilated cardiomyopathy.

<u>EXCITATION –CONTRACTION (EC) COUPLING ABNORMALITIES:</u>

- EC coupling is the ion mediated process that converts the electrical stimulus propagating through the sarcolemma to intracellular calcium release, resulting in sarcomere contraction. Calcium is the second important ion involved in EC coupling. Exposure to volatile anaesthetics causes increased release of calcium from the sarcoplasmic reticulum leading to Malignant Hyperthermia (MH). 75% of patient with MH have a pathogenic variant in *RYR1*. *STAC3* and *CACNA1S* are other structural myopathy genes at risk of MH.
- Mutations of *SCN4A*, which encodes voltage gated sodium channels in skeletal muscle, can lead to periodic paralysis. This episodic paralysis has recently been described in some CM characterized by slow progressive weakness, motor developmental delay, and cranial synostosis.

Pelin K, Wallgren-Pettersson C. Update on the Genetics of Congenital Myopathies. Semin Pediatr Neurol. 2019 Apr; 29: 12-22.

Next generation sequencing methods such as whole exome sequencing, targeted gene panels and whole genome sequencing have resulted in an exponential discovery of novel genes causing CM. There are currently mutations in 27 genes known to cause CM. The use of custom high-density oligonucleotide arrays for comparative genomic hybridization has led to the discovery of large copy number variations (CNVs). The mode of inheritance of CM can be autosomal recessive, autosomal dominant or X-linked. Clear genotype and phenotype correlations are rare. More than 1 clinical phenotype can be the result of mutations in the same gene. Similarly the same clinical phenotype result from mutations in several different genes.

The most important genes will be discussed in this review. Refer to the article for a more comprehensive review of all the genes.

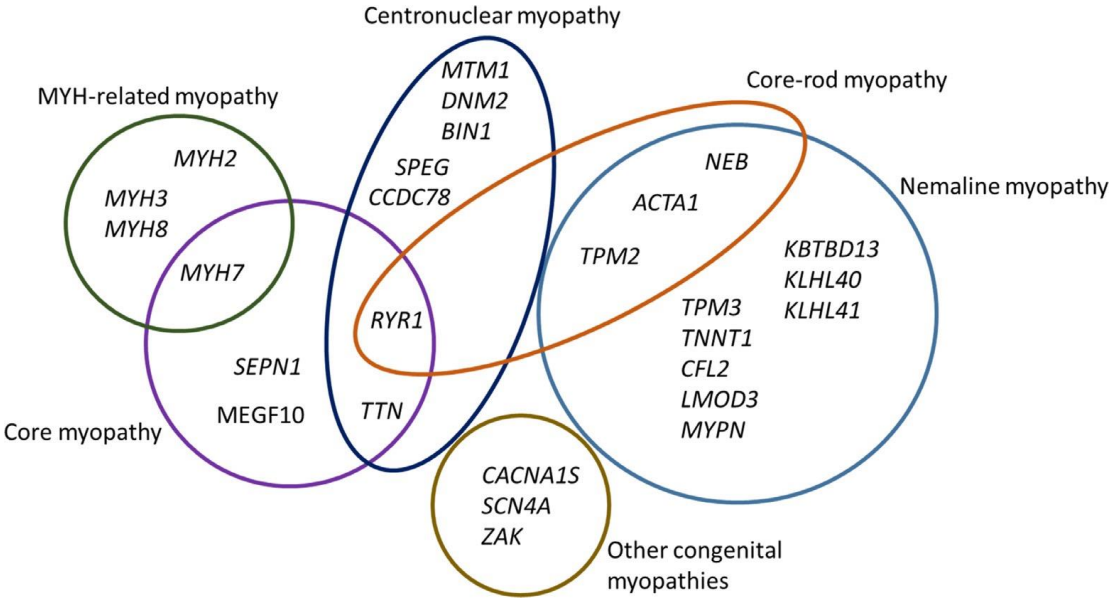

Figure 1: Congenital myopathy-causing genes. The diagram shows 27 genes implicated in various forms of the congenital myopathies, and the overlap between different entities. Core-rod myopathy was included to illustrate the overlap between nemaline myopathy and core myopathy. (Extrapolated from: Pelin K. Update on the Genetics of Congenital Myopathies. Semin Pediatr Neurol. 2019 Apr; 29: 12-22).

<u>NEMALINE MYOPATHIES</u> – Including Cap Myopathy and Fiber-Type disproportion

NM myopathies and Cap myopathies are considered overlapping entities. Fiber-type disproportion (FTD) may be caused by the same genes as NM and Cap myopathies. There are 11 NM-causing genes described.

- *ACTA1*, *NEB*, *TMP2*, *TMP3*, *TNNT1*, *LMOD3*, and *MYPN*: encode structural proteins of the skeletal muscle sarcomere.
- *CFL2*: regulates actin filament dynamics and is essential for muscle maintenance.
- *KBTBD13*, *KLHL40*, *KLHL41*: encode proteins involved in the maintenance of sarcomere integrity.

NEB – The Nebulin Gene: disease causing variants in the *NEB* gene are the most common cause of autosomal recessive NM. These account for about 50% of NM cases and the most common case of the typical form. Most of the patients are compound heterozygous for 2 different *NEB* mutations. Point mutations are the most common mutation types in *NEB*. A large pathogenic CNV in *NEB* is estimated to be present in 10-15% of NM patients. Missense variants are common in *NEB*. The current recommendation from the authors is that only variants affecting conserved actin and trypomysin binding sites in NEB should be considered as pathogenic. Recessive disease causing variants of *NEB* can cause classic NM, distal nebulin myopathy without nemaline rods, core-rod myopathy, distal forms of NM and lethal multiple pterygium syndrome. Only 1 clearly dominant *NEB* variant has been described.

ACTA1 – Alpha Actin gene: causes 23% of NM cases. Most pathogenic variants causing severe NM are dominant missense variants. Sporadic cases with *ACTA1* variants are caused by de novo missense variants. Autosomal recessive variants resulting in null alleles are rare. De novo disease causing variants in *ACTA* can also cause cap, FTD, core-rod, intranuclear

rod, zebra body, progressive scapuloperoneal myopathies, and distal myopathy with nemaline rods.

CORE MYOPATHIES

This includes Central Core disease, Minicore myopathy and Multiminicore disease, from the historical definition. Due to the pathologic, clinical and histologic overlap, Core myopathy is now the preferred term.
RYR1, *SEPN1*, *MEGF10*, *TTN* and *MYH*7 are the 5 genes reported to cause core myopathies.

RYR1 – Ryanodine Receptor 1 gene: is the major core-myopathy causing gene. The skeletal specific ryanodine receptor is a calcium release channel involved in EC coupling activating muscle contraction. It is a large gene with 106 exons, with more than 200 reported mutations reported. Dominant mis-sense variants are responsible for most mutations that cause core myopathies and malignant hyperthermia. Recessive *RYR1* mutations are associated with more severe phenotypes compared to dominant mutations. Recessive mutations include null mutations and combination of missense variants. In addition to core myopathies, both dominant and recessive mutations also cause core-rod, congenital FTD, centronuclear myopathies and Malignant Hyperthermia susceptibility.

SEPN1 – Selenoprotein N gene: is the second most common core myopathy causing gene. Recessive loss of function mutations cause rigid spine muscular dystrophy, core myopathy, CFTD and desmin related myopathy with Mallory body-like inclusions. These disorders are now collectively referred to as *SEPN1*-related myopathies due to the overlap in the clinical and histological features.

CENTRONUCLEAR MYOPATHIES

MTM1, *DNM2*, *RYR1* and *TTN* are the most common genes causing centronuclear myopathies (CNM). *BIN1*, *CCDC78* and *SPEG* are minor causative genes.
Autosomal recessive CNM (ARCNM) is caused by mutations in *RYR* and *TTN*.
MTM1 – Myotubularin gene, cause an X-linked myotubular myopathy (XLMTM). Majority of the patients are neonatally severely affected boys. There is evidence of females manifesting with a less severe variable clinical phenotype.
DNM2 – Dynamin 2 gene, causes an autosomal dominant CNM (ADCNM). This presents in childhood or early adolescence with ptosis, distal weakness and contractures, radial strands on biopsy and Charcot-Marie-Tooth peripheral neuropathy (*CMTDIB* and *CMT2M*). However the mutations causing ADCNM are distinct from those causing CMT.

MYOSIN (MYH) -RELATED MYOPATHIES

Myosin heavy-chains genes affect skeletal and cardiac muscle. *MYH2* – usually cause a mild CM with external opthalmoplegia. *MYH3* and *MYH8* cause distal arthrogryphosis syndromes.

OTHER –GENES CAUSING CM

Previously known channelopathy-causing genes, *CACNA1S* and *SCN4*, have now been implicated in CM.
Mutations in mitogen-activated protein triple kinase encoding gene, *ZAK*, have been shown to cause a congenital myopathy with FTD.

Carlier R, Quijano-Roy S. Myoimaging in Congenital Myopathies. Semin Pediatr Neurol. 2019 Apr; 29: 30-43.

Muscle imaging has become a useful non invasive tool in the diagnosis of congenital myopathies. This imaging modality assesses skeletal muscle edema and fatty infiltration. While there is enormous genetic heterogeneity and clinicopathologic overlap, myo-imaging offers homogeneity in gene mutated myopathies. Myo-imaging can guide the diagnosis when interpreting challenging results in certain genes that have a large size (*NEB*, *TTN*, and *RYR1*). This has led to muscle imaging being a first line diagnostic tool. Whole Body MRI (WBMRI) is recommended as it offers comprehensive analysis. Both 1.5T and 3T magnet systems can be used. MRI protocols should aim to detect fibroadipose tissue and muscle water content abnormalities. It is essential for the radiologist to do systematic and standardized scoring of the different muscles and muscle groups. Signal intensities, texture and volume abnormalities of the muscles should be identified and scored. There are various semiquantitative visual rating scales that have been established for clinical and research purposes. The assessment of muscle volume is more challenging than the assessment of signal intensities. Muscle atrophy is more diagnostic than muscle hypertrophy, especially in mildly affected patients. Differing muscle textures such as bands, porcelain-like, punctated, water color fatty infiltration can be described. The presence or absence of inflammation or edema must also be assessed.

Providing the radiologist with clinical and histological information, and identifying the genes most likely to be involved in the myopathy helps to reduce the number of patterns to compare from. Degree of severity is important when addressing the pattern analysis. In a positive pattern, early affected muscles will be useful in mildly affected patients. In a negative pattern, muscles that are better preserved will be more relevant in advanced disease. Using serial analysis of semiquantitative or automated muscle scorings from a series of patients with the same gene mutation, but at different stages of the disease leads to a better definition of myopathy patterns. A major step in pattern recognition techniques is identifying imaging fingerprints in different myopathies. Heatmaps representation using statistical representation provides a precise and global view of the MRI muscle semiquantitative scoring. Regional heatmaps are very intuitive and useful in clinical practice. In this approach, muscles are listed through the different regions of the body. While hierarchical heatmaps which are less evident and intuitive, asses muscles according to similarities and differences. They allow processing and classification of large numbers of muscles.

This article goes through the pattern recognition patterns taking into account the involved genes. An example is the *RYR1*-muscle pattern recognition. There is very prominent lower limb pattern involvement, even in mildly affected patients. There is diffuse involvement of the thigh with distinct sparing of the gracilis, adductor longus, rectus femoris and semitendinosus muscles. The soleus muscle is selectively and markedly involved in the leg. Whole body imaging shows wasting of the masticator muscle. There is also fatty infiltration of the neck extensors, biceps brachii, lumbar paravertebral muscles and gluteus maximus. Refer to the article for a more comprehensive review of other muscle MRI patterns.

Radke J, Stenzel W, Goebel HH. Recently Identified Congenital Myopathies. Semin Pediatr Neurol. 2019 Apr; 29: 83-90.

More than 20 new congenital myopathies have been reported in the last 5 years. Majority of these are from mutational analysis of new genes, some are from multicenter cohorts, while others are in single patients. The initial definition of CM was based on pathognomonic myopathic features such as rods or cores. Among the recently identified congenital myopathies (RICM), few new myopathological features have been reported. Some RICM share certain myopathological phenomena with already well established classical CM and others have nonspecific myopathology. This represents the shift from the diagnosis of CM being based on myopathologic criteria but now on clinical and genetic ones. If a component of the definition of a CM is its myopathologic hallmark, then RICM that lack any disease-specific morphologic hallmark should not be considered a CM. This nosologic evolution illustrates how the definition of CM needs to change when the reality changes. Therefor the RICM currently only expand the definition of CM.

Entry of a CM in the annual Gene Table suggests its recognition as a nosologic entity. The article lists the RICM that have been published but have not been entered in the latest "Gene Table of Monogenic Neuromuscular Disorders". Some RICM are recognized with mutations from new genes, others as genetic variants genetically known CM, and others are variants of other non-CM entities.

There are very few new morphological characteristics in the RICM. Examples of these are corona fibres in CM with mutation in the *SCN4A* gene, and honeycomb myonuclei described in *SYNE1*. This lack of CM-specific myopathologic features in RICM makes the individual diagnosis more complex, as the myopathologic hallmark is an important component in the definition of CM. This absence and scantiness of special myopathological features in RICM, may render muscle biopsy an unrewarding diagnostic procedure. The biopsied tissue may reveal non specific myopathic pattern, such as rods and central nuclei, but not a gene-suspecting pattern. This presents a challenge in which future diagnostic omission of a muscle biopsy will reduce the complete nosography of future RICM. The diagnostic confirmation of RICM in single patients without entity-specific myopathic features may be delayed and impaired due lack of proper clinical and myopathic features which will lead to proper genetic analysis.

Not all CM are muscle-limited entities. They may occasionally be components of multiorgan disorders such as cardiomyopathies or syndromes. These nonmuscular clinical symptoms and signs may be the nosological foreground, leading to a neglect in the assessment of the skeletal muscle. Muscle biopsies are often omitted due oversights in their indication, resulting in disease specific myopathic features going undetected.

Immunohistochemistry (IH) is a new introduction into the diagnostic myopathology of RICM. It is often corroborated or modified by immunoblotting. IH demonstrates reduction or absence of the mutant protein in the biopsied muscle, or the aggregation of mutant protein. It has been widely used when investigating autosomal recessive muscular dystrophies. IH has demonstrated protein aggregation in RICM such as autosomal dominant *CCDC78* CM (aggregates of *CCDC78* protein together with desmin, *RYR1* protein and actin). Reduction or absence of beta-IV spectrin has been seen in autosomal recessive *SPTBN4* CM. IH has also been applied to CM animal models such as zebrafish. IH is an important diagnostic addition in the myopathologic armamentarium of RICM, due to the scarcity of new specific myopathic features in RICM.

The increasing number of newly published CM may lead to revisitation of the classification and nomenclature of CM. Especially as the RICM do not carry a precise name and lack a precise myopathology. The new classification will allow inclusion of both earlier and recently reported congenital myopathies.