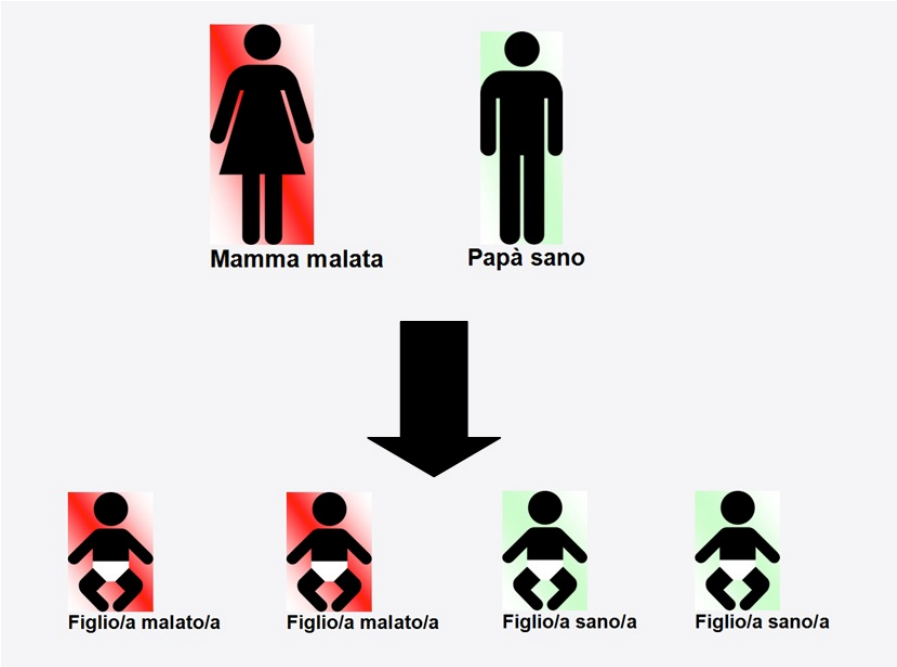
Genetic Pathology #2

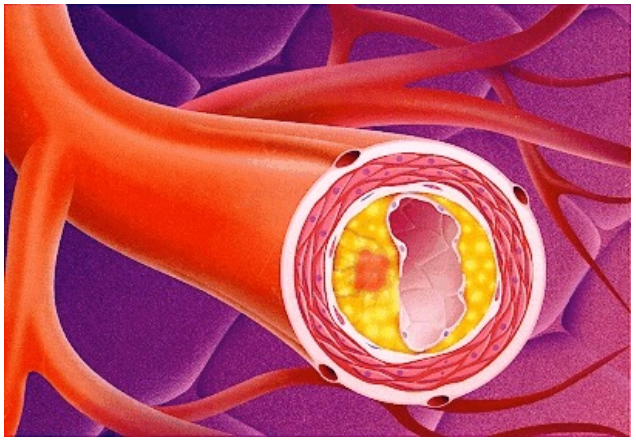
AUTOSOMAL DOMINANT DISEASES

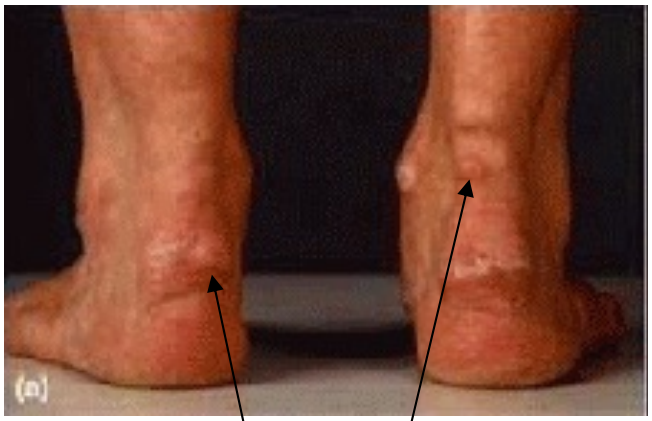


-Achondroplasia
-Marfan Syndrome
-Familiar Hypercholesterolemia
-Neurofibromatosis
-Familial Adenomatous Polyposis
-Polycystic kidney disease
-Myotonic Dystrophy

Familiar hypercholesterolemia

- Caused by **mutations of the LDL receptor** involved in cholesterol metabolism
- One of the most frequent mendelian diseases
- **Heterozygous**: 1:500 - Plasma cholesterol 2-3 times higher than average* and precocious atherosclerosis in adult age.
- **Homozygous**: 1:10^6 - Cholesterol levels 5-6 times higher than normal. Very precocious cutaneous xanthomas, coronary and cerebral atherosclerosis. Myocardial infarction before 20 years of age.





Cutaneous Xanthomas
(deposition of yellowish cholesterol)

Cholesterol Levels

(mg/dl)

DANGEROUS

Total Cholesterol	LDL Cholesterol	HDL Cholesterol
240 and higher	**160 and higher**	**Under 40** (male) **Under 50** (female)

AT-RISK

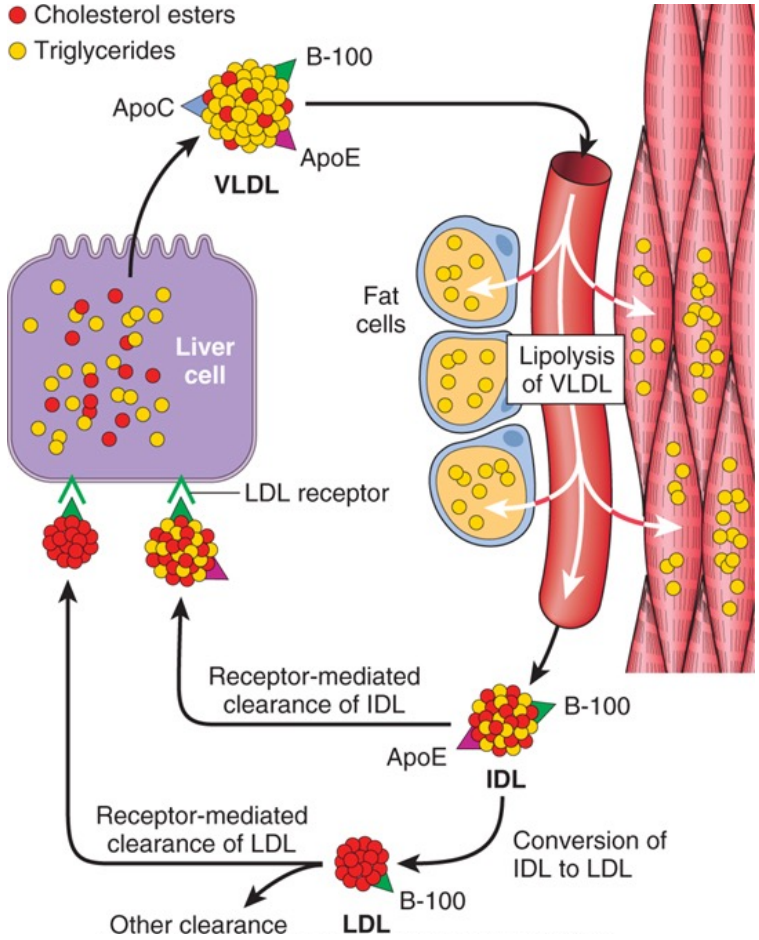
Total Cholesterol	LDL Cholesterol	HDL Cholesterol
200 – 239	**100 – 159**	**40-59** (male) **50-59** (female)

HEART-HEALTHY

Total Cholesterol	LDL Cholesterol	HDL Cholesterol
Under 200	**Under 100**	**60 and higher**

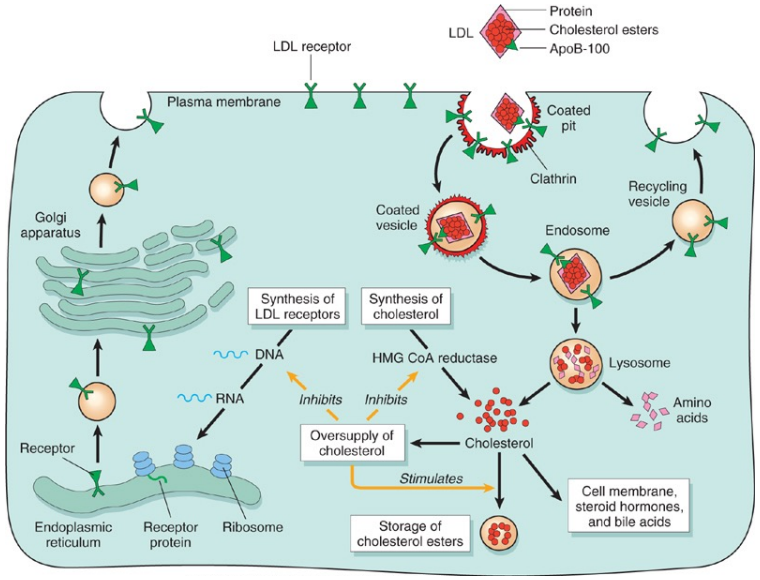
Transport and clearance of cholesterol

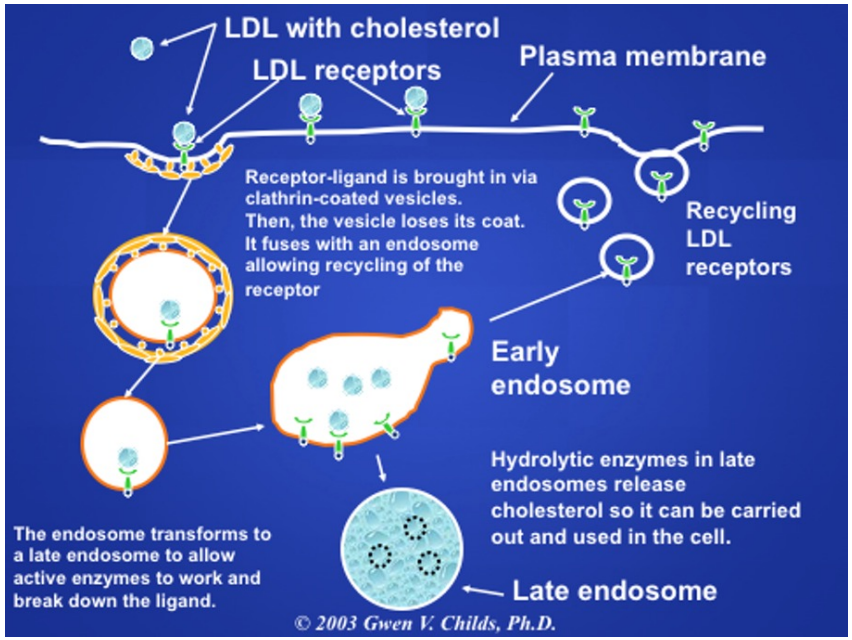
- Liver produces and releases in the bloodstream **VLDL,** containing triglycerides (TG), cholesterol and **apoproteins** ApoC, ApoE, ApoB-100
- In fat and muscle cells TG of VLDL are cleaved by the *lipoprotein lipase* with release of free fatty acids and formation of IDL
- **IDL** have less triglycerides, are enriched of of cholesteryl esters and lack ApoC.
- 50% of IDL is taken up by the liver (same receptor of LDL)
- In the other 50%, remaining triglycerides and ApoE are removed, yielding cholesterol-rich **LDL**.
- LDL are taken up by the liver (70%) or other tissues by **the LDL receptor, interacting with ApoB-100**



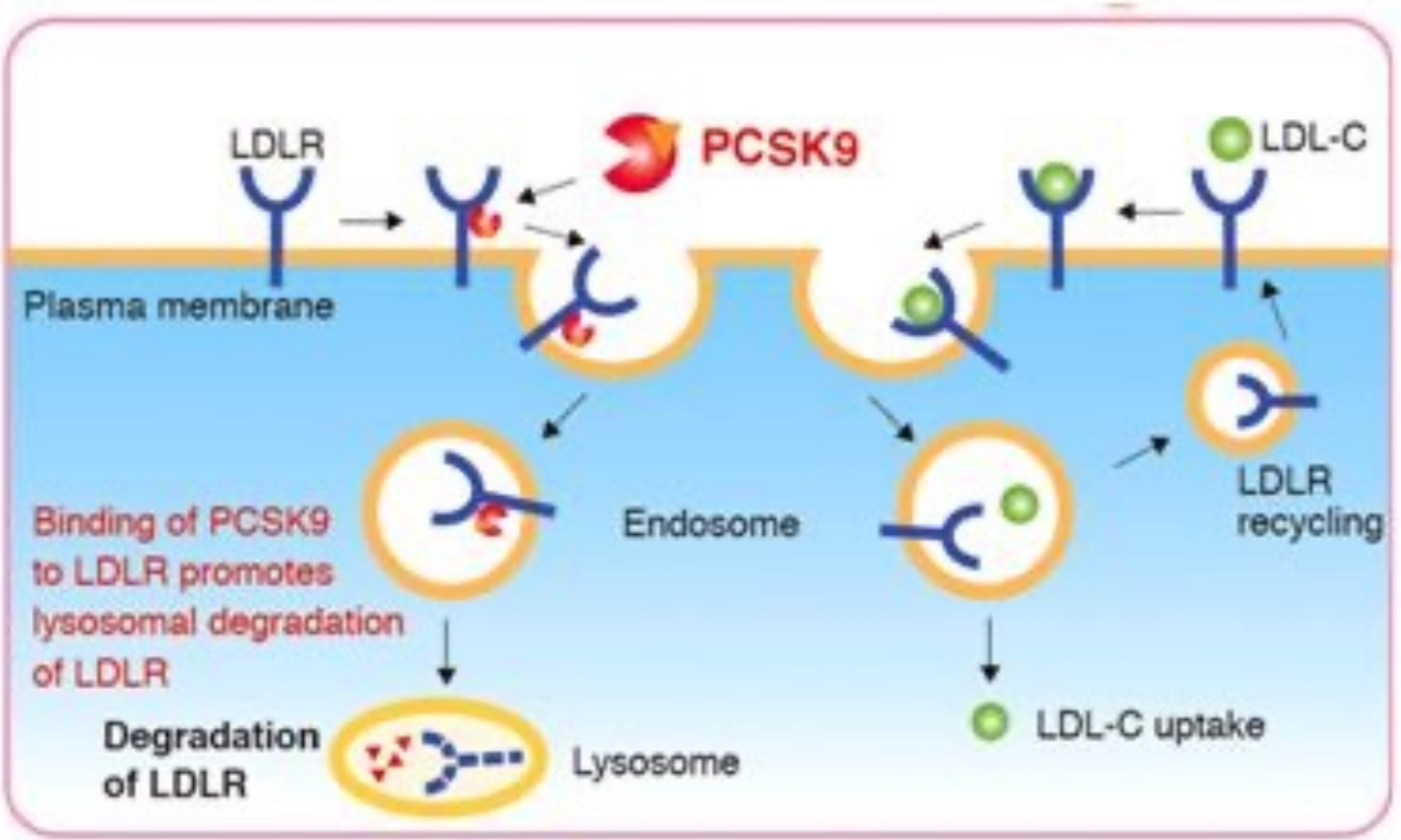
Hepatic Turnover of LDL

- LDL are taken up by the hepatocytes through surface receptors, clustered in the **coated pits**
- Coated pits bind LDL and are internalized, forming **clathrin coated vescicels** which fuse with the endosomes.
- In the **early endosome,** receptor dissociates from LDL and is recycled in the membrane
- In the **late endosome** the apoproteins are hydrolyzed into amino acids and cholesteryl esters are broken down to free cholesterol, which can be:
  - Utilized for the cell membrane or as precursor (hormones, etc)
  - Secreted in the bile
  - Stored in the cell





PCSK9* and LDL degradation

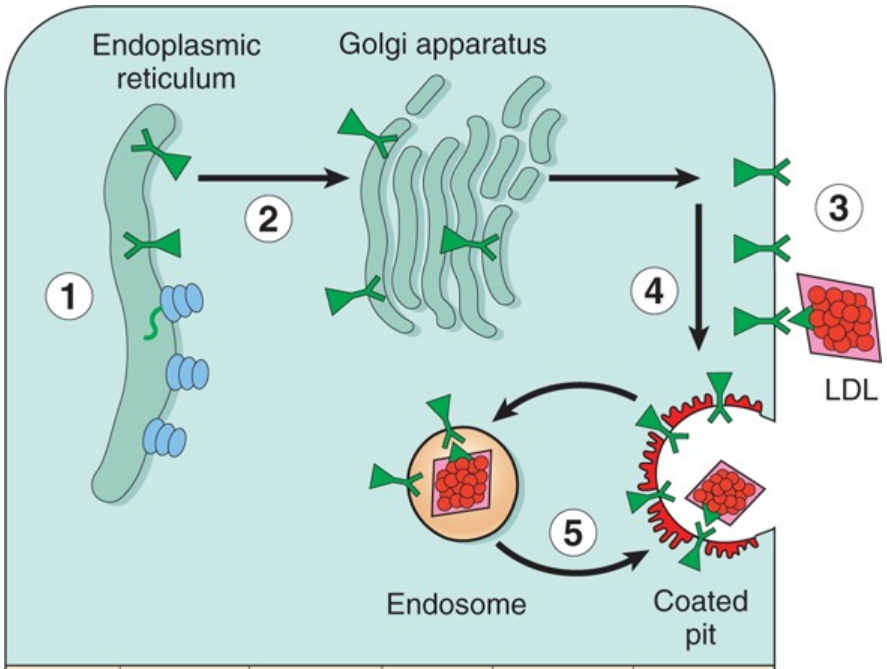


**Proprotein Convertase Subtilisin/Kexin type 9*

Mutations of LDL receptor

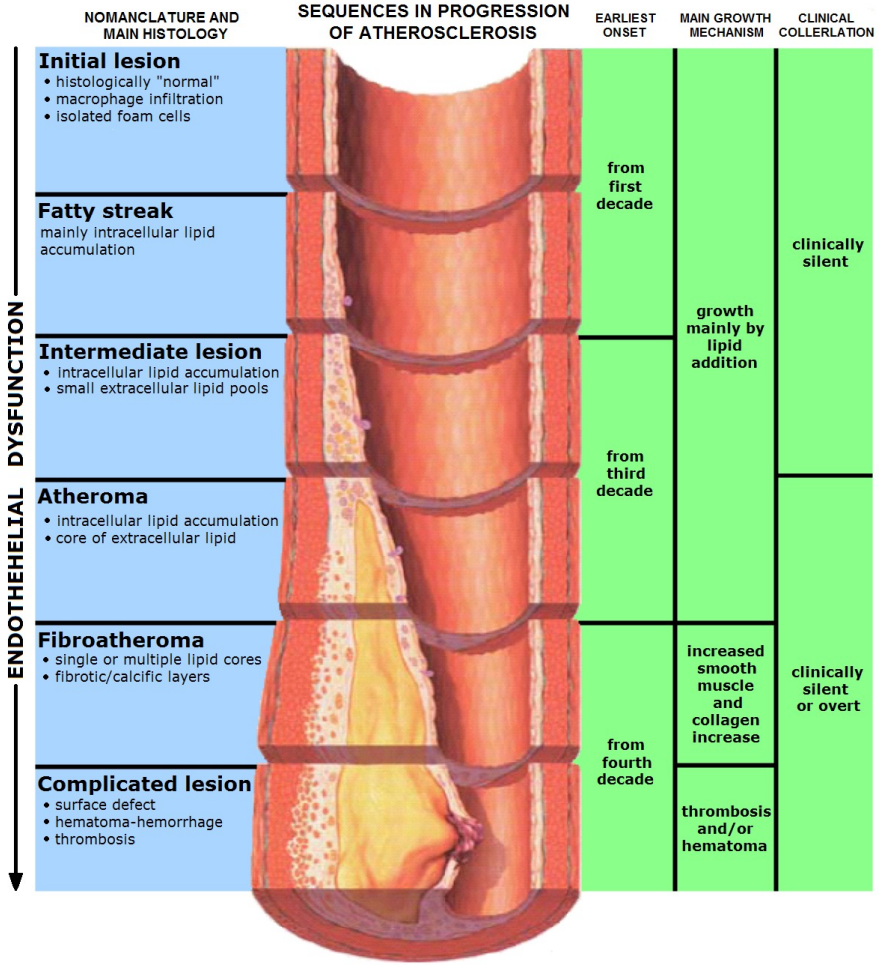
- More than **900 mutations** of the gene encoding LDL receptor have been identified, including insertions, deletions and missense or non-sense mutations.
- Can be divided into 5 groups

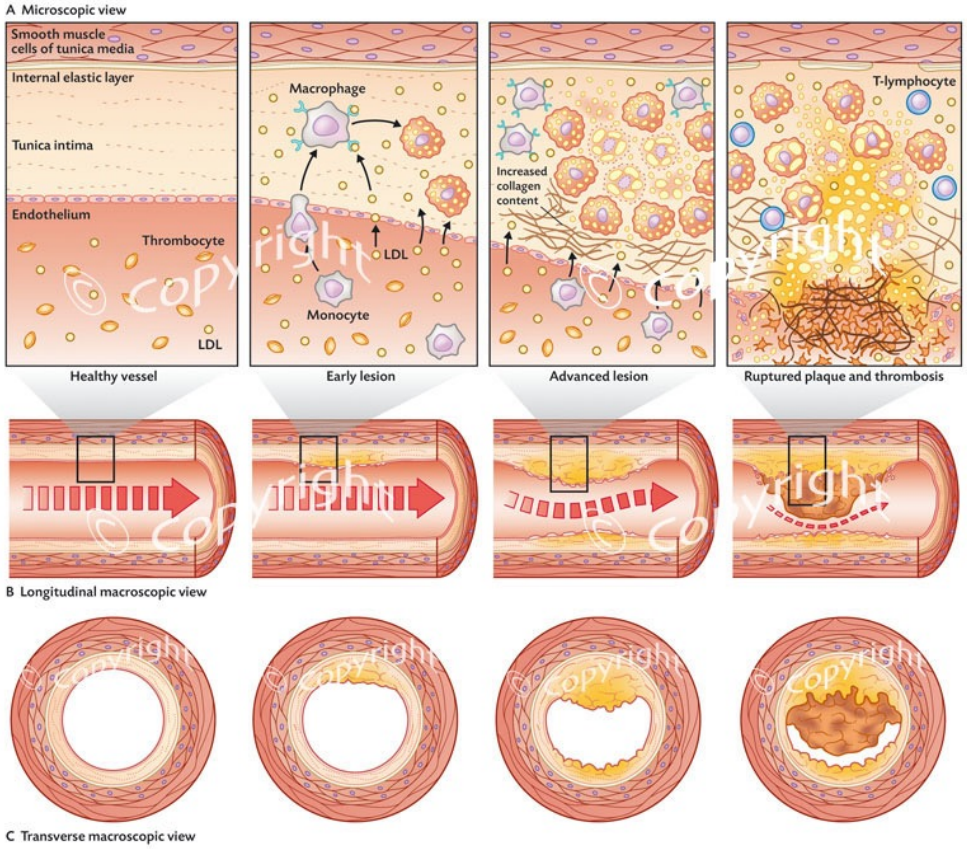
  - **Class 1:** Complete absence of receptor biosynthesis. Uncommon.
  - **Class 2**: Receptors cannot be transported in the Golgi and accumulate in the Endoplasmic Reticulum. Common.
  - **Class 3**: Receptors bind with low or no affinity to LDL
  - **Class 4**: Proteins are normal but fail to localize in the coated pits; LDL are not internalized.
  - **Class 5**: Receptors do not dissociate from LDL in the endosomes and are not recycled but degraded.



Mutation class	Synthesis	Transport	Binding	Clustering	Recycling
I	X				
II	→	X			
III	→	→	X		
IV	→	→	→	X	
V	→	→	→	→	X

Hypercholesterolemia and atherosclerosis

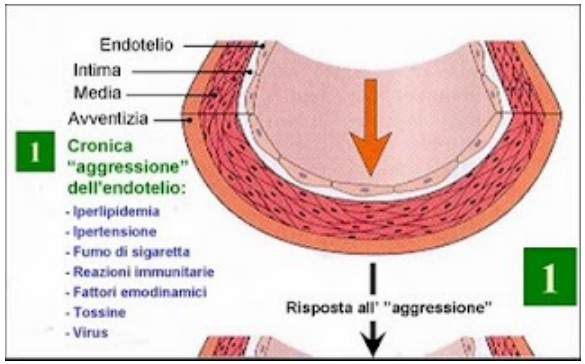


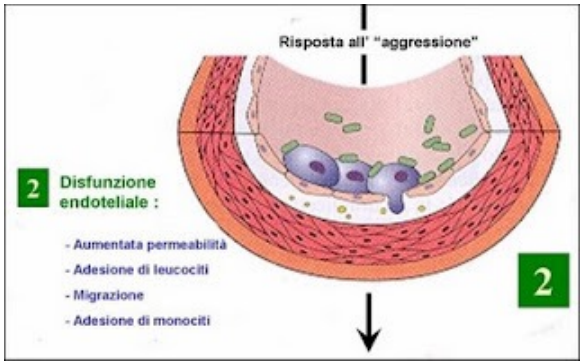


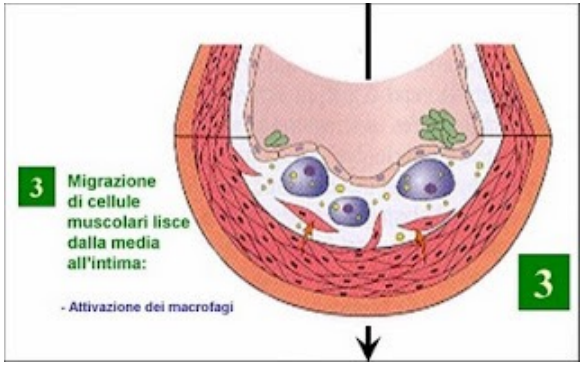
23% of the population

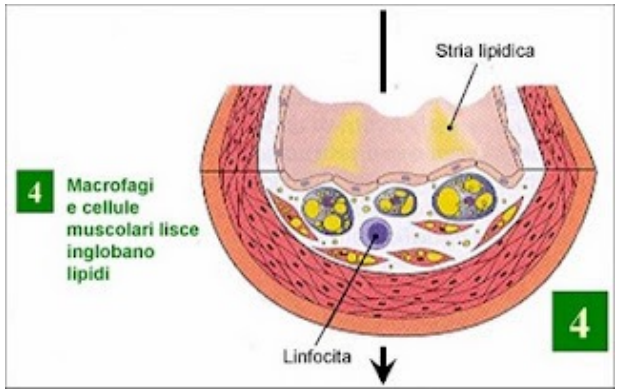
Large and medium arteries

Evolution of atherosclerosis

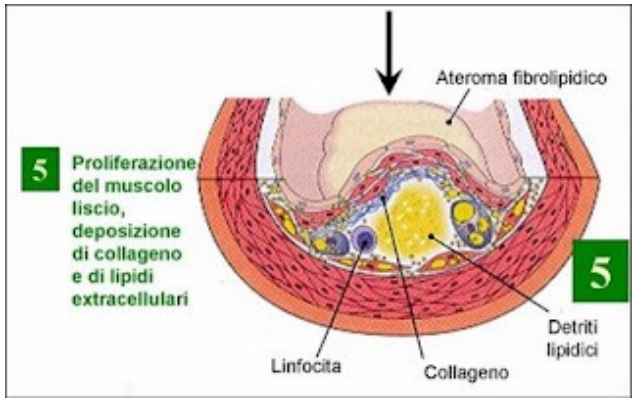




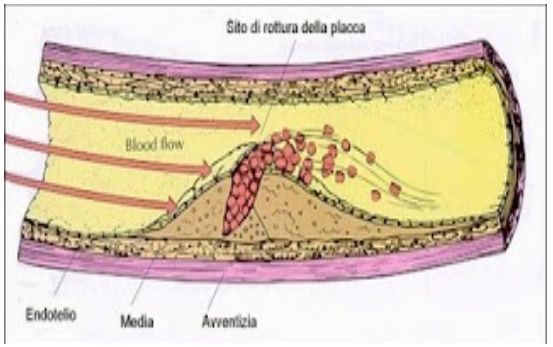




Fatty streak



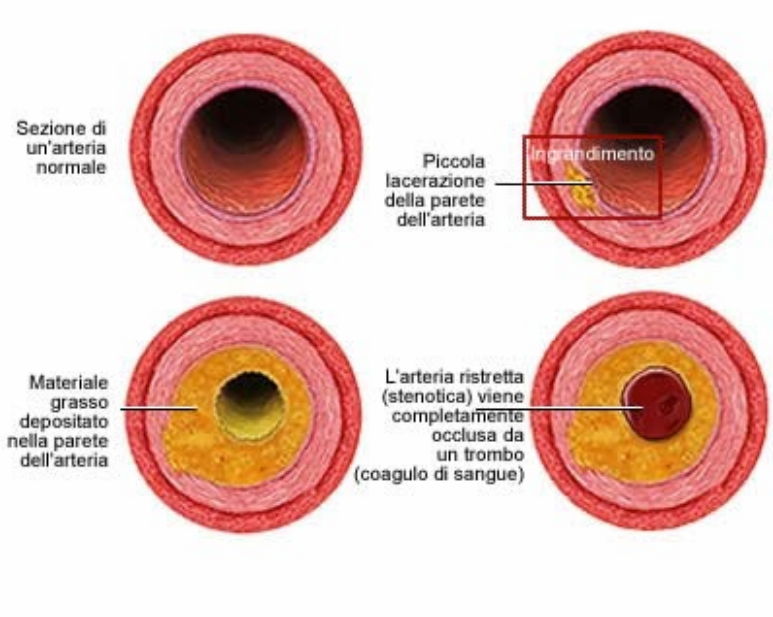
Atherosclerotic plaque



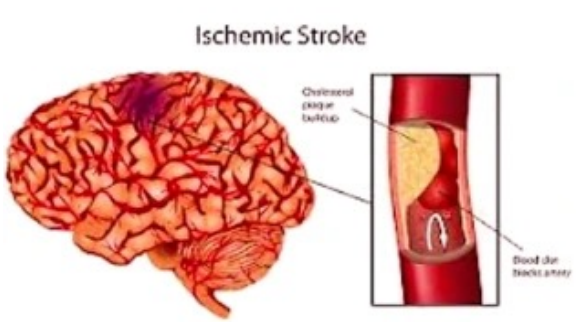
Complicated lesion

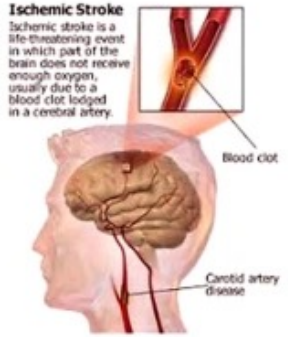
Consequences of atherosclerosis

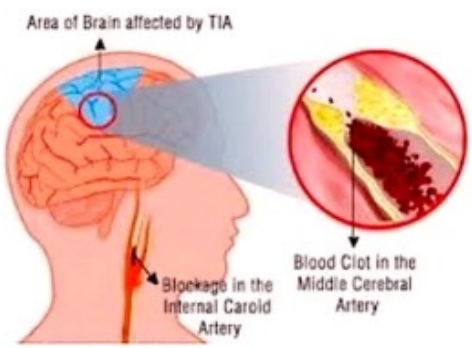
- **Stenosis** reduction of the caliber of the vessel with ischemia of the perfused territory. This occurrence causes:
  - Ischaemic Cardiomyopathy (IC): *coronary arteries,* angina pectoris.
  - Chronic encephalopathy: *carotid artery,* TIA: disturbances of brain functions , dizziness, loss of consciousness
  - Peripheral artery disease (PAD) in the lower limbs with pain on walking that forces to stop: *intermittent claudication, cramps in the calves*
- **Thrombosis** with occlusion of the vessel and necrosis of the tissues:
  - Infarction: heart, brain, spleen, intestine
  - Gangrene: if it affects a segment of skeletal muscle
- **Hemorrhage** due to *rupture of the artery*. Cerebral haemorrhage is particularly serious. The rupture of the aneurysm of the aorta is very serious

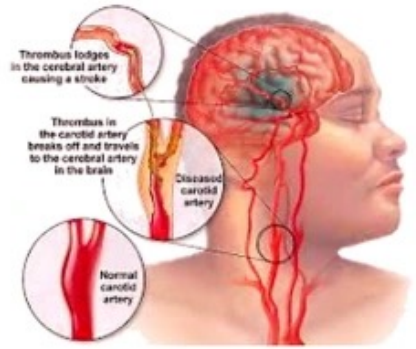


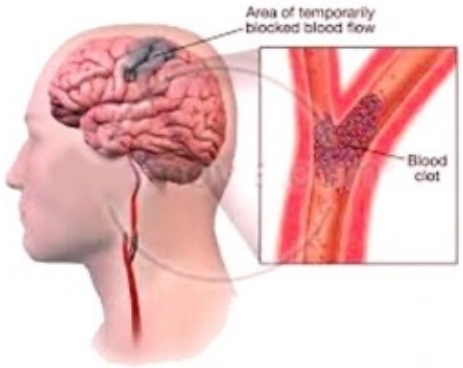
ORGANS AFFECTED BY ISCHEMIA

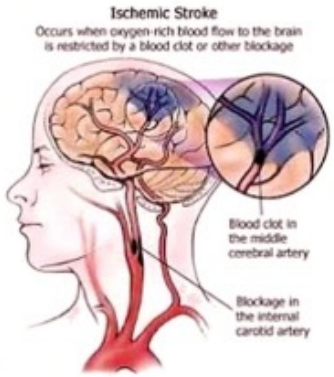


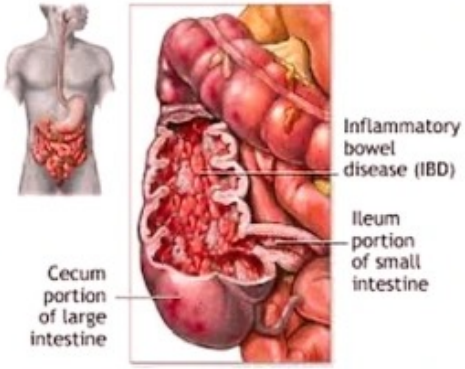


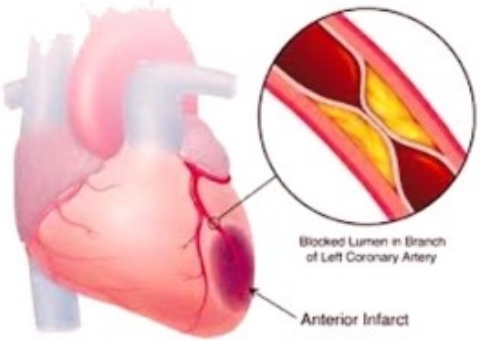


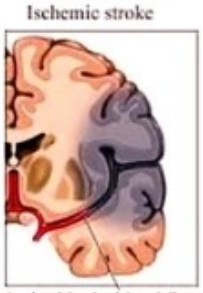


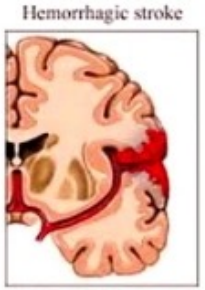


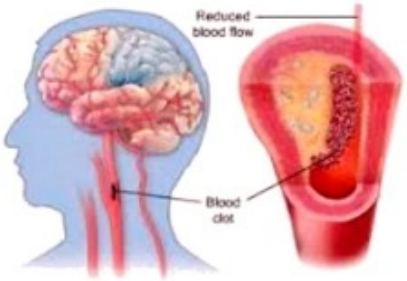


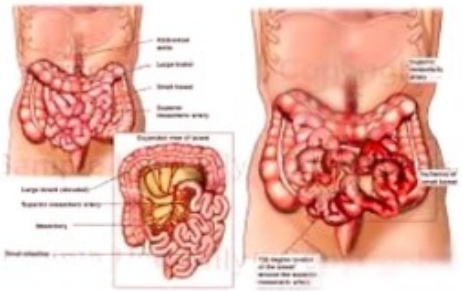


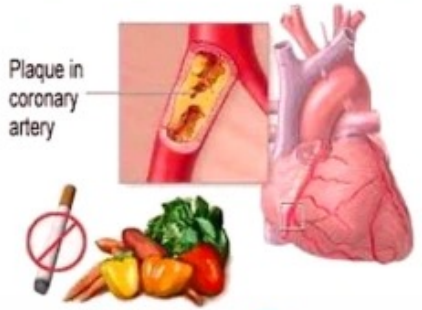








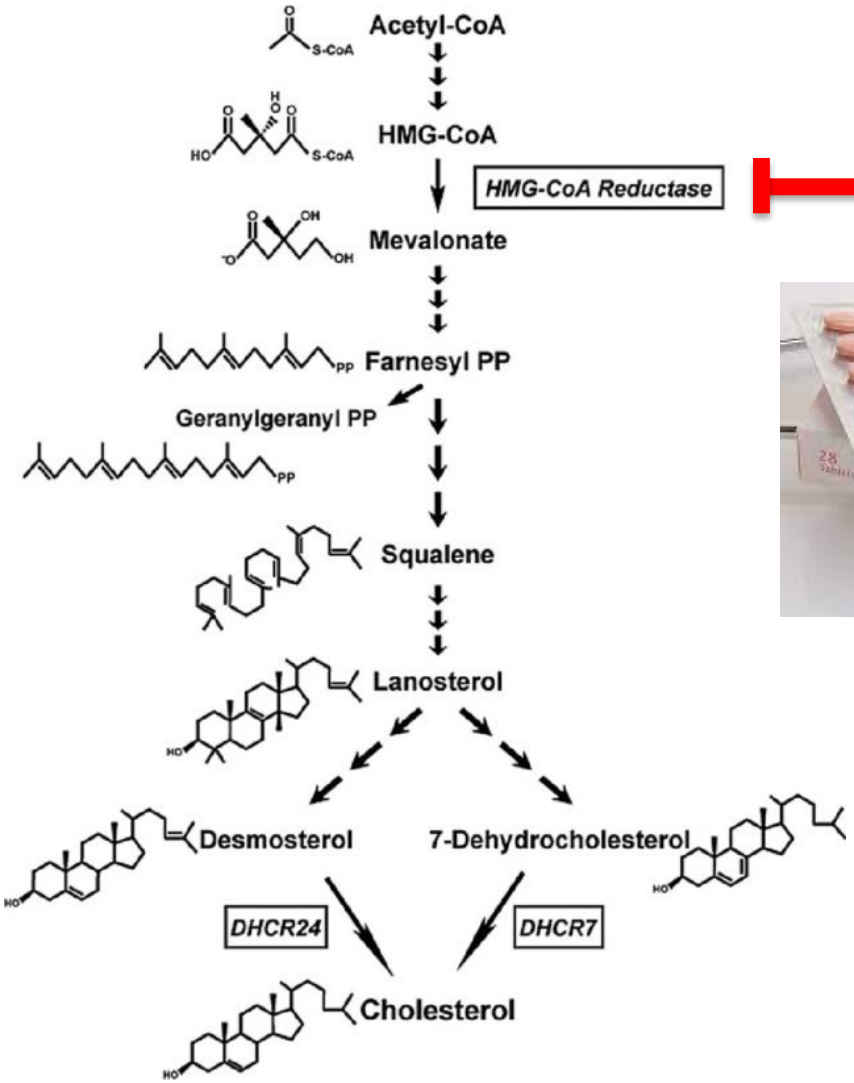


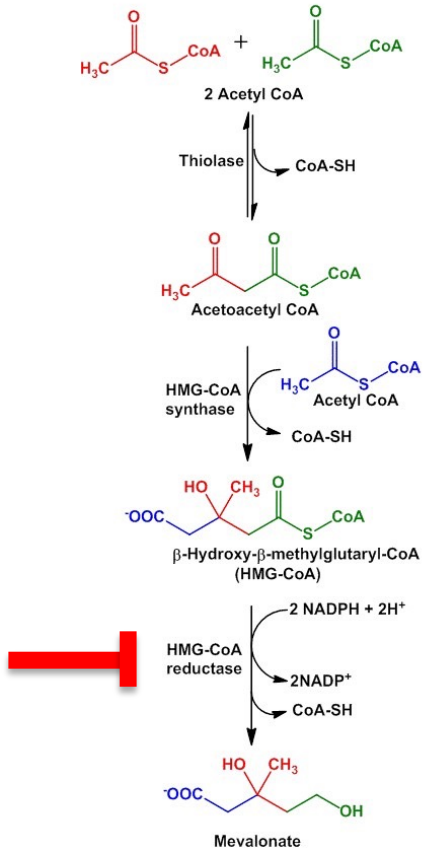


THERAPY of FH

- **Lifestyle correction** (low-calorie and low-fat diet low in saturated fatty acids, exercise, stop smoking)
- **Treatment of risk factors**: high blood pressure or diabetes mellitus.
- Coronary **angioplasty and stent, bypasses**
- **LDL Apheresis**: machine that removes LDL from the blood (like dialysis)
- **Drugs**
  - Interacting with cholesterol turnover:
    - **statins** (synthesis inhibitors),
    - Phenofibrates, absorption inhibitors.
    - **Inclisiran** (stabilization of LDL receptor)
  - Antiplatelet drugs:
    - Aspirin (low doses: COX-1 inhibition)

STATINS

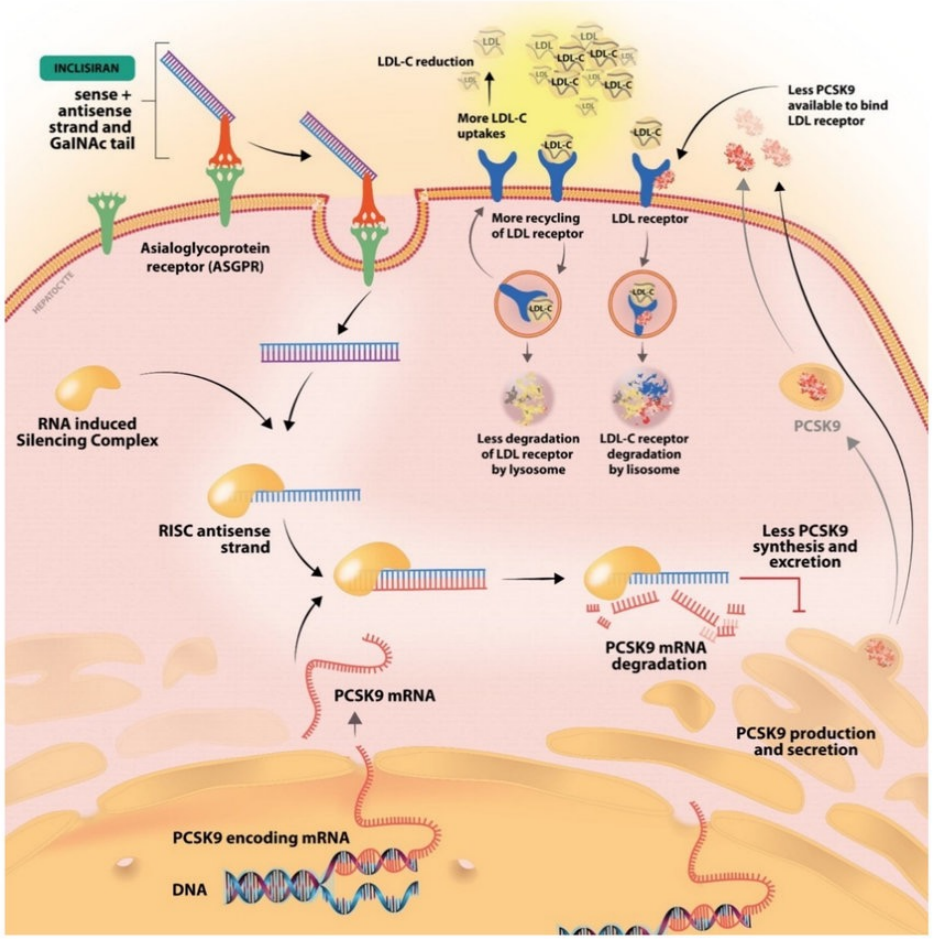




Side effects: allergy, muscle pain, Cramps, rashes, nausea, vomiting, Headache etc

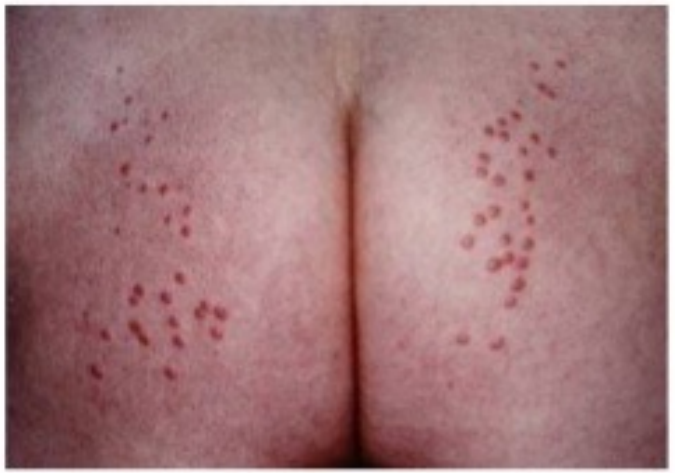
INCLISIRAN (Leqvio®)

- Approved by **AIFA 3-10-2022** to treat heterozygous familiar or non familiar hypercholesterolemia
- Prescribed in association to statins, or in case of statin intolerance
- DS **siRNA directed against PCSK9**, conjugated to triantennary-acetylgalactosamine *GalNAc* (binds to **ASGPR** (asialoglycoprotein receptor), highly expressed in hepatocytes)
- **Prevents LDLR degradation**
- Injected subcutaneously twice a year

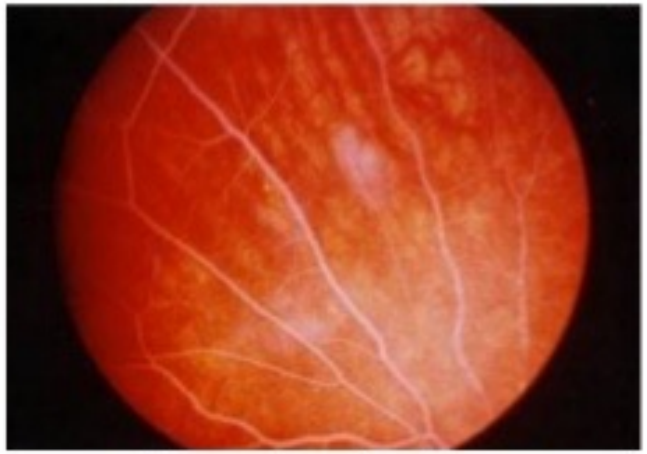


LPLD

- Lipoprotein lipase (LPL) deficiency.
- **LPL gene** mutation: short arm of *chromosome 8* in position 22. More than 220 mutations in the gene.
- **Autosomal recessive**, rare (1: 1,000,000)
- **Accumulation of lipoproteins (chylomicrons) in the blood**, hypertriglyceridemia, **deposits of fat under the skin**, toxicity to some organs (**pancreas, liver**) and diabetes.

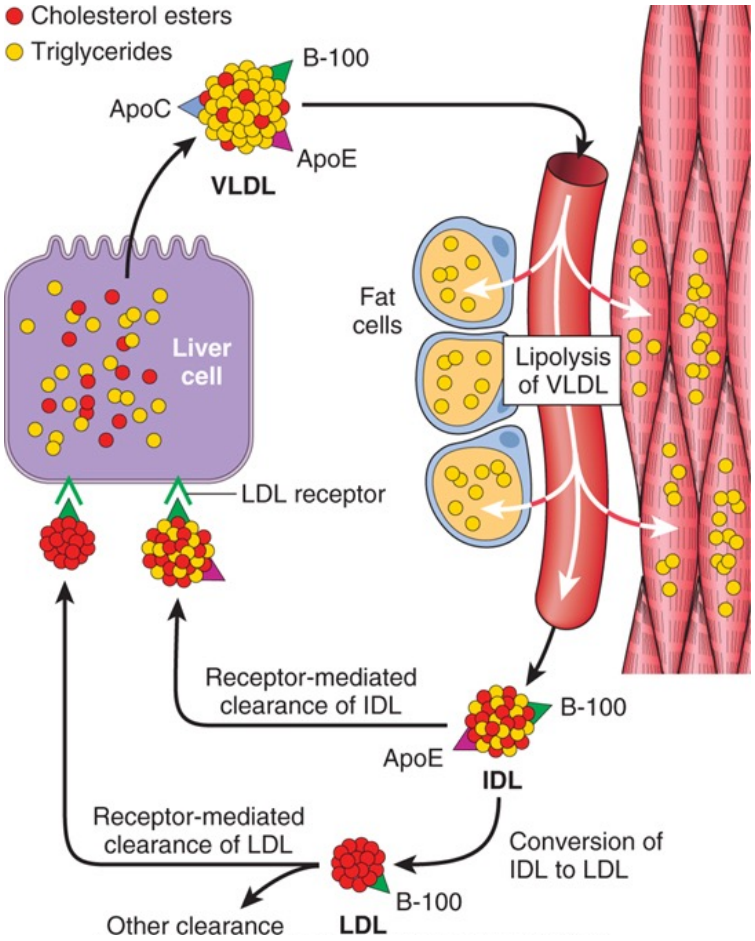


Eruptive xanthomas

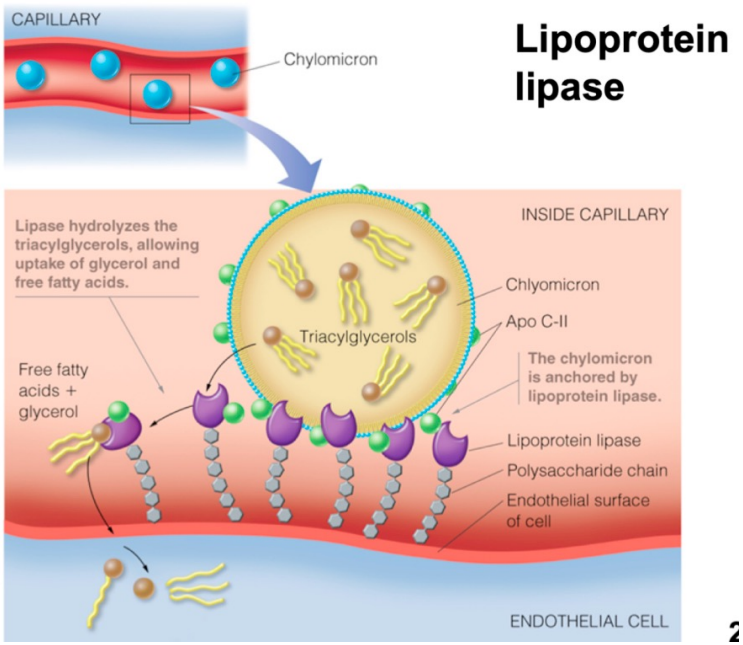


Lipemia retinalis

LPL: lipid turnover



Lipoprotein lipase is found mainly in the capillaries within the muscles or adipose tissue. The enzyme breaks down triglycerides (ester bonds) transported in the VLDLs to be used by these tissues



TRIGLYCERIDES

Triglyceride levels

Classification	Triglyceride level*
Normal	Less than 150
Borderline high	150–199
High	200–499
Very high	500 or higher

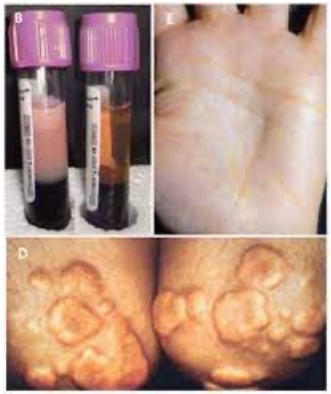
*Values in milligrams per deciliter (mg/dL)

Levels ranging from **7,000 to 10,000 mg / dL** can be reached in LPLD patients. The main consequence of the disease is the risk of developing acute pancreatitis (especially for patients with plasma triglyceride levels above 1,000 mg / dL)

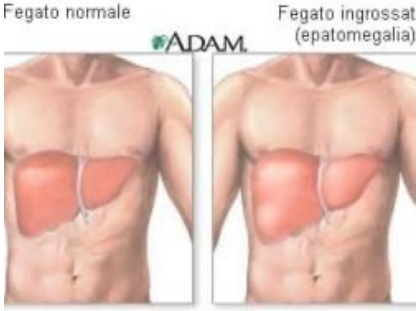
Clinical manifestations

- The most common clinical manifestation is **recurrent abdominal colicky pain** (often appears in childhood) and **acute pancreatitis.** The pain can be epigastric, with radiation to the back, or it can be widespread, with the appearance of an acute abdomen.

- Other typical symptoms are **eruptive xanthomas** (in about 50% of patients) and **hepatosplenomegaly**.



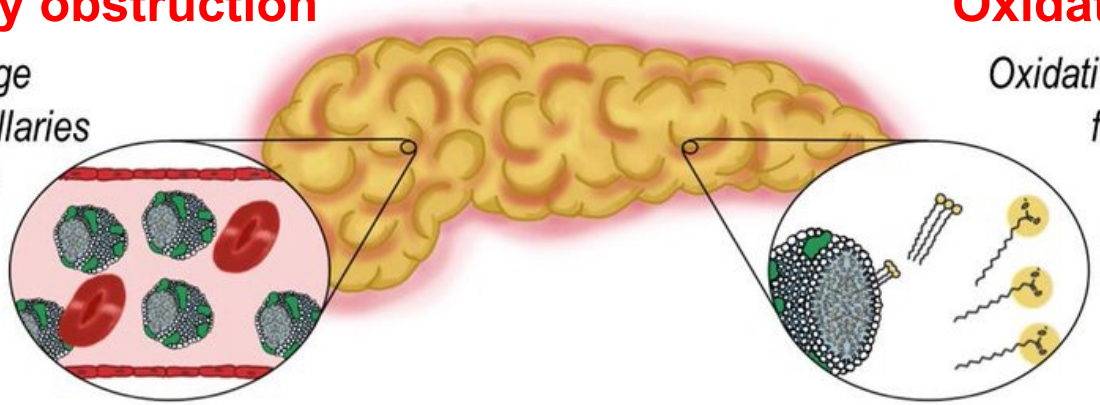
- Complications: LPLD patients are at high risk for **severe acute pancreatitis**, which can be life-threatening and can lead to chronic pancreatic insufficiency and **diabetes**. Increased risk of **atherosclerosis** and coronary heart disease.



Pathogenesis of Hypertriglyceridemia-Induced Pancreatitis

Sludging/capillary obstruction

Sludging from large molecules in capillaries causing ischemia



Oxidative Stress

Oxidative stress from free fatty acids generated by breakdown of triglycerides

Treatment: Clearance of Triglycerides

Insulin accelerates breakdown of triglycerides

Plasmapheresis removes triglycerides

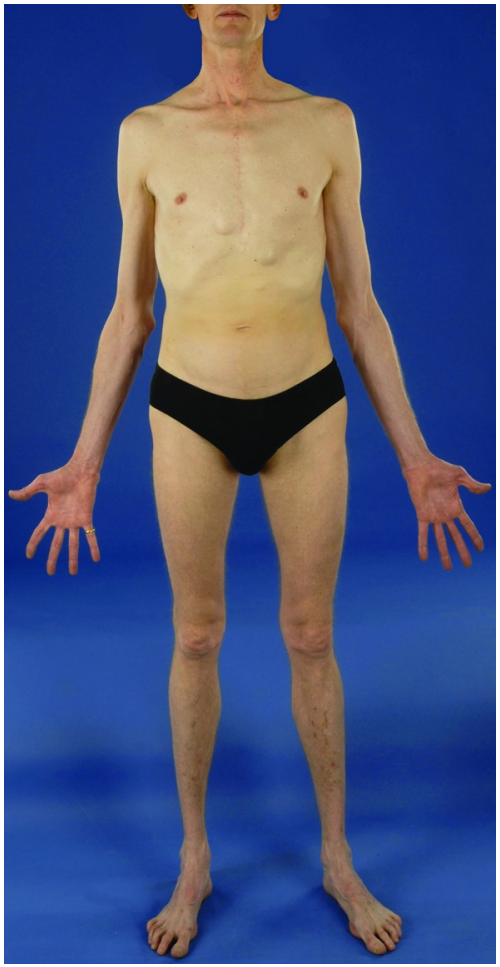
LPLD: therapy

- Low fat and simple carbohydrate **diet**
- Lipid-lowering drugs (e.g. **orlistat**)
- Lipid-lowering agents such as **fiber and omega-3 fatty acids** can be used to lower TG levels in LPLD;
- **Additional measures**: abstention from agents known to increase endogenous triglyceride levels, such as alcohol, estrogens, diuretics, antidepressants (for example sertraline) and beta blockers.
- **GENE THERAPY: AAV-LPL**

Marfan Syndrome

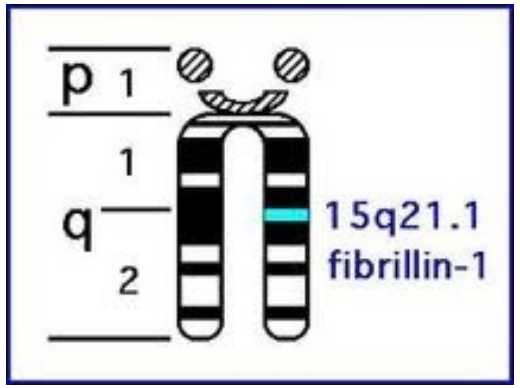
- Disorder of connective tissues characterized by alterations of skeleton, eyes and cardiovascular system.

- Prevalence: 1:5,000
  - In 80% of cases is familial
  - In 20% is sporadic (novel mutations)



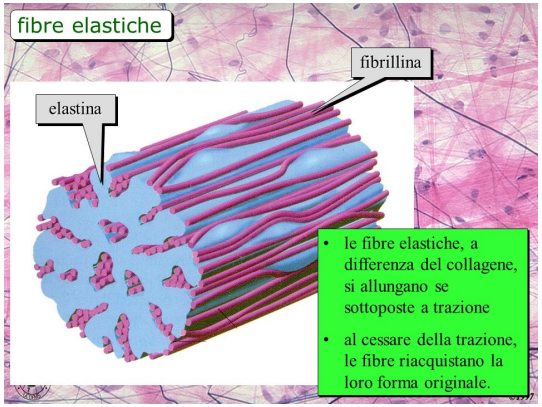
Genetics

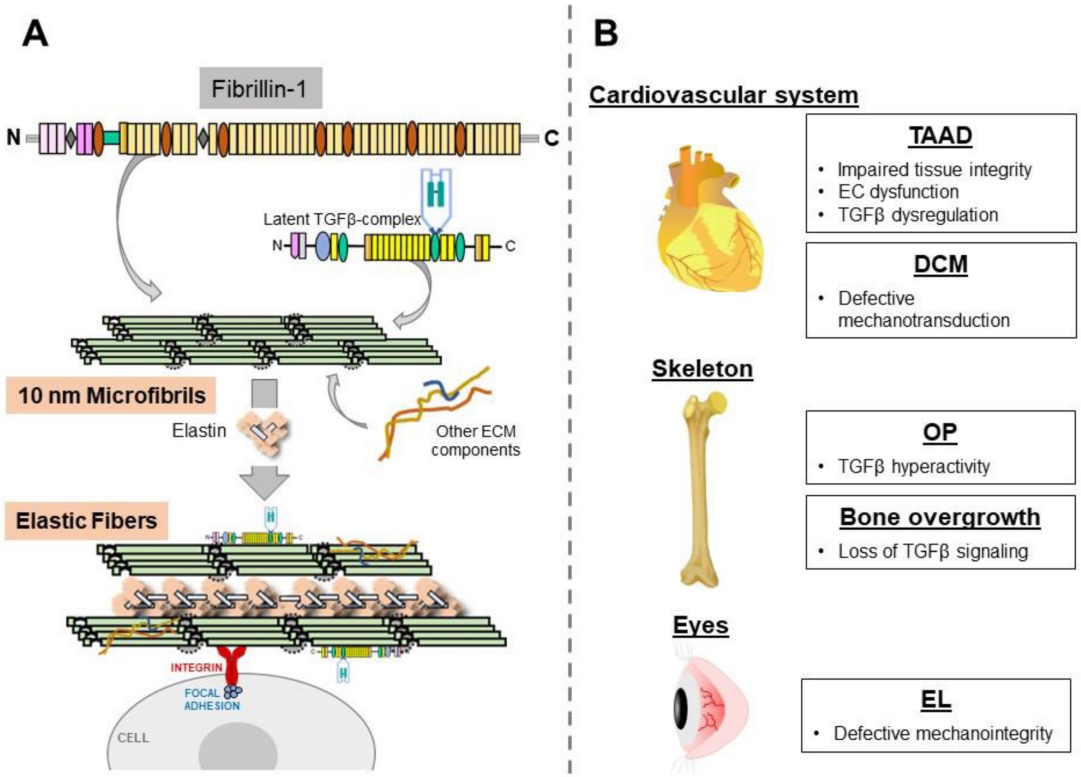
- Caused by mutations of the ***FBN1*** *gene* encoding **fibrillin-1,** an extracellular glycoprotein
- The gene maps on chromosome **15q21**
- There **are 600 different mutations** of *FBN1*
- The majority are **missense** mutations that cause production of altered fibrillin-1.



Fibrillin

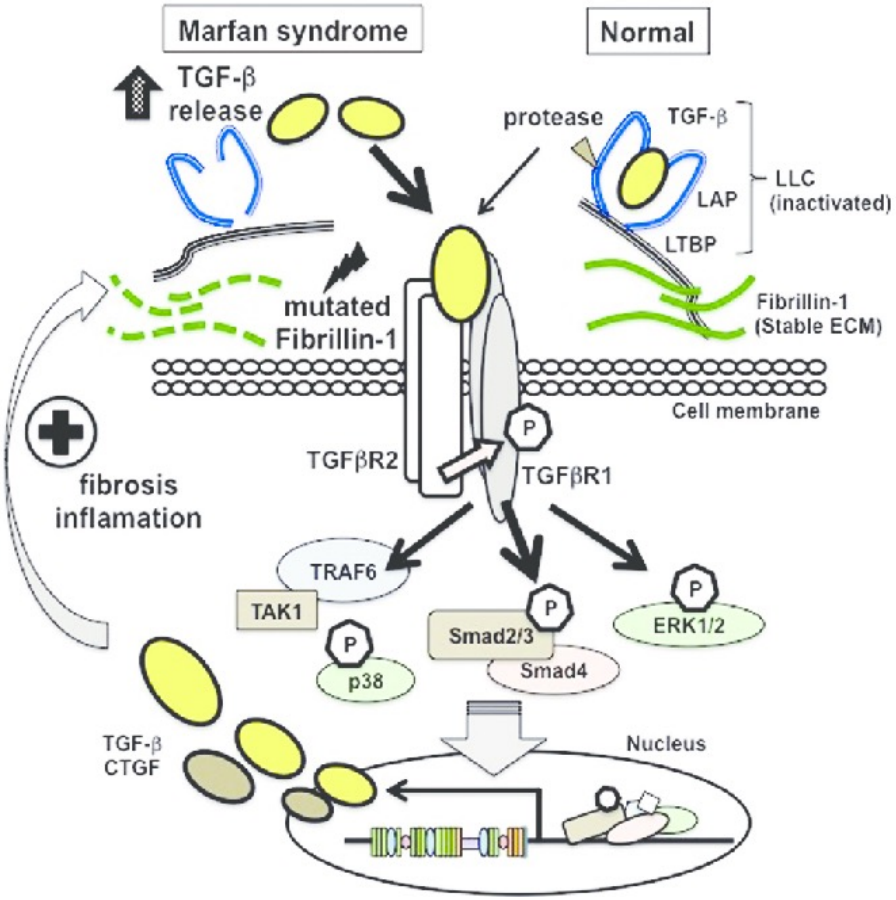
- Extracellular **glycoprotein**
- Assembles into **microfibrils** of the extracellular matrix.
- Main component of the connective tissues
- Microfibrils provide a scaffolding on which tropoelastin is deposited to form **elastic fibers.**
- Microfibrils are very abundant in the **<u>aorta, ligaments and lens</u>**.





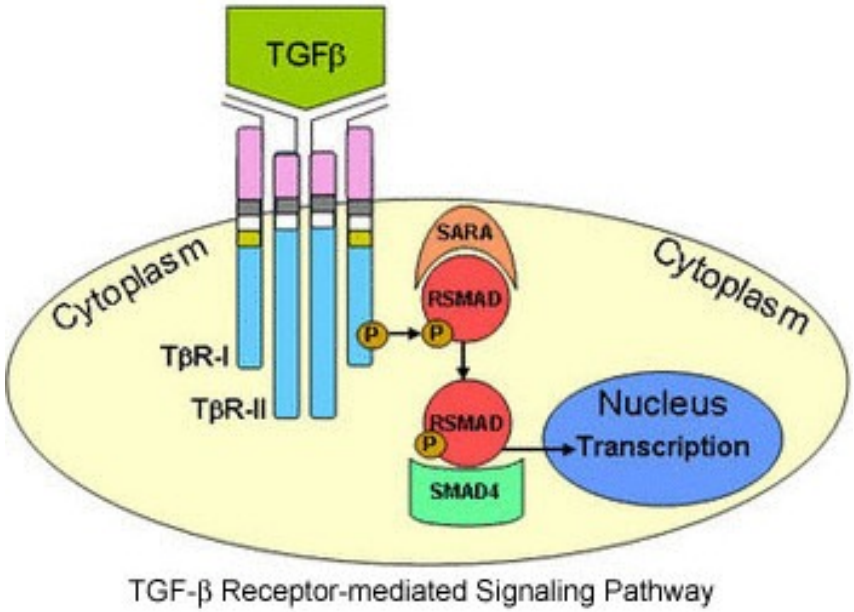
TAAD=thoracic aortic aneurysms and acute aortic dissection
DCM=dilated cardiomyopathy
OP=osteopenia
EL=ectopia lentis

Fibrillin and TFGbeta

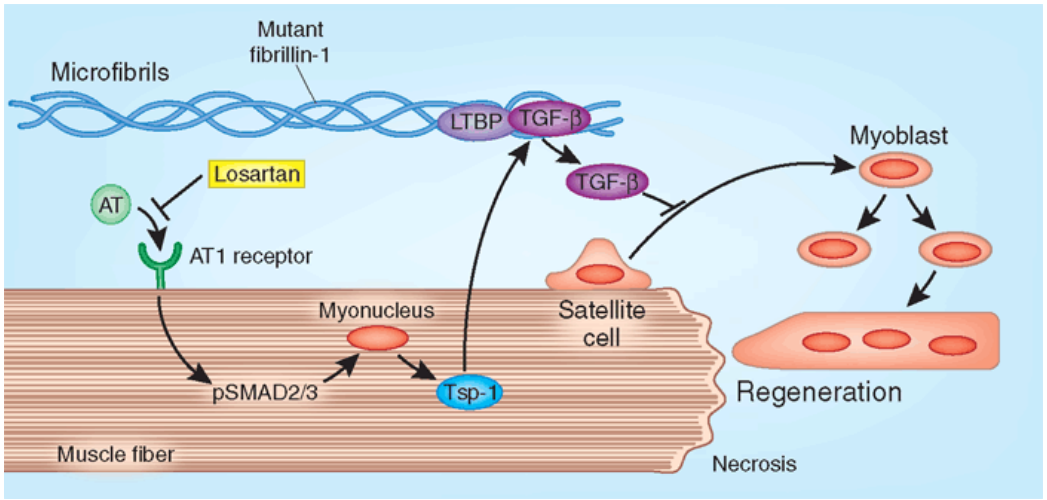


- Fibrillin1 sequesters and controls the bioavailability of transforming growth factor beta (TGFbeta)
- **Mutated Fibrillin causes an abnormal and excessive activation of TGFbeta signalling.**
- Deleterious effects on the *development of smooth muscle of the vessels, bone development* and on the integrity of extracellular matrix

TGFβ Pathway and Muscle

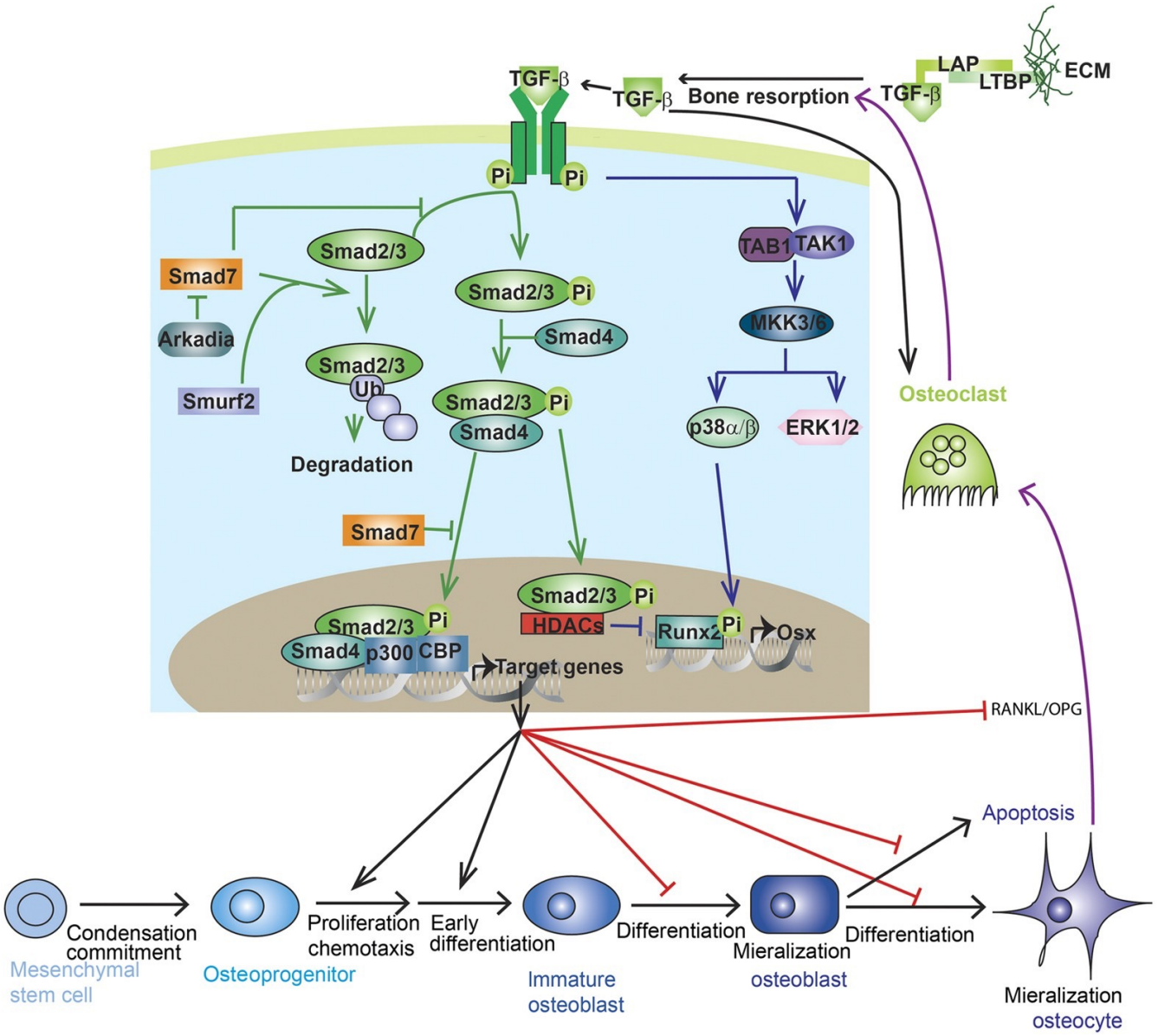


TGF-β Receptor-mediated Signaling Pathway



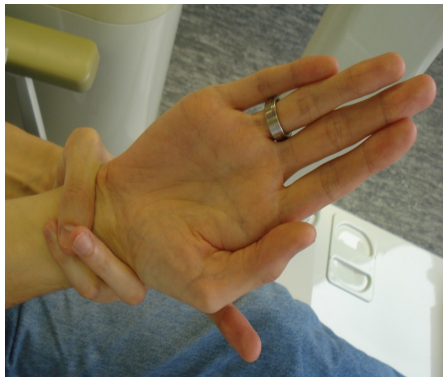
TGF beta inhibits muscle cells differentiation

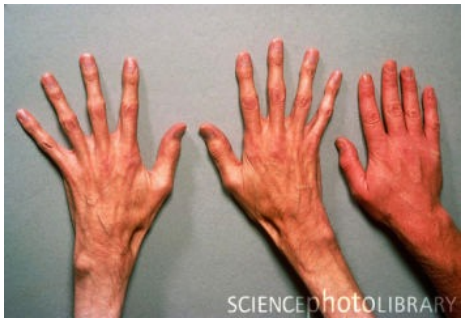
TGFβ Pathway and Bone

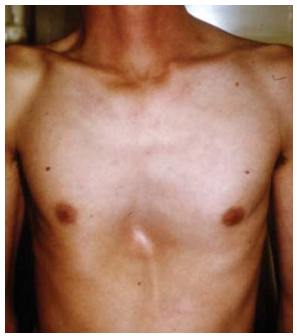


Skeletal Abnormalities

- Patients are tall, with long and slender fingers
- Abnormal joint flexibility (thumb can be bend to touch the wrist)
- Arachnodactyly
- Dolichocephaly (long head)
- Scoliosis, pectus excavatum or carinatum



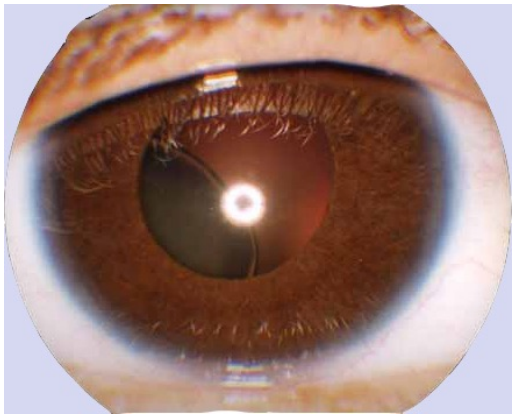




Ocular abnormalities

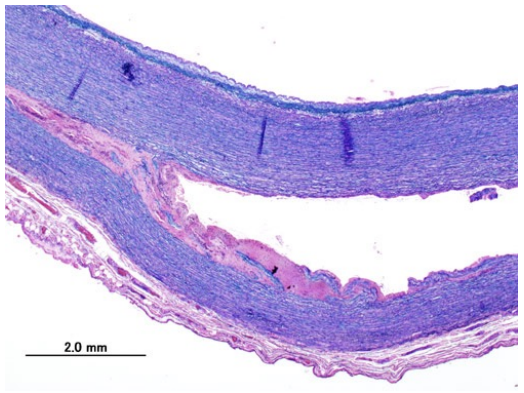
- **Myopia** (100%)

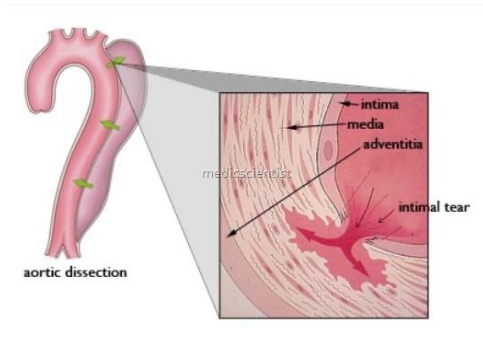
- Subluxation (dislocation) of the crystalline lens in one or both eyes (***ectopia lentis***) (in 50% of patients). Causes blindness

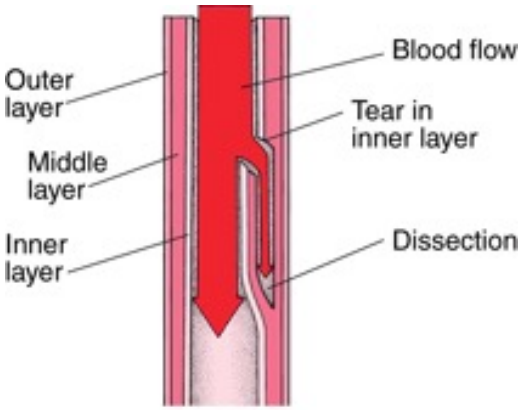


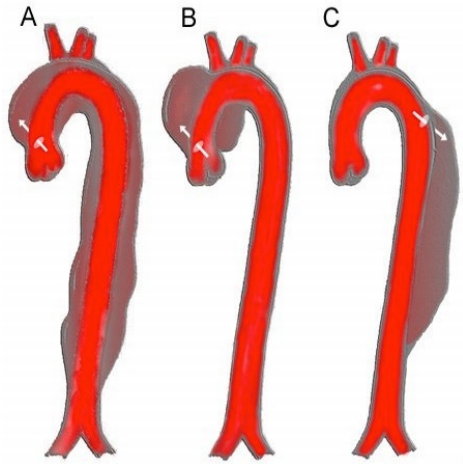
Cardiovascular Abnormalities

- **Aortic dissection:**
  - **cystic medial necrosis (CNM)**: pathology of the tunica media, with diffuse cysts and necrosis, which may lead to
  - **Aortic root dilatation and dissection**: a tear in the intima allows the blood to enter and split the inner and middle layers of the aorta, forming a channel within the aortic wall
  - Dramatic and lethal event causing death in 40% of Marfan patients
- **Aneurysm of the aortic arch**









THERAPY

- Currently there is **no specific therapy** but measures to reduce the associated risks (aortic dissection)

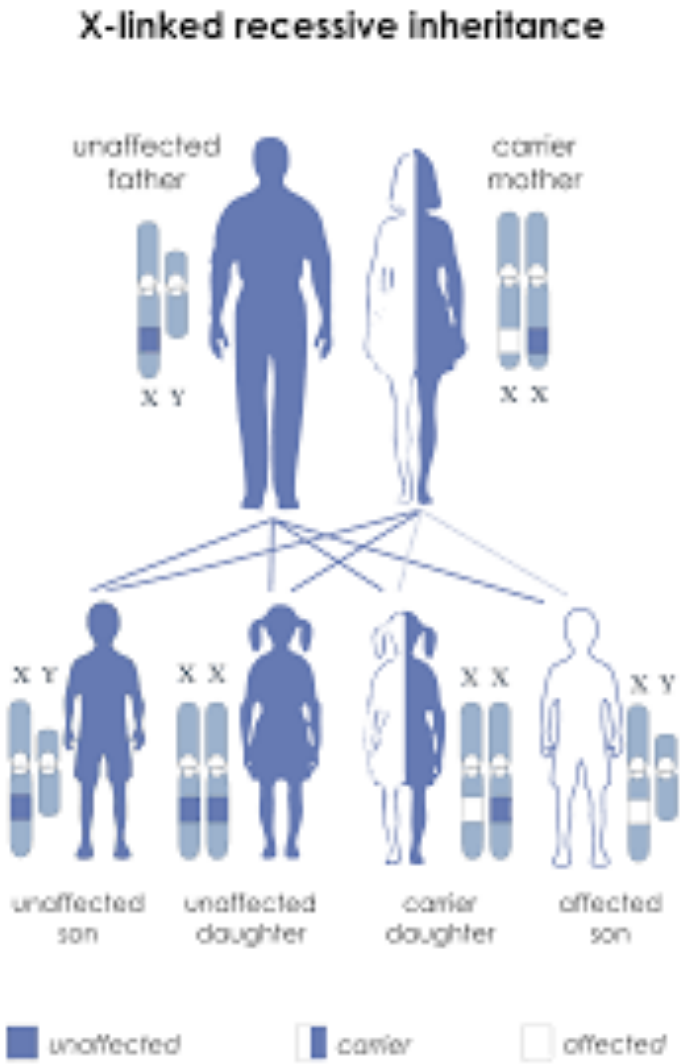
- **Beta-blockers (**Atenolol, etc) slow the heart rate and decrease the strength of heart contractions. These drugs are administered to decrease the intensity of blood flow in the aorta.

- **Angiotensin II receptor blockers** (such as losartan and candesartan) may also be given to lower blood pressure.

- In the presence of a dilated aorta or an aneurysm, the altered part can be repaired or replaced surgically. Severe valve regurgitation is also surgically repaired.

- The subluxation of the lens can usually be treated surgically.

Recessive X-linked diseases



Recessive
-Duchenne Muscolar Distrophy
-X-SCID
- OTC Deficiency
-G6PD deficiency (favism)
-Emophilia A and B

Duchenne muscular dystrophy (DMD)

1/3,500 boys worldwide

Absence of dystrophin, a cell membrane protein (approximately 0.01 % of skeletal muscle protein)

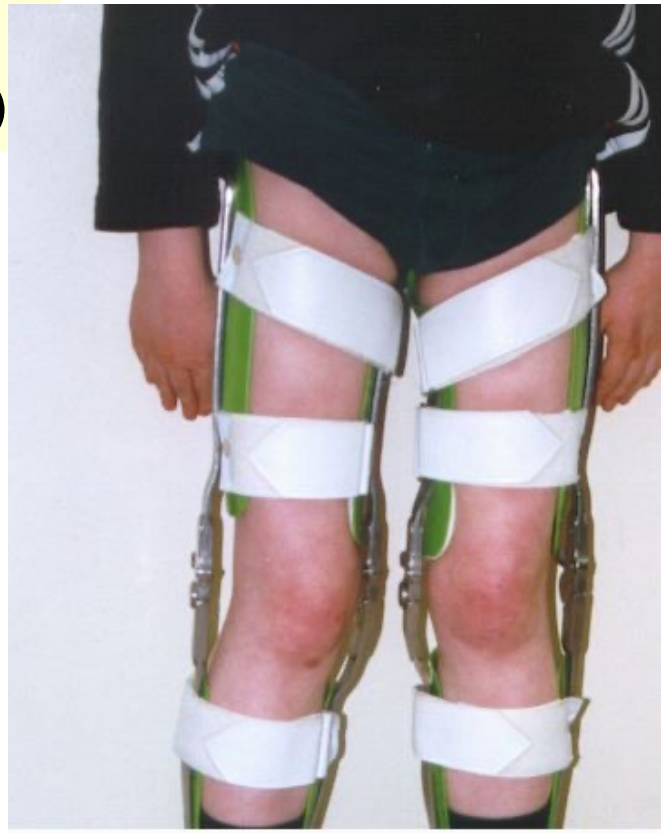
All muscles involved

Generalized muscle degeneration

Wheelchair at 12 y.o.

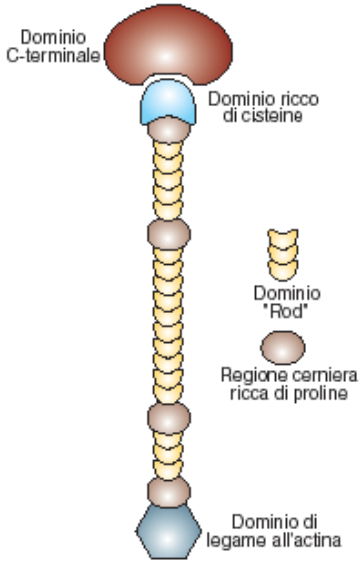
Death by 10-20 y.o. respiratory failure

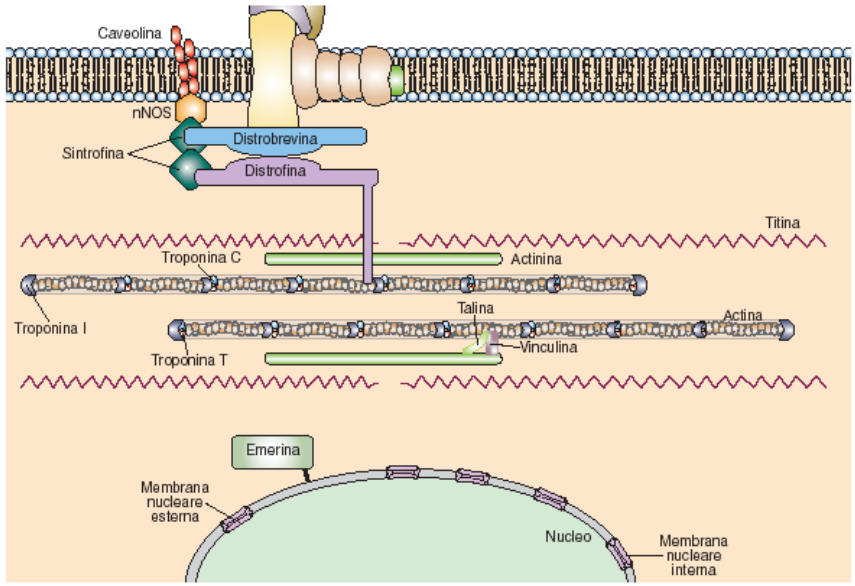
Life threatening **dysrhythmia or heart failure** develops in about 10 %.



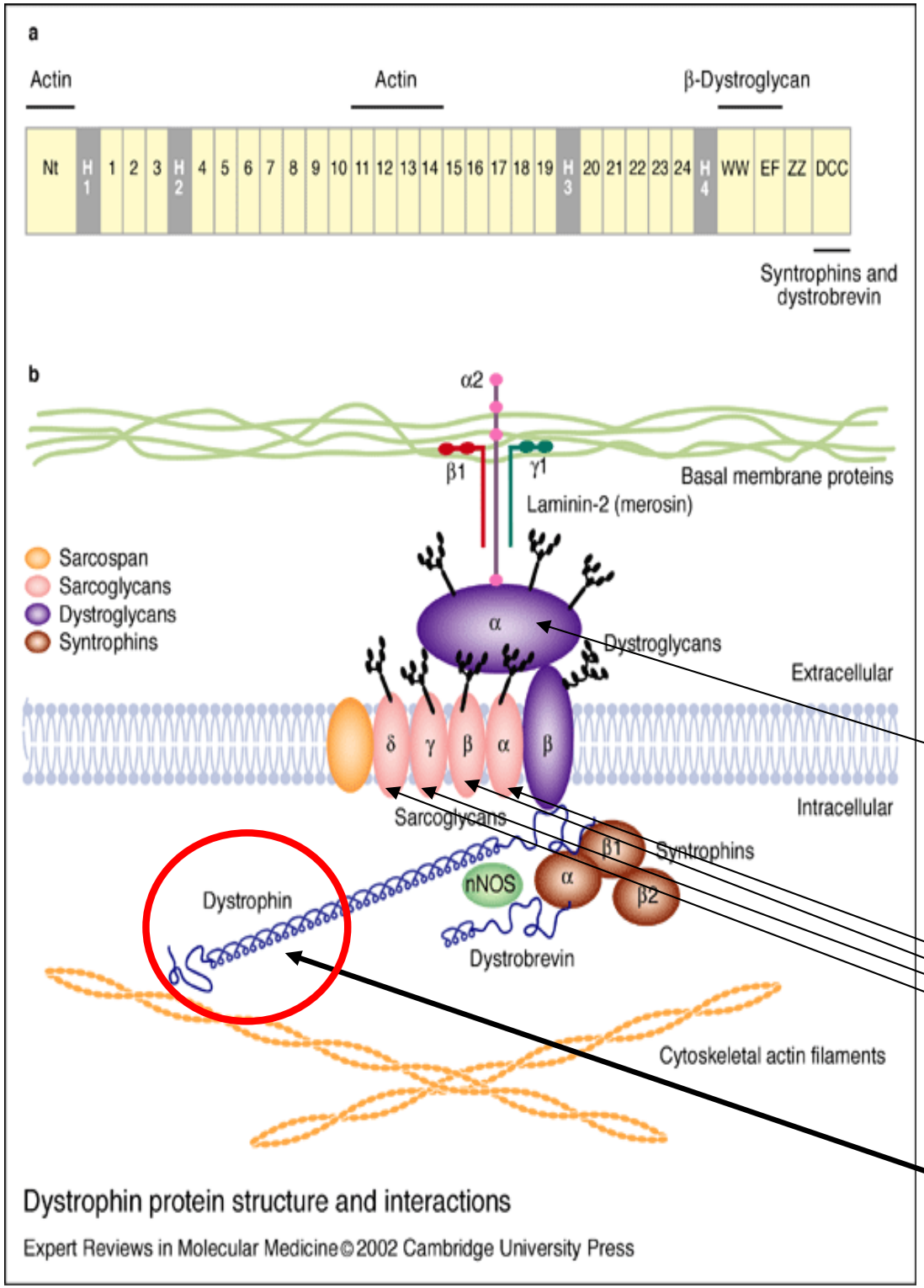
Distrophin gene

- Chromosome Xp21
- 2.4 million of bps, 79 exons
- mRNA: 14,000 bases
- Protein: 3600 aa
- Expressed in cardiac and skeletal muscle
- Contains many domains like those of cytoskeletal proteins





Dystrophin



Dystrophin protein structure and interactions

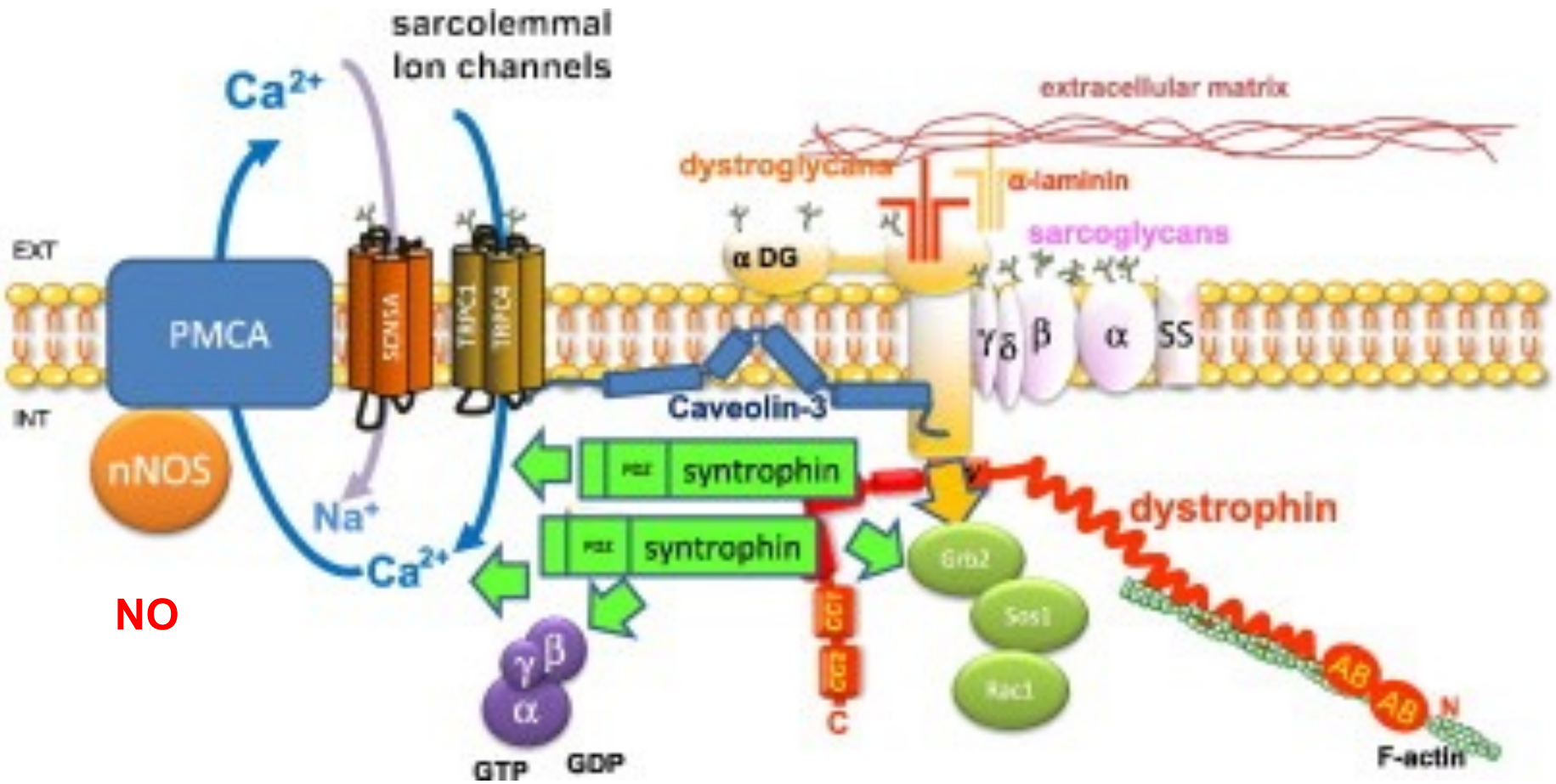
Provides **links between**
the intracellular actin filaments
of the **cytoskeleton**
with the extracellular matrix

Whole complex
stabilizes the membrane

Laminin2α2:
congenital MD chr 6

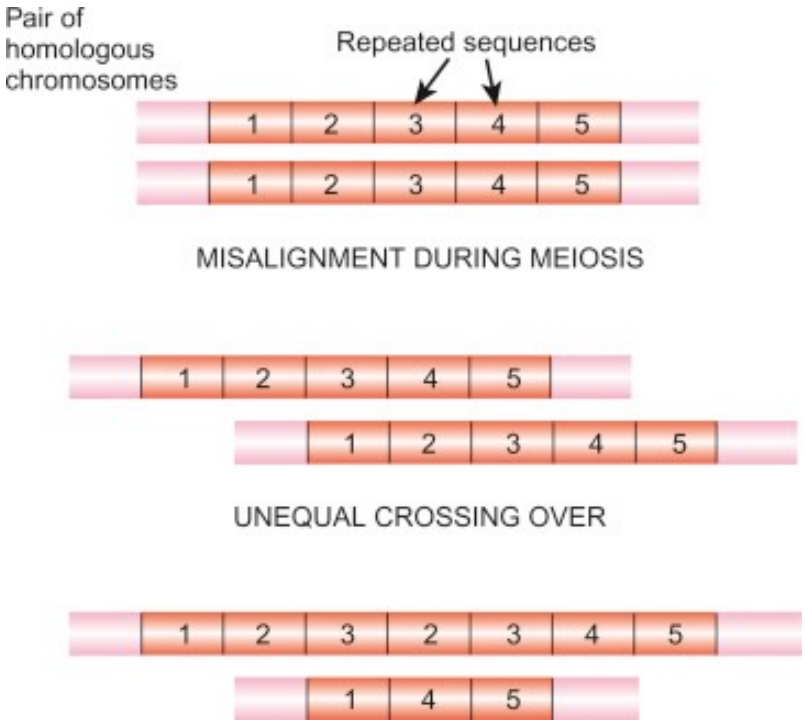
Sarcoglicans:
Limb Girdle MDs (4 types)

Duchenne and Becker MDs

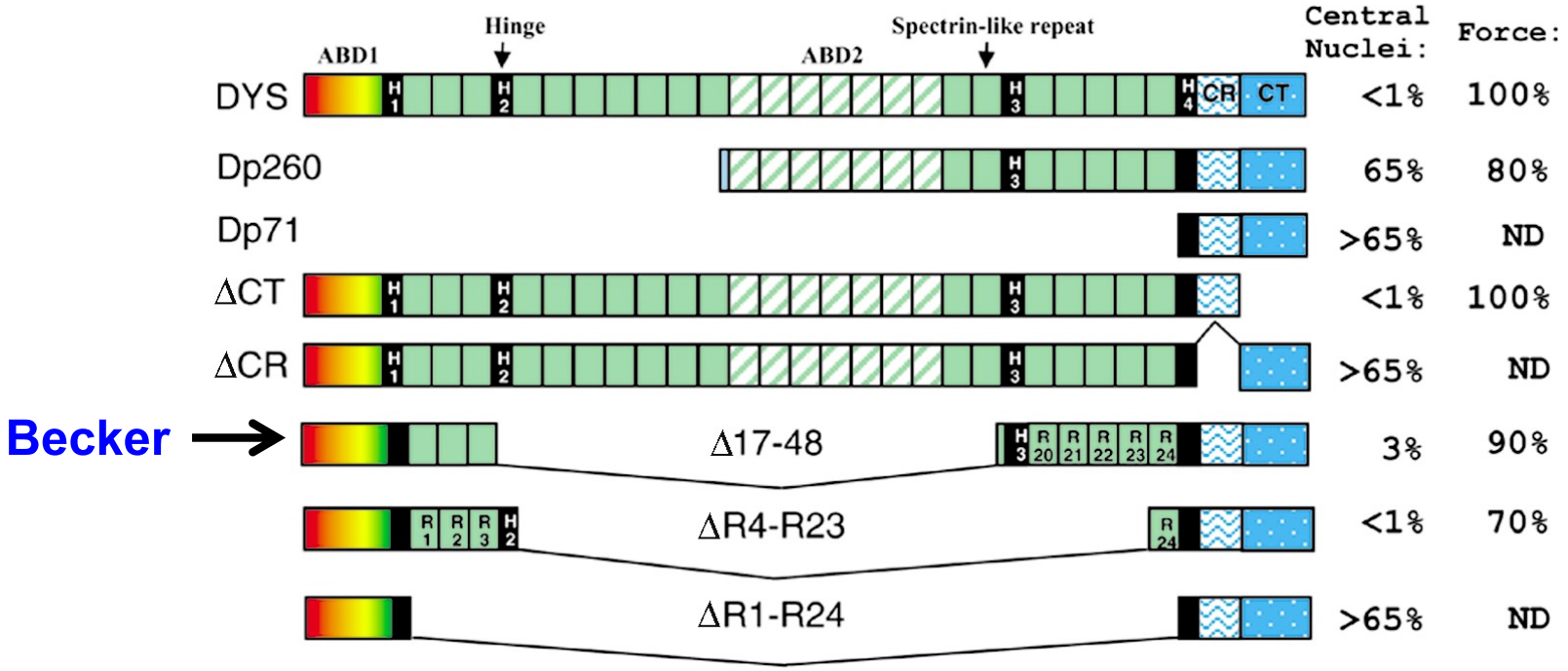

sarcolemmal
Ion channels
Ca2+
extracellular matrix
dystroglycans
α-laminin
sarcoglycans
α DG
EXT
INT
PMCA
nNOS
Na+
Ca2+
Caveolin-3
γδ β α SS
syntrophin
syntrophin
dystrophin
Grb2
Sos1
Rac1
AB AB
N
C
F-actin
γ β
α
GTP GDP
NO
GTPase
MAPK

Mutations of the dystrophin gene

- The length and structure of the gene predispose to mutations (over 700 mutations known)
- In 70% of cases there are deletions
- Il remaining 30% are point mutations
- Presence of **hot spot** sites in the gene that favor deletions or duplications because of **unequal crossing over** due to interspersed Alu elements
- **The severity of the phenotype does not correlate with the length of the deletion, but with the loss of the reading frame.**
- **Big deletions with unaltered frame** may not affect the function (such as in the **Becker phenotype**).
- In the **DM** there are non-sense **mutations or frameshift** that do not allow the proper protein synthesis

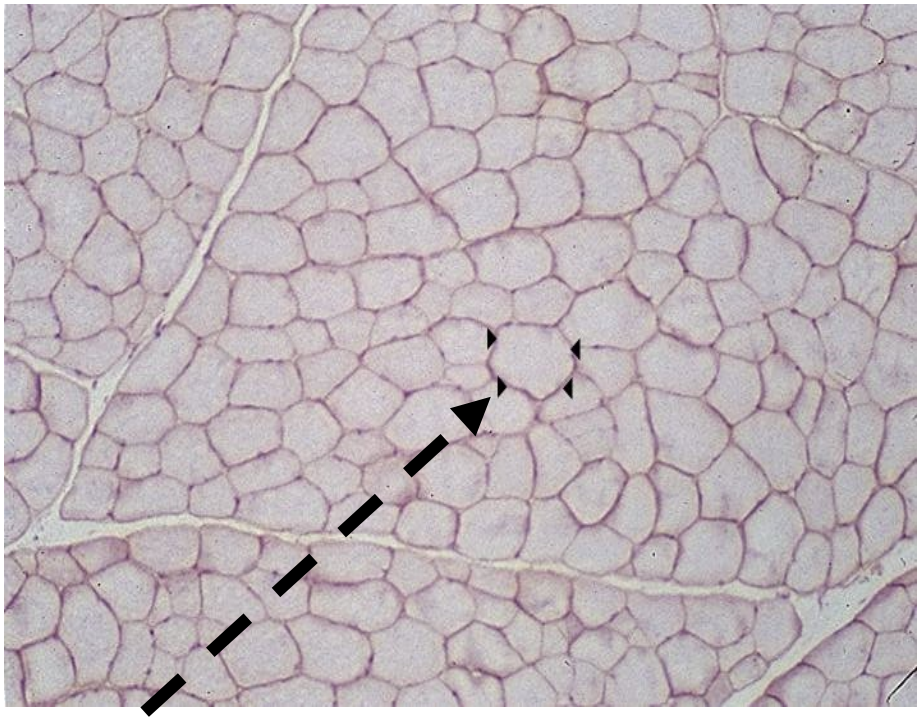


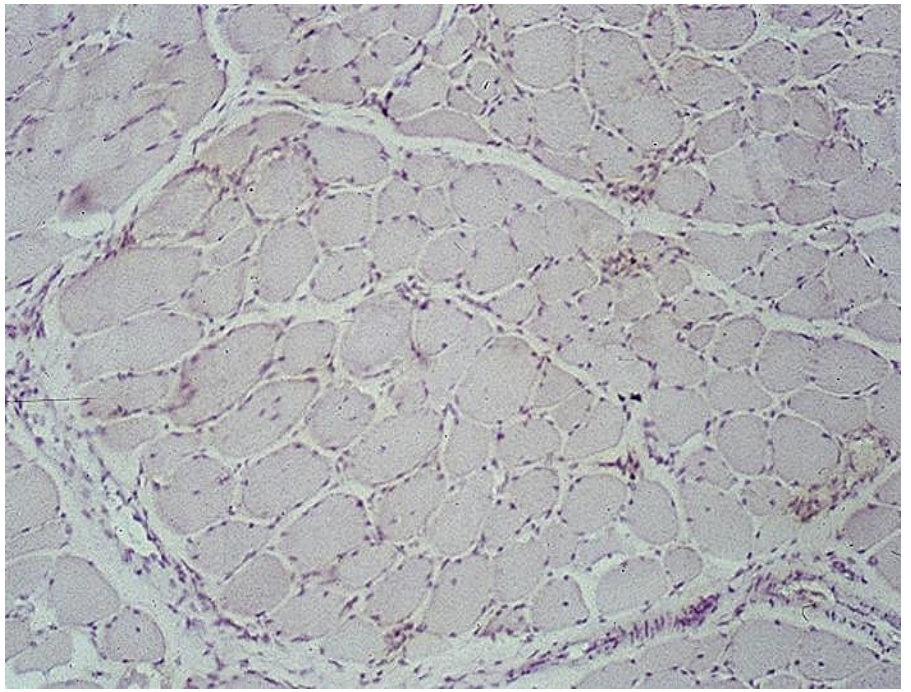
Deletion variants of dystrophin



Most, but not all, of the spectrin-like repeats are dispensable for the function of dystrophin.

Normal muscles and DM muscle





muscles stained for dystrophin with monoclonal antibodies

myofibers are **circumscribed**
by the darkly-staining dystrophin

dystrophin is not evident

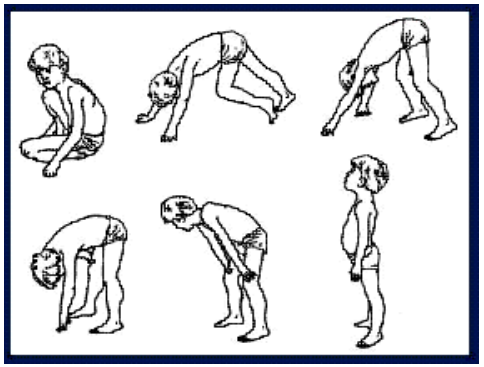
wider **variation**
in myofiber diameters

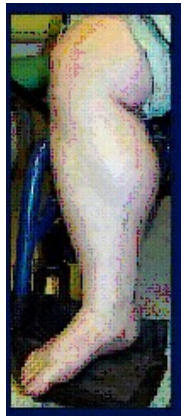
increased connective tissue

Clinical manifestations

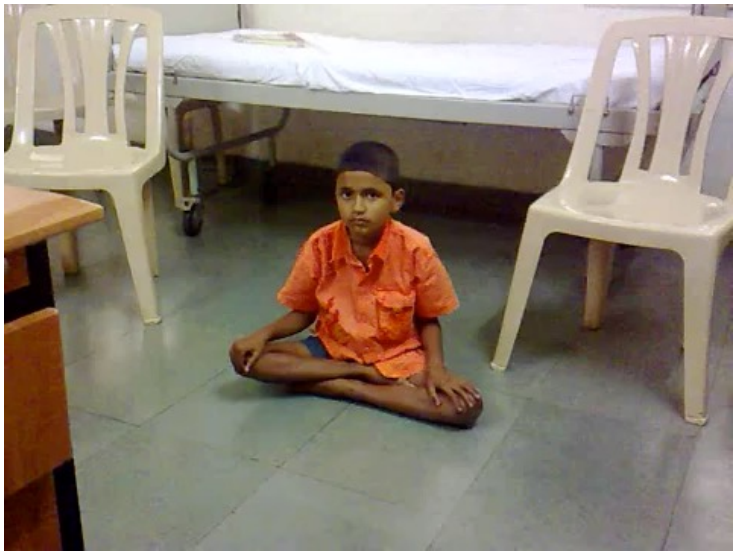
- The diagnosis usually occurs during the first year of life , when the children start walking.
- **Gower sign** (the patient must use his hands and arms to reach the standing position)
- **Reduced motor activity** (walking, playing, all movements).
- Frequent falls
- **Big calfs**
- Thin bones
- **DEATH**: respiratory failure, pulmonary infections or heart failure.

- **Life expectation** has improved in the last years (assisted mechanical ventilation). Some patients have survived until the VI decade of life.



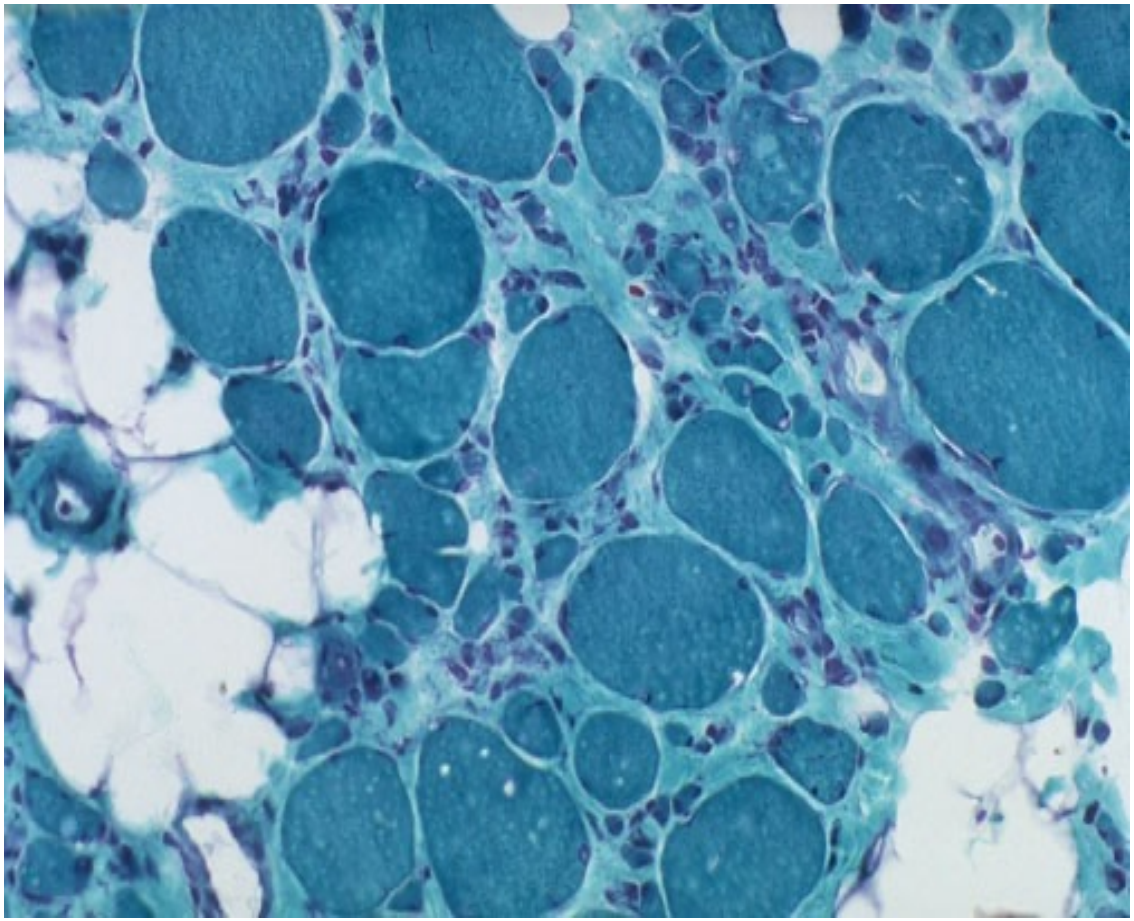


Gower sign



Why are muscles enlarged in DM patients?

Increased fibrous connective tissue revealed by this trichrome stain.
There are larger overly contracted muscle fibers
with scattered small degenerating or regenerating fibers



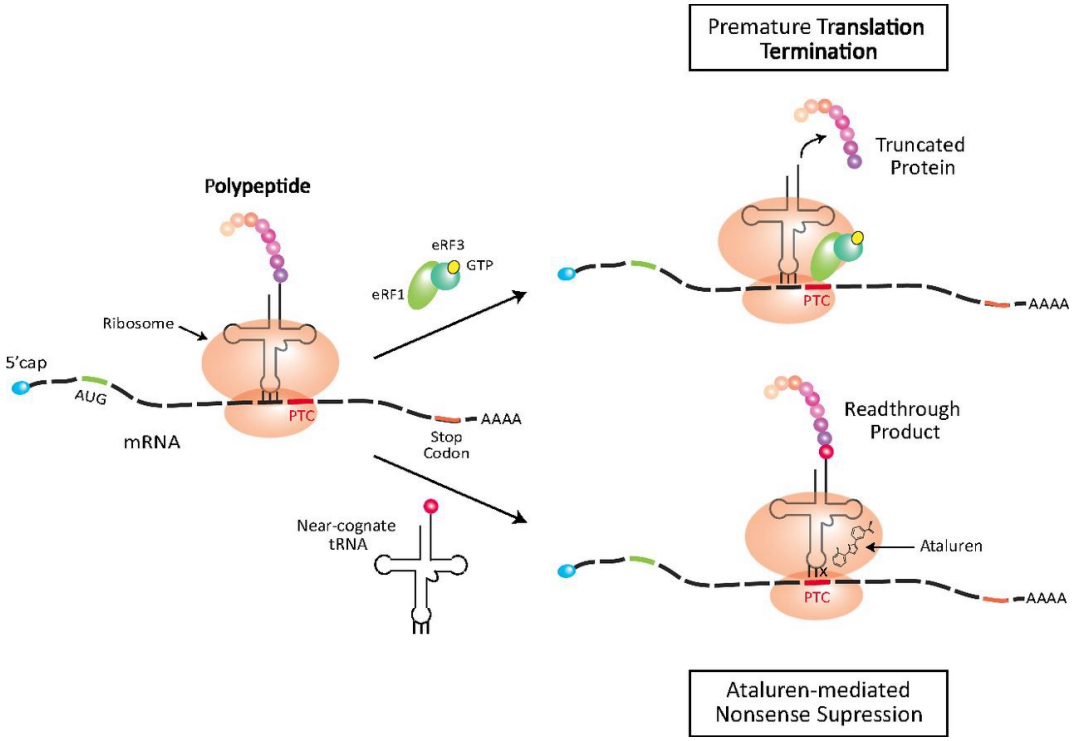
DM muscles
contain abundant
connective and adipose tissue

Therapy

- **Corticosteroids:** prednisone
- **Assistance:** physical, occupational, supportive, swallowing, respiratory, psychological
- **New "intelligent" drugs**:
  - Ataluren
  - Eteplirsen
  - SRP-9001/**ELEVIDYS** (gene therapy) Just approved (June 2023)!! Stay tuned…

New drugs: Ataluren

- **PTC124** (PTC Therapeutics): approved by EMA in 2014 for DMD in patients over 5 years old.
- The effects in DMD are noticeable only <u>when the subject is still able to walk</u>.
- The drug **prevents the arrest of dystrophin synthesis at the level of a premature stop codon and allows the continuation of the reading by promoting the insertion of near cognate tRNAs.**
- Usable only in cases where the mutation is a **premature nonsense mutation (15% of DMD pts).**
- Also used in Cystic Fibrosis



New Drugs: Eteplirsen

- **Exondys 51** (Sarepta Therapeutics: approved in 2016 by the FDA)
- 30 nt **antisense morpholino oligonucleotide** that promotes the cutting and exclusion of mutated exon 51 (premature stop) during the splicing process of the dystrophin pre-mRNA. By binding to the exon, it blocks the binding of a protein that regulates splicing
- In this way (**exon skipping**) the exon is lost but the reading frame is restored.
- Administered by venous infusion
- Indicated for patients with exon 51 mutation, which affects about **13% of patients with DMD**.
- Two other drugs act in a similar way:
  - **Golodirsen**: FDA approved in 2019
  - **Viltolarsen**: FDA approved in 2020

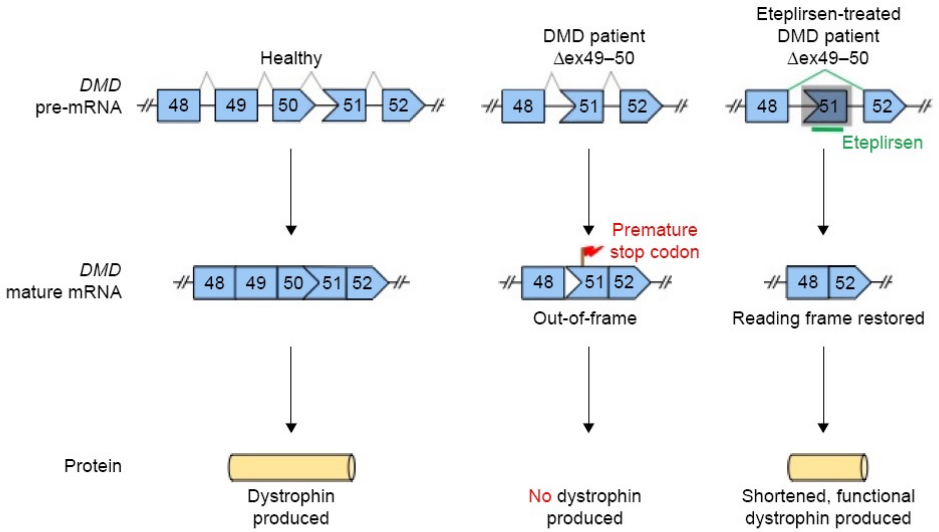


Figure 1 Eteplirsen is an exon-skipping therapeutic.
Notes: Eteplirsen (green bar) specifically recognizes exon 51 of the *DMD* gene. Upon binding, it influences the splicing machinery to skip exon 51 from the mature mRNA transcript. This restores the reading frame of *DMD*, allowing for successful translation of a shortened but functional dystrophin protein. Shown above is a case where eteplirsen is used to treat a DMD patient with a deletion spanning exons 49 and 50. This creates an out-of-frame frameshift that introduces a premature stop codon and results in nonproduction of dystrophin.
Abbreviations: DMD, Duchenne muscular dystrophy; mRNA, messenger RNA.

Severe Combined Immunodoficiency (SCID)

- The most serious human immunodeficiency disorder. It is a group of congenital disorders in which both the humoral and cell-mediated immunity fail to work properly. Children with SCID suffer from recurrent severe infections, retarded growth, and early death

- **Lymphopenia (absolute lymphocyte count is less than 200)**

Types of monogenic SCID

1) X-SCID: Mutation of interleukin receptor γC (*chromosome X*)
(SCID X-linked)

2) ADA-SCID: Linked to *chromosome 2*0 (mutation of ADA);
25% of all cases. **Autosomal recessive**.
1/100.000 newborns

X-linked SCID (bubble disease)

"bubble boy" disease, named after David Vetter, a Texan born in 1971 who lived out his 12 years in a plastic, germ-free bubble.

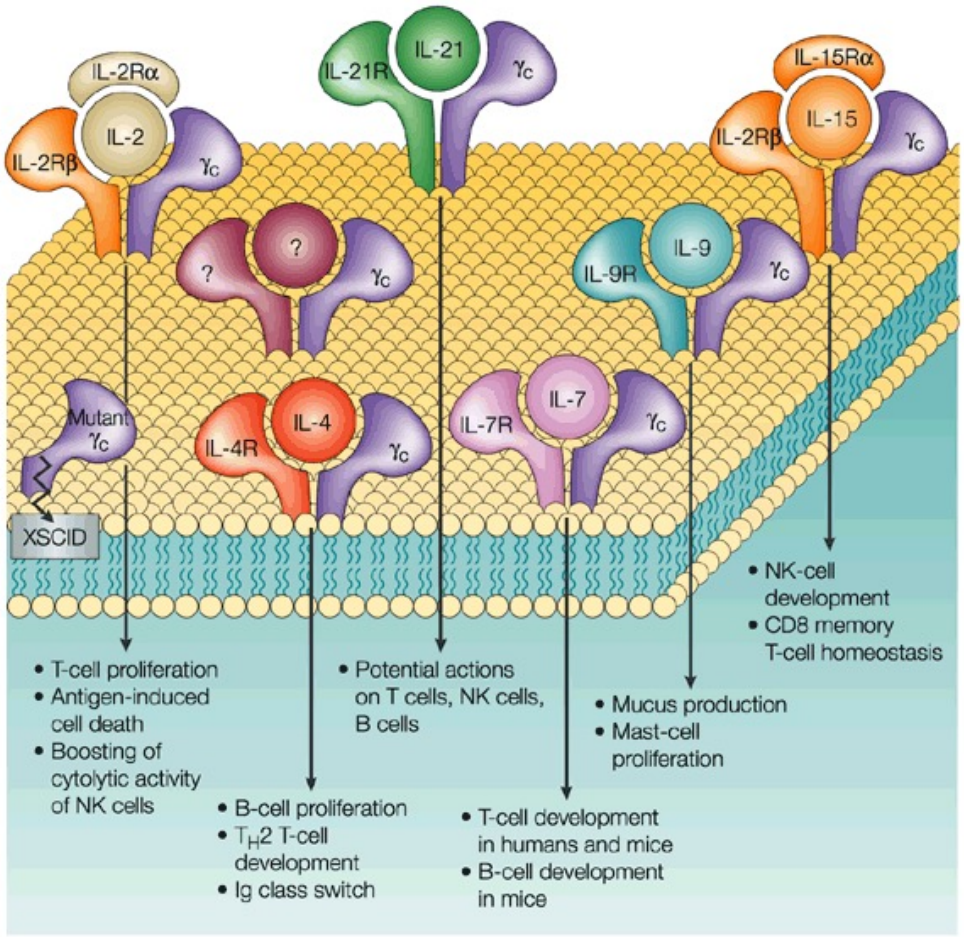
More severe than ADA-SCID,
as X-SCIDs have no B-, T-, NK cells

David received bone marrow
from his sister; **she was EBV positive**
David died in 1983

Photo: Courtesy of Duke Medical Center News Office

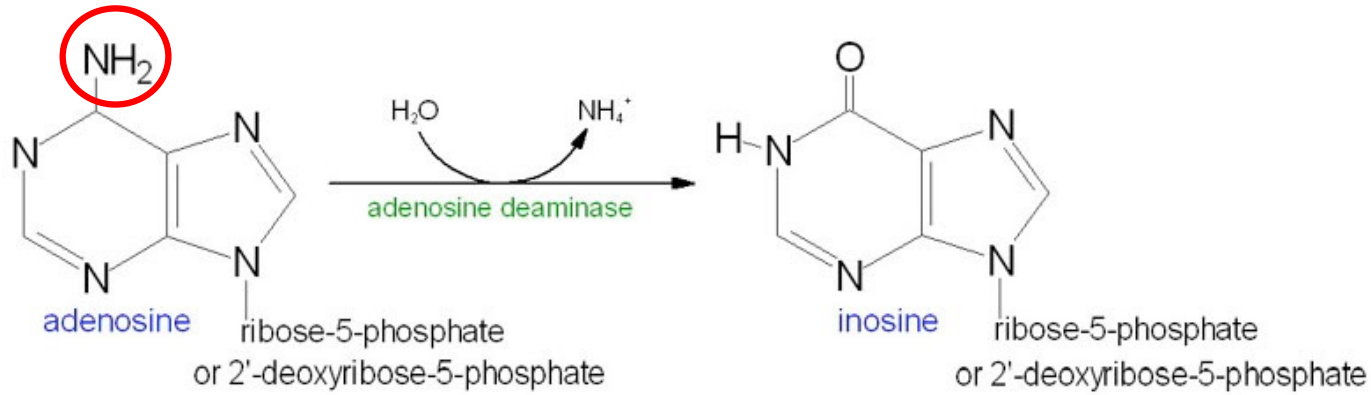
Genetics of X-SCID

- Mutations of ***IL2RG*** **gene encoding the gamma subunit (γ_c)**, common to interleukin receptor 2, 4, 7, 9, 15, 21.
- Cromosome Xq13. 8 exons and 7 introns, the mRNA is 3.6 Kb, the protein 369 aminoacids.
- The activation of these receptors promotes the **proliferation and differentiation of B, T, NK cells, monocytes.**
- **Deletions or point mutations:** γ_c chain unable to interact with the subunits of the other receptors.



ADA SCID

Adenosine deaminase is a glycoprotein
and acts as a hydrolase,
catalyzing the deamination of adenosine into inosine + ammonia.



Nitrogen base,
One of the four
nucleotides
of the nucleic acids

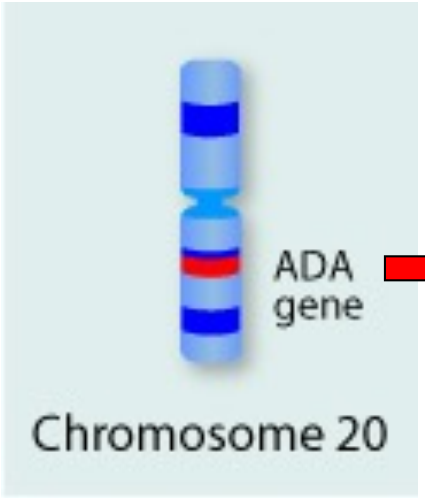
$Adenosine + H_2O = Inosine + NH_4^+$

Adenosine is toxic for B- and T-cells

ADA is essential for the proper growth and function of infection-fighting T and B lymphocytes.

ADA SCID

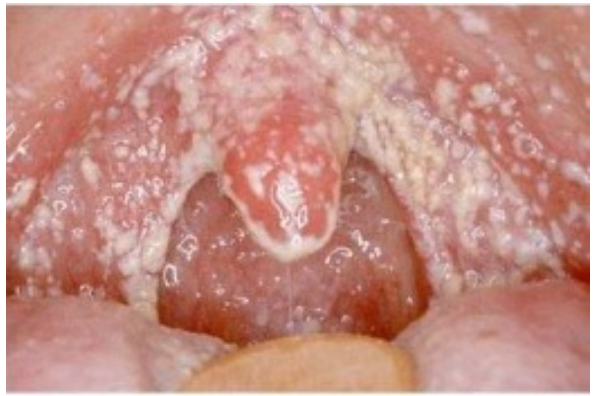
1:200,000-1,000,000 birth
14 newborns / year in Europe
Very severe: death within the first year of life



-Point mutations in the CDS
-Promoter Deletions

Syntoms of SCID

- Precocious recurrent opportunistic infections (viruses, bacteria, fungi, and parasites) within the first three months of life
- Severe infections of upper and lower airways and GI tract
- **X-SCID: moniliasis**, typical fungal infection, skin, mouth, respiratory tract. Difficulties to swallow and lesions of the oral cavity.
- **Death occurs within the first year of life**.



Diagnosis

-Lymphopenia (B, T, NK)

-Lack of antibody response to vaccination

-Low circulating levels of immunoglobulins

-Genetic tests

Cell type	Normal lymphocyte count average (range)	SCID count average (range)
T-cells	3,680 (2,500–5,500)	200 (0-800)
B-cells	730 (300–2,000)	1,300* (44 - >3,000)
NK cells	420 (170–1,100)	<100 (X.SCID)
Total	0–3 months: 5,400 (3,400–7,300)	<2,000

* Non functional

THERAPY

- **Ordinary treatment** develops on two main fronts:
  - **prevention of serious infections** using prophylaxis measures that include the infusion of **immunoglobulins**, antimicrobial prophylaxis (**antibiotics, antifungals and antivirals**). The infusion of immunoglobulins intravenously or subcutaneously every 2-3 weeks represents a fundamental aid against infections. Vaccinations with live attenuated microbes (measles, chicken pox, rotavirus, Bacillus-Calmette Guérin) are absolutely contraindicated while other vaccinations, although not contraindicated, are often not effective
  - **Early and intensive treatment of intercurrent infectious episodes**.
- The **resolutive treatment** of the disease involves **hematopoietic stem cell transplantation from a compatible family member (possible in 20% of cases)** or, more recently, **gene therapy.**