JUNE 2022

SAVOIR & COMPRENDRE
AVANCÉES DE LA RECHERCHE

ADVANCES in limb-girdle muscular dystrophy

> *limb-girdle muscular dystrophy*
> *LGMD* (limb-girdle muscular dystrophy)
> *calpainopathy*
> *dysferlinopathy*
> *sarcoglycanopathy*

Limb-girdle muscular dystrophy (LGMD) is a heterogeneous group of rare genetic muscle diseases. These diseases manifest as a deficit and atrophy of the pelvis muscles (pelvic girdle) and the shoulder muscles (pectoral girdle). The disease manifestations vary significantly, from simple muscle fatigue to forms that can result in an inability to walk, with or without cardiac and/or respiratory complications.

This document, published for the AFM-Téléthon 2022 General Meeting, presents news from the past year regarding research into limb-girdle muscular dystrophy: international symposia, ongoing studies or clinical trials, scientific and medical publications, etc.

It can be downloaded from the AFM-Téléthon website, where other information can also be found regarding scientific, medical, psychological, social or technical fields relating to limb-girdle muscular dystrophy:

WEB www.afm-telethon.fr

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Acknowledgments
- We would like to thank all the individuals with an interest in this disease who have taken the time to review or amend all or part of this document.

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131 scientific articles
published between June 2021 and May 2022
(Source: PubMed)

5 clinical trials 3 of which gene therapy

16 observational studies
underway worldwide
(Source: ClinicalTrials.gov)

What is limb-girdle muscular dystrophy ?

Characteristics

▪ Limb-girdle muscular dystrophy (LGMD) is a heterogeneous group of rare genetic muscle diseases characterised by gradual weakening, primarily, of the proximal muscles, i.e. the shoulder muscles (pectoral girdle) and the hip muscles (pelvic girdle), due to a loss of muscle fibres. The clinical manifestations vary, but are generally limited to the skeletal muscles.
Today, **32 forms of LGMD have been identified**. They are the result of defects in proteins acting at different muscle fibre locations.

*The term "**Limb-Girdle Muscular Dystrophy**" was introduced in 1954 by Walton and Nattrass, two doctors from King's College Durham, now known as Newcastle University, to designate patients who develop weakness and atrophy of the muscles of the limb girdle before they reach 30 years of age, without facial manifestations and who did not experience a fast progression of their disease*

*A **disease** is said to be **rare** if it affects less than 1 in 2000 people (0.05%). Rare diseases are subject to common public health policy in the areas of research, information, and therapeutic management.*

Varying manifestations

▪ The symptoms of LGMD are not present at birth and can **affect individuals of any age**, from children at an early age to adults at an advanced age.
The seriousness of LGMD often correlates to the age of the patient at the time the disease appears.
There are adult forms presenting mild clinical signs and a slow progression, forms that start in childhood, much more serious, with early severe disability, with or without respiratory and/or heart failure, and many intermediary forms.
A combination of clinical, pathophysiological and genetic testing is often needed to achieve a diagnosis with certainty.

Number of people affected

▪ Although the prevalence is difficult to assess and varies significantly depending on the geographical region and form of LGMD, it is estimated that, for all forms of LGMD together, the prevalence is between 1/44,000 and 1/123,000*. LGMD R1 and LGMD R2 are the most common forms, at least in France.

Mode of inheritance

▪ The majority of cases of LGMD are usually transmitted in an **autosomal recessive** manner, and much more rarely in an **autosomal dominant** manner. Currently, 32 LGMD genes have been identified as causing 32 different forms of LGMD.

Classification and nomenclature

▪ In 2017, a workshop bringing together international experts and patient representatives was organised by the European Neuromuscular Centre (ENMC) at Naarden in the Netherlands, to review the nomenclature and classification of LGMD. The results of this work were published in 2018 and the new names and classifications proposed are those that are now used by the majority of the scientific and medical community.

Proximal muscle involvement

The proximal muscles are muscles that are close to the spinal column: the shoulder and arm muscles for the upper limbs, the muscles of the hips and thighs for the lower limbs.

➤➤ The musculoskeletal system, Knowledge & Understanding reference documents, AFM-Téléthon.

* Orphanet data for limb-girdle muscular dystrophy: https://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=FR&Expert=263.

In this publication, the different types of **limb-girdle muscular dystrophy are defined as**:

"[…] hereditary genetic diseases that primarily affect the skeletal muscles and that manifest as progressive muscle weakness, with proximal predominance, linked to a loss of muscle fibres.

In order to be considered an authentic case of limb-girdle muscular dystrophy, a case of LGMD must have been reported in at least two non-related families, the affected members of which have achieved independent walking. The patients must present an elevated CPK level, images of muscle degeneration during the progression of the disease, and dystrophic deterioration under muscle histology, all of which can lead eventually to complete degeneration of the most severely affected muscles."

***Muscular dystrophy** is characterised by a gradual weakening and shrinkage of certain muscle groups. Examining a sample of these dystrophic muscles under the microscope shows a degeneration of the muscle cells accompanied by the presence of young regenerating cells that tend to counterbalance the cell loss due to degeneration.*

A new naming system for limb-girdle muscular dystrophy

▪ The naming of limb-girdle muscular dystrophy now uses the following format: *"LGMD, D (dominant) or R (recessive) for the method of transmission, order of discovery (number), name of the protein causing the disease*".

▪ Thus, the disease formerly referred to as LGMD2A becomes LGMD R1 calpain-3-related.

Angelini, C. Acta Myol. 2020 ; Liang, W. C. et al. Orphanet J Rare Dis. 2020 ; Liewluck, T. et al. Muscle Nerve. 2018 ; Mah, J. K. et al. Can J Neurol Sci. 2016 ; Straub, V. et al. Neuromuscul Disord. 2018 ; Taghizadeh, E. et al. J Cell Physiol. 2019 ; Walton, J. N. et al. Brain. 1954.

Recessive LGMDs: 27 recognised forms

▪ The recessive forms are by far the most common: they represent almost 90% of patients with LGMD.

Five groups are commonly used when talking about recessive LGMD:

- **Anoctaminopathy:** LGMD R12
- **Calpainopathy:** LGMD R1
- **Dysferlinopathy:** LGMD R2
- **Sarcoglycanopathies:** LGMD R3, R4, R5, and R6
- **Dystroglycanopathies:** LGMD R9, R11, R13, R14, R15, R16, R19, R20 and R24
- **Plectinopathy:** LGMD R17

Calpainopathy is the most common of the autosomal recessive forms, followed by dysferlinopathy, then anoctaminopathy, then the sarcoglycanopathies. However, this distribution can vary significantly from geographical area to geographical area.

Main name	Synonym(s)	ORPHA code[†]	Gene	Protein
LGMD R1	LGMD 2A; LGMD R1 calpain-3-related; Calpainopathy[‡]	*267*	*CAPN3*	Calpain-3
LGMD R2	LGMD 2B; LGMD R2 dysferlin-related	*268*	*DYSF*	Dysferlin
LGMD R3	LGMD 2D; LGMD R3 α-sarcoglycan-related	*62*	*SGCA*	α-sarcoglycan
LGMD R4	LGMD 2E; LGMD R4 β-sarcoglycan-related	*119*	*SGCB*	β-sarcoglycan
LGMD R5	LGMD 2C; LGMD R5 γ-sarcoglycan-related	*353*	*SGCG*	γ-sarcoglycan
LGMD R6	LGMD 2F; LGMD R6 δ-sarcoglycan-related	*219*	*SGCD*	δ-sarcoglycan
LGMD R7	LGMD 2G; LGMD R7 telethonin-related; Telethoninopathy	*34514*	*TCAP*	Telethonin
LGMD R8	LGMD 2H; LGMD R8 TRIM 32-related	*1878*	*TRIM32*	TRIM32
LGMD R9	LGMD 2I; LGMD R9 FKRP-related	*34515*	*FKRP*	FKRP
LGMD R10	LGMD 2J; LGMD R10 titin-related	*140922*	*TTN*	Titin
LGMD R11	LGMD 2K; LGMD R11 POMT1-related	*86812*	*POMT1*	POMT1
LGMD R12	LGMD 2L; LGMD R12 anoctamin-5-related	*206549*	*ANO5*	Anoctamin 5
LGMD R13	LGMD 2M; LGMD R13 Fukutin-related	*206554*	*FKTN*	Fukutin
LGMD R14	LGMD 2N; LGMD R14 POMT2-related	*206559*	*POMT2*	POMT2
LGMD R15	LGMD 2O; LGMD R15 POMGnT1-related	*206564*	*POMGnT1*	POMGnT1
LGMD R16	LGMD 2P; LGMD R16 α-dystroglycan-related	*280333*	*DAG1*	α and β dystroglycans
LGMD R17	LGMD 2Q; LGMD R17 plectin-related	*254361*	*PLEC*	Plectin
LGMD R18	LGMD 2S; LGMD R18 TRAPPC11-related	*369840*	*TRAPPC11*	TRAPPC11
LGMD R19	LGMD 2T; LGMD R19 GMPPB-related	*363623*	*GMPPB*	GMPPB
LGMD R20	LGMD 2U; LGMD R20 ISPD-related	*352479*	*ISPD*	ISPD
LGMD R21	LGMD 2Z; LGMD R21 POGLUT1-related	*480682*	*POGLUT1*	Protein O-glycosyltransferase 1
LGMD R22	Bethlem myopathy recessive; LGMD R22 collagen 6-related	*610*	*COL6A1, COL6A2, COL6A3*	Collagen 6
LGMD R23	Laminin α2-related muscular dystrophy; LGMD R23 laminin α2-related	*565837*	*LAMA2*	Laminin α2 (merosin)
LGMD R24	POMGNT2-related muscular dystrophy; LGMD R24 POMGNT2-related	*565899*	*POMGNT2*	POMGNT2
LGMD R25	LGMD 2X; LGMD R25 POPDC1-related	*476084*	*BVES*	POPDC1
LGMD R26	LGMD R26 POPDC3-related	-	*POPDC3*	POPDC3
LGMD R27	LGMD R27 JAG2-related	-	*JAG2*	Jagged-2

[†] Disease number in the Orphanet database. This numerical identifier is unique, remains the same over time and is never reused once attributed.

[‡] The term "calpainopathy" (in reference to the *CAPN3* causal gene) is commonly used in everyday language to specifically designate the recessive form, LGMD R1, even though the dominant form, LGMD D4, is also caused by a mutation in the *CAPN3 gene*.

Dominant LGMDs: 5 recognised forms

▪ The autosomal dominant forms (LGMD D) are much rarer than the autosomal recessive forms (LGMD R), with sometimes only a few families reported to be affected within one subtype.

Main name	*Synonym(s)*	*ORPHA code*	*Gene*	*Protein*
LGMD D1	LGMD1D; LGMD D1 DNAJB6-related	*34516*	*DNAJB6*	DNAJB6
LGMD D2	LGMD1F; LGMD D2 transportin-3-related	*55595*	*TNPO3*	Transportin-3
LGMD D3	LGMD1G; LGMD D3 HNRNPDL-related	*55596*	*HNRNPDL*	hnRNPDL
LGMD D4	LGMD 1I; LGMD D4 calpain-3-related	*565909*	*CAPN3*	Calpain-3
LGMD D5	Bethlem myopathy dominant; LGMD D5 collagen 6-related	*610*	*COL6A1, COL6A2, COL6A3*	Collagen alpha-1, 2, 3 (VI) chain

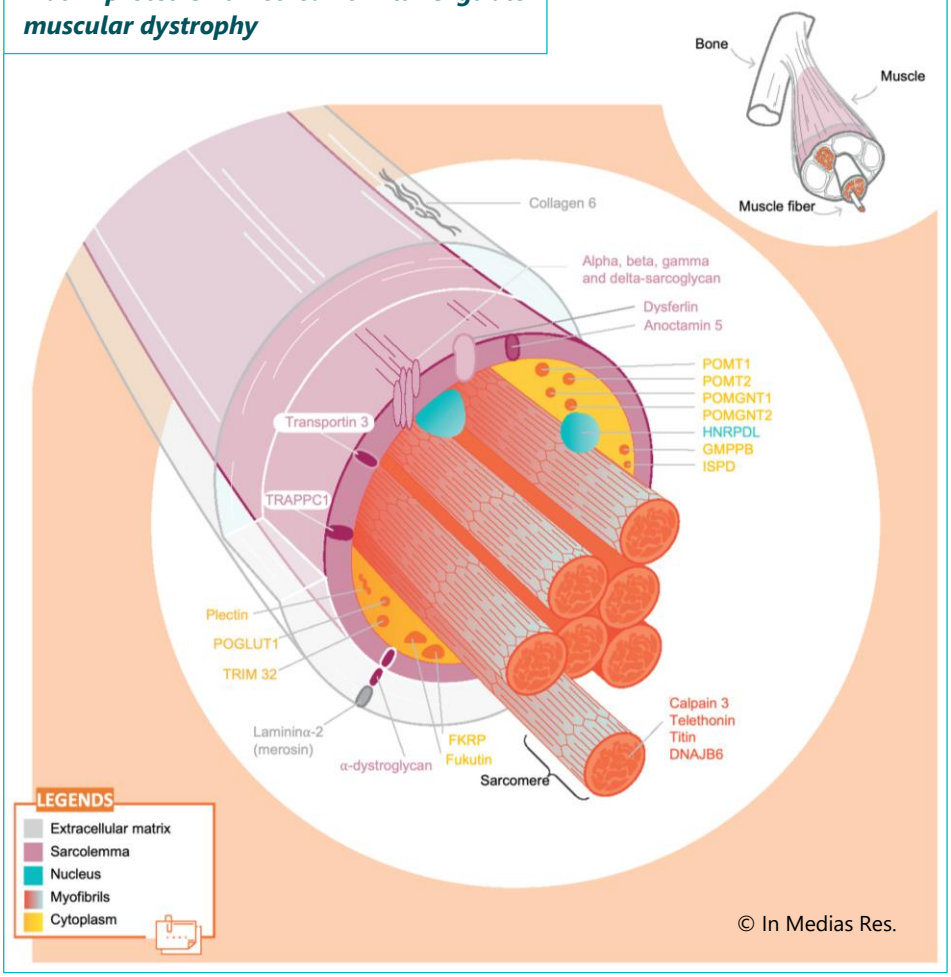
Causes of the disease

▪ The 32 genes involved in the LGMDs that are currently recognised, code for proteins that play a distinct role in terms of different constituents of the muscle cells (or fibres):

- Cell membrane (sarcolemma);
- Intracellular fluid (cytosol);
- Contractile filaments of the muscle fibre (myofibrils) and their functional units, the sarcomeres;
- Nuclear envelope;
- Sarcoplasmic reticulum;
- Cytoskeleton;
- Extracellular matrix.

The sarcolemma glycoproteins and the proteins responsible for its repair or for the transportation of materials into the cell, are the parts most frequently affected by LGMD.

Main proteins involved in limb-girdle muscular dystrophy



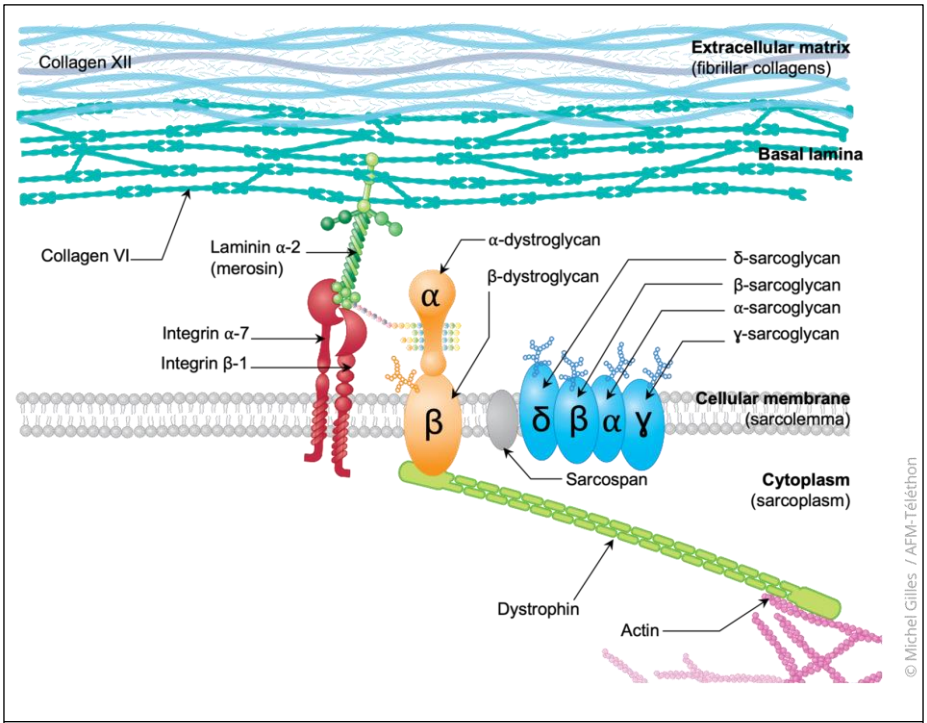
Cell membrane (sarcolemma) and extracellular matrix

▪ The **α, β, γ and δ sarcoglycan proteins (sarcoglycanopathies)** form a protein complex located in the membrane of the muscle cells, which contributes to the stability and the mechanical resistance of the muscle fibres during muscle contraction.

▪ **Dysferlin (LGMD R2)** and **anoctamin 5 (LGMD R12)** are involved in muscle fibre membrane repair.

*The **myofibrils** are the contractile system of the muscle cells. They extend along the entire length of the muscle fibre. They are divided into small contractile units, **sarcomeres**.*

*The **extracellular matrix** is a complex network of proteins in which cells are immersed. It ensures cell cohesion within a tissue, and plays an essential role in cell creation, maintenance, adhesion, movement and regulation. The extracellular matrix of the muscle specialises in responding to the mechanical constraints inherent to the contractile activity of the muscle fibres.*



From cytoskeleton to extracellular matrix
An essential bond between the inside of the muscle cell and the extracellular matrix protects the muscle cell membrane from lesions caused by alternate contraction/relaxation.

- A lack of certain sugars on the surface of **alpha-dystroglycan (dystroglycanopathies)** breaks its bond with the extracellular matrix proteins, weakening the muscle fibre membrane.
- The **POPDC proteins (LGMD R25 and LGMD R26)** contain a **"Popeye"** domain, named in this way due to their preferential expression in the skeletal and cardiac striated muscles. **POPDC1** and **POPDC3** are located in the cell membrane and the T-tubules, but are also found in the nuclear membrane of the striated muscle cells. They would appear to play a role in the membrane trafficking of certain proteins.
- **Laminin α2 (LGMD R23)** belongs to a network of proteins at the interface between the muscle fibre membrane and the muscle support tissue (conjunctive tissue). This network, called the basal lamina, surrounds each muscle fibre.
- **Collagen VI (LGMD R22 and LGMD D5)** is one of the constituents of the conjunctive tissue that surrounds the muscle fibres (the extracellular matrix), that supports them and that ensures the fibres connect to one another.

__Membrane trafficking__ is the collection of mechanisms that allow a cell to distribute materials from one cell compartment to the next, using small bags enclosed by a membrane (vesicles).

The __basal lamina__ (or basal membrane) is a particular form of extracellular matrix specific to certain tissues. It is made up of proteins that are entangled together to form a network that surrounds one or more cells.

Maturation of the muscle fibres

- **Caveolin-3 (LGMD1C[§])** would appear to play a key role in fusion of the myoblasts into myotubes during the muscle fibre maturation process.

[§] LGMD1C was excluded from the LGMD classification in 2018, and reclassified as Rippling muscle disease: Straub, V. et al. Neuromuscul Disord. 2018.

The sarcomere

▪ Several proteins of the sarcomere are involved in limb-girdle muscular dystrophy.

▪ **Calpain-3 (LGMD R1), telethonin (LGMD R7)** and **titin (LGMD R10)** are muscle-specific intracellular proteins and are involved in the development and structure of the sarcomere.

Did you know?

The sarcomere: the fundamental contractile structure of the muscle

▪ A sarcomere is delineated by two Z discs. Within the sarcomere, thick myosin filaments and fine actin filaments are arranged alternately.

▪ When the muscle contracts, these filaments slide into each other, and the distance between two Z discs decreases. The shortening of all the sarcomeres means that the cell muscles contract, which causes the contraction of the entire muscle.

LGMD news

Congresses and meetings

The International LGMD Conference 2021

▪ The **International LGMD Conference**, also organised by the Speak Foundation, took place virtually from 17 to 20 September 2021. This clinical and scientific conference, sponsored by, inter alia, the Muscular Dystrophy Association (MDA) and AFM-Téléthon, was dedicated exclusively to the limb-girdle myopathies, and was free and open to all. Of particular note, doctors, researchers and other LGMD experts, and pharmaceutical companies, reviewed the latest research, clinical trials and emerging treatments in LGMD.

The programme and the video recordings for the four days of conference are available on the Speak Foundation website. A special edition of LGMD News, a journal edited by the Foundation, was also produced to mark the occasion.

The next edition of the International LGMD Conference will be held in 2023.

WEB https://thespeakfoundation.com

WEB https://nationallimbgirdlemusculardystrophyconference.com

The 2022 LGMD global summit

▪ On 20 May 2022, an American association, the Speak Foundation, organised a global summit for LGMD, the **2022 LGMD Global Advocacy Summit**, where patients with all forms of LGMD had the opportunity to interact directly with researchers and pharmaceutical companies (AskBio, ML Bio Solutions, Sarepta, Vita, Atamyo, Edgewise, etc.) developing clinical trials and treatments for these conditions. In particular, these companies presented their pipelines and the clinical trials that were being planned. AFM-Téléthon was represented there by Mandine Casado and Mélanie Bordes, respectively the Head and the Scientific Manager of the AFM-Téléthon LGMD (Limb-Girdle Myopathies) Interest Group. They presented AFM-Téléthon and the LGMD interest group, and also their missions and their activities. All the presentations were recorded and are accessible on the Speak Foundation website.

WEB https://thespeakfoundation.com/global-advocacy-summit

*The **Speak Foundation** was created in 2008 by Kathryn Bryant two years after her LGMD R9 diagnosis. The Foundation's mission is to improve the quality of life of patients with muscular dystrophy and to be a voice for all those living with a rare disease.*

WEB www.thespeakfoundation.com

The 2022 MDA Clinical & Scientific Conference

▪ The **2022 MDA Clinical & Scientific Conference**, organised by the Muscular Dystrophy Association (MDA), took place from 13 to 16 March 2022, both online and in-person at Nashville, Tennessee, in the United States. Similar to the previous year's event, it welcomed more than 1200 participants and around a hundred speakers across more than 20 sessions, the themes of which were preclinical and translational research, and clinical research. The exhibitors included over 30 pharmaceutical companies (AskBio, BridgeBio, Novartis, Pfizer, Sarepta, etc.) and around 10 patient associations (LGMD Awareness Foundation, Speak Foundation, Jain Foundation, etc.). In particular, BridgeBio (ML Bio Solutions) and Sarepta Therapeutics presented their latest data relating to ongoing LGMD trials.

WEB https://www.mdaconference.org
WEB https://www.mdaconference.org/2022-abstract-search
WEB https://musculardystrophynews.com/mda-2022/

TREAT-NMD is focusing on the natural history of LGMD

TREAT-NMD
is an international network dedicated to neuromuscular diseases, encompassing specialist scientists and clinicians and patient associations, created in 2012 with the initial support of the European Commission. One of its objectives is to speed up the transitioning of the most promising research into clinical applications.
WEB www.treat-nmd.org

▪ On 18 June 2019, the TREAT-NMD network organised a meeting bringing together clinicians, physiotherapists, researchers, patient groups and pharmaceutical industry representatives, from eight different countries. The objective of this meeting, the proceedings of which were published in August 2021, was to survey the current state of affairs of natural history studies, whether ongoing or completed, to share experiences, and to present the new TREAT-NMD LGMD taskforce.

▪ Ten natural history studies were presented at this meeting. All of the participants were in agreement regarding the need to harmonise the design of the different studies and the collection of data, in order to allow a better comparison to be made between these studies.

▪ The participants agreed on the fact that standardised diagnostic procedures should be developed, to acquire and interpret data from clinical examinations, for the reading of muscle biopsies by immunohistochemistry and western blot, muscle imaging and genetic tests. Given the difficulty in interpreting genetic results for many clinicians, it was suggested that panels of expert clinicians and genetics specialists be developed regionally and nationally, to provide less experienced practitioners with guidance for difficult cases.

▪ Finally, the **TREAT-NMD LGMD taskforce** was presented. It was created to provide support in preparing clinical trials for LGMD, to avoid fragmentation of research efforts, and to ensure optimal collaboration between stakeholders, in order to speed up the development of therapies.
Guglieri, M. et al. Neuromuscul Disord. 2021.

The training of healthcare professionals

TREAT-NMD provides LGMD training

▪ Treat-NMD organised, on 11 and 12 May 2022, its third annual LGMD Masterclass, the **TREAT-NMD Limb-Girdle Muscular Dystrophy Australasia Expert Masterclass**. The goal of this free online event was to train doctors in the specifics of LGMD diagnosis, management and care. The objective of these meetings was to create a forum for discussion where healthcare professionals could be informed of the latest advances in LGMD, share ideas and the difficulties they have experienced, and share successes regarding the care provided to patients.

WEB https://treat-nmd.org/what-we-do/treat-nmd-education/previous-masterclasses

ENMC: workshops on dysferlinopathy and the dystroglycanopathies

▪ In 2022, the **262nd and 257th workshops of the European Neuromuscular Centre** (ENMC) were dedicated respectively to "Standards of care for the dysferlinopathies" and "The 3rd workshop on dystroglycan and the dystroglycanopathies". The 262nd workshop took place on February 2022, and the 257th workshop, which was initially supposed to occur in September 2020, took place in June 2022. Professor Volker Straub, an expert on LGMD, was an organiser for both workshops.

WEB https://www.enmc.org/wp-content/uploads/2022/02/NEW-ENMC-Workshop-planning-year-2022-WEBSITE-VERSIE-8-feb-22-1.pdf

WEB https://www.enmc.org/workshops/upcoming-workshops/

*The **European Neuromuscular Centre (ENMC)** is an international organisation that aims to support research in the field of neuromuscular disease. It regularly organises international meetings bringing together scientists and clinicians on a specific topic.*

WEB www.enmc.org/

Associations

A new LGMD Association is launched

▪ The Australian **Daniel Ferguson LGMD 2A Foundation** was launched on 20 February 2022, marking the International Rare Disease Day. This association is named after Daniel Ferguson, an Australian patient with LGMD R1.

WEB https://treat-nmd.org/news-events/newsletter-signup/newsletter-march-2022

WEB https://www.dffoundation.com.au

Review and lessons of the COVID-19 pandemic

Epidemiology and vaccine

Neuromuscular patients were less affected

▪ Patients with neuromuscular diseases in France were, proportionally, less affected by COVID-19 than the rest of the population during the first lockdown: 17/10,000 versus 26/10,000 in the general population. According to the study reporting these figures, this difference could be due to greater attention paid to self-isolation and sanitary measures, and to the actions taken by the French National healthcare network, FILNEMUS, and the neuromuscular patient associations.

▪ A majority (58%) of the 84 patients in the study with neuromuscular disease developed COVID-19 with a sufficiently mild intensity that they were able to be cared for at home. Nevertheless, hospital admission was more common than among the general population, but the mortality rate was lower (11% versus 14%). The results obtained up to now from different studies do not allow a link to be established between the development of a severe form of COVID-19 and the use of corticosteroids.

FAI2R/SFR/SNFMI/SOFREMIP/CRI/IMIDIATE consortium and contributors. Ann Rheum Dis. 2021 ; Pisella, L. I. et al. Orphanet J Rare Dis. 2021.

Atrophied muscle does not preclude the efficacy of the vaccine

▪ A study measured antibody levels, which reflect the immune response to the vaccine, 14 days after the administration of the second vaccine dose among 14 patients aged 20 to 60 years, with Duchenne muscular dystrophy or Becker muscular dystrophy (9/14), SMN1-gene-related proximal spinal muscular atrophy (SMA) (1/14), or limb-girdle muscular dystrophy (2/14). The results showed that, despite a low muscle mass and long-term treatment with corticosteroids, the antibody levels measured were similar in the group of vaccinated patients with neuromuscular diseases and in the vaccinated control group with no neuromuscular disease.
Demonbreun, A. R. et al. Neuromuscul Disord. 2022.

Mobilisation in the face of COVID-19

FILNEMUS and AFM-Téléthon by the side of patients

*The **FILNEMUS rare neuromuscular diseases healthcare network** is hosting, coordinating and encouraging interactions between actors participating in the diagnosis, treatment and research of neuromuscular diseases. It was created in February 2014, as part of the second Rare Diseases French National Plan, 2011-2014.*
WEB www.filnemus.fr
➤➤ Organisation of care and neuromuscular diseases, Knowledge & Understanding reference documents. AFM-Téléthon

▪ The **FSMR (French Rare Diseases Healthcare Networks)** published, in December 2021, their 2020 activity report, in which they noted the impact of COVID-19 on the world of rare diseases. The different surveys conducted by the FSMR revealed, overall, a discontinuation of care due to fear of the virus or hospital saturation, but the development of virtual consultations was the only area where a decrease in activity was not seen.
Ministère des Solidarités et de la Santé [French Ministry of Solidarity and Health]. (2021).

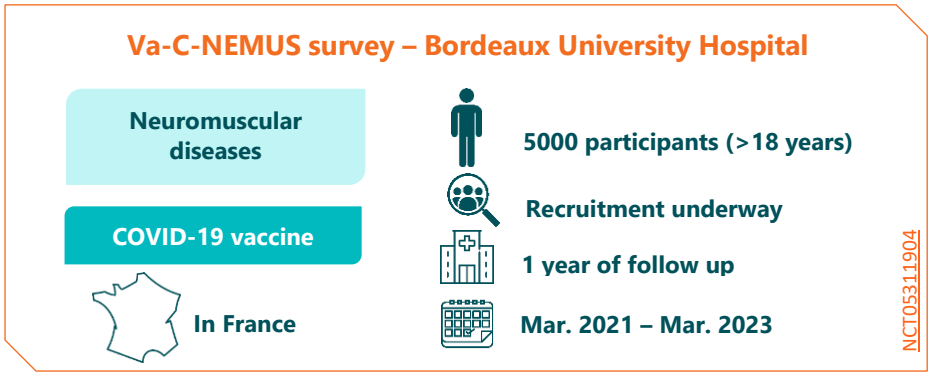
▪ In this respect, the **FILNEMUS** network, which is dedicated to neuromuscular diseases, greatly supported patients, as demonstrated by 12 surveys and guidelines regarding neuromuscular disease and COVID-19 (self-rehabilitation at home, COVID-19 in paediatrics, etc.), a webinar and a video, making them one of the networks that created the largest amount of content during this period.
WEB https://www.filnemus.fr/

▪ **AFM-Téléthon** was very active in supporting patients with neuromuscular disease during this health crisis, in particular by uploading 17 practical guideline documents on COVID-19, by publishing 16 information articles and by providing answers to more than 50 questions from patients. It was also able to develop its support model in order to adapt to COVID-19 constraints and maintain remote psychological support: videoconferencing at home, listening to and answering questions, a psychological support cell, remote training, etc., almost 12,000 families benefited from this support.
WEB https://www.afm-telethon.fr/coronavirus
WEB https://www.afm-telethon.fr/vaccination-covid19

Coronavirus research is continuing

▪ The national **Va-C-NEMUS** study (efficacy and safety of COVID-19 vaccination in neuromuscular patients) launched mid-March 2021 by Bordeaux University Hospital and coordinated by FILNEMUS, is still ongoing. Its main objective is to learn more about the effects of the COVID-19 vaccines in neuromuscular patients. Any person with neuromuscular disease, aged 18 years or over, whether vaccinated or not, may answer this survey online. The investigators are aiming for 5000 participants.

WEB https://www.chu-bordeaux.fr/Patient-proches/Maladies-rares/Recherche-clinique/Va-C-NEMUS



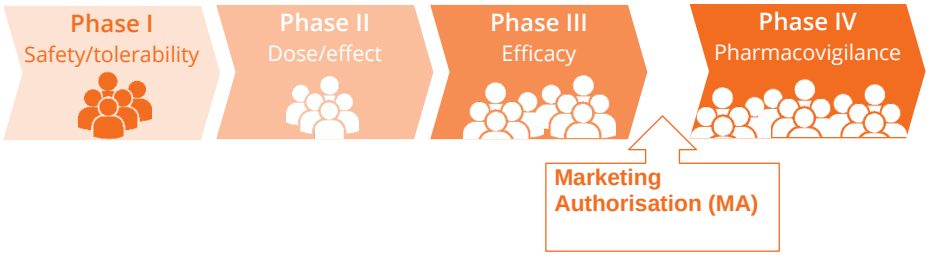
▪ The **CANNEMUSS** study, co-piloted by Bordeaux University Hospital and AFM-Téléthon, aims to assess the efficacy of COVID-19 vaccination in patients with neuromuscular disease and major muscle atrophy. The study is still ongoing.

WEB https://www.chu-bordeaux.fr/Professionnels-recherche/Recherche-clinique-et-Innovation/Participer-à-une-recherche-clinique/Essais-Cliniques-en-cours-au-CHU-de-Bordeaux/

Clinical trials

What is a clinical trial?

▪ **Clinical trials** consist of assessing a potential treatment (candidate medicine, medical device, etc.) in order to ensure that it is well tolerated and efficacious in treating a disease.



*INSERM defines **clinical research** as: "[...] scientific studies conducted on humans, with a view to developing biological or medical knowledge. This is prospective research, involving the follow up of patients or healthy volunteers.". Clinical research comprises **clinical trials** (interventional) and **observational studies** (non-interventional).*

WEB https://www.inserm.fr/our-research/clinical/la-recherche-clinique

The 4 phases of a clinical trial

The candidate medicine is assessed through the course of successive trials, corresponding to different phases: I, II, III and IV.

- **Phase I: Safety/tolerability**

A candidate medicine is tested for the first time on a small group of individuals (often healthy volunteers), to assess its safety/tolerability and its distribution throughout the body (pharmacokinetics).

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

Phase II, conducted on a homogeneous group of volunteers with the disease, studies the safety and efficacy of the product and determines 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 clarify the product's therapeutic efficacy compared to an existing treatment or a placebo. At the end of this trial, the medicine may obtain marketing authorisation.

- **Phase IV: Pharmacovigilance**

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

International clinical trials underway in LGMD at a glance

TRIAL TITLE	FORM OF LGMD	THERAPEUTIC APPROACH	CLINICAL DEVELOPMENT		
			PHASE I	PHASE II	PHASE III
WSiMD trial *(Northwestern University, United States)*	**LGMD R1 (*CAPN3*), R2 (*DYSF*), R4 (*SGCB*), R5 (*SGCG*), R6 (*SGCD*), R9 (*FKRP*), R12 (*ANO5*)**	**Pharmacotherapy**	**Prednisone** Recruitment completed		
SRP-9003 trial *(Sarepta Therapeutics, United States)*	**LGMD R4 (*SGCB*)**	**Gene therapy**	**SRP-9003** Recruitment completed		
GNT0006 trial (ATA-100) *(Généthon – Atamyo Therapeutics, France)*	**LGMD R9 (*FKRP*)**	**Gene therapy**	**ATA-100** Recruitment not yet started		
LION-101 trial *(AskBio, United States)*	**LGMD R9 (*FKRP*)**	**Gene therapy**	**LION-101** Recruitment not yet started		
BBP-418 trial (ribitol) *(BridgeBio Pharma, United States)*	**LGMD R9 (*FKRP*)**	**Pharmacotherapy**	**BBP-418** Recruitment completed		

Clinical trials relating to more than one LGMD

Pharmacotherapy: prednisone (steroids)

- Weekly Steroids in Muscular Dystrophy (WSiMD), a North American clinical trial financed by ***PTC Therapeutics***, assessed the effects of a corticosteroid treatment (**prednisone**), at a low dose to limit the risk of side effects, over a period of six months among 19 adults with **LGMD R1, R2, R4, R5, R6, R9 or R12** (and one patient with Becker muscular dystrophy). The participants, who were 18 to 60 years of age, ambulatory and non-ambulatory, took a dose of 0.75 to 1 mg of prednisone per kilogram of bodyweight once a week for six months.
- The **corticosteroids at this dose are well tolerated**, without significant side effects (no significant hypertension, hyperglycaemia, weight gain or harmful effects on bone density).

This treatment seems to protect the muscles

- Creatine phosphokinase decreased after six months of treatment and participants saw their grip strength increase by 6.5%. Those who were able to remain ambulatory were able to walk faster and for longer. Almost all the patients (19/20) chose to continue the treatment at the end of the trial.

Zelikovich, A. S. et al. J Neuromuscul Dis. 2022.

Phase II trial (pharmacotherapy) – PTC Therapeutics

LGMD R1, R2, R4, R5, R6, R9, R12; BMD

Prednisone

In the United States

20 participants (18–65 years)

Data currently being analysed

6 months of follow up

Jul. 2019 – Dec. 2022

NCT04054375

Physiotherapy: physical exercise and quality of life

- A British study conducted by the Manchester Metropolitan University analysed the psychological effects of 12 weeks of resistance training (moderate intensity) among 17 ambulatory patients, **six with LGMD R1, R2, R9 and R12**, six with Facioscapulohumeral muscular dystrophy (FSHD) and five with Becker muscular dystrophy (BMD).
- Twice a week, the participants performed exercises such as squats, hip flexion, plantar flexion, etc. At the end of the 12 weeks of training, **the beneficial effects on health** were apparent in these patients, with a 19% decrease in depressive symptoms and a 10% decrease in anxiety scores. Self-esteem and global physical self-worth increased respectively by 10% and 20%, and quality of life improved significantly for several domains (social function, vitality, general health, bodily pain, etc.).

To stay motivated and measure one's activity, why not use an electronic wristband?

- For those who are very keen on technologically, another recent study, conducted among 110 patients with neuromuscular disease, demonstrated that the Fitbit® wristbands are well tolerated and are efficacious in terms of patients becoming more attentive to their daily activity and more diligent.

O'Dowd, D. N. et al. Disabil Rehabil. 2021 ; Roberts-Lewis, S. F. et al. Disabil Rehabil. 2021.

Medical device: a brace for scapular winging

▪ Two researchers from the Zürich Federal Polytechnic School (ETH Zürich) developed an orthosis that braces the scapula against the thorax. This device was tested on eight patients with neuromuscular diseases, including **one patient with LGMD R1**.

Increased daily comfort

▪ Patients saw improvements in the angle of arm elevation, both forward and to the side. The orthosis also reduced the perceived effort when elevating a filled bottle.

▪ This orthosis resembles an adjustable brace (or a very short tank top), that leaves the arms and shoulders free. It includes a rigid plate in relation to the scapula, to which it applies pressure, the intensity which can be modulated depending on the scapular winging to be corrected. If its efficacy and convenience are confirmed, it could represent a non-invasive and completely reversible solution, unlike the surgical fixation of the scapula.

Georgarakis, A. M. et al. J Neuroeng Rehabil. 2021.

LGMD R1 (*CAPN3* – calpainopathy)

VTA-100 clinical trials anticipated

▪ Vita Therapeutics, a pharmaceutical company that is developing cell therapy products for patients with muscular dystrophy, announced in 2021 that it had raised $32 million to advance its research work. The company intends to sponsor additional preclinical studies on VTA-100, Vita Therapeutics's primary candidate medicine for LGMD R1. VTA-100 combines gene-editing technology with stem cell technology. The treatment was developed to correct gene mutations within cells collected from a patient, that are then re-administered to this patient so they can differentiate into functional muscle fibres. The company intends to ask for approval from the FDA (Food and Drug Administration) to launch clinical trials for VTA-100 very quickly.

Vita Therapeutics. Press Release. 2021.

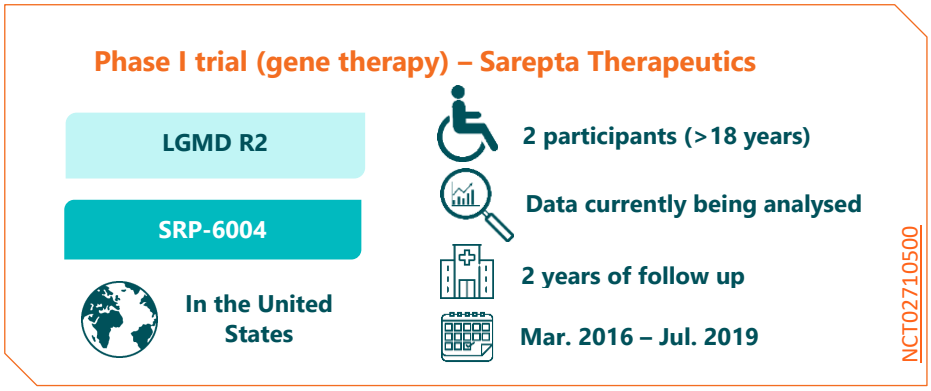
LGMD R2 (*DYSF* – dysferlinopathy)

Gene therapy: SRP-6004 (rAAVrh74.MHCK7.DYSF.DV)

▪ SPR 6004 (rAAVrh74.MHCK7.DYSF.DV) is a gene therapy product administered by the intramuscular route. It provides treated patients with the dysferlin gene, which is mutated in LGMD R2, to help produce a functional protein. A clinical trial sponsored by Sarepta Therapeutics was conducted with the aim of assessing the safety and tolerability of SRP-6004. The results of this study have not yet been published.

▪ However, Jerry Mendell, the principal investigator for this clinical trial, did comment in a publication from last September that the intramuscular injection in the two patients enrolled in the trial did not raise any particular concerns regarding the product's safety.

Pozsgai, E. et al. Neurodegener Dis Manag. 2021.

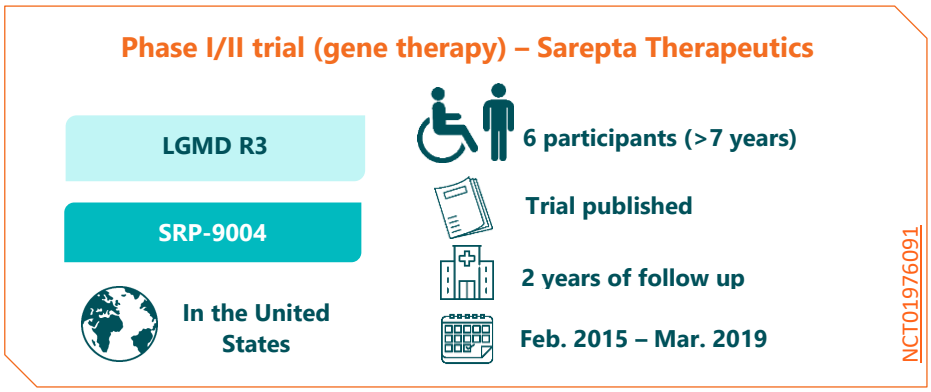


LGMD R3 (*SGCA* – alpha-sarcoglycanopathy)

Gene therapy: SRP-9004 (scAAVrh74. tMCK.hSGCA)

- In 2019, a US team from the Nationwide Children's Hospital Gene Therapy Centre, Columbus (Ohio), published the results of the first administration of a gene therapy product in humans, **SRP-9004 (scAAVrh74. tMCK.hSGCA)**, developed by Sarepta Therapeutics. The product was administered using a limb-targeted infusion technique (ILI, or Isolated Limb Infusion).
- The data showed that this **approach was well tolerated**. Control muscle biopsies showed that there was a re-expression of the gene and the protein, and for some of the participants, a distinct clinical improvement.

Mendell, J. R. et al. Hum Gene Ther. 2019



Sarepta Therapeutics anticipates the continued development of SRP-9004

- In a letter to the LGMD community, published on 25 February 2022, Sarepta Therapeutics announced it had already started the next stages of development of SRP-9003, a gene therapy product intended for LGMD R4 (SGCB), and of SRP-9004: toxicological studies for LGMD R3 and R5, and natural history studies for LGMD R3, R4 and R5 (NCT04475926) are thus underway, the goal being the collection of information for the design of future clinical trials.

Sarepta Therapeutics. Sarepta Community Letter. 2022.

WEB https://lgmd.afm-telethon.fr/nouvelles-de-sarepta-lettre-a-la-communaute-lgmd-du-25-02-2022

LGMD R4 (*SGCB* – beta-sarcoglycanopathy)

Gene therapy: SRP-9003 (rAAVrh74.MHCK7.SGCB)

▪ At the 2022 annual Muscular Dystrophy Association (MDA) conference, which took place in Nashville, United States from 13 to 16 March 2022, Sarepta Therapeutics presented interim results for the **SRP-9003 gene therapy** trial (rAAVrh74.MHCK7.SGCB), three and a half years after it had started.

This open-label trial is due to last for five years, and its goal is to assess whether SRP-9003 is well tolerated among six patients with LGMD R4. SRP-9003 is a gene therapy product that delivers the *SGCB* gene coding the β-sarcoglycan protein. The patients were divided into two cohorts, with the second cohort receiving a four-times-greater treatment dose than the first. The preliminary data from the first and second years of treatment showed that SRP-9003 was well tolerated.

Its beneficial effects are confirmed

▪ The synthesis of the β-sarcoglycan protein and its presence in the cell membrane increased for the long term in both cohorts. The sarcoglycan complex (an assembly of transmembrane proteins) is restored for up to 2 years after injection (third year not yet measured).

▪ The patients presented a distinct improvement in muscle function (walking, ability to get up, etc.), an improvement that has lasted for three years in the first cohort. While the untreated patients who were followed up saw their motor skills decrease over time, the treated patients showed a distinct improvement in these skills in the first year, followed by a stabilisation in the next two years.

In 2021, the investigators extended the clinical trial by two years, and it is now scheduled to end in 2025.

Rodino-Klapac, L. R. et al. MDA Conference. 2022.

Phase I/II trial (gene therapy) – Sarepta Therapeutics

LGMD R4

SRP-9003

In the United States

6 participants (4 to 13 years)

Recruitment completed

5 years of follow up

Oct. 2018 – Feb. 2025

NCT03652259

LGMD R9 (*FKRP*– dystroglycanopathy)

Gene therapy: ATA-100 – Trial in preparation in France

▪ **GNT0006 (ATA-100)** is a gene therapy product administered intravenously that would appear to help rebalance an FKRP deficiency in patients with LGMD R9.

A first clinical trial in preparation

▪ A clinical trial should start this year to test the safety and efficacy of the product. This trial is sponsored by Atamyo Therapeutics, a Généthon spin-off company. The trial is in preparation in three countries (Denmark, France and the United Kingdom) and the recruitment of the 39 patients intended to make up the cohort has not yet started.

A product stemming from research by Généthon

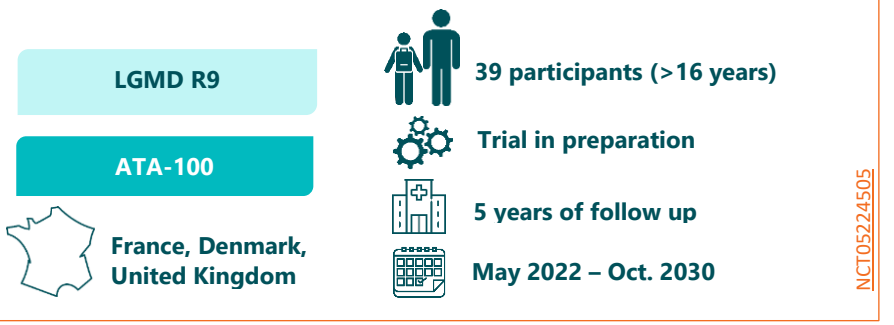
▪ The development of ATA-100 is based on research work conducted by a team headed by Isabelle Richard, who is a researcher at Généthon. In 2017, she published results showing a correction in both functional and tissue manifestations of the disease among mice modelling the disease after injection of the rAAV2/9 vector expressing the functional FKRP protein.

▪ Atamyo Therapeutics, a biotechnology company, was created in 2020 by Généthon, itself an AFM-Téléthon laboratory, to conduct gene therapy programmes stemming from its research into limb-girdle muscular dystrophy. Doctor Isabelle Richard is the Scientific Director of the company.

WEB https://atamyo.com/press-releases/2022/atamyo-therapeutics-franchit-dimportantes-etapes-reglementaires-et-financieres-pour-ata-100-sa-therapie-genique-destinee-a-traiter-la-dystrophie-musculaire-des-ceintures-de-type-2i-r9

Gicquel, E. et al. Hum Mol Genet. 2017.

Phase I/II trial (gene therapy) – *Atamyo Therapeutics*



Gene therapy: LION-101 – Trial in preparation

▪ **LION-101** is a gene therapy product designed to be administered intravenously in *FKRP*-related LGMD R9.

Asklepios BioPharmaceutical (AskBio), the company developing this candidate medicine, will launch a phase I/II multicentre trial in the first quarter of 2022, among adults and adolescents presenting LGMD R9. Recruitment for this clinical trial has not yet started.

LION-101 has demonstrated good tolerability and dose-proportional efficacy in mice modelling the disease.

Asklepios BioPharmaceutical. Press Release. 2021.

Phase I/II trial (gene therapy)
Asklepios Biopharmaceuticals

LGMD R9

LION-101

In the United States

10 participants (18-65 years)

Trial in preparation

1 year of follow up

May 2022 – Dec. 2028

NCT05230459

Pharmacotherapy: ribitol (BBP-418)

▪ Administered orally, **ribitol** provides additional substrate that helps to saturate the FKRP muscle enzyme, leading to an **increase in the glycosylation of the alpha-dystroglycan protein**. Ribitol thus helps to compensate for hypo-glycosylation of the alpha-dystroglycan in patients with LGMD R9 due to a mutation of the *FKRP* gene.

▪ The phase II pharmacotherapy trial (MLB-01-003) involving ribitol and sponsored by ML Bio Solutions (affiliated to BridgeBio), began in February 2021 and is still in progress.

At the annual 2022 Muscular Dystrophy Association (MDA) conference, BridgeBio presented the results of two ribitol trials: a phase I trial among 85 healthy volunteers, and an phase II open-label trial among 14 patients with LGMD R9, ambulatory and non-ambulatory, aged 11 to 55 years.

__Glycosylation__ is a protein modification process, consisting of the adding of sugar groups (glycans). The adding of these sugar molecules occurs in the endoplasmic reticulum and in the Golgi apparatus, two cell compartments where the formation of glycoproteins is completed and ends.

Successful phase I trial

▪ The results show that BBP-418 was very well tolerated, with no adverse effects, even at doses higher than intended therapeutic doses.

Very good phase II results

▪ Three months after the start of treatment, an increase in α-dystroglycan glycosylation of more than 40%, a decrease in blood CPK enzyme levels of 70%, and an increase in speed in the 10-metre walk test were observed. In comparison, a decrease in walking speed was observed over the same period, during the natural history study conducted before ribitol was taken. Following these first positive results for BBP-418, BridgeBio is planning the phase III trial for the second quarter of 2022.

WEB https://bridgebio.com/news/bridgebio-pharma-announces-positive-phase-2-data-for-limb-girdle-muscular-dystrophy-type-2i-lgmd2i

Harper, A. et al. MDA Conference. 2022 ; Rodriguez, H. et al. MDA Conference. 2022.

Phase II trial (pharmacotherapy) – ML Bio Solutions

LGMD R9

Ribitol

In the United States

14 participants (12-55 years)

Recruitment completed

5 years of follow up

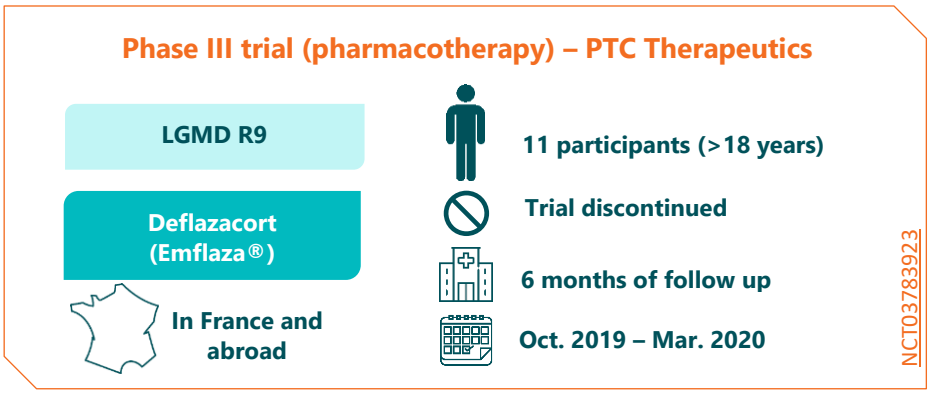
Feb. 2021 – Nov. 2026

NCT04800874

Pharmacotherapy: deflazacort (Emflaza®) – Trial discontinued

▪ A phase III multicentre trial, sponsored by PTC Therapeutics, aiming to assess the tolerability and efficacy of deflazacort (Emflaza®), a steroidal anti-inflammatory, in LGMD R9, was discontinued early, during the course of 2020. The assessment of the treatment efficacy had, indeed, been made impossible due to the low number of participants and the irregular medical follow up, caused by COVID-19. Only 11 patients participated in the trial, compared to the 30 patients originally anticipated. In a statement made to the American association, *CureLGMD2i*, PTC Therapeutics announced their decision to end the study, explaining that those patients wishing to do so could continue to take the treatment for up to 6 months after the end of the trial, with the agreement of the treating physician.

WEB https://curelgmd2i.com/news



Pharmacotherapy: EDG-5506

▪ EDG-5506 is a small drug molecule developed by Edgewise Therapeutics to protect the muscles by reducing muscle atrophy and fibrosis. Taken orally, the drug blocks fast myosin ATPase action, which limits the recruitment of the fast-twitch fibres, that are particularly affected in muscular dystrophy, thus reducing the degradation.

Although developed mainly for patients with Becker muscular dystrophy (BMD), EDG-5506 could also be used in patients with LGMD R9. The product is undergoing a phase II trial among patients with Becker muscular dystrophy.

WEB https://edgewisetx.com/science/211

Observational studies

What is an observational study ?

▪ Studies referred to here as "observational studies" are **so-called "non-interventional" studies** (also called "clinical" studies); any procedures performed or products used in the context of such studies do not differ from the usual therapeutic management provided to participants.

A distinction can be made between four different types of clinical study:

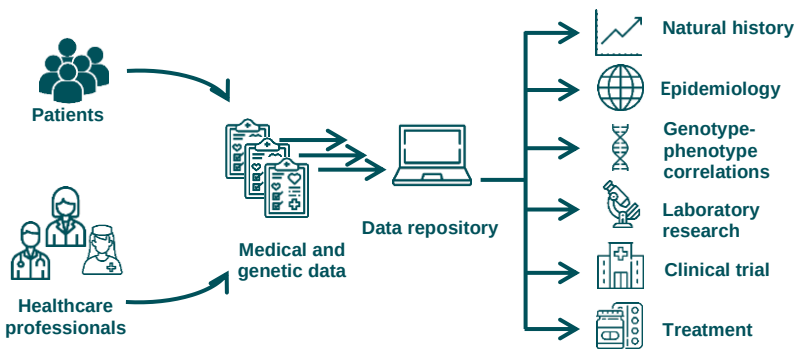
- **Cross-sectional**: focuses on subjects at the time the study is conducted, to describe the characteristics (frequency, morbidity, risk factors, etc.).
- **Prospective**: follows the progression of the subjects enrolled in the study over time. This includes, for example, disease natural history studies.
- **Retrospective**: studies past data, collected from the records of certain patients.
- **Permanent**: data collection is "endless", such as patient registries.

These studies help us to learn more about and to better describe a disease, and to identify better diagnostic and follow-up tools. They are essential in understanding disease epidemiology, in improving patient therapeutic management and in preparing future clinical trials.

LGMD patient registries

▪ Medical data repositories, or patient registries, collect information about patients and/or help, inter alia, to quickly find potential participants for a clinical trial. Registries can be national; but given the rarity of certain diseases, they are often international, in order to aggregate a greater number of patients.

Genotype/phenotype correlation *studies investigate the existence of a relationship between genetic characteristics, the genotype, and characteristics expressing themselves in an observable manner, the phenotype (height, eye colour and shape, hair colour, disease manifestation, etc.). This helps to identify whether a relationship exists, to a greater or lesser extent, between the presence of genetic abnormalities and the manifestations of a*



Medical data repositories

The medical databases or data repositories consolidate medical and genetic data from patients with the same disease, often without any time limit.
The analysis of collated data helps to clarify the natural history of the disease, to establish genotype/phenotype correlations and to facilitate the recruitment of participants for clinical trials.

Two French National LGMD registries

▪ Two French registries on LGMD, supported by AFM-Téléthon, are currently in the process of being developed:
- A **calpainopathy** registry, coordinated by Prof. E. Malfatti (Neuromuscular Pathology Reference Centre, Henri Mondor Hospital, Paris) and I. Richard (Généthon, Évry).
- A registry on the **sarcoglycanopathies**, coordinated by Prof. P. Laforêt (Neuromuscular Pathology Reference Centre, Nord-Est-Ile-de-France [North-East-Ile-de-France], Raymond Poincaré Hospital, Garches).

Eight international LGMD registries

▪ The AFM-Téléthon LGMD Interest Group has surveyed eight patient registries dedicated to the limb-girdle myopathies.
WEB https://lgmd.afm-telethon.fr/registres-internationaux-lgmd/

Disease(s)	*Gene(s)*	*Registries (coordinating country)*
LGMD D1	DNAJB6	*The LGMD-1D DNAJB6 Foundation and International Registry (USA)*
LGMD R1	CAPN3	*LGMD2A/R1 Global Patient Registry (USA)*
LGMD R2	DYSF	*The Dysferlin Registry (USA)*
LGMD R3	SGCA	LGMD2D Foundation Registry (USA)
LGMD R5	SGCC	*Kurt+Peter Foundation Registry (USA)*
LGMD R9	FKRP	*Global FKRP Registry (USA)*
LGMD R12	ANO5	*LGMD2L Foundation Registry (USA)*
LGMD D1, LGMD D5, LGMD R7, LGMD R8, LGMD R9, LGMD R10, LGMD R11, LGMD R13, LGMD R14, LGMD R15, LGMD R16, LGMD R17, LGMD R18, LGMD R19, LGMD R20, LGMD R22, LGMD R23, LGMD R24	COL6A1, COL6A2, COL6A3, CRPPA (ISPD), DAG1, DNAJB6, FKRP, FKTN, GMPPB, LAMA2, PLEC (PLEC1), POMGNT1, POMGNT2, POMT1, POMT2, TCAP, TRAPPC11, TRIM32, TTN	*The Congenital Muscle Disease International Registry (CMDIR) (USA)*

The *Global FKRP Registry* – *LGMD2i Research Fund*, *CureLGMD2I*

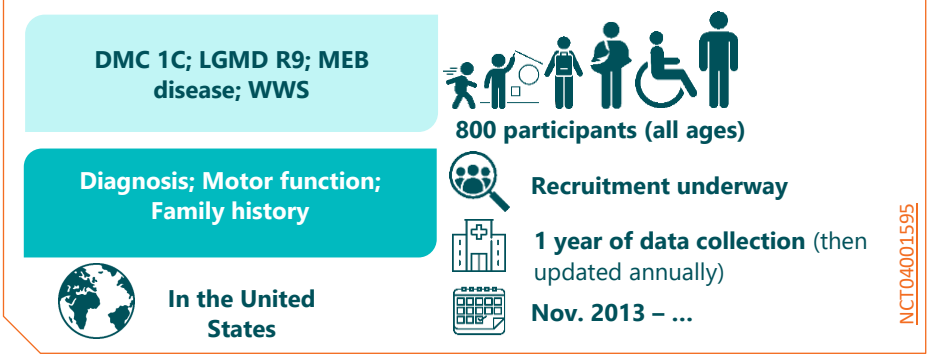
▪ Initiated in 2011 and sponsored by Newcastle University (United Kingdom), the Global FKRP Registry is a medical data repository that collects data online from patients with diseases caused by abnormalities (mutations) in the *FKRP* gene, specifically **LGMD R9** FKRP-related, but also congenital muscular dystrophy type 1C (DMC1C), muscle eye brain (MEB) disease and Walker-Warburg syndrome (WWS).

A database to learn more about FKRP-related diseases

▪ The objective is to better understand the natural history and the incidence of these diseases, and to help identify individuals who are affected by these diseases and who are likely to join a particular clinical study or trial.
▪ The collection of certain data is initiated by the patient, on a secure web portal: **WEB** www.fkrp-registry.org.
▪ The data collected relate to the age of the patient when the disease started, the first symptoms, family history, motor function and muscle strength, cardiac and respiratory function, treatments received, quality of life, and pain experienced.
▪ Whether these data are stored indefinitely or deleted on request, they need to be updated once a year.

WEB https://www.fkrp-registry.org

Global FKRP Registry – LGMD2i Research Fund, CureLGMD2I

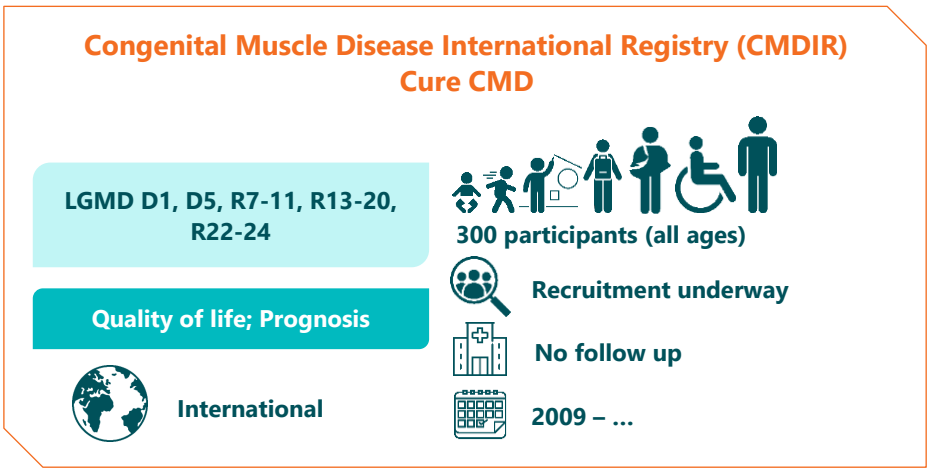


The Congenital Muscle Disease International Registry (CMDIR)

▪ The CMDIR international database, sponsored by the American patient association, *Cure CMD*, aims to help in identifying patients with **LGMD D1, D5, R7-11, R13-20, R22-24** for clinical trials, to improve diagnosis and therapeutic management, and to collect global LGMD data.

WEB https://www.cmdir.org

Congenital Muscle Disease International Registry (CMDIR) Cure CMD

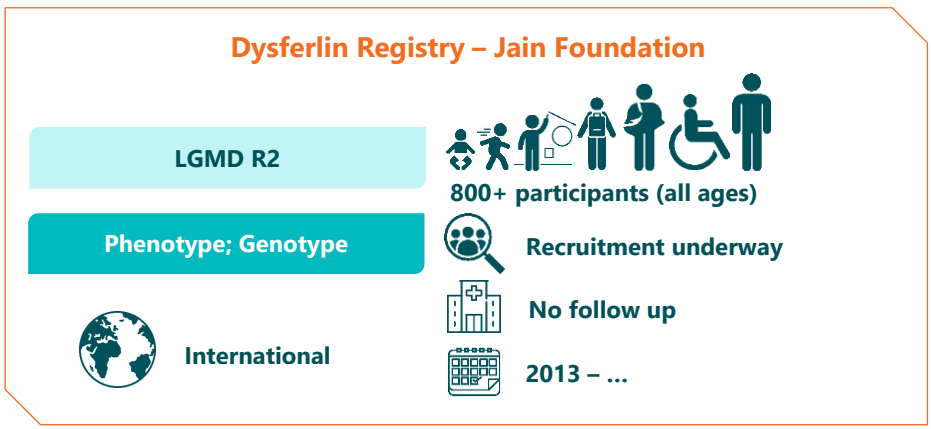


The *Dysferlin Registry* – *Jain Foundation*

▪ The Jain Foundation international dysferlinopathy database collects data from patients with **LGMD R2**. The registry aims to better understand and learn more about the frequency of genetic abnormalities related to the *DYSF* gene, and to identify patients for clinical research.

WEB https://www.jain-foundation.org/dysferlin-registry/inquire

Dysferlin Registry – Jain Foundation



The UMD-DYSF database

The Jain Foundation also finances the **UMD-DYSF** database, which is also supported by AFM-Téléthon. The goal of this database is to provide up-to-date information regarding *DYSF* gene mutations. UMD-DYSF is currently a database of genetic variants, and in the longer term should become a patient registry.

WEB http://www.umd.be/DYSF/

UMD-DYSF database

International

Created in 2002

Recruitment underway

The Global Registry for COL6-related dystrophies

▪ The Global Registry for collagen VI-related muscular dystrophies, sponsored by Newcastle University (United Kingdom), is a prospective online collection of medical data from patients and clinicians over a 5-year period. Its aim is to identify and characterise the population with Ullrich Congenital Muscular Dystrophy (UCMD), Bethlem myopathy dominant (**LGMD D5**), Bethlem myopathy recessive (**LGMD R22**) or intermediate forms, and to describe the natural history of these diseases.

WEB www.collagen6.org

The Global Registry for COL6-related dystrophies
Newcastle University

CMD; LGMD D5; LGMD R22

Diagnosis; Phenotype; Genotype; Motor function; Quality of life; Family history

International

192 participants (all ages)

Recruitment underway

1 year of data collection

Oct. 2018 – ...

NCT04020159

Clinical studies in several LGMD conditions

Survey: quality of care

▪ A survey has been launched by TREAT-NMD to better understand current clinical practices in the diagnosis and therapeutic management of patients with LGMD. Patients may respond to the survey, as may health professionals and caregivers, in order to report their experiences. The objective of the study is to help to define and implement standards of care in LGMD. The survey is open and available in 12 languages.

WEB https://redcap.nchri.org/surveys/?s=RWEDRLHNWJ

*The **TREAT-NMD** Alliance is an international network dedicated to neuromuscular diseases, bringing together specialised scientists and clinicians, and patient associations. Originally supported by the European Commission as European Network of Excellence, the TREAT-NMD Alliance continues to promote the conditions for the transformation of the most promising research into clinical applications. It also aims to provide international recognition for current Best Practices for the care of patients with neuromuscular disease.*

WEB www.treat-nmd.eu/

Cross-sectional clinical study (survey) – TREAT-NMD

- All LGMD conditions
- Diagnosis; Quality of care
- In France and abroad
- Patients, health professionals and caregivers
- Recruitment underway
- Questionnaire
- 2021 – ...

WEB LGMD Standards of Care - Patient Survey

Epidemiology: chronic pain

▪ In January 2020, the Danish National Rehabilitation Center for Neuromuscular Diseases (Aarhus University) brought together 121 adult patients with LGMD, aged 19 to 86 years, to characterise the pain experienced and its consequences. The results of this cross-sectional study, the first one of this size, were published at the end of 2021.

Frequent chronic pain

▪ The results show that 65.7% of participants have chronic pain, i.e. pain lasting more than three months. This pain is daily or constant in almost 45% of cases, and is most commonly experienced in the lower back (57% of cases) or the neck (48% of cases).

An impact on psychological health

▪ According to the participants, persistent pain interferes moderately with their daily activities. However, this pain is associated with much more frequently experienced anxiety and depression, and with a lower quality of life, compared to the general population.

Talking about it, for optimal care

▪ Patients with LGMD who present pain are encouraged to talk about it with the healthcare professionals following them up. These healthcare professionals will be able to offer them targeted care (analgesic medicines, physiotherapy, physical exercise, etc.) intended to improve their physical and psychological comfort.

Stokholm, R. N. et al. Disabil Rehabil. 2021.

Cross-sectional clinical study (epidemiology) – Aarhus University

- Dominant and recessive LGMD
- Prevalence and characteristics of pain
- In Denmark
- 121 participants (19-86 years)
- Results published
- No follow up (questionnaire)
- Jan. 2020

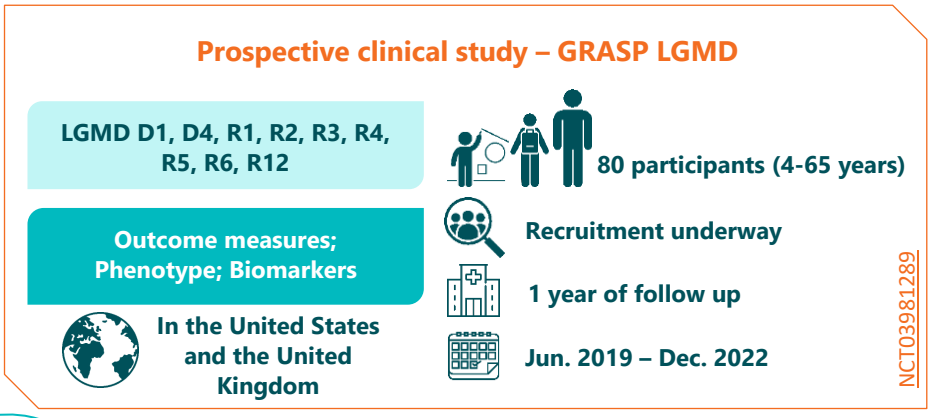
Clinical outcomes and biomarkers to assess LGMD

▪ A natural history study relating to LGMD D1 (*DNAJB6*), D4 (*CAPN3*), R1 (*CAPN3*), R2 (*DYSF*), R3 (*SGCA*), R4 (*SGCB*), R5 (*SGCG*), R6 (*SGCD*) and R12 (*ANO5*) is underway in the United States and the United Kingdom. Its objective is to identify the key clinical manifestations and biomarkers in measuring disease progression, especially in the context of clinical trials.

▪ Coordinated by the GRASP-LGMD (Genetic Resolution and Assessments Solving Phenotypes in LGMD) consortium, the study aims to provide additional data on the course of the disease by following up 80 patients over a period of one year. The results will be used to analyse future clinical trials in LGMD and will help to speed up therapeutic development.

WEB www.grasp-lgmd.org

*A **biomarker** or biological marker is a measurable characteristic that indicates a normal or pathological biological process. The identification of biological markers for a disease is very important in monitoring the progression of the disease and the efficacy of new treatments, whether these markers are physiological (change in blood pressure, heart rate, etc.), molecular (change in the expression of a protein, etc.) or imaging.*



Did you know?

An international consortium for LGMD

The **GRASP-LGMD** (Genetic Resolution and Assessments Solving Phenotypes in LGMD) **consortium**, brings together an international team of neuromuscular disease specialists, scientists, rehabilitation therapists, geneticists, information specialists and patient representatives, to speed up the transfer of research into therapies.

▪ Its objectives are to contribute to and/or improve:
- the identification of therapeutic avenues and their development;
- diagnosis accuracy;
- the validation of follow-up criteria;
- the validation of patient-reported parameters;
- the discovery of biomarkers and their validation.

The network consists of two participating sites in Europe (in the United Kingdom and the Netherlands), but the majority of its activity is located in the United States.

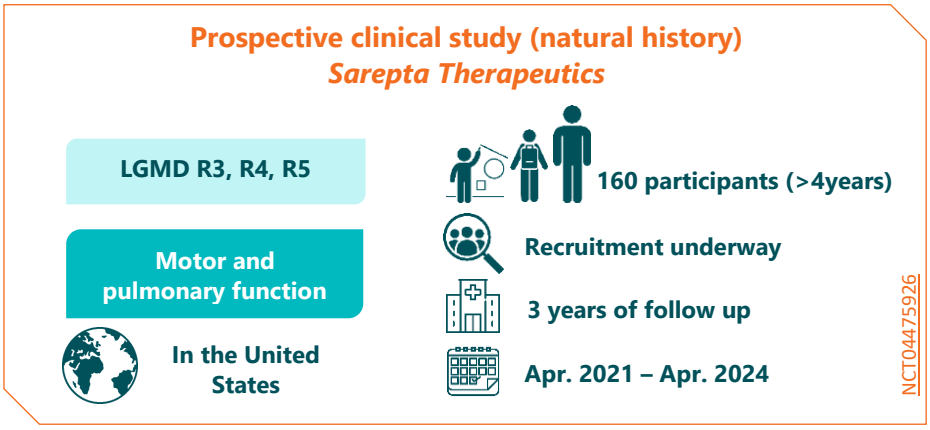
WEB www.grasp-lgmd.org

WEB www.grasp-lgmd.org/active-studies/

Motor and pulmonary function

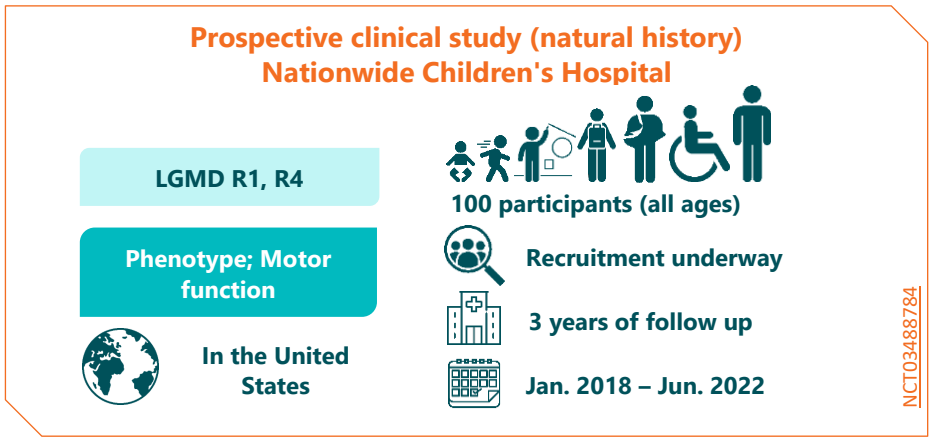
▪ A new natural history study of the sarcoglycanopathies (LGMD R3, R4 and R5), sponsored by Sarepta Therapeutics, began in April 2021 in the United States. It will monitor the progression of routine clinical parameters (NSAD motor function score, time to rise from the floor, to ascend four steps, of 10-meter walk/run, of 100-meter walk/run, PUL upper limb functional scale, measurement of vital capacity, etc.) over three years, among ambulatory and non-ambulatory patients, with LGMD R3, R4 or R5. The first results may be available as early as August 2023.

*The so-called **natural history of a disease**, as doctors refer to it, is the description of different manifestations of that disease and their progression over time without any treatment (medicines, physiotherapy, surgery, etc.).*



Clinical progression and functional impact

▪ An American single-centre natural history study sponsored by the Nationwide Children's Hospital is committed to characterising the clinical progression and functional impact of the disease among patients with LGMD R1 and R4. The study will monitor, over a five-year period, the change in muscle strength and time of 100-meter walk/run, with visits every six months during the first three years. The study, which was initially scheduled to end in 2022, will ultimately continue until 2025.



Development of biomarkers targeting sarcolemma

▪ An American study is currently underway in the United States, searching for biomarkers in the muscular dystrophies presenting fragility of the muscle cell membrane (LGMD R2 dysferlin-related, Miyoshi distal myopathy, sarcoglycanopathy (LGMD R3 to R6), LGMD R9 *FKRP*-related, LGMD R12 anoctamin-related and Becker muscular dystrophy).

Prospective clinical study (development of biomarkers) National Institutes of Health (NIH)

LGMD R2, R3, R4, R5, R6, R9, R12

Biomarkers

In the United States

11 participants (>18 years)

Recruitment completed

1 year of follow up

Nov. 2014 – Dec. 2022

NCT01851447

Fast troponin in recessive LGMD: a useful biomarker

▪ A Danish study sponsored by Edgewise Therapeutics has measured the levels of fast and slow troponin following exercise using a cycle ergometer, among patients with recessive LGMD, Becker muscular dystrophy and McArdle disease. The study's objective is to observe changes in the fast troponin biomarker in response to exercise.
The end of the study is scheduled for April 2022

Prospective clinical study (natural history) Edgewise Therapeutics

LGMD R1-R27

Biomarkers (troponin)

In Denmark

50 participants (18-50 years)

Recruitment by invitation

Measurements over 24 hours

Jun. 2020 – Apr. 2022

NCT04349566

Motor skill progression

▪ In April 2021, the French natural history study, **EIDY**, was launched by the Motion Analysis Laboratory, Raymond Poincaré Hospital, APHP (Paris Public University Hospital Trust). This prospective study is performing a 2-year follow up of motor parameters in 40 patients with LGMD who were initially able to walk at least 6 minutes consecutively without help, and 40 healthy volunteers.

▪ Besides the measurement of muscle strength, an optoelectronic device is also being used to measure joint range of motion during walking (gait), arm (upper limb) spacial exploration and the lifting of a glass of water to the mouth (drinking task). The participants will complete questionnaires about their daily activities, quality of life, fatigue and number of falls, at each six-monthly visit.

Prospective clinical study (natural history)
APHP (Paris Public University Hospital Trust)

LGMD D1-D5, R1-R27

Motor function; Quality of life

In France

80 participants (18-70 years)

Recruitment underway

2 years of follow up

Apr. 2021 – Jan. 2025

NCT04772027

Diagnosis and progression of the disease

▪ A Chinese study sponsored by the Huashan Hospital in Shanghai was launched in July 2021 in order to collect clinical, genetic, physiological and histological data among Chinese patients with LGMD. Once the patients have been accurately diagnosed, these patients will be followed up for a period of three years. The investigators will measure motor skill changes and structural muscle changes in patients at the end of the first year, then at the end of the third year, after their inclusion in the study. The first results are expected in July 2024.

Prospective clinical study (natural history) – *Huashan Hospital*

LGMD D1-D5, R1-R27

Diagnosis; Phenotype; Genotype; Physiology; Histology

In China

350 participants (>10 years)

Recruitment by invitation

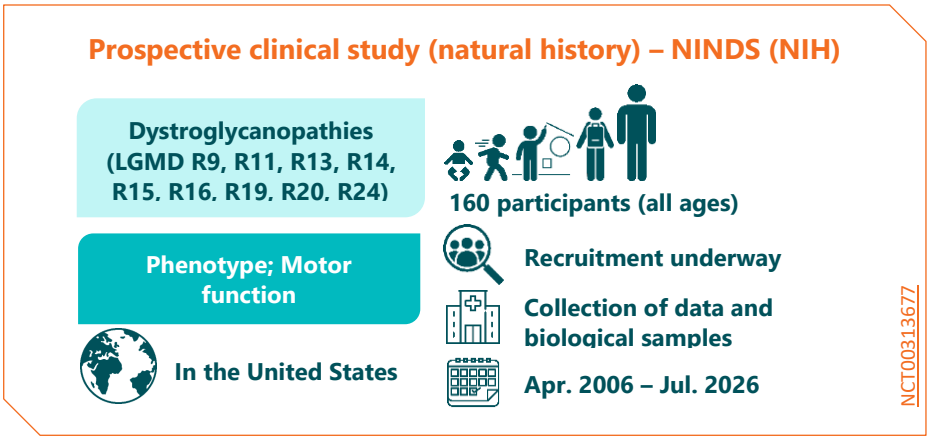
3 years of follow up

Jul. 2021 – Dec. 2024

NCT04989751

Clinical characteristics of the dystroglycanopathies

▪ Since 2006, an American study sponsored by the National Institute of Neurological Disorders and Stroke (NINDS), a branch of the NIH, has been collecting clinical data relating to the dystroglycanopathies, including LGMD R9, R11, R13, R14, R15, R16, R19, R20 and R24. The study investigators are particularly focused on the early signs and symptoms of these diseases, motor and pulmonary function, and quality of life. The collected data will be used to define the standards for the preparation of future clinical trials. Any individual with a confirmed mutation in one of the known dystroglycanopathy genes (*CRPPA* (*ISPD*), *DAG1*, *FKRP*, *FKTN*, *GMPPB*, *POMGNT1*, *POMGNT2*, *POMT1* and *POMT2* for the LGMD conditions) may participate in this study. Currently, five publications (2015-2021) have resulted from the use of these data.



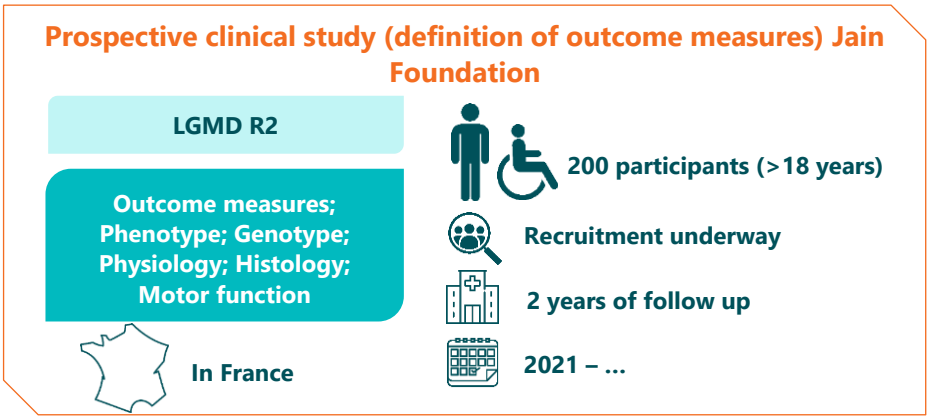
LGMD R2 (*DYSF*– dysferlinopathy)

Outcome measures to validate clinical measurements

▪ The objective of the international study, COS 2 (Clinical Outcome Study for Dysferlinopathy), sponsored by the Jain Foundation, is to identify the most relevant **outcome measures** (biomarkers, measurement tools, tests, etc.) with a view to conducting clinical trials in the dysferlinopathies (Miyoshi distal myopathy and limb-girdle muscular dystrophy type R2).

▪ The recruitment of participants started in 2021 and ended in June 2022. Once every six months, a series of medical examinations is performed to monitor disease progression. The trial is being conducted in Chile, South Korea Denmark, Spain, the United States, France, Italy, Japan and the United Kingdom. In France, an investigating site is open at the Institute of Myology (Paris).

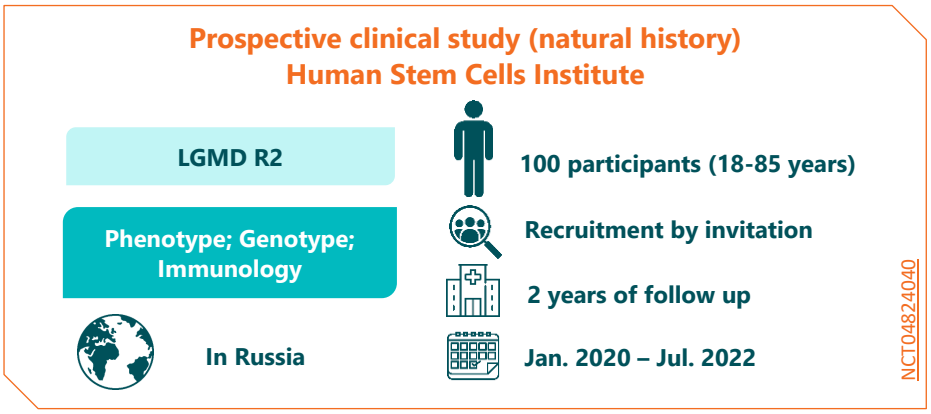
WEB https://www.jain-foundation.org/patients-clinicians/how-to-take-action/clinical-trials-studies-and-surveys/cos2



WEB The COS2 clinical outcome study

Russian patients with LGMD being studied

▪ The Russian biotechnology company, Human Stem Cells Institute, is sponsoring a natural history study on patients from various regions of Russia, with LGMD R2. This study will, over a period of two years, follow up 100 patients from the Russian dysferlinopathy registry (DYSF Russian registry), in order to assess the special clinical features of the disease in the Russian population. The study is looking at many variables: phenotype, genotype, immunological profile, motor and cardiac function, etc.



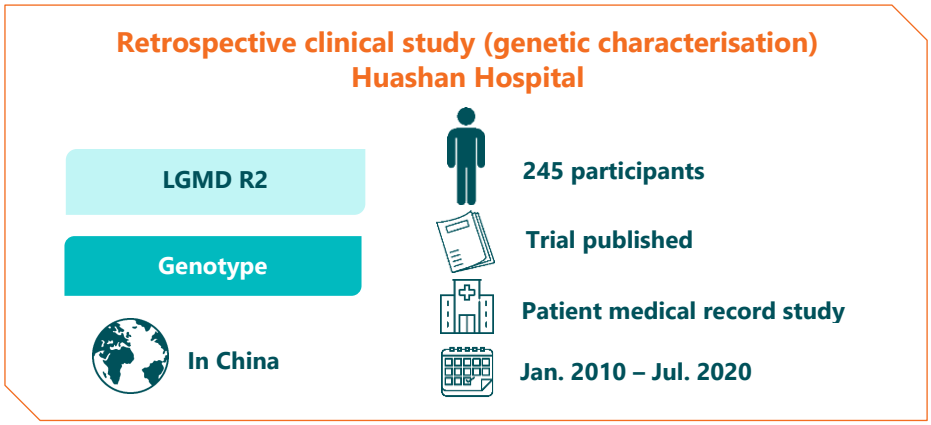
Which *DYSF* gene variants are found in a cohort of Chinese patients?

▪ A retrospective study by the Huashan Hospital in China has analysed the genetic profile of 245 patients with dysferlinopathy. The researchers have identified 222 genetic variants of *DYSF*, 40 of which are new.

▪ The authors also studied all of the 2199 variants of *DYSF* collected by LOVD (1020 variants) and ClinVar (1179 variants), two international databases of genetic variants, and they discovered two mutation hotspots in this gene: "c.2997G>T" in the international patient population, and "c.1375dup" among the Chinese patients. The pathogenic mutations are spread along the entire length of the gene.

In the Chinese population, the study results indicate that patient sex, and not genotype, influences the age at which the dysferlinopathy appears, manifesting at an earlier age in men.

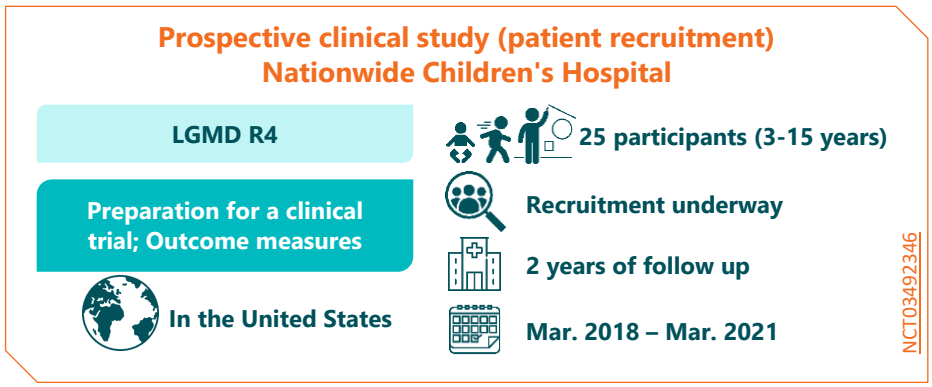
Zhong, H. et al. Hum Mutat. 2021.



LGMD R4 (*SGCB* – beta-sarcoglycanopathy)

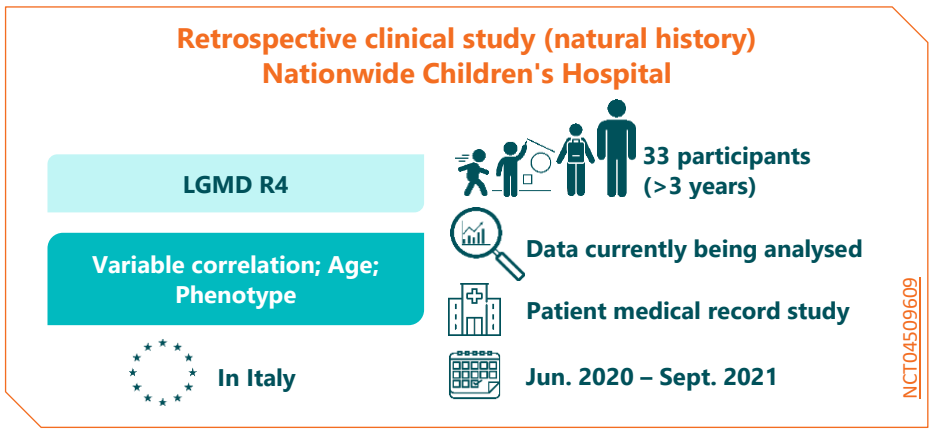
A study prior to a future gene therapy trial

▪ The Nationwide Children's Hospital (UK) and Myonexus Therapeutics (now Sarepta Therapeutics) are currently sponsoring an observational study prior to a future clinical trial. The goal of the study is to recruit subjects with LGMD R4 β-sarcoglycan-related who are potentially eligible for a gene therapy trial and to describe their disease progression over a two-year period. This pre-inclusion study is in progress and is still recruiting patients.



A correlation between clinical manifestations and age?

▪ The NeuroLGMD2E study is a retrospective study sponsored by the Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico in Milan (Italy). By collecting and analysing data from the medical records of 33 patients with LGMD R4, the study aims to determine whether a correlation exists between certain clinical observations (loss of ability to walk, first respiratory assistance, etc.) and patient age. The study ended in September 2021 and the results are pending.



LGMD R6 (*SGCD*– delta-sarcoglycanopathy)

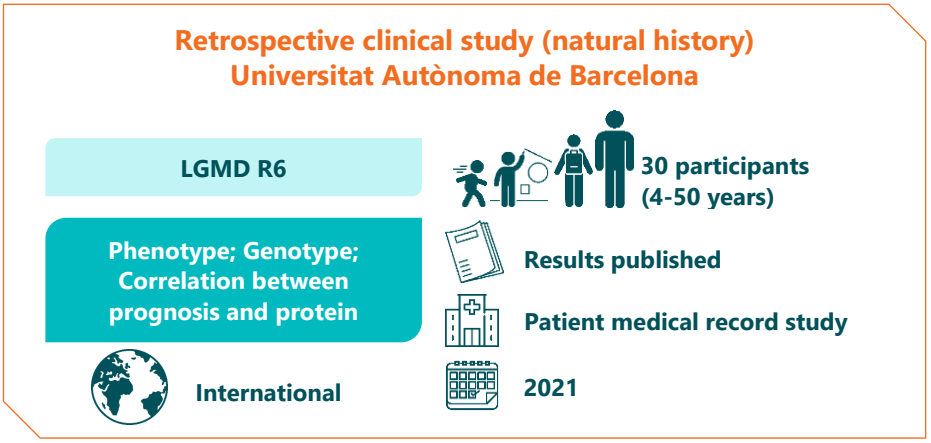
The largest cohort of patients with LGMD R6 under the microscope

▪ LGMD R6 is one of the sarcoglycanopathies; it is the least common of these conditions and is considered to be an "ultra-rare" disease. The results of a retrospective natural history study on LGMD R6, which was sponsored by the Universitat Autònoma de Barcelona, were published at the end of 2021. Its objective was to characterise the clinical and genetic spectrum of the disease and to determine whether its severity could be predicted using gene and/or protein expression data. The study, conducted by an international consortium of researchers and clinicians, has identified 30 patients with an extremely rare type of recessive autosomal limb-girdle muscular dystrophy (delta-sarcoglycan-related LGMD R6).

▪ The clinical and biological data for 23 of these patients were analysed, and showed that:

- high throughput sequencing techniques facilitate the diagnosis of LGMD in general;
- the progression of LGMD R6 seems faster than that observed for the other forms of recessive autosomal LGMD;
- it is possible to characterise the disease's progression profile based on the quantity of residual proteins visible in the muscle tissues.

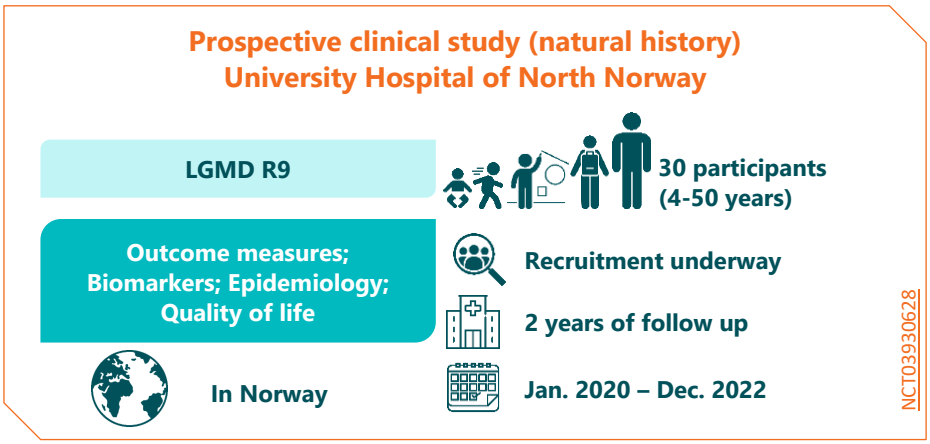
Alonso-Pérez, J. et al. Brain. 2021.



LGMD R9 (*FKRP*– dystroglycanopathy)

Learning more about the disease among Norwegian patients

▪ A natural history study sponsored by the University Hospital of North Norway is underway in Norway. Its goal is to identify and characterise the population with LGMD R9 FKRP-related and to follow up the disease's progression over a two-year period.



LGMD R9 outcome measures for future trials

▪ In anticipation of future gene therapy trials, Généthon has initiated an international natural history study of LGMD R9 FKRP-related. The objective of the **GNT-015-FKRP** study, which is being conducted in France, Denmark and the United Kingdom, is to better understand the pathophysiology and to characterise the progression of LGMD R9, using standardised evaluations that are tailored to the follow up of the progression of the disease. Another objective is to determine the best outcome measures in order to prepare future therapeutic trials.

Prospective clinical study (natural history) – Généthon

LGMD R9

Outcome measures; Physiology; Motor function

In France

52 participants (16-99 years)

Recruitment underway

2 years of follow up

Feb. 2020 – Dec. 2023

NCT03842878

New biomarkers

- In collaboration with the GRASP Consortium, ML Bio Solutions launched, at the end of 2019, a natural history study (MLB-01-001) prior to the BBP-418 trial (NCT04800874, underway) among patients with LGMD R9. This is currently being conducted at 11 centres in the United States and one centre in Denmark.
- The objective is to gather information regarding the natural progression of LGMD R9, using standardised disease progression monitoring parameters, to identify the best possible outcome measures, and to validate potential biomarkers for future clinical trials, including dystroglycan levels in the muscle.

Prospective clinical study (natural history) – ML Bio Solutions

LGMD R9

Outcome measures; Biomarkers

In the United States and Denmark

101 participants (10-65 years)

Recruitment completed

12 months of follow up

Dec. 2019 – Sept. 2022

NCT04202627

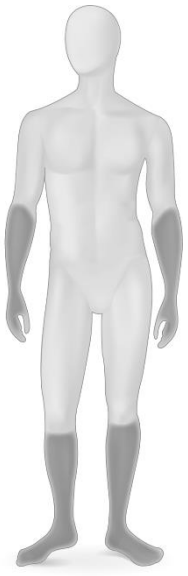
Medical imaging: involvement of the trunk muscles

- At the beginning of the year, researchers at the Copenhagen Neuromuscular Centre at Rigshospitalet, Denmark, published the results of a cross-sectional study involving 17 patients with LGMD R9, conducted between June 2018 and October 2020. The objective of the study was to describe the structural differences in the trunk muscles between patients with the disease and healthy subjects.

Magnetic resonance imaging (MRI) shows, in these patients, significant replacement of certain portions of the trunk muscles with fatty tissue. Trunk flexion and extension strength are lower compared to that of the subjects who do not have the disease. The data indicate that the axial muscles are some of the muscles that are severely impacted in LGMD R9.

Revsbech, K. L. et al. Muscle Nerve. 2022.

Distal muscle involvement



The distal muscles are the muscles that are further from the spinal column. They are located at the limb extremities: the muscles of the hands and the forearms for the upper limbs, the muscles of the feet and the lower legs for the lower limbs.

➤➤ The musculoskeletal system, Knowledge & Understanding reference documents, AFM-Téléthon.

Cross-sectional clinical study – *Rigshospitalet*

LGMD R12

Medical imaging (MRI); Motor function

In Denmark

14 participants (>18 years)

Results published

Single assessment (no follow

Jun. 2018 – Oct. 2020

What about pain?

▪ Based on the data collected by the Global FKRP Registry, an English team from the Newcastle-upon-Tyne Hospitals NHS Trust analysed the answers to a pain questionnaire involving 502 patients with LGMD R9 (formerly LGMD 2I), taken from the FKRP Registry. The chronic pain felt by patients with LGMD R9 was experienced approximately twice as frequently as in the general population. Also, a total of 87% of these patients said they experienced pain, and this pain was severe in a quarter of cases. A little over two thirds of participants (69%) reported that the intensity of their non-neuropathic pain varied over time.

Richardson, M. et al. J Clin Med. 2021.

Cross-sectional clinical study (epidemiology)
Newcastle-upon-Tyne Hospitals NHS Trust

LGMD R9

Prevalence and characteristics of pain

International

502 participants (0-9 years to 70-79 years; taken from the Global

Results published

No follow up (questionnaire)

June 2021

Magnetic resonance imaging *or* ***MRI*** *is a medical imaging technique that provides images in sections or volumetrically of an organ or an area of the human body. During the examination, the person is lying down, still, on a mobile bed that slides into a cylindrical device made up of very powerful magnets. This examination is not painful. However, the sensation of being enclosed, isolated, the noise made by the machine and the duration of the examination can be a bit overwhelming.*

➤➤ Diagnosis of neuromuscular diseases, Knowledge & Understanding reference

LGMD R12 (*ANO5* – anoctaminopathy)

Characterising the nature of the involvement using imaging

▪ A retrospective study analysing the medical records of 200 patients with LGMD R12 from around the world was launched in 2021 by the Rigshospitalet in Denmark. Its objective is to characterise the type of involvement (phenotype) among these patients, using a magnetic resonance imaging (MRI) examination, and investigating the symmetry of the muscle involvement, the difference in severity between men and women, and any correlation that exists between the involvement and the genetic abnormality responsible (phenotype-genotype correlation). The data will be collected via healthcare centres from around the world, who will share their electronic data forms, using the MyoShare platform, with the Copenhagen Neuromuscular Center.

Retrospective clinical study (medical imaging) – Rigshospitalet

LGMD R12

MRI phenotype

In Denmark

200 participants (all ages)

Recruitment underway

Patient medical record study

Apr. 2021 – Feb. 2024

NCT05102799

Understanding the progression of the muscle involvement

▪ The Rigshospitalet has also been financing, since 2018, another natural history study of patients with LGMD R12, the objective of which is to describe disease progression and to identify reliable clinical outcome measures. The investigators are following up 17 patients over a period of 3 years, and are observing, inter alia, changes in patients' state of fatigue, quality of life and motor function. The study is no longer recruiting, and should be completed in March 2023.

A first analysis of patient muscle characteristics

▪ Using the data from this cohort, John Vissing's team already published, in 2021, the results of a study that sought to measure the muscle fat fraction and muscle strength among the 17 patients with LGMD R12. From January to December 2018, the muscles of these patients, aged 31 to 73 years, were examined by magnetic resonance imaging (MRI) and their muscle strength was measured using a dynamometer. The analyses were then compared to those relating to 11 subjects who did not have the disease. The results for the patients with the disease show in particular:

- Very significant replacement of muscle tissue by fatty matter in the muscles of the thighs and calves.
- Muscle involvement that was less significant in women than in men.
- General symmetry of the muscle involvement.
- Relatively insignificant involvement of the muscles of the back.
- Hypertrophy of the muscles of the inner part of the thigh.

Khawajazada, T. et al. Eur J Neurol. 2021.

Prospective clinical study (natural history) – Rigshospitalet

LGMD R12

Outcome measures; Motor function; Quality of life

In Denmark

17 participants (>18 years)

Recruitment completed

3 years of follow up

Mar. 2018 – Mar. 2023

NCT05206617

LGMD R23 (*LAMA2*)

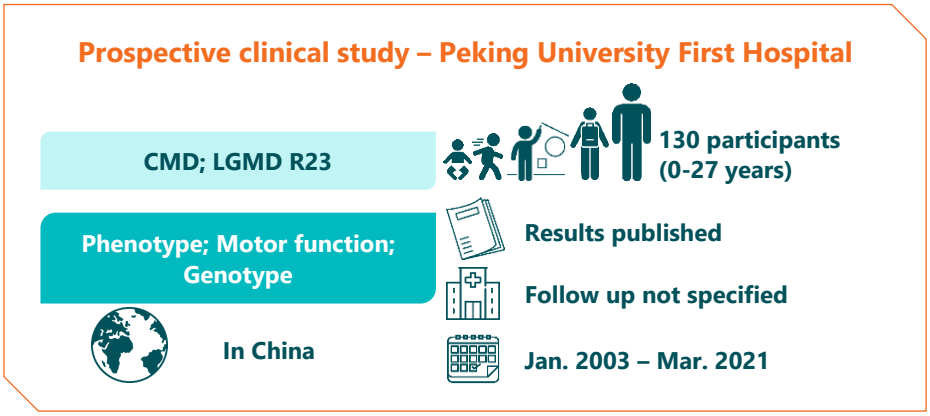
Clinical and genetic data in the Chinese population

▪ In July 2021, the results were published for a Chinese natural history study conducted by researchers at the Peking University First Hospital, on LAMA2-related muscular dystrophies. The study related to 130 individuals: 116 with a congenital form, and 14 with a form related to the limb-girdle myopathies (LGMD R23). The clinical characteristics (age of first symptom onset, ability to walk, respiratory and cardiac signs, etc.) and genetic characteristics (abnormalities) of these patients were collected and analysed.

▪ The results showed:

- age of onset of the first manifestations (decrease in muscle tone, etc.) of between 13 months and 13 years for the limb-girdle form;
- the presence of epileptic seizures in more than one third of the children with the limb-girdle form.

Tan, D. et al. Orphanet J Rare Dis. 2021.



Preclinical studies: therapeutic avenues

What is a preclinical study?

▪ Preclinical studies are the first stage of investigating/demonstrating the safety and/or efficacy of a candidate medicine, treatment or procedure, using an animal model (in vivo) or cultured cells (in vitro). If the results are successful, clinical trials on humans can be envisaged, and have to be constructed for the product in question.

In vitro *techniques (in Latin: "in the glass") are, in contrast to in vivo techniques, performed in a laboratory dish (that used to be made of glass): cell model.*

In vivo *techniques (in Latin: "in the living") are performed on a living organism: animal model.*

LGMD R1 (*CAPN3* – calpainopathy)

Gene therapy: AAVrh74.tMCK.hCAPN3: proof of concept

▪ A team from the Nationwide Children's Hospital (Columbus, United States), headed by Jerry Mendell, a specialist in gene therapy for different neuromuscular diseases, has developed a gene therapy product in LGMD R1 calpain-related (formerly LGMD2A), called "AAVrh74.tMCK.hCAPN3". This product has been tested at two different doses, one low and one high, in two LGMD R1 mouse model groups, one involving mice aged 2 months and the other involving mice aged 5 months, who do not yet have symptoms.

▪ At the end of a five-month follow up period after injection, the results demonstrated the efficacy of the product at both assessed doses, and on both groups of mice: increase in the size and contractility of the muscles, improvement in muscle function and endurance, and no cardiomyopathy.

Sahenk, Z. et al. Mol Ther Methods Clin Dev. 2021.

Pharmacotherapy: GSK-3β inhibition using tideglusib in vitro

- **Tideglusib** (VP0.7) is a medicine that is used in Alzheimer's, and that is well tolerated by humans. It is being tested in a clinical trial for the congenital and infantile forms of Steinert's disease.
- In an article published in July 2021, a Spanish team reported a significant reduction in the expression of the proteins involved in the Akt/mTOR and Wnt signalling pathways in the muscles of patients with **LGMD R1**. These signalling pathways stimulate protein synthesis, growth and regeneration of the muscle fibres, and inhibit protein degradation.
- The team showed that this reduction is related to an over-expression of the GSK-3β enzyme, which is involved in cell proliferation, the metabolism, apoptosis, etc. Inhibiting the activity of this enzyme using tideglusib restores the activity of the Wnt and mTOR metabolic pathways in vitro, in muscle cells of patients with LGMD R1 in cell culture.

Rico, A. et al. Int J Mol Sci. 2021.

***Cell signalling pathways** transmit messages within a cell in order to modulate its activity (growth, division, differentiation, death, etc.). A message can originate from other cells in the body or from the external environment. Its arrival at a cell receptor triggers a cascade of reactions that will modify the cell's behaviour.*

***Apoptosis** is physiological cell death that occurs in an ordered manner in several stages, at the end of which the entire cell and its contents are eliminated, without the neighbouring cells being damaged. Apoptosis is in constant equilibrium with cell multiplication, to ensure cell renewal.*

LGMD R2 (*DYSF*– dysferlinopathy)

Gene therapy: hASM-AAV and acid sphingomyelinase

- LGMD R2 is caused by a mutation of the *DYSF* gene that is responsible for a reduction in, or an absence of the dysferlin protein in the muscle cell membrane (sarcolemma). Dysferlin is a large protein that plays a role in cell regeneration, a cell repair process that involves an enzyme, lysosomal acid sphingomyelinase (ASM), that is secreted when the cell membrane is damaged. If there is no dysferlin, this causes a reduction in the secretion of ASM, and cell repair is therefore no longer carried out.

A size problem

- To get around the problem resulting from the large size of the *DYSF* gene, which makes it difficult to package it in the most appropriate gene therapy vectors for the targeting of the muscles, researchers from the Children's National Hospital in the United States have developed an alternative gene therapy that targets a reduction in the secretion of ASM. This approach delivers the human recombinant enzyme to the liver via the hASM-AAV vector.

hASM-AAV acts on the muscle

- A single dose of hASM-AAV injected in model mice restores muscle fibre repair capabilities. The treatment has proven to be harmless, reduces muscle degeneration, increases the size of the muscle fibres, and helps to restore muscle strength.

Using a liver-specific vector, this study provides first proof of concept of gene therapy in LGMD R2.

Bittel, D. C. et al. J Clin Invest. 2022.

Pharmacotherapy: ataluren for stop codon readthrough

Readthrough *is the reading of the genetic message beyond a message termination signal (stop codon), up to the following termination message. Certain genetic abnormalities cause the appearance of a premature stop codon, and therefore a shortening of the protein. Premature stop codon readthrough aims to restore the production of the entire protein.*

- Ataluren is a drug molecule that makes it possible to read through a premature stop codon. This substance has already shown its efficacy in Duchenne muscular dystrophy in restoring the production of functional dystrophin in patients with a stop codon-type mutation.

A Korean team from the Pusan National University tested oral Ataluren on model mice presenting one of the most common nonsense mutations among Korean patients with LGMD R2.

- After two weeks of treatment with Aataluren, the mice showed restored expression of dysferlin, which was previously absent from the skeletal muscles, and significantly improved physical performance. No improvement was observed in the mice without the nonsense mutation, showing the specificity of the treatment with respect to this type of mutation.
- These results suggest that ataluren could be efficacious in patients with LGMD R2 with a nonsense mutation.

Seo, K. et al. Mol Ther Methods Clin Dev. 2021.

Pharmacotherapy: ezetimibe prevents muscle degradation

- In a previous study, a team from the University of British Columbia showed that cholesterol could have harmful effects on the muscles of patients with dysferlinopathy. Indeed, the results of their study revealed that the muscles of mice deficient in dysferlin and apolipoprotein E also showed an accumulation of plasma lipoproteins, correlating with accelerated replacement of muscle fibres with adipose tissue. These mice presented severe impairment in terms of their ability to walk.
- In their latest study, the investigators evaluated ezetimibe, a cholesterol intestinal-absorption inhibitor, on two-month-old mice modelling DMD or LGMD R2 (*DYSF*), who were also mutated for the *APOE* (apolipoprotein E) gene.
- Treatment with ezetimibe completely prevents loss of mobility in the model mice. The muscles in the posterior limbs became significantly larger.
- The negative impact of cholesterol also underwent more in-depth investigation: multiplying the amount of cholesterol in their food by ten caused their premature death and severe worsening of the muscle damage.

Sellers, S. L. et al. J Lipid Res. *2018; White, Z. et al. J Cachexia Sarcopenia Muscle. 2022.*

Pharmacotherapy: 4-PBA restores membrane repair

- 4-Phenylbutyric acid (4-PBA), also known by the name of Buphenyl or Ravicti, is a medicine that has been approved and used for a long time in the treatment of patients with urea cycle disorders. 4-PBA is described as being a chemical chaperone, i.e. a molecule that improves the folding and/or the stability of proteins.

Among patients with LGMD R2 (*DYSF*), 30% to 40% present a missense mutation that causes a loss of function of the dysferlin protein. The latter, due to a folding defect, is degraded in the cell by the proteasome, and membrane repair is no longer correctly performed.

Proteasome *is a complex enzyme responsible for the degrading misfolded, denatured or obsolete proteins for the cell. The proteins to be degraded are marked with a protein called ubiquitin. A chain of at least four ubiquitins is needed for the proteasome to recognise the protein to be degraded.*

- A team from the Massachusetts Institute of Technology (MIT) in the United States has tested, in vitro, the impact of 4-PBA on the presence of dysferlin in the cell membrane. Based on a sample of 327 patients from the Jain Foundation registry, researchers have identified 64 mutations that had a high probability of causing dysferlin folding defects and of resulting in dysferlin degradation. They showed, in vitro, that 4-PBA partially restores dysferlin in the muscle cell membrane for 25 of the 64 mutations.

▪ Tests in mice presenting one of these mutations show that the oral administration of 4-PBA over a period of 2 days helps to restore muscle fibre membrane repair. As a result, 4-PBA could be a possible therapy for a subset of patients with dysferlinopathy.
Tominaga, K. et al. iScience. 2022.

LGMD R3 (*SGCA* – alpha-dystroglycanopathy)

Pharmacotherapy: Givinostat and Bortezomib: a winning pair

▪ The work of the team headed by Isabelle Richard, a researcher at Généthon, an AFM-Téléthon laboratory, has helped to demonstrate that certain types of LGMD R3 are caused by the degradation of the alpha-sarcoglycan protein, that is functional but malformed, once recognised by the cell's quality control. Based on this work, the "Pharmacology of the muscular dystrophies" team headed by Xavier Nissan, at I-Stem, another AFM-Téléthon laboratory, has sought to identify a drug molecule that prevents this degradation. The team developed a cell model, then tested, using high throughput screening, the efficacy of nearly 1000 candidate chemical molecules.

▪ Once this screening had been performed, the team collaborated with a Belgian start-up company, Kantify, specialising in artificial intelligence, to identify the most promising molecules. This work, performed by Lucile Hoch, also a researcher at I-Stem, thus showed that Givinostat, a histone-deacetylase (HDAC) enzyme inhibitor (involved in gene expression regulation via epigenetic modifications), paired with bortezomib, which blocks proteasome (involved in the destruction of cell's proteins), was the most efficacious combination in preventing the degradation of the malformed R77C alpha-sarcoglycan protein. This combination would appear to help prevent the appearance of the disease.
Hoch, L. et al. Frontiers in Pharmacology. *2022.*

Pharmacotherapy: the repositioning of CFTR corrector C17

▪ The LGMD R3 conditions are caused, for the most part, by missense mutations that produce a misfolded defective alpha-sarcoglycan protein, a characteristic that leads to its detection and destruction by specific enzymes in the cell.

▪ An Italian team at the University of Padua (Italy) has hypothesised that drug molecules used to correct a protein folding defect in cases of cystic fibrosis, could also work in LGMD R3. The researchers have created a mouse model presenting the LGMD R3 dystrophic phenotype in the rear legs, and treated it with CFTR corrector C17 (or C17), a member of the group of CFTR correctors, CFTR being the protein implicated in cystic fibrosis. By binding to this protein, C17 gives it a correct three-dimensional shape, which prevents its degradation.

▪ The results of the study show that C17 does not present any toxicity in treated mice. Analyses at the end of five weeks of treatment indicate a salvaging of the complex associated with dystrophin (to which alpha-sarcoglycan belongs), a sign of alpha-sarcoglycan function restoration. The complex is stable and improves the integrity of the sarcolemma, despite the presence of a defective subunit. Muscle strength is restored in treated mice and returns to the levels found in healthy mice.
Scano, M. et al. Human Molecular Genetics. 2022.

***Epigenetic** factors are factors that regulate genetic information by acting on the organisation of the DNA molecule (more or less condensed, whether methylated or not, etc.), and not its contents (the nucleotide sequence is maintained). Gene expression epigenetic modifications can occur spontaneously, in response to the environment, and can be reversible.*

***Repositioning** is the investigation of the possible extension of a compound that has already been perfectly characterised, to other diseases. This often involves a medicine that has already been brought to the market.*
WEB *Martinat, C. et al. médecine/sciences. 2018.*

LGMD R7 (*TCAP*– telethoninopathy)

Gene therapy: rAAV.TCAP works in mice

▪ A team from the Nationwide Children's Hospital (Columbus, United States) has developed a gene therapy product for LGMD R7 *TCAP*-related (TCAP is also known as telethonin), called "rAAV.TCAP". Its expression improves the disease phenotype and prevents its progression in animals.

▪ The data collected following the injection of the product into mice modelling the disease, aged 6-7 weeks, show TCAP protein expression is restored, by up to 350% in the triceps. Immunofluorescence tests indicate that telethonin is, indeed, present in the titin-telethonin complex, embedded in the Z disc of the muscle fibre contractile structures, the sarcomeres. These results mean that the replacement of the defective *TCAP* gene via a recombinant adeno-associated virus (AAV) could be considered a viable therapeutic approach for LGMD R7.

Gushchina L. et al. MDA Conference. Mars 2022

Fundamental research

What is fundamental research?

▪ According to the definition by INSERM (French National Institute of Health and Medical Research), fundamental research is exploratory research that can be responsible for the emergence of new concepts. Its main objective is to generate knowledge and to understand natural phenomena. In the field of human health, it elucidates the body's mechanisms and functions, especially the pathological causes and processes that result in the manifestation of a disease.

Fundamental research is therefore, in general, the first stage in the process of developing new treatments. It precedes preclinical and clinical research, and helps to produce a knowledge base upon which preclinical and clinical research can be built.

WEB https://www.inserm.fr/nos-recherches/recherche-fondamentale

LGMD R2 (*DYSF*– dysferlinopathy)

The role of exon 40a of the *DYSF* gene in the disease

▪ Fourteen transcripts of the dysferlin gene are known to date, allowing the same number of isoforms of the dysferlin protein to be produced. These different forms of dysferlin, which are obtained by alternative splicing or the use of different promoters, do not all have the same biological importance, or the same distribution in the tissues.

▪ Marc Bartoli's team, supported by financing from AFM-Téléthon, as part of the Marseille strategic centre, has investigated the role of dysferlin transcript 11, which contains exon 40a. This exon codes for a part of dysferlin that is necessary for the cleaving of the protein by the calpains, an essential step in the activation of dysferlin in muscle fibre membrane repair. The results show that dysferlin transcript 11 is a key element in muscle cell membrane repair.

Ballouhey, O. et al. Front Cell Dev Biol. 2021; Pramono, Z. A. et al. Hum Genet. *2009*.

***Splicing** is one of the steps in protein production. In the first step, transcription, the gene message is "transcribed" into messenger RNA (a bit like a photocopy of the part of the DNA carrying the gene). In the second step, namely "splicing", the messenger RNA is spliced: certain parts (the introns) are cut and the remaining pieces (the exons) are recombined into a single mature messenger RNA strand that contains only the information needed to guide protein synthesis.*

Annexin and dysferlin in sarcolemma repair

▪ In a summary article about annexin and sarcolemma repair dysfunction, the team headed by Alain Brisson and Anthony Bouter, at the University of Bordeaux, and supported by AFM-Téléthon, describes the interdependent roles of annexin and dysferlin. It has been shown that annexin A1 and annexin A2 interact with dysferlin in sarcolemma membrane repair mediation. Work has shown that these annexins are over-expressed in patients with dysferlinopathy.

Annexin is an aggravating factor in dysferlinopathy

This over-expression of annexins could represent a method of compensating for the lack of dysferlin and restoring membrane repair capabilities. However, it has been observed that excess annexin A2 could escape from the damaged muscle cell and activate adipocyte differentiation. These adipocytes gradually replace the muscle fibres, and this leads to muscle generation in patients.

Annexin A6's genetic modifier role has also been described: annexin A6 expression extinction in a zebrafish model of dysferlinopathy accentuates the dystrophy.

Croissant, C. et al. Int J Mol Sci. 2021.

Involvement of dysferlin in muscle contraction

▪ The dysferlin protein, the gene of which is mutated in LGMD R2, plays a part in the muscle fibre membrane repair process: this mechanism is defective in patients with the disease.

▪ Researchers at Northwestern University in the United States have shown that dysferlin also played a role in muscle excitability and contraction. Compared to mice without the disease, mice that are mutated for dysferlin present, at the surface of the sarcoplasmic reticulum (SR), a greater density of ryanodine receptors, the calcium channels allowing calcium to pass from the SR towards the cytosol.

The data collected in the study suggest that the loss of dysferlin amplifies the excitation-contraction coupling prrocess: the time between muscle stimulation and peak intracellular calcium release is shortened.

Barefield, D. Y. et al. Sci Rep. 2021.

*The **sarcoplasmic reticulum** is a complex network of cavities inside the muscle cell, forming a cell compartment in which reserves of calcium needed for muscle contraction are built up.*

LGMD R4 (*SGCB*– beta-sarcoglycanopathy)

A new line of induced pluripotent stem cells

▪ From a sample taken from a seven-year-old child with LGMD R4, a Jordanian team has generated a new line of induced pluripotent stem cells carrying the homozygous missense mutation, "c.859delC", in the SGCB gene, which is involved in LGMD R4. The cells resulting from this line show a normal morphology and karyotype. They express all the characteristics needed to be able to differentiate into adult muscle cells, thus providing a new cell model to better understand LGMD R4.

Ababneh, N. A. et al. Stem Cell Res. 2021.

***Induced pluripotent stem cells** (iPSCs) are cells that can self-renew indefinitely in a culture, and differentiate into any specialised cell in the body.*

LGMD R9 (*FKRP* – dystroglycanopathy)

CRISPR-Cas9, a tool to replace exon 4 of the *FKRP* gene

*The **CRISPR-Cas9** system is an approach that acts like a molecular pair of scissors and cuts DNA at precise locations in the genome, in any cell. Thus, the goal is to target a DNA sequence or a gene, in order to remove it, repair it or modify it.*

▪ The CRISPR-Cas9 genome editing technique is being studied in different neuromuscular diseases. However, in theory, its application must be tailored to each abnormality located in different regions of the gene.

▪ The *FKRP* gene has the special characteristic that it contains the sequence coding the FKRP protein in a single region, exon 4. American researchers at the University of Minnesota used the CRISPR-Cas9 technique to replace the entire exon 4 in the pluripotent stem cells of patients with an abnormality of the FKRP gene. In these cells, corrected in this way, the glycosylation of α-dystroglycan was once again functional. Furthermore, when transplanted into mice presenting an *FKRP* gene mutation, this also restored α-dystroglycan glycosylation. It remains to be demonstrated whether this approach, exon 4 replacement, applicable to any *FKRP* gene mutation, improves muscle function in these mice.

Dhoke, N. R. et al. Cell Rep. 2021.

▪ CRISPR-Cas9 helps to locate the target region, using a small RNA guide. The therapeutic strategies used have proliferated. They allow different results to be achieved, such as removing a piece of DNA, correcting a mutation, changing the reading frame for a gene or a splicing site to perform exon skipping, or adding a piece of DNA to a gene.

FKRP appears to play a role in cell homeostasis

▪ The role of FKRP in the glycosylation (adding a molecule of sugar) of alpha-dystroglycan is well known. In patients with LGMD R9 *FKRP*-related, the loss of this glycosylation function prevents the binding of dystroglycan to the extracellular matrix laminin, which is essential for muscle fibre integrity. However, some patients do not show a correlation between levels of hypo-glycosylation of alpha-dystroglycan and disease severity, indicating that other possible mechanisms may be responsible for the disease.

▪ Researchers at the University of Minnesota have attempted to discover these other mechanisms, and have observed, in vitro, that *FKRP*-mutant myotubes showed a rate of apoptosis (cell death) that is greater than that of the healthy cells. The results also show a reduction in the autophagy-lysosome system for the degradation of intracellular macromolecules. This work opens the door to new therapeutic strategies in the dystroglycanopathies, targeting autophagy modulation or apoptosis inhibition.

Ortiz-Cordero, C. et al. Stem Cell Reports. 2021.

***Autophagy** is a process that allows a cell to cause a part of its contents to be degraded. Autophagosomes are vacuoles containing cell debris to be degraded. They merge with lysosomes, where their contents are "digested".*

LGMD R12 (*ANO5* – anoctaminopathy)

A new mouse model for LGMD R12

▪ An international collaboration between researchers at the Children's National Health System (United States) and the team at Généthon (France) headed by Isabelle Richard has made it possible for a new mouse model for LGMD R12 to be created. This model, obtained by the deletion of exons 10-12 of the *ANO5* gene, shows muscle weakness, muscle fibre lesions and gradual muscle loss. This mouse, which reproduces the clinical manifestations described among patients with LGMD R12, could be used to investigate the functional role of the ANO5 protein in the muscle, and to conduct therapeutic development preclinical studies for LGMD R12.

Thiruvengadam, G. et al. J Neuromuscul Dis. 2021.

Summaries of limb-girdle myopathies

Overview and therapeutic outlook

▪ A dossier dedicated to the limb-girdle muscular dystrophies appeared in the November 2020 edition of the *Cahiers de myologie* publication, and still serves as a reference. Three summary articles review what we know about calpainopathy, the sarcoglycanopathies and LGMD R9 FKRP-related.

Calpainopathy (LGMD R1)

▪ The involvement of genetic abnormalities of the *CAPN3* gene, in the occurrence of an autosomal recessive form of limb-girdle myopathy (LGMD R1), which was described for the first time in 1884 by the German neurologist, Wilhelm Heinrich Erb, was discovered in 1995.

▪ In 2016, a dominant form of limb-girdle myopathy (LGMD D4), less severe and more asymmetric compared to the recessive form, was found to be connected to dominant abnormalities of the *CAPN3* gene. A copy of the mutated gene produces abnormal calpain 3, which not only cannot perform its function, but also prevents normal calpain, produced by the other copy of the gene, exempt of any abnormalities, to play its role.

▪ LGMD R1 is the most common form of limb-girdle muscular dystrophy. Individuals with LGMD R1 present proximal and axial deficit with variable progression, appearing between the patient's first and second decade of life. There is no cardiac involvement.

▪ The possibility of a gene therapy providing the *CAPN3* gene via an adeno-associated virus (AAV) has been demonstrated in mouse models. While we await its application in humans, natural history studies need to be conducted in order to prepare future clinical trials.

Malfatti, E. et al. Med Sci (Paris). 2020.

*A particular gene can exist in several different forms, called **alleles** (or gene copies). The different alleles of the same gene are composed of a DNA sequence that has small variations. They are found at exactly the same place on the chromosome and have the same function.*
In each individual, there are two alleles for each gene. In the case of a dominant genetic disease, a single mutated allele (presenting a genetic abnormality) is sufficient to cause the disease – the presence of a correct copy of the gene does not allow the manifestation of the disease to be prevented. In the case of a recessive genetic disease, both alleles must be mutated to cause the symptoms of the disease to manifest.

Sarcoglycanopathies (LGMD R3, R4, R5, R6)

▪ The sarcoglycanopathies are the third most common cause of limb-girdle myopathy.

▪ Involvement of the muscles of the pelvic girdle (pelvis), of the scapula (junction between the trunk and shoulders) and of the trunk is generally symmetric. More-or-less severe cardiorespiratory manifestations are commonly observed.

▪ The disease appears in the first 10 years of life, and patients often lose the ability to walk during the second decade of their lives.

▪ CPK enzyme blood levels are elevated and the histological data show dystrophic-type lesions upon muscle biopsy, and a reduction in, or complete lack of sarcoglycan protein (α, β, γ, or δ depending on the mutated gene).

▪ At present, no curative treatment exists, but gene therapy clinical trials are underway.

Fernández-Eulate, G. et al. Med Sci (Paris). 2020.

LGMD R9 *FKRP*-related

- LGMD R9 is characterised by a mainly proximal limb-girdle (iliopsoas, adductor, gluteus maximus, quadriceps) muscle deficit, starting with the lower limbs. Cardiorespiratory manifestations may be observed and can be life-threatening.
- CPK enzyme serum levels are elevated and the histological data show dystrophic lesions upon muscle biopsy, and a reduction in α-dystroglycan glycosylation.
- At present, treatment of LGMD R9 is purely symptomatic, and requires a multidisciplinary approach.
- Several gene therapy trials are underway, following the demonstration of the efficacy of the approach involving the correction of α-dystroglycan glycosylation defects in animal models. Ribitol, a chemical compound, has also demonstrated its efficacy in preclinical studies, and it is currently undergoing clinical development.

Villar Quiles, R. N. et al. Med Sci (Paris). 2020.

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WEB www.afm-telethon.fr > See all the news