UNIVERSITEIT GENT

FACULTEIT GENEESKUNDE EN GEZONDHEIDSWETENSCHAPPEN

Disorders of structural proteins

Postgraduate course Human Genetics

08/12/2023

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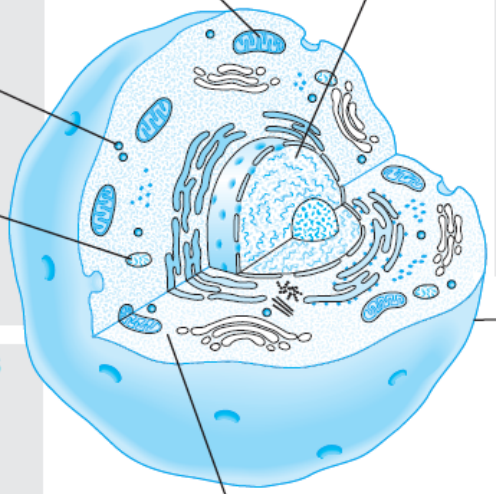


ORGANELLES

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
- α_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*

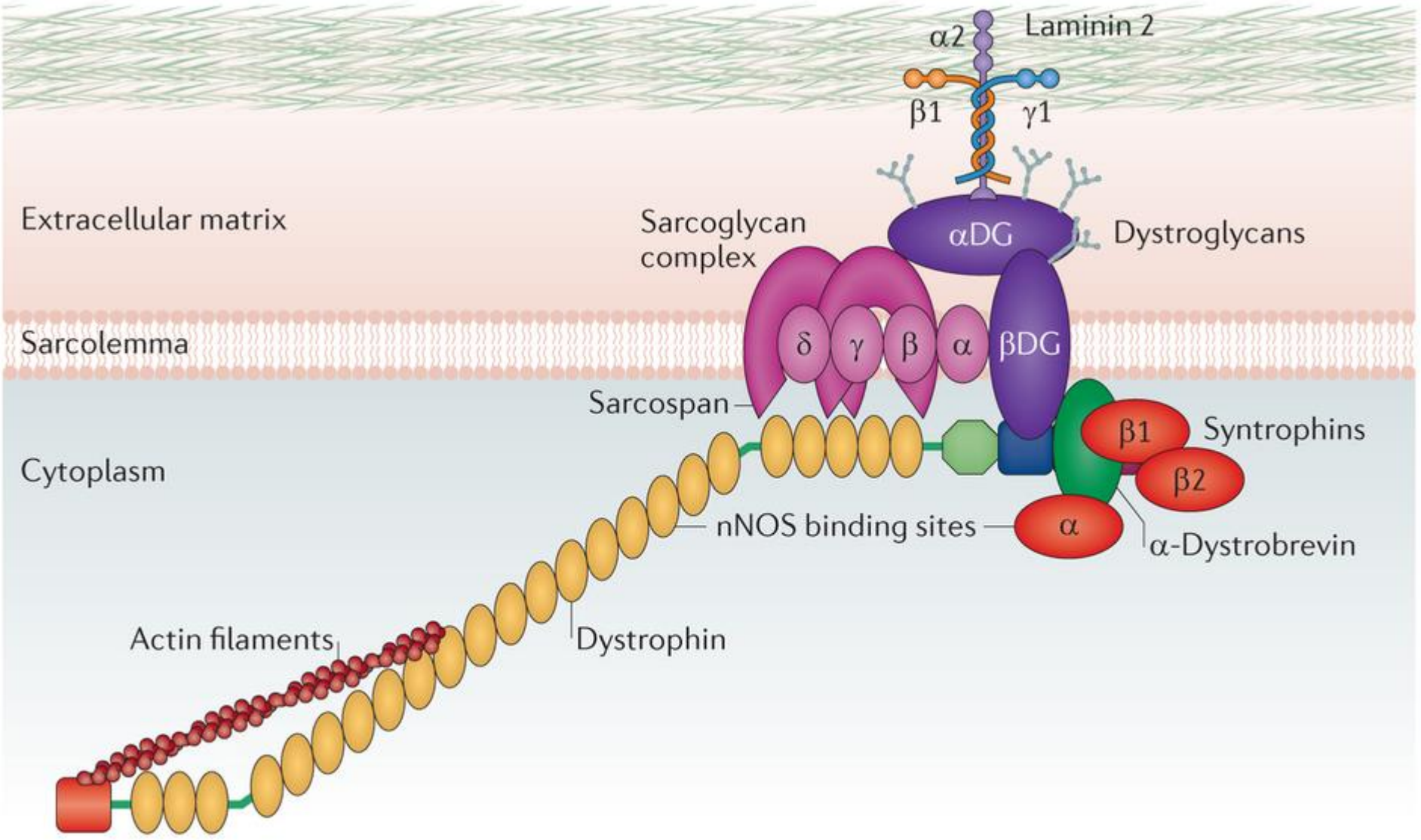
Ion transport
- CFTR
  - *cystic fibrosis*

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

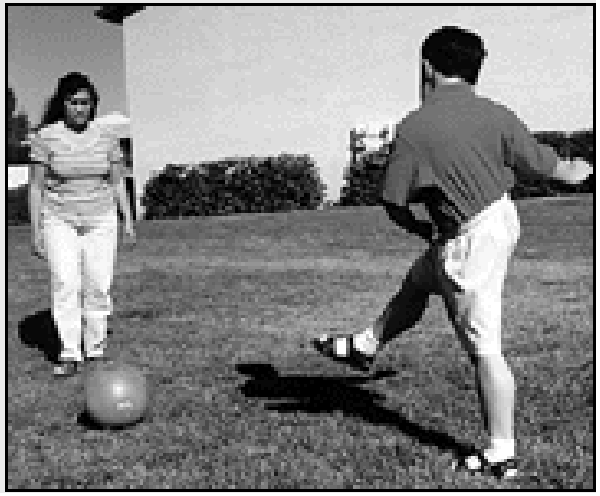
- asymptomatic increase in serum concentration of creatine phosphokinase (CK)
- muscle cramps with myoglobinuria
- isolated quadriceps myopathy

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

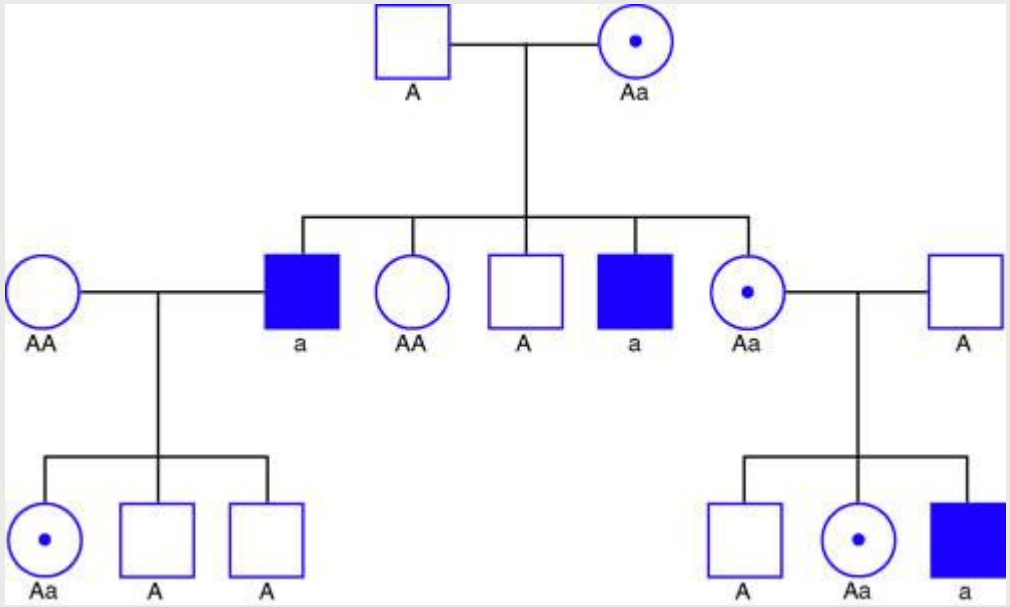
Molecular Genetics: inheritance

- 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

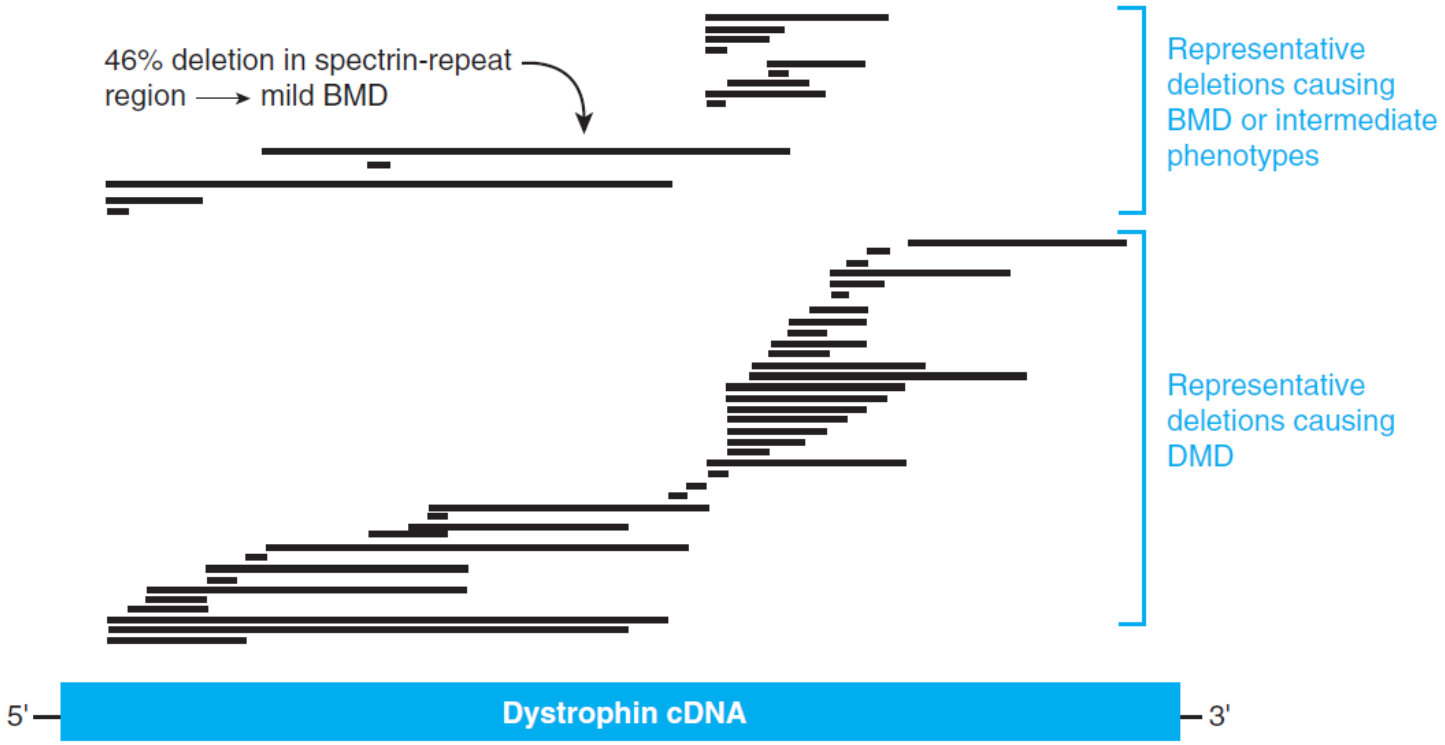
lack of dystrophin expression: DMD

- 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

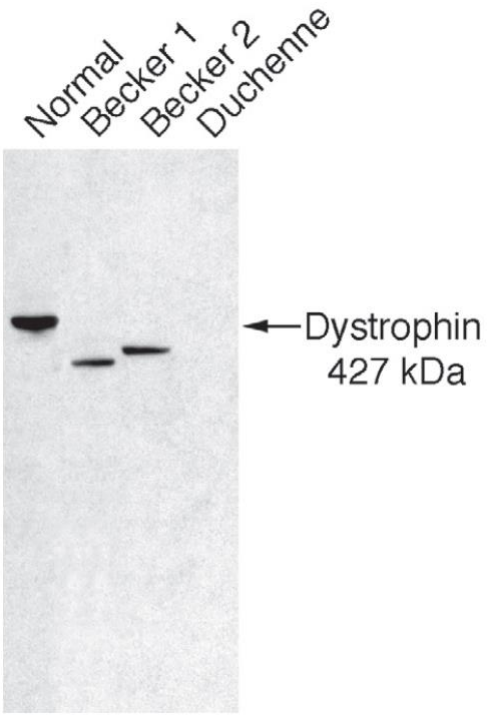
	phenotype	% of affected individuals	Serum CK conc.
Males	DMD	100%	> 10x normal
	BMD	100%	> 5x normal
	DMD-associated DCM	Most individuals	“increased”
Female carriers	DMD	~50%	2- 10x normal
	BMD	~30%	2- 10x normal

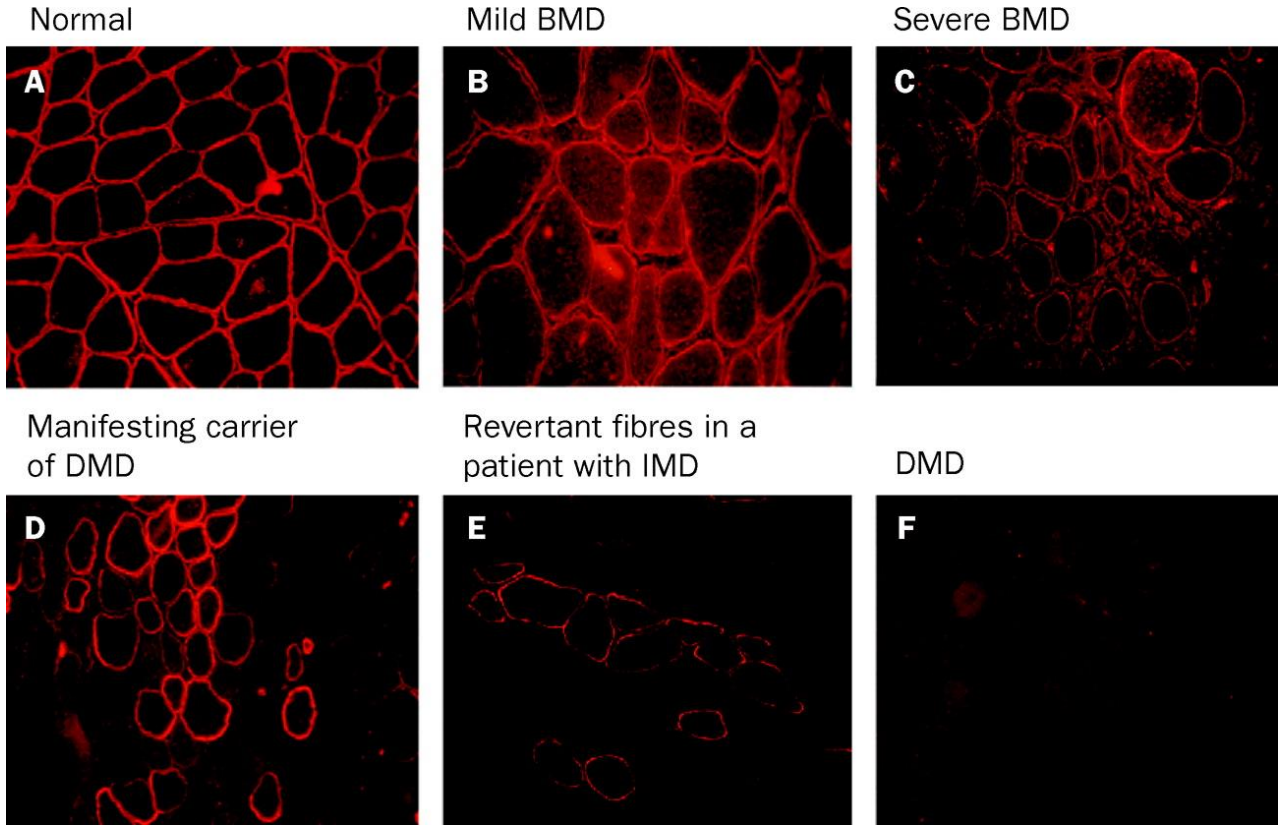
Testing

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 Abnormal	20%-50% 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

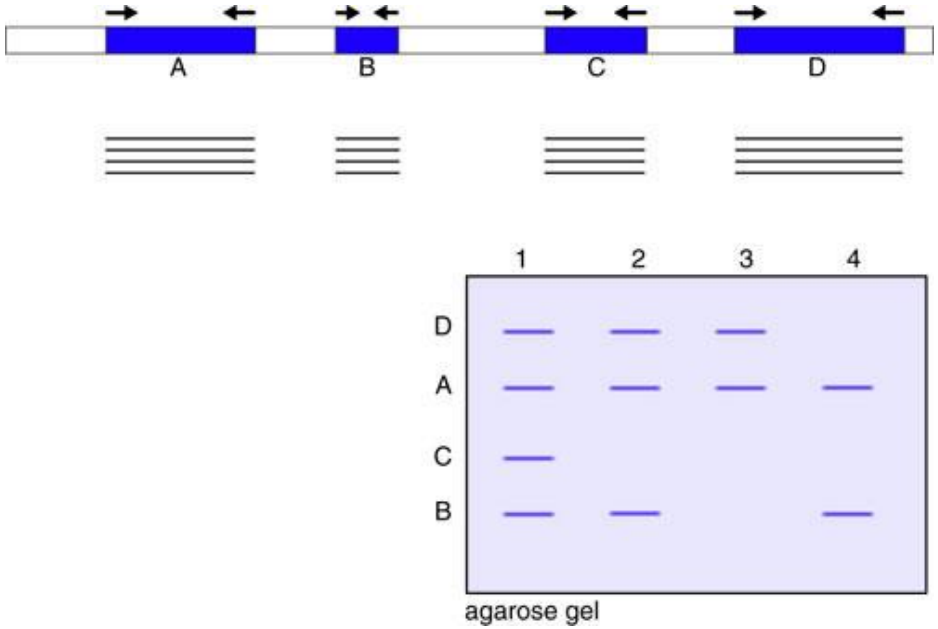
Molecular genetic testing

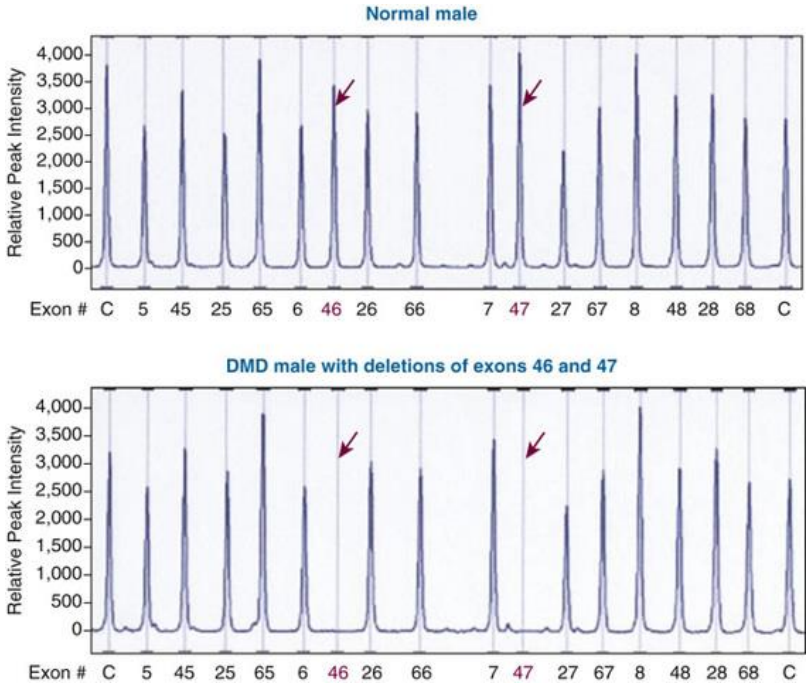
- *Deletion/duplication analysis*
  - Multiplex PCR, southern blotting and FISH (deletions)
  - Southern blotting and quantitative PCR (duplications)
  - MLPA (deletions/duplications), arrayCGH

- *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

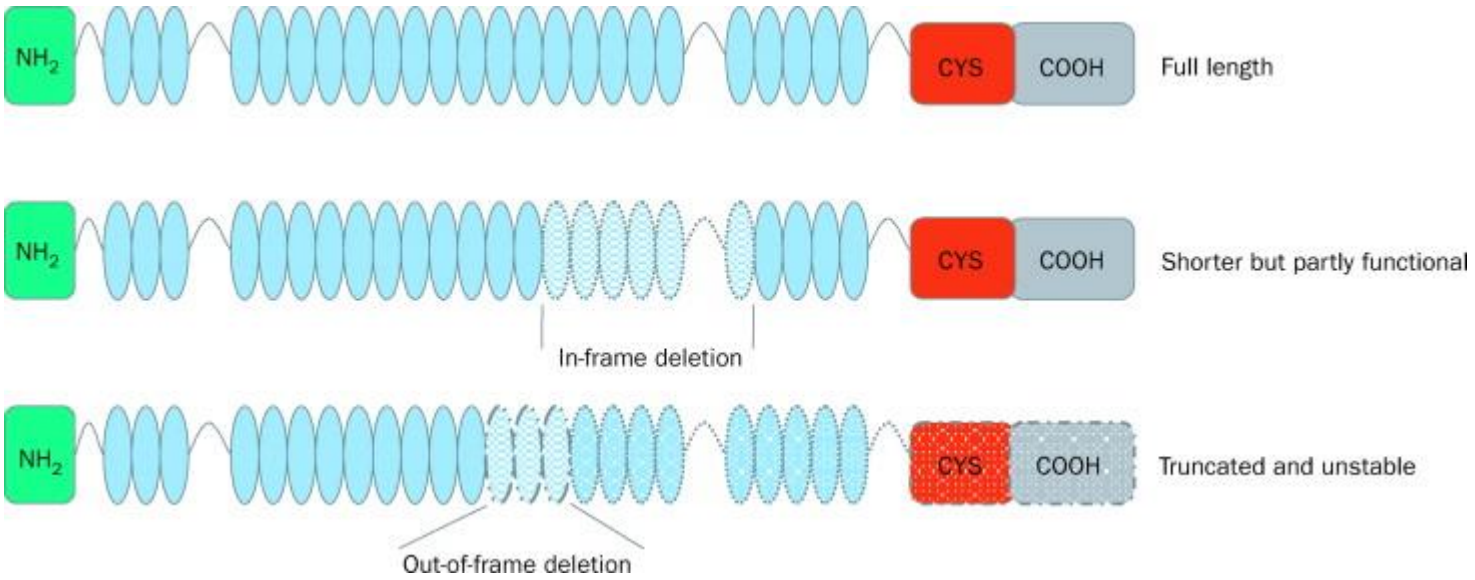
Multiplex PCR - MLPA

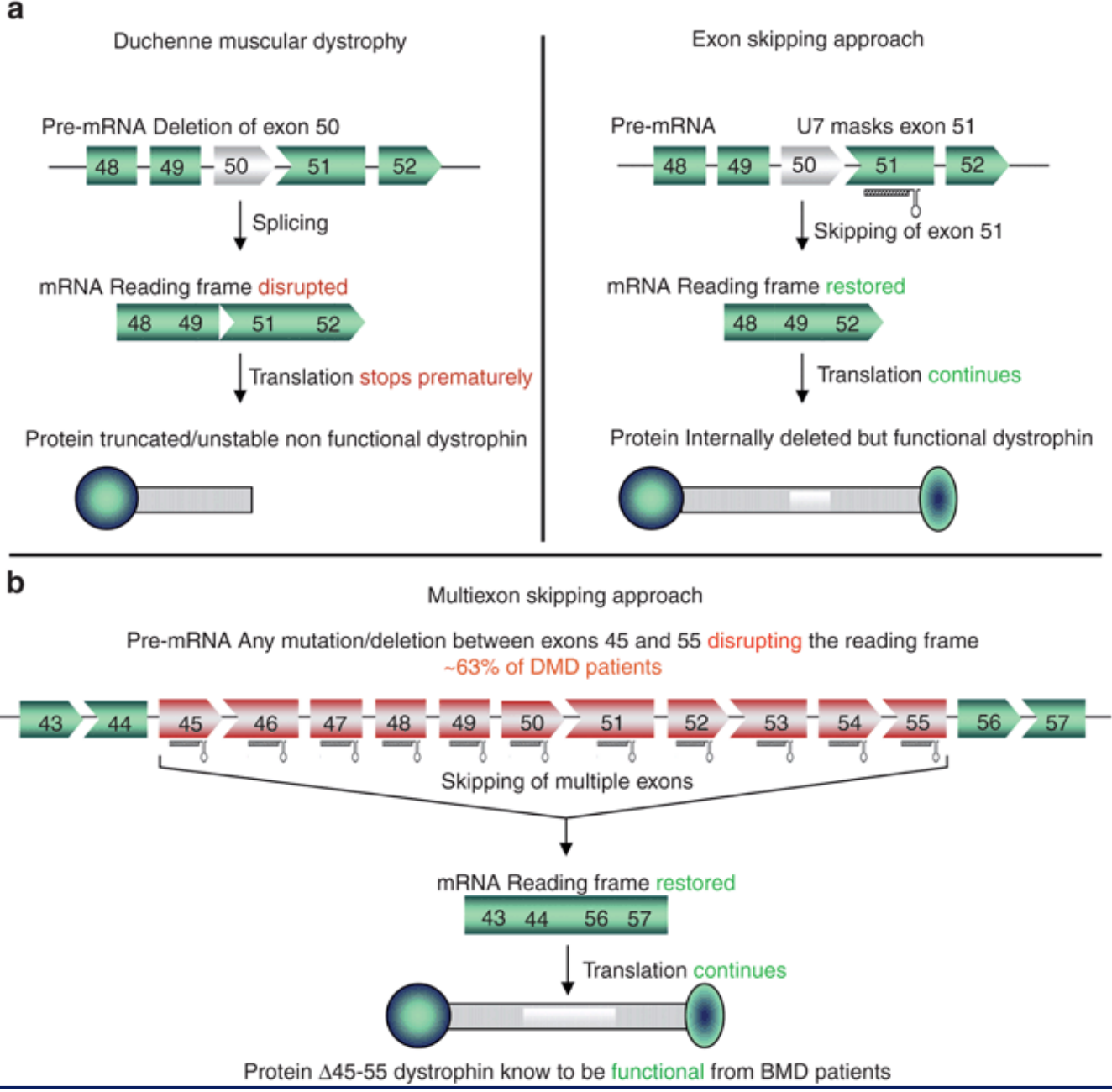




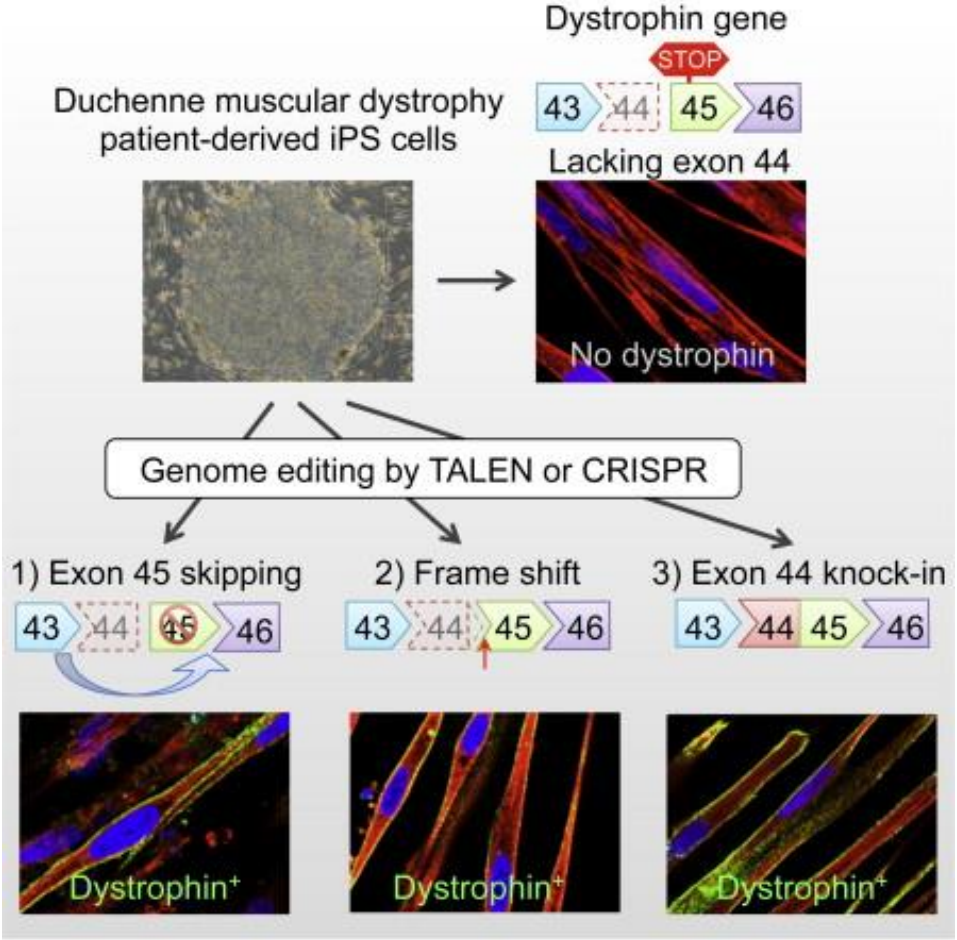
Multiplex PCR analysis of dystrophin gene deletions. Exons A, B, C, and D are amplified in a single PCR reaction (arrows indicate PCR primers). The products (shown below each exon) are separated by size on an agarose gel and are visualized.

Reading frame hypothesis

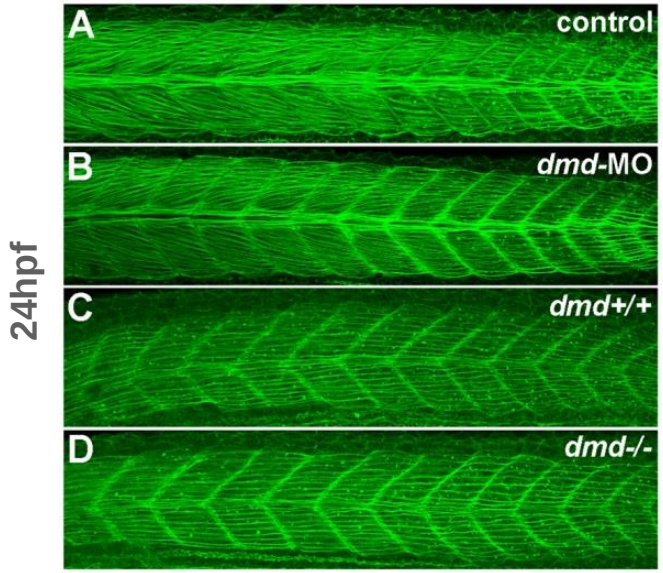


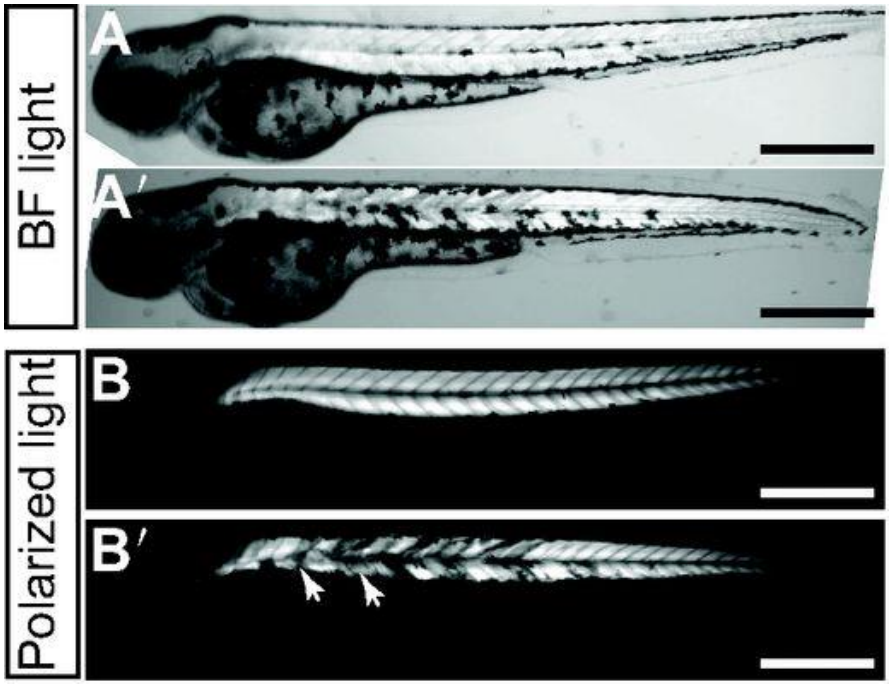

a
Duchenne muscular dystrophy
Pre-mRNA Deletion of exon 50
48
49
50
51
52
Splicing
mRNA Reading frame disrupted
48 49 51 52
Translation stops prematurely
Protein truncated/unstable non functional dystrophin
Exon skipping approach
Pre-mRNA
U7 masks exon 51
48
49
50
51
52
Skipping of exon 51
mRNA Reading 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

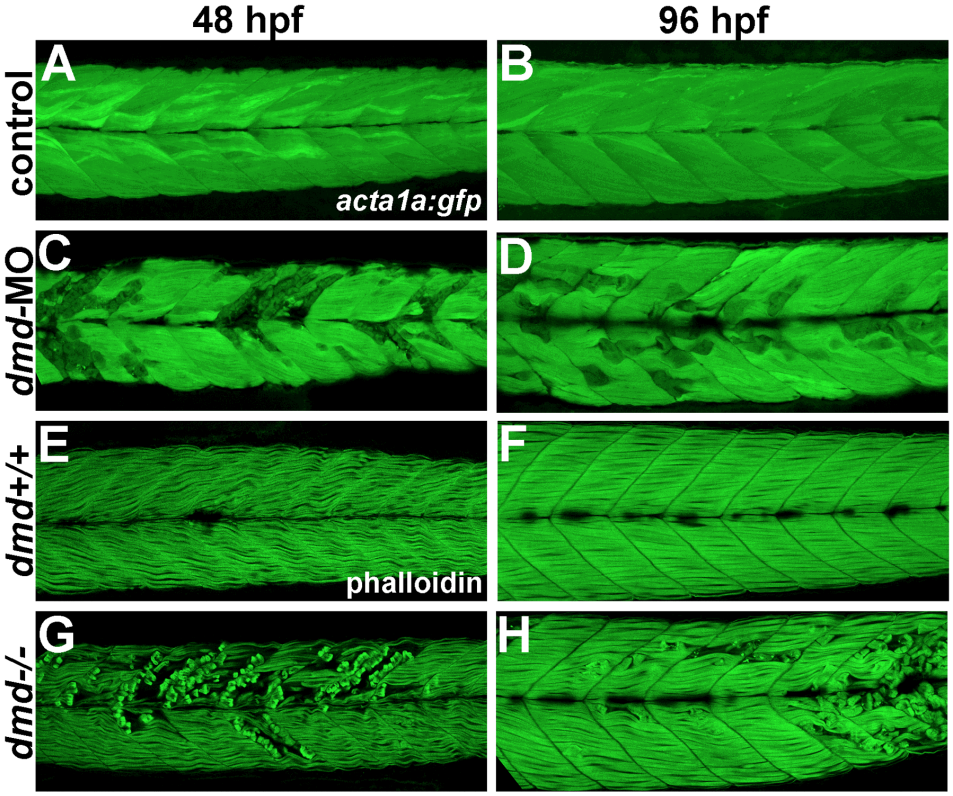
Correction of *DMD* in Patient Induced Pluripotent Stem Cells by TALEN and CRISPR-Cas9

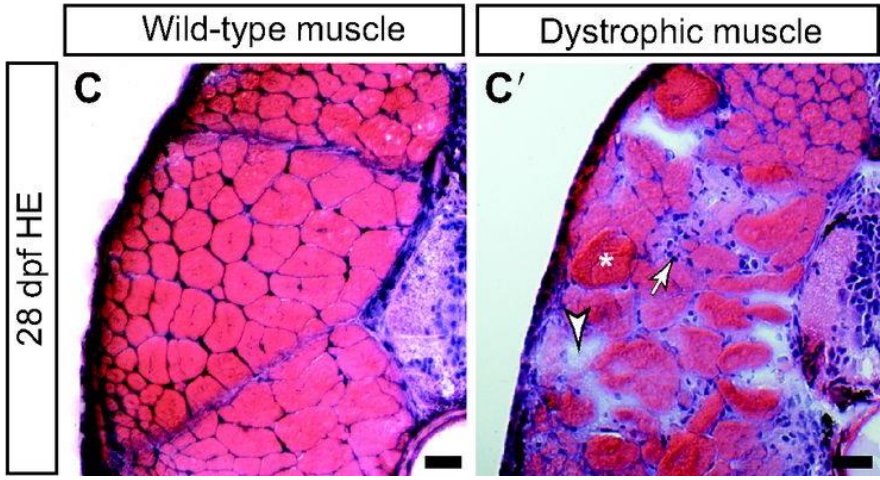


dmd zebrafish







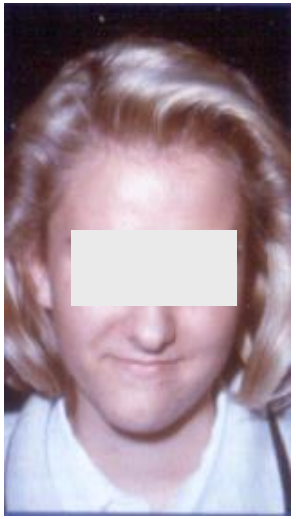


Mutations in collagen structural genes: Osteogenesis imperfecta

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

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

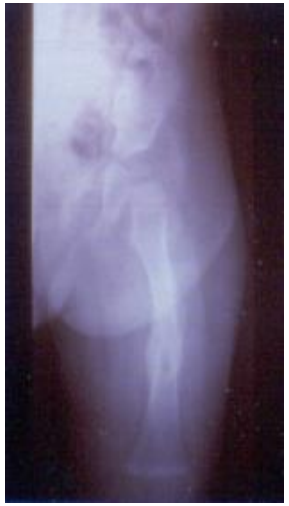
- Defects of type I collagen
- Due to mutations in *COL1A1/COL1A2*

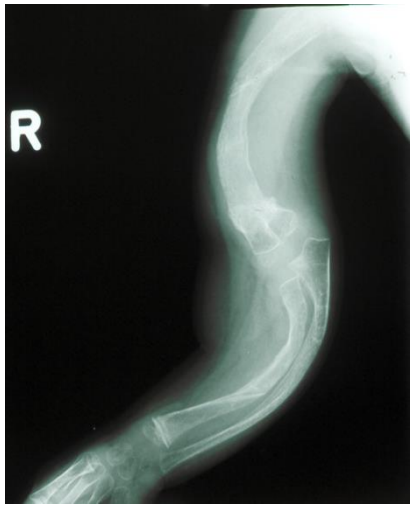


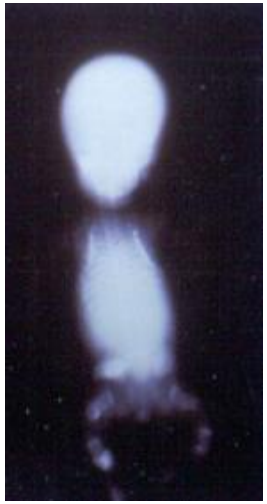
Mild OI

Severe OI

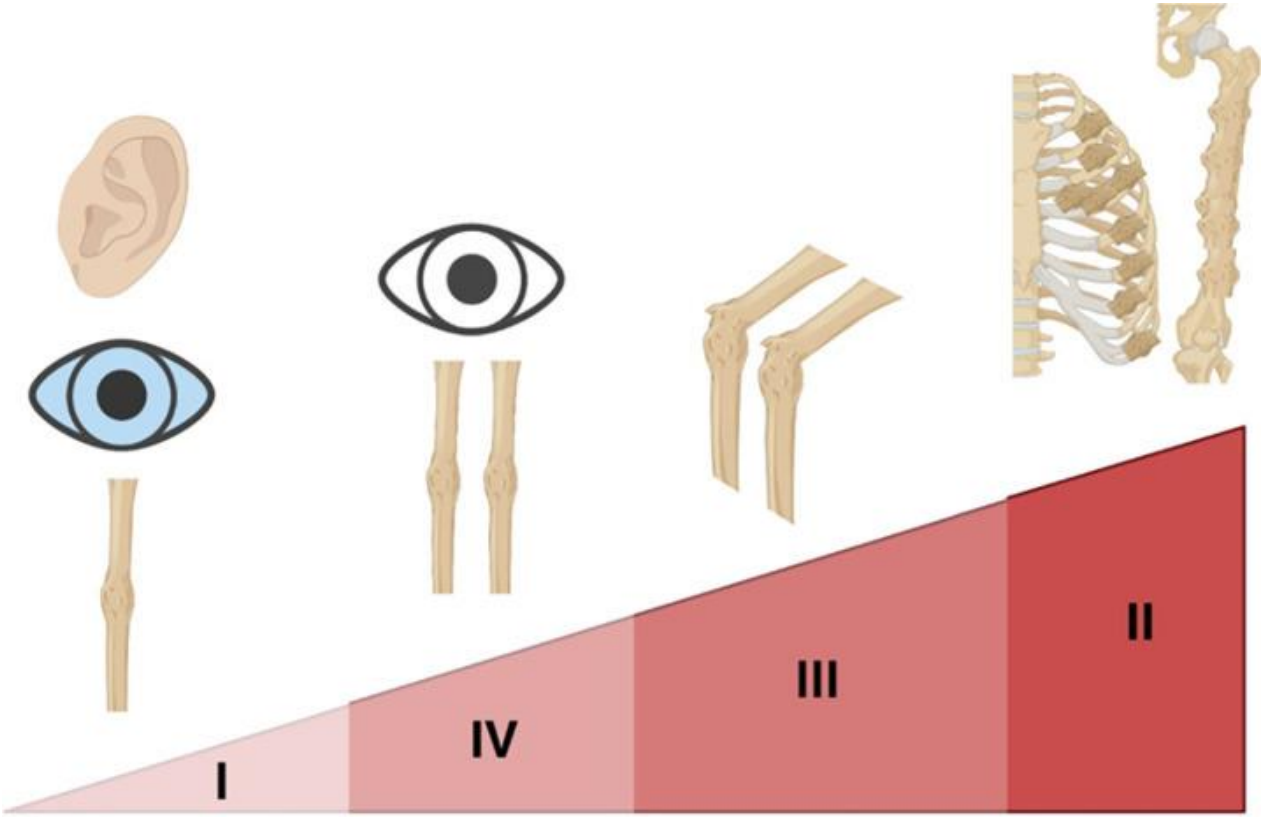
Lethal OI







Phenotypic spectrum OI

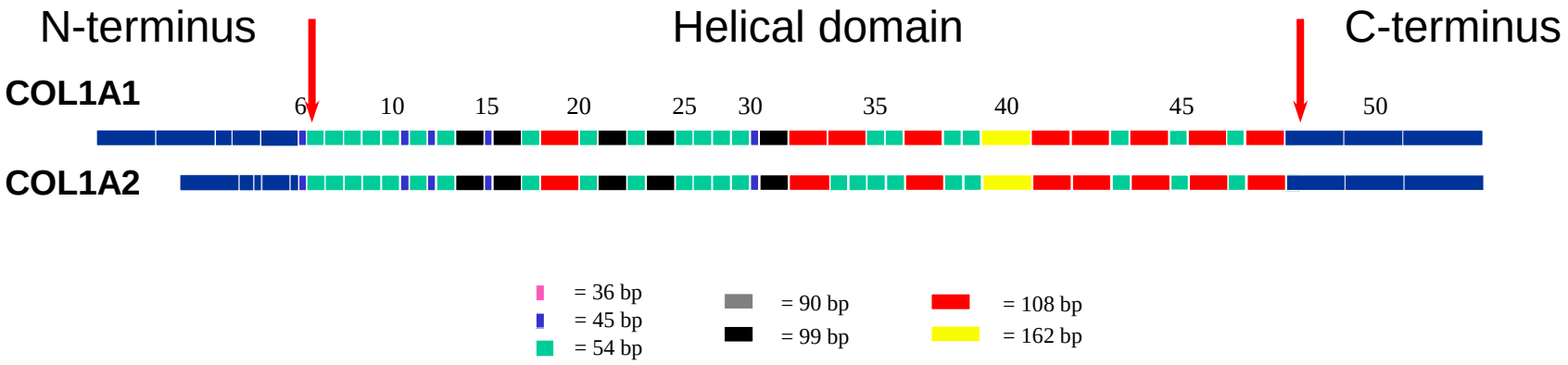


DOI: 10.12998/wjcc.v11.i12.2604

PMID: 37214584

Type I (pro)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)

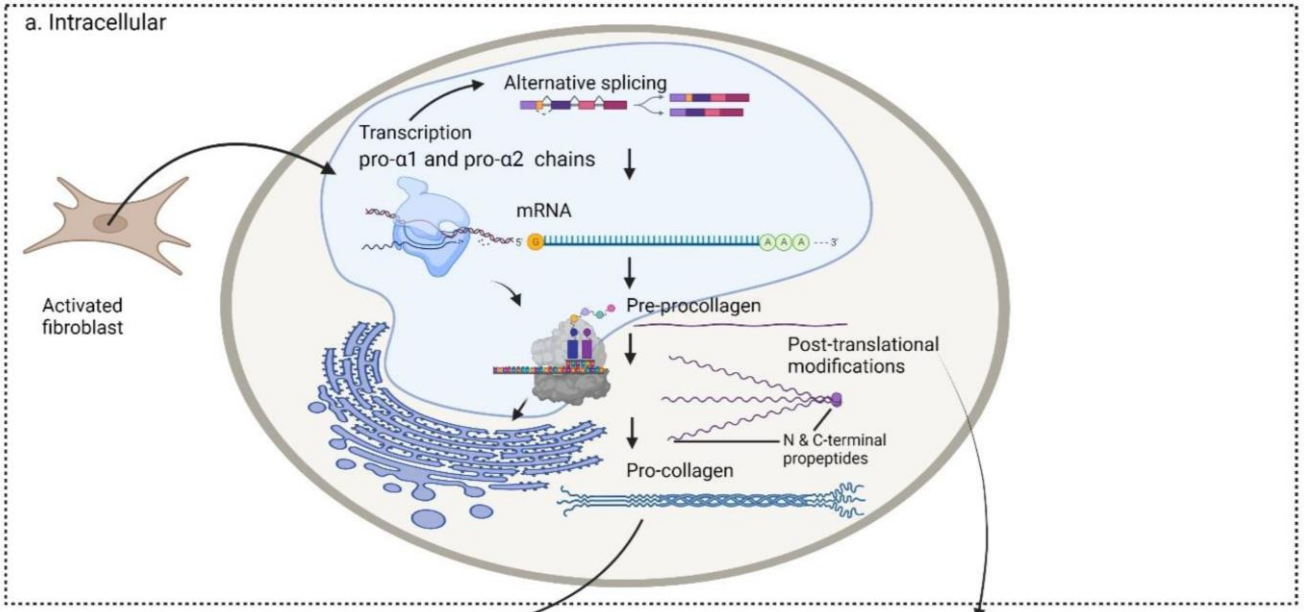


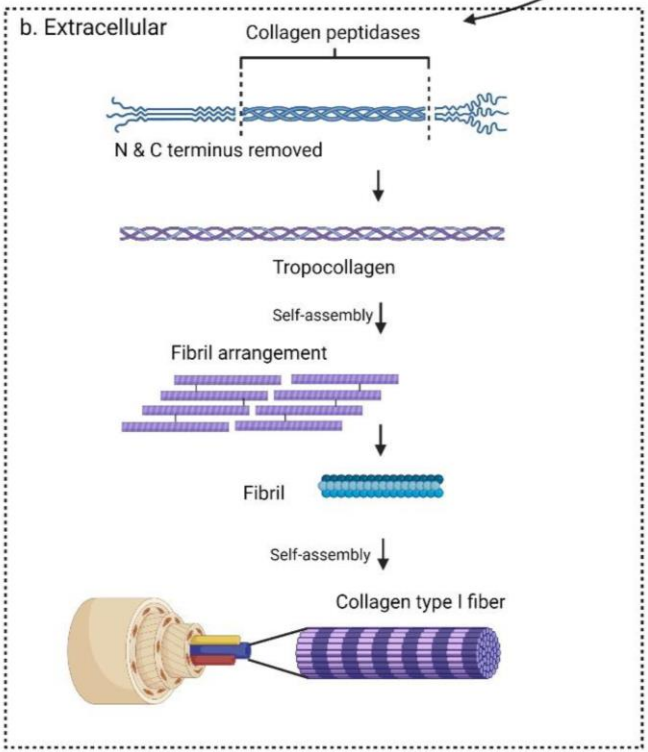
Complex gene structure

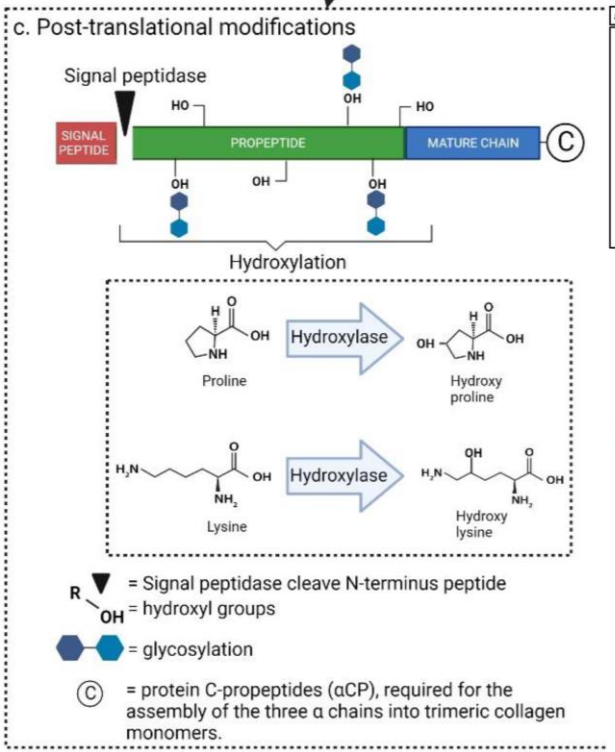
Great potential for mutations

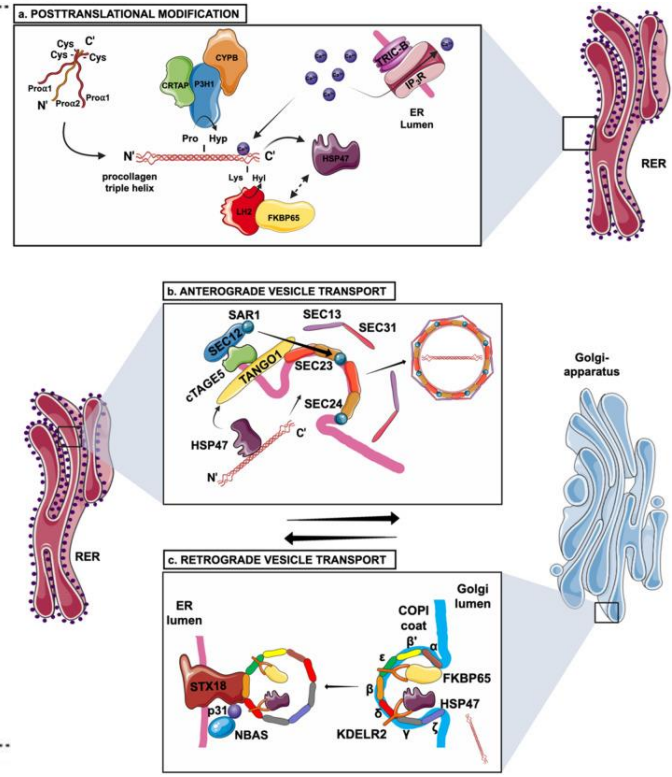
Collagen biosynthesis

PMID: 36140407 and 34169326

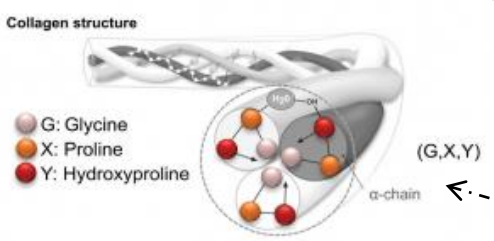




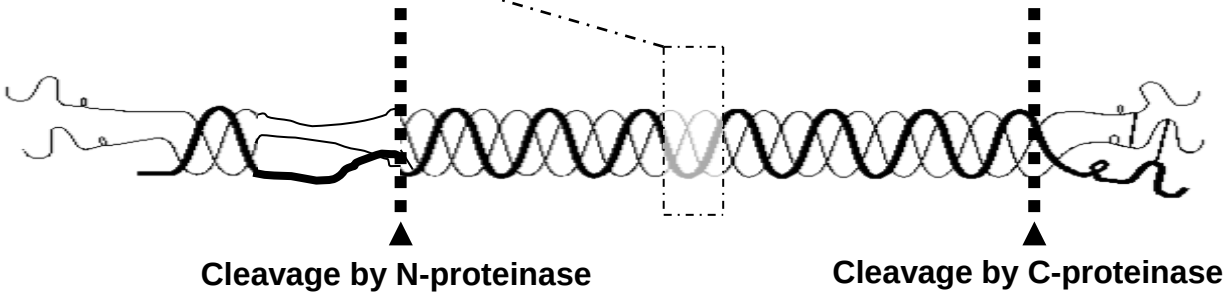




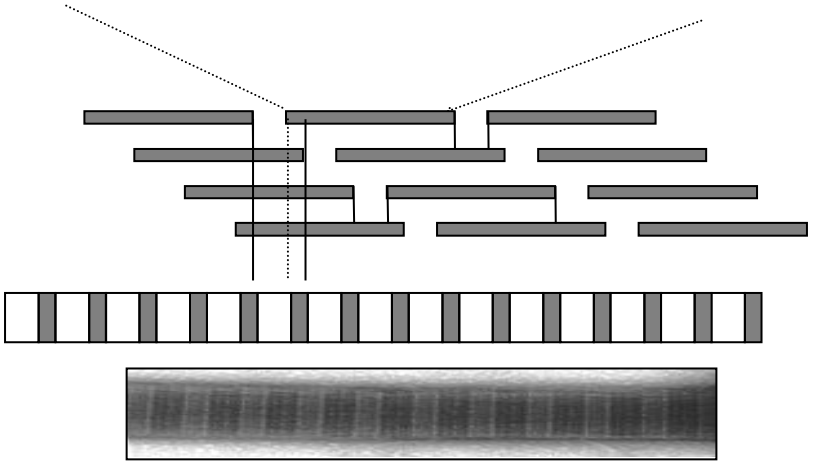
Collagen Fibrillogenesis



AMINO ACID SEQUENCE



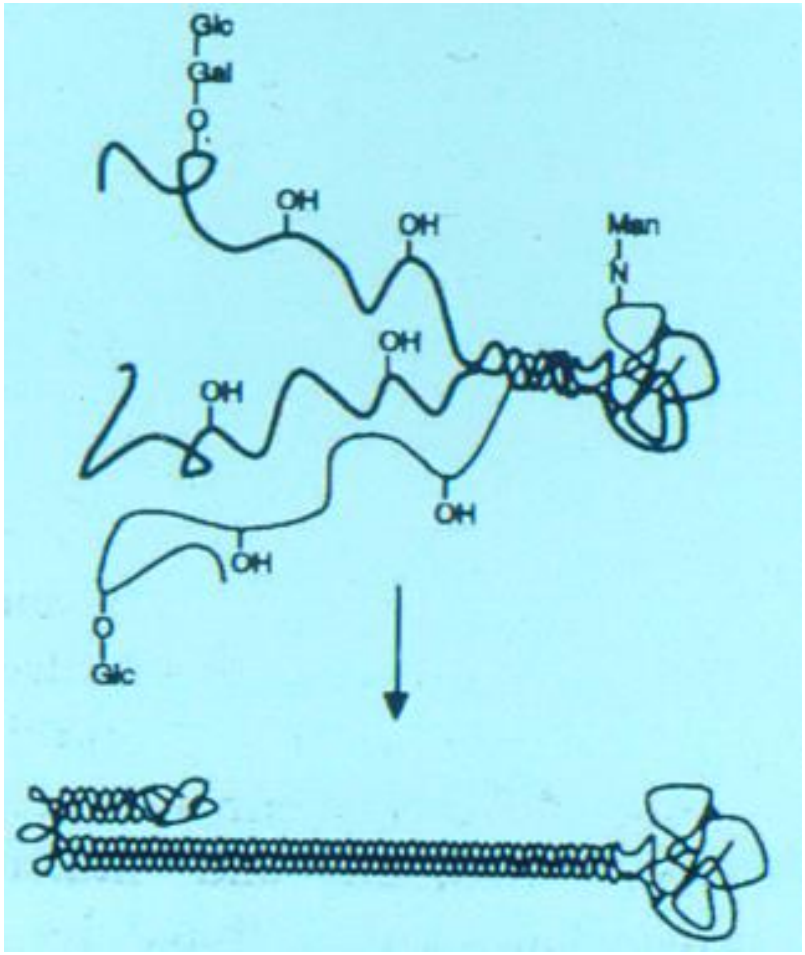
MOLECULE
Type I procollagen

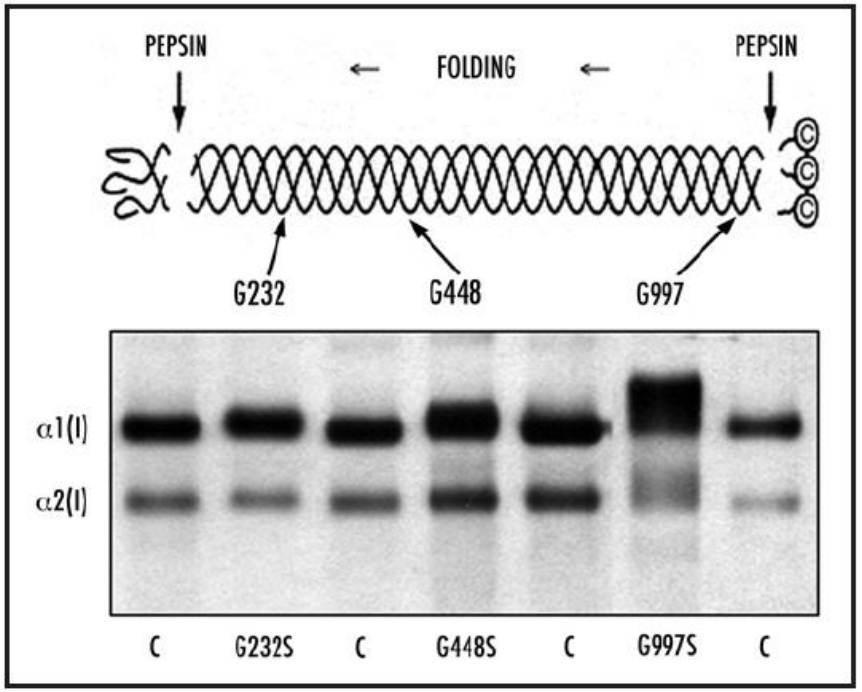


MOLECULAR PACKING

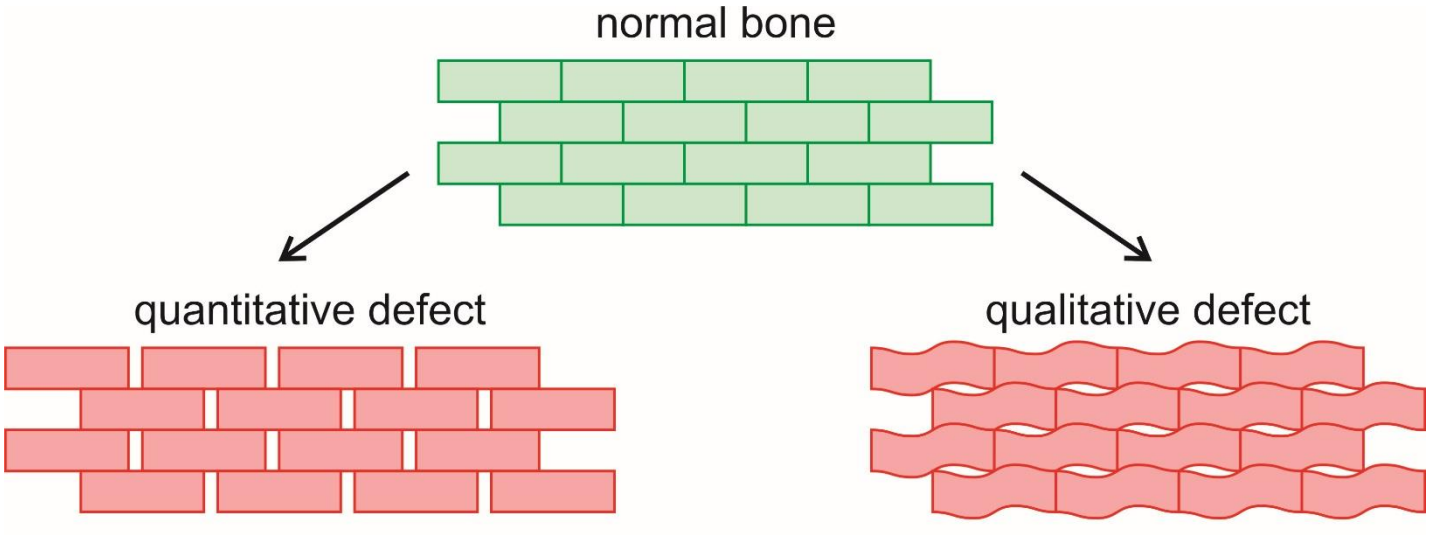
COLLAGEN FIBRIL

Collagen fibrillogenesis





Molecular pathogenesis of dominant OI



Nonsense, frameshift, ...
Variant 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)

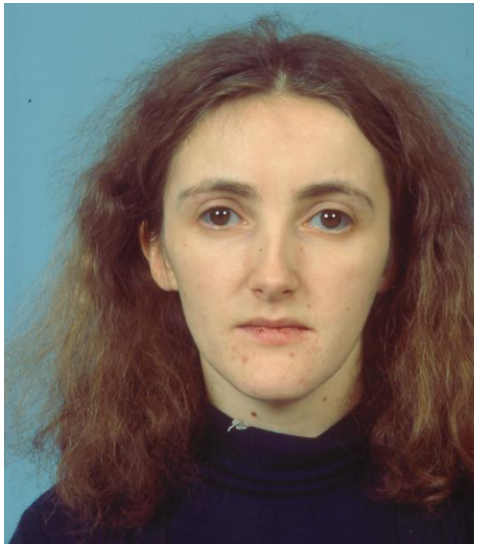
↓

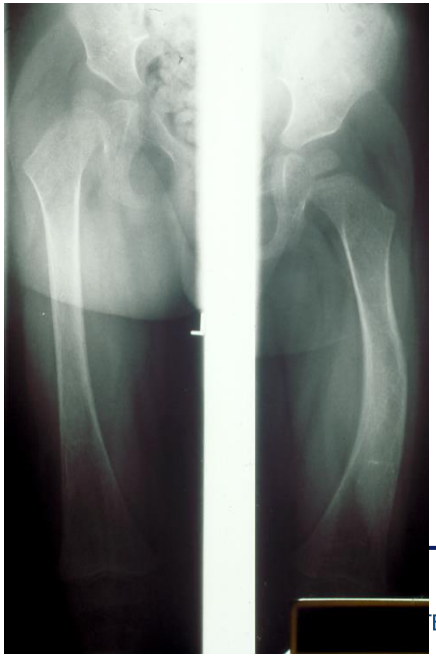
Production of an abnormal protein

↓

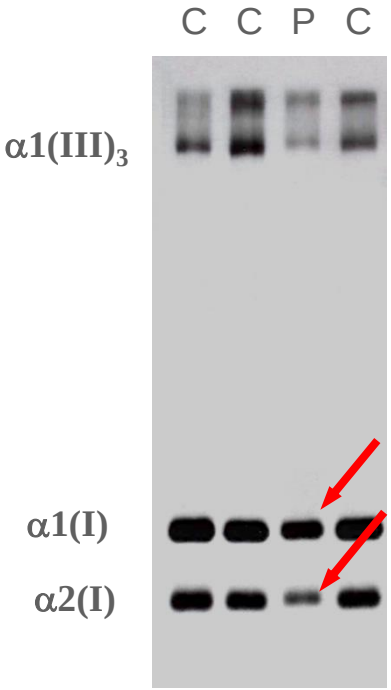
Severe to lethal phenotype (OI type II, III & IV))

Osteogenesis Imperfecta type I

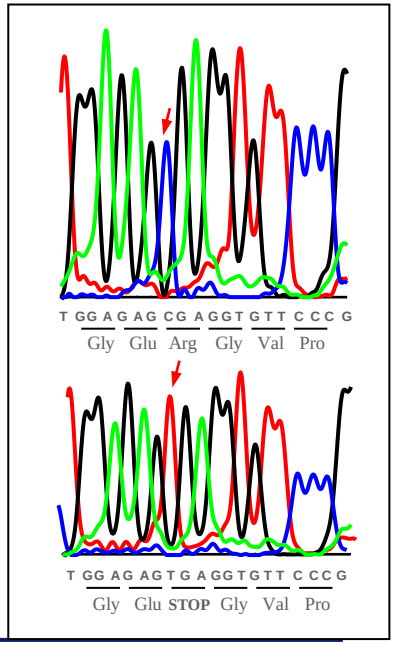




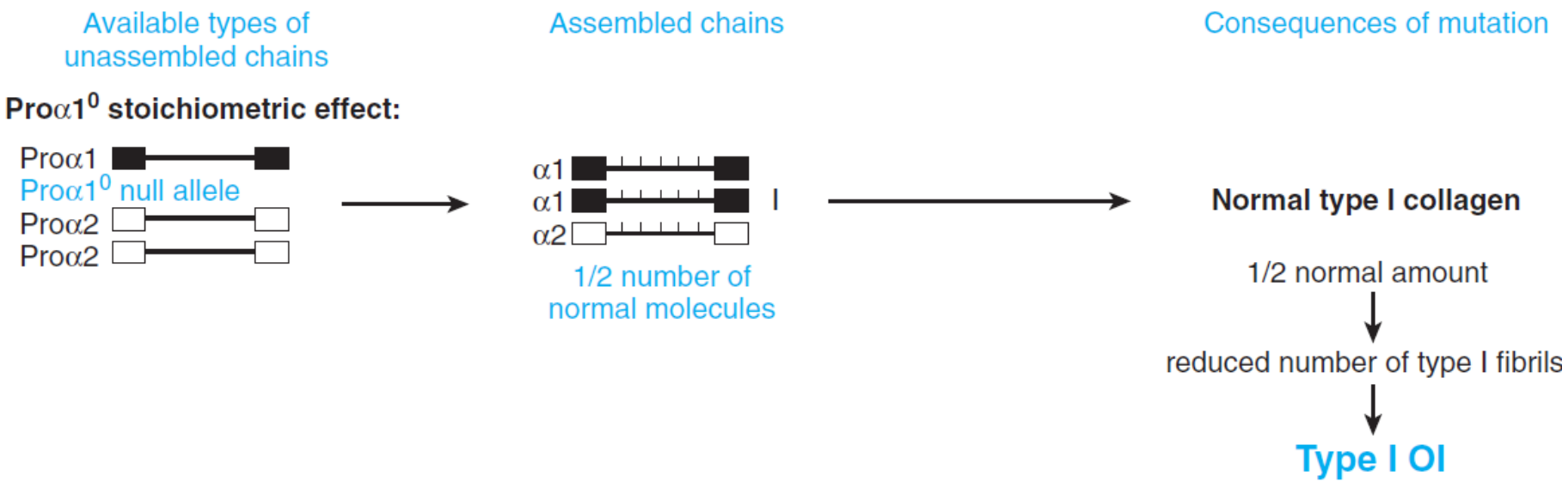
- Bone fragility (mild to moderate)
- Varying number of bone fractures
- Blue sclerae
- Hearing loss



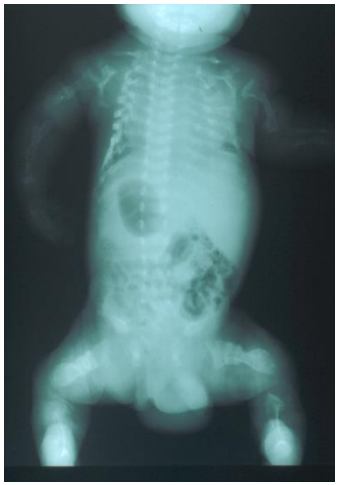
α1(I) - Arg 240 Stop
(CGA → TGA)

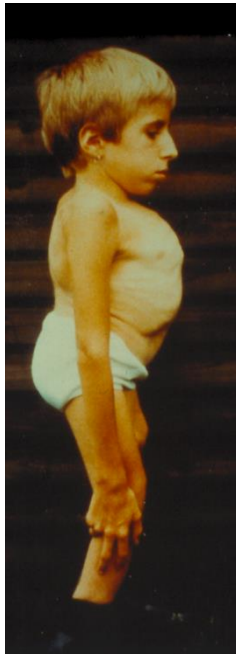


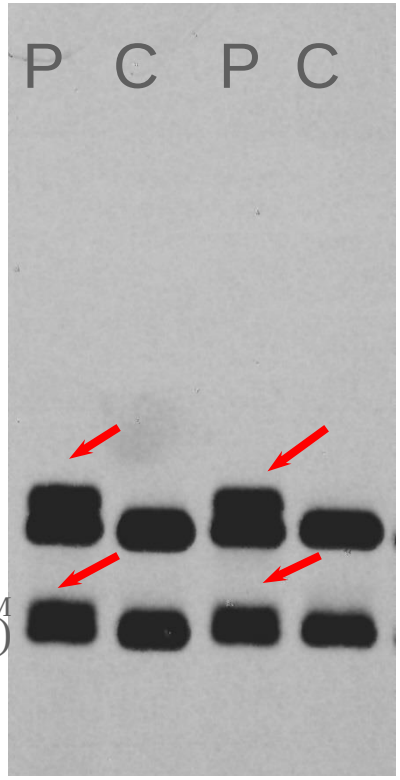
Osteogenesis imperfecta type I (mild)



Osteogenesis imperfecta type II-III-IV



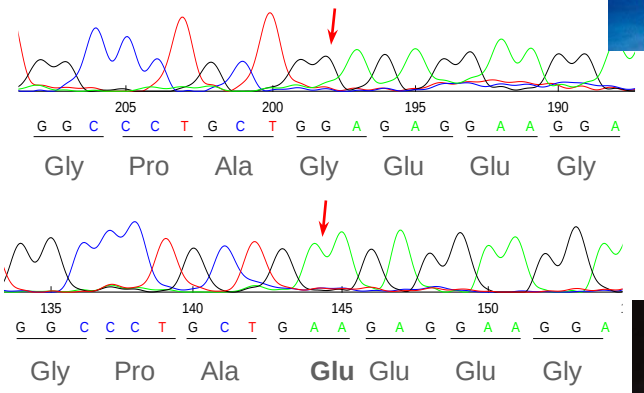


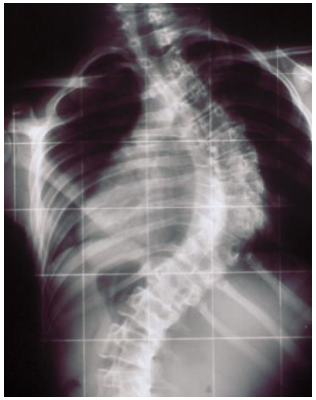


medium collagens

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

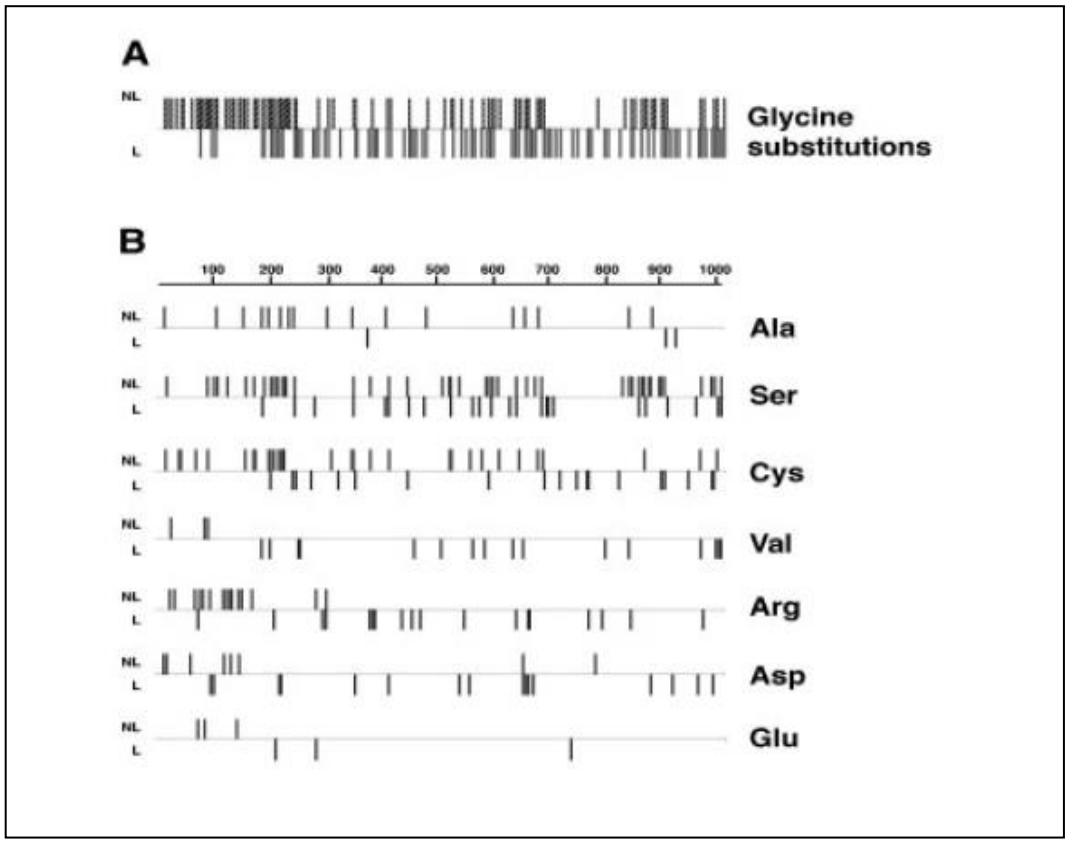
The phenotype is determined by the type of chain involved, the nature and position of the substituting amino acid

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

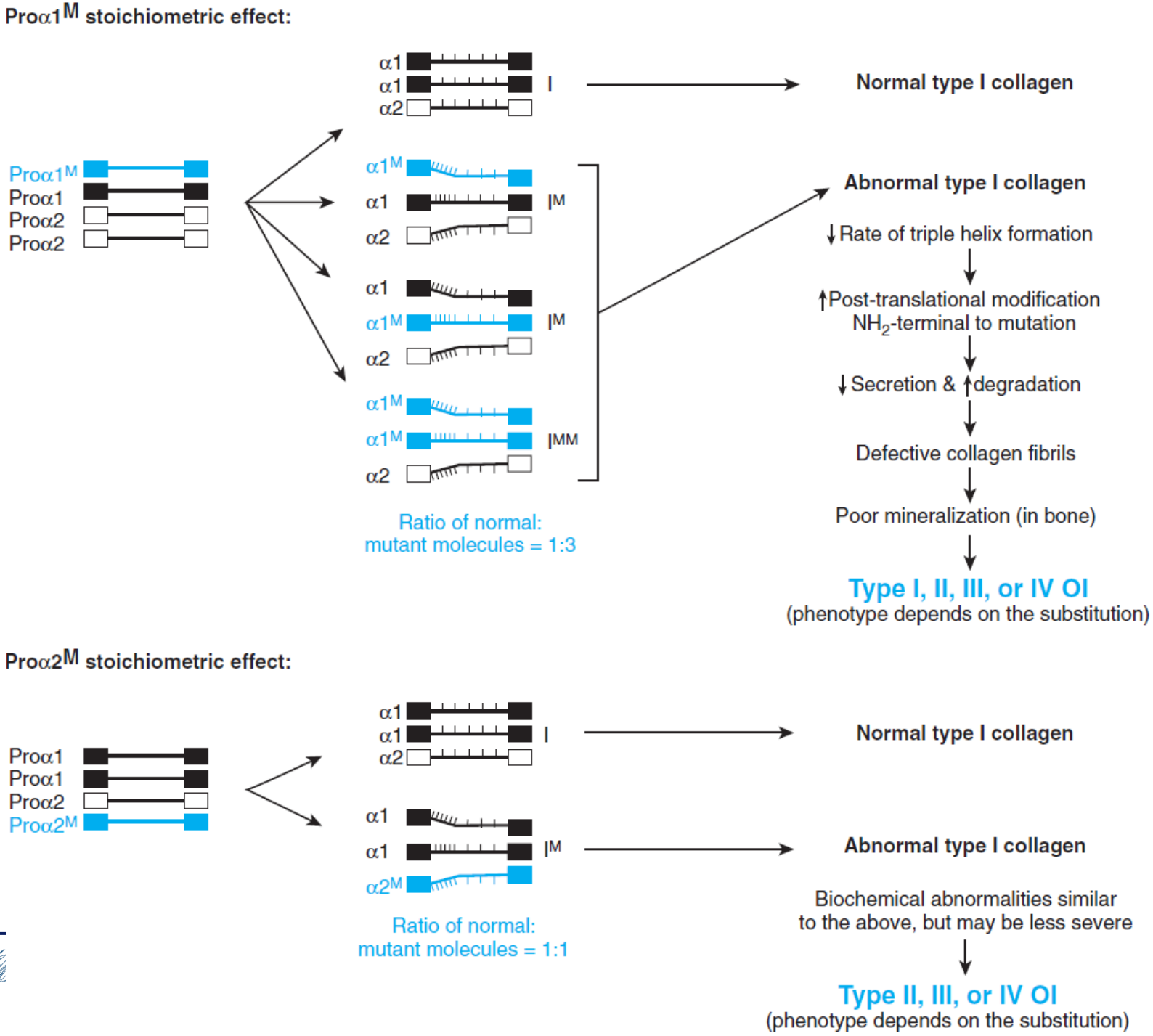
Distribution of mutations along α2(I)-collagen chain

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.

Name of disorder	MOI	OMIM No.	Gene	Molecular diagnosis OMIM
OI type 1	AD	166200	*COL1A1, COL1A2*	OI type I
OI type 2	AD	166200	*COL1A1, COL1A2*	OI type II
	AR	610854	*CRTAP*	OI type VII
	AR	610915	*LEPRE1*	OI type VIII
	AR	259440	*PPIP*	OI type IX
	AR[a]	607723[a]	*SUCO*[a]	SUN1[a]
OI type 3	AD	259240	*COL1A1, COL1A2*	OI type III
	AR	613982	*SERPINF1*	OI type VI
	AR	610682	*CRTAP*	OI type VII
	AR	610915	*LEPRE1*	OI type VIII
	AR	259440	*PPIB*	OI type IX
	AR	613848	*SERPINH1*	OI type X
	AR	610968	*FKBP10*	OI type XI
	AR	615066	*TMEM38B*	OI type XIII
	AR	112264	*BMP1*	OI type XIV
	AR/AD	615220	*WNT1*	OI type XV
	AR	616229	*CREB3L1*	OI type XVI
	AR[a]	616507[a]	*SPARC*[a]	OI type XVII[a]
	AR[a]	617952[a]	*TENT5A(FAM24A)*[a]	OI type XVIII[a]
	AR[a]	607783[a]	*MESD*[a]	OI type XX[a]
OI type 4	AD	166220	*COL1A1, COL1A2*	OI type IV
	AD	615220	*WNT1*	OI type XV
	AR	610854	*CRTAP*	OI type VII
	AR	259440	*PPIB*	OI type IX
	AR	610968	*FKBP10*	OI type XI
	AR	606633	*SP7*	OI type XII
OI type 5	AD	610967	*IFITM5*	OI type V
Osteoporosis X-linked form[a]	XL	300294	*PLS3*	OI type XIX
	XL		*MBTPS2*	
Osteporosis AD form[a]	AD	615220	*WNT1*	OI type XV
	AD		*LRP5*	

PMID: 32901963

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

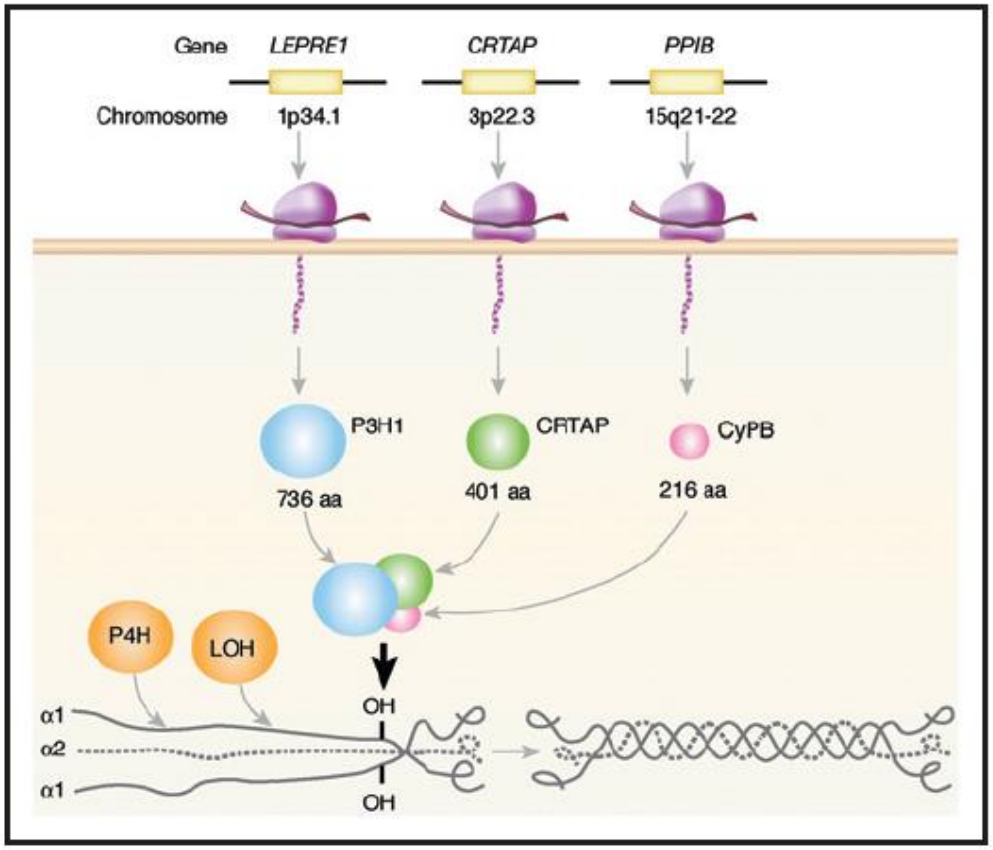
- *LEPRE1* (6)
- *CRTAP* (2)
- *FKBP10* (5)
- *SERPINH1* (1)
- *SERPINF1* (2)
- *PLOD2* (1)
- *CREB3L1* (1)
- *TAPT1* (2)

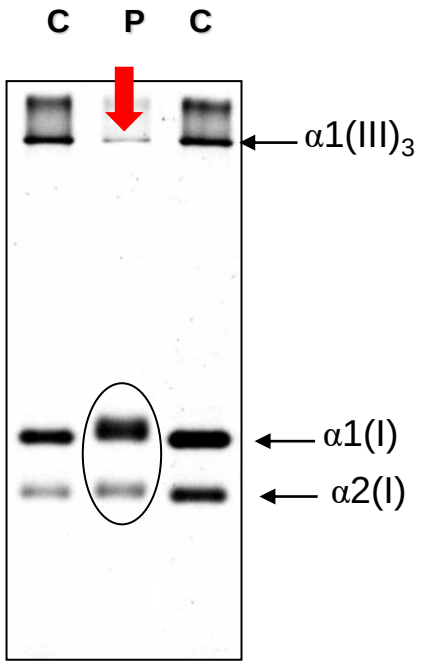
17(~4%) "unsolved" after screening all OI genes

Diagnostics nowadays:

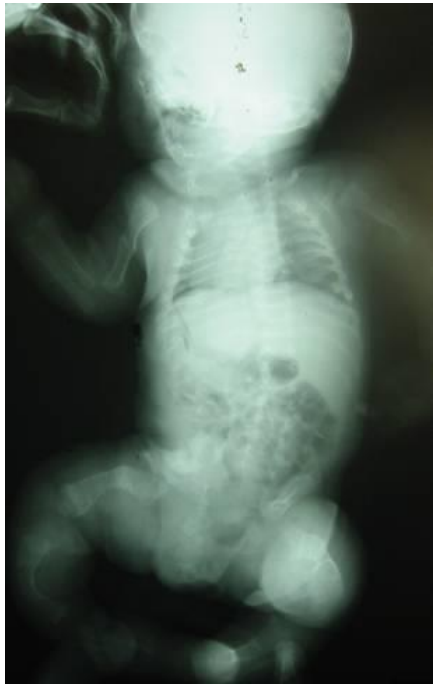
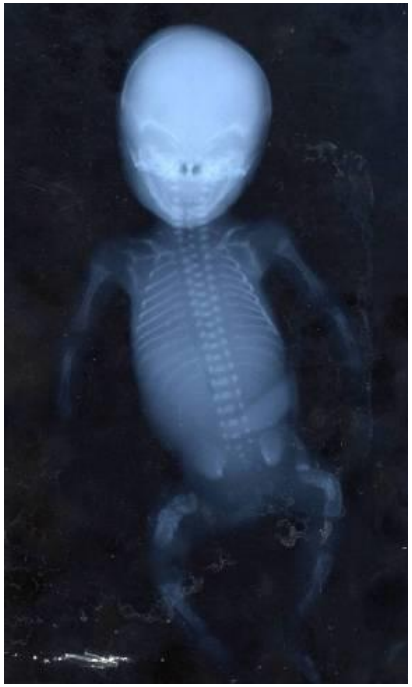
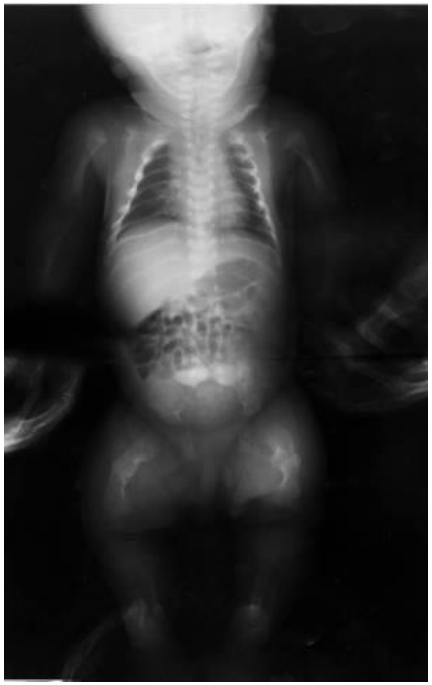
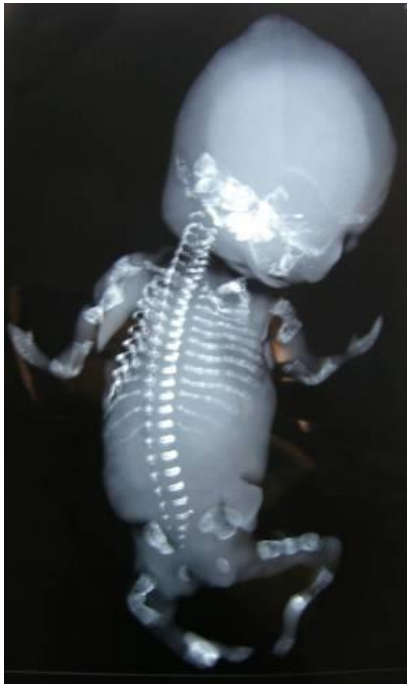
- **NGS gene panel: targeted OI**
- **WES skeletal dysplasia**
- **(MLPA, arrayCGH)**
- **CNV: Exome Depth**

Recessive OI: CRTAP/LEPRE/PPIB complex





Causal genetic defects in *P3H1*: 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*) Het. c.2055+18G>A, Intron 14	Hom. c.2055+18G>A, Intron 14
			

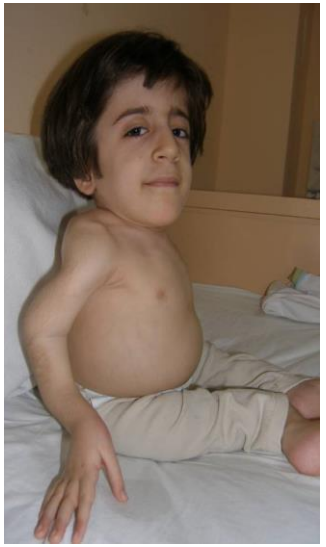
- Lack of calvarial ossification
- Beaded ribs with multiple fractures
- Platyspondyly
- Shortened, wide, bowed and fractured large tubular bones

→ **Based on the phenotype: no distinction between AR OI or Severe/Lethal AD OI ??**

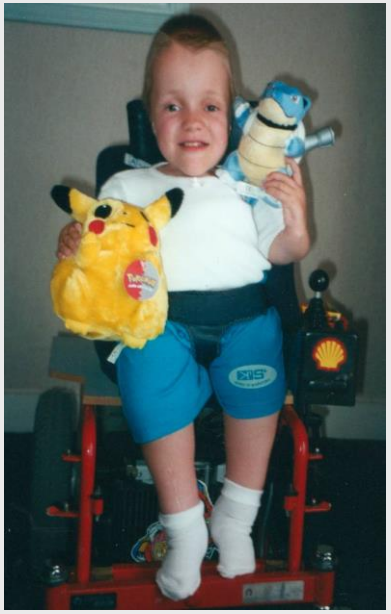
Mutations in *LEPRE1*: OI type VIII

- *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

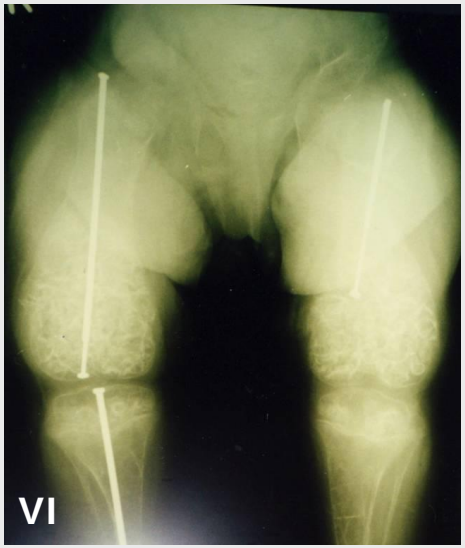
P4, 8 yrs

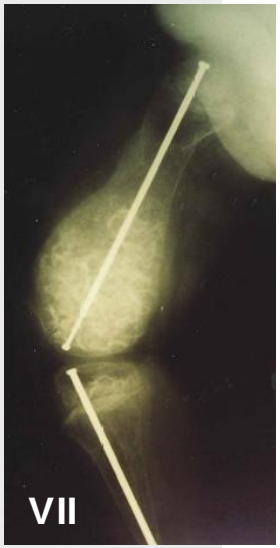


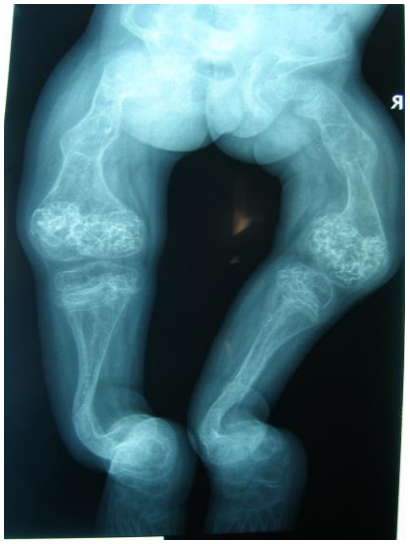
P3 ,10 yrs



P3, X-rays at 5 yrs and 4 mths





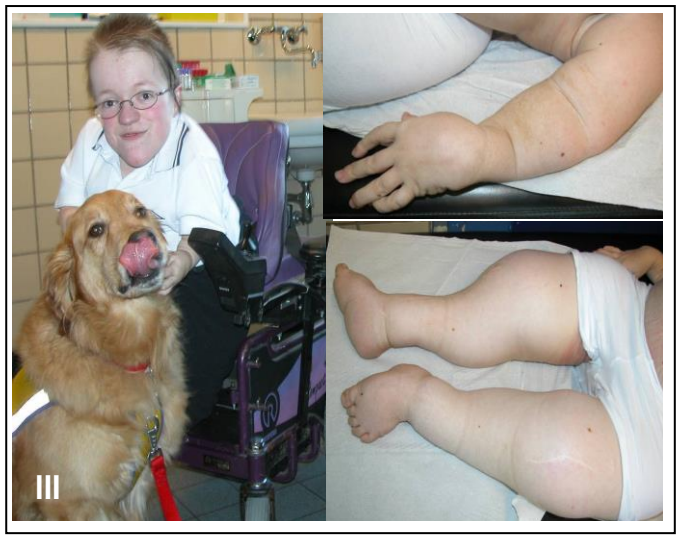


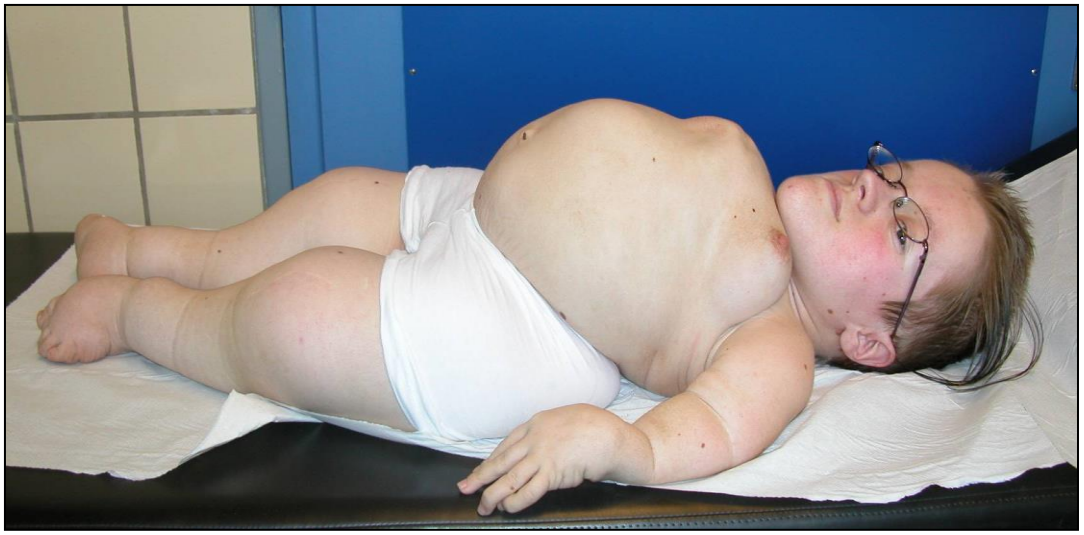
P4

Mutations in *LEPRE1*: OI type VIII

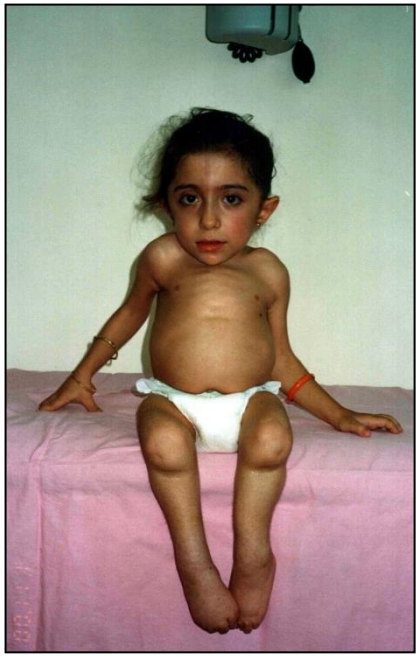
- *Adolescence/early adulthood (> 15 yrs)*
- Extreme short stature, very severe osteoporosis
- Disappearance of the popcorn-like structures
- Additional widening of the rhizomelic diaphyses
- Progressive narrowing and bowing of the mesomelic diaphyses
- Reduced knee joint spaces
- Long hands & fingers

P3 at age 17 ½ yrs

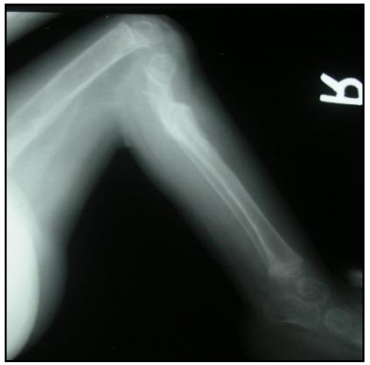


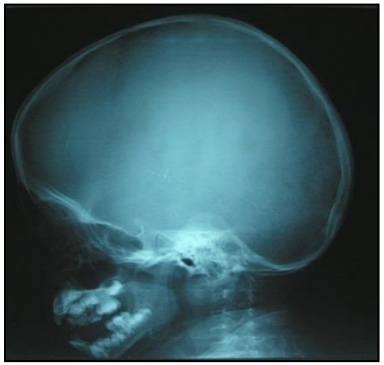


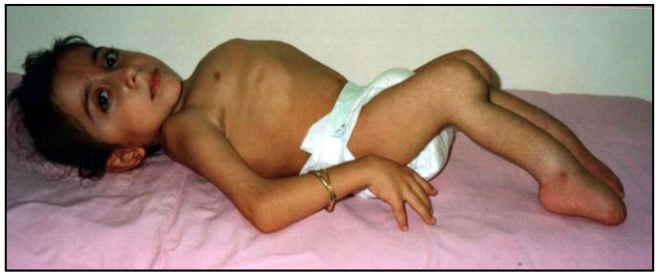
Mutations in *FKBP10*: OI type XI

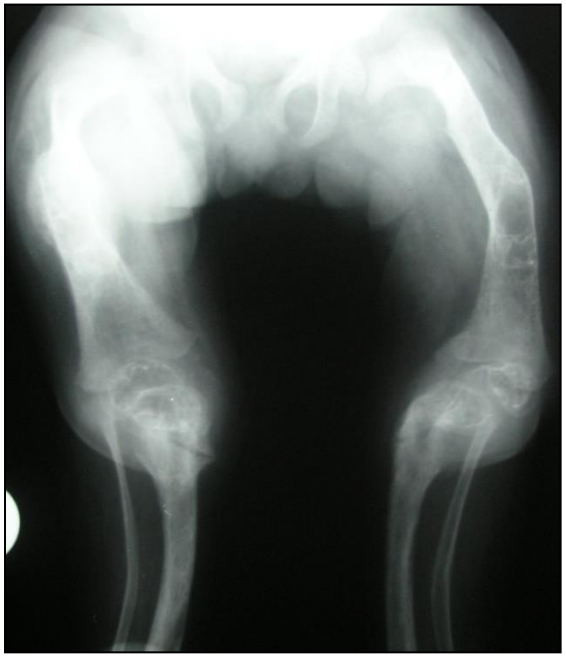


P4 at age 7 yrs









Hom. p.(Gly278Argfs*95)

Consanguineous parents of Turkish origin

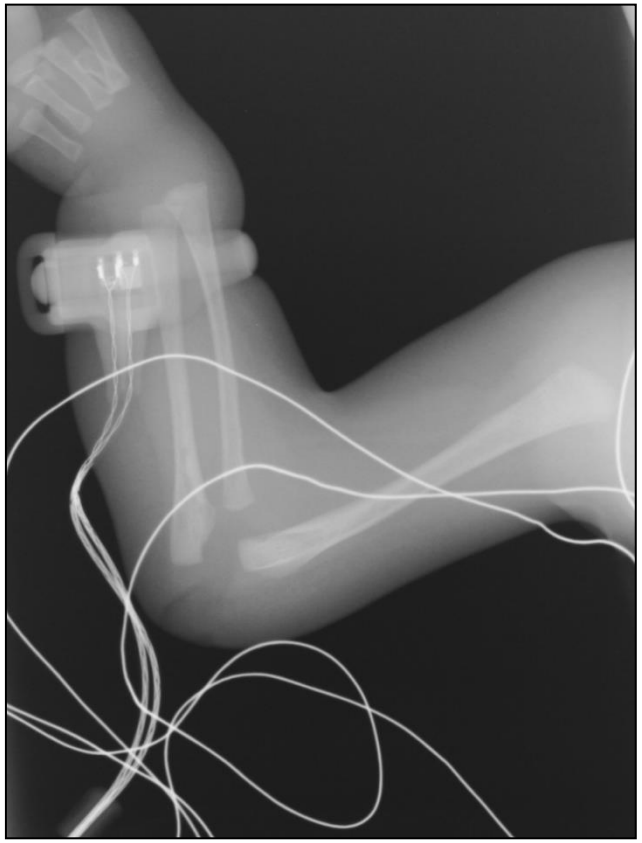
Congenital contractures of knees and ankles, wormian bones

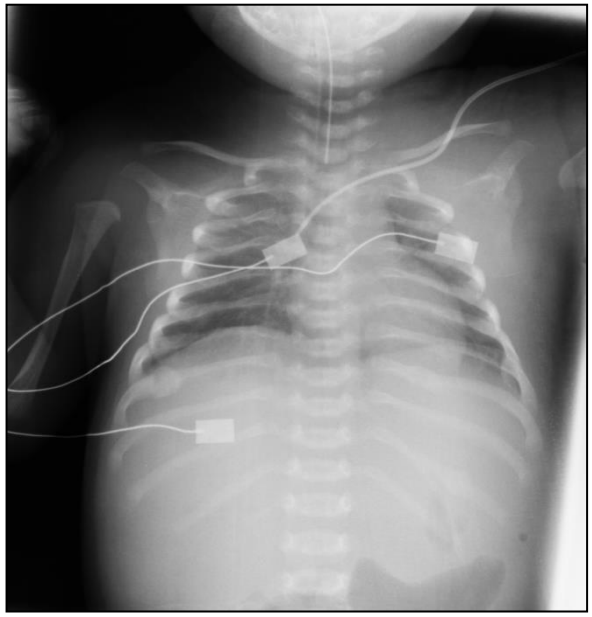
Since age 2 months recurrent costal and femoral fractures

Triangular face, normal dentition & hearing, white sclerae

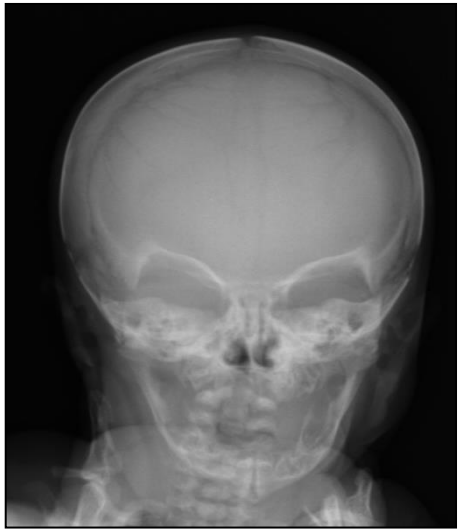
At age 9 yrs greatly restricted limb movement, wheelchair-bound

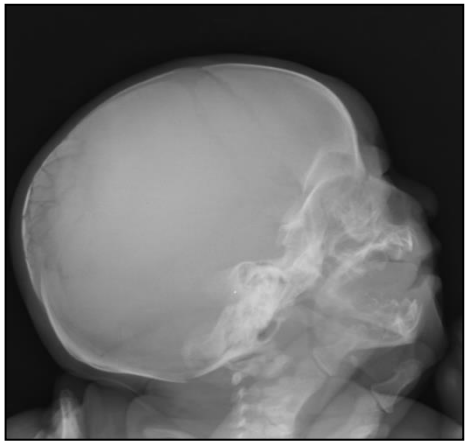
Mutations in *FKBP10*: OI type XI





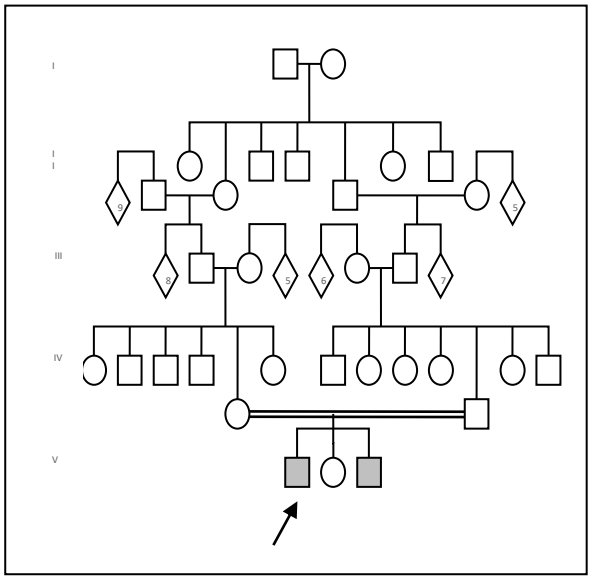
Rib fractures





wormian bones

Tricky case

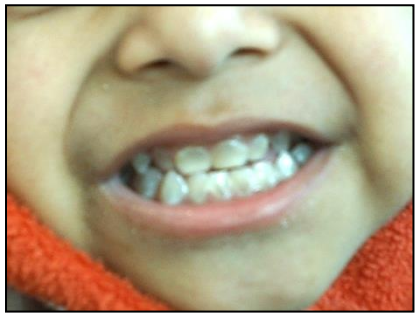


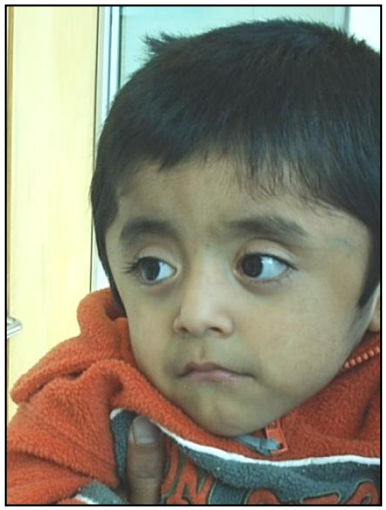
- Proband : 1st child from healthy consanguineous parents of aboriginal Chilean origin

- 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)

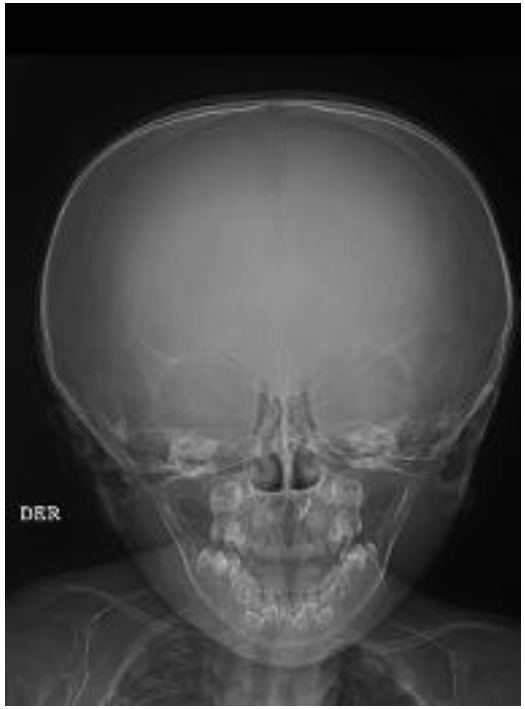
- Brother of P1: similar clinical history

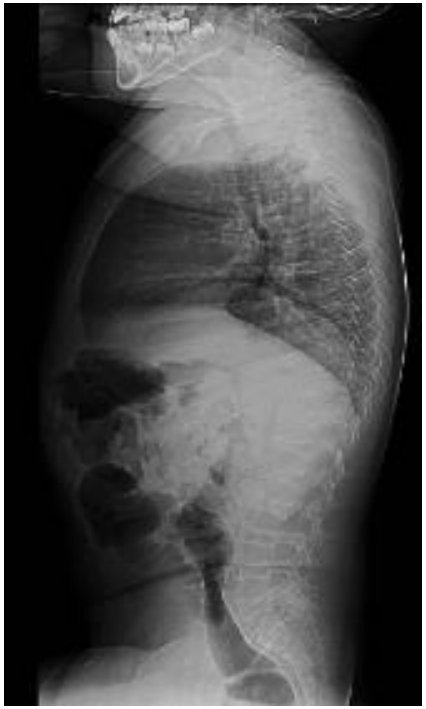
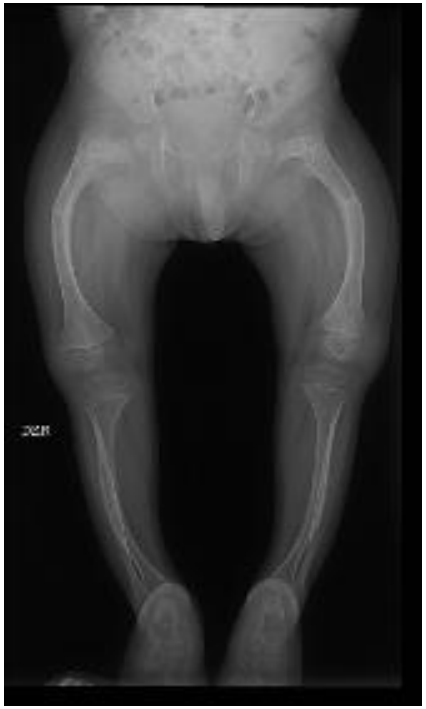




- *CRTAP* excluded in another center

Tricky case



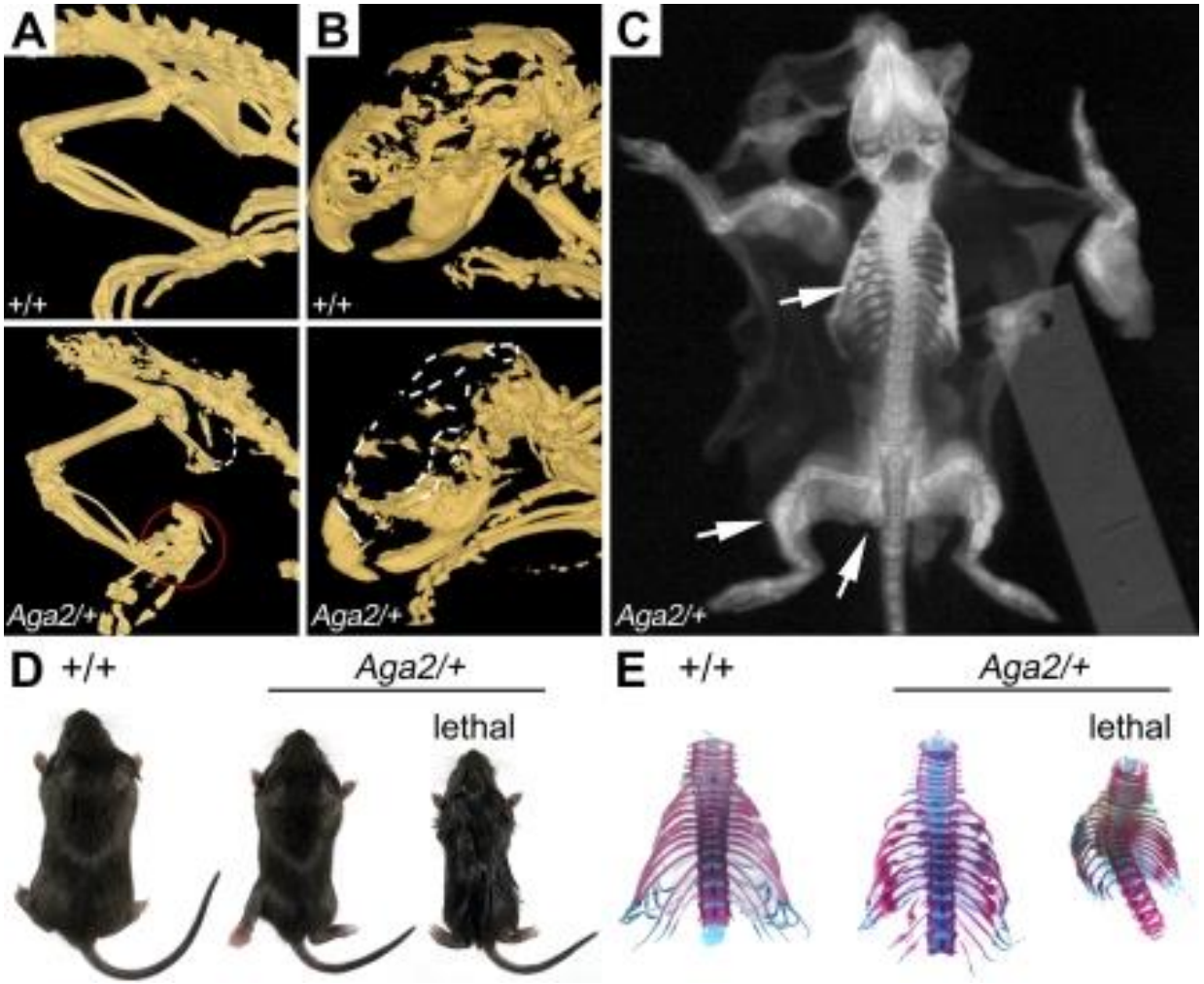




- Exclusion of *LEPRE1* and *PPIB*

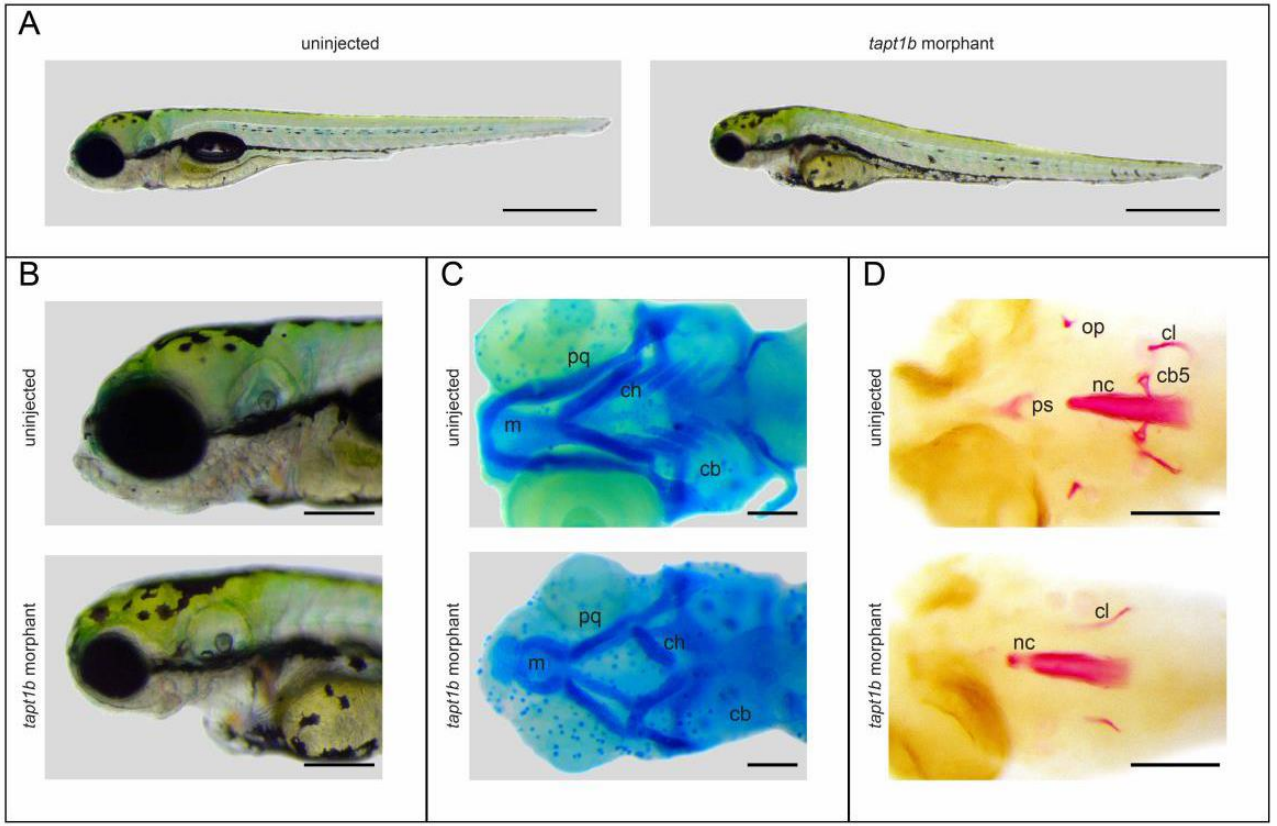
- *COL1A2:* heterozygous c.2565+1G>A
  - Present in 2 affected children
    - Absent in healthy parents

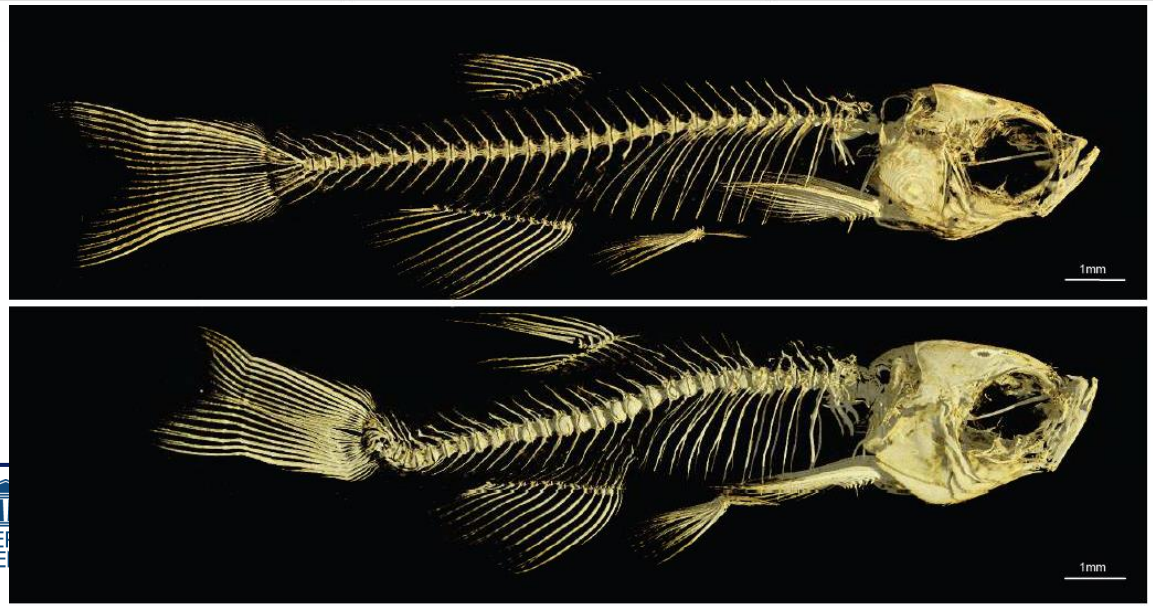
→ Parental mosaicism

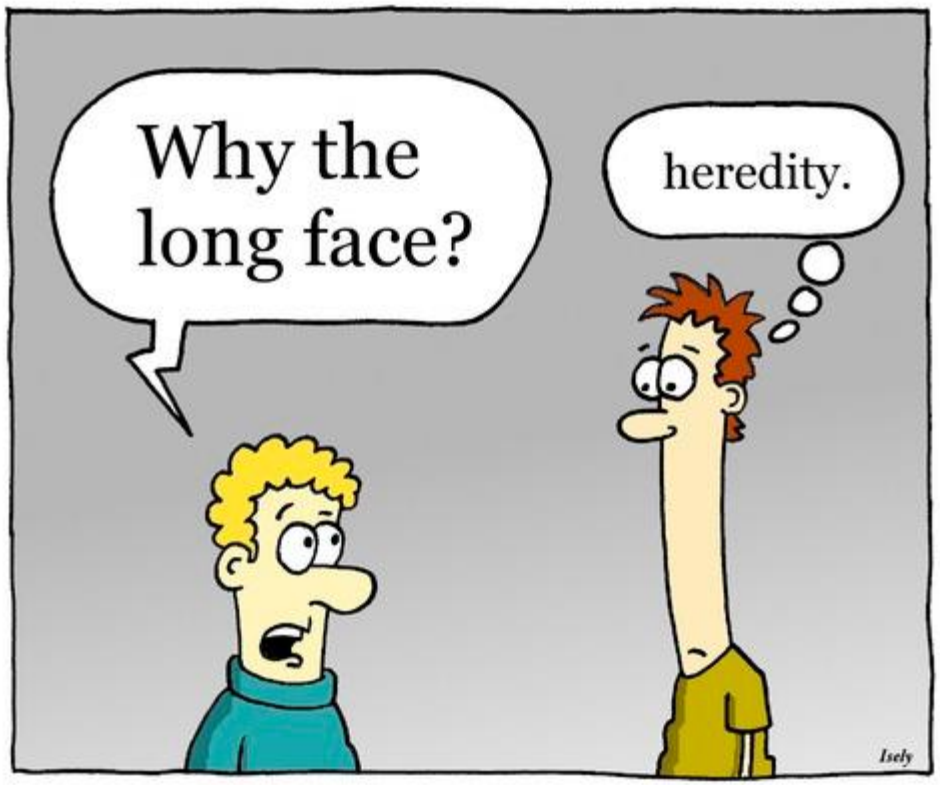
OI mice



OI zebrafish






Why the long face?
heredity.
Isely