UNIVERSITEIT GENT

FACULTEIT GENEESKUNDE EN GEZONDHEIDSWETENSCHAPPEN

# Disorders of structural proteins

Postgraduate course Human Genetics

06/12/2019

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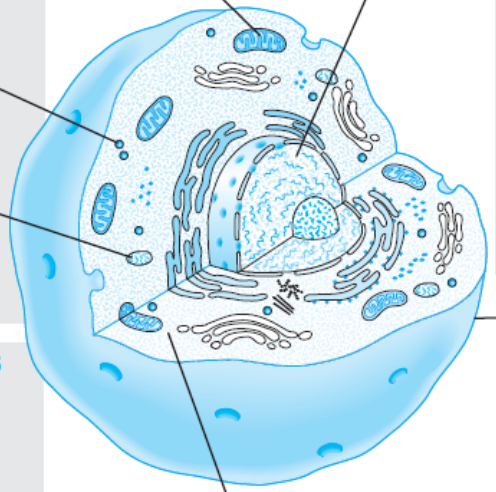


## ORGANELLES

**Mitochondria**
Oxidative phosphorylation
- ND1 protein of electron transport chain
  - *Leber hereditary optic neuropathy*

Translation of mitochondrial proteins
- $tRNA^{leu}$
  - *MELAS*
- 12S RNA
  - *sensorineural deafness*

**Peroxisomes**
Peroxisome biogenesis
- 8 proteins
  - *Zellweger syndrome*

**Lysosomes**
Lysosomal enzymes
- Hexosaminidase A
  - *Tay-Sachs disease*
- α-L-iduronidase deficiency
  - *Hurler syndrome*

## EXTRACELLULAR PROTEINS

Transport
- β-globin
  - *sickle cell disease*
  - *β-thalassemia*

Morphogens
- Sonic hedgehog
  - *holoprosencephaly*

Protease inhibition
- $\alpha_1$-Antitrypsin
  - *emphysema, liver disease*

Hemostasis
- Factor VIII
  - *hemophilia A*

Hormones
- Insulin
  - *rare forms of type 2 diabetes mellitus*

Extracellular matrix
- Collagen type 1
  - *osteogenesis imperfecta*

Inflammation, infection response
- Complement factor H
  - *age-related macular degeneration*

## NUCLEUS

Developmental transcription factors
- Pax6
  - *aniridia*

Genome integrity
- BRCA1, BRCA2
  - *breast cancer*
- DNA mismatch repair proteins
  - *hereditary nonpolyposis colon cancer*

RNA translation regulation
- FMRP (RNA binding to suppress translation)
  - *Fragile X syndrome*

Chromatin-associated proteins
- MeCP2 (transcriptional repression)
  - *Rett syndrome*

Tumor suppressors
- Rb protein
  - *retinoblastoma*

Oncogenes
- BCR-Abl oncogene
  - *chronic myelogenous leukemia*

## CELL SURFACE

Hormone receptors
- Androgen receptor
  - *androgen insensitivity*

Growth factor receptors
- FGFR3 receptor
  - *achondroplasia*

Metabolic receptors
- LDL receptor
  - *hypercholesterolemia*

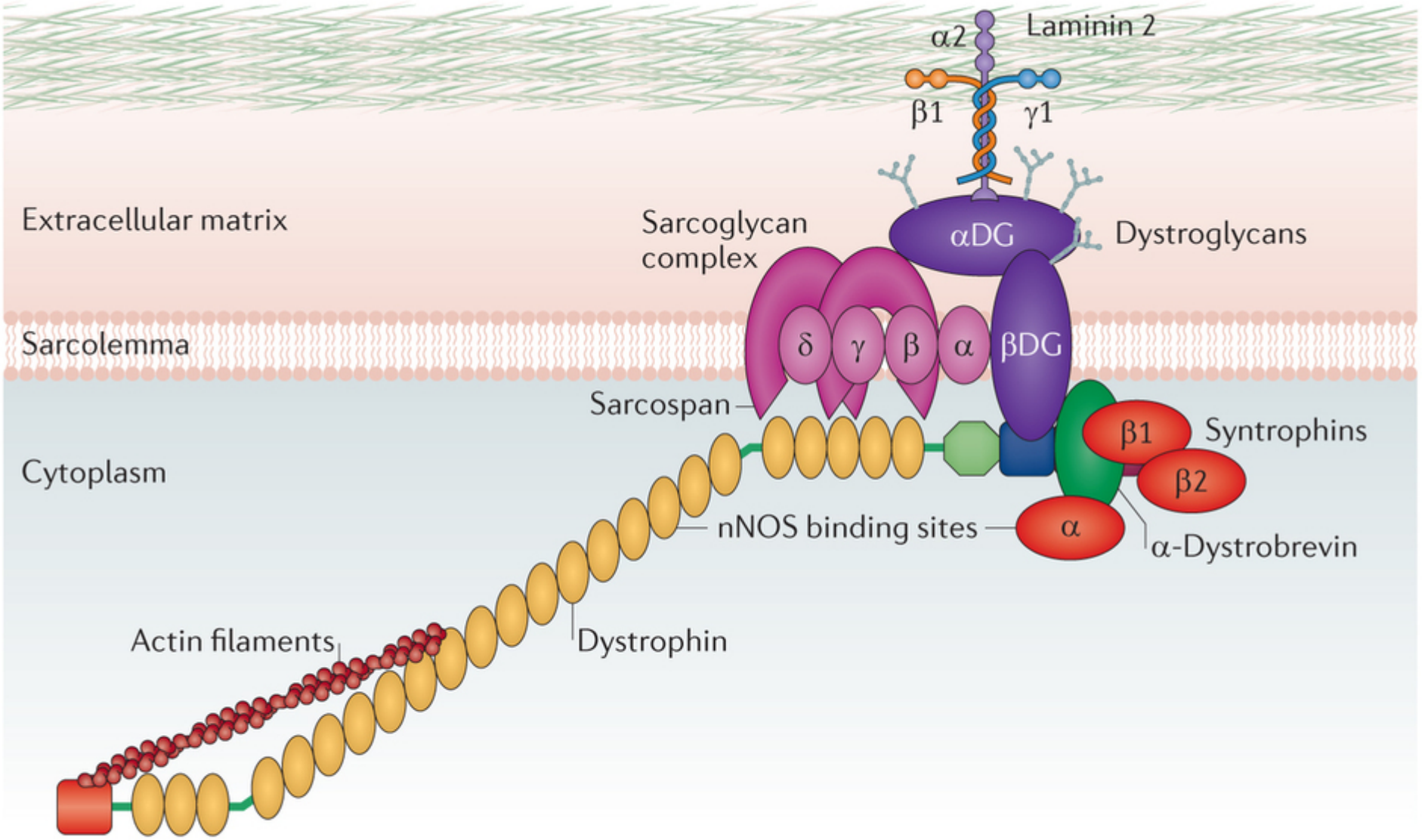
Ion transport
- CFTR
  - *cystic fibrosis*

Antigen presentation
- HLA locus DQβ1
  - *Type 1 diabetes mellitus*

## CYTOPLASM

Metabolic enzymes
- Phenylalanine hydroxylase
  - *PKU*
- Adenosine deaminase
  - *severe combined immunodeficiency*

Cytoskeleton
- Dystrophin
  - *Duchenne muscular dystrophy*




Nature Reviews | Genetics

# Dystrophin complex:major functions

- maintenance of muscle membrane integrity
- correct positioning of proteins in the complex, so that they function correctly
- ion channels and signaling molecules → participation in cell-cell and/or cell-substrate recognition

# Molecular Genetics

## Gene: *DMD*

- the largest known human gene (1,5% of X-chromosome)
- 2.4 Mb of DNA
- comprises 79 exons
- 7 tissue-specific promoters
- differential splicing → tissue-specific, developmentally regulated isoforms (18 different isoforms)

## Protein: dystrophin

- part of a protein complex that links the cytoskeleton with membrane proteins that in turn bind with proteins in the extracellular matrix
- expressed in skeletal and cardiac muscle, brain

# Defects of dystrophin

A *spectrum of muscle disease* caused by mutations in the *DMD* gene, which encodes the protein dystrophin.

The mild end of the spectrum

- asymptomatic increase in serum concentration of creatine phosphokinase (CK)
- muscle cramps with myoglobinuria
- isolated quadriceps myopathy

The severe end of the spectrum: progressive muscle diseases

- Duchenne/Becker muscular dystrophy (skeletal muscle)
- *DMD*-associated dilated cardiomyopathy (heart)

# Duchenne muscular dystrophy (DMD)

- Normal for the first two years of life
- Symptoms present before age 5 years
- Progressive symmetrical muscular weakness, proximal greater than distal, often with calf hypertrophy
- Wheelchair-dependency before age 13 years
- Unlikely to survive beyond age of 20 years
- Die of respiratory failure or cardiomyopathy
- Modest decrease in IQ (~20 points)
- Prevalence: 1/3,500 males

# Becker muscular dystrophy (BMD)

- Progressive symmetrical muscle weakness and atrophy, proximal greater than distal, often with calf hypertrophy (weakness of quadriceps femoris may be the only sign)
- Activity-induced cramping (present in some individuals)
- Flexion contractures of the elbows (if present, late in the course)
- Wheelchair dependency (if present, after age 16 years)
- Preservation of neck flexor muscle strength (differentiates BMD from DMD)
- Prevalence: 1/18,000 males

# DMD-associated dilated cardiomyopathy

- Dilated cardiomyopathy (DCM) with congestive heart failure, with males typically presenting between ages 20 and 40 years and females presenting later in life
- Usually no clinical evidence of skeletal muscle disease; may be classified as "subclinical" BMD
- Rapid progression to death in several years in males and slower progression over a decade or more in females

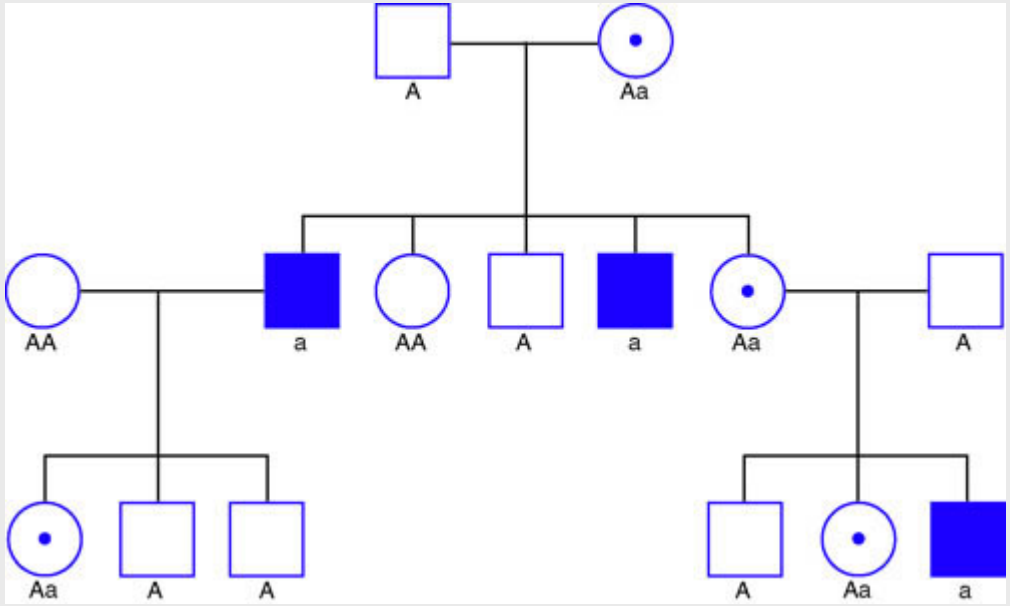
# Molecular Genetics: inheritance

- Incidence DMD:
  - 1:3,500 live male births
  - Calculated mutation rate $10^{-4}$
  - Given a sperm production rate of $8x10^{7}$ sperm/day: sperm with new mutation is produced every 10 seconds by normale male!

# Molecular Genetics: inheritance

## X-linked recessive disorder (Xp21.2)

- 1/3 of cases: new mutations
- 2/3 have carrier mother



# Molecular Genetics: inheritance

## Carrier mother:

- majority: no clinical manifestations
- 70 % has slightly elevated serum creatine kinase
- Random inactivation of X-chromosome →
  - ~19% of adult female carriers have some muscle weakness
  - 8% has life-threatening cardiomyopathy and severe muscle weakness

## Females with DMD (rare):

- Nonrandom X-inactivation
- Turner syndrome (45,X)
- X; autosome translocation

# Molecular Genetics

**Mechanisms of Mutation in Duchenne or Becker Muscular Dystrophy**

| Molecular or Genetic Defect | Frequency | Phenotype |
|---|---|---|
| IN AFFECTED MALES | | |
| Gene deletion (1 exon to whole gene) | ~60% | DMD or BMD |
| Point mutations | ~34% | DMD or BMD |
| Partial duplication of the gene | ~6% | DMD or BMD |
| Contiguous gene deletion | Rare | DMD plus other phenotypes, depending on other genes deleted |
| IN AFFECTED FEMALES | | |
| Nonrandom X inactivation | Rare | DMD |
| Turner syndrome (45,X) | Rare | DMD |
| X;autosome translocation | Rare | DMD |

# Molecular Genetics: inheritance

## DMD

Lethal

↓

gene is not transmitted

↓

1/3 of cases: new mutations

2/3 have carrier mother

## BMD

Non-lethal

↓

gene is transmitted

↓

high proportion of BMD cases is inherited, only 10% new mutations

# Genotype-phenotype correlations

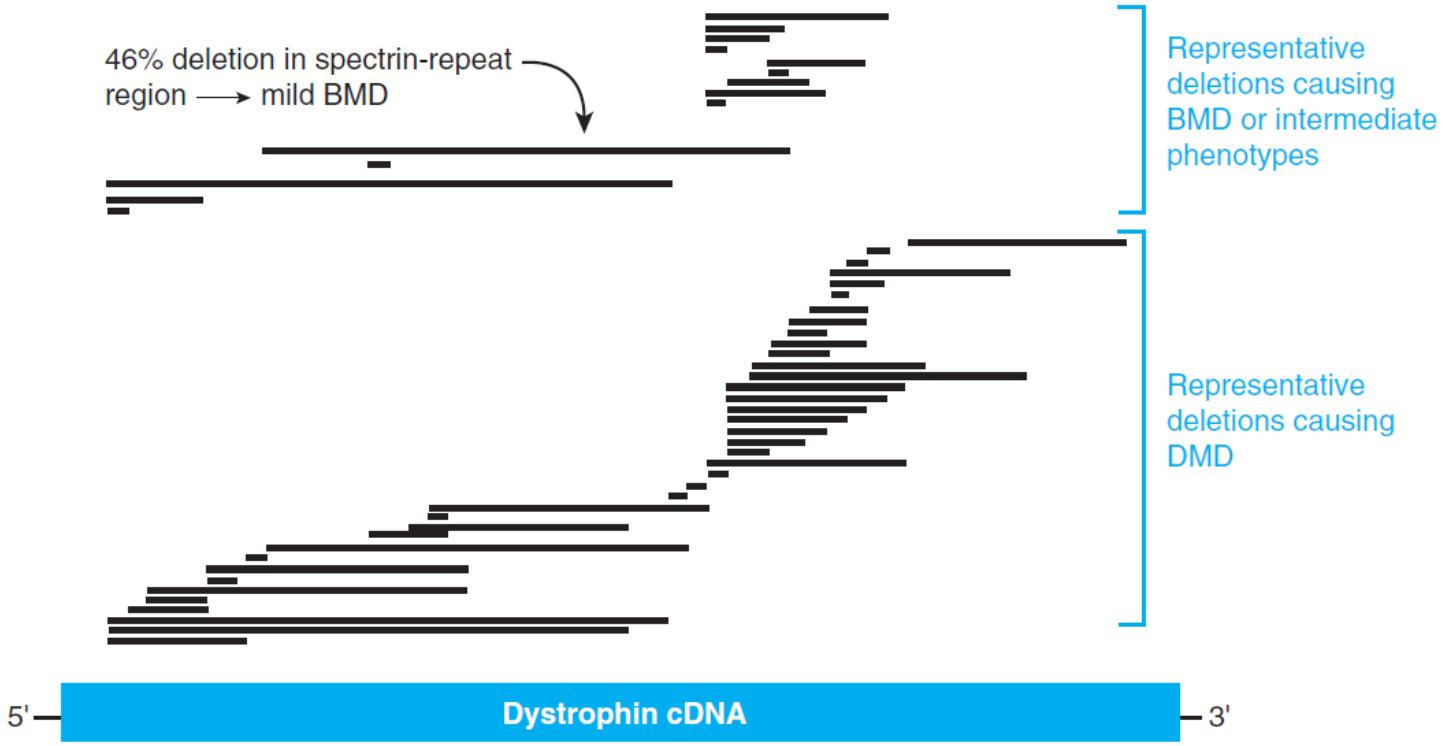
*lack of dystrophin expression: DMD*

- very large deletions→ absence of dystrophin expression
- mutations that disrupt reading frame (stop mutation, splicing mutation, deletion, duplication) → severely truncated dystrophin that is degraded

*remaining dystrophin production (abnormal quality or quantity): BMD*

- deletions or duplications that juxtapose in-frame exons
- some splicing mutations
- most non-truncating single-base changes that result in translation of a protein product with intact N and C termini.

# Molecular Genetics




# Testing

Electromyography: to differentiate between myopathy and neurogenic disorder

Serum Creatine Phosphokinase (CK) Concentration

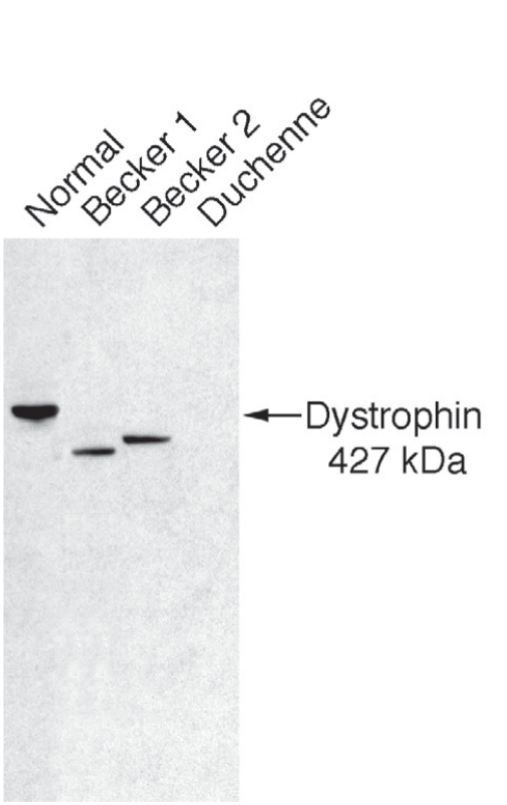
| | phenotype | % of affected individuals | Serum CK conc. |
|---|---|---|---|
| Males | DMD | 100% | > 10x normal |
| | BMD | 100% | > 5x normal |
| | DMD-associated DCM | Most individuals | “increased” |
| Female carriers | DMD | ~50% | 2- 10x normal |
| | BMD | ~30% | 2- 10x normal |

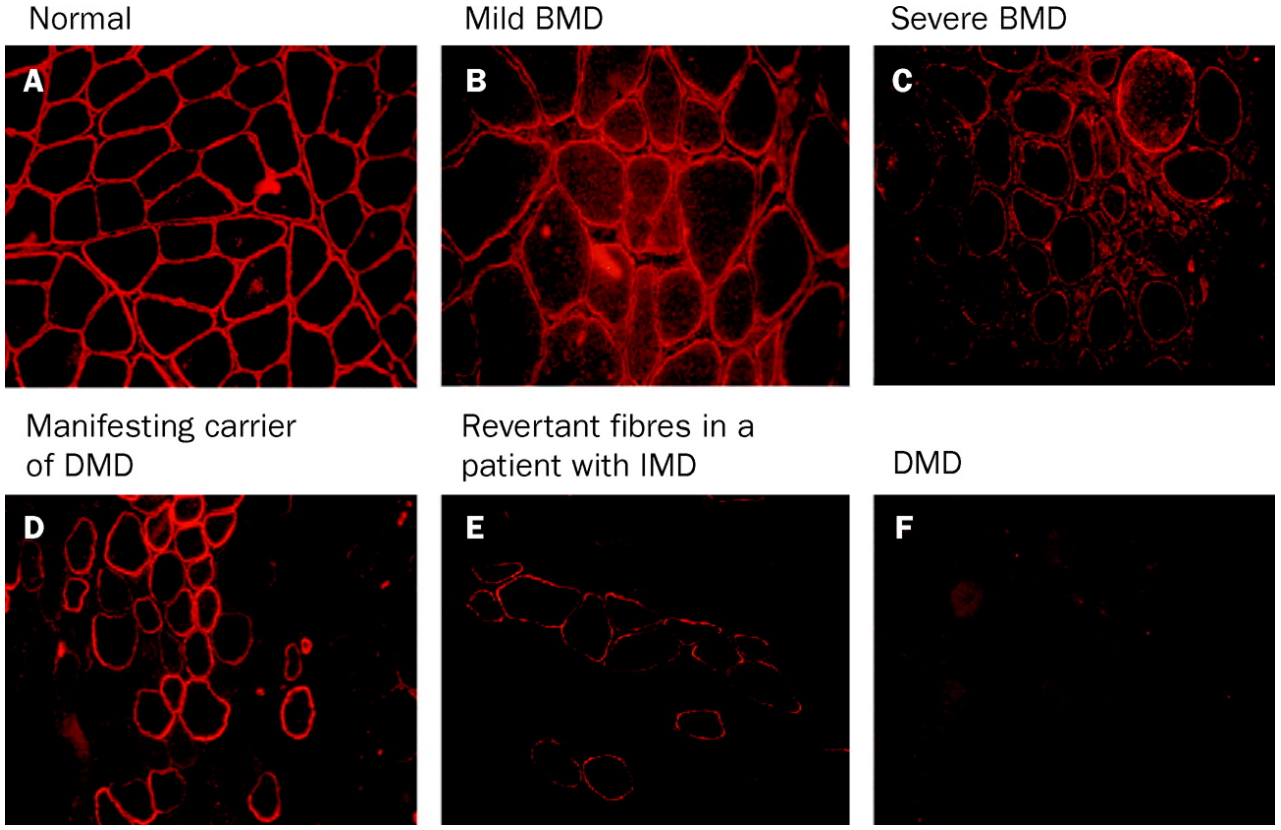
# Testing

## Western Blot and immuno-histochemistry

| | Phenotype | Western Blot | | Immunohistochemsitry |
|---|---|---|---|---|
| | | Dystrophin | Dystrophin quantity | |
| Males | DMD | Non-detectable | 0%-5% | (almost) complete absence |
| | Intermediate | Normal/Abnormal | 5%-20% | |
| | BMD | Normal<br>Abnormal | 20%-50%<br>20%-100% | Normal appearing or reduced intensity ± patchy staining |
| Female carriers | DMD Random XCI | Normal/Abnormal | > 60% | Mosaic pattern |
| | DMD Skewed XCI | Normal/Abnormal | < 30% | Mosaic pattern |

# Western blot and Immuno-histochemistry



# Testing

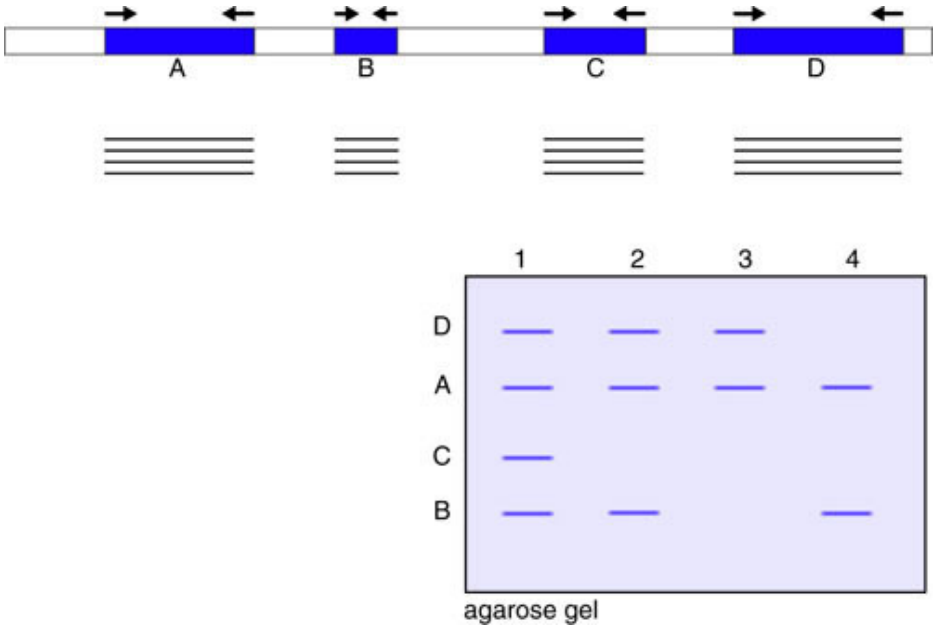
## Molecular genetic testing

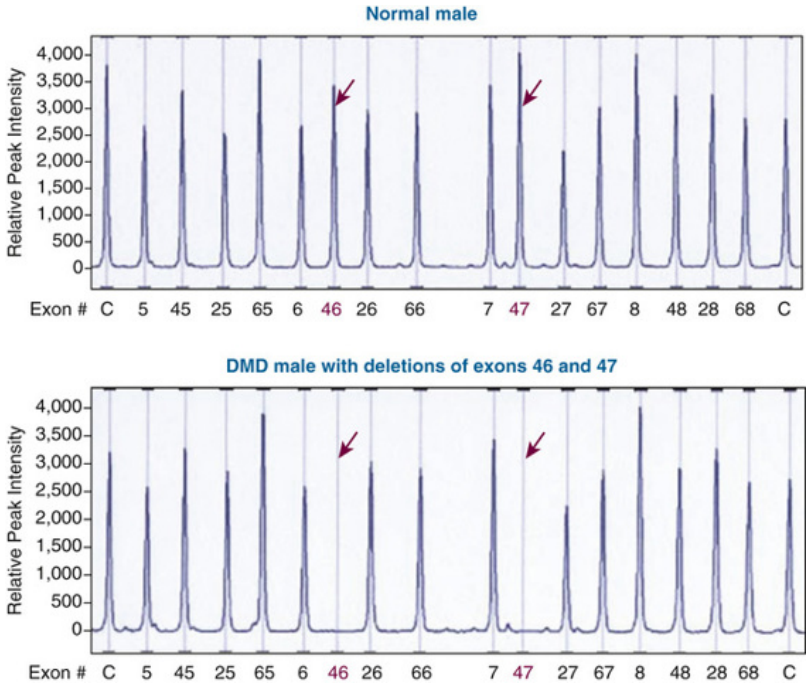
- *Deletion/duplication analysis*
  - Multiplex PCR, southern blotting and FISH (deletions)
  - Southern blotting and quantitative PCR (duplications)
  - MLPA (deletions/duplications), arrayCGH

- *Mutation scanning and sequence analysis*
  - Small deletions/insertions, single base changes, splice mutations

- *NGS approaches*
  - Amplicon-based targeting NGS technique (Multiplicon DMD MASTR™ assay – 122 amplicons) or other NGS platform: specific for DMD *gene*
  - Gene panels for neuromuscular disease (varying from 12 to 579 different genes) – challenge: update gene panels – bioinformatic pipelines

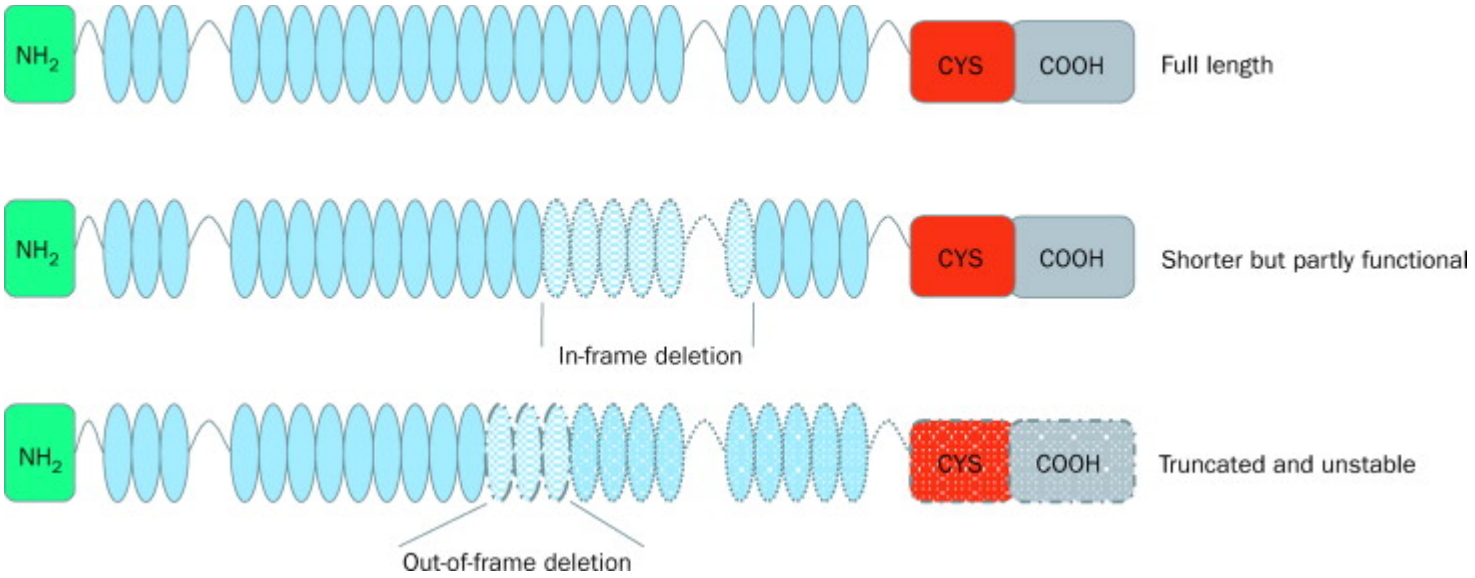
# Multiplex PCR - MLPA

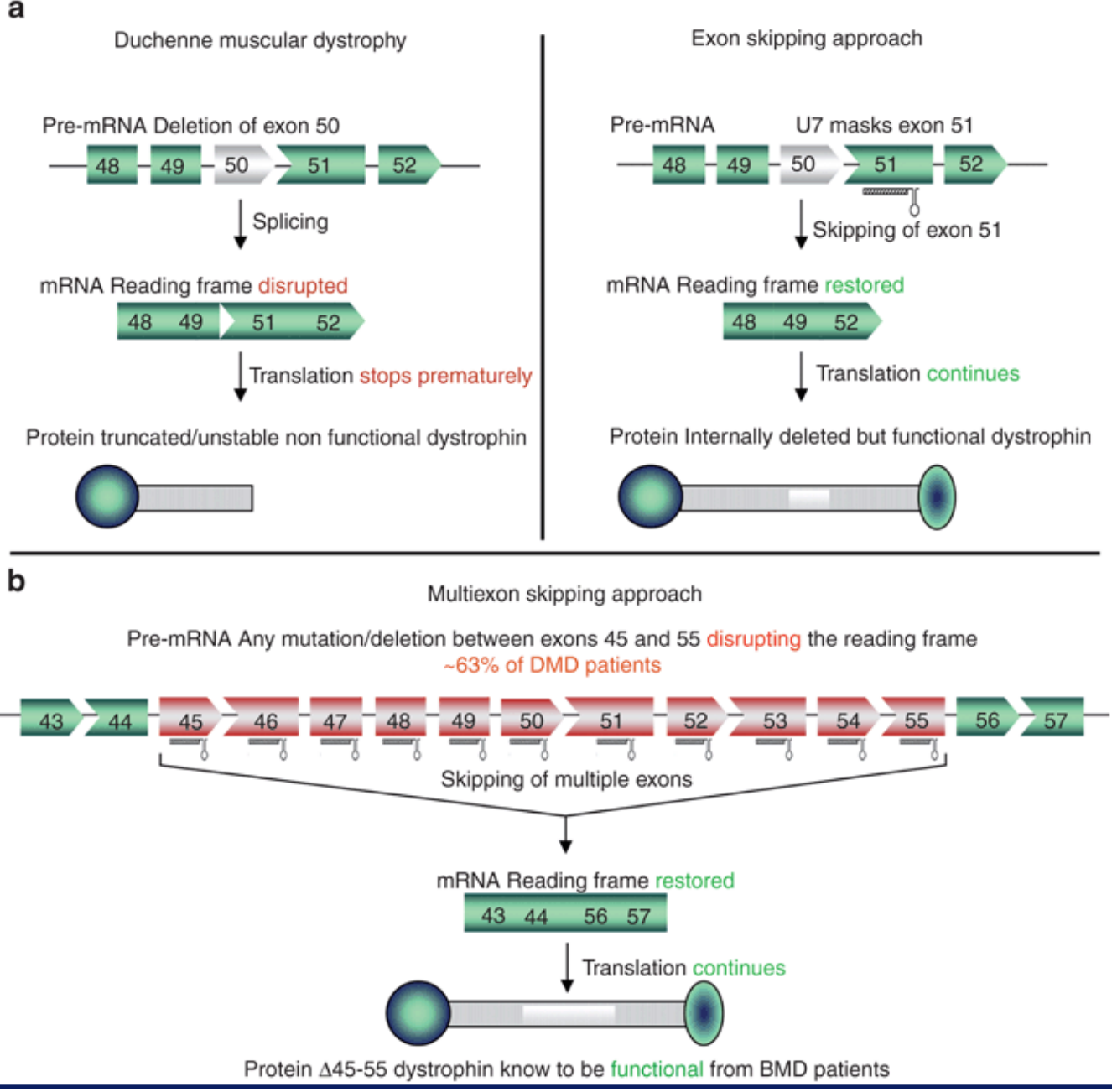


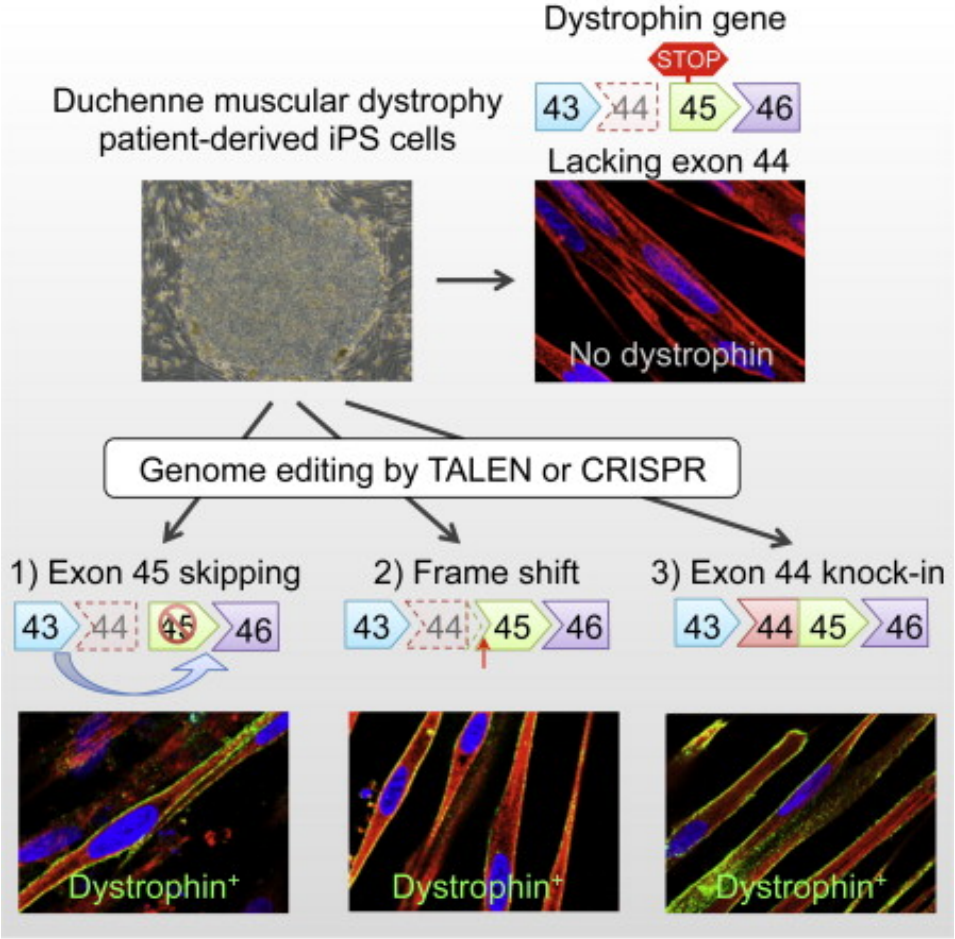

Multiplex PCR analysis of dystrophin gene deletions. Exons A, B, C, and D are amplified in a single PCR reaction (arrows indicate PCR primers). The products (shown below each exon) are separated by size on an agarose gel and are visualized.

# Reading frame hypothesis

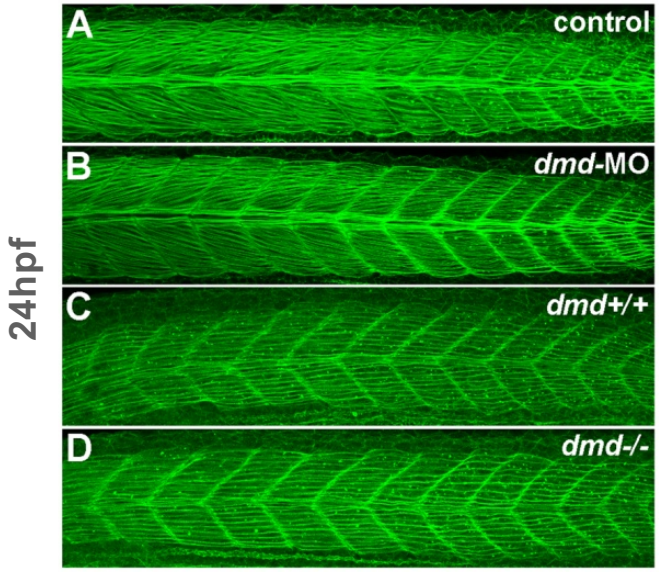


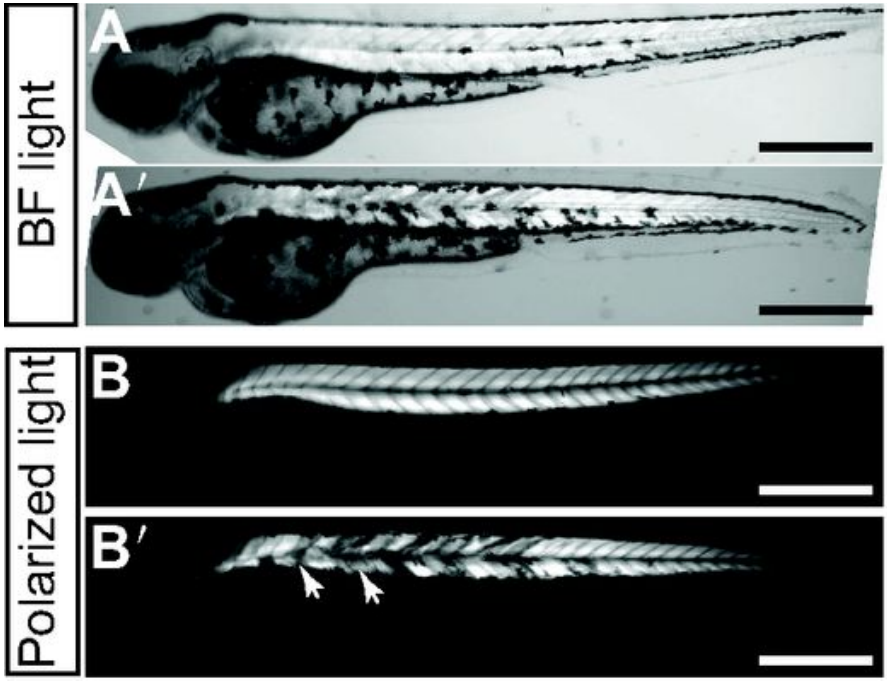

a
Duchenne muscular dystrophy
Pre-mRNA Deletion of exon 50
48
49
50
51
52
Splicing
mRNA Reading frame disrupted
48 49 51 52
Translation stops prematurely
Protein truncated/unstable non functional dystrophin
Exon skipping approach
Pre-mRNA
U7 masks exon 51
48
49
50
51
52
Skipping of exon 51
mRNA Reading frame restored
48 49 52
Translation continues
Protein Internally deleted but functional dystrophin
b
Multiexon skipping approach
Pre-mRNA Any mutation/deletion between exons 45 and 55 disrupting the reading frame
~63% of DMD patients
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
Skipping of multiple exons
mRNA Reading frame restored
43 44 56 57
Translation continues
Protein Δ45-55 dystrophin know to be functional from BMD patients

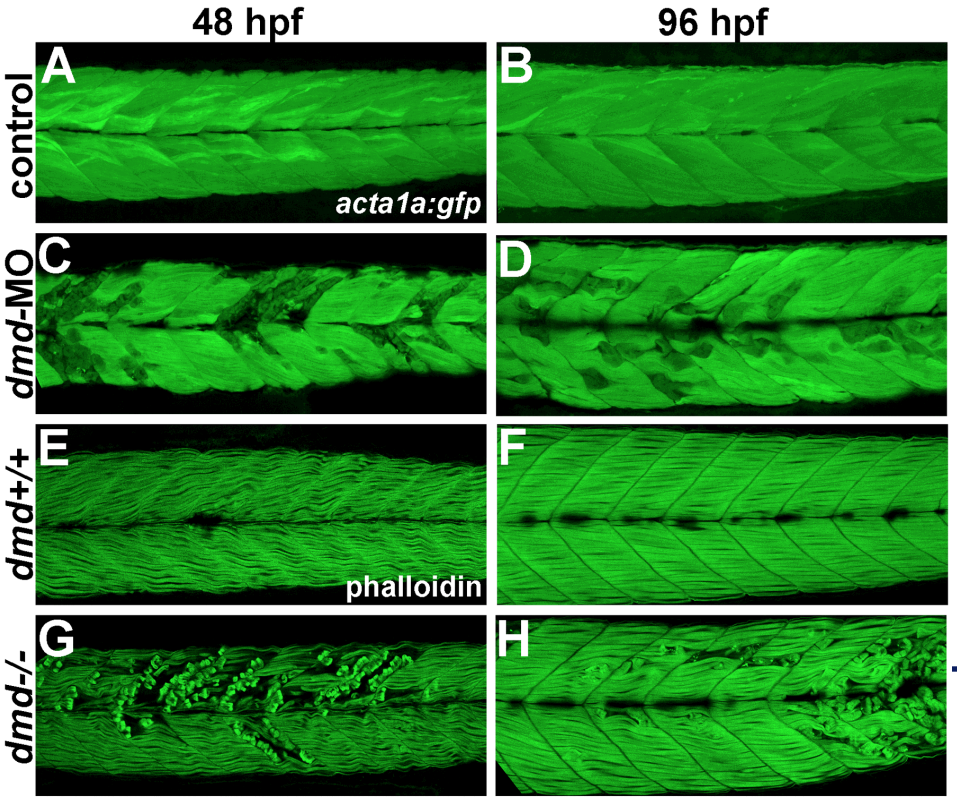
# Correction of *DMD* in Patient Induced Pluripotent Stem Cells by TALEN and CRISPR-Cas9

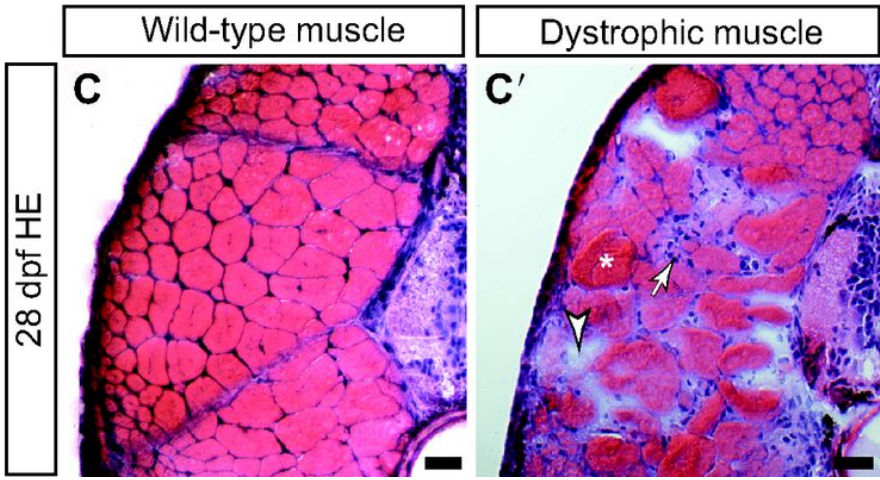


# *dmd* zebrafish



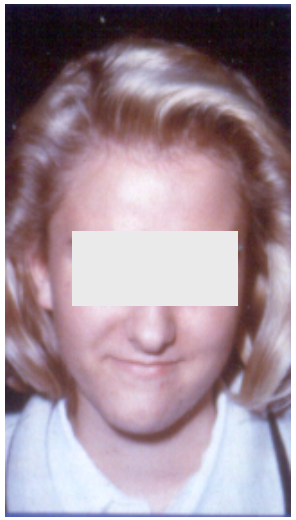



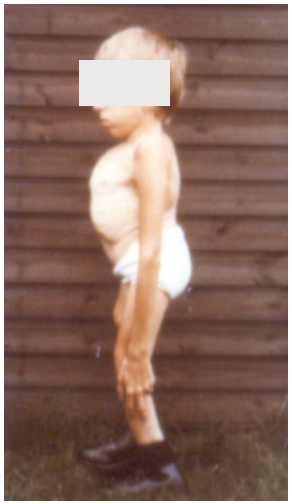
# Mutations in collagen structural genes: Osteogenesis imperfecta

- Variable degree of bone fragility
- 4 subtypes (Sillence et al. 1979, 1984)

| | |
|---|---|
| Type I | Mild |
| Type II | Lethal |
| Type III | Severe |
| Type IV | Moderate |

- Defects of type I collagen
- Due to mutations in *COL1A1/COL1A2*

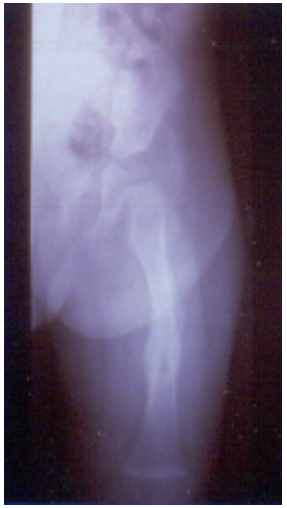


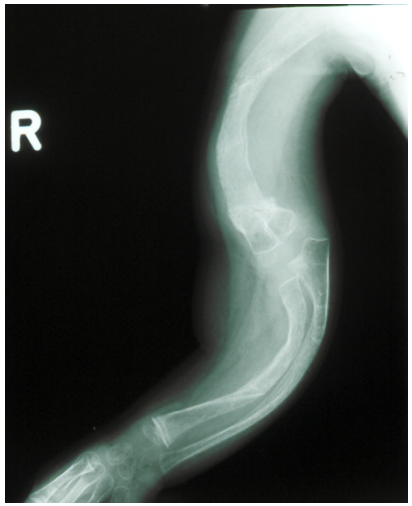


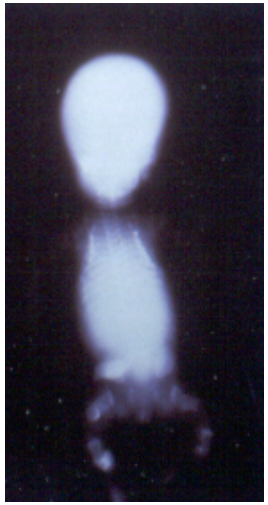
Mild OI

Severe OI

Lethal OI

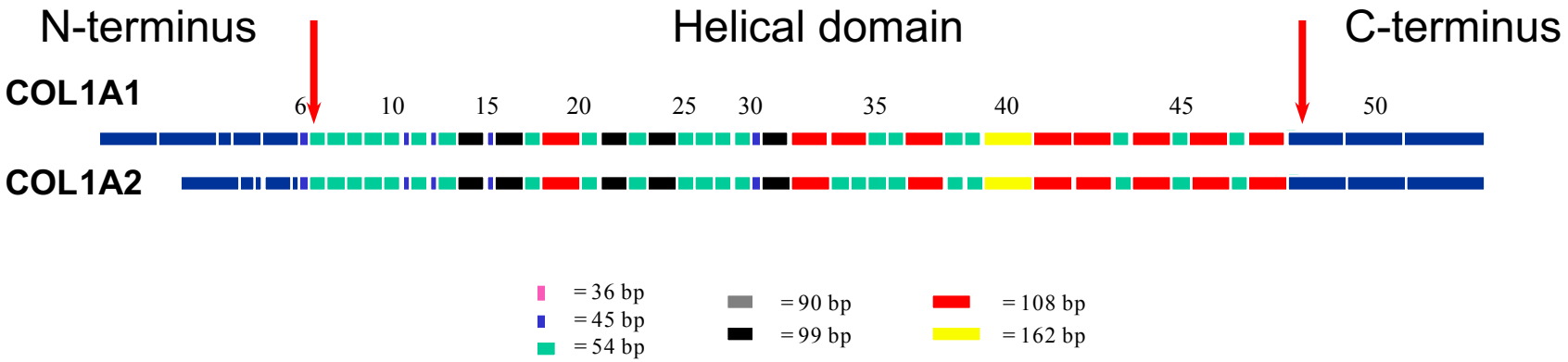




# Type I collagen

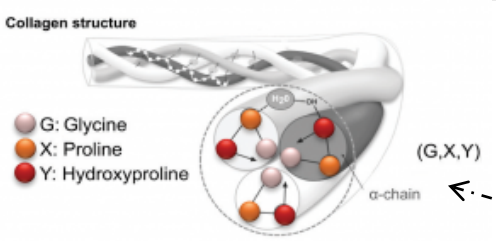
- Most abundant fibrillar collagen in body
- Widely expressed in bone, tendon, skin, other tissues
- Heterotrimer: 2 α1 chains → *COL1A1* (chr 17)
  1 α2 chain → *COL1A2* (chr 7)




Complex gene structure

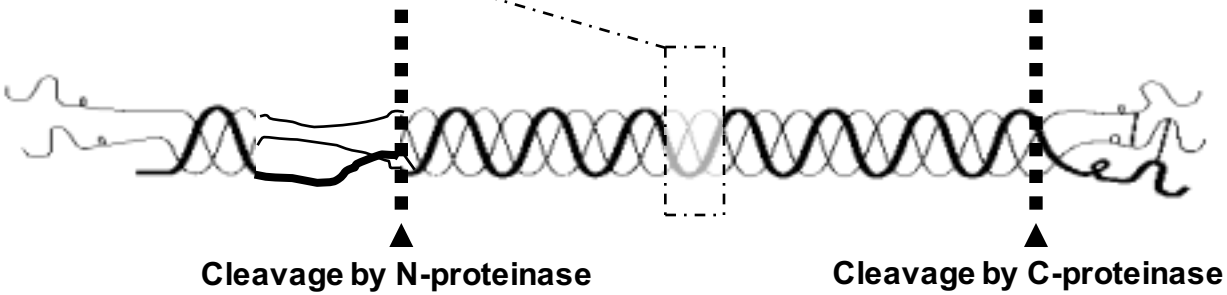
Great potential for mutations

# Collagen Fibrillogenesis

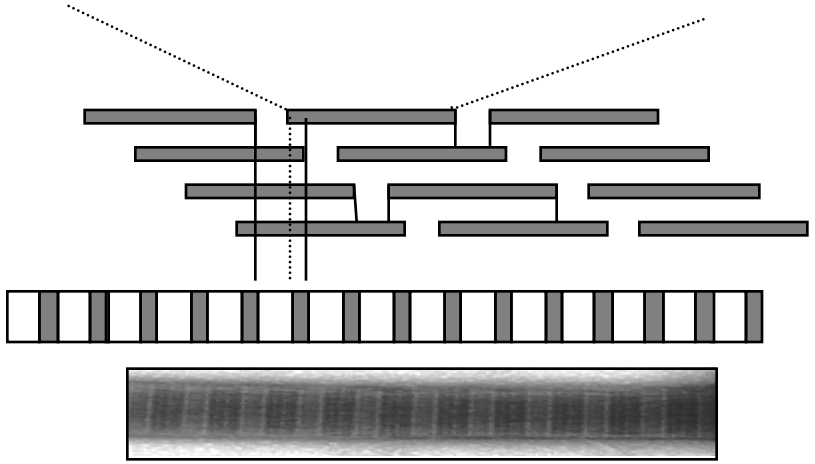




**AMINOACID SEQ**



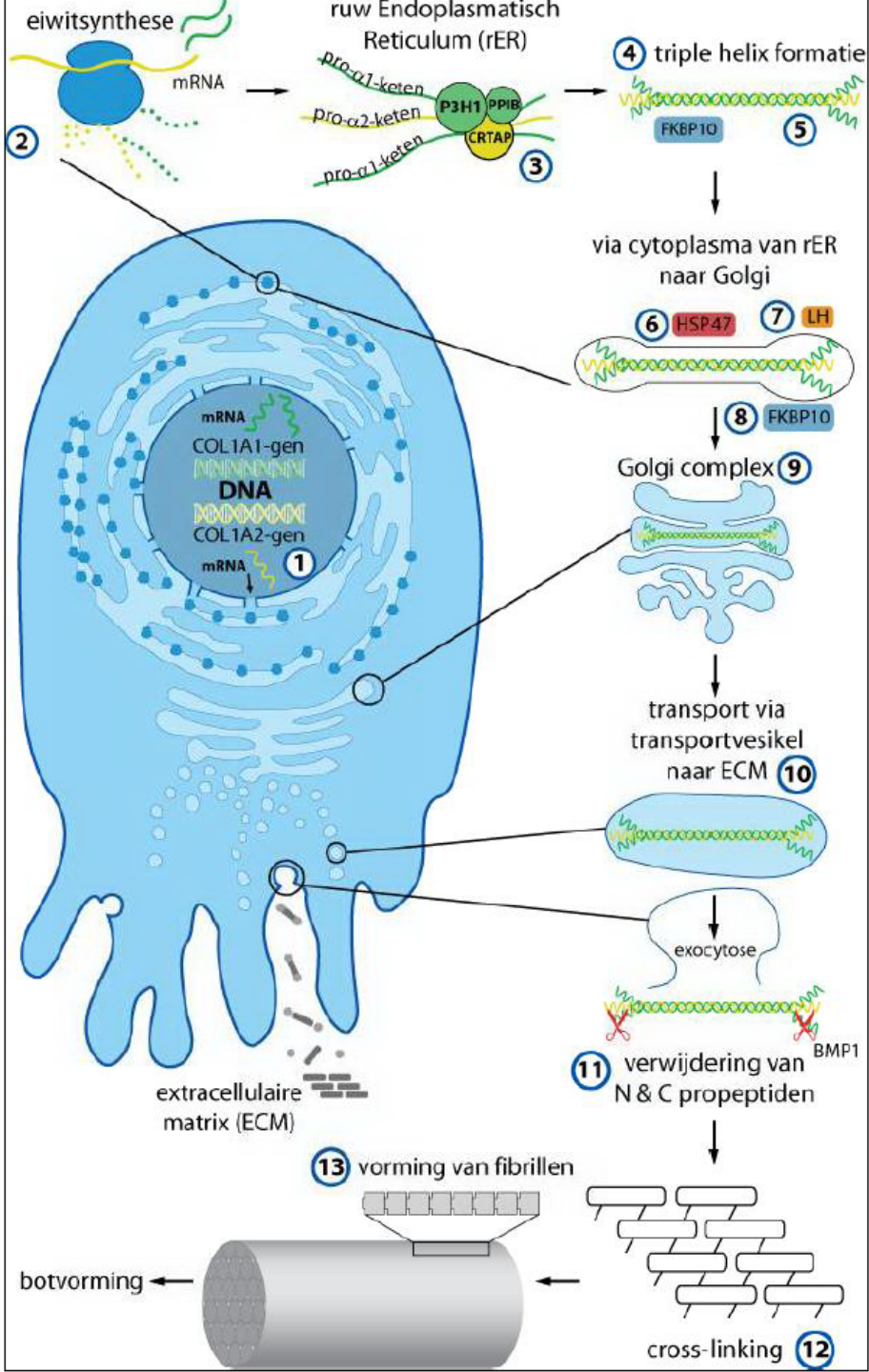

**MOLECULE**
**Type I procollagen**



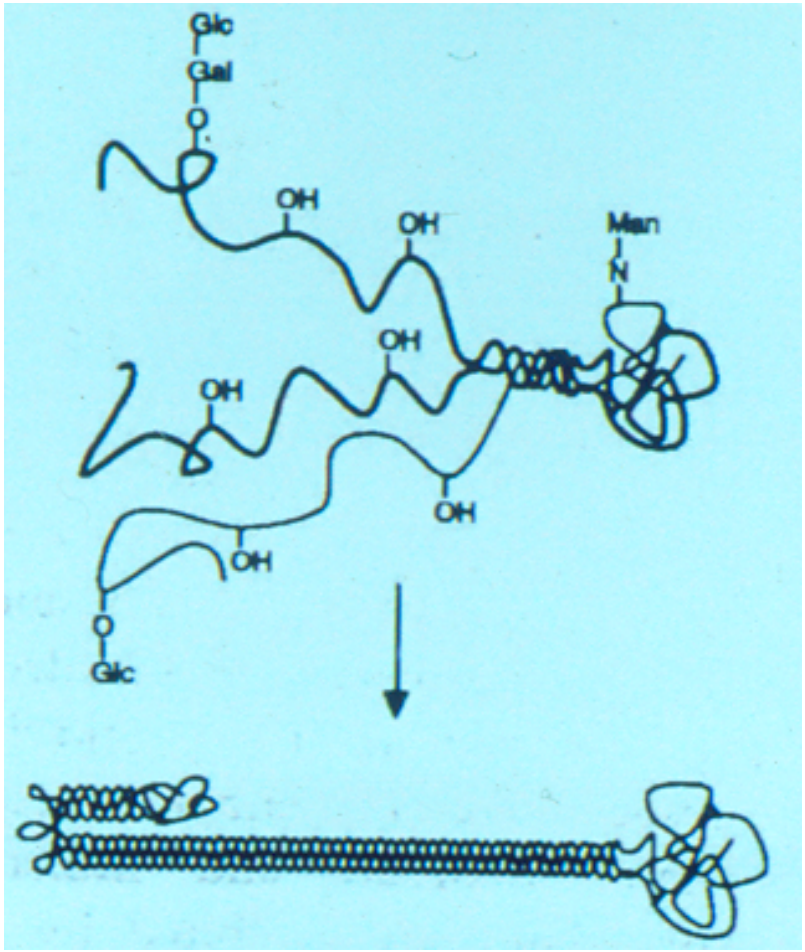
**MOLECULAR PACKING**

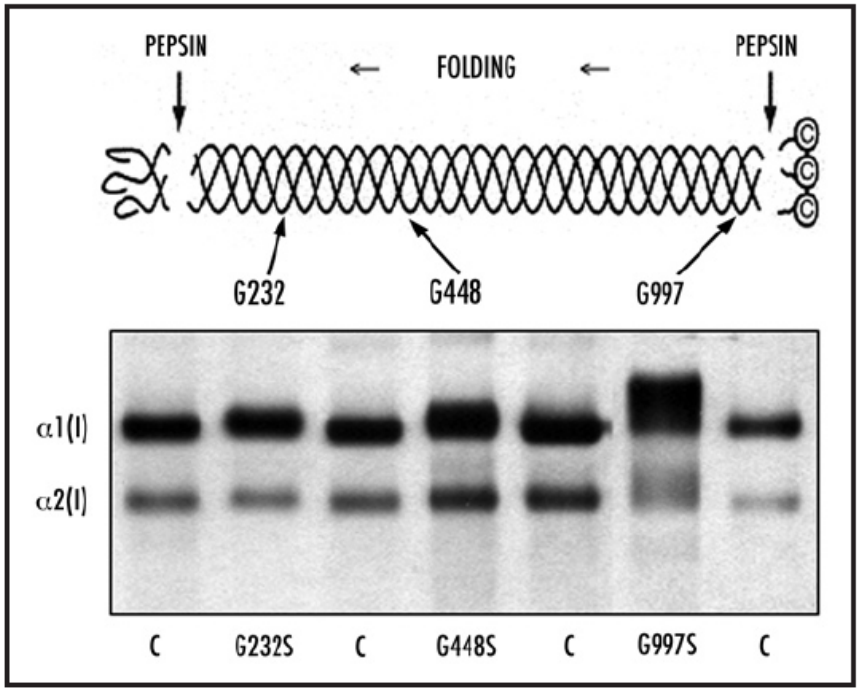
**COLLAGEN FIBRIL**

# Collagen biosynthesis

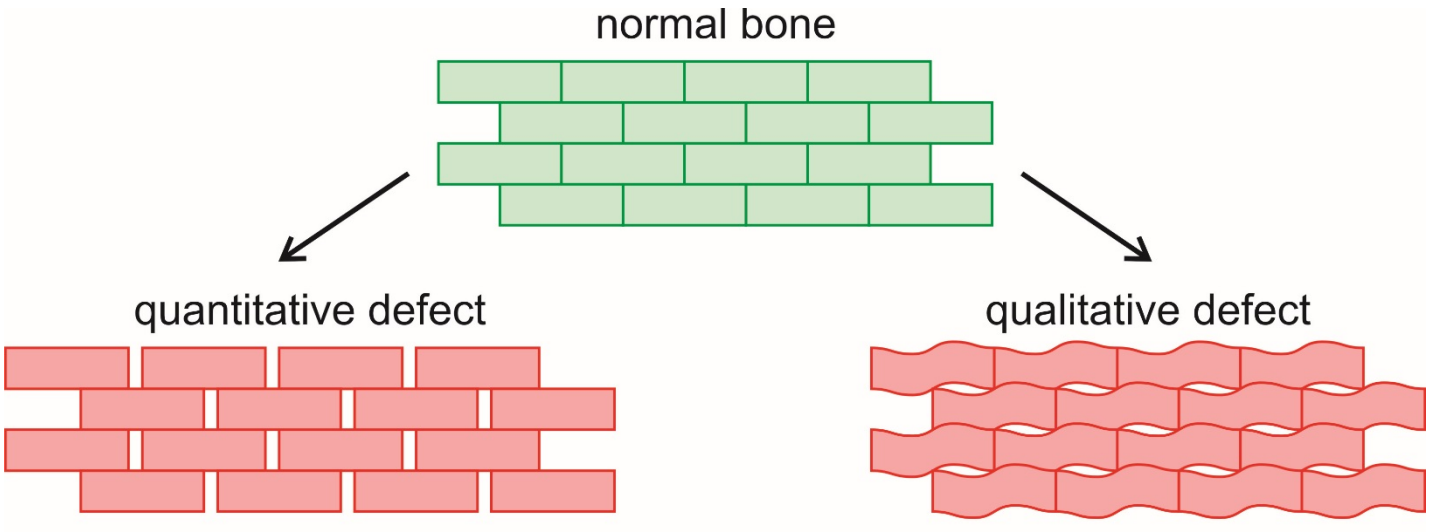


# Collagen fibrillogenesis



# Molecular Pathogenesis of dominant OI




Nonsense, frameshift, ... mutation
Leading to introduction of premature stopcodon
'haplo-insufficiency'

↓

Diminshed production of normal protein
(from the wild type allele)

↓

Mild phenotype (OI type I)

Missense mutation (mostly glycine substitutions)

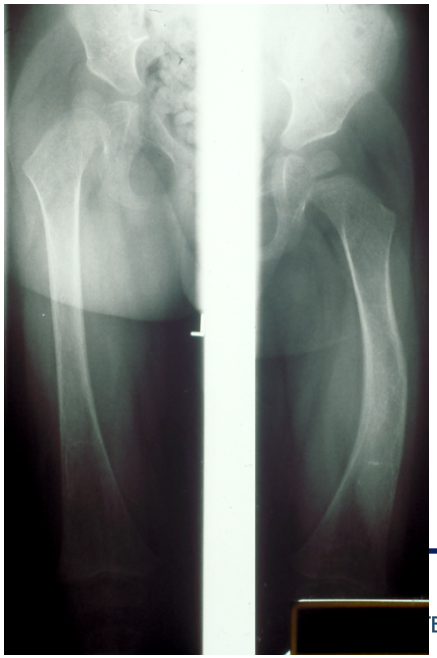
↓

Production of an abnormal protein

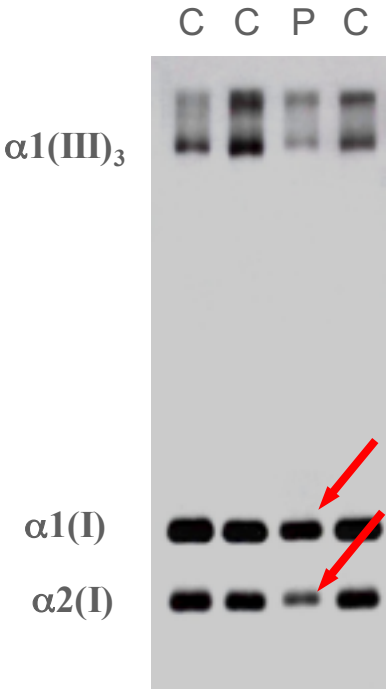
↓

Severe to lethal phenotype (OI type II, III & IV))

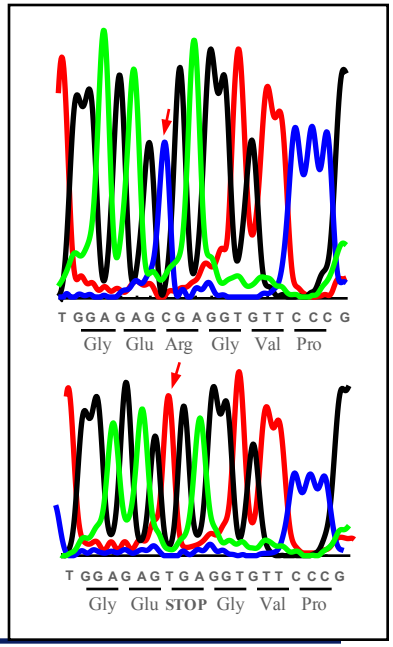
# Osteogenesis Imperfecta type I



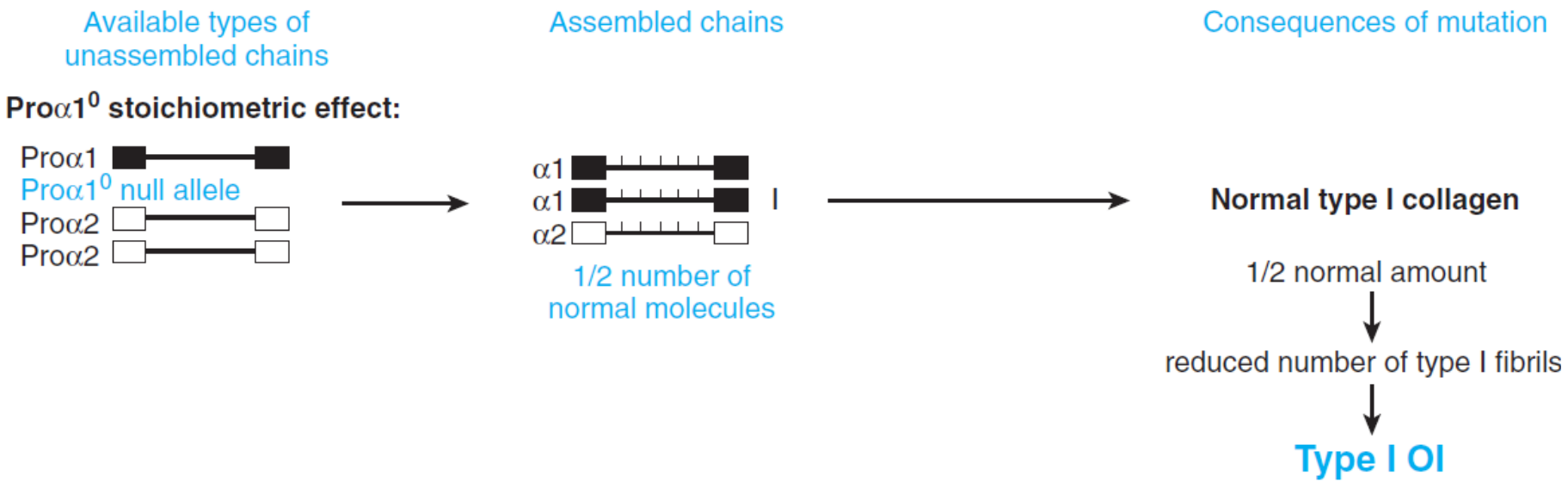
- Bone fragility (mild to moderate)
- Varying number of bone fractures
- Blue sclerae
- Hearing loss



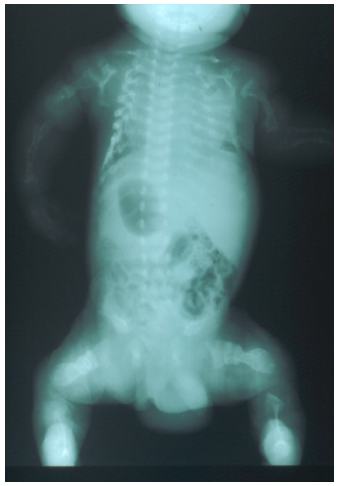

α1(I) - Arg 240 Stop
(CGA → TGA)

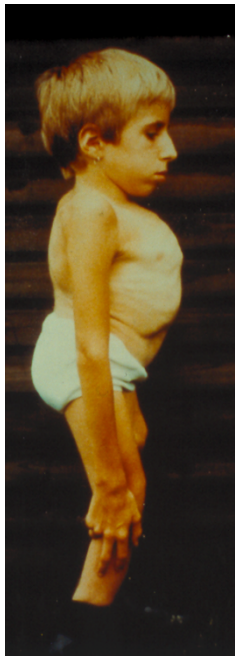


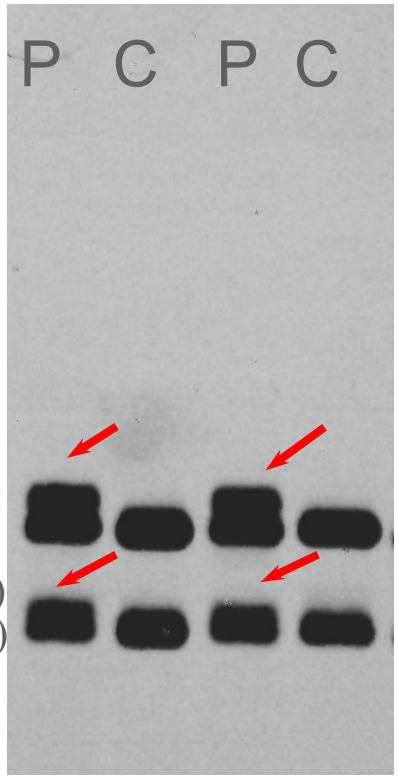
# Osteogenesis imperfecta type I (mild)



# Osteogenesis imperfecta type II-III-IV



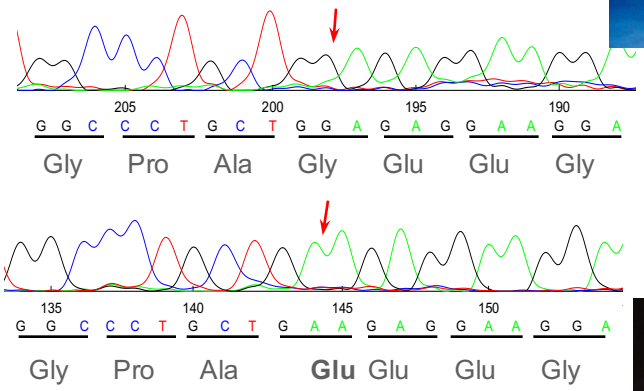


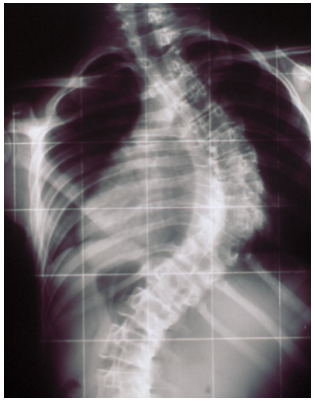



medium collagens

GGA > GAA

α1(I) - Gly286Glu



# Dominant OI

Most prevalent form of OI; caused by primary defects in type I collagen

Over 1500 mutations identified in *COL1A1* or *COL1A2*

The majority are glycine substitutions in the triple helical domain of either the pro-α1 or pro-α2 chain of type I collagen

Mutations alter the structure or quantity of type I collagen and cause a skeletal phenotype that varies from subclinical to lethal

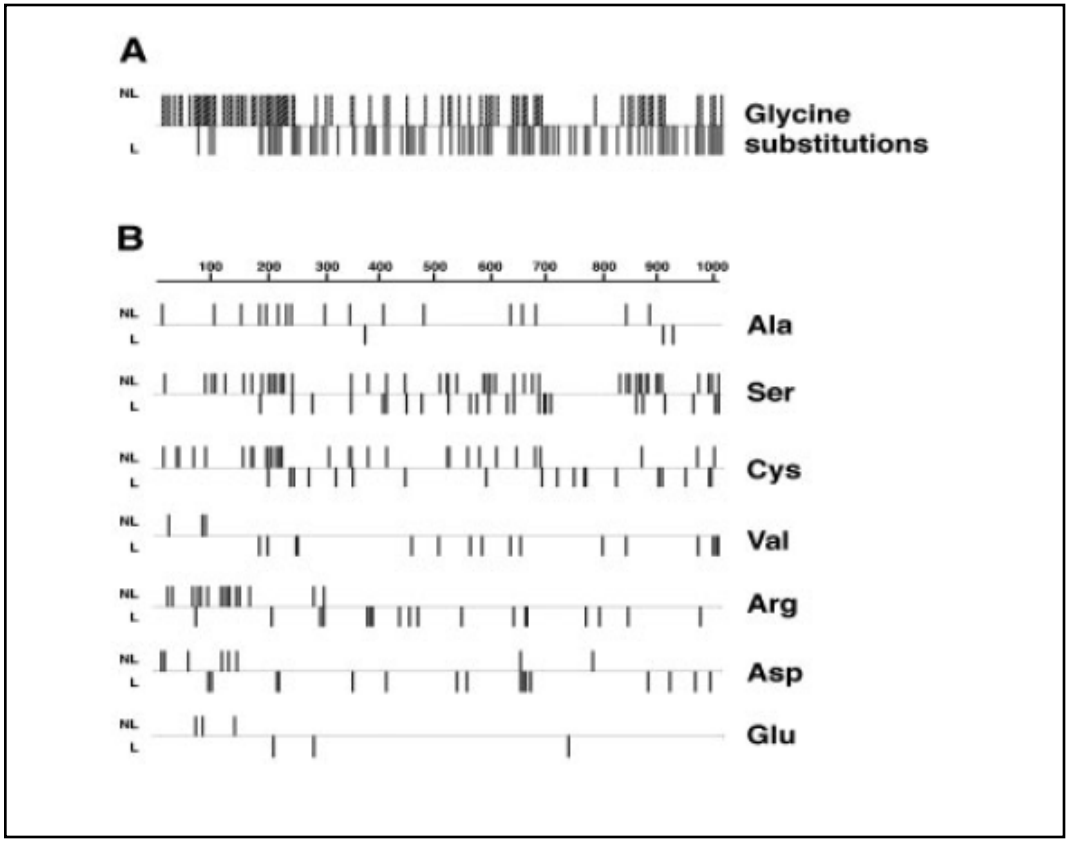
The phenotype is determined by the type of chain involved, the nature and position of the substituting amino acid

Multiple contributing mechanisms including intracellular stress, disruption of interactions between collagen and noncollagenous proteins, compromised matrix structure, abnormal cell-cell and cell-matrix interactions, tissue mineralization

# Genotype-phenotype correlations

- α1(I)-chain:
  - Glycine-substitutions in N-terminal 200 residues are associated with non-lethal phenotype
  - C-terminal glycine substitutions are associated with severe to lethal phenotype
  - Two exclusive “lethal regions”
- α2(I)-chain
  - 80 % of glycine substitutions is non-lethal
  - 8 “lethal regions”

# Distribution of mutations along α1(I)-collagen chain



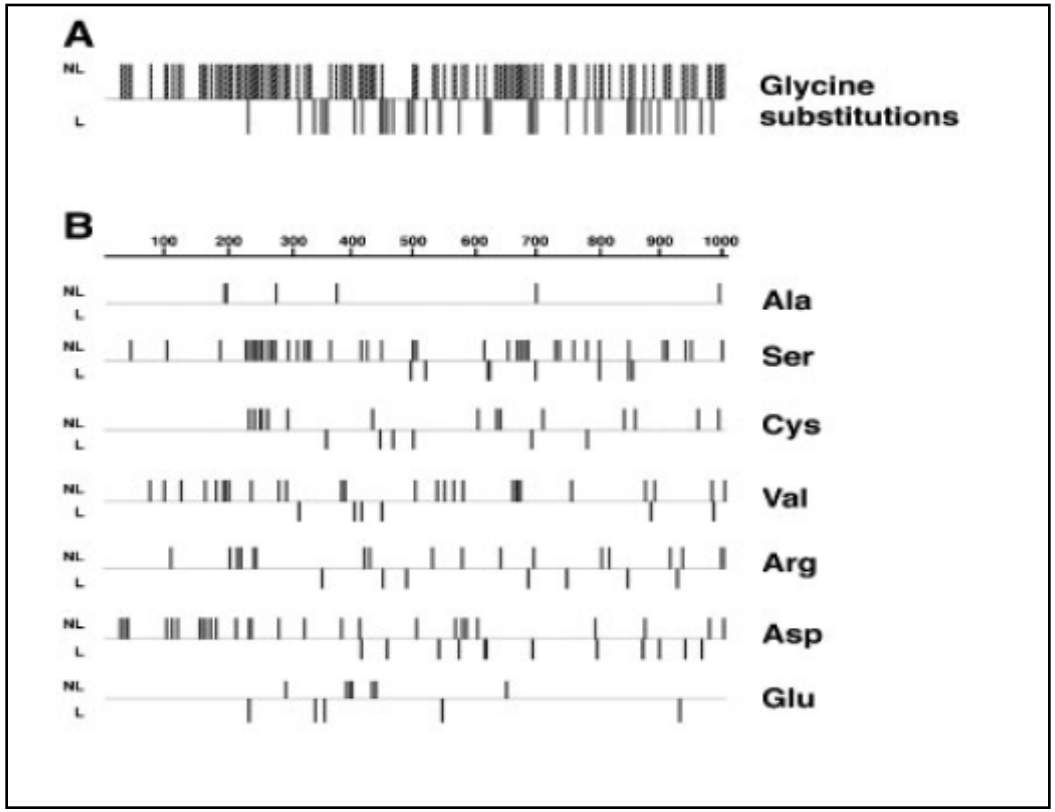

- Valine: branched non-polar side-chain

- Arg, Asp, Glu Charged AA

↓

Overrepresentation of lethal phenotypes

# Distribution of mutations along α2(I)-collagen chain

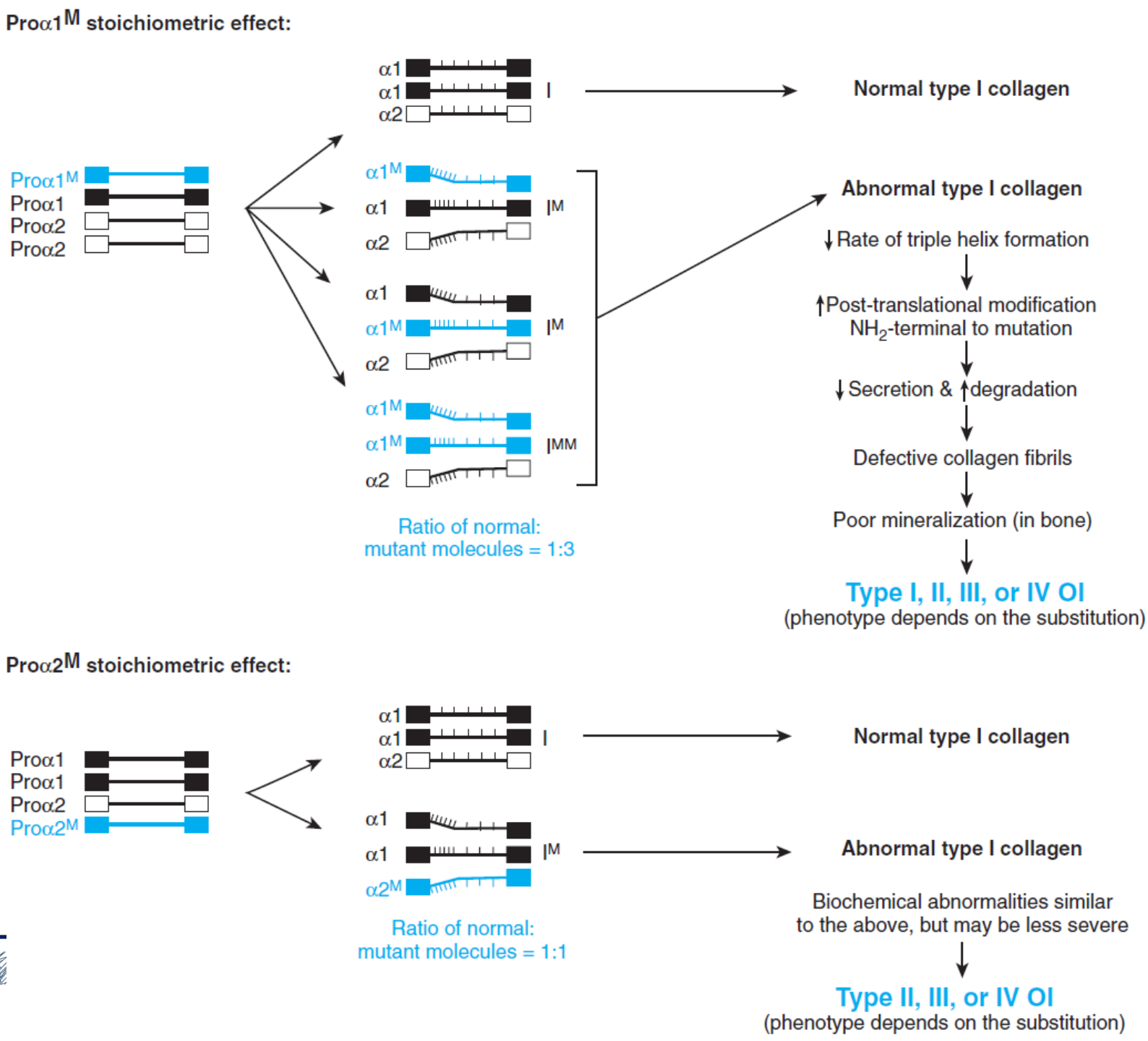



Arg, Asp, Glu
Charged AA

↓

Overrepresentation of lethal phenotypes

# Dominant negative effect



# Genetic counseling

Mild OI: ~60% of individuals with mild OI have *de novo* mutations

Severe (type III) and lethal (type II) OI: virtually 100% of individuals with *de novo* mutations.

**Table 1** | Nosology of osteogenesis imperfecta

| Osteogenesis imperfecta type | Inheritance | Phenotype | Gene defect |
|---|---|---|---|
| ***Classical Sillence types*** | | | |
| I | AD | Mild | Null *COL1A1* allele |
| II | AD | Lethal | *COL1A1* or *COL1A2* |
| III | AD | Progressive deforming | *COL1A1* or *COL1A2* |
| IV | AD | Moderate | *COL1A1* or *COL1A2* |
| ***Unknown etiology*** | | | |
| V | AD | Distinctive histology | Unknown |
| ***Mineralization defect*** | | | |
| VI | AR | Mineralization defect, distinctive histology | *SERPINF1* |
| ***3-hydroxylation defects*** | | | |
| VII | AR | Severe (hypomorphic) Lethal (null) | *CRTAP* |
| VIII | AR | Severe to lethal | *LEPRE1* |
| IX | AR | Moderate to lethal | *PPIB* |
| ***Chaperone defects*** | | | |
| X | AR | Severe to lethal | *SERPINH1* |
| XI | AR | Progressive deforming (Bruck syndrome 1) | *FKBP10* |
| ***Unclassified osteogenesis imperfecta-like or collagen-based disorders*** | | | |
| Bruck syndrome 2 | AR | Joint contractures | *PLOD2* |
| Caffey disease | AD | Cortical hyperostosis | *COL1A1* |
| Osteoblast maturation defects | AR | Moderate | *SP7* |

Abbreviations: AD, autosomal dominant; AR, autosomal recessive.



Forlino et al. Nat Rev Endocrinol, 2011

# The Ghent experience for OI: 1990-2015

417 referrals for OI: biochemichal and/or molecular collagen studies

383 (~92%) heterozygous mutations in type I collagen

- 270 *COL1A1*
- → 120 with *COL1A1* null allele
- 113 *COL1A2*

34 (~8%) no type I collagen defect

17 ( ~4%) homozygous/compound heterozygous mutations in

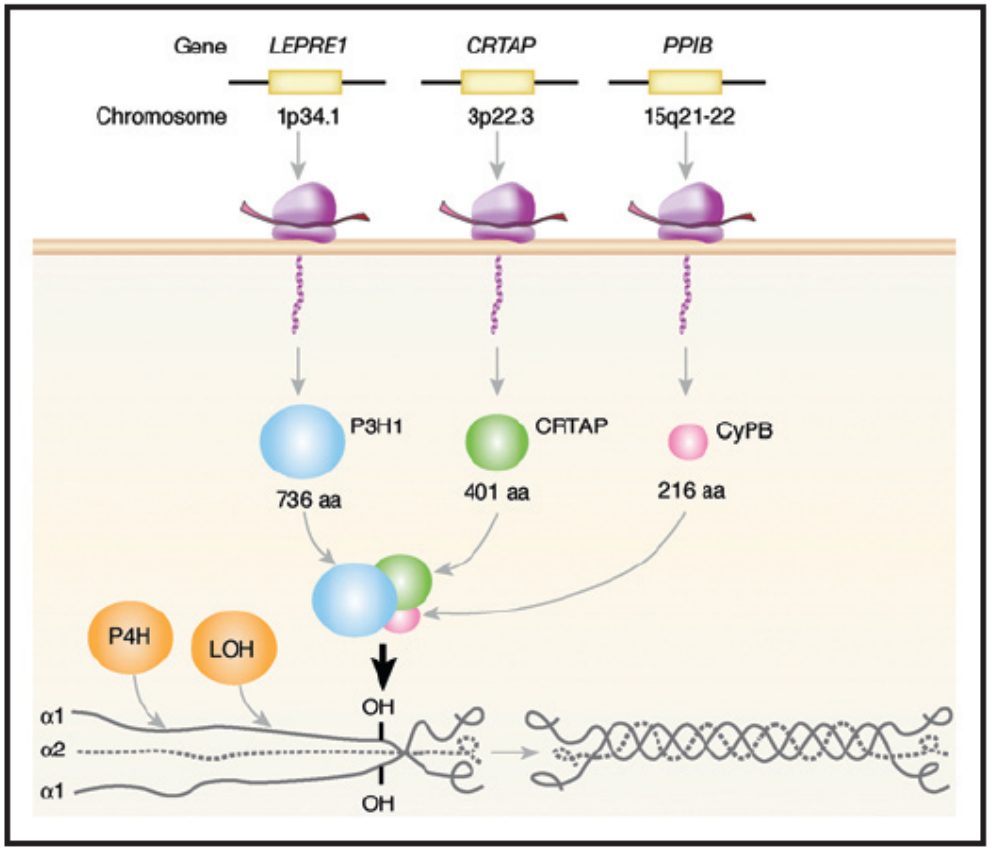
- *LEPRE1* (6)
- *CRTAP* (2)
- *FKBP10* (5)
- *SERPINH1* (1)
- *SERPINF1* (2)
- *PLOD2* (1)
- *CREB3L1* (1)
- *TAPT1* (2)

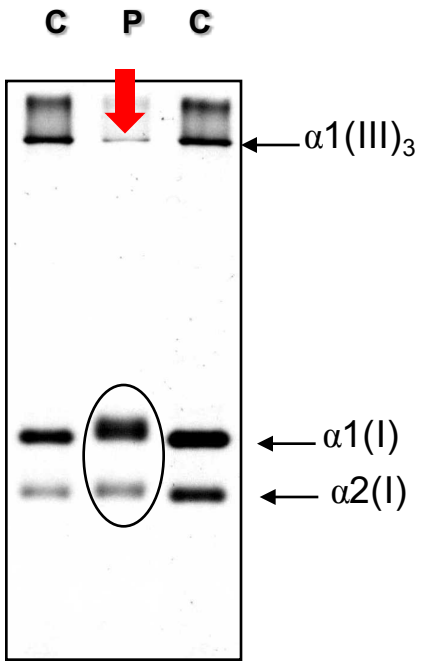
17(~4%) "unsolved" after screening all OI genes

**Diagnostics:**

- **NGS gene panels**
- **MLPA, arrayCGH**

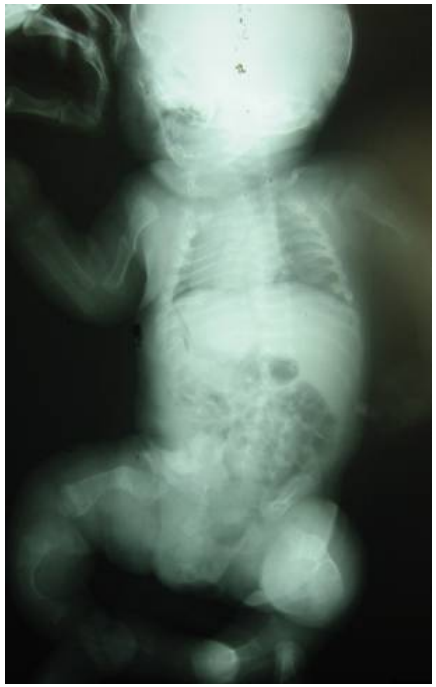
# Recessive OI: CRTAP/LEPRE/PPIB complex

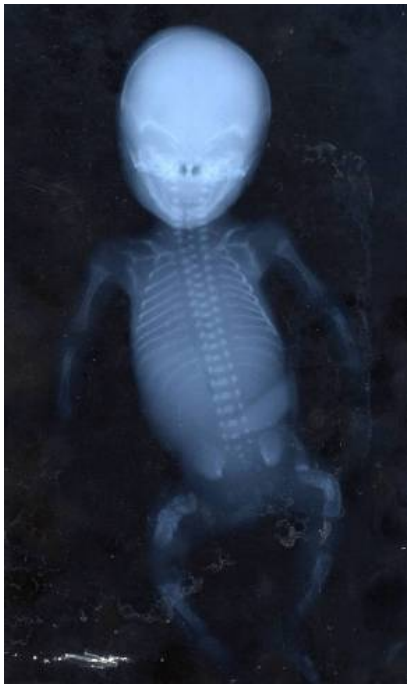


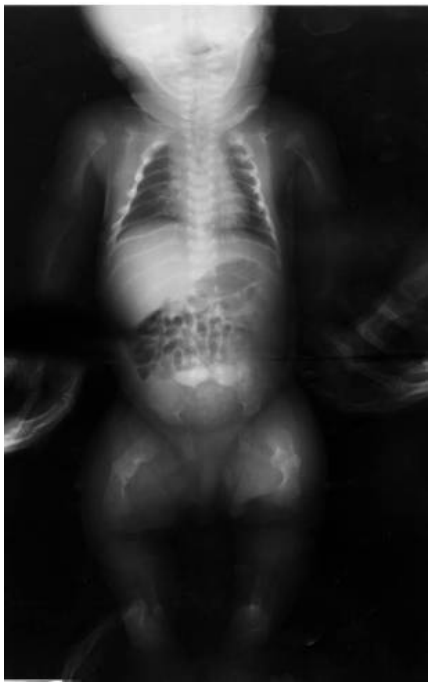



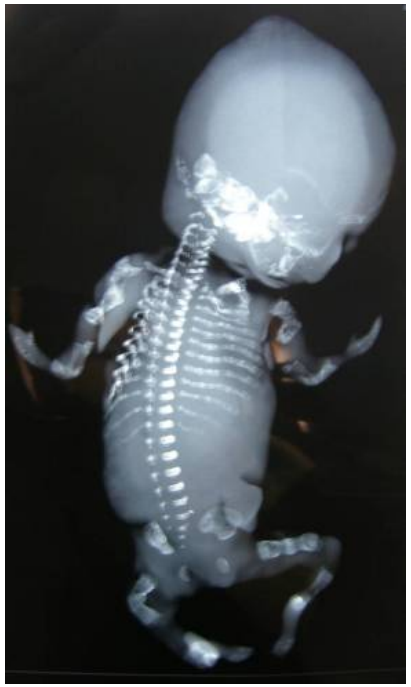
# Mutations in *LEPRE1*: OI type VIII

| Proband 1 | Proband 2 | Proband 3 | Proband 4 |
|---|---|---|---|
| Hom. c.1365-1366delAGinsC (p.Glu455fs) | Hom. c.628C>T (p.Arg210*) | Het. c.1102C>T (p.Arg368*)<br>Het. c.2055+18G>A, Intron 14 | Hom. c.2055+18G>A, Intron 14 |









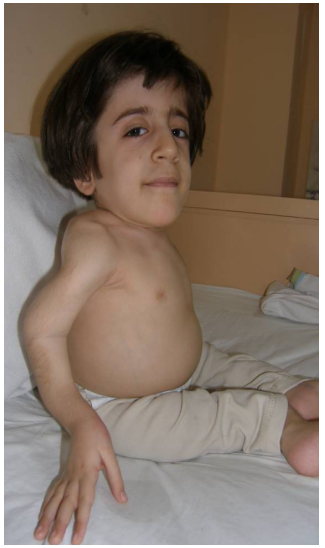
- Lack of calvarial ossification
- Beaded ribs with multiple fractures
- Platyspondyly
- Shortened, wide, bowed and fractured large tubular bones

→ **Recessive OI or Severe/Lethal Autosomal Dominant OI ??**

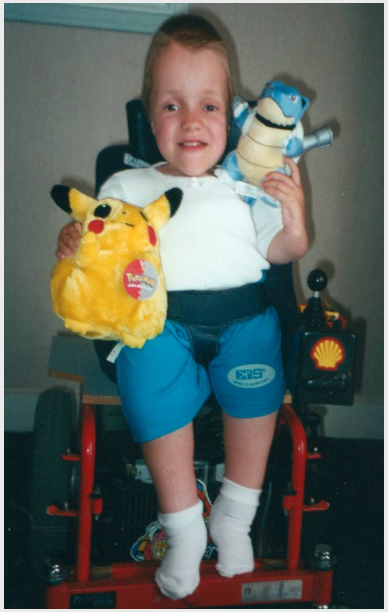
# Mutations in *LEPRE1*: OI type VIII

- *Childhood to adolescence*
  - Severe growth deficiency & extreme bone fragility
  - Very short, wide bowed and fractured tubular bones
  - Popcorn-like 'epiphyses' and round cyst-like translucencies
  - Barrel-shaped chest, short ribs, platyspondyly, thoracic scoliosis
  - Tall prominent forehead, narrow head, round face
  - Long, gracile hands with joint hyperlaxity

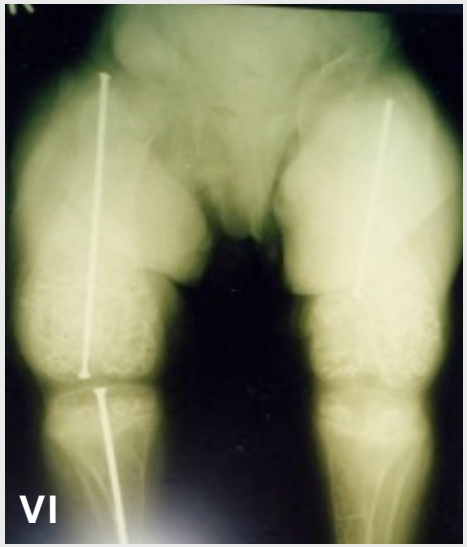
P4, 8 yrs

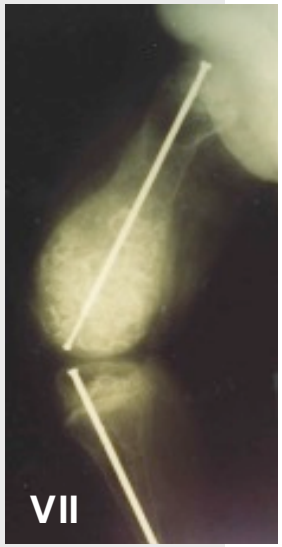


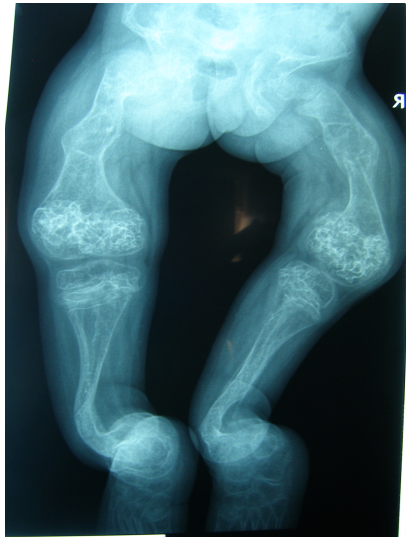
P3 ,10 yrs



P3, X-rays at 5 yrs and 4 mths



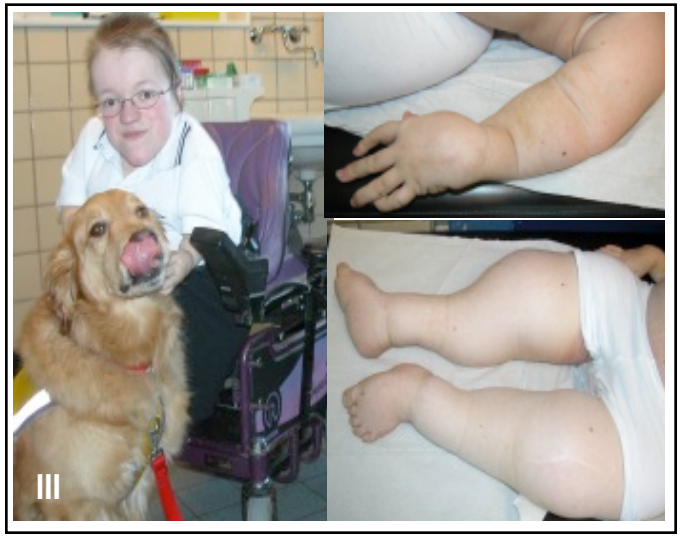



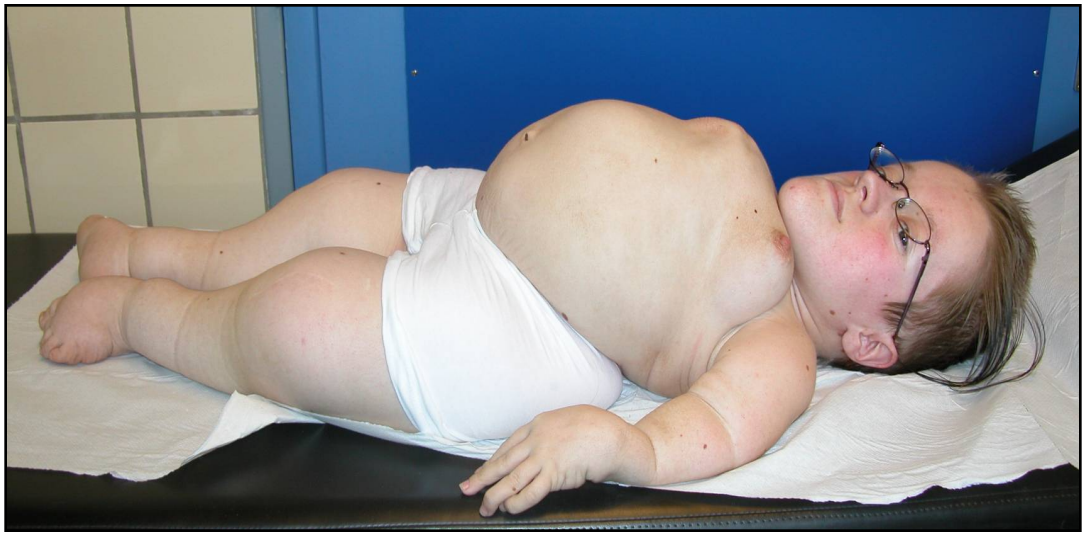
P4

# Mutations in *LEPRE1*: OI type VIII

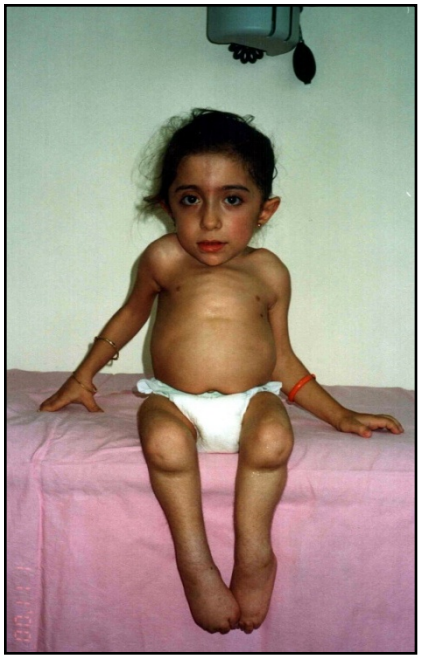
- *Adolescence/early adulthood ( > 15 yrs)*
- Extreme short stature, very severe osteoporosis
- Disappearance of the popcorn-like structures
- Additional widening of the rhizomelic diaphyses
- Progressive narrowing and bowing of the mesomelic diaphyses
- Reduced knee joint spaces
- Long hands & fingers

P3 at age 17 ½ yrs

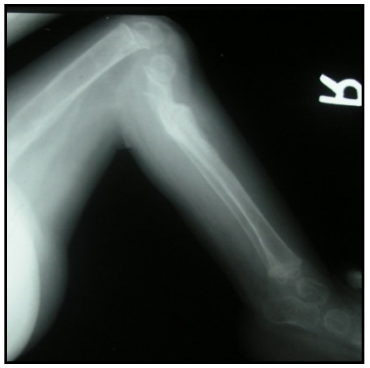


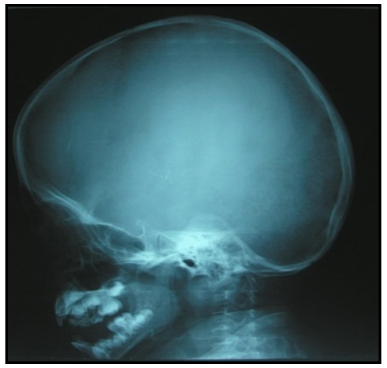



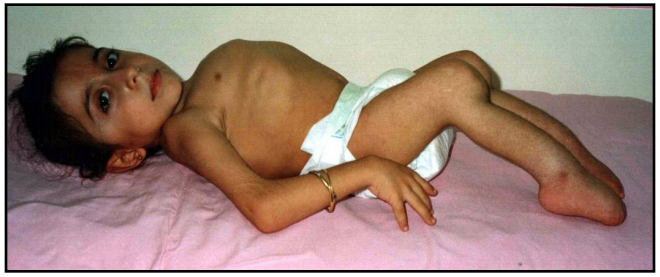
# Mutations in *FKBP10*: OI type XI

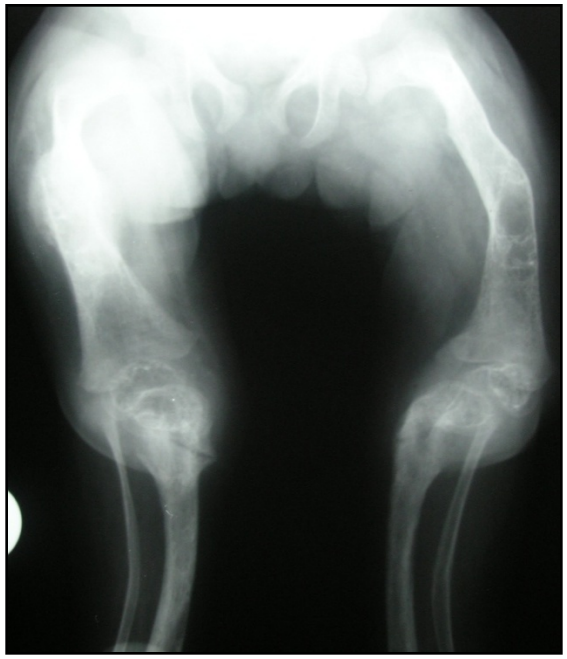


P4 at age 7 yrs









Hom. p.Gly278Argfs*95

Consanguineous parents of Turkish origin

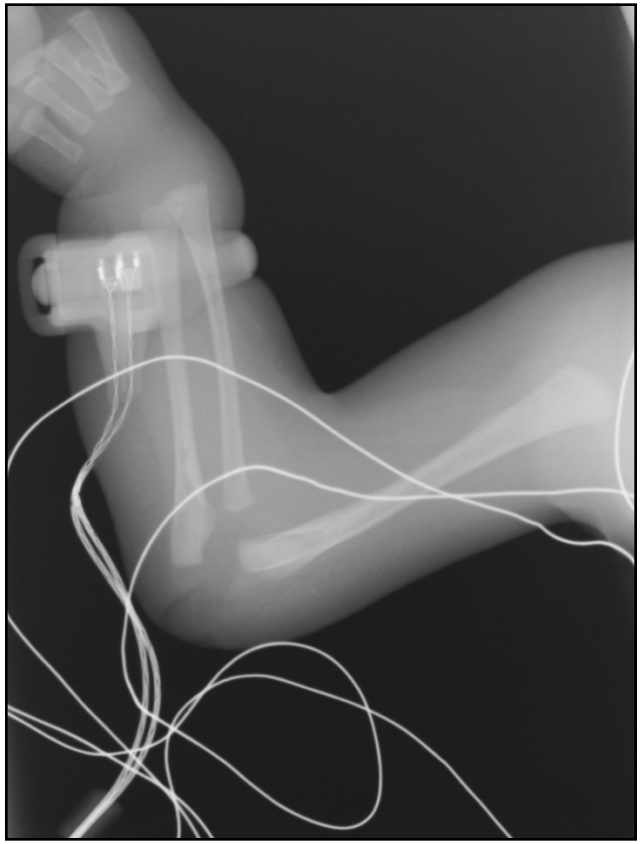
Congenital contractures of knees and ankles, wormian bones

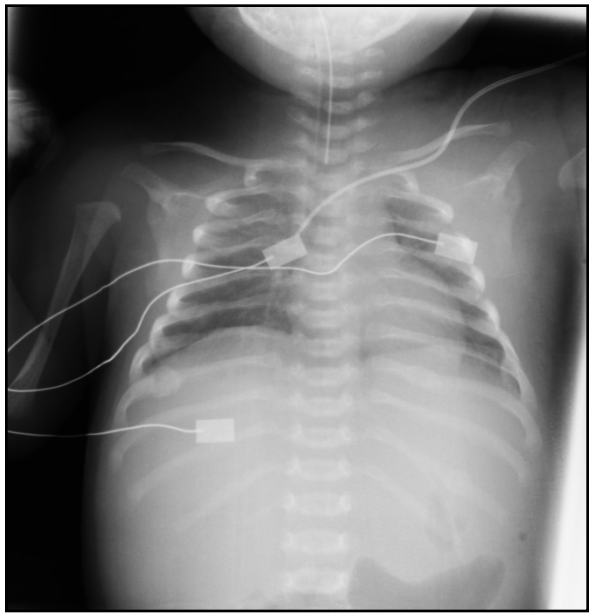
Since age 2 months recurrent costal and femoral fractures

Triangular face, normal dentition & hearing, white sclerae

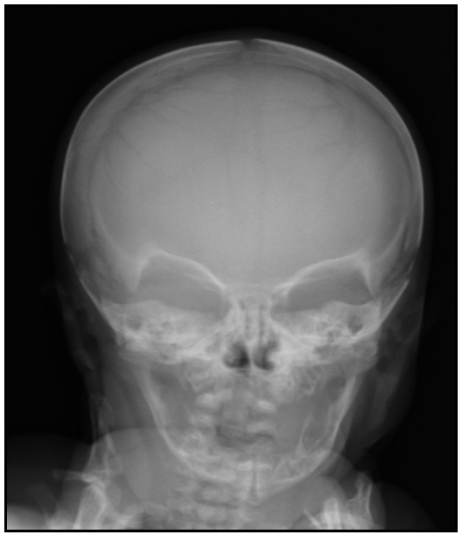
At age 9 yrs greatly restricted limb movement, wheelchair-bound

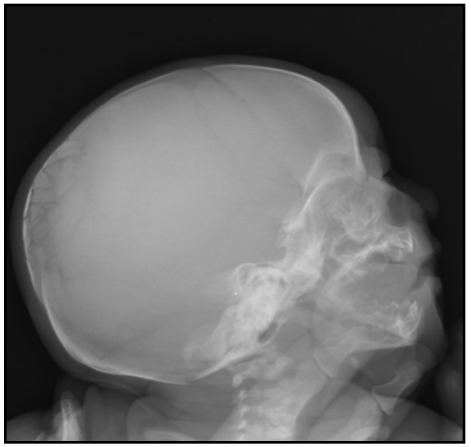
# Mutations in *FKBP10*: OI type XI





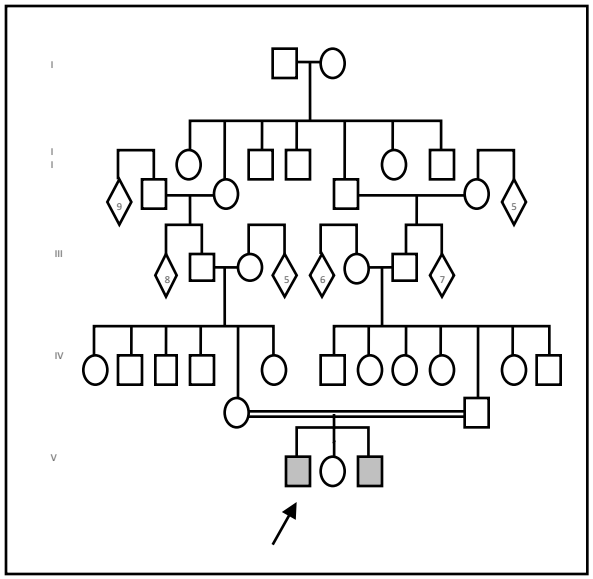
Rib fractures





wormian bones

# Tricky case



- Proband : 1st child from healthy consanguineous parents of aboriginal Chilean origin

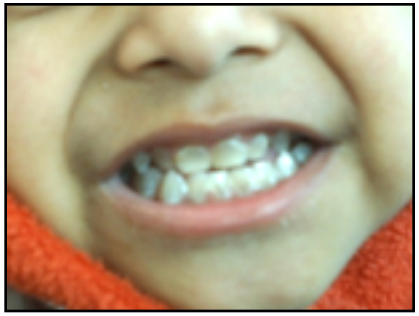
- Referred with suspicion of OI type VII:

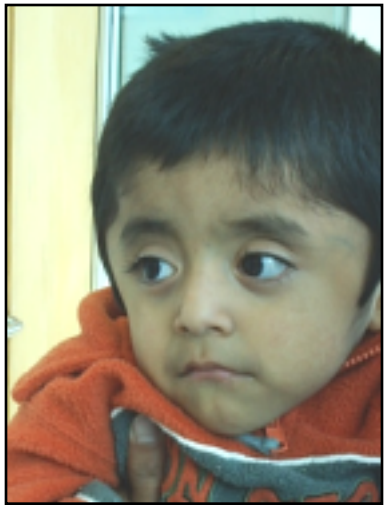
➢ At birth: small stature, rhizomelia, multiple fractures, bowing of limbs,

➢ walked at age 2 yrs, stopped walking at age 6 yrs

➢ severe osteoporosis ( Z-score -6.65)

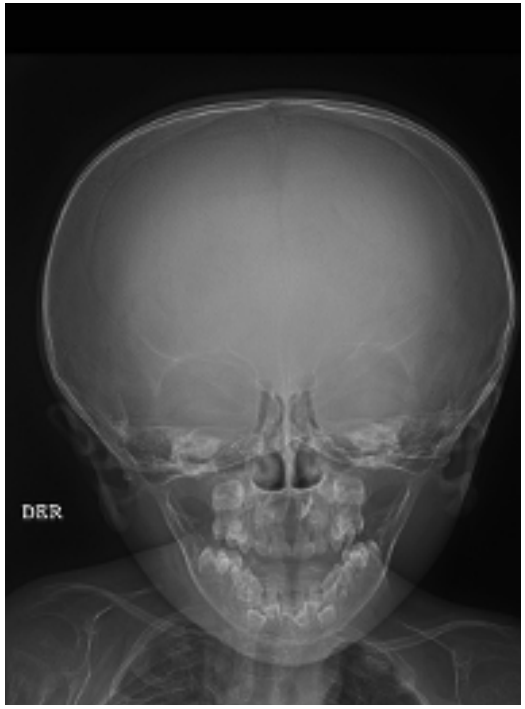
- Brother of P1: similar clinical history

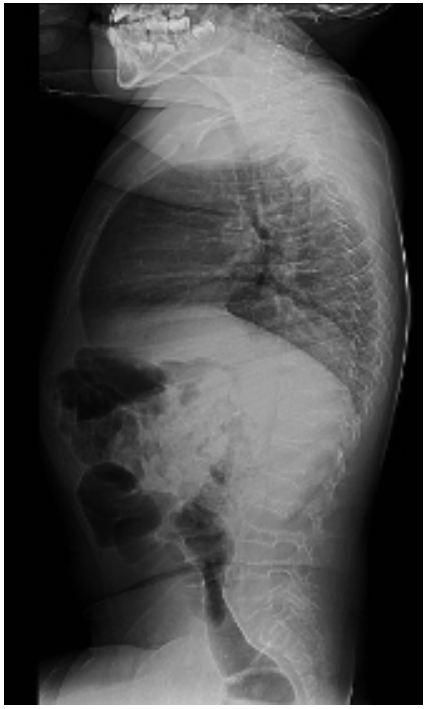


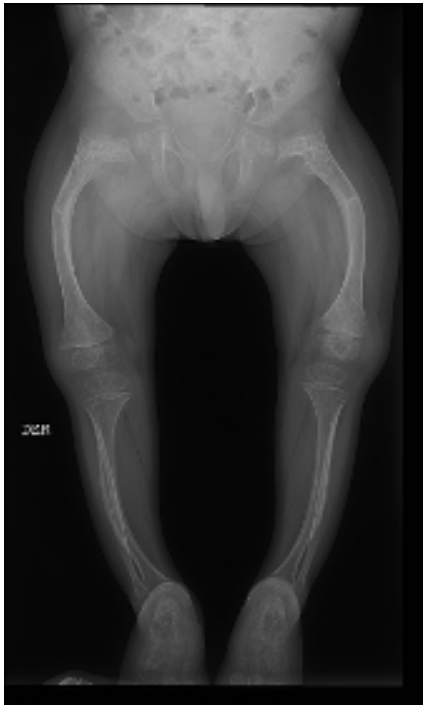


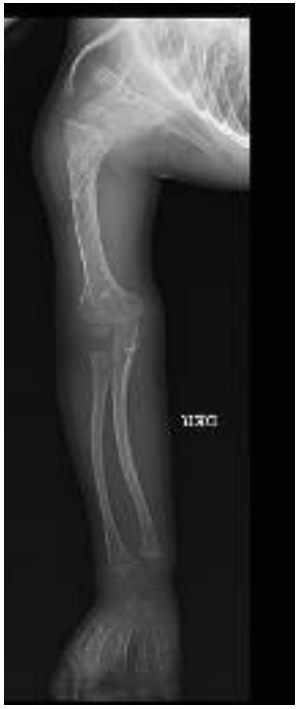
- *CRTAP* excluded in another center

# Tricky case





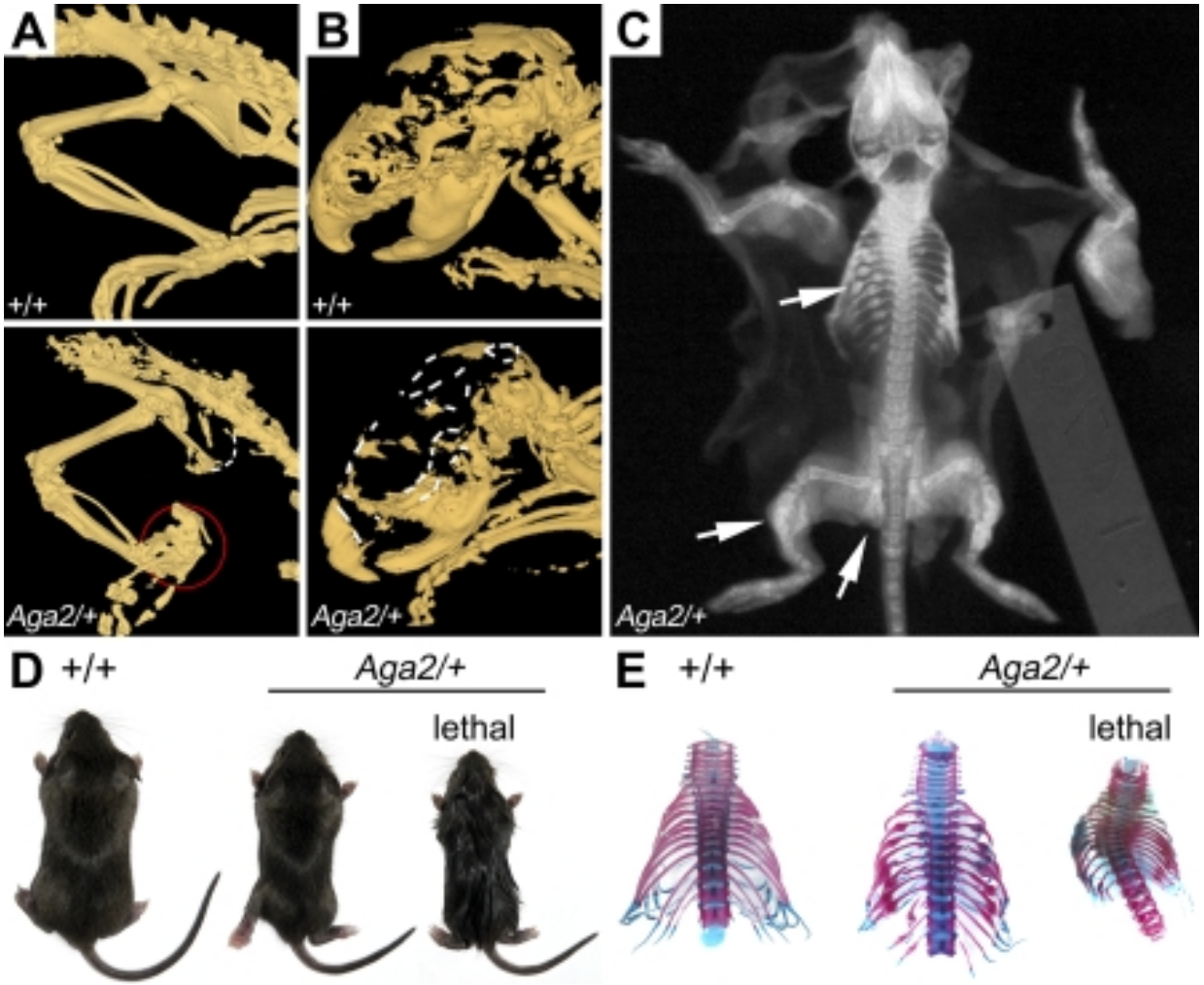


- Exclusion of *LEPRE1* and *PPIB*

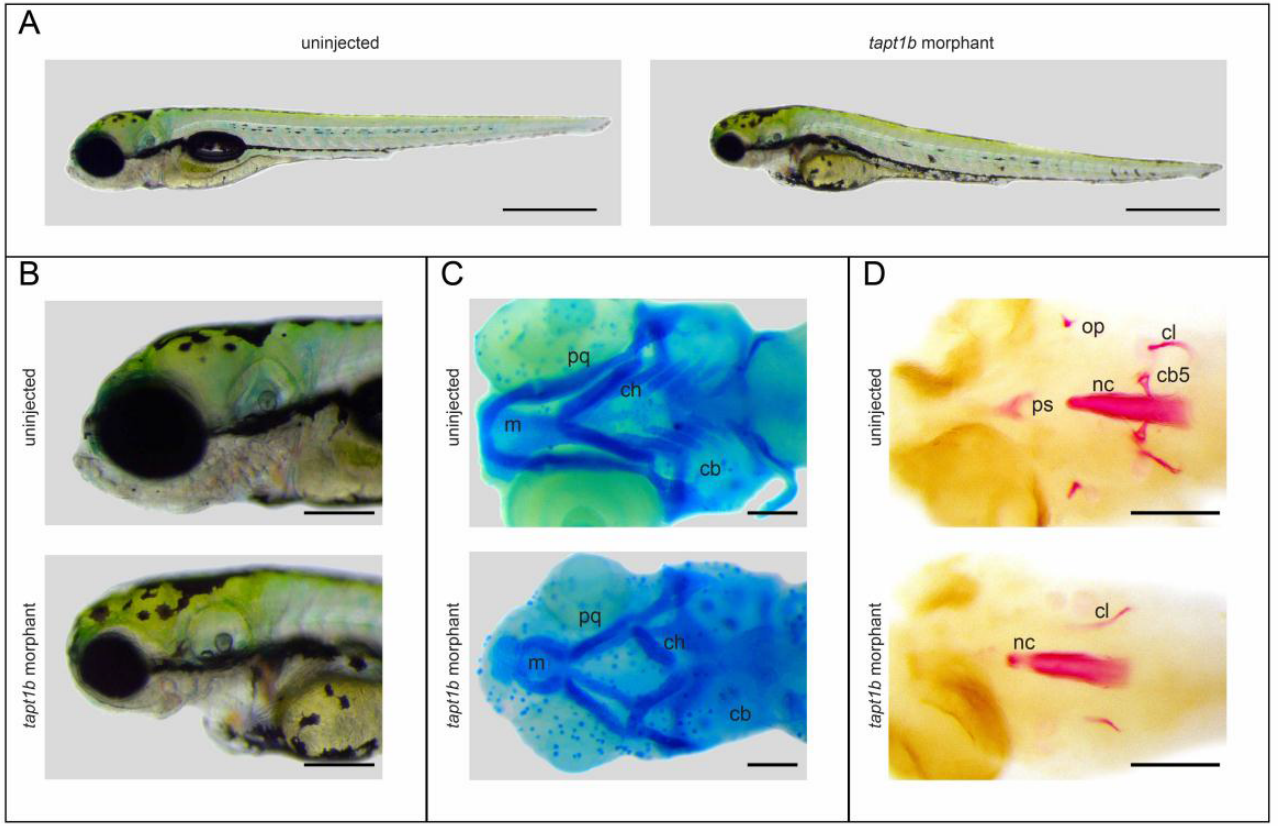
- *COL1A2:* heterozygous c.2565+1G>A
  - Present in 2 affected children
    - Absent in healthy parents

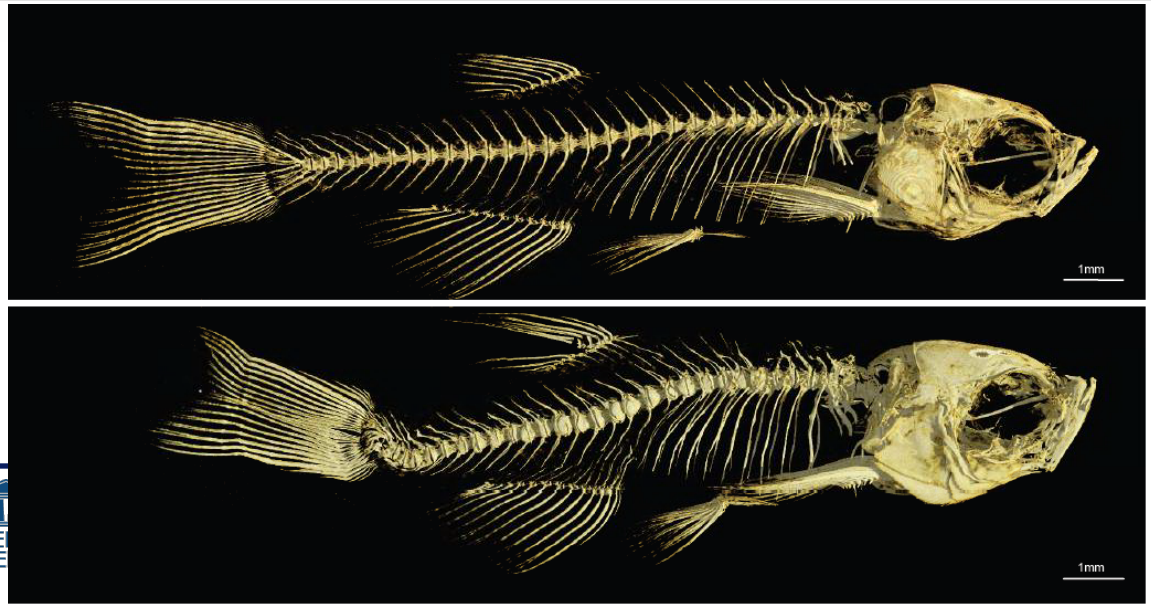
→ Parental mosaicism

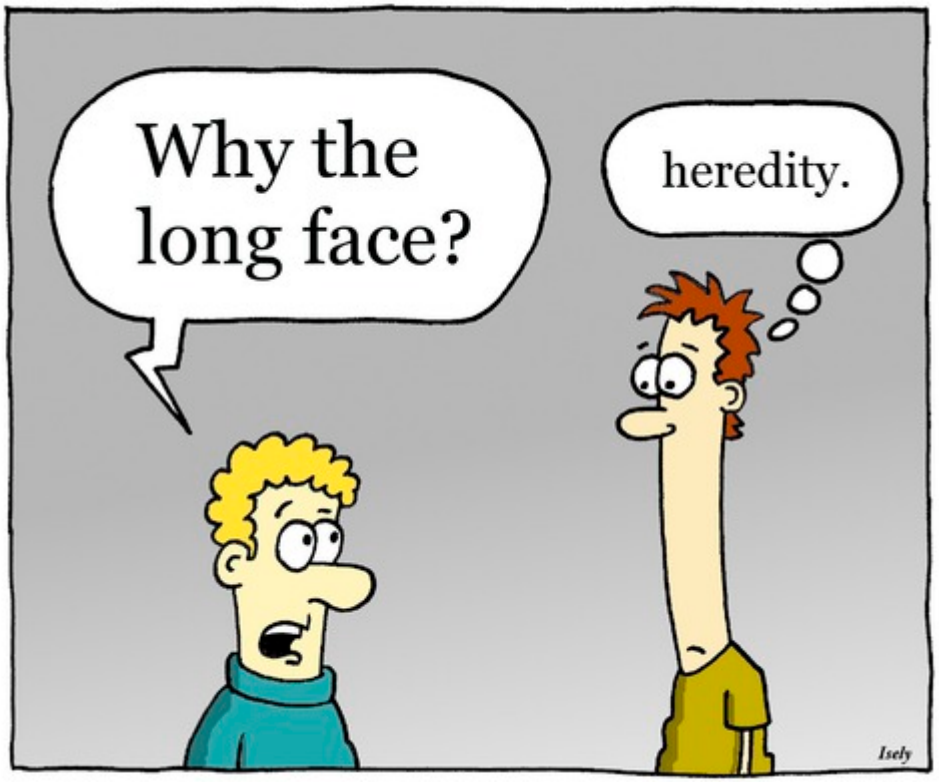
# OI mice



# OI zebrafish




Why the long face?
heredity.
Isely