JUNE 2023

SAVOIR & COMPRENDRE
AVANCÉES DE LA RECHERCHE

ADVANCES in facioscapulo-humeral muscular dystrophy

> facioscapulohumeral muscular dystrophy (FSHD or FSH)

Facioscapulohumeral muscular dystrophy (FSHD or FSH) is a rare genetic disease. The first symptoms (wasting and weakness of muscles in the face and upper limbs) usually appear in adolescence or adulthood. The muscle involvement in FSHD is often asymmetric and varies greatly from person to person. It is a slowly progressive disease in most cases with stable periods that vary in their duration.

This document, published to coincide with the AFM-Téléthon General Meeting 2023, presents facioscapulohumeral muscular dystrophy research news from the past year (ongoing studies and clinical trials, scientific and medical publications, etc.).

It can be downloaded from the AFM-Téléthon website where further information in the scientific, medical, psychological, social and technological fields relating to facioscapulohumeral muscular dystrophy can be found:

WEBSITE www.afm-telethon.fr

AFMTÉLÉTHON
INNOVER POUR GUÉRIR

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A very active research field

96 scientific articles

published between May 2022 and May 2023

(Source: PubMed)

15 clinical trials

underway or in preparation worldwide as of 31 May 2023

(Source: ClinicalTrials.gov)

A new AFM-Téléthon document to help you learn out more about facioscapulohumeral muscular dystrophy

The "Ma Maladie" ["My Disease"] reference document on FSHD provides information on the disease as well as how to access better medical monitoring, improve your everyday life, further your independence and even travel!

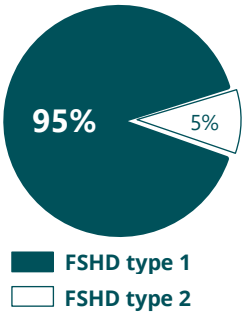
This document is available at www.afm-telethon.fr/Repères [in French]

What causes facioscapulohumeral muscular dystrophy?

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

Facioscapulohumeral muscular dystrophy (FSHD or FSH) is a "rare" muscle disease. It only affects five to seven people in every 100,000.

Two types of FSHD

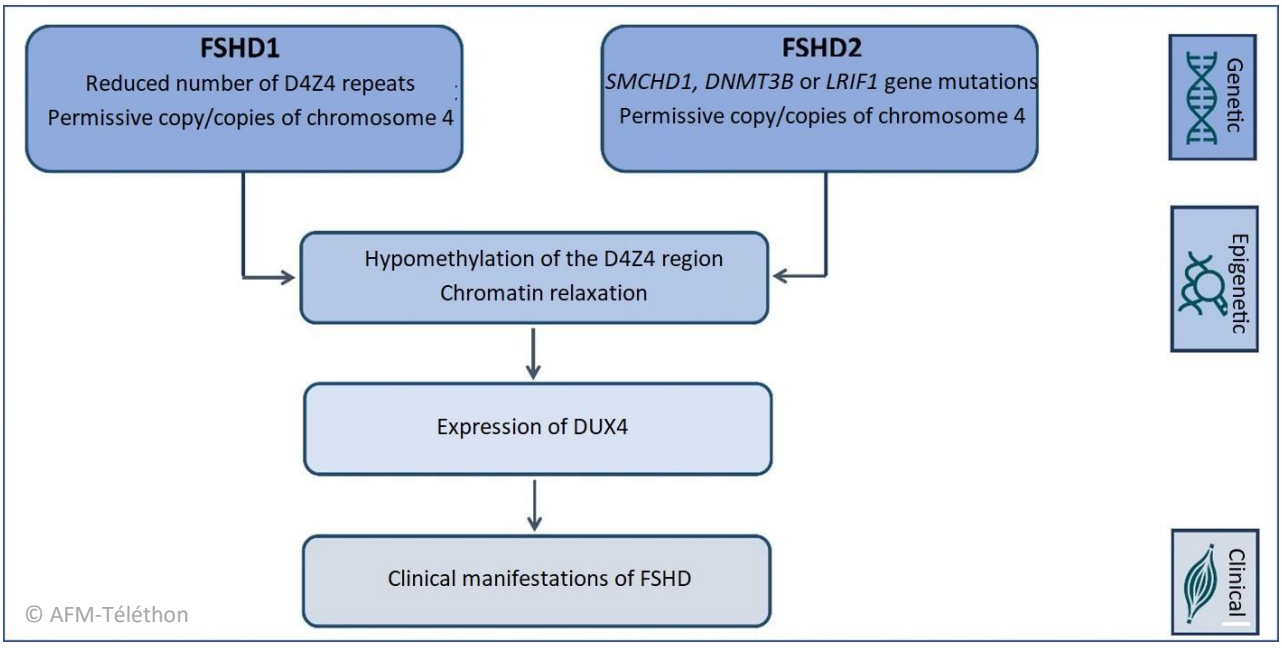


Epidemiological data on FSHD shows that:
*- **the most common type is FSHD1,** which is associated with a decrease in the number of D4Z4 repeats (chromosome 4),*
*- **FSHD2 is much rarer,** which is associated with mutations in the* SMCHD1 *gene (chromosome 18),* DNMT3B *gene (chromosome 20) or* LRIF1 *gene (chromosome 1).*

__Chromatin__ is a substance found in cell nuclei. It is made up of both DNA (a cell's genetic material) and proteins which organise and protect this DNA. When a cell divides, chromatin condenses into little rods (chromosomes).

▪ **FSHD type 1 (FSHD1)** is caused by a **decrease in the number of repeats in the D4Z4 region** (containing one to 10 repeats instead of the usual 11 to 100 repeats) on chromosome 4, leading to a chromatin structure which is less compact in this region (scientists refer to this as "chromatin relaxation") and a decrease in DNA methylation (hypomethylation). This type of FSHD involves another genetic mutation on chromosome 4 which are referred to as "permissive" copies of chromosome 4.

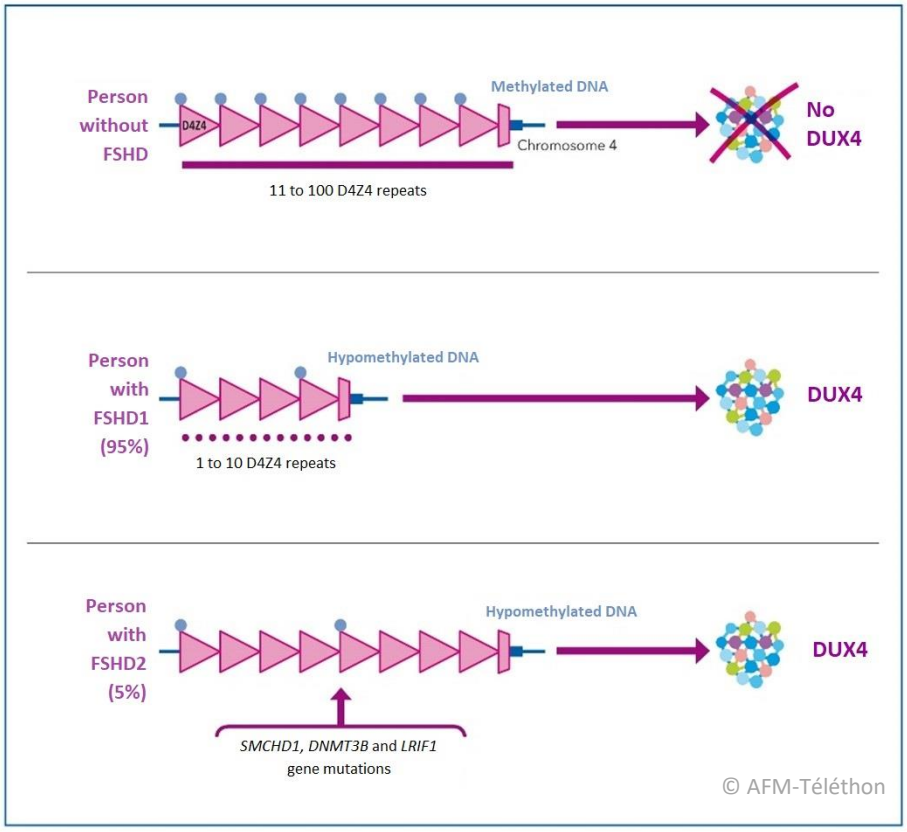
▪ People with **FSHD type 2 (FSHD2)** do not have a reduced number of D4Z4 repeats on chromosome 4, but they do have chromatin relaxation and hypomethylated DNA in this region of chromosome 4.
This chromatin relaxation is found in 85% of FSHD2 patients with ***SMCHD1* gene mutations** on chromosome 18 (and in a small number of FSHD2 patients who do not have *SMCHD1* gene mutations), ***DNMT3B* gene mutations** on chromosome 20 or ***LRIF1* gene mutations** on chromosome 1 (identified in 2020).
Like in FSHD1, these patients also need to have at least one permissive copy of chromosome 4 for the disease to occur.



Inappropriate DUX4 expression

There is a gene (the ***DUX4* gene**) in the D4Z4 region which codes for the DUX4 transcription factor. The *DUX4* gene is inactive in people who do not have FSHD.

The *DUX4* gene is abnormally expressed in FSHD
In both types of FSHD, the *DUX4* gene is abnormally expressed in muscle.
It codes for the DUX4 protein which participates, by activating a cascade of other genes, in the degradation of muscle fibres.



Expression of the *DUX4* gene is not always related to the severity of the disease. The abnormal expression of the *DUX4* gene has even been occasionally found in people who do not have FSHD. These findings have led researchers to suggest that the *DUX4* gene is necessary but not enough to cause FSHD.

The expression levels of a gene *correspond to the amount of protein made from that gene. High levels of gene expression lead to a large amount of proteins being produced, while low levels of gene expression lead to small quantities being produced.*

Clinical trials

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

Four phases

Did you know?

The four phases of clinical trials

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

- **Phase I: Safety/tolerability**

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

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

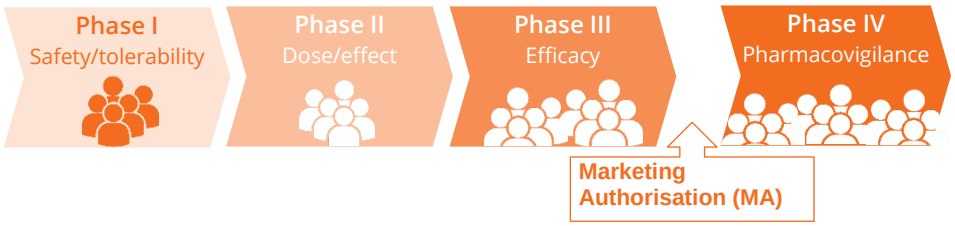
Phase II, conducted on a comparable group of volunteers with the disease, studies the safety and efficacy of the product and will determine the optimum dose to be used.

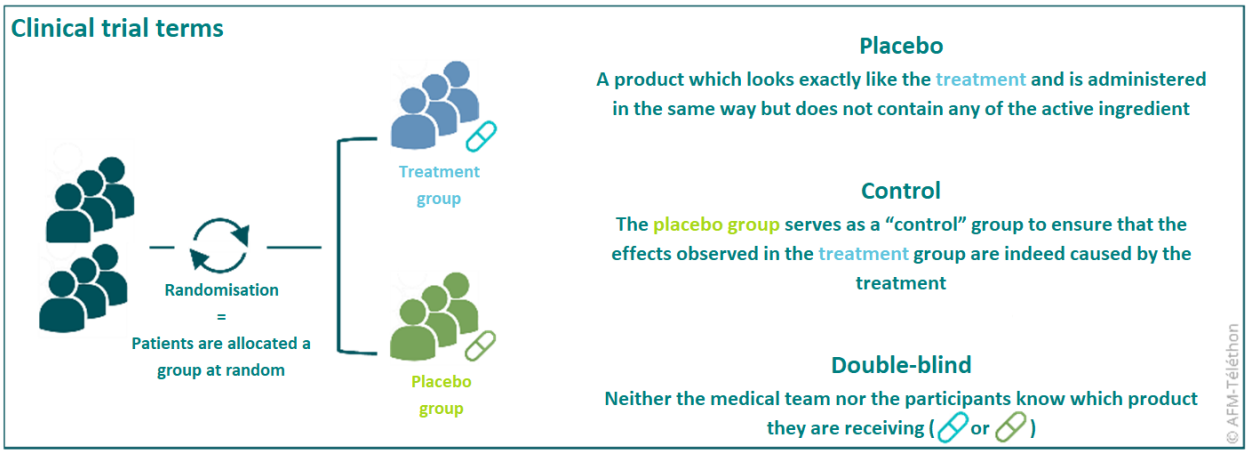
- **Phase III: Therapeutic efficacy**

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

- **Phase IV: Pharmacovigilance**

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





Losmapimod

Available in tablet form, losmapimod **inhibits an enzyme called p38 MAPK (mitogen-activated protein kinase).**

p38 MAPK or p38

The p38 MAPK enzyme is involved in inflammation and regulates cell cycle checkpoints.
It is expressed mainly in endothelial cells (vessel walls) and myocytes (muscle cells).

- Since 2008, over 20 clinical trials of losmapimod have been conducted in various conditions such as myocardial infarction, chronic obstructive pulmonary disease (COPD) and neuropathic pain. Losmapimod has not been shown to be effective but is well tolerated.
- Losmapimod was granted **orphan drug designation** for the treatment of FSHD by the American health authorities in March 2020.

An orphan drug

An orphan drug is a drug developed to treat an "orphan" disease, that is, a disease for which no treatments are available. These are often rare diseases.
This designation is granted to drug candidates that have not been proven to be effective yet. Its aim is to encourage pharmaceutical companies to develop and market drugs for patients with rare and neglected diseases using economic and methodological measures that facilitate the different stages of drug development.
WEBSITE https://www.eurordis.org/ > What is an orphan medicine?

Preclinical results

- Researchers from the pharmaceutical company Fulcrum Therapeutics identified losmapimod as a compound capable of reducing *DUX4* gene expression by carrying out screening in cell models.
- A study conducted by another American team confirmed that inhibiting p38 reduces *DUX4* expression in cell and animal models of the disease.

Oliva J et al. J Pharmacol Exp Ther. 2019 August.

A phase I trial

Phase I
Safety/tolerability

- Encouraged by these findings, Fulcrum Therapeutics conducted a phase I trial of losmapimod in **20 people with FSHD1** and 10 healthy volunteers. The aim of this trial was to evaluate the safety, tolerability, pharmacokinetics and target engagement (receptor binding) of oral losmapimod (7.5 or 15 mg/day) administered twice daily for 14 days, and compared to a placebo in some participants.

The results of this trial showed that losmapimod was well tolerated, and appeared to be active in the muscles of the FSHD patients (p38 was inhibited in their muscle biopsies).

Mellion ML et al. Br J Clin Pharmacol. 2021 Apr.

Phase II trials

The results of the phase I trial backed the choice of using the 15 mg dose, which was tested in a phase II trial called ReDUX4.

- Its objective was to evaluate the safety and efficacy of losmapimod vs placebo in **80 FSHD1 patients** aged 18 to 65 years old in four countries including France (CHU de Nice [Nice University Hospital]) over one year.

*An **open-label trial** is a clinical trial in which the doctors and participants know what treatment is being administered.*

▪ All of the participants in the ReDUX4 trial were able to take part in its open-label extension which is evaluating the safety and efficacy of long-term losmapimod use (up to five years).

Initial results at different stages

▪ **After four months of treatment**, no significant difference in *DUX4* gene expression was observed in 29 participants who were on losmapimod or placebo (the primary endpoint of the trial). However, participants with high levels of *DUX4* expression in their muscle biopsies at the start of the trial showed a significant reduction in this expression following treatment with losmapimod.
Fulcrum Therapeutics. Press release 11 August 2020.

▪ **After 11 months of treatment**, losmapimod delayed loss of strength, and even improved it, in certain shoulder and ankle muscles in the FSHD patients compared to the placebo. It also significantly slowed down the rate at which muscle was being replaced by fat in the "intermediate" muscles as shown by MRI scans. However, yet again no significant difference in the expression of genes regulated by *DUX4* in muscle was found between the placebo and losmapimod (the primary endpoint of the trial).
Tawil R et al. Neuromuscular Disorders 31 (2021).
Fulcrum Therapeutics. Press release 24 June 2021.

Phase II
Dose/effect

Phase II FSHD1 trial (ReDUX4)

In France and abroad

80 participants (18 to 65 years old)

Data currently being analysed

1 year of follow-up

August 2019 – January 2021

NCT04003974

▪ Initial results from the trial's extension seem to show that losmapimod leads to a decrease in fat infiltration in the intermediate muscles.
Fulcrum Therapeutics. Press release 12 October 2022.

Phase II
Dose/effect

Open-label extension of the ReDUX4 trial in FSHD1

In France and abroad

76 participants (18 to 65 years old)

Not recruiting

5 years of follow-up

February 2020 - February 2025

NCT04264442

▪ Losmapimod is also the subject of another phase II, open-label trial which is being conducted by Fulcrum Therapeutics in the Netherlands in 14 participants with FSHD1.

Phase II trial of losmapimod in FSHD1

Phase II
Dose/effect

In the Netherlands

14 participants (18 to 65 years old)

Not recruiting

1 year of follow-up

August 2019 – January 2024

NCT04004000

A phase III trial

Given the positive results obtained during the phase II ReDUX4 trial of losmapimod, Fulcrum Therapeutics launched a phase III trial.

▪ This double-blind, randomised, placebo-controlled trial called REACH needs to recruit **230 adults with FSHD1 or FSHD2.** It will evaluate the safety and efficacy of losmapimod over a period of 48 weeks.

Fulcrum Therapeutics. Press release 5 July 2022.

Phase III trial of losmapimod in FSHD1 or FSHD2 (REACH)

Phase III
Efficacy

In France and abroad

230 participants (18 to 65 years old)

Recruiting

1 year of follow-up

June 2022 - January 2026

NCT05397470

AOC 1020

Developed by Avidity Biosciences, AOC 1020 is a small interfering RNA molecule which **targets *DUX4* mRNA.**

▪ In animal models, a single IV injection of AOC 1020 slowed the progression of muscle weakness.

An RNA-based drug

AOC 1020 is a small interfering RNA (siRNA) molecule which was created to bind specifically to a target RNA molecule, the RNA of the *DUX4* gene, which destroys it.

▪ AOC 1020 has been granted **orphan drug status** for FSHD by the European and American health authorities, as well as **"fast track"** designation, a process which accelerates certain stages of a drug's development.

Messenger RNA (mRNA) *is a replica of a region of DNA corresponding to a gene, which serves as a template for the synthesis of a protein. It is composed of a nucleotide sequence which determines the protein's amino acid sequence, i.e., its composition and structure.*

▪ A phase I/II, placebo-controlled trial (FORTITUDE) is currently taking place in the United States to study the safety, tolerability, pharmacokinetics, pharmacodynamics and efficacy of AOC 1020 in FSHD1 and FSHD2.

Phase I
Safety/tolerability

Phase II
Dose/effect

FORTITUDE trial

In the United States

72 participants (18 to 65 years old)

Recruiting

1 year of follow-up

April 2023 – September 2025

NCT05747924

RO7204239

RO7204239 is a drug candidate developed by the pharmaceutical company Hoffmann-La Roche to inhibit myostatin. It is being tested in a phase II trial (MANOEUVRE) in order to evaluate its effects on the contractile muscle volume of quadriceps muscles in ambulatory adults with FSHD.

An anti-myostatin antibody
RO7204239 is an anti-myostatin antibody, i.e., it blocks myostatin which naturally inhibits muscle growth.

Phase II
Dose/effect

MANOEUVRE trial

In the United States and Denmark

48 participants (18 to 65 years old)

Recruiting

2.5 years of follow-up

February 2023 – December 2025

NCT05548556

Antioxidants

A team at CHU de Montpellier [Montpellier University Hospital] analysed mitochondrial function and oxidative stress in the blood and muscles of FSHD patients and healthy volunteers.

Did you know?

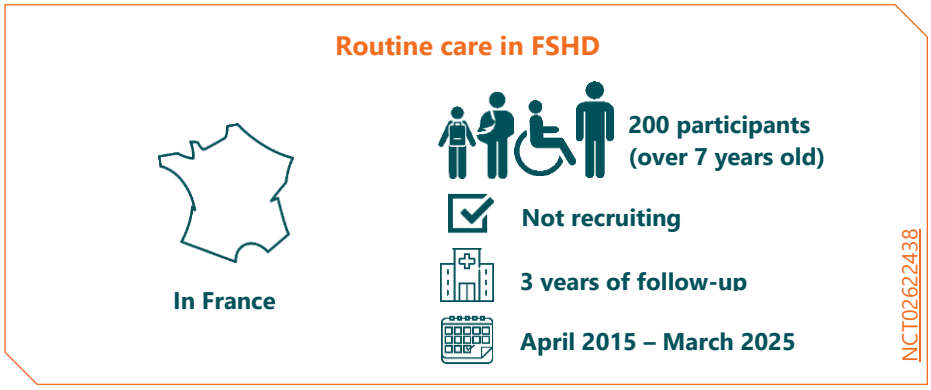
Oxidative stress
Oxidative stress is a situation in which a cell no longer controls the presence of excessive toxic molecules which are mainly produced by cellular respiration (free radicals).
Free radicals are produced by the transformation of the oxygen which is used by cells to function via mitochondria (cellular respiration). They are toxic as they oxidise other molecules and damage cells.

▪ Published in July 2012, the Montpellier team's results showed an increase in oxidative stress in the FSHD patients as well as a correlation between this oxidative stress and muscle weakness in the quadriceps (endurance and maximal voluntary contraction).
Turki A et al. Free Radic. Biol. Med. 2012 Sep.

▪ The same team conducted the first clinical trial to evaluate the effects of a combination of antioxidants (vitamin C, vitamin E, zinc and selenium) vs placebo in 53 FSHD patients.
The results of this trial, published in September 2014, showed a reduction in oxidative stress, but also an improvement in the endurance and maximal voluntary contraction strength of the quadriceps in the patients on antioxidants compared to the placebo group after four months of treatment. However, no significant statistical difference was observed between the two groups in the two-minute walk test.
Passerieux E et al. Free Radic Biol Med. 2014 Sep.

▪ These results still needed to be confirmed over time and in a larger number of patients. This is the objective of a second clinical trial which is an open-label trial running for three years conducted by the team in Montpellier in 151 FSHD patients. The criteria used to evaluate the effect of the antioxidants are maximal voluntary contraction of the quadriceps, quality of life, daily physical activities and oxidative stress markers.

*An **open-label trial** is a clinical trial in which the doctors and participants know what treatment is being administered.*



▪ The evaluation of the nutritional status of 74 women and 85 men with FSHD showed vitamin and mineral deficiencies despite a varied and balanced diet. The average dietary intake of zinc and vitamin C and E was lower than the recommended daily intake. Daily energy and protein intake were also lower in FSHD. As for daily calorie intake, this was lower in the women than in the men. These results were published by the team in Montpellier which have been studying the role of oxidative stress and antioxidants (vitamins, minerals, etc.) in FSHD for several years.
Amzali S et al. Nutrients. 2023 March.

Creatine monohydrate

Creatine monohydrate is a compound used by athletes to increase their muscle mass.

- A phase II trial sponsored by the Murdoch Childrens Research Institute evaluated the effects of creatine monohydrate on muscle mass and strength in children with FSHD.

Phase II
Dose/effect

Creatine monohydrate trial

In Australia

20 participants (5 to 18 years old)

Trial completed, data currently being analysed

3 months of follow-up

October 2017 – July 2022

NCT02948244

Testosterone and human growth hormone

- In the United States, the University of Rochester conducted the STARFISH trial to evaluate testosterone combined with human growth hormone in men with FSHD. The data is currently being analysed.

Phase I
Safety/tolerability

STARFISH trial

In the United States

20 participants (18 to 65 years old)

Trial completed, data currently being analysed

9 months of follow-up

December 2017 – March 2022

NCT03123913

Filgrastim

- In Poland, a phase I trial is currently recruiting in order to evaluate the effects of filgrastim, a granulocyte colony-stimulating factor (G-CSF) which acts on the proliferation of satellite cells as well as muscle regeneration and membrane repair.

Did you know?

Satellite cells

Satellite cells are stem cells found near muscle cells. Stem cells possess both the ability to multiply to produce identical new stem cells and the ability to give rise, under specific conditions, to differentiated cells (blood cells, liver cells, muscle cells, etc.). When skeletal muscle is damaged, satellite cells are rapidly activated to regenerate the muscle.

▪ This trial, conducted by the Medical University of Bialystok, includes children and adolescents with FSHD but also Duchenne or Becker muscular dystrophy.

Filgrastim trial

In Poland

27 participants (5 to 15 years old)

Recruiting

7 years of follow-up

March 2013 – December 2022

NCT02814110

Phase I
Safety/tolerability

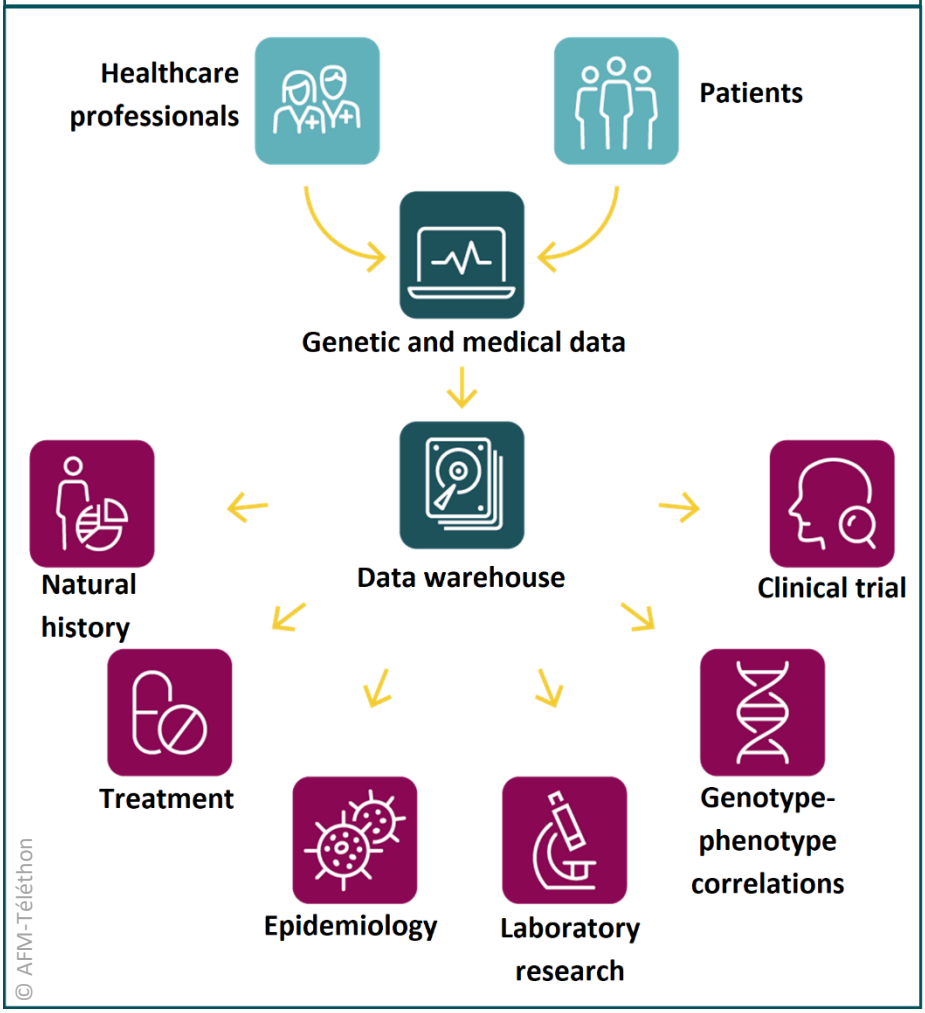
Databases

Databases collect medical and genetic data from people with the same disease, often for an unlimited period of time.
Analysing this data helps to determine the natural history of the disease, establish genotype-phenotype correlations and recruit participants to clinical trials.

*The so-called **natural history** of a disease, as doctors refer to it, is the description of the different manifestations of a disease and their progression over time without treatment.*

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

Data warehouse
A data warehouse is a collection of genetic and medical data from people with the same disease (with their consent).



A national registry

Did you know?

A French registry

The Observatoire National Français des patients atteints de DMFSH [French National Registry for patients with FSHD] was created in June 2013. Funded by AFM-Téléthon, this data warehouse aims to gather genetic and clinical information from as many people as possible with FSHD in order to improve our understanding of the disease and accelerate the development of drugs.

▪ This registry also takes into account contributions made by both doctors and patients through self-administered questionnaires.
The genetic and clinical data collected using **self-administered questionnaires** and/or **clinical assessment forms** completed with a doctor during consultations is entered by doctors and scientists into a computer database which has been specially developed so that this data can be used easily.
Quality control is carried out by a curator. Access to the data for scientific purposes is subject to authorisation from the registry's steering committee, which is made up of medical specialists and patient representatives.

▪ As of 20 December 2022, the registry contained **data from 1,025 adults**, the vast majority of whom had FSHD type 1 (FSHD1). The goal of reaching 1,000 patients by 2022 has been achieved; now, the registry's new goal is to collect data from 1,500 patients by 2027.

▪ The registry's coordinator for adults is Prof. Sabrina Sacconi (Centre de Référence des maladies neuromusculaires et SLA [neuromuscular diseases and MND reference centre], CHU de Nice - Hôpital Archet 1 [Nice University Hospital - Archet 1 Hospital]). This registry is also open to children over the age of six years old. The coordinators for children are Prof. François Rivier (CHU de Montpellier), Dr Silvana de Lucia (I-Motion, Paris), Dr Marine Guichard (CHU de Tours [Tours University Hospital]) and Dr Georgia Quérin (Institut de Myologie [Institute of Myology], Paris).
WEBSITE www.fshd.fr

The Observatoire National Français des patients atteints de DMFSH

In France

Created in June 2013

Recruiting

1,025 adults as of 20 December 2022

Registries outside of France

The United Kingdom, the United States and China, among others, have also developed national FSHD registries.

The United Kingdom Facioscapulohumeral Muscular Dystrophy Patient Registry

In the United Kingdom

Created in May 2013

Recruiting

937 patients as of May 2022

NCT04001582

WEBSITE www.fshd-registry.org.uk/

National Registry for Myotonic Dystrophy and Facioscapulohumeral Dystrophy

In the United States

Created in September 2000

Recruiting

Target: 3,000 patients by June 2023

NCT00082108

WEBSITE www.urmc.rochester.edu/neurology/national-registry.aspx

The China FSHD1 Patient Registry

In China

Created in January 2001

Recruiting

Target: 1,000 patients by December 2031

NCT04369209

Observational studies

Observational studies allow researchers to gain a better understanding of a disease, identify better monitoring or diagnostic tools, monitor the effect of a treatment over a somewhat long period of time, etc.

Observational studies currently taking place

In order to make evaluating new drugs during clinical trials easier and more reliable, it is essential to use reliable assessment tools.

▪ **The ReSOLVE study** is working to substantiate the merit of two of these tools - a FSHD-specific functional rating scale (FSHD-COM) and a non-invasive muscle exam (electrical impedance myography).

ReSOLVE study

In the United States

160 participants (18 to 75 years old)

Not recruiting

2 years of follow-up

March 2018 – March 2023

NCT03458832

The ReSOLVE study is being conducted at eight American research centres which are part of the FSHD Clinical Trial Research Network. Three European research centres, including CHU de Nice (coordinated by Prof. Sabrina Sacconi), joined this network in May 2019 and participate in its research programmes.

▪ CHU de Nice is conducting the French part of the study (**ReSOLVE_France**) which is evaluating the reliability of the FSHD-COM scale, but not that of electrical impedance myography.

ReSOLVE_France study

In France

100 participants (18 to 75 years old)

Recruiting

2 years of follow-up

September 2019 – March 2025

NCT04038138

▪ CHU de Nice is also conducting another study on using digital facial analysis technology for automated diagnosis (algorithm) of FSHD. This "eHealth" tool could facilitate the diagnosis of the disease (especially moderate cases) and monitoring of its progression using telemedicine for patients who live far away from research centres.

CV4DIAGNOSIS pilot study

In France

100 participants (over 18 years old)

Recruiting

18 months of follow-up

May 2020 – March 2021

NCT04377217

▪ Since January 2018, CHU de Nice has also been conducting a pilot study on pro-inflammatory molecules as a potential therapeutic target in FSHD1.

CYTOKINE-FSH pilot study

In France

30 participants (18 to 75 years old)

Not recruiting

21 months of follow-up

January 2018 – March 2022

NCT04694456

▪ In the United States, a study is currently taking place to detect and study muscle changes in FSHD patients using MRI and magnetic resonance spectroscopy.

***MRI** or **magnetic resonance imaging** is a medical imaging technique which can obtain cross-sectional or volumetric images of an organ or area of the human body. The exam involves lying still on a bed which slides into a cylindrical machine made from a very powerful magnet. MRI scans are not painful. However, being in a confined space, the length of the scan, being on your own and the noise from the machine can be a little bit scary.*

➤➤ Diagnostic des maladies neuromusculaires [Diagnosis of neuromuscular diseases], Savoir & Comprendre references documents, AFM-Téléthon.

Study of imaging and spectroscopy biomarkers for FSHD

In the United States

75 participants (over 12 years old)

Not recruiting

21 months of follow-up

March 2012 – June 2023

NCT01671865

▪ In Turkey, the impact of scapulothoracic arthrodesis surgery on balance and gait was studied in 24 participants with FSHD.

Study of the effects of scapulothoracic arthrodesis surgery on balance and gait

In Turkey

24 participants (18 to 65 years old)

Trial completed, data currently being analysed

One visit

November 2021 – August 2022

NCT05022355

▪ Another Turkish study evaluated the effects of upper limb rehabilitation on balance and gait in FSHD.

Study of the effects of rehabilitation on balance and gait

In Turkey

36 participants (18 to 50 years old)

Trial completed, data currently being analysed

2 months of follow-up

February 2022 – June 2022

NCT05178706

▪ A British study aimed to identify factors for shoulder instability, i.e., partial or complete dislocation of the shoulder joint, in 14 FSHD patients.

*A **dislocation** is the separation of two bones where they meet at a joint.*

Study of the factors for shoulder instability in FSHD

In the United Kingdom

14 participants (over 18 years old)

Trial completed, data currently being analysed

One day

March 2022 – October 2022

NCT05239520

Other treatment avenues being studied

Some avenues being explored in FSHD
- **VivoPMO-PACS4, an optimised antisense oligonucleotide** which is able to inhibit the *DUX4* gene in mouse models of FSHD.
- **Flavones** which have been shown to block DUX4-induced toxicity in cell models of FSHD.
- **A cell therapy approach** which is able to replace diseased muscle in mouse models of FSHD.
- **Genome editing techniques** which target the *DUX4* or *SMCHD1* gene.

Targeting *DUX4* expression

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

In order to combat the abnormal expression of the *DUX4* gene, an English team developed **vivoPMO-PACS4, an antisense oligonucleotide that has been optimised to better target muscle cells,** which reduces the expression of the *DUX4* gene as well as genes activated by the DUX4 protein.

- In a new study, this team administered long-term (eight weeks) vivoPMO-PACS4 to a mouse model in which the disease had already been present for several weeks. *DUX4* expression was considerably reduced (by 60%) while muscle strength and mass increased and fibrosis decreased.
Lu-Nguyen N et al. Biomedicines. 2022 July.

Targeting processes downstream of DUX4 activity

Treatment strategies developed in FSHD usually target the expression of the *DUX4* gene or the protein that it codes for. Therapies targeting processes downstream of DUX4 activity have not been studied as much.

- In 2019, an American team identified an aberrant and toxic accumulation of hyaluronic acid induced by DUX4. Treatment with a hyaluronic acid synthesis inhibitor did not have a direct impact on DUX4 in a cell model of FSHD, but did prevent the accumulation of hyaluronic acid, thereby reducing DUX4-induced toxicity.

- This team also screened molecules in order to identify those that might be able to reduce DUX4-induced toxicity in cell models of FSHD. **Five compounds (flavones) that function downstream of DUX4 activity to inhibit DUX4-induced toxicity** were identified in cell cultures of FSHD.
Cohen J et al. Res Sq [preprint]. 2023 Feb.

Replacing diseased muscle with healthy stem cells

__Stem cells__ possess both the ability to multiply to produce identical new stem cells (self-renewal) and the ability to give rise, under specific conditions, to differentiated cells (blood cells, liver cells, muscle cells, etc.).

Cell therapy
Cell therapy consists of replacing diseased cells with stem cells. In FSHD, stem cell transplantation in small muscles is performed with the aim of promoting the regeneration of these muscles and improving their motor function.

▪ A new American study demonstrated the feasibility of **a cell therapy approach which replaces diseased muscle with mouse pluripotent stem cell-derived progenitor cells** in mouse models of FSHD. These cells are able to differentiate into many different cells, including muscle cells.
The leg muscles of the mice which were injected with stem cells had reduced fibrosis and improved contractile force compared to the non-transplanted mice.
Azzag K et al. NPJ Regen Med. 2022 Sept.

Modifying the genome

Genome editing
The term "genome editing" refers to a set of techniques used to modify the DNA sequences that make up the genome, such as nucleases, TALENs, etc. Today, the most well-known technique is the **CRISPR/Cas9 system** which cuts DNA at a specific place in the genome in any cell in a very simple, quick and efficient way. This means that it can deactivate or correct a defective gene. Since its discovery, the CRISPR/Cas9 system has evolved a lot and can now also used to alter DNA bases without having to cut the DNA.

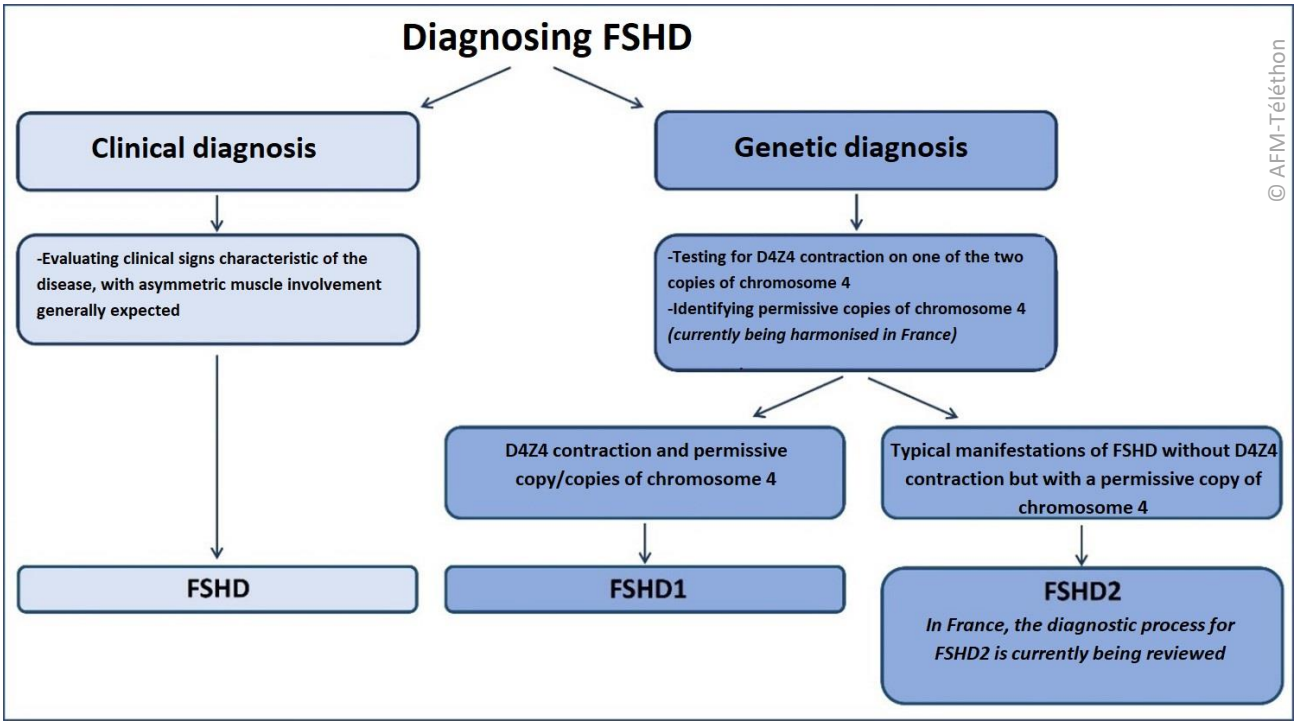
▪ Many teams use these approaches in FSHD to inhibit or sequester DUX4 at every stage of its production (at the DNA, mRNA or protein level). Other teams are attempting to target the *SMCHD1* gene. In a recently published review, two researchers **assessed all of the genome editing strategies currently being investigated for FSHD,** detailing the initial successes but also the challenges linked to their development and use.
Mariot V et al. Front Genome Ed. 2022 July.

▪ During the 26th Annual Meeting of the American Society of Gene & Cell Therapy (ASGCT) which took place between 16 and 20 May 2023 in Los Angeles (United States), the pharmaceutical company Epic-Bio presented **preclinical data on its drug candidate, EPI-321.** This product is based on an approach derived from CRISPR technology which does not involve cutting DNA. It targets the methylation of the D4Z4 region and suppresses the expression of the *DUX4* gene.
A single administration of EPI-321 inhibited the expression of the *DUX4* gene and the genes that it activates in FSHD patient-derived cell models, as well as in a humanised mouse model of the disease. Other results showed a 55% increase in muscle cell survival in this mouse model 24 days after the injection was administered.
Epic-Bio. Press release 19 May 2023.

▪ Another team also presented their work during this annual meeting which involved using **a product that combines a viral vector (AAV) with a small RNA molecule to target *DUX4* expression (AAV-shDUX4).** When injected into mouse models of FSHD, this treatment led to an increase in myostatin levels as well as a decrease in the expression of genes activated by DUX4 and those involved in fibrosis.
26th Annual Meeting of the American Society of Gene & Cell Therapy (ASGCT). Molecular Therapy. May 2023.

Improving the diagnosis of FSHD

FSHD is currently diagnosed by evaluating clinical signs that are characteristic of the disease together with genetic parameters (e.g. testing for D4Z4 contraction and identifying permissive copies of chromosome 4). However, making genetic diagnosis requires the use of complex techniques, such as molecular combing, a technique created by a team in Marseille which stretches DNA molecules, making it easier to detect genetic mutations in them.



▪ In order to complete or improve the diagnosis of this disease, a German team proposed **using epigenetic parameters based on the methylation profile of the D4Z4 region.**
This technique determines the methylation level of D4Z4 repeats, as well as the most distal D4Z4 repeat unit, meaning that not only is it able to distinguish FSHD patients from healthy individuals, but also FSHD1 patients from FSHD2 patients. This new diagnostic marker could also be useful for monitoring the severity of the disease, which appears to be related to the level of D4Z4 methylation.
Erdmann H et al. Brain. 2023 Apr.

▪ Japanese researchers have developed a new technology based on the use of the CRISPR/Cas9 system. It enables **very long fragments of genomic DNA to be sequenced, including D4Z4 repeats,** and the degree of hypomethylation of these regions of interest to be studied at the same time. Hypomethylation of the D4Z4 region and a reduced number of D4Z4 repeats were thus confirmed in FSHD1. In FSHD2, the size of the D4Z4 region did not vary but the hypomethylation was very significant. This technology could help simplify the molecular diagnosis of FSHD.
Hiramuki Y et al. J Transl Med. 2022 Nov.

Molecular and cellular interactions

A new molecular partner for DUX4

The DUX4 protein, coded for by the *DUX4* gene, activates a cascade of other genes which are involved in inflammation and the destruction of muscle fibres.

▪ An Italian team, supported by AFM-Téléthon, showed that the production of a non-coding RNA molecule called DBE-T (short for "D4Z4 binding element transcript") was involved in the aberrant expression of the *DUX4* gene.
As they continued to work on this project, the team identified a direct interaction between DBE-T and WDR5, a chromatin remodelling protein. **WDR5** is believed to be recruited to the D4Z4 region by DBE-T to activate the expression of the *DUX4* gene in muscle cells. It could constitute a potential therapeutic target since its inhibition in FSHD cell cultures restores muscle differentiation and extends cell viability.
Mocciaro E et al. Nucleic Acids Res. 2023 Apr.

Interactions between cells

A team of French and Russian researchers showed that myoblasts that express the *DUX4* gene stimulated abnormal mesenchymal stem cell migration. Mesenchymal stem cells can differentiate into fat cells (adipocytes) and contribute to the formation of fibrosis. They could therefore be involved in the fibrosis observed in FSHD.
▪ By studying these cells more closely, this team was able to highlight the importance of the interactions between myoblasts and mesenchymal stem cells in the development of FSHD. Myoblasts attract mesenchymal stem cells to them, stimulating them to multiply and secrete collagen (a component of connective tissue and fibrosis) which prevents myoblasts from fusing and myotubes from forming - essential stages in muscle formation.
Kiseleva E et al. Cell Physiol. 2022 August.

__Myoblasts__ are the precursor cells (stem cells) of muscle cells.

A __myotube__ is a maturing muscle cell. They are formed by the fusion of several myoblasts (muscle stem cells). Myotubes are elongated ("tube") cells that have several nuclei like the mature muscle fibres that they will become.

Other clinical study results

A large-scale survey conducted by FSHD Europe

▪ A survey on the expectations of patients and caregivers regarding future clinical trials in FSHD was conducted by FSHD Europe, an organisation of which AFM-Téléthon is a member.
Over one thousand participants (1,147 to be precise) with an average age of 50 years old from 26 European countries, including France, responded to this survey. The number of male and female respondents was fairly equal.

The main findings

▪ Most of the respondents had FSHD1 (68%) vs 7% who had FSHD2, with the remaining 25% not having received a genetic diagnosis.
▪ Just over half of the respondents used walking aids or a wheelchair.
▪ Initial symptoms started between the ages of 11 and 20 years old in 40% of the participants, with scapular winging being the highest reported initial symptom (31%), followed by lower limb weakness (22%) then upper limb weakness (19%).
▪ The average time from onset of the first symptoms to diagnosis was 7.9 years.
▪ Physiotherapy, occupational therapy, exercise and mobility aids were the means used most frequently to help manage their condition.
▪ The main factors motivating patients to participate in clinical trials were access to treatment and trial results as well as being able to collectively provide benefits to other FSHD patients.
▪ However, distance and accessibility of trial sites as well as the fear of side effects would make patients less likely to participate in clinical trials.
▪ The patients also emphasised the importance of receiving clear information throughout their participation in a clinical trial as well as being informed of the trial's results.
This data could help encourage FSHD patients to participate in future clinical trials.
FSHD European Patient Survey report. 2022.

Guidelines for heart rhythm disturbances

▪ Neuromuscular disease specialists from around the world, together with cardiologists, created practical guidelines for evaluating and managing heart rhythm disturbances found in diseases such as facioscapulohumeral muscular dystrophy.
The conclusions of the document were reached by analysing literature on the subject but were also the result of discussions on the practices and expertise of specialists in the field. The authors highlighted the importance of a joint decision being made between the patient, their GP and their family, especially when it comes to invasive treatments, and the frequent dissociation between motor and cardiac symptoms, hence the need for regular routine heart rhythm monitoring.
Groh WJ et al. Heart Rhythm. 2022 Apr.

Monitoring sleep quality

To date, little work has been conducted on sleep in FSHD.
▪ An American team carried out a survey of 690 FSHD patients between the ages of 12 and 74 years old on the subject of sleep. The survey showed that two thirds of the patients suffered from reduced sleep quality, regardless of their age or sex, which was usually linked to nocturnal pain, and 15% had

excessive daytime sleepiness. This team suggested that doctors should monitor the sleep quality of their FSHD patients.
Hoffmann HM et al. Muscle Nerve. 2022 Oct.

A survey on pregnancy and childbirth

The vast majority of pregnancies in women with neuromuscular diseases go smoothly. However, certain complications can arise.

▪ An international study published in December 2022 analysed the pregnancy and childbirth experiences of 305 women with neuromuscular diseases (721 pregnancies in total). This data was collected using online questionnaires. Twenty-six neuromuscular diseases were featured in this survey, and 42% of the women surveyed had FSHD.
The results of this survey showed that 21% of the pregnancies ended in miscarriage and 8% in abortion. Of the pregnancies carried to term, 38% resulted in a caesarean section and 19% involved an assisted vaginal birth, significantly higher averages than those in the general population.
A deterioration in muscle strength was recorded during 43% of the pregnancies, with women who were symptomatic at the start of their pregnancy being at a significantly higher risk of this occurring. After giving birth, 23% of the women indicated that they had never returned to their previous state of health; conversely, the majority of the participants completely recovered after three months, including 75% of the women who had lost their ability to walk before their pregnancy. If breastfeeding was possible (sometimes with assistive technology) for the 52% of the women who considered it, one in five had to give it up (fatigue, pain, lack of milk, etc.).
The authors recommended considering perinatal monitoring, a specialist obstetric review and an occupational therapy consultation before and after giving birth.
Ursula Moore et al. Neuromuscul Disord. 2022 Dec.

Using MRI to evaluate FSHD

Magnetic resonance imaging (MRI)
MRI is a medical imaging technique which is useful for examining muscles. For example, it makes it possible to quantify the degree of muscle fat infiltration (when muscle cells are destroyed, they are replaced by fat cells).

▪ An international consortium of researchers designed novel techniques and algorithms to study muscle degeneration in 17 adult FSHD patients. These patients underwent whole-body musculoskeletal MRI scans several weeks apart. The researchers used muscle fat infiltration and muscle fat fraction as relevant endpoints, all compared to clinical severity items. This type of imaging could prove to be a valuable biomarker for monitoring the course of the disease but also in future clinical trials.
Mellion ML et al. Neurology. 2022 June.

*A biological marker (or **biomarker** for short) is a measurable characteristic that indicates a normal or pathological biological process. Identifying new biological markers for a disease is very important for monitoring the course of the disease and the efficacy of new treatments. These markers can be physiological (change in blood pressure, heart rate, etc.) or molecular (change in the expression of a protein, etc.).*

▪ An American team performed MRIs of the lower limbs as well as muscle biopsies in the tibialis anterior muscles (the front of the shin) in 34 FSHD patients. The results showed a trend towards a strong association between the MRI data, the expression of genes regulated by DUX4 in the biopsies and the disease's activity.
Wong CJ et al. bioRxiv [preprint]. 2023 Feb.

A function scale to evaluate facial muscle weakness

Facial muscle weakness is part of the classic description of FSHD, particularly around the eyes and mouth.

▪ Dutch researchers created a new scale in order to assess its significance and functional impact. Preliminary questionnaires for patients regarding their experiences of the disease in this area of the body enabled the researchers to create and improve the scale. A new approach derived from the Rasch method was used and significantly saved time during the analysis. This scale could thus be used as part of clinical monitoring or in clinical trials.
Mul K et al. Disabil Rehabil. 2022 May.

A closer look at some conferences

*The **European Neuromuscular Centre (ENMC)** is an international organisation which aims to support research in the field of neuromuscular diseases. It regularly organises meetings on a given topic which bring together scientists and clinicians from around the world.*
WEBSITE www.enmc.org/

FSHD featured in workshops and conferences

▪ **The ENMC** organised two workshops, one on muscle imaging in FSHD with the goal of planning future clinical trials (22-24 April 2022), and the other on genetic diagnosis, clinical classification, outcome measures, and biomarkers for the implementation of future clinical trials in FSHD (30 September-2 October 2022). These two workshops were set up at the request of the FSHD European Trial Network, a network which was created with the aim of bringing together European FSHD experts, facilitating the implementation of clinical trials and making future treatments more accessible.
Monforte M et al. Neuromuscul Disord. 2023 Jan.
Montagnese F et al. Neuromuscul Disord. 2023 May.

▪ The **2022 FSHD International Research Congress**, organised by the FSHD Society and held on 16-17 June 2022 in the United States, allowed attendees to reflect on their understanding of the disease and discuss the therapeutic strategies currently being studied. The **2023 FSHD International Research Congress** was held on 15-16 June in Milan (Italy) and included discussions on clinical trials, research and genetics and infantile FSHD.
Leung DG et al. Neuromuscul Disord. 2023 Feb.

▪ At the **Myology 2022** congress, organised by AFM-Téléthon and held on 12-16 September in Nice, a session was dedicated to FSHD and included topics such as inflammatory responses, induced pluripotent stem cells and clinical and genetic heterogeneity.

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