

Myotubular Trust conference - 070908

Causes and Diagnoses of myotubular / centronuclear myopathies

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Illkirch – Strasbourg



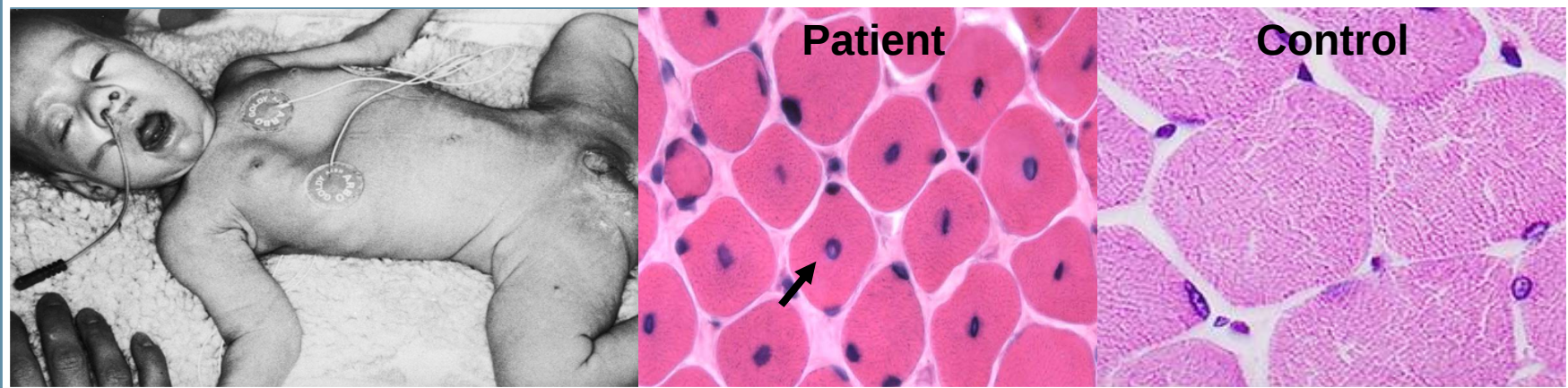
IGBMC

IGBMC

(Institut de Génétique et de Biologie Moléculaire et Cellulaire)



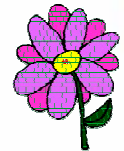
Centronuclear / myotubular myopathies (CNM)



Muscle weakness

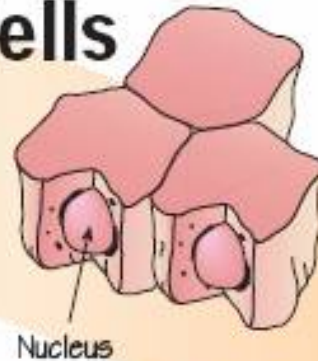
Smaller fibers with central nuclei

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



Chromosomes



DNA



Amino acids

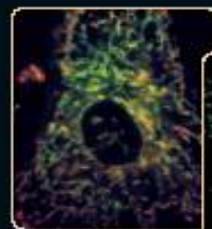


Protein

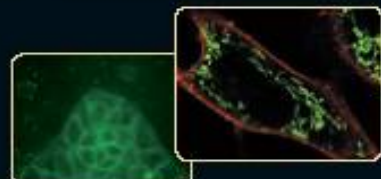
Most of the 100 million million cells from which a human is made contain 23 pairs of chromosomes. The DNA from which they are composed includes 50 to 100 thousand genes, which are the instructions required for assembling proteins from amino acids.

The Illuminated Cell

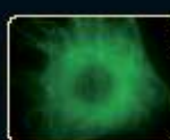
Product Guide for Fluorescence Imaging



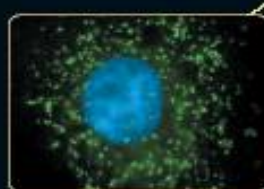
Mitochondria
M17512 MitoProbe dyes Red CMXRos
M17514 MitoProbe dyes Green F64
M17510 MitoProbe dyes Orange CMXRos
S25115 SelectPro™ Alexa Fluor® 488 Cytokeratin 8 Labeling Kit
T3168 JC-1
A6405 anti-cytokeratin cocktail antibody



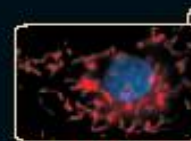
Plasma Membrane
D2565 FM1-43FX™ Double analog of FM1-43 membrane dye
V25655 MitoProbe dyes cell labeling solution
V25656 MitoProbe dyes cell labeling solution
V25657 MitoProbe dyes cell labeling solution
W11265 Alexa Fluor® 594 wheat germ agglutinin



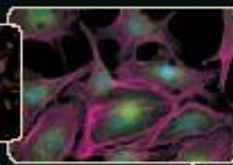
Cytoskeleton/Tubulin
F22218 Dragon Green® 488 stain
A11126 anti-galectin



Peroxisomes
S24201 SelectPro™ Alexa Fluor® 488 Peroxisome Labeling Kit



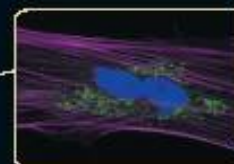
Nucleus
D1205 DAPI
H2500 Hoechst 33258
S2028 SYTO® Green
S11548 SYTO® Orange
T2605 TO-PRO-3 stain



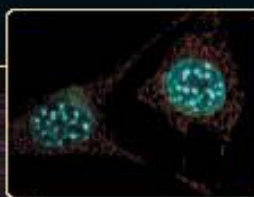
Nucleolus
S2703 SYTO® RNASelect™ green fluorescent cell stain



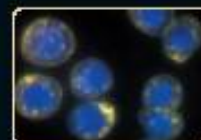
Endoplasmic Reticulum
S12512 DE-Tracker™ Blue-WB dye DAPI
S24280 SelectPro™ Alexa Fluor® 488 Endoplasmic Reticulum Labeling Kit
S27440 Invitrogen™ Alexa Fluor® 555/568 conjugated "donor" 1



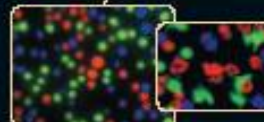
Golgi
A21276 anti-golgin-97
N25051 NBD C₆-carboxy-labeled to BSA
S25058 NBD C₆-carboxy-labeled to BSA
S24408 NBD C₆-carboxy-labeled to BSA



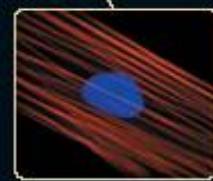
Cytosolic Biosensors
Cytosolic Ca²⁺
F1222 In de-1, AM
F1221 In de-2, AM
F14261 In de-4, AM
B13458P In de-2, AM
Cytosolic Mg²⁺
M14280 mag-fluo-4, AM
M1202 mag-fluo-2, AM
Zn²⁺
ZS4185 fluoZn™-2, AM
E36251 fluoZn™-1, AM
Cytosolic pH
B1150 BCECF, AM
C1222 SNARF-1, acetoxymethyl ester, acetate
Cytosolic ROS
O882P CM-H₂DCFDA (RO15 gaward)
D11247 4-BiPNS (hydroxide) (1000x)
Cytosolic RNAs
A21285 anti-ribosomal, rabbit IgG fraction
D25942 DAPI stain



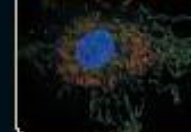
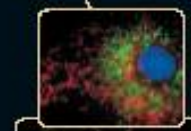
Lipid Rafts
B12930 1,6-Diphenyl-1,3,5-hexatriene
V24402 Vybrant™ Alexa Fluor® 488 Lipid Raft Labeling Kit
V24404 Vybrant™ Alexa Fluor® 555 Lipid Raft Labeling Kit
V24405 Vybrant™ Alexa Fluor® 594 Lipid Raft Labeling Kit



Cytosol
C21084 F-actin, AM
C2925 CellTracker™ Green CMFDA
C4552 CellTracker™ Red CMPTX



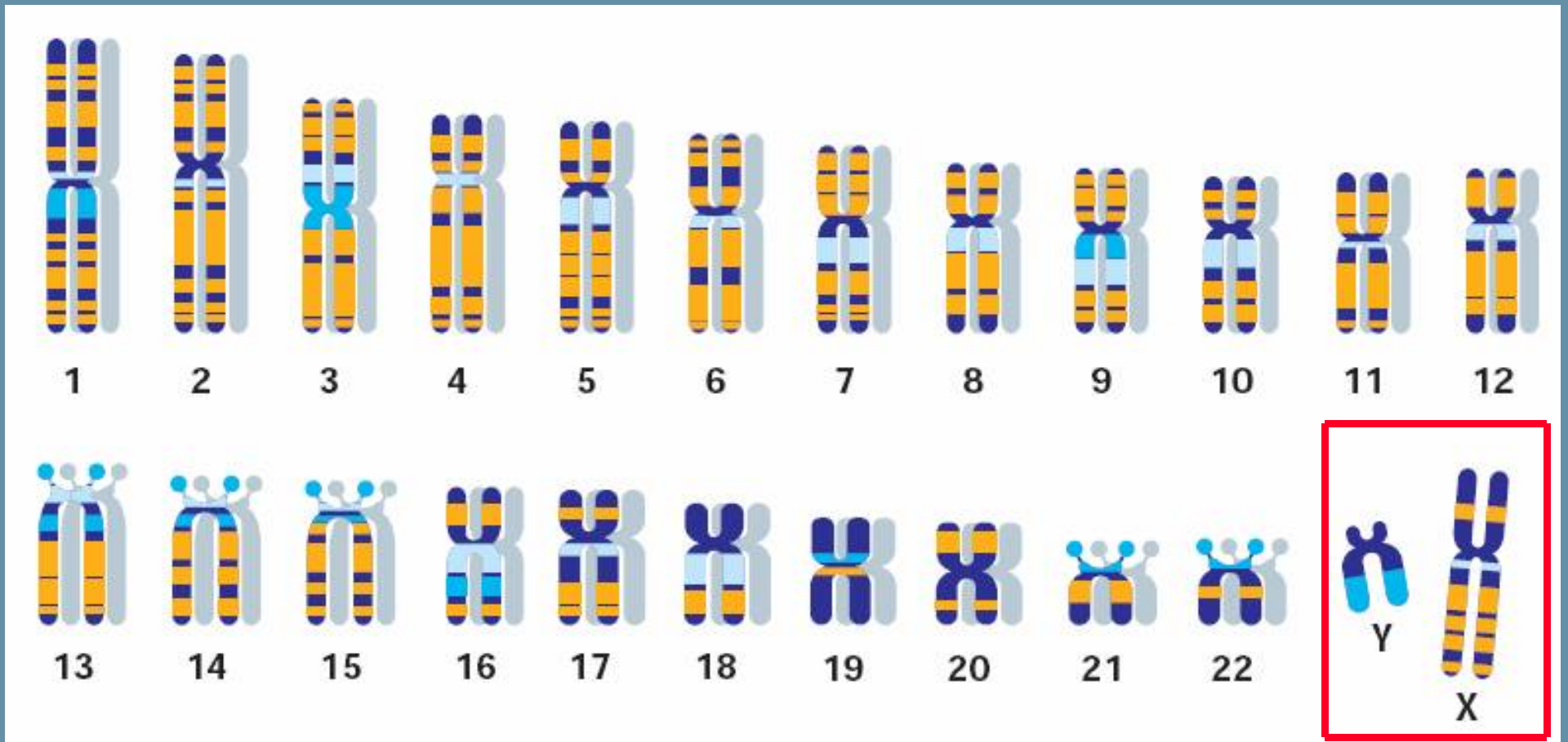
Cytoskeleton/Actin
A12279 Alexa Fluor® 488 phalloidin
M1515 Alexa Fluor® 555 phalloidin
A12281 Alexa Fluor® 594 phalloidin



Lysosomes
L7528 LysoTracker™ Red DND-89
L7526 LysoTracker™ Green DND-26
L7545 LysoSensor™ Yellow/Blue DND-159

Molecular Probes
Invitrogen detection technologies
www.probes.com | www.invitrogen.com

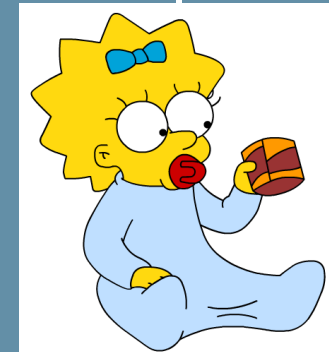
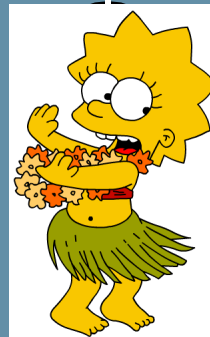
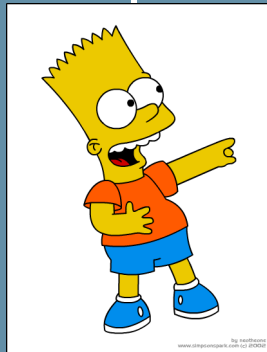
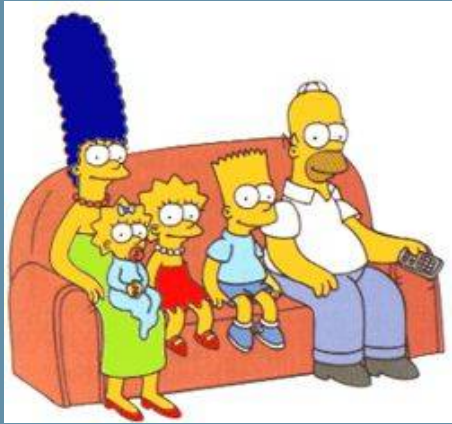
Chromosome pairs



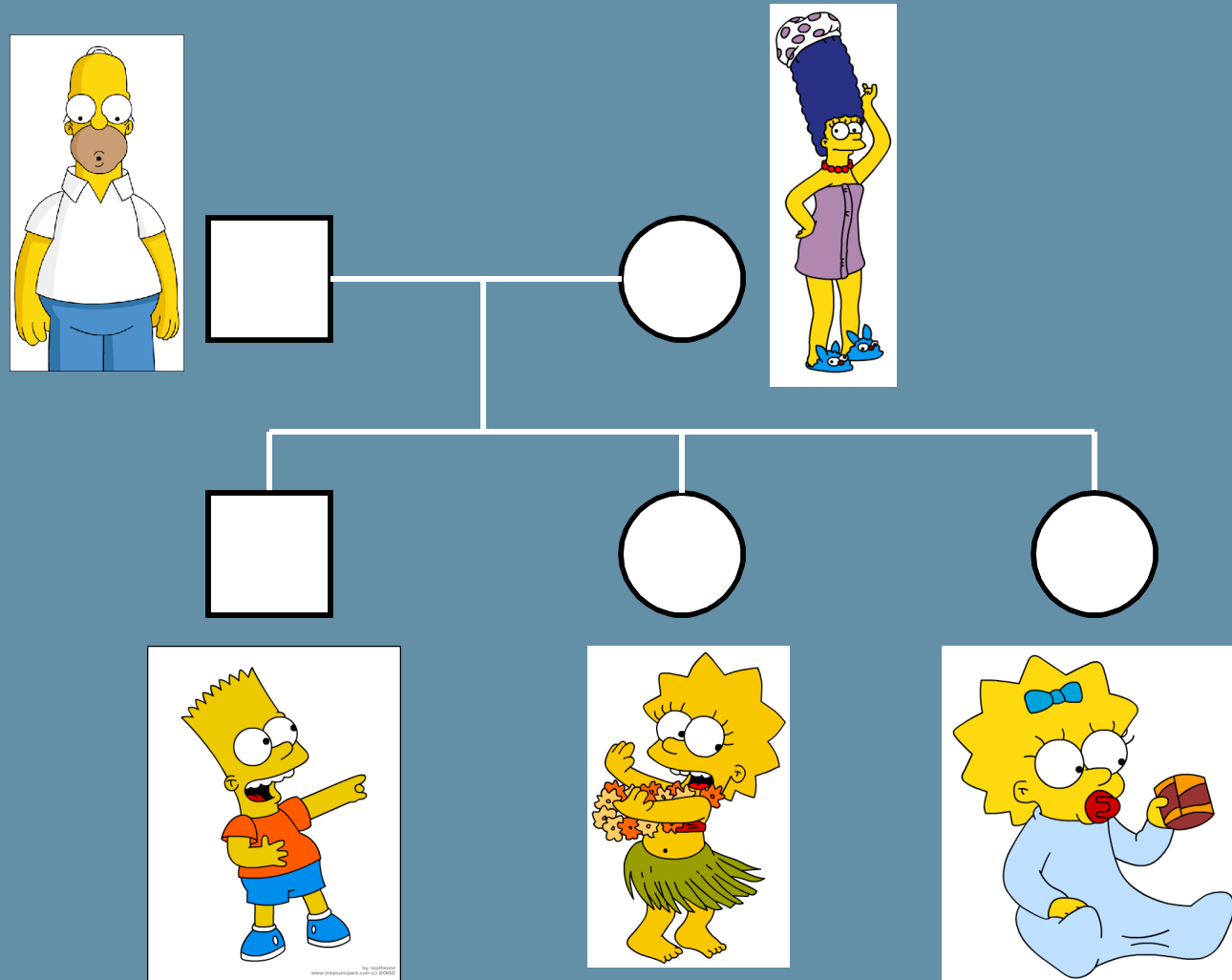
<http://www.eibe.info/>

Sexual chromosomes = determine sex

The Simpsons

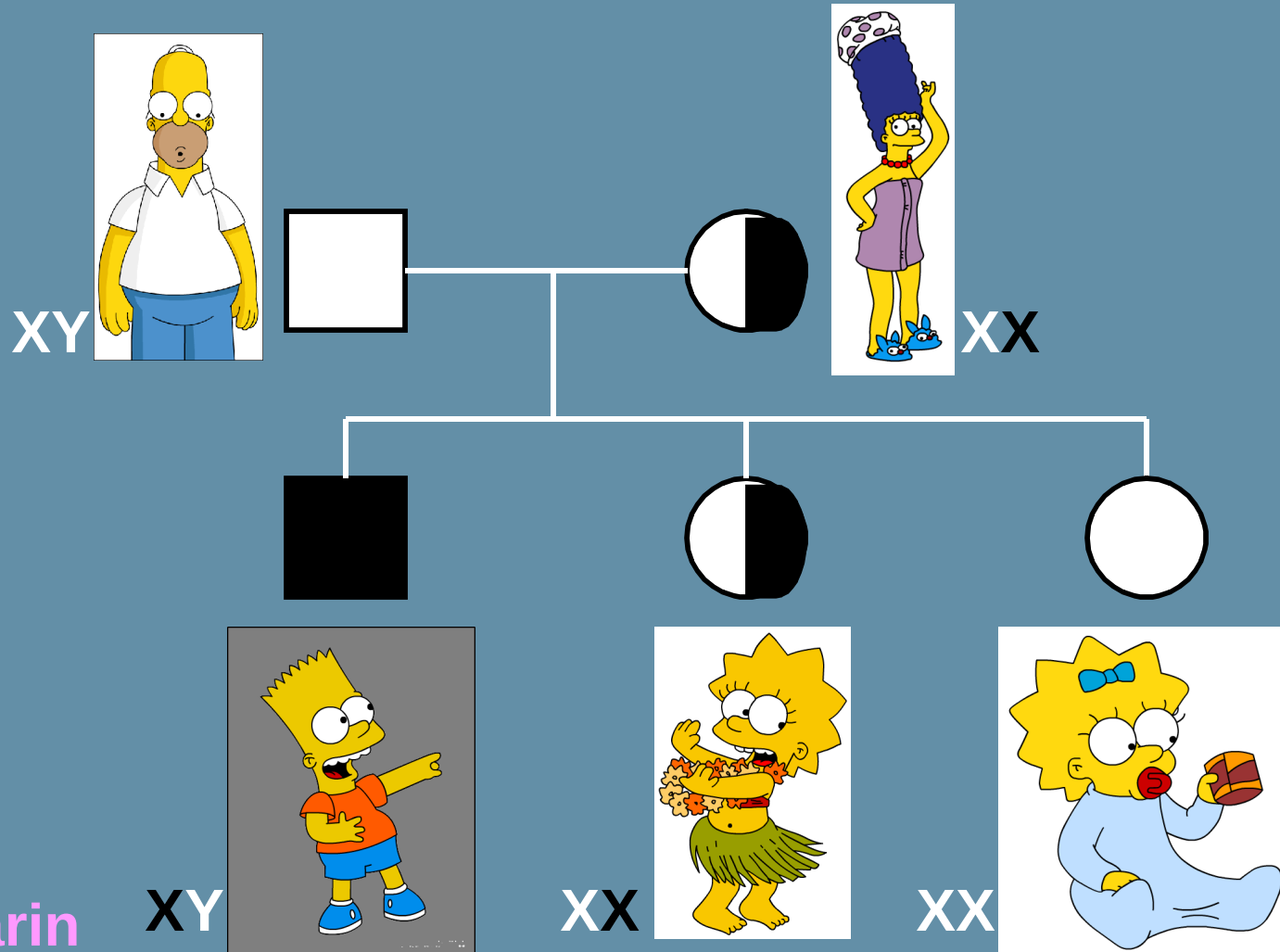


The Simpsons



X-linked centronuclear myopathy (= myotubular myopathy)

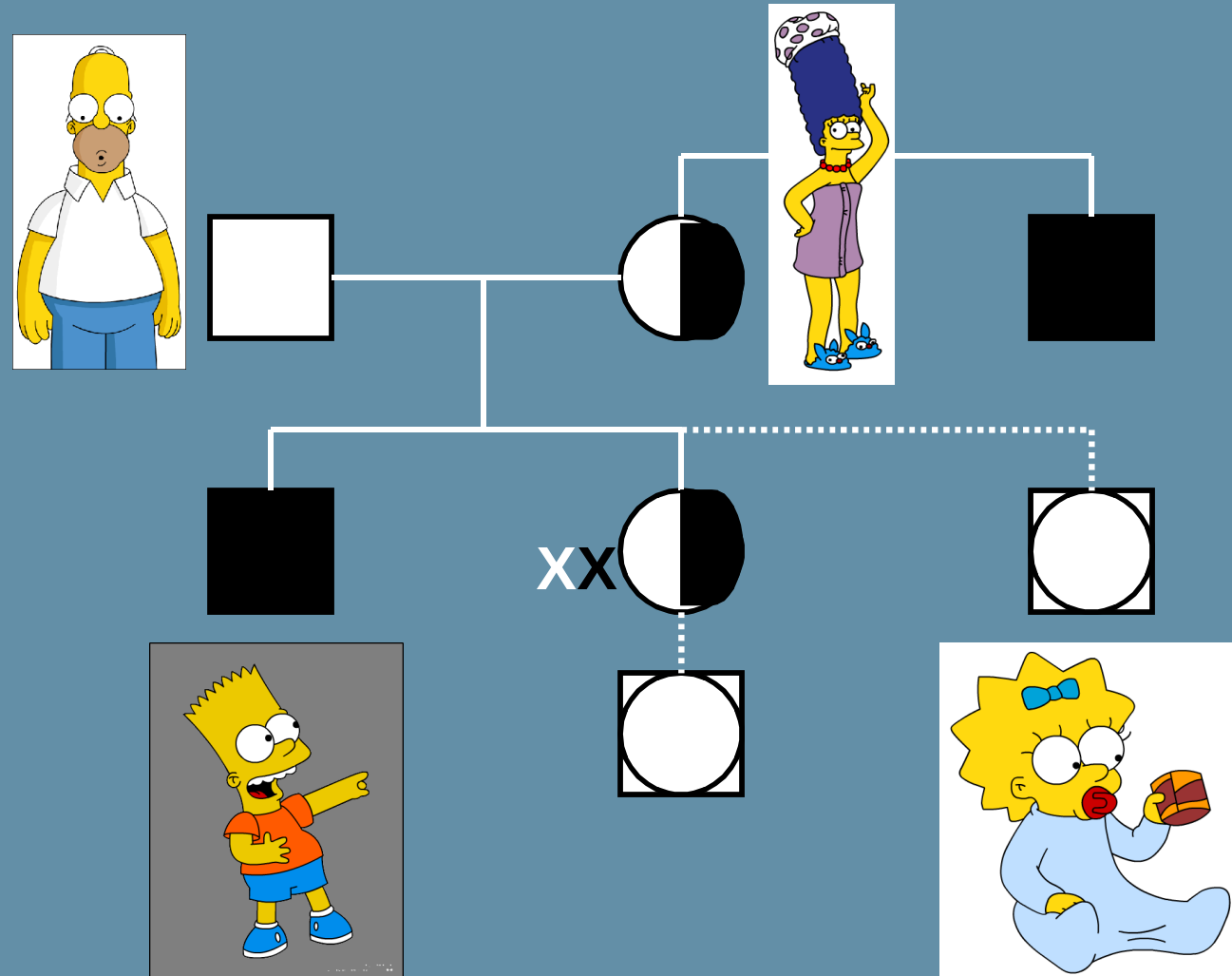
X chromosome



Myotubularin
(MTM1)

X-linked centronuclear myopathy (= myotubular myopathy)

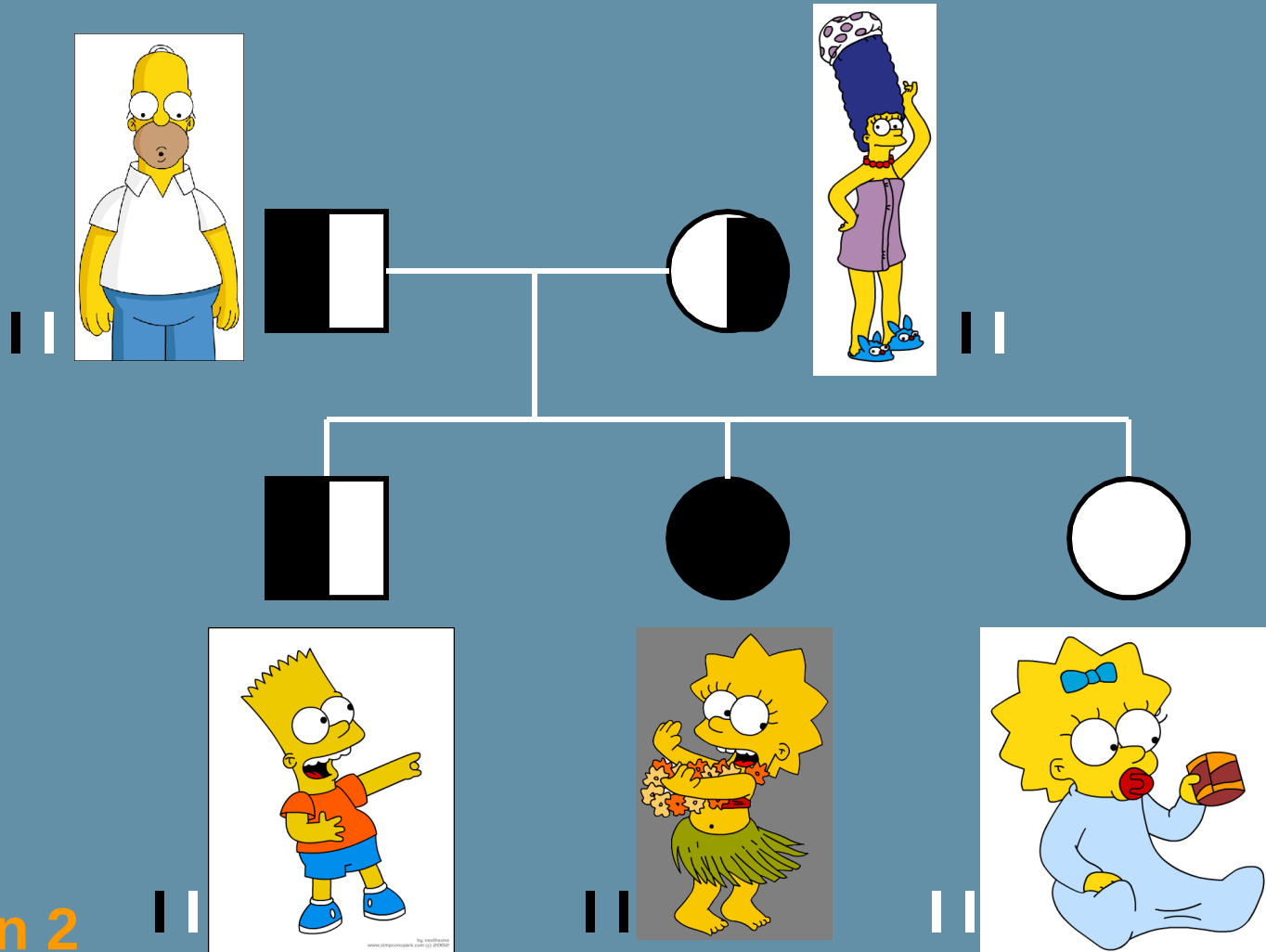
X chromosome



Myotubularin
(MTM1)

Autosomal recessive centronuclear myopathy

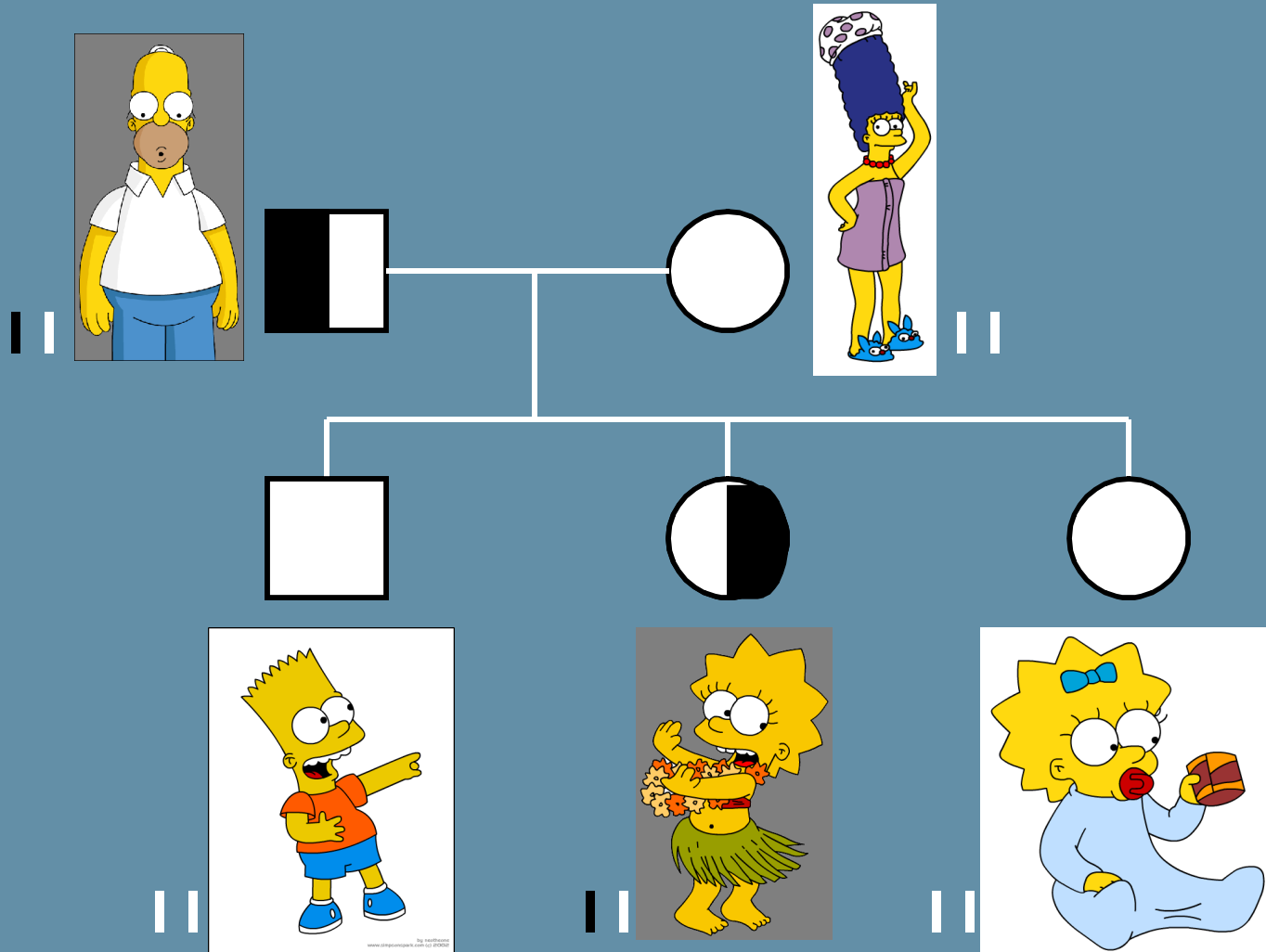
« Weak » mutation



Amphiphysin 2
(BIN1)

Autosomal dominant centronuclear myopathy

« Strong » mutation

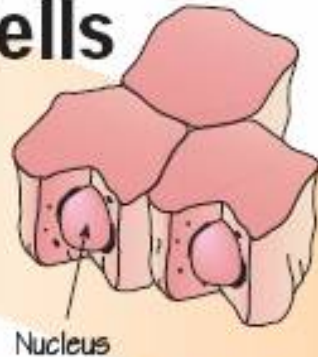


**Dynamin 2
(DNM2)**



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

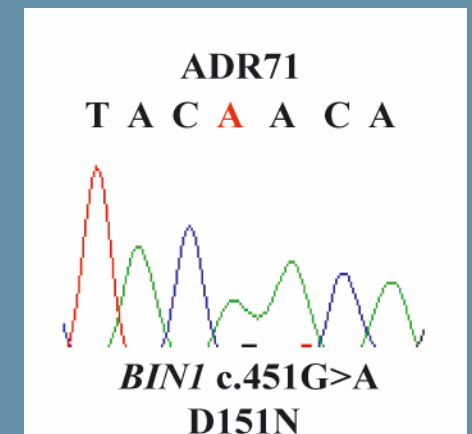


Letter change:
unnoticed
beneficial
mutation => disease

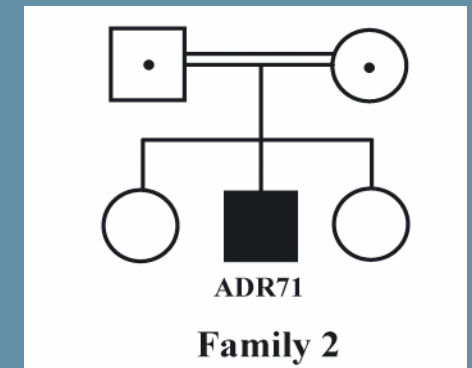
Patients samples



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

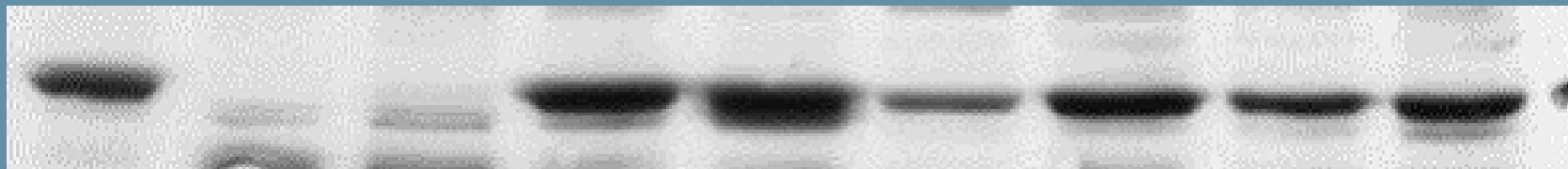
Reading DNA



© Applied Biosystems

Use of cells and biopsies:

α -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

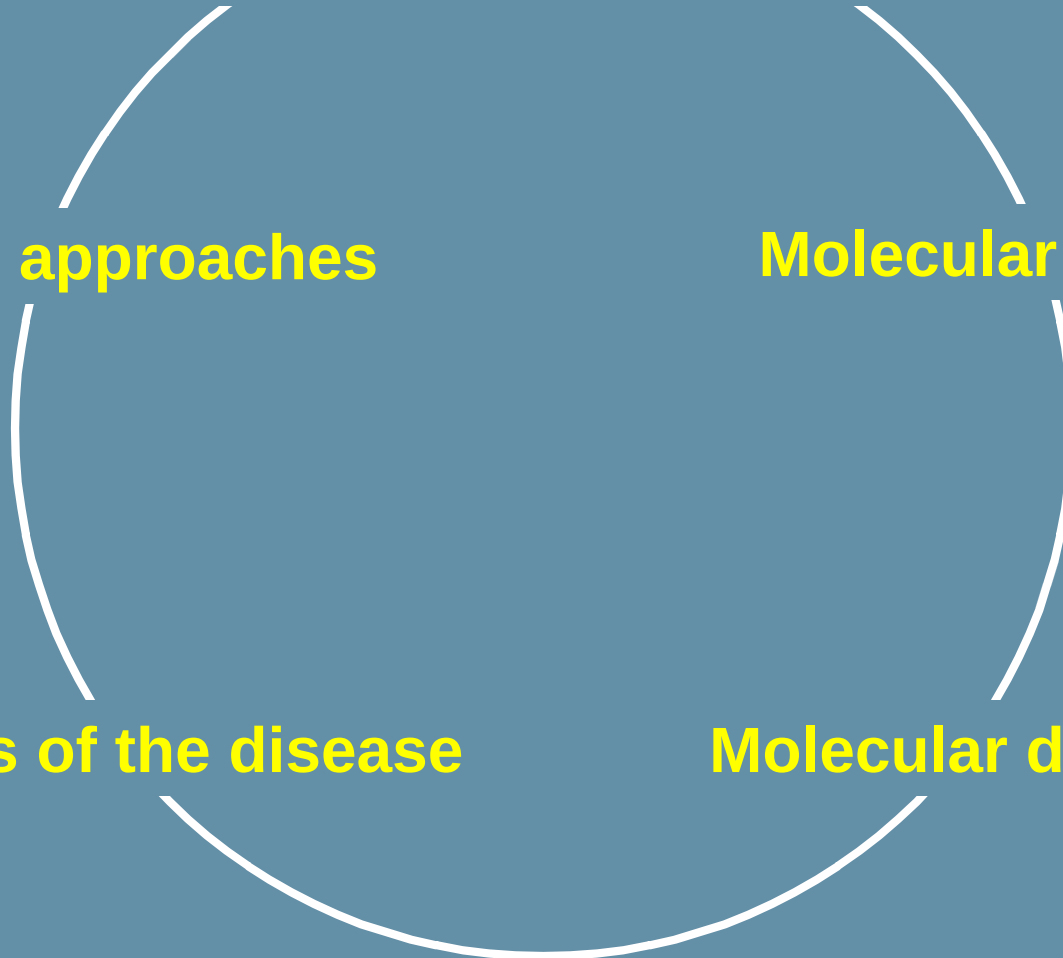
Myotubular / centronuclear myopathies

Therapeutic approaches

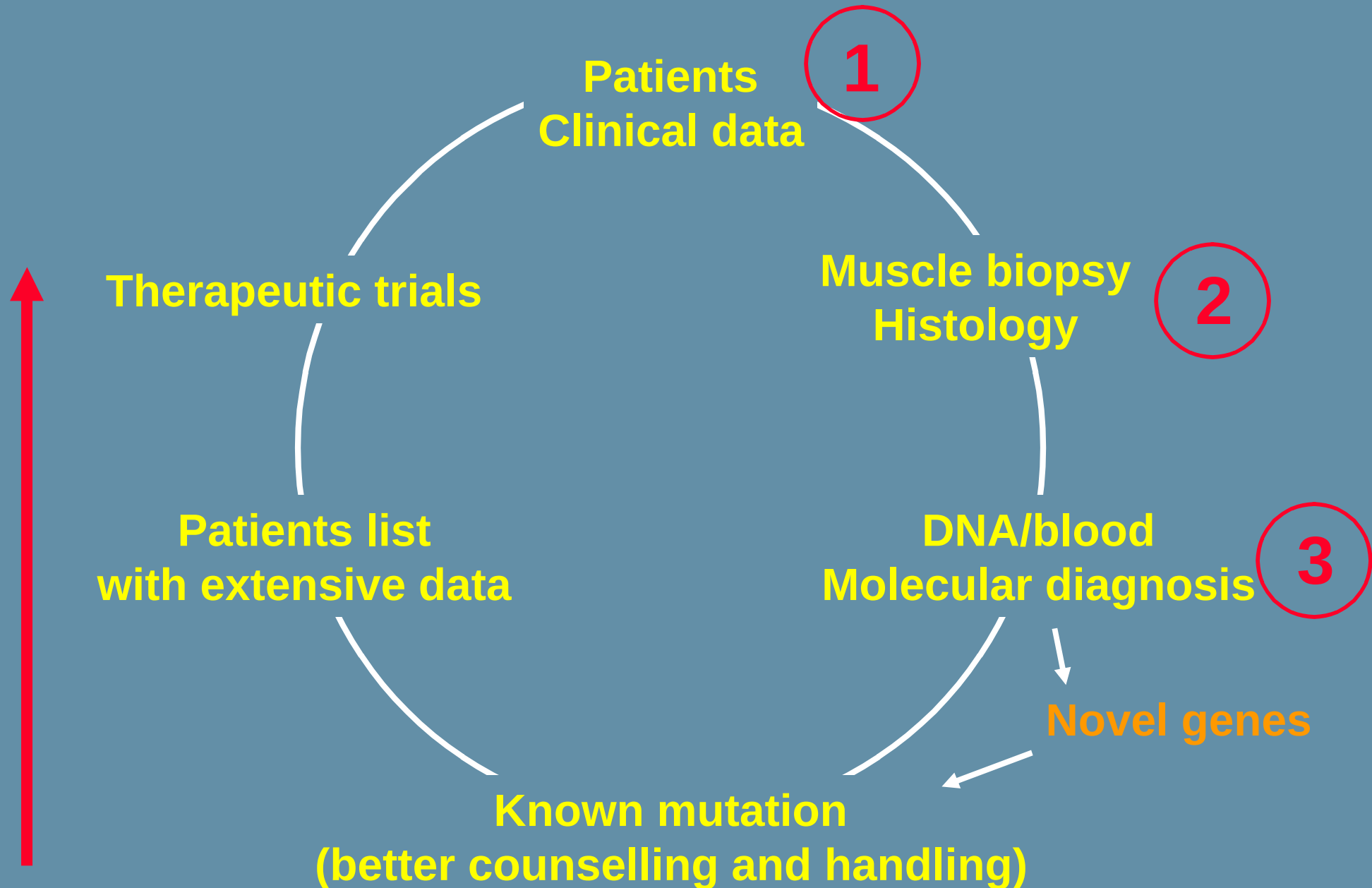
Molecular causes

Mechanisms of the disease

Molecular diagnosis



Extensive Patients and Data recruitment



Acknowledgements

TEAM

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Anders Oldfors
Carina Wallgren-Pettersson

Patients, families and clinicians

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- Association Française contre les Myopathies (AFM)