

Myotubular Trust conference - 070908

# **Causes and Diagnoses of myotubular / centronuclear myopathies**

Jocelyn Laporte  
IGBMC – Illkirch - France



# Illkirch – Strasbourg



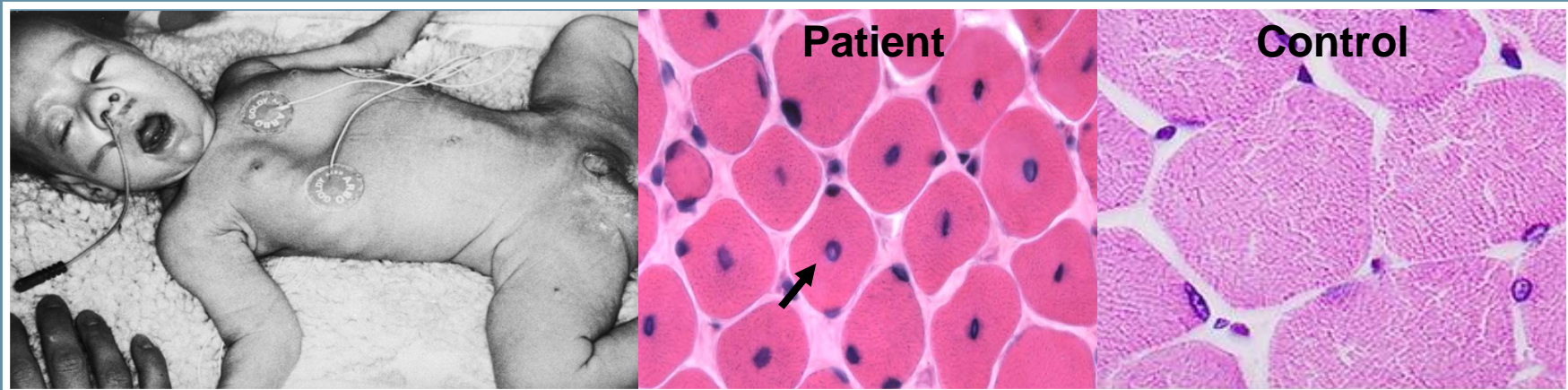
**IGBMC**

# IGBMC

(Institut de Génétique et de Biologie Moléculaire et Cellulaire)



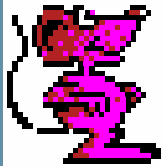
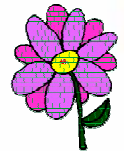
# Centronuclear / myotubular myopathies (CNM)



Muscle weakness

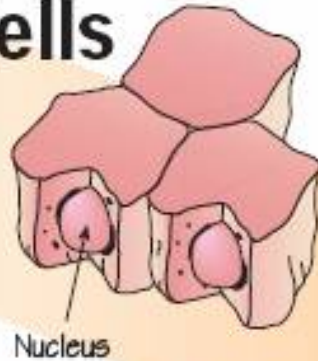
Smaller fibers with central nuclei

CNM	X-linked (XLCNM)	Autosomal recessive (ARCNM)	Autosomal dominant (ADCNM)	Sporadic
Protein	Myotubularin	Amphiphysin 2	Dynamin 2	hJUMPY, RYR1
Severity /onset	+++ / birth	++ / childhood	+ / adulthood	++



**Body**

**Cells**



*Most of the 100 million million cells from which a human is made contain 23 pairs of chromosomes. The DNA from which they are composed includes 50 to 100 thousand genes, which are the instructions required for assembling proteins from amino acids.*

**Chromosomes**



**DNA**



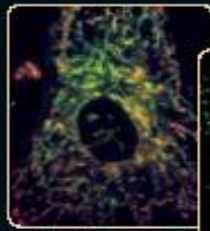
**Protein**

**Amino acids**

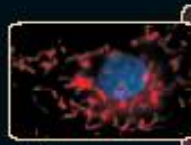


# The Illuminated Cell

Product Guide for Fluorescence Imaging



**Mitochondria**  
 M7512 MitoPro<sup>™</sup> dextr<sup>™</sup> Red C100Fluo  
 M7514 MitoPro<sup>™</sup> dextr<sup>™</sup> Green Fluo  
 M7510 MitoPro<sup>™</sup> dextr<sup>™</sup> Orange CMT10Fluo  
 S25115 SelectPro<sup>™</sup> Alexa Fluor<sup>™</sup> 488 Cytocrome c Labeling Kit  
 T2108 JC-1  
 A6405 anti-cytochrome c oxidase subunit I



**Nucleus**  
 D1205 DAPI  
 H2500 Hoechst 33258  
 S2028 SYTO<sup>™</sup> R Green  
 S11548 SYTO<sup>™</sup> R Orange  
 T2605 TO-PRO-3<sup>™</sup> iodide



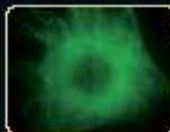
**Nucleoli**  
 S22700 SYTO<sup>™</sup> RNASelect<sup>™</sup> green fluorescent cell stain



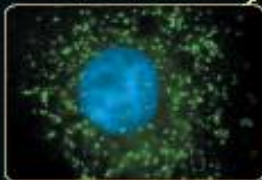
**Endoplasmic Reticulum**  
 S12552 DR-Tracker<sup>™</sup> Blue-WB Fluo  
 S24280 SelectPro<sup>™</sup> Alexa Fluor<sup>™</sup> 488 Endoplasmic Reticulum Labeling Kit  
 S27447 Invitrogen<sup>™</sup> Alexa Fluor<sup>™</sup> 555/568 conjugated "fluorose" 7



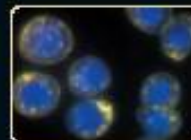
**Plasma Membrane**  
 S25165 FM1-43FX<sup>™</sup> Thapsigargin analog of FM1-43 membrane stain<sup>™</sup>  
 M28455 MitoPro<sup>™</sup> dextr<sup>™</sup> cell labeling solution  
 M28460 MitoPro<sup>™</sup> dextr<sup>™</sup> cell labeling solution  
 M28467 MitoPro<sup>™</sup> dextr<sup>™</sup> cell labeling solution  
 W11262 Alexa Fluor<sup>™</sup> 594-wheat germ agglutinin



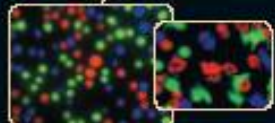
**Cytoskeleton/Tubulin**  
 F22218 Oregon Green<sup>™</sup> 488 Taxol  
 A11126 anti-galectin



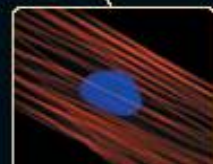
**Peroxisomes**  
 S24201 SelectPro<sup>™</sup> Alexa Fluor<sup>™</sup> 488 Peroxisome Labeling Kit



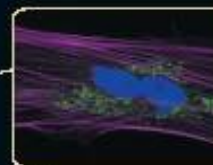
**Lipid Rafts**  
 B12930 1:00 Biotinylated Cytochrome c  
 V24400 Vybrant<sup>™</sup> Alexa Fluor<sup>™</sup> 488 Lipid Raft Labeling Kit  
 V24404 Vybrant<sup>™</sup> Alexa Fluor<sup>™</sup> 555 Lipid Raft Labeling Kit  
 V24405 Vybrant<sup>™</sup> Alexa Fluor<sup>™</sup> 594 Lipid Raft Labeling Kit



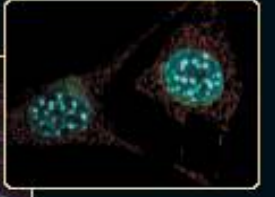
**Cytosol**  
 C210847 calcein AM  
 C2925 CellTracker<sup>™</sup> Green CMFDA  
 C4552 CellTracker<sup>™</sup> Red CMFDA



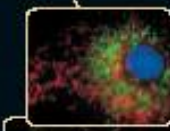
**Cytoskeleton/Actin**  
 A12279 Alexa Fluor<sup>™</sup> 488 phalloidin  
 R415 rhodamine phalloidin  
 A12881 Alexa Fluor<sup>™</sup> 594 phalloidin



**Golgi**  
 A21276 anti-galectin-37  
 N62651 NBD-Carboxy-1,5-ansamycin complexed to BSA  
 S22658 NBD-PP1FLC<sub>2</sub>-carboxy-1,5-ansamycin complexed to BSA  
 S24408 NBD-PP1FLC<sub>2</sub>-carboxy-1,5-ansamycin complexed to BSA



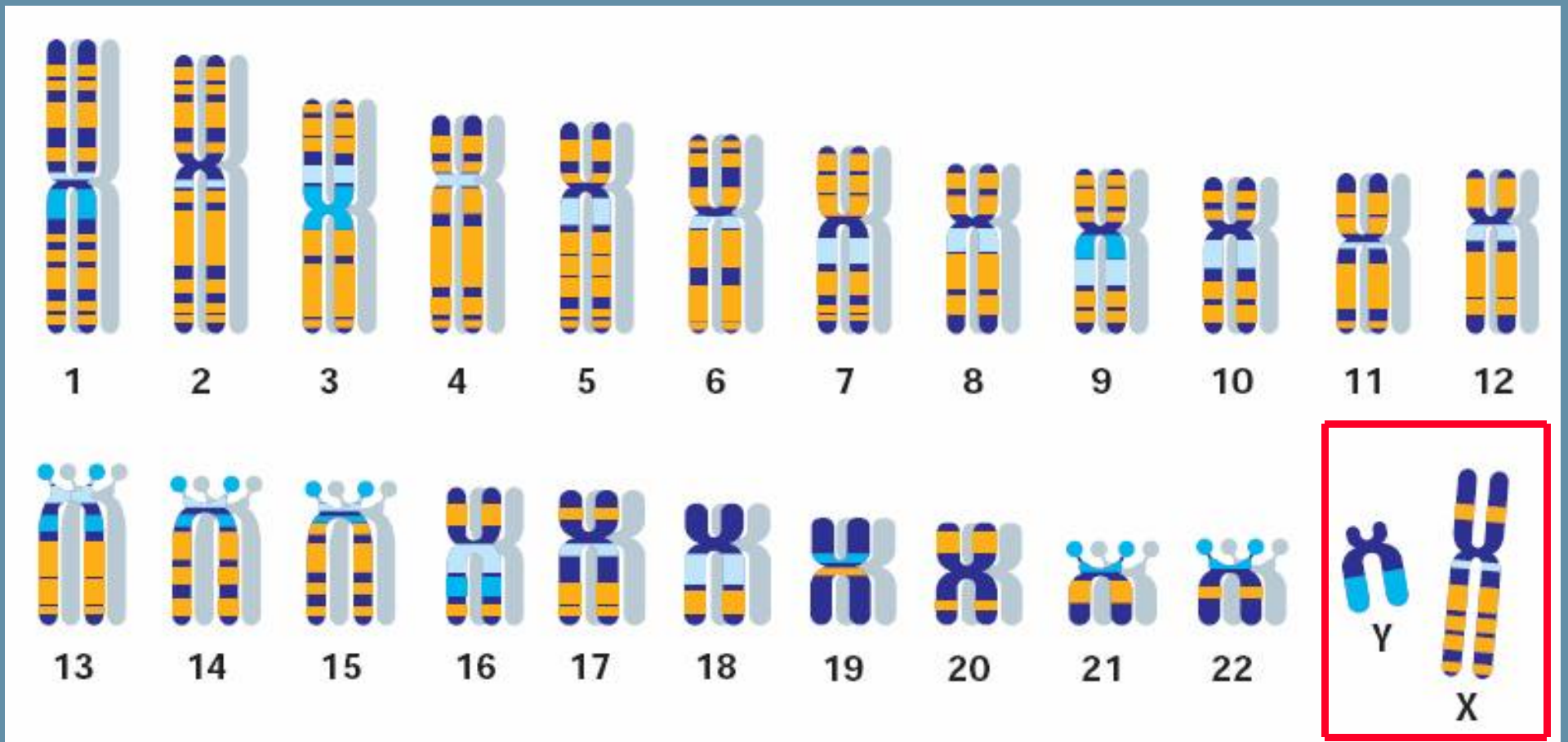
**Cytosolic Biosensors**  
**Cytosolic Ca<sup>2+</sup>**  
 F1229 fura-2, AM  
 F1221 fura-2, AM  
 F14261 fura-4, AM  
 B12458P fura-2, AM  
**Cytosolic Mg<sup>2+</sup>**  
 M14286 mag-fura-4, AM  
 M1200 mag-fura-2, AM  
**Zn<sup>2+</sup>**  
 S24185 fluoZn-2, AM  
 E30251 fluoZn-1, AM  
**Cytosolic pH**  
 B1150 BCECF, AM  
 C1222 SNARE<sup>™</sup>-1, acetoxyethyl ester, acetate  
**Cytosolic ROS**  
 D882P Cl-1,2-DCE DA (DCE 1-galactose)  
 D11247 4-BiFC-1,2-DCE DA (hydroxyl radical) (1-galactose)  
**Cytosolic RNAsi**  
 A21245 anti-ribonuclease, rabbit IgG fraction  
 D28942 DAPI nuclear stain



**Lysosomes**  
 L7528 LysoTracker<sup>™</sup> Red DND-99  
 L7526 LysoTracker<sup>™</sup> Green DND-26  
 L7545 LysoSensor<sup>™</sup> Yellow/Blue DND-100

**Molecular Probes**  
 Invitrogen detection technologies  
[www.probes.com](http://www.probes.com) | [www.invitrogen.com](http://www.invitrogen.com)

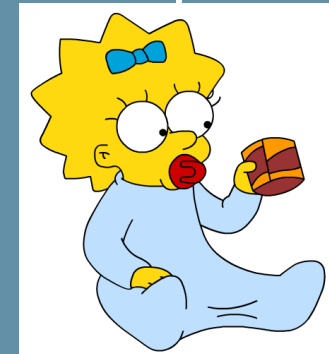
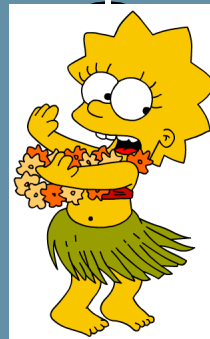
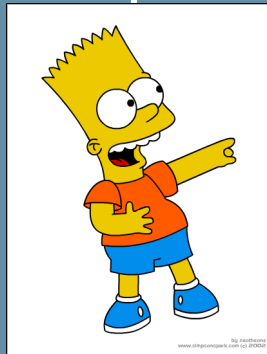
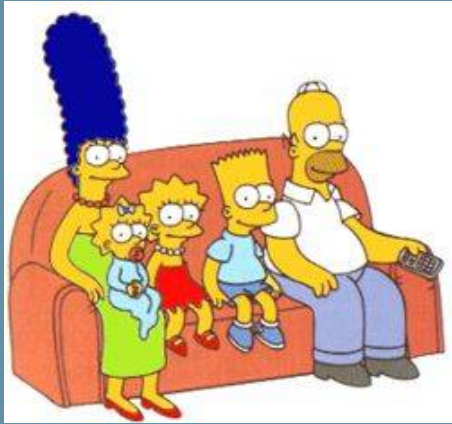
# Chromosome pairs



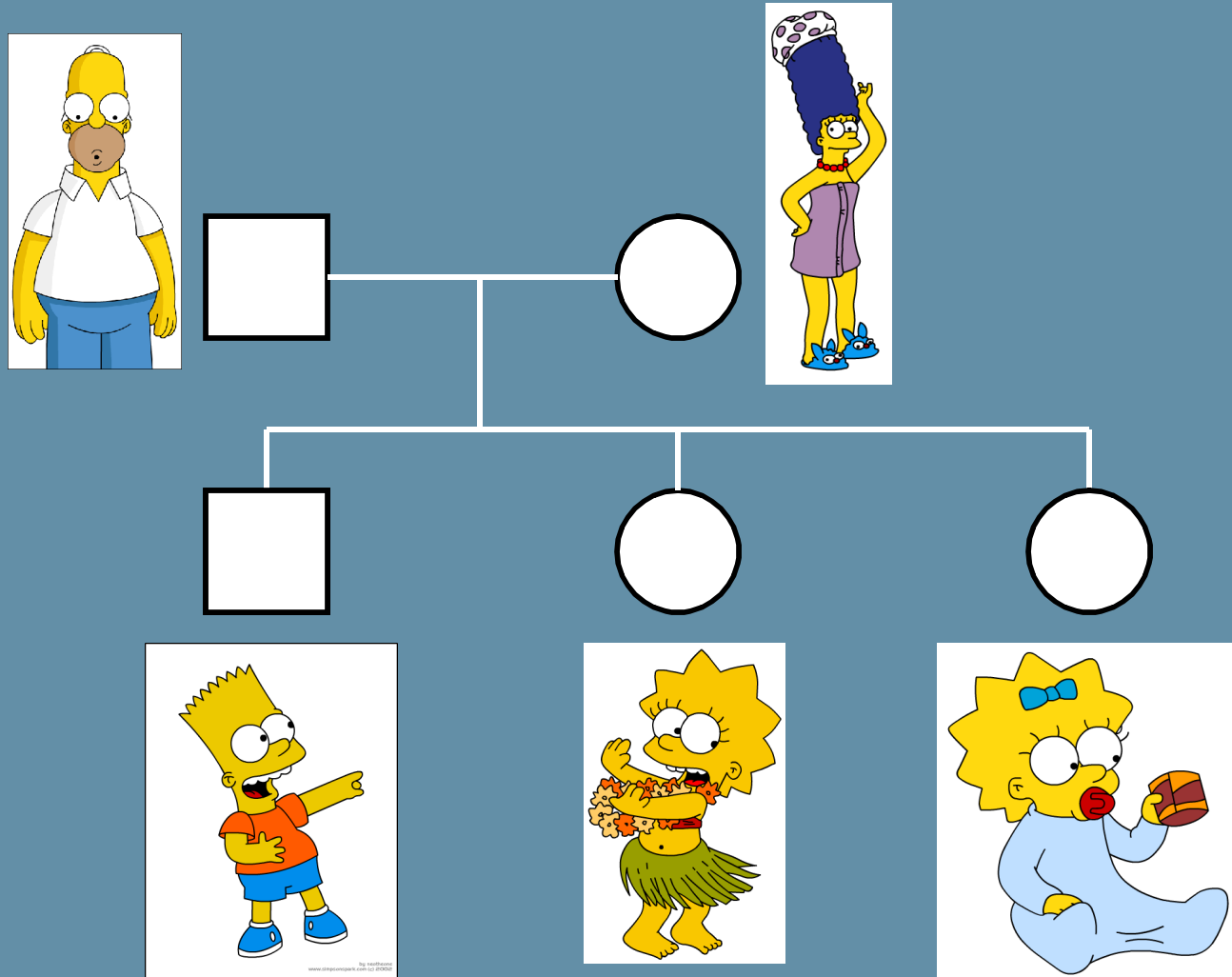
<http://www.eibe.info/>

Sexual chromosomes = determine sex

# The Simpsons

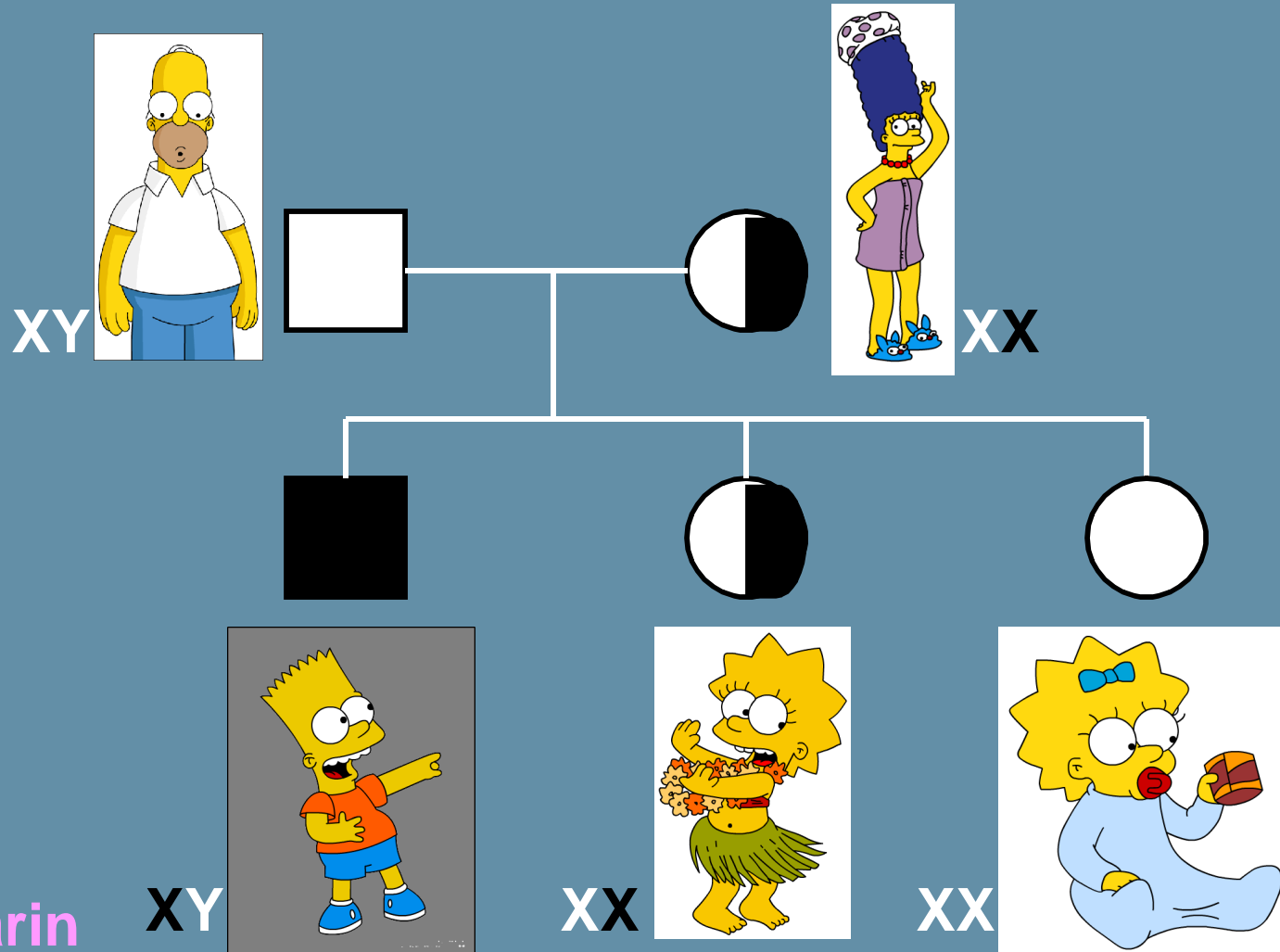


# The Simpsons



# X-linked centronuclear myopathy (= myotubular myopathy)

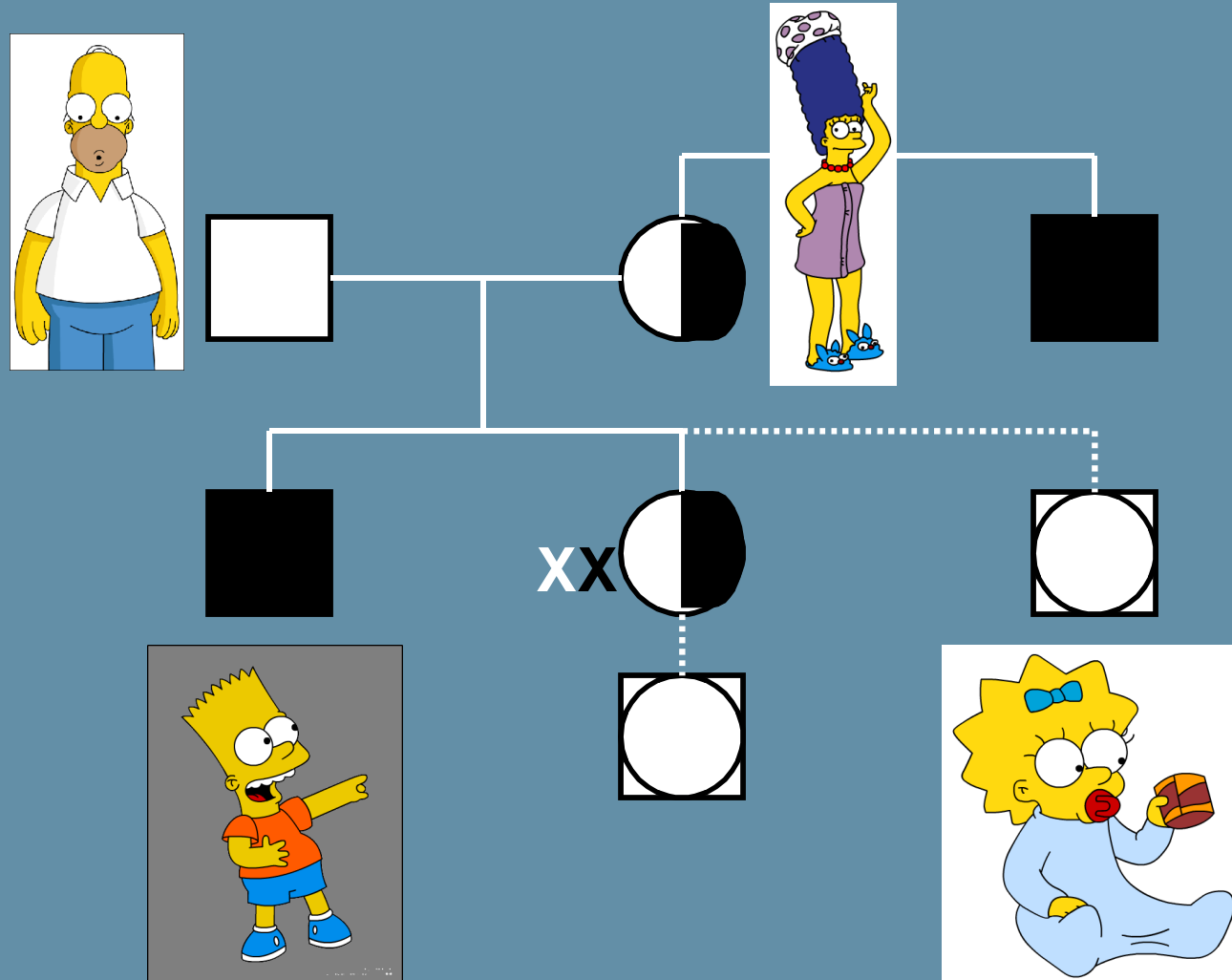
X chromosome



Myotubularin  
(MTM1)

# X-linked centronuclear myopathy (= myotubular myopathy)

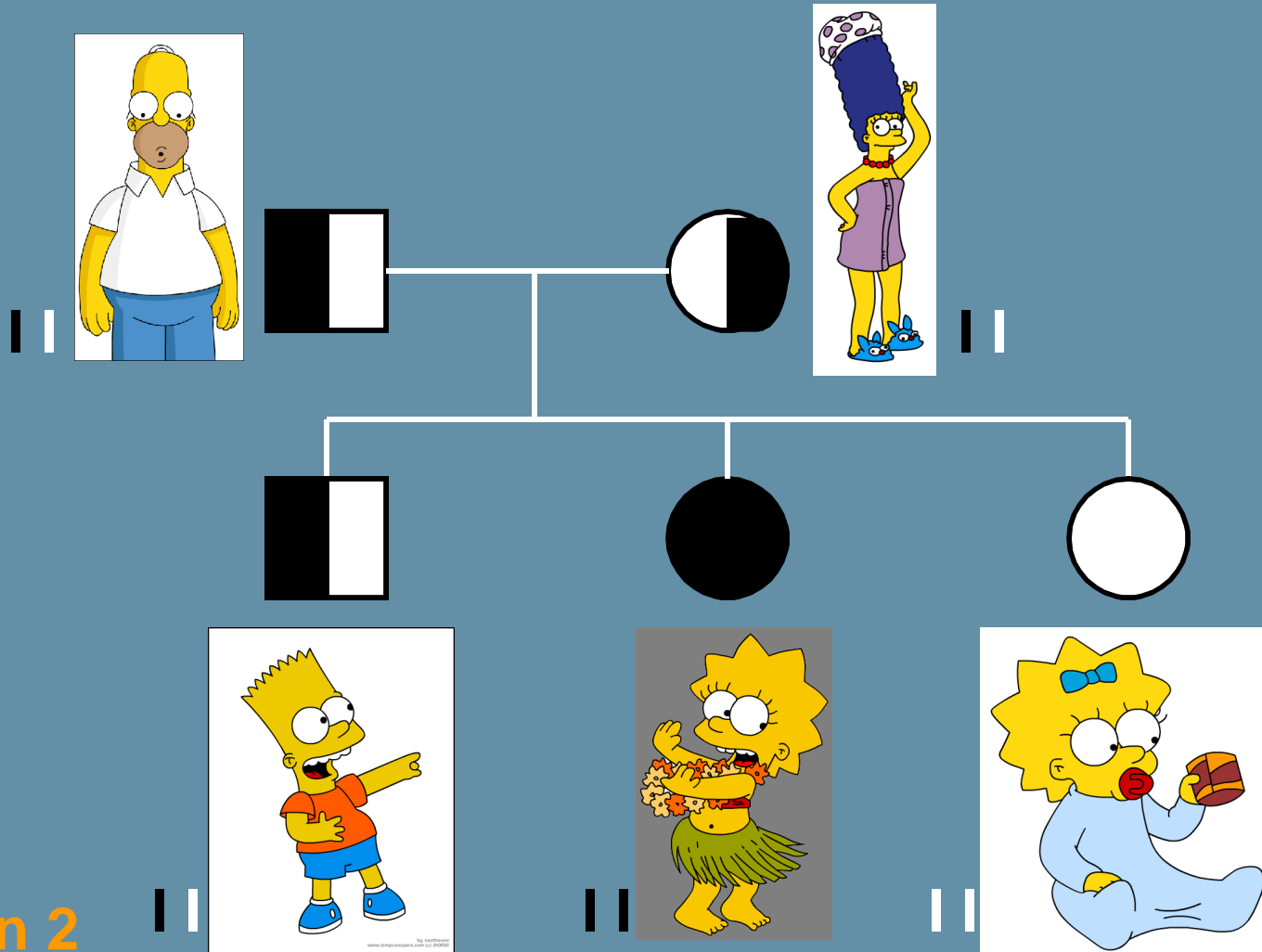
X chromosome



Myotubularin  
(MTM1)

# Autosomal recessive centronuclear myopathy

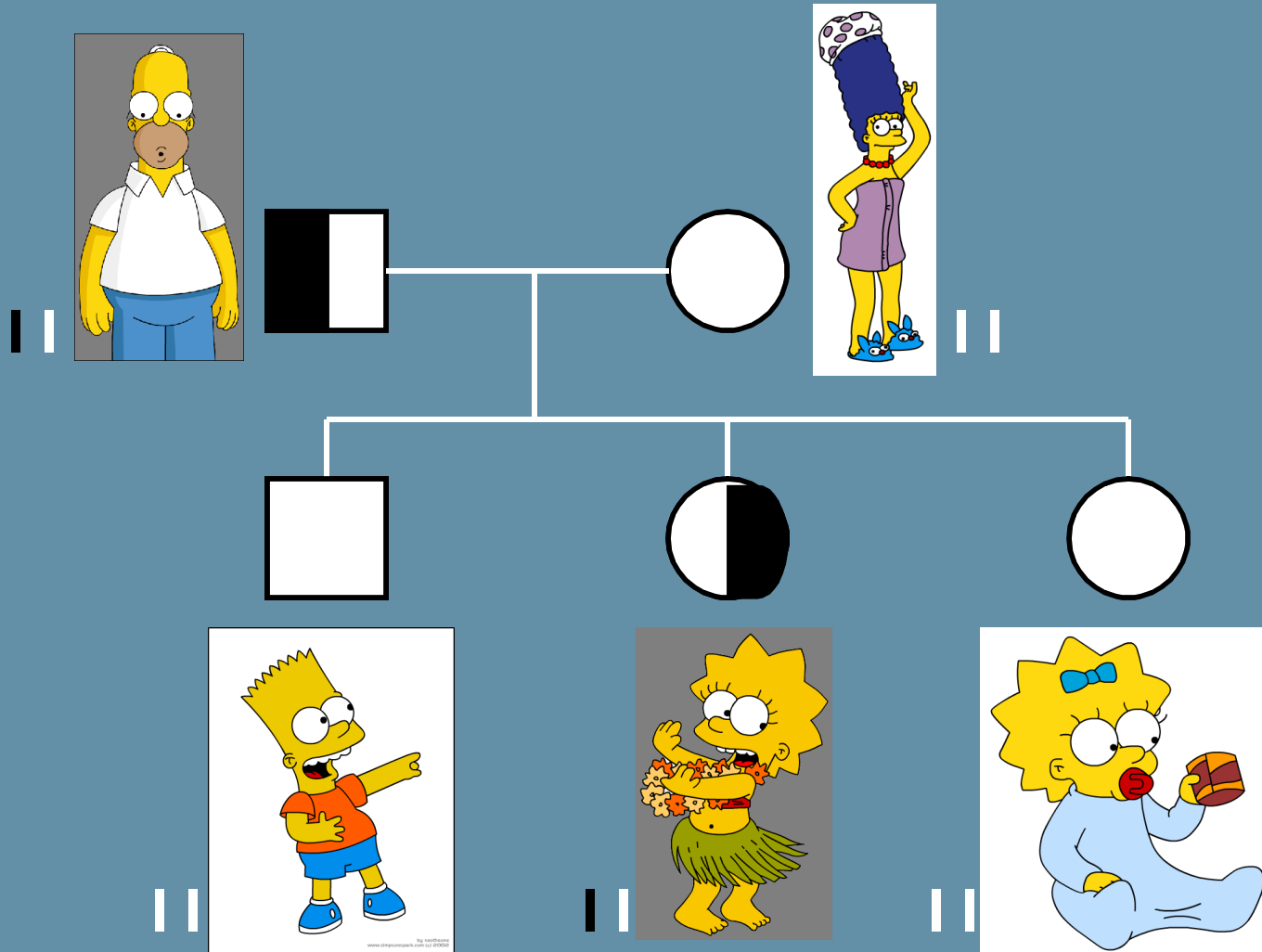
« Weak » mutation



Amphiphysin 2  
(BIN1)

# Autosomal dominant centronuclear myopathy

« Strong » mutation

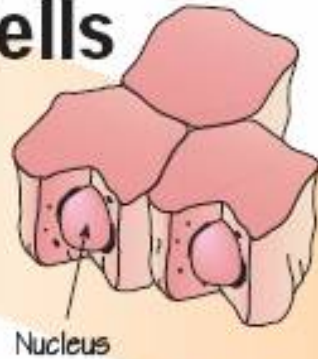


**Dynamin 2  
(DNM2)**



**Body**

**Cells**



Nucleus

*Most of the 100 million million cells from which a human is made contain 23 pairs of chromosomes. The DNA from which they are composed includes 50 to 100 thousand genes, which are the instructions required for assembling proteins from amino acids.*

**Chromosomes**



**DNA**



**Protein**

**Amino acids**



# Mutations and implicated genes

GATTACA ATAG CTTA



GATTACA ATAC CTTA

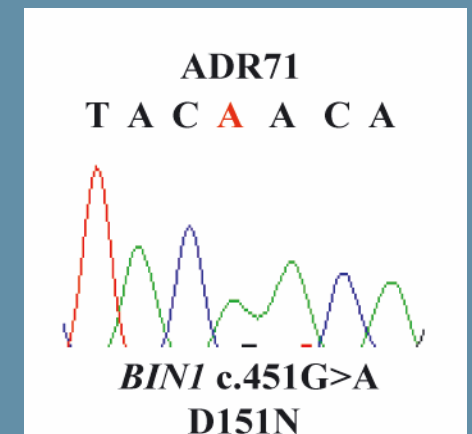


Letter change:  
unnoticed  
beneficial  
mutation => disease

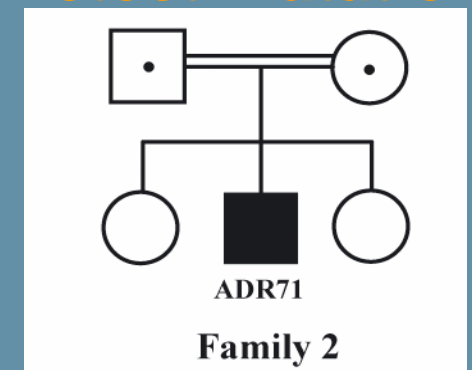
Patients samples



Reading DNA



Detect mutation



# Gene identification

Data from molecular analysis  
(research lab)

DNA/samples from patients  
55 patients without mutations in known genes

Hypothesis

Identification of amphiphysin 2 as a causative gene

Data released in the journal Nature Genetics (september 2007) :

- First link between 2 forms of the disease (recessive + dominant)
- Hypothesis on the mechanisms of centronuclear myopathy

# Molecular diagnoses

- Blood sample : DNA
- Blood or skin : cells (DNA and protein)
- Muscle biopsy : histology and proteins

350 patients received :

220 mutated in MTM1 - myotubularin

15 mutated in DNM2 - dynamin

3 mutated in BIN1 - amphiphysin

~100 without known cause (**1/3**), mainly sporadic or recessive

=> Other unknown genes

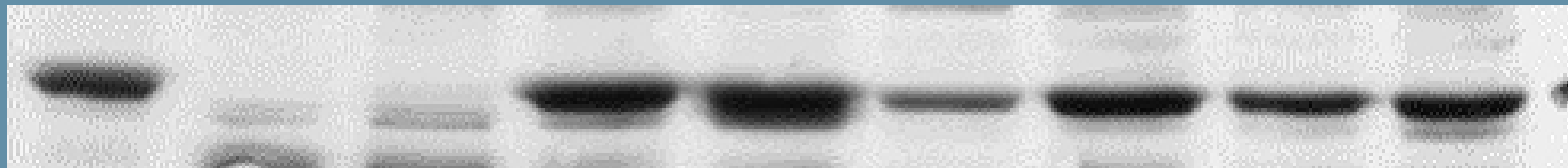
# Reading DNA



© Applied Biosystems

## Use of cells and biopsies:

$\alpha$ -MTM1



# Molecular diagnoses

- Preimplantation
- Prenatal
- Confirmation of clinical diagnosis
- Primary care : minimizing the effects of the disease
- Prognosis (requires exhaustive molecular / clinical comparison)
- Prerequisite to apply specific therapeutics

# Research projects

**Patients**

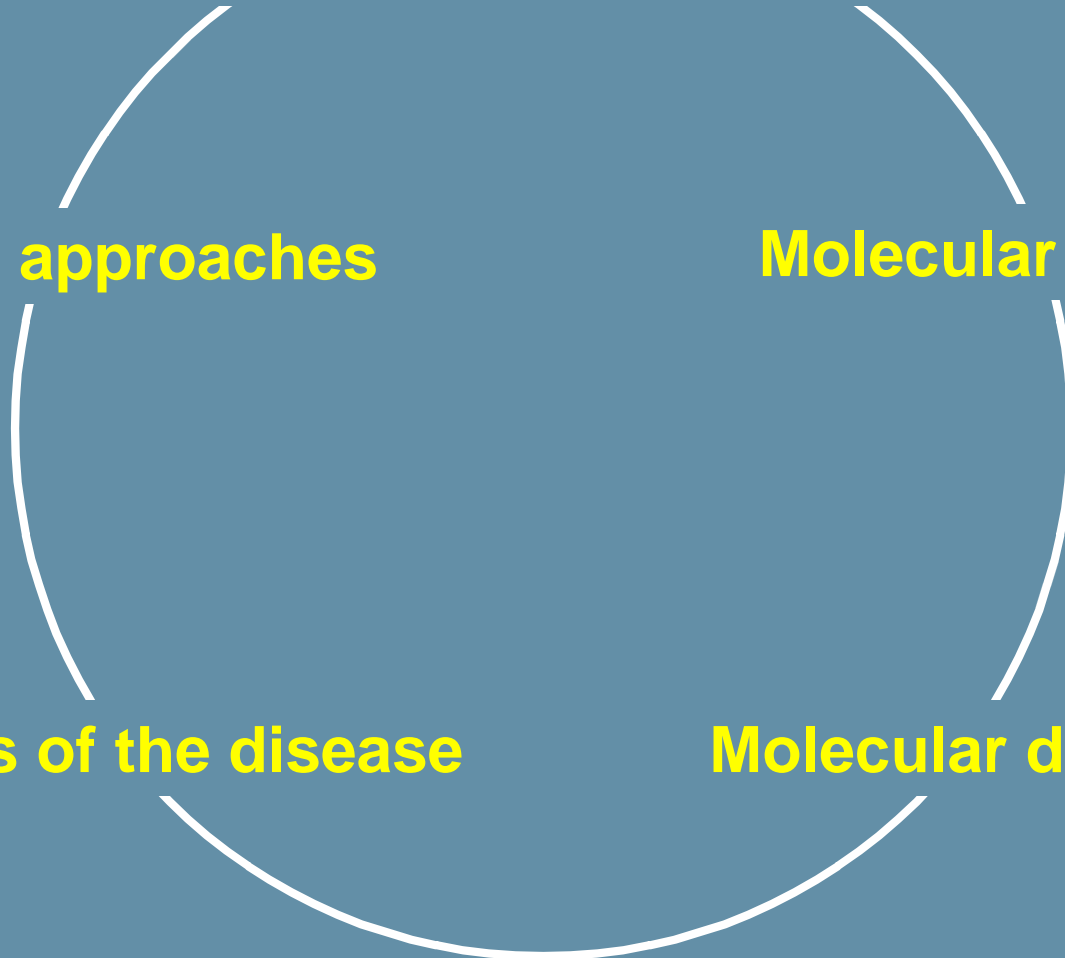
**Myotubular / centronuclear myopathies**

**Therapeutic approaches**

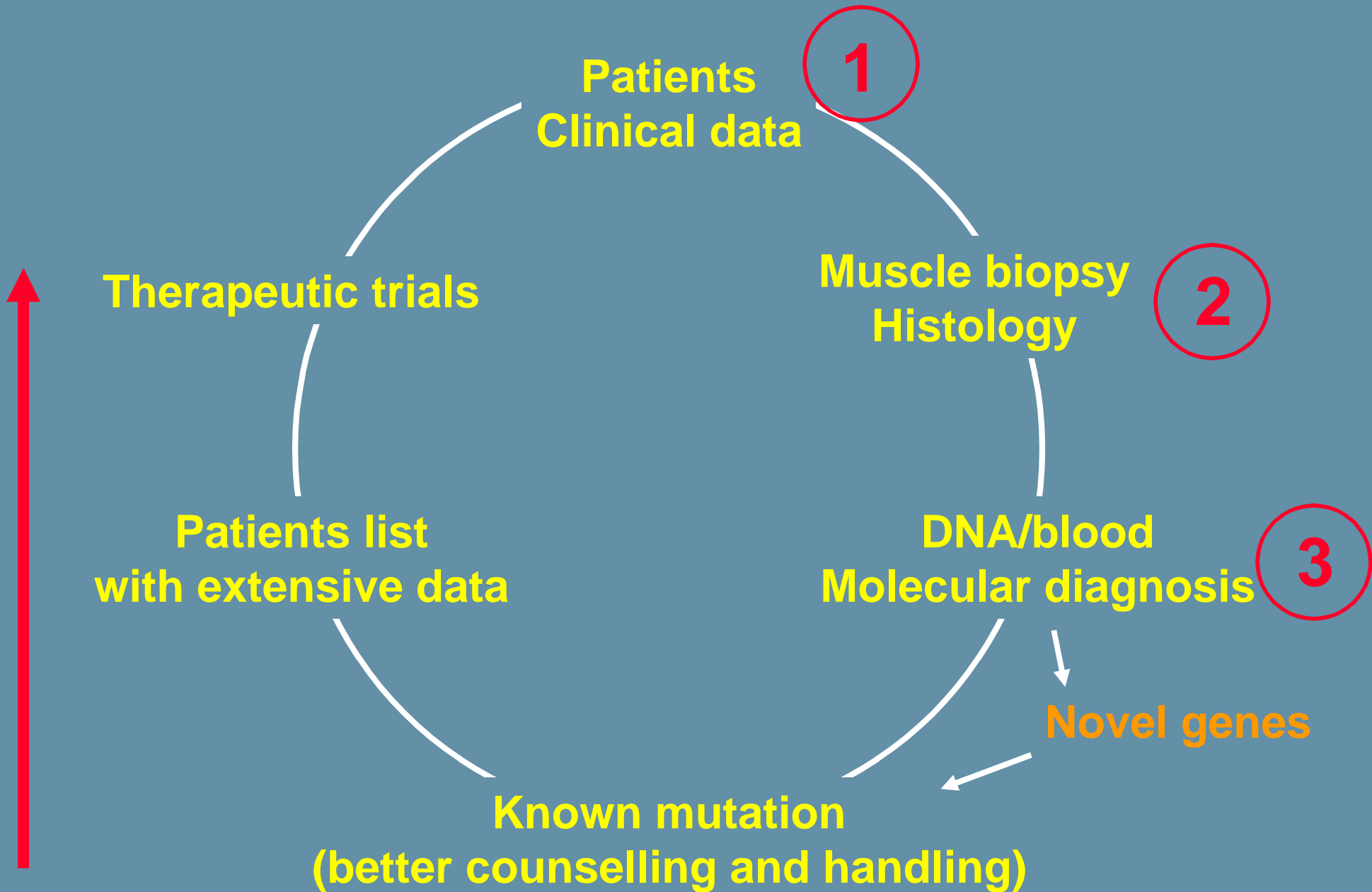
**Molecular causes**

**Mechanisms of the disease**

**Molecular diagnosis**



# Extensive Patients and Data recruitment



# Acknowledgements

## TEAM

**Mandel Jean-Louis**  
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**Nicot Anne-Sophie**  
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**Tosch Valérie**  
**Toussaint Anne**

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**Helen Kingston**  
**Anders Oldfors**  
**Carina Wallgren-Pettersson**

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- **Agence Nationale de la Recherche (ANR)**
- **Association Française contre les Myopathies (AFM)**