JUNE 2023

SAVOIR & COMPRENDRE
AVANCÉES DE LA RECHERCHE

ADVANCES in congenital myopathies

> Core myopathy
> Central core disease
> Multiminicore myopathy
> Core-rod myopathy
> Nemaline myopathy
> Rod myopathy
> Cap myopathy
> Centronuclear myopathy
> X-linked myotubular myopathy
>Myosin storage myopathy
> Myosinopathy
> Congenital fibre-type disproportion myopathy

Congenital myopathies are a heterogenous group of rare diseases characterised by structural abnormalities in muscle fibres which are most often detected at a young age. These abnormalities lead to muscle weakness (hypotonia and impaired motor skills) which generally manifests at birth or during the first few months of life ("congenital").

This document, published to coincide with the AFM-Téléthon General Meeting 2023, presents research news on congenital myopathies from the past year (ongoing studies and clinical trials, scientific and medical publications, etc.).
It can be downloaded from the AFM-Téléthon website where further information in the scientific, medical, psychological, social and technological fields relating to congenital myopathies can be found:
WEBSITE www.afm-telethon.fr

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69 scientific publications

between May 2022 and May 2023

(Source: PubMed)

14 clinical trials, 6 of which

are underway or in preparation worldwide as of 30 May 2023

(Source: ClinicalTrials.gov)

What are congenital myopathies?

Congenital myopathies are a heterogenous group of rare diseases characterised by abnormalities in the structure and architecture of muscle cells (also called muscle fibres) which are most often detected at a young age.

- These abnormalities lead to muscle weakness (hypotonia and impaired motor skills) which generally manifests at birth or during the first few months of life ("congenital"). The severity of these diseases varies from one congenital myopathy to another.
- Treatment is multidisciplinary and is carried out by a team which specialises in myology. Current treatments aim to prevent possible complications and improve the quality of life of patients affected by these conditions.
- The main mechanisms affected are those involved in muscle contraction (excitation-contraction coupling, intracellular calcium movements, interactions between thin and thick filaments).

*A **disease** is said to be **rare** if it affects less than 1 in 2,000 people. Rare diseases are subject to common public health policy in the areas of research, information and therapeutic management.*

Treatments at different stages of development

- Some are in clinical trials:

- gene therapy with AAV-MTM1 or tamoxifen in X-linked myotubular myopathy;
- the Rycal® ARM210 in RyR1-related myopathies.

- Others are still being studied in animals:

- calcium-release modifiers (dantrolene, AICAR, Rycals (JTV519 and S107)) in RyR1-related myopathies;
- troponin activators (tirasemtiv, reldesemtiv, levosimendan, omecamtiv mecarbil) in α-actin- or nebulin-based myopathies;
- enzyme replacement (Valerion's 3E10Fv-MTM1) in X-linked myotubular myopathy;
- decreasing the amount of abnormal dynamin 2 with an AAV-shRNA;
- inducing the overexpression of cardiac actin to compensate for a deficiency in skeletal muscle actin;
- preventing the formation of abnormal protein aggregates using a chemical chaperone such as 4-phenylbutyric acid.

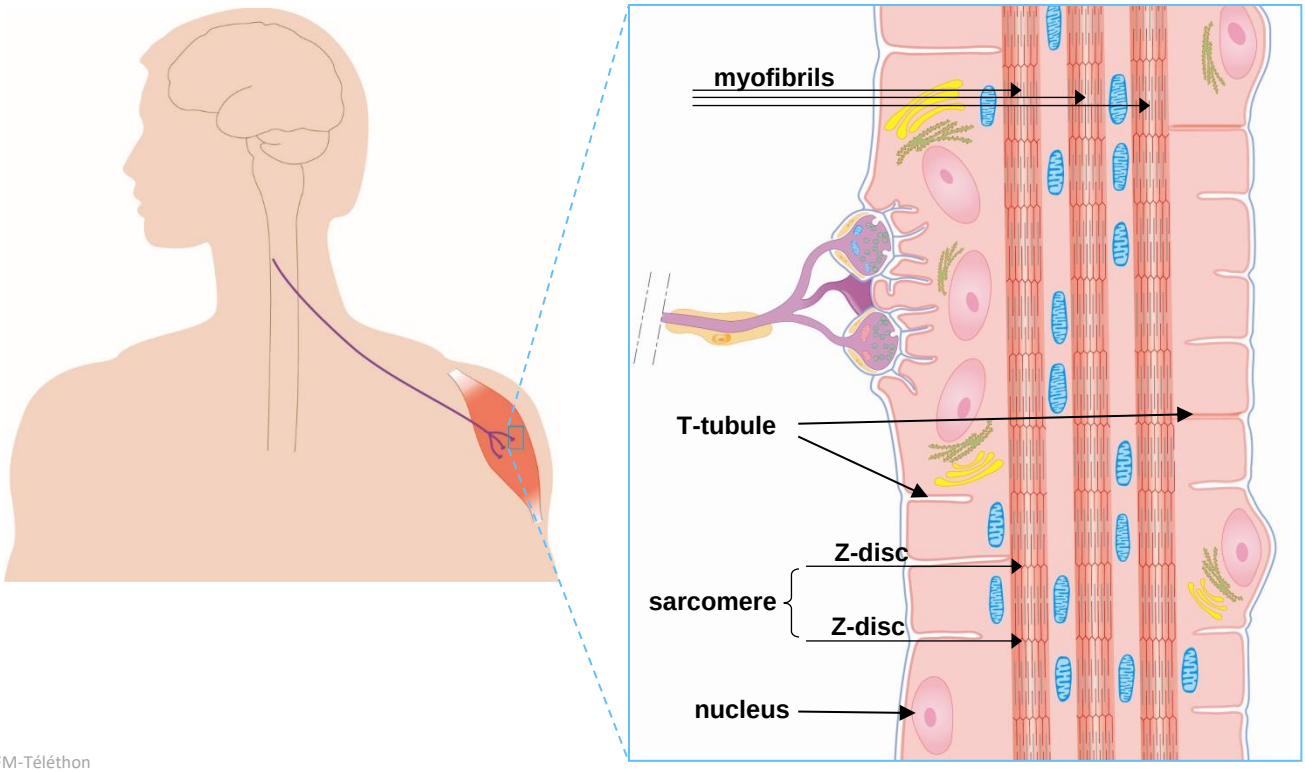
- Certain drugs, such as salbutamol in patients with RyR1-related myopathies, cholinesterase inhibitors in some cases of RyR1-related multiminicore myopathy and KLHL40-related myopathy, and L-tyrosine in a small number of patients with nemaline myopathy, have been used successfully.

Beaufils M et al. Curr Pharm Des. 2022
Gineste C et al. Curr Opin Pharmacol. February 2023

Classification of congenital myopathies

Congenital myopathies can be classified into six main types based on the predominant structural abnormalities observed under a microscope during a muscle biopsy:

- core myopathies
- nemaline myopathies
- centronuclear myopathies
- congenital fibre-type disproportion myopathies
- myosin storage myopathy
- ultra-rare congenital myopathies



Myofibrils are composed of repeating sections of sarcomeres

Myofibrils run lengthwise through muscle cells from end to end. They are divided into small contractile units called sarcomeres. A sarcomere is the distance between two Z-discs. Within a sarcomere, thick myosin filaments and thin actin filaments are arranged in an alternating pattern. When a muscle contracts, these filaments slide along each other and the distance between the two Z-discs decreases. When all of the sarcomeres shorten, it causes the muscle cells to contract.

Core myopathies

Core myopathies are the most common type of congenital myopathy. They are characterised by the presence of abnormal regions of structural disorganisation called "cores" in muscle fibres.

What are "cores"?

"Cores" are regions in a cell which are not coloured by stains typically used to see the interior of cells under a microscope. They are disorganised areas, devoid of mitochondria, where proteins such as desmin, αB-crystallin, filamin C, myotilin, ryanodine receptors (RyR1), triadin and dihydropyridine receptors (DHPR) accumulate in an abnormal manner.

The size and location of these cores, observed under an electron microscope, are what is used to distinguish the different types of these congenital myopathies:

- **Central core disease**, which is characterised by centrally-positioned cores which span the length of the muscle fibre or, occasionally, peripheral cores under the membrane; less frequently, the cores are different sizes with blurry edges; this disease is caused by dominant or recessive *RYR1* gene mutations;
- **Multiminicore myopathy**, which is characterised by multiple smaller cores that are spread throughout the muscle fibre; it is often linked to recessive *RYR1* or *SELENON* gene mutations or, less frequently, dominant *MYH7, ACTA1, CCDC78* or *ACTN2* gene mutations or recessive *TTN, MEGF10* or *FXR1* gene mutations;

▪ **Core-rod myopathy**, where muscle fibres that contain cores and others that contain rods are present in the same muscle; the genetic mutations found in this myopathy are dominant *RYR1* and *KTBD13* gene mutations and recessive *ACTA1, NEB, TNNT1,* and *CFL2* gene mutations.

Nemaline myopathies

Nemaline myopathies are characterised by the abnormal presence of protein aggregates in the muscle fibres.

▪ **Rod myopathy** (nemaline myopathy) is characterised by the presence of rod-liked protein masses in the muscle fibres.

▪ **Cap myopathy** is characterised by cap-like structures at the periphery of muscle fibres.

▪ To date, fifteen genes involved in nemaline myopathies have been identified. Most of these code for components of thin filaments or proteins that regulate the stability or renewal of thin filaments.

A variation in any of these proteins results in poor interaction between actin and myosin, the mechanism behind muscle contraction.

The contraction of myofibrils

Sarcomeres are the basic units of myofibrils and the cellular structures responsible for the contraction of muscle fibres.

▪ Every sarcomere is delimited by two Z-discs and is formed by two types of myofilament: thin (actin) filaments and thick (myosin) filaments.

▪ Sarcomeres contract when the thick filaments slide along the thin filaments. This results in the myofibrils and, consequently, the muscle cell contracting.

▪ The most common genetic mutation responsible for nemaline myopathies is a recessive *NEB* gene mutation, with the second most common being a dominant *ACTA1* gene mutation. They are found in over 80% of people with a nemaline myopathy.

▪ In Japan, the *ADSSL1* gene is most frequently responsible for nemaline myopathies.

▪ Other mutations such as recessive or dominant *TPM3* or *TPM2* gene mutations or recessive *KLHL40, KLHL41, LMOD3, TNNT3, MYO18B, RYR3, TNNT1, CFL2* or *MYPN* gene mutations are much rarer; they are sometimes involved in very severe types of neonatal nemaline myopathy, with or without cardiac involvement.

▪ *ADSSL1* gene mutations are recessive and lead to a distal muscle deficit starting during childhood or adulthood, a decrease in grip strength after puberty and heart conditions in 25% of cases; there are fewer rod-containing muscle fibres than in other nemaline myopathies.

▪ *KBTBD13* gene mutations lead to a type of dominant congenital myopathy with rods, cores and protein aggregates which causes a characteristic slowness of movements.

Centronuclear myopathies

While muscle cell nuclei are usually found all along the periphery of the cell, **centronuclear myopathies**, including **X-linked myotubular myopathy**, are characterised by chains of nuclei located at the centre of muscle cells. X-linked myotubular myopathy is also characterised by the presence of muscle fibres that resemble muscle precursor cells (myotubes), which are found instead of mature muscle fibres.

- Centronuclear myopathies can be classified into three groups:
- the most severe type is the one that is linked to the X chromosome known as **X-linked myotubular myopathy**; it is caused by *MTM1* gene mutations.
- **autosomal dominant** centronuclear myopathy is caused by *DNM2* or *BIN1* mutations or, less frequently, by *MYF6* or *CCDC78* mutations.
- the **autosomal recessive** type is caused by *BIN1* or *RYR1* mutations, or sometimes *TTN, SPEG* or *ZAK* mutations.
- Other genes such as *RYR1, TTN, SPEG, CACNA1S* and *ZAK(MAP3K20)* or *CCDC78* are involved in congenital myopathies which manifest as centronuclear myopathy with other clinical and histopathological characteristics.
- Around 16% of centronuclear myopathy cases are caused by unknown genetic mutations.

***Histopathology** involves using a microscope to examine tissue in order to characterise and identify abnormalities linked to a disease.*

Congenital fibre-type disproportion myopathies

Congenital fibre-type disproportion myopathies are characterised by smaller type I muscle fibres (hypotrophy) compared to type II muscle fibres.

Slow- or fast-twitch muscle fibres depending upon the activity required

In skeletal muscles, there are different types of muscle fibres whose appearances differ under a microscope.
- **Type I (slow-twitch) muscle fibres** are small in diameter, rich in mitochondria and myoglobin and highly vascularised. They are also known as red fibres. They are slow to fatigue and are therefore used during sustained and less powerful activities (maintaining posture, etc.).
- **Type II (fast-twitch) muscle fibres** are larger in diameter and are somewhat vascularised. They contain few mitochondria and are rich in glycogen. They are also known as white fibres. They are very powerful and fatigue quickly, therefore, they are used during short and intense activities.
- The proportion of fast-twitch muscle fibres in a muscle depends on the type of effort that the muscle exerts. It is possible to change this proportion depending on training and type of exercise performed.

- Ten genes are involved in congenital fibre-type disproportion myopathies: dominant *TPM3* gene mutations are the most common, recessive *RYR1* gene mutations are responsible in one in five cases, while dominant *ACTA1* gene mutations are the cause in 5% of cases; less frequently, dominant *TPM2* or *MYH7* gene mutations or recessive *SELENON, MYL2, HACD1, TTN, SCN4A* or *ZAK* gene mutations are responsible.

Myosin storage myopathy

- This myopathy is characterised by the presence of protein clumps, which contain abnormal myosin, within muscle fibres. It is caused by dominant mutations in the *MYH7* gene which codes for the beta myosin heavy chain.

Other congenital myopathies

- Other very rare muscle diseases, which have only been reported by doctors a handful of times, have been "classified" as suspected congenital myopathies, mainly due to the presence of abnormal structures within muscle fibres, such as fingerprint body myopathy and cylindrical spirals myopathy.

Ogasawara M et al. J Hum Genet. March 2023.

What causes congenital myopathies?

All congenital myopathies are genetic disorders. They are caused by mutations in the DNA which have generally been inherited from one parent (autosomal dominant, X-linked recessive) or both parents (autosomal recessive). The genetic mutations that cause congenital myopathies affect proteins that have essential roles in the functioning of muscle cells by inducing a deficiency in a particular protein or the formation of an abnormal protein.

Nearly 40 genes involved in congenital myopathies

With advances in high-throughput molecular diagnostic techniques (whole exome sequencing and whole genome sequencing), we now have a better understanding of the genetic basis of congenital myopathies, although this does complicate classification.

***Genetic disorders** are diseases caused by mutations in the DNA, i.e. the information that determines the biological functioning of our bodies. This information exists in our cells in the form of chromosomes which we inherit from our parents, and which our children inherit from us. This is why genetic disorders are often familial, i.e. several members of the same family may be affected by the genetic disorder.*

Type of congenital myopathy	Mode of inheritance[1]	Gene	Protein
Core myopathies			
Central core disease	AD, AR	*RYR1*	ryanodine receptor 1
Multiminicore myopathy	AR	*SEPN1*	selenoprotein N
		RYR1	ryanodine receptor 1
		MEGF10	membrane protein involved in cell proliferation and differentiation
		FXR1	RNA-binding protein, autosomal homologue of the fragile-X protein 1
		UNC45B	myosin-specific chaperone protein
		MYH7	myosin heavy chain 7
	AD	*ACTA1*	skeletal α-actin
		CCDC78	coiled-coil domain-containing protein 78
		MYH2	myosin heavy chain IIa
		ACTN2	actinin α2
		ACTA1	skeletal α-actin
		CCDC78	coiled-coil domain-containing protein 78
	AD, AR	*TTN*	titin
Other myopathy with focal structural disorganisation and alveolar aspect of the intermyofibrillar network	AR, AD	*CACNA1S*	subunit of the dihydropyridine receptor
Core-rod myopathies			
Core-rod myopathies	AD	*RYR1*	ryanodine receptor 1
		KBTBD13	protein from the BTB/Kelch family
	AR	*ACTA1*	skeletal α-actin
		NEB	nebulin
		TNNT1	slow skeletal muscle troponin T
		CFL2	cofilin-2

[1] autosomal dominant (AD); autosomal recessive (AR); X-linked recessive (XLR)

Nemaline myopathies			
Rod myopathy	AD, AR	*TPM3*	slow muscle α-tropomyosin
		ACTA1	skeletal α-actin
	AD	*TPM2*	β-tropomyosin
		KBTBD13	protein from the BTB/Kelch family
	AR	*NEB*	nebulin
		TNNT1	slow skeletal muscle troponin T
		CFL2	cofilin-2
		KLHL40	Kelch-like family member 40
		KLHL41	Kelch-like family member 41
		LMOD3	leiomodin 3
		MYO18B	myosin XVIIIB
		MYPN	myopalladin
		RYR3	ryanodine receptor 3
		CAP2	cyclase-associated protein
Cap myopathy	AD	*TPM2*	β-tropomyosin
		TPM3	slow muscle α-tropomyosin
	sporadic	*ACTA1*	skeletal α-actin
	AR	*MYPN*	myopalladin
Centronuclear myopathies			
X-linked myotubular myopathy	XLR	*MTM1*	myotubularin
Centronuclear myopathy	AD	*DNM2*	dynamin 2
	AD, AR	*BIN1*	amphiphysin 2
	AR	*SPEG*	SPEG protein
	AD	*CCDC78*	CCDC78
	AR	*RYR1*	ryanodine receptor 1
		TTN	titin
Congenital fibre-type disproportion myopathies			
Congenital fibre-type disproportion myopathy	AD	*TPM3*	slow muscle α-tropomyosin
		ACTA1	skeletal α-actin
		TPM2	β-tropomyosin
		MYH7	myosin heavy chain 7
	AR	*RYR1*	ryanodine receptor 1
		HACD1	3-hydroxyacyl-CoA dehydratase 1
		SELENON	selenoprotein N
		MYL2	myosin light chain 2
		TTN	titin
		SCN4A	sodium voltage-gated channel alpha subunit 4
		ZAK	ZAK
Myosin-related myopathies			
Myosin storage myopathy	AD	*MYH7*	myosin heavy chain 7
Myosin-related myopathies	AD, AR	*MYBPC1*	myosin-binding protein
	AD	*MYH2*	myosin heavy chain IIa
		MYH3	embryonic myosin heavy chain
		MYH8	perinatal myosin heavy chain
Rare congenital myopathies			
Contactin deficient myopathy	AR	*CNTN1*	contactin-1
Tubular aggregate myopathy	AD	*STIM1*	stromal interaction molecule 1
	AD	*ORAI1*	membrane calcium channel
	AD	*CASQ1*	calsequestrin 1
Zebra body myopathy	Unknown	*ACTA1*	skeletal α-actin
Classic congenital myopathy	AR	*SCN4A*	sodium voltage-gated channel alpha subunit 4
KY-related congenital myopathy with core targetoid defects	AR	*KY*	KY
STAC3-related congenital myopathies including Bailey-Bloch congenital myopathy	AR	*STAC3*	STAC3, which facilitates mechanical coupling between DHPR and RyR1
Congenital myopathy with fast-twitch (type II) fibre atrophy	AR	*MYL1*	myosin light chain 1
PAX7-related congenital myopathy	AR	*PAX7*	PAX7, a transcription factor which enables the differentiation of satellite cells into myoblasts
UNC45B-related congenital myopathy	AR	*UNC45B*	myosin chaperone B
Congenital amyotrophy	AR	*CACNA1H*	type T calcium voltage-gated channel subunit alpha1 H
TNNC2-related congenital myopathy	AD	*TNNC2*	fast skeletal muscle troponin C

[1] autosomal dominant (AD); autosomal recessive (AR); X-linked recessive (XLR)

Complex genotype-phenotype correlations

▪ Most congenital myopathies are caused by mutations in different genes. For example, nine genes are involved in core myopathy, around 12 genes are responsible for nemaline myopathy and there are seven different causative genes involved in centronuclear myopathies.

▪ **Some genes are responsible for several types** of congenital myopathy, such as the *RYR1* gene, whose mutations can lead to central core disease, multiminicore myopathy or centronuclear myopathy.

Did you know?

RYR1 mutations are the most common

To date, *RYR1* gene mutations are the mutations that have been most frequently found to be involved in congenital myopathies.

▪ Furthermore, **the same genetic mutation can lead to** different types of muscle damage in the same family, even in the same person at different ages.

▪ It is increasingly more common to find the same type of structural abnormalities in muscle fibres in the same type of congenital myopathy, although with manifestations that vary greatly in severity. This is the case for mutations in the *DNM2* gene for dynamin 2, the *RYR1* gene for ryanodine receptors and the *MYH7* gene for myosin which can each cause congenital myopathies that can be either more or less severe.

Goebel HH et al. Indian J Pathol Microbiol. May 2022.
Ogasawara M et al. J Hum Genet. March 2023.

***Genotype-phenotype correlation** studies are looking for links between genetic characteristics (genotype) and physical characteristics (phenotype, i.e. height, eye colour and shape, hair colour, disease manifestations, etc.). A somewhat close connection can therefore be identified between the presence of a specific genetic mutation and certain manifestations of a genetic disease.*

There are still more genes to be discovered

While the development of next-generation sequencing techniques has made it easier to identify new types of genetic mutations, the search for other causative genes is still relevant. As a matter of fact, in many people with a congenital myopathy, none of the genetic mutations known to be involved in these diseases are found.

High-performance DNA sequencing

New molecular genetic techniques have been developed to search for new genes or to identify new genetic mutations. Quicker and more accurate, these next-generation sequencing (NGS) techniques enable thousands of genes, even all the known genes of an individual (their genome), to be read "word for word" simultaneously (what researchers call "sequencing").

▪ The biggest issue with using these techniques is that they increase the risk of finding variations in an individual's DNA sequence, called genetic variants, which are not pathological (researchers call these "variants of uncertain significance" or "VUS"). Some variations have no consequences while others (genetic mutations) lead to the onset of a genetic disease.

▪ In order to differentiate between the two, geneticists need to see whether everyone who has the disease in the same family has the same variant. They also refer to medical literature and genetic databases (where numerous genetic mutations are recorded) and study the suspected gene in a laboratory (in cell or animal models) to confirm whether the variant is pathological or not.

▪ This requires geneticists and clinicians to work closely together, discussing each case in order to confidently establish a direct link between the genetic mutation detected by NGS and the individual's clinical manifestations.

The role of proteins in congenital myopathies

There are numerous modified proteins involved in congenital myopathies. Their pathological implications are mostly exerted through multiple mechanisms.

An intact cell membrane
In contrast to congenital muscular dystrophies, the integrity of the muscle cell membrane is preserved in congenital myopathies, which explains the normal or moderately increased levels of muscle enzymes (CPK) in the blood as they do not escape from muscles.

The pathological mechanisms involved in congenital myopathies affect intracellular processes which ensure that muscle cells continue to function normally such as excitation-contraction coupling, the interactions between thin and thick filaments induced by calcium, the molecular basis of muscle contraction, the formation of muscle tissue (myogenesis), membrane trafficking, oxidative stress and the quality control of proteins.

The mechanisms involved in some congenital myopathies are not yet understood, such as those linked to actinin α2, myopalladin and sodium voltage-gated channel alpha subunit 4.

Excitation-contraction coupling

This mechanism involves a large number of muscle cell proteins and structures.

▪ **Ryanodine receptor 1 (RyR1)** plays a major role in muscle contraction during the **excitation-contraction coupling** of muscle fibres.

Excitation-contraction coupling
This mechanism is the process by which a nerve impulse (the order to contract sent by the nerve) is transformed into a muscle cell contraction through the controlled release of calcium by the sarcoplasmic reticulum.
▪ The diffusion of the electrical signal (nerve impulse) across the entire cell membrane of the muscle fibre (**excitation**) triggers calcium to flow via RyR1 channels from the sarcoplasmic reticulum where it is stored to the cytoplasm of the muscle cell.
▪ This release of calcium into the cytoplasm activates the enzymes responsible for sliding the myofilaments over one another, causing the sarcomeres to shorten and therefore the **contraction** of the muscle fibre.
▪ The calcium is then reabsorbed by the sarcoplasmic reticulum, resulting in muscle relaxation.

*The **sarcoplasmic reticulum** is a complex network of cavities inside muscle cells in which the calcium required for muscle contraction is stored. The sarcoplasmic reticulum releases and reabsorbs this calcium, playing an essential role in muscle contraction.*

*An **ion channel** is a protein that is integrated into the membrane of a cell or cell compartment which allows certain molecules called ions (sodium, potassium, calcium, chloride) to enter or leave the cell or cell compartment in response to a signal. These channels play a very important role in the activity of "excitable" cells such as nerve and muscle cells.*

Ryanodine receptor 1 (RyR1) is a skeletal muscle calcium release channel. It is situated on the terminal cisternae of the sarcoplasmic reticulum, which are located near the T-tubules with which they form a "triad".
The part of the RyR1 which is situated on the cytoplasm side interacts with calcium, magnesium, caffeine, ATP and ryanodine.
RyR1 also plays a role in regulating the concentration of intracellular calcium (outside of any contractions) and may be a modulator of oxidative balance and gene expression.

▪ **CACNA1S** is the main subunit of **dihydropyridine receptors (DHPRs)** which are localised in transverse tubules.

Changing DHPR opens RyR1

- The depolarisation of the muscle cell membrane triggered by nerve stimulation elicits a conformational change of the dihydropyridine receptor (DHPR) in the membrane.
- This conformational change of the DHPR triggers the opening of RyR1, resulting in a significant amount of calcium being released into the cytoplasm.

- The **STAC3** protein facilitates the essential mechanical coupling of the CACNA1S subunit of the DHPR, which is integrated into the muscle cell membrane, and that of RyR1, which is integrated into the membrane of the endoplasmic reticulum.

- When the calcium concentration in the sarcoplasmic reticulum is low, the **membrane calcium channel ORAI1** is activated and allows calcium to enter cells. Its opening is controlled by **stromal interaction molecule 1**, coded for by the *STIM1* gene and localised to the membrane of the sarcoplasmic reticulum.

- The **CCDC78** protein plays a role in muscle contraction which is still unclear (possibly in the regulation of triad structure and function).

- **Calsequestrin 1** is a protein that stores calcium in the sarcoplasmic reticulum; it interacts with ryanodine receptor 1 (RyR1) and triadin, which anchors it close to RyR1. Calcium is reabsorbed into the endoplasmic reticulum via the SERCA1 calcium pump.

The components of sarcomeres

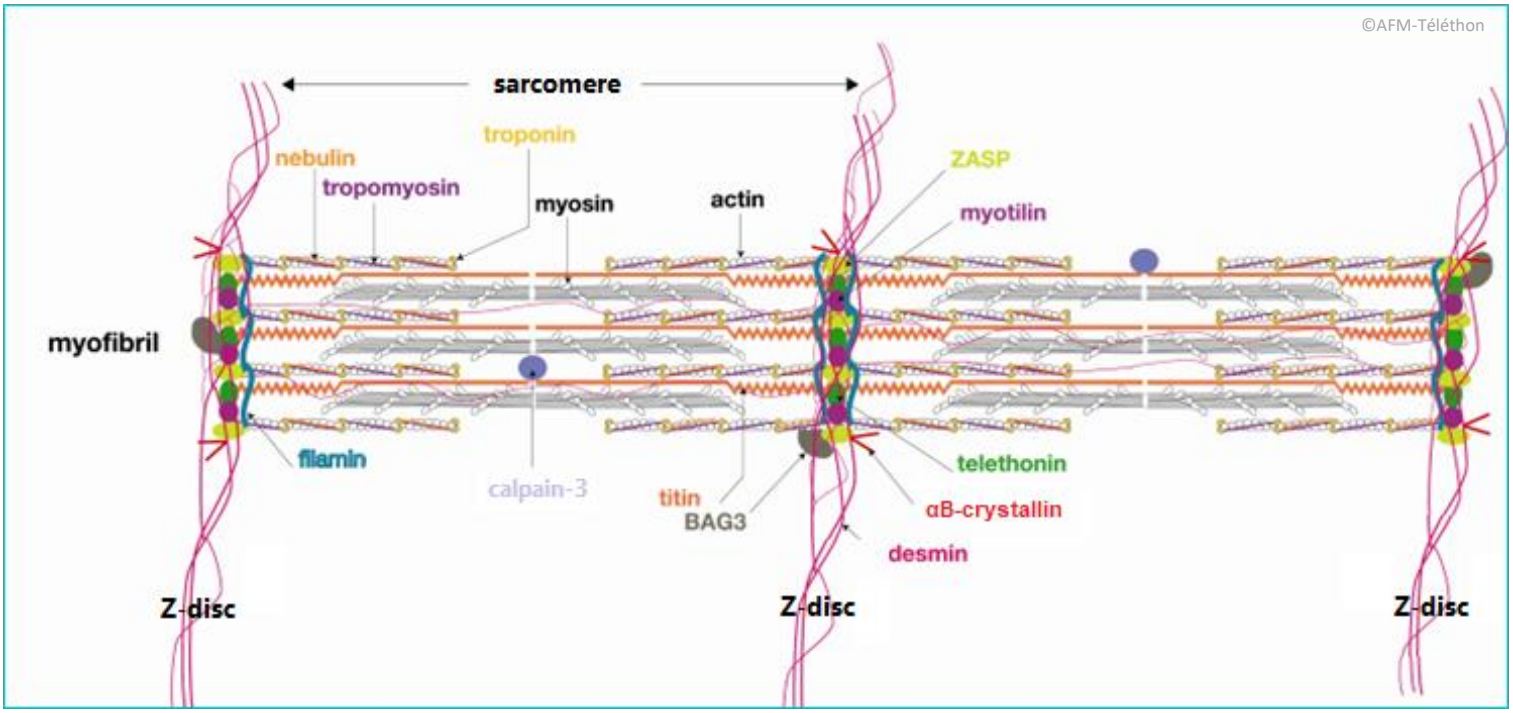
- **Nebulin, α-actin, α-tropomyosin, β-tropomyosin, KBTBD13, troponin complex** and **myopalladin** are all sarcomere proteins and components of thin filaments.

- **Cofilin-2** and **leiomodin-3** are proteins that regulate the stability and renewal of thin filaments.

- **KLHL40 and KLHL41** belong to the Kelch-like (KHL) protein family. They are involved in muscle formation (myogenesis) and play a role in the assembly of myofibrils.

Transverse tubules, *or* ***T-tubules,*** *are thin invaginations which are found at regular intervals over the entire muscle fibre membrane. Like fingers on a glove, they penetrate deeply into the muscle fibre membrane so that they come into contact with the sarcoplasmic reticulum which surrounds myofibrils.*
The sarcoplasmic reticulum plays an essential role in muscle contraction by releasing the calcium that it stores (which causes the myofibrils to contract) and then reabsorbing it (which triggers relaxation).
➤➤ Le muscle squelettique [Skeletal muscle], Savoir & Comprendre references documents, AFM-Téléthon.

A ***triad*** *is formed by a T-tubule and two terminal cisternae of the sarcoplasmic reticulum. It is the cellular structure where the excitation-contraction coupling of muscle cells takes place.*



__Chaperones__ are proteins that enable proteins being synthesised to take their shape in space (adequate three-dimensional folding).

A __myoblast__ is a muscle stem cell. During muscle formation (myogenesis), the fusion of several myoblasts leads to the formation of a long cell (a tube) called a __myotube__ which will continue to differentiate to become a mature muscle fibre.

- **KBTBD13** is a protein that binds to the actin in thin filaments, which is necessary for myofibrils to relax properly.
- **UNC45** is a chaperone involved in the assembly of myosin in skeletal and cardiac muscle fibres; it plays a role in the fusion of myoblasts and the organisation of sarcomeres in myofibrils.
- **α-actinin 2** is localised to the Z-discs of cardiac and skeletal muscles where it anchors thin actin filaments.
- **Titin** is a giant sarcomere protein that spans half the length of a sarcomere; its main role is to organise and stiffen cardiac and skeletal muscle sarcomeres.
- Myosin heavy chains are known to be involved in myosin-related myopathies ("myosinopathies"). **Myosin** heavy chain mutations have been found in distal myopathies with multiminicores (a type of congenital myopathy), myopathy with eccentric cores and myopathies with axial contractures.
Unconventional **myosin XVIIIB**, which is found at the Z-discs of muscle fibres and which may participate in the function and maintenance of the contraction mechanism, has been identified in a nemaline myopathy with cardiac involvement.
- Slow skeletal muscle myosin-binding protein C, **MYPC1,** helps stabilise thick myosin filaments and regulate actin-myosin cross-bridges in striated muscle. In 2019, a new type of early-onset myopathy caused by a dominant *MYBPC1* gene mutation was described. It manifests as a myogenic tremor of the head and hands whose molecular mechanisms are in the process of being clarified.

Molecular transport

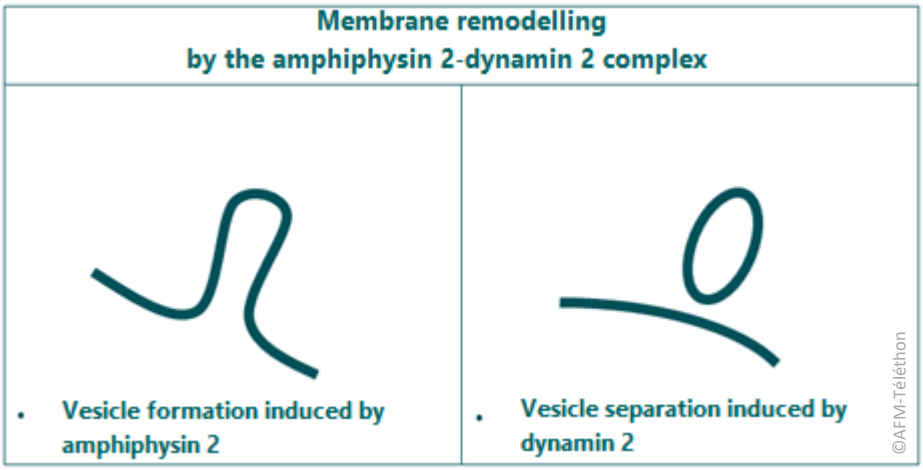
Myotubularin, dynamin 2 and amphiphysin 2 are involved in a type of molecular transport which takes place inside cells called membrane trafficking or intracellular transport.

Intracellular transport
Membrane trafficking is the collection of mechanisms that enable a cell to circulate material from one cell compartment to another by using small, membrane-bound sacks called vesicles.

- **Myotubularin** is a protein coded for by the *MTM1* gene which is expressed in all tissues. This enzyme regulates the sorting and trafficking of intracellular vesicles.

*An **enzyme** is a protein that specifically allows, facilitates or accelerates a particular chemical reaction in our bodies (cell digestion, protein synthesis, DNA replication, etc.).*

- **Amphiphysin 2** (coded for by *BIN1*) and **dynamin 2** (coded for by *DNM2*) work together during membrane remodelling and tubule and vesicle formation; amphiphysin 2 causes the membrane to curve to form a protrusion (future membrane tubule/vesicle), then it binds to dynamin 2 which causes the vesicle to separate from the membrane via membrane fission.



Oxidative stress

Selenoprotein N, coded for by the *SELENON* gene, belongs to a group of proteins called selenoproteins which contain selenium. Its function in cells is still unknown. It is localised in the endoplasmic reticulum in close contact with mitochondria, suggesting its involvement in one or several processes that take place there (protein synthesis, lipid synthesis, muscle contraction, etc.). Selenoprotein N has a protective effect against oxidative stress. It is also essential for ryanodine receptor 1 to function well.

***Oxidative stress** is a situation in which a cell no longer controls the presence of excessive toxic molecules produced by cellular respiration (free radicals). In excess, these free radicals can damage cells and DNA.*

Ion channel

- The **Nav1.4 sodium (Na^+) channel,** whose alpha subunit is coded for by the *SCNA4* gene, is involved in muscle contraction; during synaptic transmission at the neuromuscular junction, Nav1.4 sodium channels are activated which allows the nerve impulse to spread to the whole muscle cell which then contracts.

Muscle growth and development

▪ The **SPEG protein** is a protein kinase which is mainly expressed in striated muscle. It plays a role in regulating the growth and differentiation of vascular smooth muscle cells.

▪ **MEGF10** is a membrane receptor involved in the differentiation and proliferation of muscle stem cells (satellite cells). Exercise or trauma activates satellite cells; the overexpression of MEGF10 transforms them into myogenic cells, i.e. future muscle fibres.

▪ Unlike those present in other tissues, **FXR1 proteins** in cardiac and skeletal muscle integrate a piece of protein coded for by exon 15 of the *FXR1* gene; it is the recessive genetic mutations of this exon that cause multiminicore myopathy in humans.

▪ The **TRIP4** and **ASSC1 proteins** are subunits of the ASC1 complex which regulates the differentiation and growth of muscle fibres.

▪ The **MYOD1** protein is a transcription factor which is only expressed in skeletal muscle and is essential for its differentiation and repair.

__Smooth muscle__ is found in the walls of blood vessels, the digestive tract, the urinary tract and some organs. This type of muscle contracts involuntarily. Its organisation is different to that of skeletal muscle.

Every gene is organised into an alternating arrangement of coding sequences (__exons__) and non-coding sequences (introns). The term "coding" is used to refer to the portions of genes that are used by cell machinery as a blueprint for making proteins, therefore only exons are translated into proteins.

Treatment avenues

The different treatment avenues in congenital myopathies consist of:
- improving or correcting genetic defects (primarily through gene therapy),
- fixing mechanisms altered by the disease or treating general muscle imbalance with drugs

Treatment avenues explored in congenital myopathies

Gene	*Approach*[1]	*Mechanism*	*Compound*	*Stage*
Core myopathies				
RYR1	GT	modification of RyR1 expression using exon skipping	U7 antisense oligonucleotide	Cell model (2013)
		RYR1 knockdown	siRNA	Preclinical (2012)
	D	reduction of calcium release	RyR1 inhibitors: dantrolene, FKBP12 stabilisers	ARM_210_RYR1-RM trial (NCT04141670)
		reduction of oxidative stress	N-acetylcysteine	NCT02362425 trial (2020)
		neuromuscular junction transmission enhancement through acetylcholinesterase inhibition	pyridostigmine	Case study (2014)
		epigenetic action: histone deacetylase (HDAC) and DNA methyltransferase (DNMT) inhibition	TMP269, 5-Aza	Preclinical (2022)
		abnormal protein aggregate prevention by impacting endoplasmic reticulum stress	chemical chaperone: 4-phenylbutyric acid (4-PBA)	Preclinical (2017)
		neuromuscular junction enhancement using an adrenergic receptor agonist	salbutamol	Clinical (2004)
		p38MAPK inhibition	SB203580, SB202190	Cell model (2020)
SEPN1	D	reduction of oxidative stress	N-acetylcysteine	SelNac trial (NCT02505087)

Treatment avenues explored in congenital myopathies *(continued)*				
Gene	*Approach*[1]	*Mechanism*	*Compound*	*Stage*
Nemaline myopathies				
ACTA1	GT	ACTC expression	genetic cross	Preclinical (2013)
		MYL4 overexpression	AAV6-*Myl4*	Preclinical (2016)
	D	myofilament interaction increase through troponin activation	tirasemtiv (calcium sensitiser)	Preclinical (2021)
		amino acid supplementation	L-tyrosine	Preclinical (2008, 2011, 2018)
		myostatin inhibition	ActRIIB-mFc, mRK35	Preclinical (2019)
NEB	D	myofilament interaction increase through troponin activation	troponin activators/calcium sensitisers: tirasemtiv, levosimendan, CK-2066260	Preclinical (2013, 2015, 2019)
		myofilament interaction increase through MYH7 activation	omecamtiv mecarbil	Preclinical (2019)
		myostatin inhibition	ActRIIB-mFc	Preclinical (2019)
		amino acid supplementation	L-tyrosine, L-carnitine, taurine, creatine	Preclinical (2018)
TPM2/3	D	myofilament interaction increase through troponin activation	troponin activators/calcium sensitisers: EMD 57033, CK-1909178	Preclinical (2008, 2012)
TPM3	D	amino acid supplementation	L-carnitine	Preclinical (2021)
			L-tyrosine	Clinical (2008)
KLHL40	D	neuromuscular junction transmission enhancement through acetylcholinesterase inhibition	pyridostigmine	Case report (2016)
Centronuclear myopathies				
MTM1	GT	myotubularin expression increase through gene replacement	AAV8-*MTM1*	ASPIRO trial (NCT03199469)
		myotubularin expression increase through enzyme replacement	3E10Fv-MTM1	Preclinical (2013)
		myotubularin homologue overexpression	AAV9-*MTMR2*	Preclinical (2017, 2018)
		dynamin 2 expression reduction	RNAi *DNM2* (AAV2/9-shRNA, ASO)	DYN101 Unite-CNM trial (NCT04033159)
		phosphatidylinositol 3-phosphate (PI3P) level reduction	genetic cross	Preclinical (2016)
		amphiphysin 2 overexpression	AAV9-*BIN1*	Preclinical (2019)
		myostatin inhibition	AAV-PropD76A	Preclinical (2017)
	D	PI3K inhibition to reduce PI3P level	wortmannin	Preclinical (2016)
		neuromuscular junction transmission enhancement through acetylcholinesterase inhibition	pyridostigmine	Clinical and preclinical (2011, 2012)
		myostatin inhibition	ActRIIB-mFc	Preclinical (2011, 2014)
		epigenetic action: HDAC inhibition	valproic acid	Preclinical (2022)
		unknown	tamoxifen	TAM4MTM trial (NCT04915846)
		autophagy activation	mTOR pathway inhibitors: RAD001, AZD8055	Preclinical (2013)
DNM2	GT	dynamin 2 level reduction	CRISPR/Cas9	Cell model (2019)
			RNAi *DNM2* (AAV2/9-shRNA, ASO, allele-specific AAV1-shRNA)	DYN101 Unite-CNM trial (NCT04033159)
		amphiphysin 2 overexpression	AAV9-*BIN1*	Preclinical (2022)
	D	neuromuscular junction transmission enhancement through acetylcholinesterase inhibition	pyridostigmine	Clinical and preclinical (2013)
BIN1	GT	dynamin 2 level reduction	RNAi *DNM2* (ASO)	Preclinical (2022)

[1] GT: gene therapy; D: drug

Treatment avenues explored in congenital myopathies *(continued)*				
Gene	*Approach*[1]	*Mechanism*	*Compound*	*Stage*
Other congenital myopathies				
various	D	neuromuscular junction enhancement using an adrenergic receptor agonist	salbutamol	COMPIS trial (NCT05099107)
various	exercise	unknown	aerobic exercise	Preclinical (2004) and clinical (2016, 2020)
TNNC2	D	myofilament interaction increase through troponin activation	tirasemtiv (calcium sensitiser)	Preclinical (2021)
STIM1	GT	*ORAI1* knockdown	RNAi *Orai1* (AAV9-shRNA)	Preclinical (2022)

Based on Gineste C, Laporte J. Curr Opin Pharmacol. 2023 Feb;6 :102328.

[1] GT: gene therapy; D: drug

Gene therapy

The genetic diagnosis of congenital myopathies is essential, not just for treatment but also because it is a prerequisite for gene therapy.
Various gene therapy techniques aim to modify the expression or functions of genes:
- gene transfer provides a replacement gene;

Gene transfer
Gene transfer introduces a gene to compensate for an existing genetic defect.
▪ It is particularly useful in autosomal recessive and X-linked diseases which lead to a loss of gene expression.
▪ Gene transfer causes temporary common side effects such as a decrease in platelet levels and an increase in certain liver enzyme levels, which are controlled by immunosuppressants.
▪ These approaches use a viral transporter, an AAV (adeno-associated virus), to deliver the therapeutic molecule to cells.
The limited transport capacity of AAVs means that the gene being transferred cannot be a large size (*RYR1, TTN* and *NEB* are too big).
▪ Furthermore, 30-60% of children have a natural immunity to AAV9 (the viral vector used to target muscles), preventing them from successfully receiving a gene transfer. Researchers are trying to find ways to get around this AAV9 immunity.

*An **antisense oligonucleotide** (ASO) is a fragment of RNA that is usually synthesised in a laboratory which can bind specifically to natural messenger RNA molecules. The nucleotide sequence (its chemical formula) of antisense oligonucleotides is complementary to that of the messenger RNA molecules which it targets. They can therefore modify messenger RNA (skip or incorporate exons by intervening during its maturation stage (splicing)).*

- genome editing modifies the "writing" of a gene by altering its DNA sequence which is achieved by using a tool such as the CRISPR/Cas9 system
- a simple, fast and effective system for cutting DNA in a specific place in the genome;
- RNA modulation is achieved using oligonucleotides which interfere with the synthesis or translation of RNA into proteins, for example exon skipping to get around a genetic mutation or neutralisation of a dominant mutation.
These techniques are making progress towards improving the muscle specificity of antisense oligonucleotides, the safety of AAVs and the sizes of DNA that AAVs can transport. Regarding the CRISPR/Cas9 system technique, targeting the mutation, the size and the efficiency of the genome editing still need to be improved.
Findlay AR, Weihl CC. Continuum (Minneap Minn). 2022 Dec 1;28(6):1800-1816.

In centronuclear myopathies

- Two clinical trials are currently underway in X-linked myotubular myopathy - a gene therapy trial (ASPIRO trial) and a tamoxifen trial.
- In centronuclear myopathies, RNA interference (RNAi) targeting mutated dynamin (R465W) was found to be effective in mice.

In nemaline myopathies

- The *NEB* gene is one of the largest genes in the genome and its size prevents it from being inserted into an AAV. One solution could be to use mini-nebulin which, although incomplete, would be functional.
- *ACTA1* gene mutations can affect practically every amino acid of skeletal α-actin, which therefore involves developing an individual strategy for each mutation.
- The upregulation of α-cardiac actin induced by CRISPR/Cas9 genome editing is currently being studied in *ACTA1*-related nemaline myopathies.
- In recessive forms of nemaline myopathy, the size of the *LMMOD3, CFL2, KLHL40* and *KLHL41* genes allows them to be inserted into AAVs.

Fisher G et al. Expert Opin Ther Targets. October 2022.

In RyR1-related myopathies

- The size of the *RYR1* gene means that it cannot be inserted into a viral vector, preventing direct gene replacement.
- There are no functional truncated RyR1 proteins which would enable a "mini-RyR1" to be transferred.
- The recent "prime editing" technique enables a base to be replaced (i.e. a letter of the genetic message to be corrected); its advances and application in RyR1-related myopathies may be able to correct 97% of *RYR1* mutations.
- The use of small interfering RNA (siRNA) molecules enables dominant RyR1 mutations to be neutralised, with subsequent improved muscle function in two mouse models;
- Exon skipping is only applicable when extra exons are created by the genetic mutation, as none of the 106 exons of the *RYR1* gene can be deleted.
- Antisense oligonucleotides can also be used to retain an exon that is abnormally eliminated due to a genetic mutation; this may constitute 6% of *RYR1* mutations.
- Finally, trans-splicing replaces the abnormal RNA segment with a normal RNA segment, with the advantage of being able to address all the mutations present in the same region of the RNA; however, it has been found to be ineffective in a mouse model of Duchenne muscular dystrophy.

Marty I et al. Curr Opin Pharmacol. 2023 Feb;68:102330.

In other congenital myopathies

- The *SELENON* gene has the right characteristics for gene therapy, whereas the complexity and large size of the *TTN* gene mean that it is a real challenge to use this gene in gene therapy.
- Dominant *MYH7* mutations could be subjected to silencing techniques (oligonucleotides, CRISPR/Cas9, etc.). The first projects of this kind were carried out in mice in 2020.

Clinical trials in congenital myopathies

Clinical trials consist of evaluating the effects of a potential treatment (drug candidate, medical device, etc.) for a disease in order to ensure that it is well tolerated and effective.

Did you know?

The four phases of a clinical trial

A drug candidate is evaluated during four successive phases: I, II, III and IV.

- **Phase I: Safety/tolerability**

A drug candidate is tested for the first time on a small group of individuals (often health volunteers) to evaluate its safety/tolerability and its movement through the body (pharmacokinetics).

- **Phase II: Optimum dose/Effect**

Phase II, conducted on a comparable group of volunteers with the disease, studies the safety and efficacy of the product and will determine the optimum dose to be used.

- **Phase III: Therapeutic efficacy**

Phase III is conducted on a larger number of participants who have the disease in order to determine the treatment's therapeutic efficacy compared to an existing treatment or a placebo. At the end of this phase, the drug may obtain marketing authorisation (MA).

- **Phase IV: Pharmacovigilance**

The goal of phase IV, which is conducted after the drug has been launched on the market, is to fine-tune knowledge of the product and to identify any serious and/or unexpected side effects caused by its administration.

Ongoing clinical trials in France

TRIAL TITLE	THERAPEUTIC APPROACH	CLINICAL DEVELOPMENT			REGULATORY STATUS
		PHASE I	PHASE II	PHASE III	
ASPIRO trial X-linked myotubular myopathy	**Gene therapy** *MTM1* gene transfer	**AT132** Not recruiting			
Exo-NMD1 trial Congenital myopathies	**Exoskeleton**	**Myosuit™** Recruiting			**Medical device**
Exo-KGO1 trial Congenital myopathies	**Exoskeleton**	**Keeogo™** Recruiting			**Medical device**

Ongoing clinical trials around the world

In congenital myopathies

COMPIS trial

Salbutamol (Ventoline®), which is already marketed, is used by asthmatics to help dilate their airways. It also affects muscles, which makes it a doping product.

In congenital myopathies, case reports and studies on a small number of subjects have reported an increase in muscle strength with oral salbutamol.

- The COMPIS trial is currently evaluating whether six months of oral salbutamol increases muscle strength in patients with congenital myopathies. This is measured using the MFM-32 scale.

COMPIS trial: an open-label, crossover, randomised evaluation of salbutamol taken for six months

In Sweden

20 participants (6 to 30 years old)

Enrolling by invitation

19 months of follow-up

October 2021 – December 2025

NCT05099107

Assessment of two exoskeletons

Conducted by the Institut de Myologie [Institute of Myology] in Paris, the Exo-NMD1 and Exo-KGO1 trials aim to evaluate the safety and immediate effects of using a knee-hip powered soft exoskeleton (**MyoSuit™**) and a lower-limb powered dermoskeleton (**Keeogo™**) in 52 people with muscular dystrophy, congenital myopathies, inflammatory myopathy, mitochondrial myopathies or glycogen storage disease.

These trials also aim to create guidelines for using the MyoSuit™ and Keeogo™ devices efficiently and safely in people with neuromuscular diseases.

They are a prerequisite for future studies that will evaluate the benefits of long-term use of such devices at home.

Exo-NMD1 trial

In France

52 participants (18 to 70 years old)

Recruiting

1 month of follow-up

January 2022 – July 2023

NCT05200702

Exo-KGO1 trial

In France

52 participants (18 to 70 years old)

Recruiting

1 month of follow-up

January 2022 – July 2023

NCT05199246

*The **MFM** (Mesure de Fonction Motrice [Motor Function Measurement]) is a quantitative scale that measures motor function abilities in people (adults and children) with a neuromuscular disease. It is reproducible, easy to use (35 minutes) and is adapted so that it can be used regardless of impairment severity (able or unable to walk). It comprises 32 items (MFM-32) classified into three domains - D1: standing and transfers, D2: axial and proximal motor function and D3: distal motor function.*

A pediatric version, which comprises 20 items (MFM-20), was approved in 2009 for children under seven years old.

WEBSITE mfm-nmd.org/

In RyR1-related myopathies

S 48168 (ARM 210)

Drugs derived from benzothiazepines, such as S107 and its derivatives, called Rycals, which are developed by ARMGO Pharma, prevent the unbinding of RyR1 and FKBP12, favouring the closed position of the RyR1 calcium channel.

Did you know?

Stabilising the closed position of RyR1

By binding to ryanodine receptor 1 (RyR1), calstabin 1 (or FKBP12) stabilises the closed position of the calcium channel and reduces "leaks" from the channel.

▪ In the United States, ARMGO Pharma is conducting an open-label trial of two doses of S48168 or ARM210 (another Rycal) administered over four weeks in 10 subjects whose muscle fibres have leaky RyR1 channels which responds to *ex vivo* administration of S48168.

Phase I
Safety/tolerability

S48168 or ARM210 trial: safety and tolerability, pharmacokinetics, effects on muscle strength

In the United States

10 participants (over 18 years old)

Data currently being analysed

6 weeks of follow-up

August 2020 – June 2023

NCT04141670

In centronuclear myopathies

ASPIRO trial placed on hold but monitoring continues

The ASPIRO trial aims to evaluate the safety, tolerability and efficacy of two different doses (a lower dose in the first participants, then a dose three times higher in the following participants) of AT132 administered via a single intravenous infusion in 24 boys under the age of five with X-linked myotubular myopathy.

AT132

AT132 is a gene therapy product which enables a normal *MTM1* gene to be transferred using a viral vector - an inactive AAV8.

▪ This phase I/II clinical trial, which started in 2017, was placed on hold in August 2020 due to the death of three participants who received the higher dose of AT132. However, the analysis of these serious complications in consultation with the FDA allowed the AT132 trial to resume at the end of December 2020 with only the lower dose being offered. It also excluded children with preexisting hepatobiliary disease and/or those over the age of five. It was then placed on hold again on 24 September 2021 by the American regulatory authorities (Food and Drug Administration - FDA) following the death of a fourth participant.

Astellas press release from 14 September 2021

ASPIRO trial: *MTM1* gene transfer

In France and abroad

26 participants (under 5 years old)

Recruitment

10 years of follow-up

August 2017 – October 2030

NCT03199469

Phase I
Safety/tolerability

Phase II
Dose/effect

Phase III
Efficacy

▪ To date, 24 children have received AT132 - seven at the lower dose (1.3 x 10^{14} vg/kg) and 17 at the higher dose (3 x 10^{14} vg/kg) and will continue to be monitored closely for 10 years.

WEBSITE www.afm-telethon.fr/

Termination of the Unite-CNM trial

DYN101 is an oligonucleotide which targets *DNM2* pre-messenger RNA in order to decrease the amount of dynamin 2 expressed in cells.

The **Unite-CNM trial** aimed to study the pharmacokinetics and pharmacodynamics of DYN101 and evaluate its tolerability and safety at different doses (low, middle, high) administered via intravenous infusion in 18 subjects over the age of 16 with centronuclear myopathy caused by *DNM2* or *MTM1* gene mutations.

▪ While the first dose was well tolerated, the second dose, which was the lowest dose at which any potential efficacy had been expected, led to liver enzyme abnormalities and low platelet levels. It is for this reason that, in collaboration with an independent committee, Dynacure decided to end the DYN101 trial in July 2022. The participants are still being monitored and have seen their liver enzyme and platelet levels return to normal.

https://myotubulartrust.org/dyn101-unite-cnm-anti-sense-programme-ends/

Tamoxifen in X-linked myotubular myopathy

Tamoxifen is an anti-oestrogen drug which has been used for a number of years to treat some types of cancer, in particular breast cancer.

In 2018, two teams demonstrated that tamoxifen improves overall motor function and significantly increases the life span of mouse models of X-linked myotubular myopathy.

Maani N et al. Nat Commun. November 2018.
Gayi E et al. Nat Commun. November 2018.

▪ The TAM4MTM trial is evaluating the tolerability, safety and efficacy of tamoxifen (Apo-Tamox®) to improve motor and respiratory function in 16 subjects over the age of two with X-linked myotubular myopathy. It is being conducted in Canada, the United States and the United Kingdom.

Muscle strength is evaluated using the MFM-32 scale and a 10 meter walk test.

*The **MFM** (Mesure de Fonction Motrice [Motor Function Measurement]) is a quantitative scale that measures motor function abilities in people (adults and children) with a neuromuscular disease. It is reproducible, easy to use (35 minutes) and is adapted so that it can be used regardless of impairment severity (able or unable to walk). It comprises 32 items (MFM-32) classified into three domains - D1: standing and transfers, D2: axial and proximal motor function and D3: distal motor function.*

WEBSITE mfm-nmd.org/

Phase I
Safety/tolerability

Phase II
Dose/effect

TAM4MTM trial: double-blind, crossover, placebo-controlled evaluation of tamoxifen taken for six months

Abroad (outside France)

16 participants (over 2 years old)

Recruiting

15 months of follow-up

December 2020 – December 2023

NCT04915846

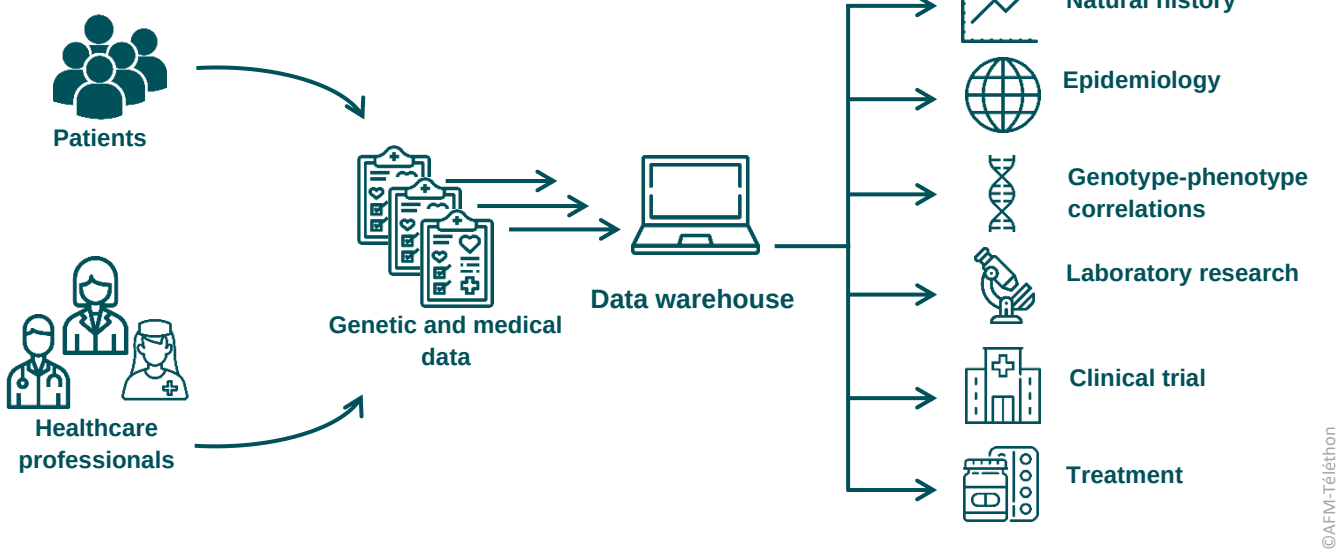
Clinical research

Observational studies and databases that detail diseases are essential for clarifying diagnostic approaches, improving treatment and planning future clinical trials.

These clinical research tools collect data on:

- the topography of affected muscles using muscle imaging techniques;
- the type of lesions observed during muscle biopsies;
- disease manifestations and their variability in the same family and between unrelated families;
- the parameters for monitoring the course of muscle damage, which are essential for clinical trials;
- the results of genetic diagnoses and genotype-phenotype correlations.

***Genotype-phenotype correlation** studies are looking for links between genetic characteristics (genotype) and physical characteristics (phenotype, i.e. height, eye colour and shape, hair colour, disease manifestations, etc.).*



Medical data warehouses

Medical databases and data warehouses collect medical and genetic data on people with the same disease, often without a time limit.

The analysis of this data helps to determine the natural history of the disease, establish genotype-phenotype correlations and recruit participants to clinical trials.

There are two types of **observational studies:**
- **cross-sectional studies** which describe how a disease manifests in a group/population of patients at a single point in time;
- **longitudinal studies** which describe the course of a disease over time (natural history protocol, for example).

In several neuromuscular diseases

Remote consultations

The recent COVID-19 pandemic has been the basis for important changes in the doctor-patient relationship which involve using new remote medical technologies. It increased the use of telemedicine, which was previously reserved for areas where there are few doctors ("healthcare deserts").

A rather positive change according to healthcare professionals

The European Reference Network for Rare Neuromuscular Diseases (EURO-NMD) carried out a survey among its members which revealed that:
- all of the 42 out of 76 centres who responded to the survey had increased their use of remote medical technologies,
- this comprised mainly of telephone consultations and teleconsultations (video),
- the vast majority of the centres were satisfied with this alternative way of working,
- these technologies, useful and satisfactory as they are, were not really suitable for initial consultations or patients with cognitive impairment.

However, many believe that remote medicine should be used mainly for monitoring or administrative management purposes, and should never replace face-to-face appointments.

El-Hassar L et al. J Neuromuscul Dis. 2023.

But patients prefer practice-based consultations

The majority of neuromuscular disease patients are comfortable with telemedicine (video or telephone consultations) but 50% prefer in-person consultations at a doctor's surgery. This is what was revealed by a telephone survey conducted by 10 specialist centres in Canada and the United States in 520 neuromuscular disease patients.

- Although neither the technology (92% were comfortable with the technology), cost, nor confidentiality (84% thought that virtual consultations were sufficiently private) were a concern, the majority of people interviewed (50%) preferred face-to-face consultations. Notably, 40% of those interviewed were worried about the lack of physical examination, while 20% were concerned about the lack of vital signs evaluation (pulse, respiration rate, etc.).

On the whole, teleconsultations were seen more as a complementary option rather than a substitute for in-person consultations.

Hafeez K et al. Muscle Nerve. August 2022.

In several congenital myopathies

Molecular and genetic studies of congenital myopathies

This study, conducted at Boston Children's Hospital (United States) and supported by the Muscular Dystrophy Association (MDA), aims to identify the causative genes of congenital myopathies and define the clinical characteristics associated with these diseases.

Molecular and genetic studies of congenital myopathies

In the United States

4,000 participants (all ages)

Recruiting

Blood and saliva samples + clinical exams

August 2003 – January 2050

NCT00272883

The CMDIR

- The Congenital Muscle Disease International Registry (CMDIR) aims to carry out a census of the global congenital muscle disease community. It is a space where people affected by a congenital muscle disease, with or without genetic confirmation, can register and follow clinical news.
- The organisers of this registry are directors of academic institutions and charities. The information available on the CMDIR has been provided with the help, advice and approval of a group of experts in congenital muscle diseases.

Congenital Muscle Disease International Registry (CMDIR)

International

Created in 2009

Recruiting

2,600 registrations *(May 2020)*

NCT01403402

WEBSITE www.cmdir.org

Muscle strength and volume

A Danish study is investigating whether there is a relationship between muscle strength measured using a dynamometer and muscle volume evaluated using an MRI of the thigh and calf muscles in inherited muscle diseases, starting with congenital myopathies.

Relationship between muscle strength and cross-sectional area of muscles on an MRI in congenital myopathies

In Denmark

200 participants (18 to 80 years old)

Not recruiting

One one-hour visit to take measurements

December 2016 – December 2026

NCT03018184

Bullying in young people

▪ A Canadian, multisite, cross-sectional study aimed to evaluate bullying in young people (10 to 19 years old) with a congenital myopathy or muscular dystrophy.

What does "bullying" mean?
Bullying is repeated aggressive behaviour which is intended to hurt someone. It is characterised by an imbalance of power between the bully and their victim.

▪ In Canada, at least one in three young people report having been bullied. Studies have shown that young people with a chronic disease or disability are more likely to be bullied, especially if the disability is visible.

Bullying in young people with a congenital myopathy or muscular dystrophy

Abroad (outside France)

29 participants (10 to 19 years old)

Data currently being analysed

Questionnaire ± Interview

January 2021 – September 2022

NCT04733976

In core myopathies

A Dutch natural history of *SELENON*- and *LAMA2*-related congenital myopathies

▪ A prospective descriptive study is being conducted in the Netherlands by Radboud University (Nijmegen) in 20 participants with a congenital myopathy caused by *SELENON* gene mutations or *LAMA2*-related congenital muscular dystrophy (CMD).

▪ This natural history study will help researchers prepare for any future clinical trials as it also aims to determine relevant follow-up and evaluation parameters, as well as the optimal cardiac and respiratory monitoring schedule.

Natural history of *SELENON*-related congenital myopathies and *LAMA2*-related congenital muscular dystrophy

Abroad (outside France)

20 participants (all ages)

Recruiting

18 months of follow-up

April 2020 – April 2023

NCT04478981

In centronuclear myopathies

The Myotubular and Centronuclear Myopathy Patient Registry

*The **TREAT-NMD Alliance** is an international network for neuromuscular diseases which brings together scientists, clinicians and patient groups. Since 2012, its goal has been to maintain an infrastructure which ensures that the most promising research reaches patients quickly. It also seeks international recognition of the best current care practices for people with neuromuscular diseases. With this dual aim, the TREAT-NMD Alliance has developed tools that are essential for clinicians and treatment developers such as global patient registries and the TACT (TREAT-NMD Advisory Committee for Therapeutics), an independent evaluation platform for preclinical projects.*

WEBSITE www.treat-nmd.eu

MTM and CNM Registry

This international collection of medical data on myotubular and centronuclear myopathies is run by the John Walton Muscular Dystrophy Research Centre at Newcastle University (United Kingdom) and is part of the TREAT-NMD network. It is funded by the Myotubular Trust, the British charity Muscular Dystrophy UK, and more recently by Astellas which is developing a gene therapy product for X-linked myotubular myopathy.

WEBSITE myotubulartrust.org

WEBSITE www.musculardystrophyuk.org

▪ Launched in 2013, its aim is to collect data over time from people with X-linked myotubular myopathy or centronuclear myopathy, as well as from female carriers of X-linked myotubular myopathy.

An online questionnaire

▪ The participants respond to an online questionnaire which includes questions on their diagnosis, causative genetic mutation, motor function, ability to walk, respiratory function, ventilation type, how they eat, cardiac function, etc. and update the information every six months.

▪ The questionnaires are not only available in English, but also in French, German, Spanish, Italian, Polish, Brazilian Portuguese, Hindi, and many other languages.

▪ The data can also be collected by the doctors who are monitoring the participants.

▪ At the end of January 2023, the data warehouse had collected responses from 444 people, 20% of whom were female carriers of X-linked myotubular myopathy.

Comprehensive collection of medical data in myotubular and centronuclear myopathies

International

500 participants (all ages)

Recruiting

1 year of follow-up

March 2013 – December 2022

NCT04064307

WEBSITE mtmcnmregistry.org

NatHis-CNM study

▪ This European study which is currently underway, sponsored by the Institut de Myologie (Paris) and Dynacure, is a two-year extension of the longitudinal study of the course of X-linked myotubular myopathy linked to the *MTM1* gene (NCT02057705) which was conducted in Europe and the United States from February 2014 to June 2017.

▪ Twenty new patients with other centronuclear myopathies caused not only by *MTM1* gene mutations but also by *DNM2* and *BIN1* gene mutations were included in order to obtain data on at least 70 patients who were monitored for a year.

NatHis-CNM study: extension of the study of the natural history of *DNM2* and *MTM1*-related centronuclear myopathies

In Europe including France

70 participants (all ages)

Data currently being analysed

1 to 5 years of

May 2017 – September 2022

NCT03351270

Management of congenital myopathies

The incidence of bone fragility should be considered

▪ A literature analysis of articles published on bone health in congenital myopathies found 35 articles which had collected data on 244 children with congenital myopathies who had an average age of 4.1 years old.

▪ Bone quality was reduced in 93 of the children (37%) who had an average age of 2.6 years old. A decrease in bone density was reported in 11 children, congenital long bone fractures in 64 and long bone fractures later in life in 24. Four children received bisphosphonates or vitamin D or calcium supplementation.

Bouman K et al. J Neuromuscul Dis. 2023.

Using a vibration platform

▪ A team from New Zealand tested the feasibility and efficacy of using a vibration platform to improve the respiratory, muscle and bone health of 11 children with a congenital myopathy who had an average age of 11.5 years old. After three months of undergoing four nine-minute sessions a week, motor performance and chair rising test results had improved.

▪ This pilot study has encouraged the authors to explore the potential benefits of this therapy in congenital myopathies over longer periods of time and in more participants.

Adaikina A et al. Neuromuscul Disord. October 2022.

Understanding the mechanisms of congenital myopathies

Actin filament length adaptation mechanisms

Did you know?

Length determines the intensity of the force produced

▪ Thin filaments, together with thick filaments, constitute the contraction mechanism of myofibrils.

▪ To ensure optimal contraction efficacy, the length of thin filaments (along which thick filaments slide during contraction) needs to be adapted to the intensity of the force that a muscle needs to generate.

A Hungarian team summarised what is known about the molecular mechanisms that regulate the length of thin filaments (which are made up of actin, tropomyosin and troponin).

- Actin filaments are formed by an alignment of actin molecules which are added or removed according to physiological requirements.
- Tropomodulin prevents actin molecules from being added (elongation of thin filaments) or removed (shortening of thin filaments) by binding to the free end of actin filaments. Leiomodin, on the other hand, promotes thin filament elongation.
- While the length of thin filaments varies from muscle to muscle, it is uniform in a sarcomere. Nebulin may serve as a template for the length of thin filaments. The Lasp protein, a member of the nebulin protein family but which only contains two repeating units (nebulin contains between 22 and 29), seems to play a role in fine-tuning the length of thin filaments.
- Another theory is that the space between Z-discs, determined by the size of titin, may determine the length of thin filaments.

Szikora S et al. Int J Mol Sci. May 2022.

Common molecular abnormalities in congenital myopathies

An international team went in search of common microRNA (miRNA) and messenger RNA expression abnormalities in the muscles of people with different congenital myopathies (*RYR1*-related rhabdomyolysis, recessive and dominant *RYR1*-related myopathies, *SELENON*-related multiminicore myopathy, *KBTBD13*-related nemaline myopathy, and *MTM1*-related X-linked myotubular myopathy). They found that there was a decrease in the expression of *RYR1, ATPB2* and miRNA-22 in all of the congenital myopathies, but no expression abnormalities in cases of rhabdomyolysis.

Bachmann C et al. Brain Commun. September 2022.

Other advances in core myopathies

The *RYR1* gene is the gene that is most frequently involved in core myopathies. A growing number of other genes have also found to be responsible such as *SELENON, MYH2, MYH7, TTN, CCDC78, UNC45B, ACTN2, MEGF10, CFL2, KBTBD13* and *TRIP4*. Additionally, it has recently been discovered that mutations in genes such as *ACTA1, NEB* and *TNNT1*, which were initially associated with nemaline myopathies, are also involved in core myopathies.

Other advances in RyR1-related myopathies

Ryanodine receptor 1 (RyR1) mutations can lead to a susceptibility to malignant hyperthermia, central core disease, multiminicore myopathy, congenital fibre-type disproportion myopathy or centronuclear myopathy. *RYR1*-related myopathies make up 30% of all congenital myopathy cases.

*An **ion channel** is a protein that is integrated into the membrane of a cell or cell compartment which allows certain molecules called ions (sodium, potassium, calcium, chloride) to enter or leave the cell or cell compartment in response to a signal. These channels play a very important role in the activity of "excitable" cells such as nerve and muscle cells.*

Mutations with different consequences

There are four main types of *RYR1* gene mutations that lead to different disorders:

- hypersensitivity of the channels on activation by electrical and pharmacological stimuli, leading to a massive release of calcium in response to halogenated agents, which are used in general anaesthetic, in malignant hyperthermia;

- leaky channels leading to a depletion of calcium in the sarcoplasmic reticulum stores in central core disease with malignant hyperthermia;
- excitation-contraction uncoupling caused by a channel that is unable to trigger the release of calcium from the sarcoplasmic reticulum in central core disease without malignant hyperthermia;
- a decrease in the amount of RYR1 channels in the sarcoplasmic reticulum membrane in RyR1-related myopathies (multiminicore myopathy, centronuclear myopathy, and congenital fibre-type disproportion myopathy).

Genotype-phenotype correlation

▪ Medical and genetic data as well as the structure of abnormal ryanodine receptors (RyR1) were studied in 33 subjects with an *RYR1*-related myopathy (21 dominant and 12 recessive with a family history in 10 cases).
▪ The dominant *RYR1* mutations causing central core disease were found in three distinct regions of the gene (exons 1 to 17, 39 to 46, and 90 to 103), while the recessive mutations were spread throughout the gene and were associated with different congenital myopathies (multiminicore myopathy, centronuclear myopathy, and congenital fibre-type disproportion myopathy).
Chang X et al. Front Neurol. May 2022.

RYR1 mutations are also involved in a mild form of tubular aggregate myopathy

▪ An Italian team reported the cases of two unrelated men, aged 30 and 39, who complained of muscle stiffness after sustained physical activity or exposure to the cold and whose CPK levels were elevated.
Their muscle biopsies showed the presence of submembranous tubular aggregates in their type II muscle fibres, with no evidence of cores or other histological abnormalities.
Next-generation sequencing identified two mutations previously thought to be involved in malignant hyperthermia.
Vattemi GNA et al. Eur J Neurosci. June 2022.

RYR1-related myopathy responsive to pyridostigmine

▪ A Danish team reported the case of a 17-year-old young man who had a congenital myopathy with signs resembling those of myasthenia gravis - muscle weakness, fatigability, drooping eyelids, and paralysis of the extraocular muscles. Treatment with pyridostigmine, an acetylcholinesterase inhibitor used to treat myasthenia gravis, improved his symptoms.
The authors concluded that *RYR1*-related congenital myopathies may also resemble myasthenia gravis and that therefore it may be useful to propose treatment with pyridostigmine.
Lester EB et al. Eur J Med Genet. March 2023.

Neuromuscular symptoms in *RYR1*-related malignant hyperthermia and rhabdomyolysis

▪ People with *RYR1* gene mutations that cause malignant hyperthermia susceptibility and/or episodes of exertional rhabdomyolysis report muscle pain or cramps more often when compared with healthy subjects. The majority have normal muscle strength when they are young, but some may develop muscle weakness in the lower limbs later in life.
van den Bersselaar LR et al. Brain Commun. November 2022.

Excitation-contraction coupling and extracellular calcium entry

Maintaining calcium stores in cells

▪ The calcium needed for muscle contraction is stored in the sarcoplasmic reticulum until it is released, coupling the membrane excitation (which causes it to be released) with the contraction (triggered by its release into the cytoplasm). After contraction, the calcium is reabsorbed by the sarcoplasmic reticulum through a "calcium pump" called SERCA1 (sarcoplasmic/endoplasmic reticulum calcium ATPase).

▪ The calcium stores in the endoplasmic or sarcoplasmic reticulum are also replenished by calcium entering from outside the cell, a mechanism called SOCE (store-operated calcium entry).

▪ An article summarised the mutations affecting these two major mechanisms and the congenital myopathies they cause:
- *RYR1, CACNA1S* and *STAC3* for excitation-contraction coupling
- *STIM1* and *ORAI1* for extracellular calcium entry.
It concluded that other proteins with no link to calcium movements may be involve in these congenital myopathies and that it is necessary to better understand these pathogenic mechanisms in order to improve classification of these myopathies and identify new possible drug candidates.
Rossi D et al. J Gen Physiol. September 2022.

In search of RyR1 modulators

A review provided an update on the drug compounds being studied which are aiming to correct RyR1 activity, as well as the research tools which have been developed to discover these compounds on a large scale (high-throughput screening platforms).

Did you know?

Calcium release from RyR1 is induced by:

▪ membrane depolarisation;

▪ the presence of calcium.

▪ Various therapeutic approaches which consist of directly correcting RyR1 activity using chemical compounds (drugs) are being studied:
- **calcium-induced calcium release inhibitors** such as dantrolene are potential candidates for treating malignant hyperthermia and central core disease with malignant hyperthermia;
- **RyR1 activators**, on the other hand, would be useful in central core disease which has been caused by loss of RyR1 function;
- RyR1-related myopathies would benefit best from an **increase in RyR1 expression**, although RyR1 activators could be effective.

Known RyR1 modulators

▪ **dantrolene** prevents calcium from being released by interacting directly with RyR1, however, it can lead to prolonged muscle weakness, which has recently been resolved by a change to its formulation (nanocrystalline suspension of dantrolene - Ryanodex®)

▪ **Rycals** are derived from benzodiazepines which prevent the unbinding of RyR1 and FKBP12, a molecule which keeps RyR1 in the closed position (ARM210 trial)

▪ High-throughput screening enables thousands or even millions of chemical compounds to be tested, provided you have the appropriate platform. However, the usual techniques are not applicable in this case as RyR1 channels are situated inside cells.

▪ Two screening techniques specific to calcium-induced calcium release activity have been developed:
- the first technique tested 727 compounds and identified that cefatrizine, disulfiram, ebselen and tacrolimus increase RyR1 activity, while chloroxine decreases it;
- the second tested 1,535 compounds and found three new potential RyR1 inhibitors: oxolinic acid, 9-aminoacridine and alexidine, as well as over 50 potential activators.

Additionally, a team synthesised a compound derived from oxolinic acid which selectively inhibited RyR1 to an even greater extent. This compound prevented malignant hyperthermia in a mouse model and had the advantage of not having a prolonged effect on muscle strength.

▪ It is only recently that researchers have succeeded in designing a platform that reconstitutes the release of calcium induced by membrane depolarisation, which will make it possible for excitation-contraction coupling to be studied.
Murayama T et al. J Gen Physiol. December 2022.

▪ Recent advances in cryo-electron microscopy have made it possible to identify the sites where RyR1 would bind to its potential modulators, therefore accelerating the optimisation of their chemical structure and the development of a drug.
Murayama T et al. Curr Opin Pharmacol. April 2023.

Improving our understanding of the structure of RyR1

▪ A review of our knowledge of the structure of ryanodine receptors and their interactions with the molecules that regulate their function assessed which mechanisms trigger the opening and closing of various ryanodine receptors, how they are regulated by several modulators (ions, small molecules, regulatory proteins, etc.), and how the mutations that cause the diseases affect their structure and function.

Ryanodine receptors

▪ Ryanodine receptors are large ion channels which change conformation (open or closed) and play a crucial role in muscle excitation-contraction coupling, neuronal excitability, cell differentiation and apoptosis.
▪ They are found mainly in the membrane of the endoplasmic reticulum and sarcoplasmic reticulum in muscle and enable calcium to be released into the cytoplasm.
▪ There are three types of ryanodine receptors: RyR1 is found predominantly in skeletal muscle, RyR2 is found mainly in cardiac muscle and RyR3, initially detected in the brain, is found throughout the body, including in muscle but at a low level.

Apoptosis *is a form of programmed cell death. It is an orderly process with several stages which culminate in the whole cell and its contents being disposed of or recycled without the surrounding cells being damaged. Apoptosis is in constant balance with cell multiplication in order to ensure cell renewal.*

▪ The article also describes the contribution of this structural knowledge to the development of drugs, such as a dantrolene analogue which is easier to use and has less side effects, as well as new promising compounds.
Hadiatullah H et al. Front Pharmacol. May 2022.

▪ A cryo-electron microscopy study identified the site where the Rycal ARM210, a drug being developed, binds cooperatively with ATP to stabilise the closed position of RyR1.
Melville Z et al. Structure. July 2022.

▪ Research into the Y522S mutation of RyR1, which causes central core disease and malignant hyperthermia, revealed that this mutation triggers

channel preactivation, increasing the potency of calcium to open the channel by nearly 20-fold.
Iyer KA et al. Proc Natl Acad Sci U S A. July 2022.

Searching for biomarkers

▪ A Chinese team has identified four muscle tissue-specific genes as potential biomarkers for diagnosing *RYR1*-related myopathies: *MYH1* (which codes for adult myosin heavy chain 1), *TNNT3* (which codes for fast skeletal muscle troponin T3), *MYLPF* (which codes for myosin light chain 11) and *ATP2A1* (which codes for the sarcoplasmic reticulum calcium pump SERCA1).
Wang X et al. Dis Markers. May 2022.

Comparative proteomics of RyR1-related myopathy mice and wild-type mice

▪ A Swiss team found that, compared to normal mice, mouse models carrying recessive *RYR1* mutations had not only a reduced amount of RyR1 in their muscles, but also changes in the expression of 1,130 proteins in a leg muscle and 967 proteins in their extraocular muscles. The mutations affected the expression level of proteins involved in calcium signalling, the extracellular matrix, quality control of proteins in the endoplasmic reticulum and protein synthesis.
Eckhardt J et al. Elife. March 2023.

Other advances in selenoprotein N-related myopathies

Selenoprotein N is coded for by the *SELENON* gene and is localised in the membrane of the endoplasmic reticulum in a region which is in contact with mitochondria. It senses calcium and, through a redox-mediated mechanism, regulates the entry of calcium into the terminal cisternae of the endoplasmic reticulum using endoplasmic reticulum calcium pumps called SERCAs.

Rationale for cardiorespiratory monitoring

▪ A team of researchers at Radboud University in the Netherlands collected and analysed every case of *SEPN1*-related myopathy with information on the patients' cardiac function (192 cases including patients with an average age of 19) described in literature.
▪ Fifteen percent of these patients (who had an average age of 20) had signs of cardiac involvement.
▪ This study confirmed the importance of cardiac screening, starting in childhood. The authors also advised carrying out an ECG (including with an ECG Holter monitor) and echocardiogram every two years in order to achieve optimal monitoring of asymptomatic patients.
Bouman K et al. Neuromuscul Disord. August 2022.

*A **myoblast** is a muscle stem cell. During muscle formation (myogenesis), the fusion of several myoblasts leads to the formation of a long cell (a tube) called a **myotube** which will continue to differentiate to become a mature muscle fibre.*

Necessary regulation of selenoprotein N levels during muscle development

▪ Selenoprotein N maintains redox homeostasis and calcium concentration in the sarcoplasmic reticulum during the differentiation of myoblasts into mature muscle cells (myotubes).
▪ A Japanese team found that selenoprotein N levels are high in the early myoblast stage and gradually decrease during myotube formation, suggesting a fine regulation of selenoprotein N expression.
Noda Y et al. Nat Commun. May 2022.

Other advances in titin-related myopathies

TTN gene mutations cause a wide variety of muscle diseases called "titinopathies" such as hereditary myopathy with early respiratory failure (HMERF), core myopathies, late-onset distal muscular dystrophy and/or cardiomyopathies.

A better understanding of the characteristics of titinopathies

▪ A retrospective study of 103 cases (93 of which were published) of severe titinopathies enabled an international team to find correlations between manifestations (foetal immobility, arthrogryposis, facial abnormalities, etc.) and recessive *TTN* gene mutations that lead to the formation of truncated titin. Titin, therefore, proves to be essential for foetal development.
Di Feo MF et al. J Med Genet. March 2023.

▪ Another team was able to find a correlation between a genetic mutation concerning part of the *TTN* gene which is only expressed during foetal development and a severe type of titinopathy which manifests as neonatal hypotonia, muscle atrophy and joint contractures (arthrogryposis) which mainly affect the upper limbs and joints of the extremities. The joint contractures improved over time, but only two out of the five children studied were able to walk with the help of a walking aid at the ages of three and five years old.
Averdunk L et al. Neuropediatrics. October 2022.

▪ A French team described a congenital myopathy characterised by multiple contractures, a rigid spine, non-progressive muscle weakness and a novel *TTN* gene mutation in a metatranscript-only exon in a 35-year-old man. Studies quantifying titin from muscle biopsies surprisingly showed that titin was normal.
Studies of the muscle fibres suggested that the weakness was not linked to sarcomere abnormalities but that it was due to hypotrophy. The increased calcium sensitivity observed during force generation may be contributing to the patient's contractures and rigid spine.
Cardone N et al. Acta Neuropathol Commun. 2023 Mar 21;11(1):48.

The advantages of third-generation sequencing

The huge clinical heterogeneity of titinopathies combined with the very large size of the *TTN* gene (it contains 364 exons) is the basis of several diagnostic enigmas.

▪ Third-generation sequencing enables much longer DNA sequences to be read and is specifically adapted to searching for genetic mutations in the repeated areas of the *TTN* gene. Several complex diagnoses, including the prenatal diagnosis of titinopathy, have therefore been resolved.
Perrin A et al. J Mol Diagn. July 2022.

Biological disease models

The role of titin as well as the mechanisms involved in titin-related myopathies and cardiomyopathies are far from being completely understood. Having well-characterised animal models that include different titin mutations is essential for studying the structural properties and mechanics of the different titin domains.

▪ An article has outlined the main animal models of the titinopathies that have been characterised to date (eight mouse models and three fish models) and their contributions to the understanding of these complex diseases.

▪ 3D culture models from the cells of people with titinopathies are starting to be developed and will also eventually be a tool for modelling these diseases.
Marcello M et al. J Cell Mol Med. October 2022.

▪ A South Korean team succeeded in generating *TTN*-deficient induced pluripotent stem cell (iPSC) lines using the CRISPR/Cas9 genome editing system. These cells constitute a model that could be used to better understand the role of *TTN*.
Kang JY et al. Stem Cell Res. October 2022.

Induced pluripotent stem cells *(iPS cells) are cells that are able to multiply infinitely (stem cells), differentiate into any type of cell (pluripotent), and are produced by reprogramming adult cells (induced).*

ASC1 complex-related myopathies

ASC1 complex-related myopathy is a severe recessive myopathy with cores which was first described in 2016. The causative gene is *TRIP4* which codes for a transcriptional coactivator - ASC1 (Activating Signal Cointegrator 1).

Did you know?

ASC1 - a muscle fibre growth and differentiation regulator.

During the formation of muscle tissue (myogenesis), ASC1 regulates the proliferation of cells by slowing down cell cycles and promotes the growth of myoblasts in the proliferation phase and myotubes in the differentiation phase.

*A **myoblast** is a muscle stem cell. During muscle formation (myogenesis), the fusion of several myoblasts leads to the formation of a long cell (a tube) called a **myotube** which will continue to differentiate to become a mature muscle fibre.*

Since then, other cases of myopathies have been described that are related to *TRIP4* mutations which lead to an ASC1 deficiency. The manifestations range from lethal neonatal forms to mild forms in adults who are able to walk. These include early-onset axial and proximal muscle weakness, a stiff spine, facial abnormalities, skin involvement and respiratory impairment. Dilated cardiomyopathy was found in the oldest patients.
Muscle biopsies showed various abnormalities: multiple minicores, rods, cytoplasmic inclusions, cap-like structures, centrally-positioned nuclei, etc.
▪ An international team of researchers have identified four new subjects with *TRIP4*-related myopathy.
Marais A et al. Eur J Med Genet. August 2022.

Other advances in nemaline myopathies

Nemaline myopathies are characterised by muscle weakness associated with the presence of nemaline bodies (rods) in muscle biopsies.
To date, around 15 genes involved in the development of a nemaline myopathy have been identified: *NEB, ACTA1, TPM2, TPM3, TNNT1, KBTBD13, CFL2 (COFILIN2), KLHL40, KLHL41, LMOD3, MYO18B, MYPN, RYR3, MYH2* and *CAP2*.

The rods of nemaline myopathies

▪ These rods, which are characteristic of nemaline myopathies (or rod myopathies), may be derived from Z-discs (although it is unclear how they are formed).
▪ As a matter of fact, they may show continuity with Z-discs, have a similar lattice structure and contain the same proteins, such as alpha-actinin, actin, tropomyosin, myotilin, gamma-filamin, cofilin-2, telethonin and nebulin.

Treatment approaches still being trialled

A few dozen treatment approaches have been or are being explored in nemaline myopathies in human and mice cells.

- The sheer size of the majority of the genes that cause nemaline myopathies means that they are not able to be introduced into an AAV vector.
- A gene therapy technique aiming to switch off the production of abnormal troponin T in a mouse model is being trialled in mouse models of *TNNT1*-related nemaline myopathy.
- Oligonucleotides and an mRNA trans-splicing system which can be used to induce exon skipping are still in the very early stages of development.
- The upregulation of α-cardiac actin induced by CRISPR/Cas9 genome editing is currently being studied in *ACTA1*-related nemaline myopathies.
- Encouraging data on the upregulation of an embryonic form of light myosin chain has been obtained from mouse models of *ACTA1*-related nemaline myopathy but not from mouse models of *NEB*-related myopathy.
- Approaches targeting myostatin have been studied in *ACTA1* and *NEB*-related nemaline myopathies with mixed results.
- Under certain conditions, fast troponin activators partly improve muscle strength and contractility, however with little functional effect. They may still be a way of improving the quality of life for people with nemaline myopathy.
- Pyridostigmine may be helped in certain types of nemaline myopathy.
- The effects of L-tyrosine and L-carnitine are varied.

However, the rareness and heterogeneity of these diseases, as well as the lack of information on their natural history, make it more difficult to move from the preclinical stage to clinical trials in humans.

Fisher G et al. Expert Opin Ther Targets. October 2022.

A better understanding of nemaline myopathies

The rareness of nemaline myopathies means that every case described provides information that helps us to better understand these diseases.

A literature review

An American team analysed the observations of 101 patients with these diseases from 23 countries which were published in 385 articles on nemaline myopathies issued between January 2010 and December 2020.

- A quarter had a *NEB* gene mutation, while an *ACTA1* gene mutation was responsible in 22% of cases.
- Findings from muscle biopsies were available in nearly two thirds of cases. Three quarters had cytoplasmic nemaline rods or bodies, while a little less than 10% had intranuclear rods. Forty percent showed a variation in fibre size.
- Neonatal hypotonia affected 64% of cases, early respiratory difficulty was seen in 36% of cases and scoliosis was noted in 37% of the patients. Some cases of *MYO18B*, *TNNT1* and *ACTA1*-related myopathies had cardiac involvement. Fifty six percent of the patients had respiratory failure or weakness.
- Weakness affected the axial and proximal muscles in 31% of cases, and the distal muscles in 23%. Muscle weakness in the face was noted in nearly one third of cases.
- In all cases, treatment was multidisciplinary.

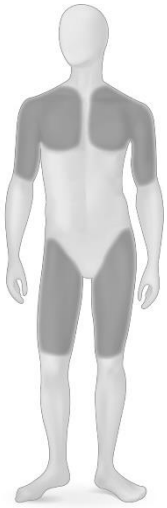
Christophers B et al. J Child Neurol. June 2022.

*An **antisense oligonucleotide** (ASO) is a fragment of RNA that is usually synthesised in a laboratory which can bind specifically to natural messenger RNA molecules. The nucleotide sequence (its chemical formula) of antisense oligonucleotides is complementary to that of the messenger RNA molecules which it targets. They can therefore modify messenger RNA (skip or incorporate exons by intervening during its maturation stage (splicing)).*

***Axial muscles** are the muscles that are situated along the centreline of the human body, i.e. the spine.*

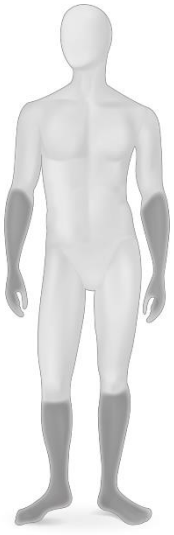
***Proximal muscles** are the muscles that are closest to the centre of the body.*

These include the shoulder, upper arm, hip and thigh muscles.



***Distal muscles** are the muscles that are furthest from the centre of the body.*

These include the forearm, hand, lower leg and foot muscles.



A Brazilian cohort

The analysis of clinical and genetic data from 30 nemaline myopathy patients from 25 Brazilian families confirmed the complex heterogeneity of this disease.

- sixteen families (64%) had a *NEB* gene mutation, five (20%) had an *ACTA1* gene mutation, two (8%) had a *KLHL40* gene mutation, one (4%) had a *TPM2* gene mutation and one (4%) had a *TPM3* gene mutation.
- the "typical" form was the most common (24/30) and was caused by mutations in the different genes.
- three patients had a mild form and another three had a severe neonatal form.
- heterogeneity of manifestations was observed among patients with mutations in the same gene, in particular *NEB* and *ACTA1*.
- respiratory involvement was common and often more significant than limb weakness (8/30 were on ventilator support but were able to walk).

Gurgel-Giannetti J et al. Int J Mol Sci. October 2022.

Respiratory involvement in nemaline myopathies

- The respiratory function of 44 patients with different forms of nemaline myopathy was studied in detail: 11 had a typical form, seven had a mild form and 24 had a form that started in childhood with slowness of movements.
- It shows that there is respiratory muscle weakness in all forms of nemaline myopathy, including those that start in childhood with slowness of movements, regardless of the degree of motor impairment.
- The authors emphasized the importance of routinely monitoring respiratory function in patients with nemaline myopathy.

van Kleef ESB et al. Neuromuscul Disord. 2022 Aug;32(8):654-663.

Oral feeding is possible

Just like an improvement in respiratory function leads to tracheostomy closure in some children with nemaline myopathy, swallowing difficulties are likely to improve with oropharyngeal rehabilitation that accompanies the child's progress.

- A South Korean team reported the cases of two children with nemaline myopathy who had swallowing difficulties which led to them being fed by nasogastric tubes. Rehabilitation, which started at 15 months for one patient and 42 weeks for the other, conducted with the help of regular video fluoroscopic swallowing studies, allowed them to achieve fully oral feeding.
- Rehabilitation sessions twice a week used massage of the cheeks, lips, and throat as well as stimulations to induce a swallowing reflex and strengthen the anterior neck muscles to better support the head and for better performance of the cheek muscles and chewing muscles.

Yoo YJ et al. Children (Basel). August 2022.

*A **nasogastric tube** is a very thin, soft tube which is inserted into the stomach through a nostril. It is used to deliver medications or liquid food complete with nutrients directly into the stomach.*

*A **sporadic** disease is a disease that affects isolated individuals as opposed to diseases that regularly occur within a region (endemic diseases), diseases that affect a large number of individuals at the same time (epidemic diseases) or diseases that affect several members of the same family (hereditary genetic diseases).*

Other advances in α-actin-related myopathies

Improving our understanding of ACTA1-related myopathies

ACTA1 gene mutations are responsible for half of all nemaline myopathy cases. An international collaboration coordinated by the Institut de Myologie in Paris detailed 10 new sporadic cases of a severe form of nemaline myopathy.

- General muscle hypotonia and breathing difficulties were present at birth. Seven of the patients died before they were three years old. As well as the

usual features found in nemaline myopathies, muscle biopsies also revealed abnormalities in the space around the cell nuclei.

▪ The levels of α-cardiac actin in the skeletal muscle of the patients who survived beyond the neonatal period were significantly higher than those of the patients who did not which suggests that α-cardiac actin plays a role in compensating for the deficiency in muscle actin and reducing its severity.

Labasse C et al. Acta Neuropathol Commun. July 2022.

Frequently occurring actin folding mutations

In order to better understand the mechanisms of the disease, a Russian team studied the distribution of 78 actin mutations that cause nemaline myopathy.

▪ These mutations were distributed throughout the length of the *ACTA1* gene. The majority of the mutations (54/78) were situated in the part of the gene that governs actin folding.

Glyakina AV et al. Biophys Rev. December 2022.

Experimental models

Researchers are developing cell models in order to study the molecular mechanisms involved in congenital myopathies and to test new treatment approaches.

▪ A French-Australian team developed two cell models of *ACTA1*-related nemaline myopathy from immortalised white blood cell lines of two infants (a one year old and a three month old), one for a severe recessive form and the other for an intermediate dominant form.

Clayton JS et al. Stem Cell Res. August 2022.
Suleski IS et al. Stem Cell Res. August 2022.

Cells for studying early processes

▪ The benefit of pluripotent stem cells in congenital diseases is that they retain their differentiation abilities.

▪ The study of the differentiation of these cells may shed some light on the early mechanisms involved in α-actin-related myopathy.

▪ An American team has developed another cell model of nemaline myopathy by using genome editing to create an early mutation in the *ACTA1* gene in induced pluripotent stem cell-derived muscle cells. The comparison of what happens in cells with this mutation and in those without it will provide a better understanding of the molecular mechanisms occurring in muscle cells in the event of an α-actin 1 deficiency.

Gartz M et al. Exp Cell Res. March 2023.

Other advances in nebulin-related myopathies

A better description of nebulin-related nemaline myopathy

A study of 33 people with nemaline myopathy caused by a *NEB* gene mutation showed a certain stability in swallowing ability and muscle strength while respiratory function and scoliosis tended to worsen over time.

▪ Of these 33 people, whose ages ranged from two to 59 years old, more than half (55%) could walk independently and 18 were on ventilator support.

▪ The onset of the disease was congenital in the vast majority of cases (94%). All of the patients had a high arched palate, a common feature of nemaline

myopathies, and 16 had a spinal deformity (most had scoliosis while a small number had a rigid spine).

- Ventilatory support was started between the ages of three and 15 and, for six patients, in the first few days of life; five (16%) required permanent ventilation.
- Nearly half of the cases where it was examined had mild or pronounced tongue atrophy, correlated with the presence of swallowing difficulties. Almost a third of the patients had a gastrostomy.
- Swallowing ability and motor function were similar among the different age groups (child, adolescent, adult), while scoliosis and respiratory dysfunction were more common in adults, suggesting disease progression in these areas.

Moreno CAM et al. Neurol Genet. 2023 Jan 25;9(1):e200056.

*A **gastrostomy** is a small surgical procedure which consists of inserting a tube through the abdominal wall and into the stomach. It helps to rebalance nutritional intake by introducing food directly into the stomach, bypassing any swallowing difficulties.*

MuRF1 increases muscle mass but not strength

Did you know?

MuRF1 and muscle atrophy

Muscle atrophy is tightly linked to the upregulation of MuRF1, an E3 ligase which targets proteins to be broken down by proteasomes.

Proteasomes are protein complexes responsible for breaking down proteins that are poorly-folded, denatured or obsolete in a cell. Proteins to be broken down are marked by a protein called ubiquitin. A chain of at least four ubiquitin proteins is required for the proteasome to be able to recognise the protein to be broken down.

- Researchers have found that MuRF1 is increased in both patients with nebulin-related nemaline myopathy and mouse models of the disease. Therefore, they created an MuRF1 deficiency in a mouse model of a typical form of nebulin-related nemaline myopathy and in a model of a severe form. Despite this deficiency leading to increased muscle mass in the mice, it did not improve their muscle strength.

Lindqvist J et al. Int J Mol Sci. July 2022.

Instability of myosin at rest increases cell energy consumption

- Various biological, biophysical and proteomic investigations performed on the muscle fibres of patients with nebulin-related nemaline myopathy and mouse models have led to the discovery of a significant impairment in the myosin stabilising conformational state known as the super-relaxed state. The loss of this stabilising state leads to an increased consumption of cell energy (ATP) at rest, explaining the apparent abnormalities in energy metabolism proteins seen in nebulin-related nemaline myopathy.
- The authors suggest investigating the potential beneficial effect of drugs that target myosin activity and/or conformation in nebulin-related nemaline myopathy.

Ranu N et al. Acta Neuropathol Commun. December 2022.

The role of NRAP in nebulin-related myopathy

Did you know?

The NRAP anchor

NRAP (nebulin-related anchoring protein) is a protein that connects terminal actin filaments in myofibrils to protein complexes situated in the membrane of muscle fibres.

- In nebulin-related nemaline myopathy, there is an upregulation of NRAP. The suppression of NRAP in a fish model with a nebulin deficiency restored sarcomere disorganisation, reduced protein aggregates and improved muscle function. These results suggest that NRAP is a modifier of nebulin-related nemaline myopathy and could be used as a treatment regardless of the *NEB* gene mutation.

Casey JG et al. Hum Mol Genet. January 2023.

Other advances in *TPM3*-related myopathies

Mutations in the tropomyosin 3 gene (*TPM3*) are mainly responsible for autosomal dominant and recessive nemaline myopathies, congenital fibre-type disproportion myopathy and cap myopathy.

Tropomyosin

▪ Tropomyosin is a filament protein located in the grooves of actin filaments.

▪ It wraps around them to stabilise them. In doing so, it covers the actin-myosin interaction sites and therefore prevents myofibrils from contracting. It binds to troponin T, a subunit of troponin complexes.

▪ When calcium binds to a troponin complex, it causes it to become displaced, which in turn causes the displacement of the tropomyosin, thus uncovering the actin-myosin interaction sites and allowing contraction to take place.

New manifestations linked to *TPM3* defect

▪ A French-Chilean team reported the case of a 47-year-old man with erythrocytosis (a higher-than-normal concentration of red blood cells in the blood), reduced vital capacity requiring noninvasive ventilation, a high arched palate and diffuse axial muscle and limb weakness. A deltoid muscle biopsy showed a pattern combining fibre-type disproportion and caps. This observation increases the diversity of manifestations related to *TPM3* mutations.

Bevilacqua JA et al. Neuromuscul Disord. August 2022.

▪ An Israeli team described the case of a child with a severe form of congenital myopathy and bilateral clubfoot manifesting at birth. At the age of three, he could not sit up and had a tracheostomy and tongue fasciculations. Brain imaging showed cerebral atrophy which was possibly linked to the recessive *TPM3*-related congenital myopathy.

Yogev Y et al. Mol Diagn Ther. September 2022.

Uncovering the molecular mechanism involved

▪ A Russian team revealed that tropomyosin uncovers actin-myosin binding sites (which generates strength) in a step by step process, ensuring the transition from weak to strong bonds between myosin and actin. Each tropomyosin position determines the balance between active and inactive actin molecules.

▪ Based on the same experimental design, the analysis of a tropomyosin mutation showed that during relaxation (low concentration of calcium), abnormal tropomyosin continues to activate actin molecules and that during contraction (high concentration of calcium), it decreased the strong bonds between myosin and actin.

Karpicheva OE et al. Int J Mol Sci. March 2023.

Other advances in *TNNT1*-related myopathies

The involvement of the *TNNT1* gene, which codes for troponin T1, was described in 2000 in 71 Amish children with severe nemaline myopathy. Since then, 23 other cases have been reported in different parts of the world.

▪ The recessive *TNNT1*-related nemaline myopathy manifests as severe motor delay, proximal joint contractures and muscle weakness (shoulders, elbows, hips, knees), a chest deformity which pushes the sternum outwards, significant stiffness of the rib cage and tremor.

Troponins - key agents in regulating muscle contraction.

- The troponin complex is composed of three subunits: troponin C, troponin I and troponin T, all coded for by different genes.
- Troponin C binds to calcium, troponin I is the one that covers actin-myosin binding sites and troponin T is the subunit that binds the troponin complex to tropomyosin.
- When calcium binds to troponin, it triggers a conformational change within the thin filament which uncovers the actin-myosin binding sites, enabling bridges to form between the two and the actin filaments to slide along the myosin filaments.

Exon skipping and *TNNT1*-related nemaline myopathy

- A Chinese team described a mild type of *TNNT1*-related nemaline myopathy with no chest deformities or respiratory dysfunction in a girl with a *TNNT1* gene mutation leading to the loss of exon 9. A muscle biopsy showed the residual presence of truncated troponin T which means that a treatment based on skipping exon 9 in certain *TNNT1*-related nemaline myopathies may be possible.

Wang G et al. J Hum Genet. February 2023.

Other advances in *KBTBD13*-related myopathy

In *KBTBD13*-related nemaline myopathy, muscle weakness is compounded by slow muscle relaxation which interferes with contraction (unrelaxed muscle cannot contract) and activities of daily living such as running or climbing stairs.

Although KBTBD13 expression in cardiac muscle is situated in roughly the same place as it is in skeletal muscle, cardiac involvement has not been described in patients with *KBTBD13*-related nemaline myopathy until now.

Monitoring heart function

- Alerted by the onset of heart failure in a patient with *KBTBD13*-related nemaline myopathy and a family history of sudden cardiac death, a team of Dutch doctors reassessed the cardiological health of 65 people from three families with the Dutch founder *KBTBD13* gene mutation.
- Sixty percent of them had cardiac abnormalities. Additionally, Kbtbd13-deficient mouse models had mild cardiac dysfunction.
- The authors concluded that it is necessary to consider *KBTBD13* gene mutations as a cause of cardiomyopathy and to start regular cardiac monitoring in patients with *KBTBD13*-related nemaline myopathy.

de Winter JM et al. Hum Mutat. December 2022.

A better understanding of *LMOD3*-related myopathies

While nemaline myopathies linked to *LMOD3* gene mutations are most often severe congenital forms, a mild form, which started with frequent falls during infancy, has been described in two adults from the same family in Spain. Prominent facial weakness and mild muscle weakness in the limbs were caused by a genetic mutation that fails to stop the synthesis of leiomodin-3.

Segarra-Casas A et al. Neuromuscul Disord. April 2023.

Other advances in *ACTN2*-related myopathies

The first *ACTN2* gene mutations discovered were related to heart diseases. It is only since 2019 that certain mutations have been found to be involved in congenital and distal myopathies. People with these *ACTN2* -related myopathies do not usually have cardiac involvement.

Actinin α2

- Actinin α2 is a structural cardiac and skeletal muscle protein coded for by the *ACTN2* gene.
- Located in sarcomere Z-discs, it acts as a link between two actin filaments.
- It also binds to one end of titin molecules, thus contributing to sarcomere stability.

Little obvious genotype-phenotype correlation

- A review of all known *ACTN2* gene mutations showed that mutations situated in the last two exons of the *ACTN2* gene lead to muscle weakness in the legs and face.
- No other clear correlations between disease manifestations (phenotype) and the type of genetic mutation involved (genotype), particularly in relation to cardiac involvement, could be established.
- Regular cardiac monitoring of people with an *ACTN2*-related myopathy is recommended in case cardiac involvement occurs during the disease's course.

Ranta-Aho J et al. Hum Mutat. December 2022.

Advances in centronuclear myopathies

The *MTM1, DNM2, BIN1, RYR1* and *TTN* genes are the genes that are most frequently involved in centronuclear myopathies. *SPEG1* and *CCDC78* may also be involved but this is less common.

MTM1, DNM2, and *BIN1* code for proteins which interact with each other and are involved in membrane remodelling and trafficking, while RyR1 plays a role in excitation-contraction coupling and titin in the assembly of sarcomeres.

Membrane remodelling defects in *DNM2* and *BIN1*-related myopathies under the magnifying glass

An article reviewed what is known about the role of dynamin 2 and amphiphysin 2 in the formation of T-tubules during skeletal muscle development, as well as the molecular mechanisms involved in membrane remodelling defects caused by *DNM2* or *BIN1* gene mutations.

- During the formation of vesicles, amphiphysin 2 makes the membrane curve to form a protrusion (future membrane vesicle) then dynamin 2 causes the vesicle to separate from the membrane through membrane fission.
- Amphiphysin 2 is also essential for maintaining T-tubules - by inhibiting the fission activity of dynamin 2, it allows dynamin 2 molecules surrounding the tubules in a helix to stabilise their conformation.
- With a *DNM2* gene mutation, the membrane fission activity of dynamin 2 is increased, altering T-tubules and excitation-contraction coupling function.

Transverse tubules, *or* ***T-tubules,*** *are thin invaginations which are found at regular intervals over the entire muscle fibre membrane. Like fingers on a glove, they penetrate deeply into the muscle fibre membrane so that they come into contact with the sarcoplasmic reticulum which surrounds myofibrils.*
T-tubules enable the membrane's electrical depolarisation signal to be brought closer to the endoplasmic reticulum calcium stores and therefore ensure the synchronisation of the release of calcium to the whole fibre responsible for the synchronous contraction of the myofibrils in the muscle fibre.

- There may be a correlation between the extent of the membrane fission overactivity of abnormal dynamin 2 and the severity of centronuclear myopathy manifestations.

Fujise K et al. Int J Mol Sci. June 2022.

Proof of concept of tamoxifen in DNM2 or BIN1 mice

Tamoxifen

Tamoxifen is a drug which is currently used to treat breast cancer. Encouraging effects have already been seen in animal models of other myopathies, such as X-linked myotubular myopathy and Duchenne muscular dystrophy.

A team in Strasbourg, supported by AFM-Téléthon, studied the effects of five weeks of tamoxifen in mouse models of two centronuclear myopathies: one linked to the *BIN1* gene (CNM-*BIN1*) and the other linked to the *DNM2* gene (CNM-*DNM2*).

- Taking tamoxifen improved muscle strength in the CNM-*DNM2* mice by 80%, while the CNM-*BIN1* mice regained similar or even greater muscle strength than healthy mice.
- However, tamoxifen does not reduce muscle atrophy, no matter the animal model.
- Tamoxifen completely corrected the disorganisation of muscle fibres in the CNM-*BIN1* mice but not in the CNM-*DNM2* mice.
- Although the treatment response differs depending on the mutation involved, these results show the therapeutic potential of tamoxifen in both early- and late-onset centronuclear myopathies.

The TAM4MTM clinical trial, which is currently testing the safety and efficacy of the drug in X-linked myotubular myopathy, will provide additional information on its application in patients with centronuclear myopathy.

Gineste C et al. Brain. December 2022.

Ubiquitous dynamin 2 involved in severe forms

Did you know?

The two forms of dynamin 2

- Two forms of dynamin 2 coexist in muscle: muscle-specific M-DNM2, and Ub-DNM2 which is also present in other tissues (ubiquitous).
- Mutations in Ub-DNM2 increase membrane fission, but those in M-DNM2 do not.

- Ub-DNM2 overexpression is correlated with severe forms of centronuclear myopathy, while M-DNM2 overexpression may be linked to mild forms.
- Additionally, Ub-DNM2 overexpression worsens X-linked myotubular myopathy manifestations.

Gómez-Oca R et al. Nat Commun. November 2022.

Mitofusin 2 and HIF1α regulate muscle fibre maturation speed

- American researchers discovered that the transition of muscle fibres from the neonatal stage to the adult stage was controlled by mitofusin 2 (Mfn2), which accelerates the process, and hypoxia-inducible factor-1α (HIF1α), which slows down the process.
- In centronuclear myopathies, HIF1α levels are high and muscle fibres appear immature. This discovery has opened up the prospect of manipulating muscle fibre maturation by inhibiting HIF1α in order to

accelerate the regeneration of muscle fibres in the event of injury or initiate their maturation in centronuclear myopathy.
Wang X et al. J Clin Invest. December 2022.
Salekeen R, Kyba M. J Clin Invest. December 2022.

Other advances in X-linked myotubular myopathy

X-linked myotubular myopathy affects one in every 50,000 newborn boys per year. It represents nearly 60% of centronuclear myopathy cases.

▪ It is a serious disease which appears before (prenatal) or at birth (neonatal) and manifests as severe hypotonia, muscle atrophy, generalised weakness, breathing difficulties requiring immediate ventilator support, and swallowing difficulties which require placement of an enteral feeding tube.

▪ The most advanced therapeutic approaches are:
- MTM1 gene transfer (ASPIRO trial);
- decreasing the overexpression of DNM2 using an antisense oligonucleotide (UNITE-CNM);
- tamoxifen (TAM4MTM trial).

Female carriers - a common condition that is often overlooked

▪ The results of an online survey published in 2021 which was answered by 76 British, German and Dutch female carriers of X-linked myotubular myopathy showed that:
- over half of the respondents (51%) had muscle weakness: mild for 39% (able to walk independently), moderate for 9% (able to walk with assistance) and severe in 3% of cases (wheelchair dependent);
- seventy percent reported fatigue and 49% reported exercise intolerance;
- the symptomatic female carriers also suffered from pain, limitations in their daily activities and reduced quality of life.
Reumers SFI. et al. Neurology. August 2021.

▪ This survey was continued in the Netherlands and examined 21 female carriers of X-linked myotubular myopathy. Eighteen of them participated in the 2021 survey.
With an average age of 44 (with ages ranging from 22 to 62 years old), the vast majority were mothers (52%), but also sisters (10%), daughters (10%), grandmothers (5%), cousins (5%) and aunties (5%) of someone with X-linked myotubular myopathy. Nearly half of them (10/21) had no symptoms.
- Out of the symptomatic women, two had severe manifestations (unable to walk independently), two had moderate signs (able to walk short distances or with assistance), three had mild manifestations (limb or axial muscle weakness but able to walk) and four had minimal signs (only facial muscle weakness). Three of these women (one with moderate signs and two with mild manifestations) had not been previously diagnosed as symptomatic carriers.
- Muscle weakness was mainly in the proximal muscles of the limbs (shoulders/arms, pelvis/thighs), and asymmetric facial muscle weakness was common (73% of symptomatic women). Three had a history of pneumothorax (collapsed lung).
This study confirmed that over half of the female carriers of X-linked myotubular myopathy had muscle weakness which still had not been diagnosed or treated.
Franken DK et al. Neurology. November 2022.

Understanding the disease's course in mice to speed up the development of drug candidates

▪ A team at the Institut de Génétique et de Biologie Moléculaire et Cellulaire (Institute of Genetics and Molecular and Cellular Biology) in Strasbourg modelled the course of X-linked myotubular myopathy in a mouse model. It was able to demonstrate that an antisense oligonucleotide which reduces the amount of dynamin 2 prevented or slowed down the course of the disease in the mouse model significantly and in a dose-dependent manner.
Buono S et al. Dis Model Mech. July 2022.

__Basic__ (studying muscle physiology, identifying immunological/genetic causes and exploring the mechanisms of each type of neuromuscular disease, etc.), __preclinical__ (experimentation/exploration, exploration of possible therapeutic approaches using biological models) and __clinical__ (natural history of the disease, improving diagnosis and treatment, trialling potential drugs and biological therapies) __research__ are all used to study neuromuscular diseases.

▪ A Canadian team produced a detailed natural history of X-linked myotubular myopathy from a mouse model in order to better understand the disease's mechanisms. As well as the course of signs of the disease over time, the researchers also studied changes in muscle structure, protein and DNA expression, etc. This detailed description of the pathological process over time can be used as a reference during preclinical trials of drug candidates.
Sarikaya E et al. Dis Model Mech. July 2022.

Exploring PI3KC2β inhibitors

Discovering the function of the PI3KC2β enzyme has opened up the prospect of a new treatment option in X-linked myotubular myopathy.

▪ **Myotubularin** is a type of enzyme called a phosphatase which removes a phosphate molecule from phosphoinositides.
▪ **Phosphatidylinositol-3-kinase C2β** (PI3KC2β) is a type of enzyme called a kinase which adds a phosphate molecule to phosphoinositides.
▪ **Phosphoinositides** are cell membrane lipids which are involved in membrane trafficking regulation.

▪ A German team, together with a researcher from the Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC) in Strasbourg, showed that PI3KC2β had an opposite action to myotubularin on membrane adhesion mechanisms in a cell model.
▪ Six months later, the IGBMC team, together with researchers from Université Toulouse III [Toulouse III University] and the UCL Cancer Institute in London, showed that the selective inactivation of the kinase activity of PI3KC2β was sufficient to prevent muscle atrophy and muscle weakness as well as sarcomere and triad disorganisation in a myotubularin-deficient mouse model.
Samsó P et al. Proc Natl Acad Sci U S A. October 2022.
Massana-Muñoz X et al. JCI Insight. March 2023.

Asynchronous calcium release

Did you know?

Myotubularin deficiencies disrupt T-tubule function and lead to a delayed release of calcium from the endoplasmic reticulum.

▪ By using mathematical modelling of muscle fibre membrane depolarisation, Hungarian researchers, in collaboration with the Institut NeuroMyoGène [NeuroMyoGène Institute] in Lyon and Généthon [a charity-funded biotherapy organisation] in Évry, have shown that alteration of the membrane invagination network (T-tubules) sufficiently explains excitation-contraction coupling dysfunctions.

▪ Hence, the first critical event involved in X-linked myotubular myopathy is the disruption of the propagation of membrane depolarisation, responsible for desynchronising the calcium release from the sarcoplasmic reticulum within the muscle fibre.
Szentesi P et al. J Physiol. January 2023.

Valproic acid ameliorates mouse models

▪ The screening of 1,280 drugs in zebra fish models of X-linked myotubular myopathy showed that histone deacetylase (HDAC) inhibitors such as valproic acid and trichostatin A improve swimming speed in a dose-dependent manner. Similarly, *Mtm1*$^{-/y}$ mouse models saw their survival increase and their motor function ameliorated by these drugs, in particular by valproic acid.
▪ This antiepileptic drug also corrected the abnormal increase of DNA methylation in the mouse models.
▪ The analysis of blood samples from 19 people with X-linked myotubular myopathy showed DNA methylation abnormalities similar to those found in the animal models, abnormalities that appear to be specific and unique to this disease.
Volpatti JR et al. Acta Neuropathol. September 2022.

The role of myotubularin in adapting to periods of fasting

One way that cells adapt to periods of fasting is by changing the shape and dynamics of their mitochondria and lysosomes.

Did you know? Myotubularin is involved in the formation of vesicular cell membrane structures such as endosomes and lysosomes and forming a network of terminal cisternae such as the endoplasmic reticulum.

▪ A German team discovered the early role of myotubularin and phosphatidylinositol 3-phosphate in the remodelling of the endoplasmic reticulum and mitochondria during periods of fasting. This could explain why people with X-linked myotubular myopathy often appear malnourished.
Jang W et al. Science. December 2022.

***Lysosomes** are small sacks (vesicles) found inside cells whose role is to digest molecules produced by cell functions into smaller molecules. These molecules are then either removed as waste, or recycled and reused by the cell. Lysosomes break down and recycle materials that come from outside the cell (heterophagy) as well as those that come from inside the cell (autophagy) using a large number of different enzymes which are able to digest large molecules.*

Other advances in *DNM2* abnormalities

Autosomal dominant *DNM2*-related centronuclear myopathy affects around 550 people in total in Europe, the United States, Australia and Japan. Its manifestations are variable, from severe neonatal onset forms to more mild adult onset forms.

The functional prevalence of abnormal dynamin 2
▪ Over 35 different mutations in the *DNM2* gene are involved in autosomal dominant centronuclear myopathy.
▪ They lead to the formation of abnormal dynamin 2 which is more stable and whose activity is higher than that of normal dynamin.

A natural history

A retrospective study of 42 people with *DNM2*-related centronuclear myopathy confirmed that this myopathy is less severe than X-linked myotubular myopathy.

- Walking difficulties appeared in childhood (38% showed initial signs before the age of two) or adulthood, even after the age of 50. Out of the 42 people, seven lost the ability to walk between the ages of 10 and 71.
- Nearly half (47%) had no respiratory involvement, while out of those that did, only a few required ventilation. Forty percent had chewing and/or swallowing difficulties. Only four children were on nutritional support.
- Over half of the participants were independent in their activities of daily living, a quarter needed help from time to time while a fifth required full-time assistance.
- The severity of the course of the disease seemed to be correlated to the localisation of the genetic mutation - some led to a more severe form with an earlier onset, while others caused a late-onset and slowly progressive form. The most common genetic abnormality seemed to be responsible in a form with slower progression and minimal respiratory or feeding impairment.

Hayes LH et al. Neurol Genet. October 2022.

A versatile siRNA to switch off the abnormal *DNM2* gene, regardless of the mutation

Did you know?

- In a recessive disease such as Duchenne muscular dystrophy and certain limb-girdle muscular dystrophies, gene therapy consists of providing a normal copy of a gene.
- In a dominant disease, the abnormal copy of the gene needs to be prevented from acting, thus leaving the other normal copy free to function.

- In February 2022, a team from the Centre de recherche de l'Institut de Myologie [Institute of Myology, Myology Research Centre] (Paris) showed that a single injection of a gene therapy product specific to the *DNM2* gene mutation (AAV-shRNA), which stops it from being expressed, enabled mouse models treated early to regain strength and increase the size of their normal muscle fibres which persisted for more than a year after receiving the injection.

Trochet D. et al. Mol Ther Nucleic Acids. February 2022.

- In August 2022, the same researchers developed versatile small interfering RNAs (siRNAs) which can be used for any causative mutation. These siRNAs were shown to be effective in cell lines derived from patients with different mutations.

This data suggests that some siRNAs could target the vast majority of mutation carriers and patients who overexpress dynamin 2.

Dudhal S et al. Mol Ther Nucleic Acids. 2022 Aug 13;29:733-748.

Other advances in SPEG-related myopathy

Abnormalities in the *SPEG* gene lead to either cardiomyopathy or centronuclear myopathy (with or without dilated cardiomyopathy). Since SPEG-related myopathy was first described in 2014, around 20 cases of people with a *SPEG* gene mutation have been reported.

Four SPEG proteins

The *SPEG* gene gives rise to four SPEG proteins: one preferentially expressed in the aorta, one preferentially expressed in the brain, and two types (SPEGα and SPEGβ) preferentially expressed in cardiac and skeletal muscle.

▪ In skeletal muscle, the SPEG protein is found at the triads in the alignment of the terminal cisternae of the sarcoplasmic reticulum. It plays an important role in maintaining the structure and number of triads as well as developing and regenerating muscles.

*A **triad** is the cellular structure where the excitation-contraction coupling of muscle cells takes place. It is formed by a T-tubule and two terminal cisternae of the sarcoplasmic reticulum.*

Lowering dynamin 2 levels

▪ An American team, together with the IGBMC team in Strasbourg, discovered that the SPEGβ protein interacts with dynamin 2 and that, like in other *DNM2*, *BIN1* and *MTM1*-related centronuclear myopathies, SPEG protein deficiency leads to an increase in dynamin 2 levels.

▪ The reduction of dynamin 2 levels increased the body weight and life span of a Speg-deficient mouse model; it improved the mouse's motor performance but not its cardiac dysfunction.

Li Q et al. JCI Insight. August 2022.

Advances in MAP3K20-related centronuclear myopathy

Mitogen-activated protein triple kinase 20 (MAP3K20) is also known as ZAK. MAP3K20-related myopathy was first described in 2017 in three unrelated families. It is inherited in an autosomal recessive manner and manifests as slowly progressive muscle weakness beginning in infancy or early childhood. It is associated with hypotonia at birth, delayed motor development and walking difficulties. The muscle weakness caused by this disease mainly affects the proximal upper limbs (shoulders, upper arms) and distal lower limbs (ankles, feet). It can be accompanied by scoliosis or mild respiratory impairment.

A fourth family

▪ A German-Pakistani team reported the case of a consanguineous family in which seven members had a slowly progressive congenital myopathy linked to a new *MAP3K20* (or *ZAK*) mutation detected using whole exome sequencing.

Ahmad I et al. J Hum Genet. February 2023.

Advances in other congenital myopathies

Other advances in congenital fibre-type disproportion myopathies

Congenital fibre-type disproportion myopathy is characterised by smaller type I muscle fibres compared to type II muscle fibres. Several different genes may be responsible for this type of congenital myopathy.

▪ Recently, mutations in the *HACD1* gene, which codes for 3-hydroxyacyl-CoA-dehydratase-1, have been associated with this disease. A new mutation in this gene has just been discovered in a 12-year-old Iranian girl, who first showed signs of congenital myopathy when she was four months old.
Jabbarpour N et al. J Genet. 2023.

Other advances in *MYH7*-related myopathy

MYH7-related myopathies are extremely rare. They are usually a distal myopathy related to abnormalities in the linear part of myosin molecules.

▪ A German team investigated the cases of two unrelated boys and discovered that abnormalities in the myosin head domain resulted in axial and proximal myopathy and/or stiffness with respiratory involvement.
Bader I et al. Orphanet J Rare Dis. July 2022.

Advances in fast myosin IIa myopathy

*The **extraocular muscles** are the muscles that move the eyes.*

The *MYH2* gene codes for fast skeletal myosin IIa. *MYH2* gene mutations cause muscle weakness, joint contractures and extraocular muscle paralysis.

New observations in a dominant form

While recessive myosin heavy chain (MyHC) IIa myopathy has been described in several cases, the dominant form has only been observed in one family and in two sporadic cases.

▪ A Swedish team found that a sister and two brothers (aged 54, 56 and 66) had muscle weakness which started in the lower limbs when they were young adults. They now have generalised proximal muscle weakness which affects their ability to walk, however, there is no extraocular muscle involvement. Muscle biopsies found type I muscle fibre predominance and whole-genome sequencing identified *MYH2* gene mutations.
Hedberg-Oldfors C et al. BMC Neurol. November 2022.

▪ An American team identified a new dominant mutation in the *MYH2* gene in three members of a family in which four generations of individuals had a slowly progressive, predominantly proximal myopathy. The individuals affected had no congenital contractures or extraocular muscle involvement.
Cassini TA et al. Neuromuscul Disord. March 2023.

Beginning to understand molecular mechanisms

▪ *MYH2* gene mutations which cause truncated type IIa myosin heavy chains to be produced impact myosin presence and functionality in adult human muscle cells by disrupting ATPase activity and actin-myosin binding.
Sonne A et al. Am J Physiol Cell Physiol. March 2023.

Other advances in *MYOD1*-related myopathy

MYOD1-related congenital myopathy has been described in three families to date. It is a severe myopathy which manifests as generalised muscle weakness with respiratory muscle involvement (particularly the diaphragm), kidney abnormalities and facial and finger deformities.

▪ An Australian team reported the case of a 38-year-old pregnant women who had slowly progressive breathing difficulties which started six years

prior. She was found to have a mild form of *MYOD1*-related congenital myopathy.
Ashton C et al. J Neuromuscul Dis. 2022.

A new *DNAJB4*-related congenital myopathy with early respiratory failure

An essential chaperone
DNAJB4 is a protein present in sarcomeres which maintains their structure, particularly during muscle contraction. Other proteins in the DNAJB family are known to be associated with various myopathies, for example, DNAJB6 is involved in limb-girdle muscular dystrophy D1.

▪ An international team of doctors and researchers have described a new "chaperonopathy" associated with the *DNAJB4* gene in four people from two families which is characterised by myofibrillar disorganisation, spine rigidity and respiratory failure caused by weakness in the diaphragm.
Weihl CC et al. Acta Neuropathol. January 2023.

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Keep up to date on neuromuscular disease research news throughout the year on the AFM-Téléthon website:
WEBSITE www.afm-telethon.fr