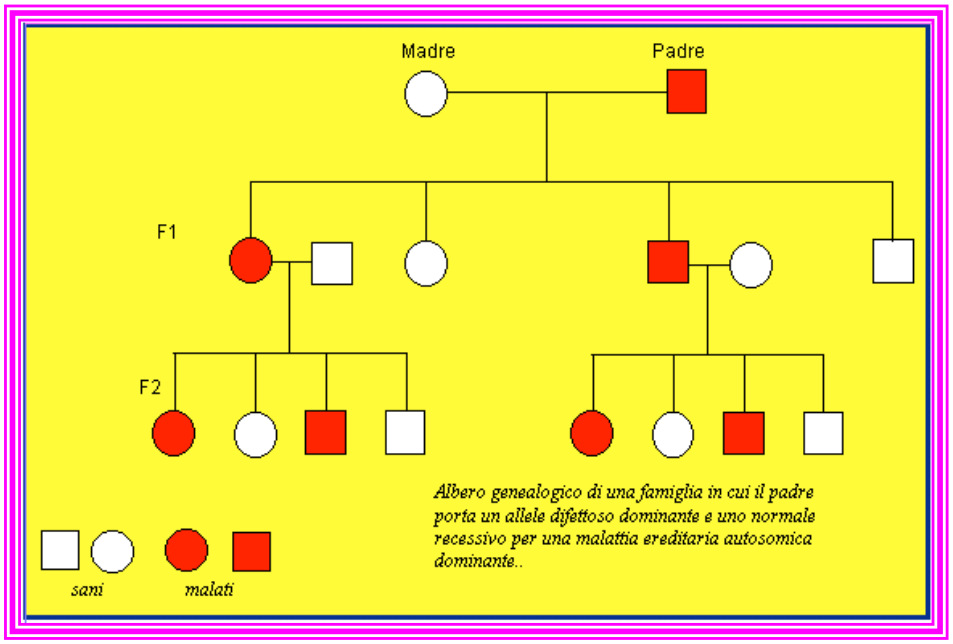
GENETIC PATHOLOGY #1



Genes and Diseases

1) Chromosomal Abnormalities
(Trismomy, monosomy, translocations etc)

2) **Mendelian diseases: monogenic**

3) Multifactorial diseases: genes + environment

Gregor Mendel

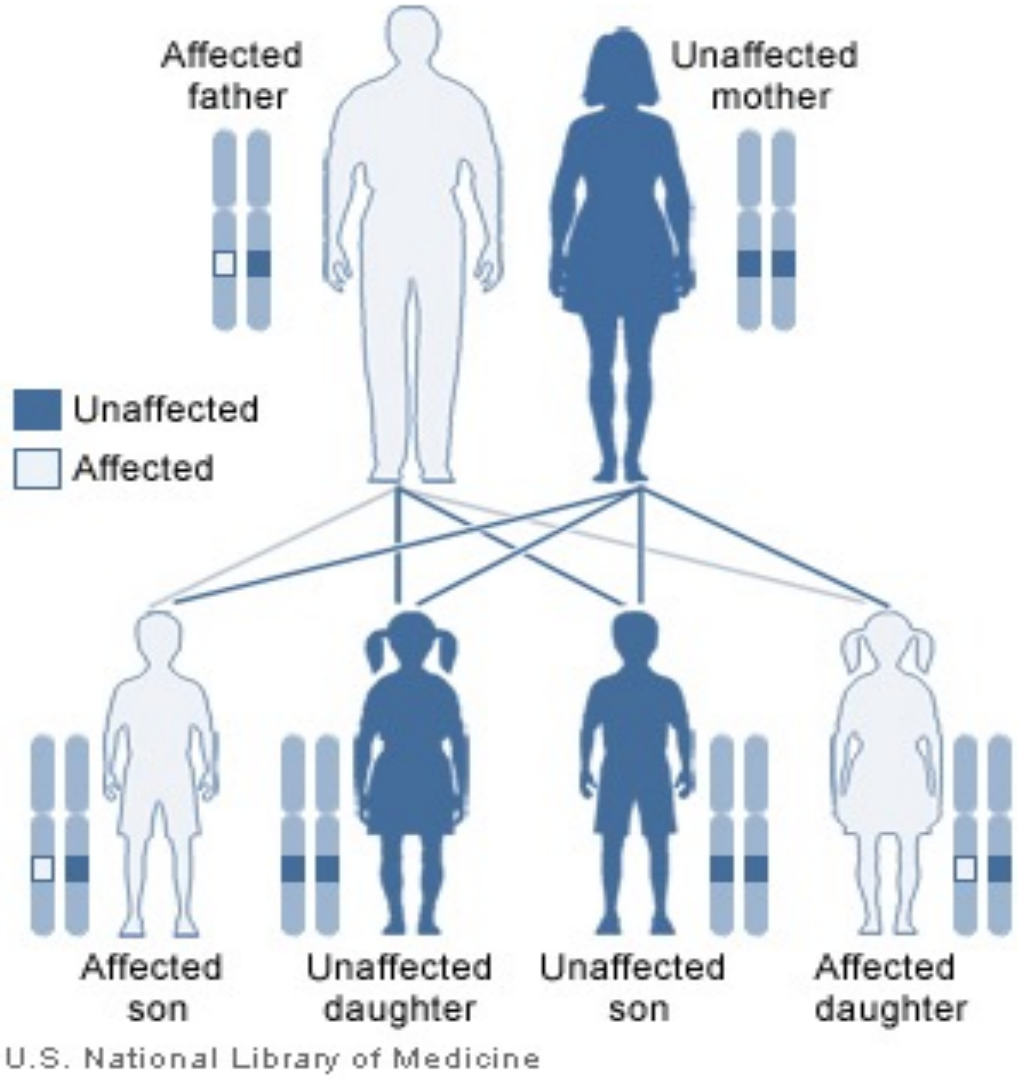
MENDELIAN DISEASES

- **Definition**: Diseases in which the phenotypes are determined by mutations at individual *loci*.

- **Rare**: 1 % of all live born individuals

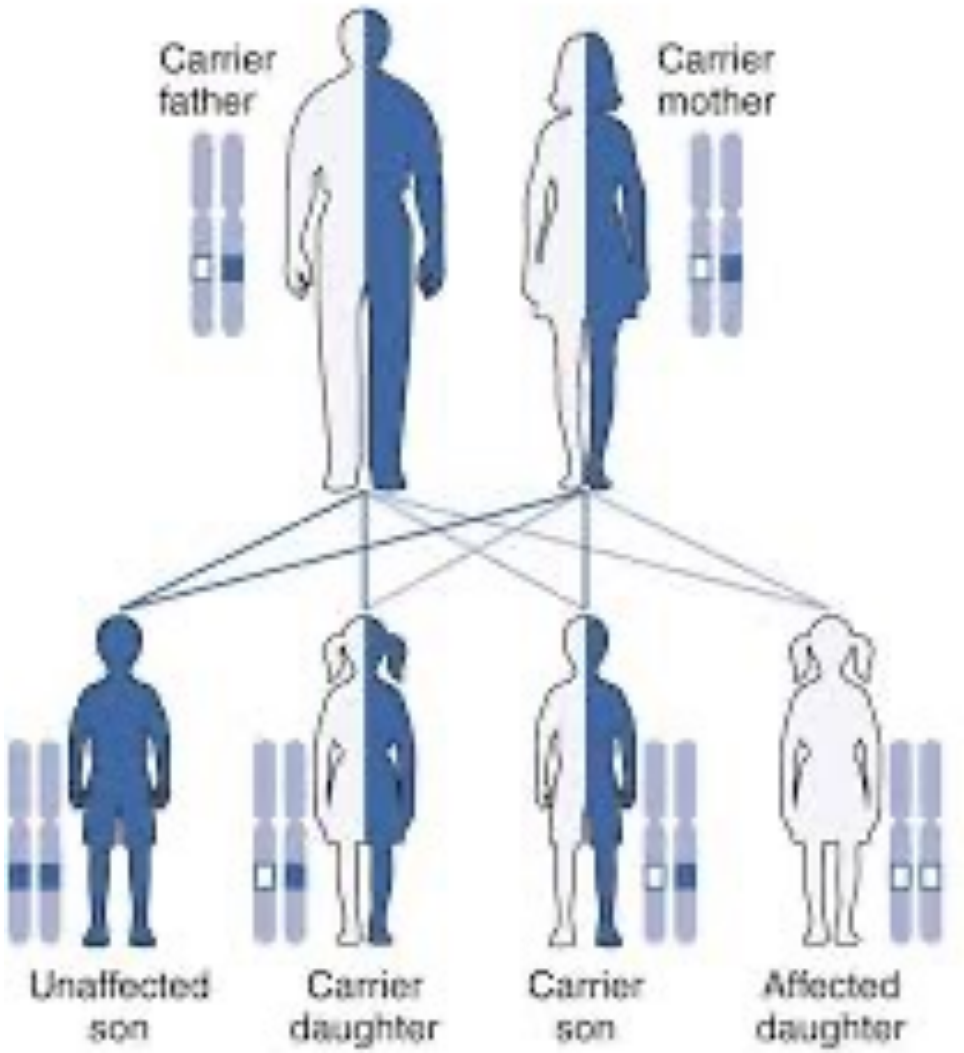
- 4 types of inheritance
- ***Autosomal***
  - *Dominant*
  - *Recessive*
- ***X-linked***
  - *Dominant*
  - *Recessive*

Autosomal Dominant



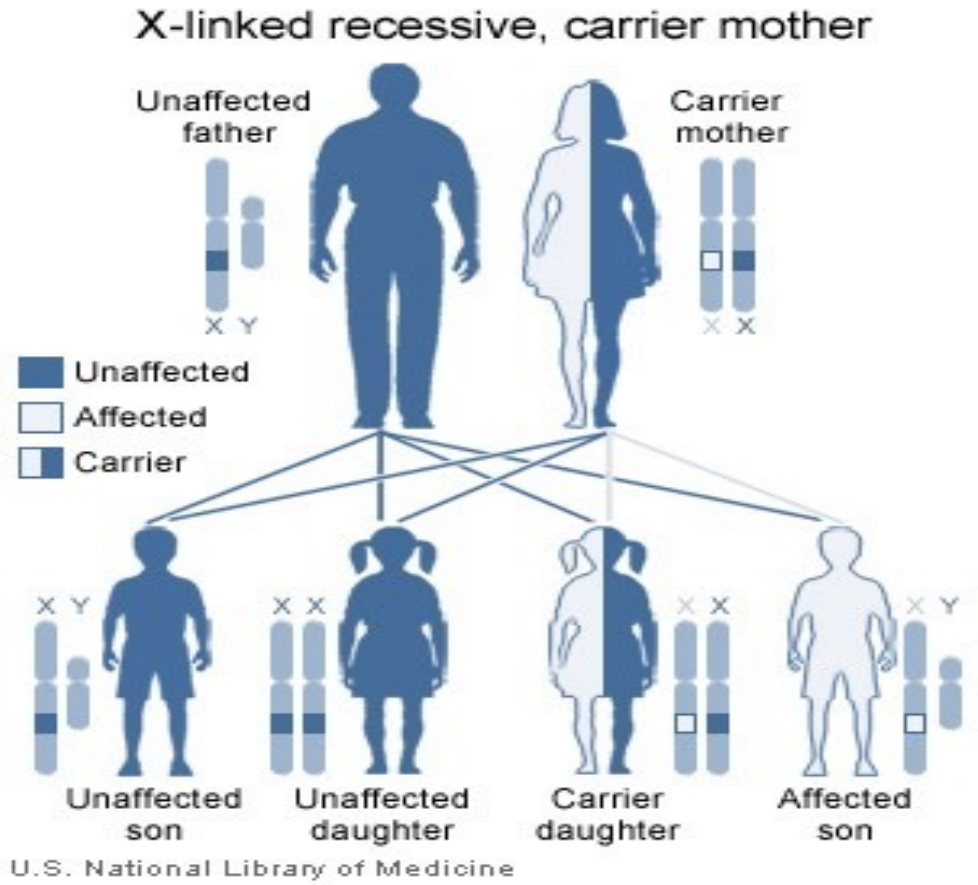
- Offspring has a 50% chance of being affected
- Both sexes equally affected
- Examples:
  - Achondroplasia
  - Marfan syndrome
  - Familial Hypercholesterolemia

Autosomal Recessive



- Probability from carrier parents:
  - 25% (1:4) affected
  - 50% carrier
  - 25% Unaffected
- Both sexes equally affected

X-linked Recessive

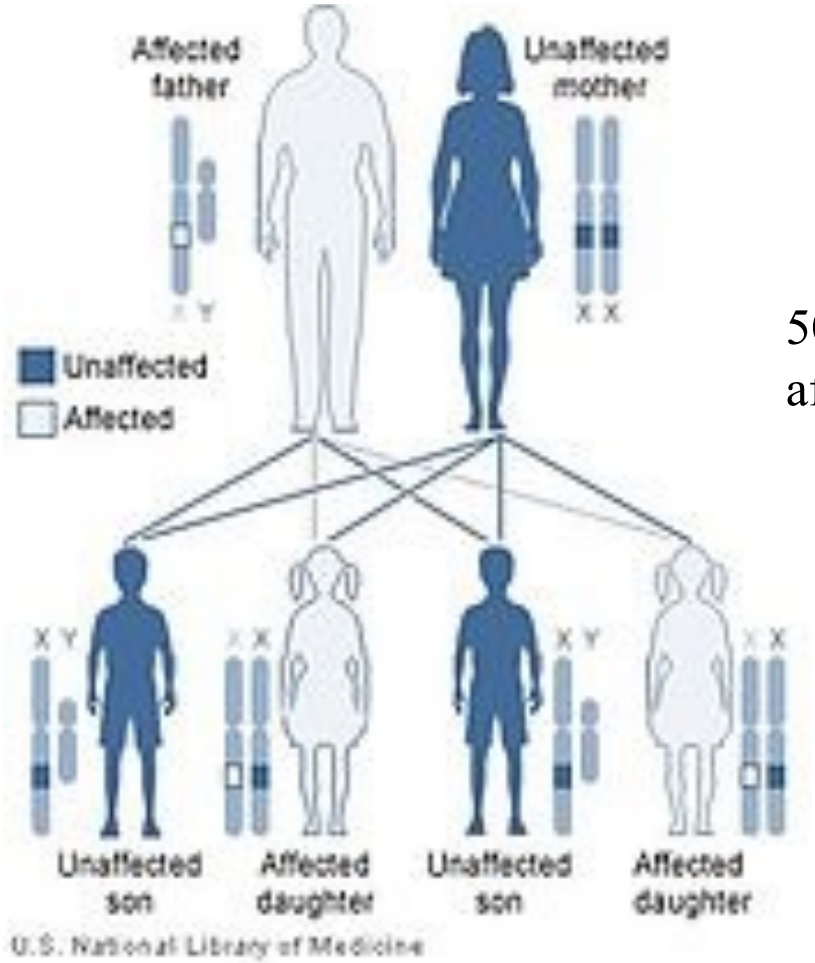


- Transmission of a mutant recessive gene on X chromosome from a carrier mother:
  - 25% of the offspring is affected.
  - Only males (50%) are affected as there will be no normal allele to compensate (**hemizygosity**).
  - **Diagynic** inheritance
  - Females are not affected since they also have the normal allele.

Males are hemizygous for the X chromosome

X-linked dominant

affected father

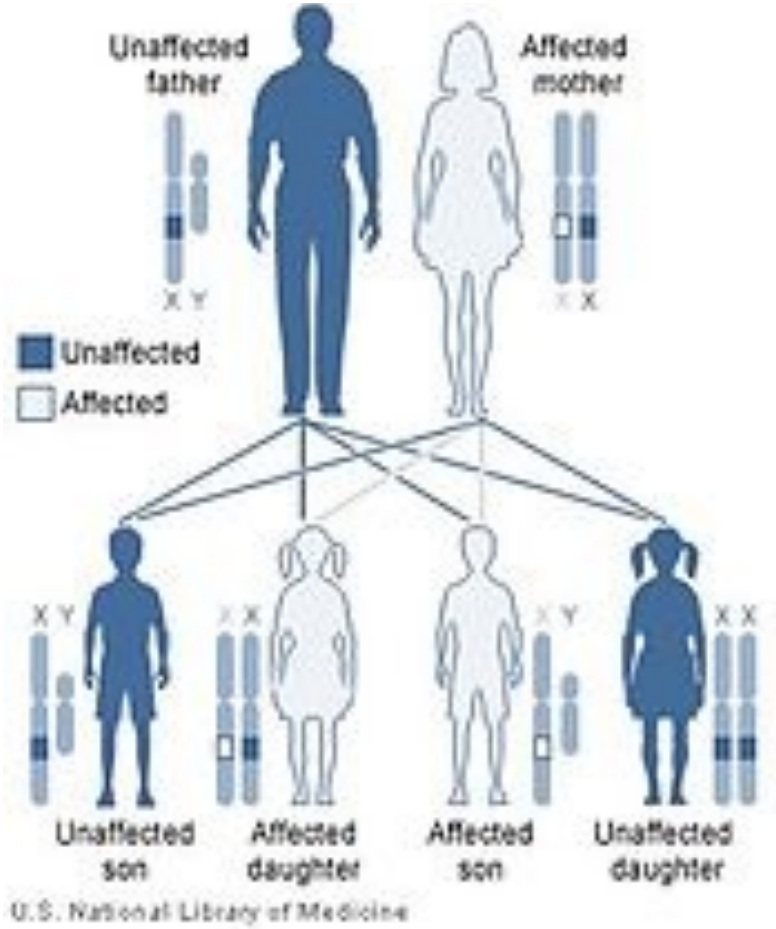


50% probability of affected female offspring

Daughters are affected, sons escape

X-linked dominant

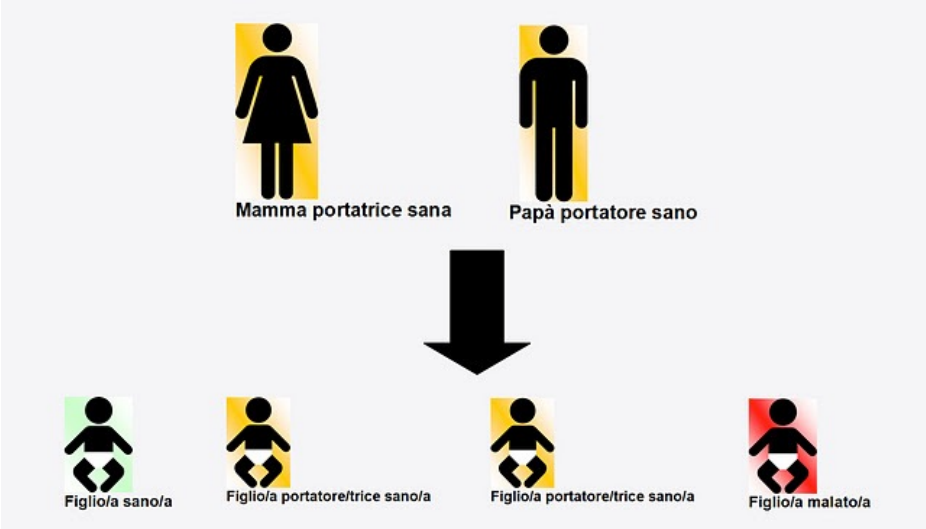
affected mother



50% probability of affected offspring

Daughters and sons are affected

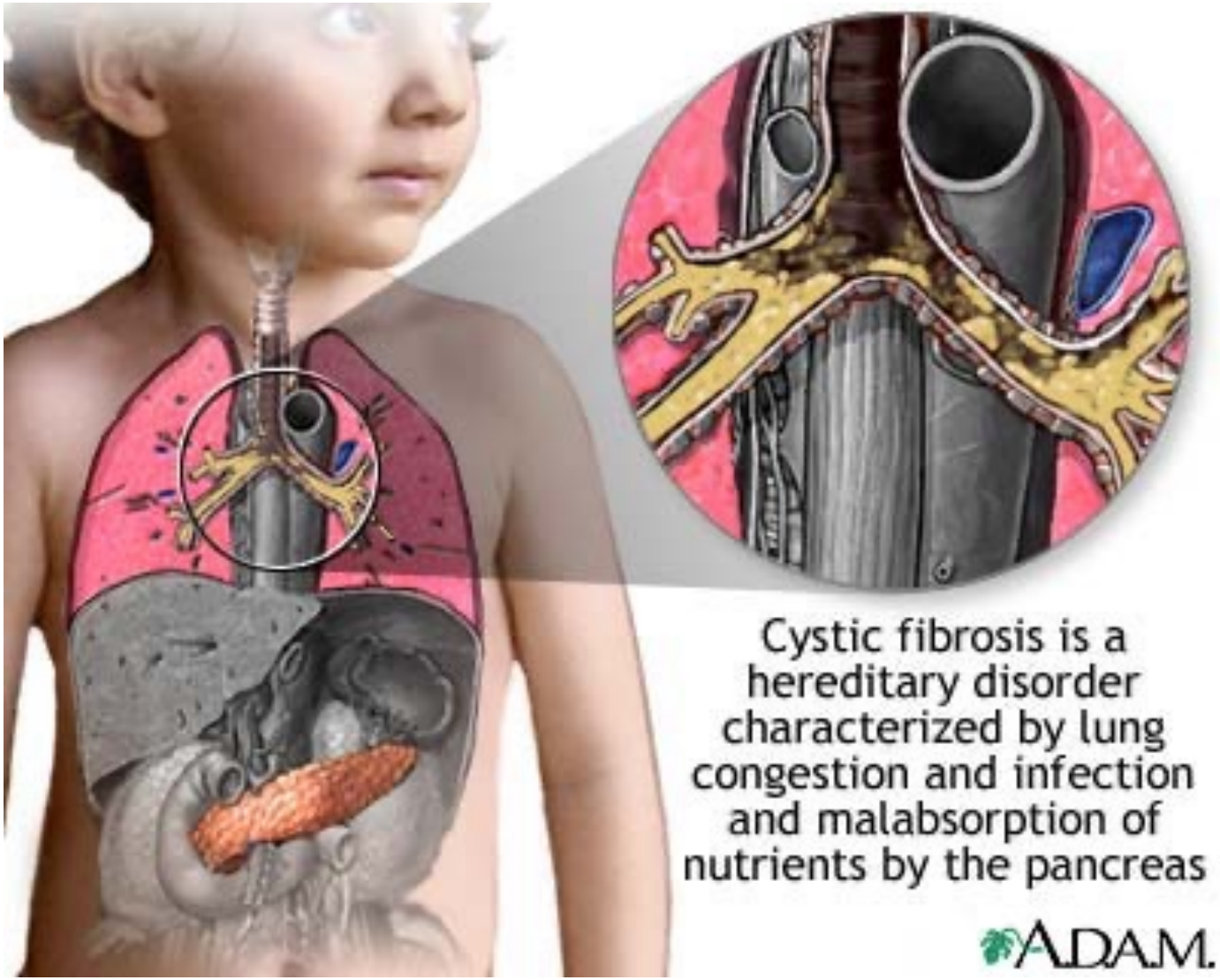
Autosomal Recessive Diseases



Examples:

-Cystic Fibrosis
-Beta thalassemia
-Sickle cell anemia
-Phenylketonuria
-ADA-SCID
-Galactosemia
-Hemochromatosis
-Gaucher Diseasae
-Laron Dwarfism
-LPLD
-Albinism, alkaptonuria
-SMA

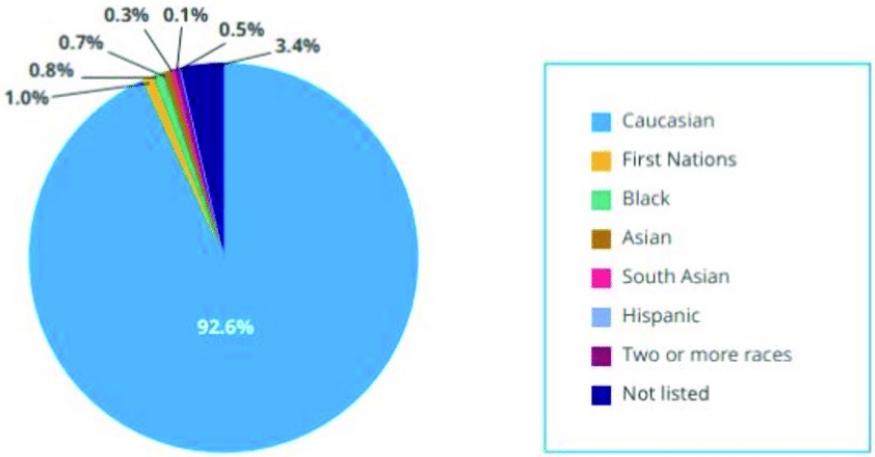
Cystic Fibrosis (mucoviscidosis)



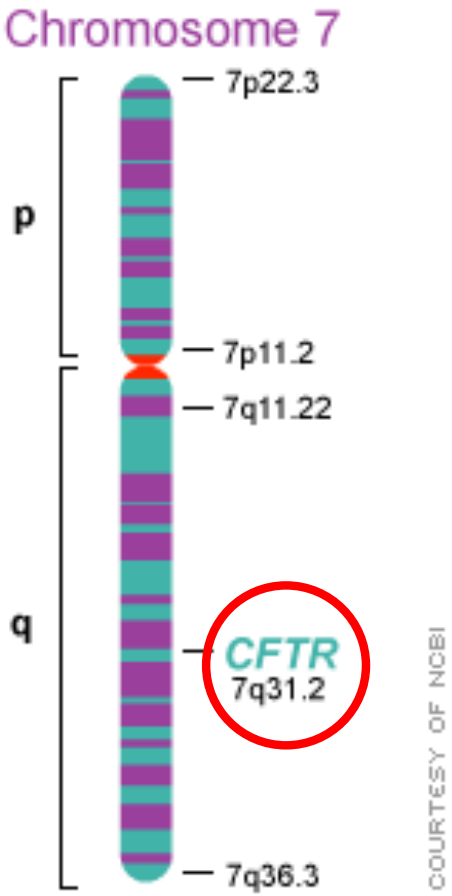
Cystic Fibrosis (CF)

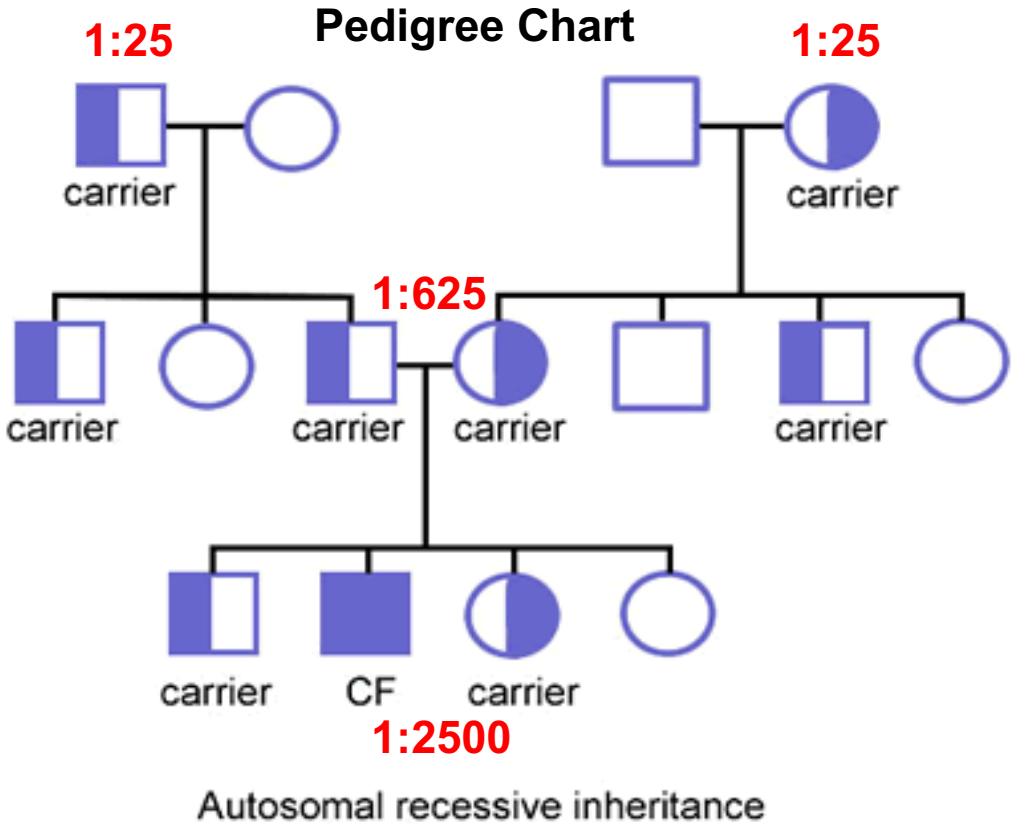
- **The most common life-shortening genetic disorder in the caucasian population (incidence: 1:2500) newborns**
- **Very rare in Africans and Asians**

Ethnic groups	Carrier risk
Ashkenazi Jewish	1 in 24
Non-Hispanic White	1 in 25
Hispanic White	1 in 58
African American	1 in 61
Asian American	1 in 94



Cystic Fibrosis: autosomal recessive inheritance (Chromosome 7)





In Italy: 2 millions of carriers

Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene

250.000 nucleotides, 27 exons, 1480 aminoacids, 168 kDa

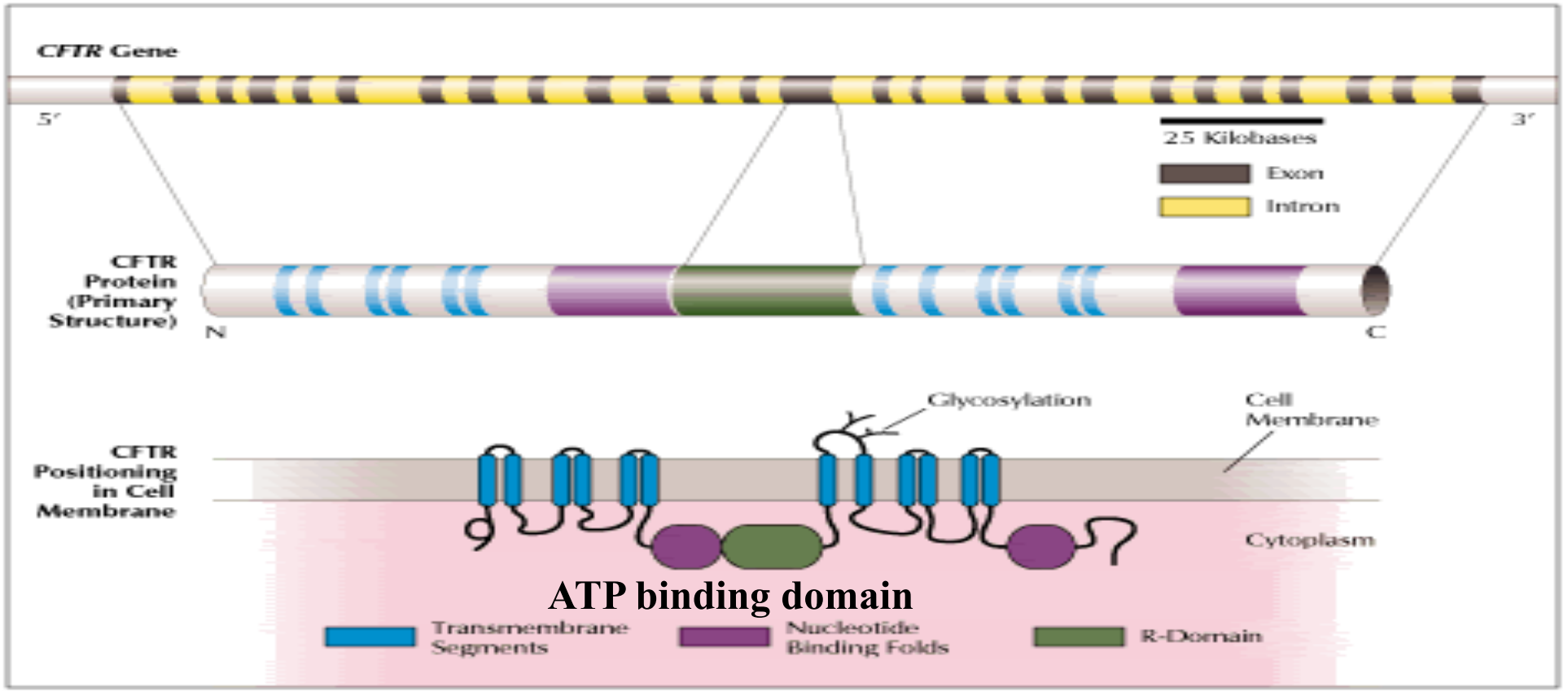
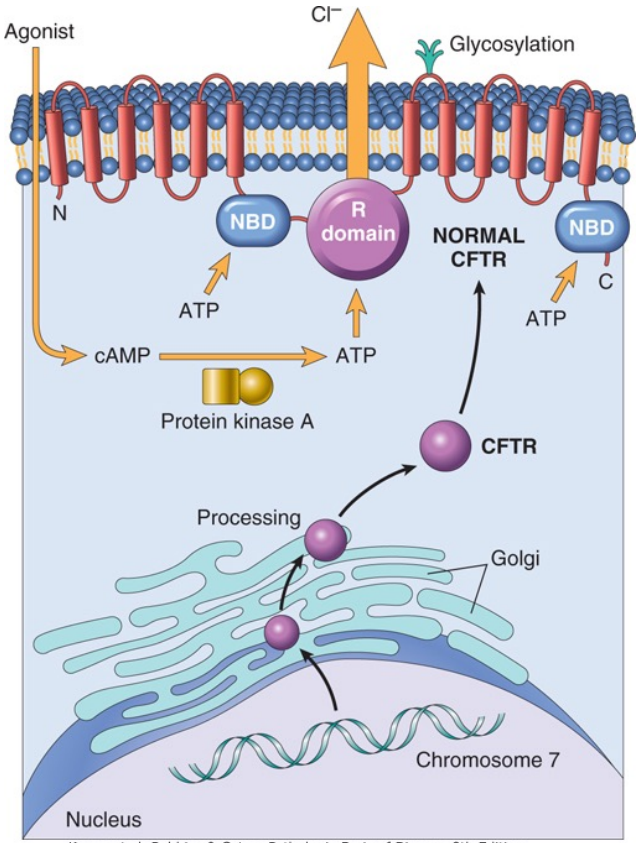
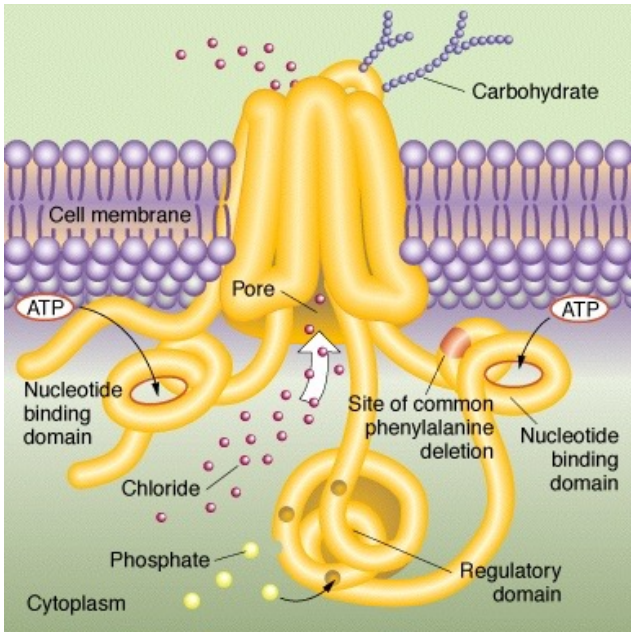


Figure 2. Human *CFTR* gene (top), identified in 1989 on the long arm of chromosome 7, uses 27 exons to specify a polypeptide consisting of 1,480 amino acids (middle). On the basis of its dual versions of a nucleotide-binding fold (NBF), the polypeptide has been classified as an ATP-binding-cassette protein (where "cassette" signifies a functional module). The polypeptide also has dual sets of six membrane-spanning segments. Unique to CFTR is a central region coded by the gene's longest exon. Suspected of having a regulatory function, it is called the R-domain. Analysis of the primary sequence of CFTR suggests that the only part of it protruding from a cell (bottom) is a short loop between transmembrane segments 7 and 8, which has attachment sites for two sidechains.

CFTR

- Cystic Fibrosis is due to an impaired function of the **chloride channel CFTR** (Cystic Fibrosis Transmembrane conductance Regulator)
  - 2 transmembrane domains (**TD**) with 6 α-helices,
  - 2 nucleotide binding domains (**NBD**)
  - 1 regulatory domain (**R**), phosphorylated by PKA and PKC.
- The transmembrane domains form a channel, crossed by the chloride
- Several cAMP inducing signals (e.g. Acetylcholine) increase PKA activity, which phosphorylates the R domain
- Phosphorylation of the R domain causes opening of the channels and passage of Cl^-



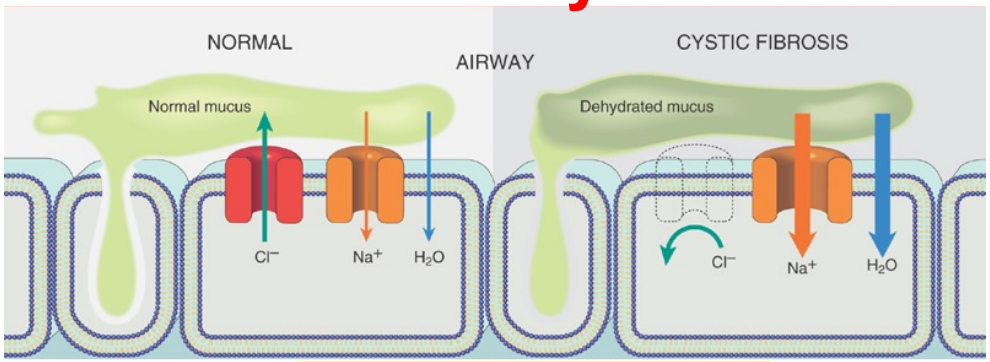


CFTR regulates ENaC

- CFTR regulates the ENaC (**Epithelial sodium Channel)** function by interacting with the NBD
- ENaC is localized on the apical surface of exocrine cells and *internalizes sodium from the lumen*, thus making the secretions hypotonic.
- In the **epithelial airways** and **digestive tract CFTR inhibits** ENaC. Thus, in CF there is an increase of ENaC function and uptake of Na and H_2O inside the cell with consequent *mucus dehydration*.
- Conversely, in the **sweat gland ducts CFTR stimulates** the ENaC.

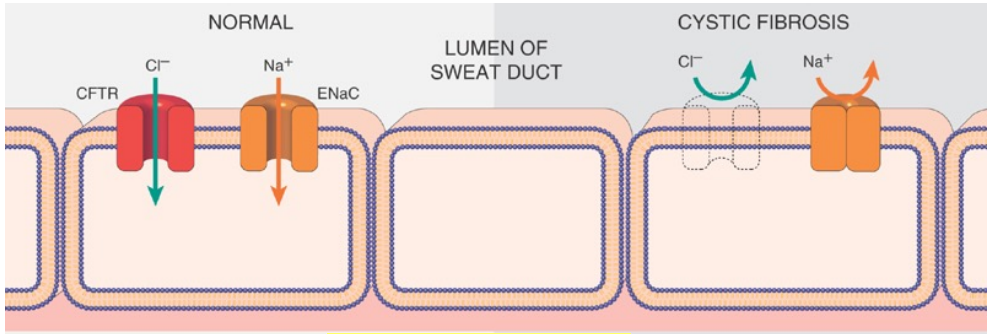
  In the CF there is a decreased ENaC activity and increase of *NaCl content in the sweat. "*Salty sweat disease"

Airways --



Dehydrated Mucus

Sweat ducts ++



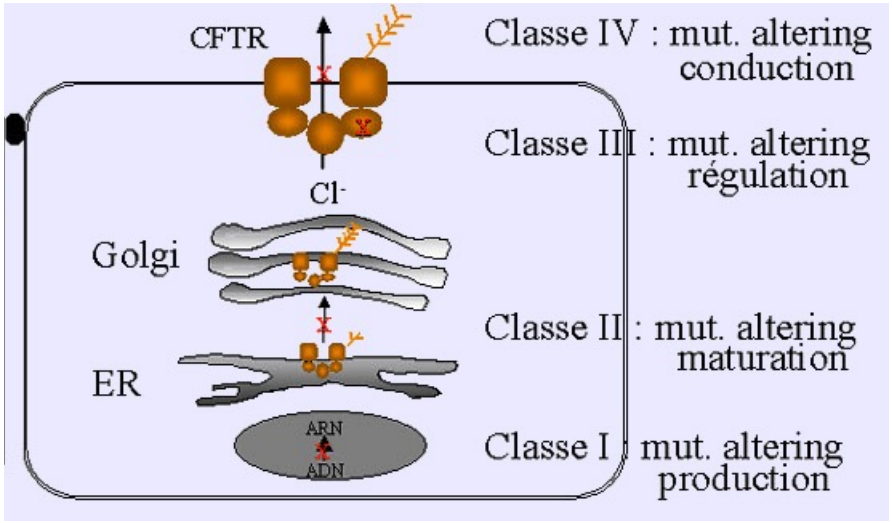
Salty sweat

"Unfortunate is the child who tastes salty when kissed"

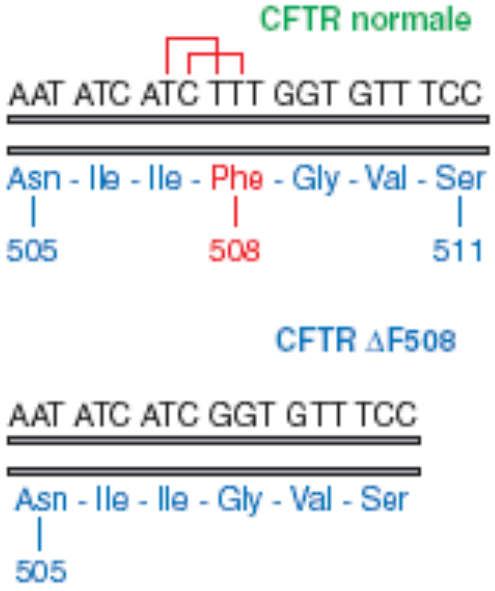
German proverb - XVII century

Molecular variants

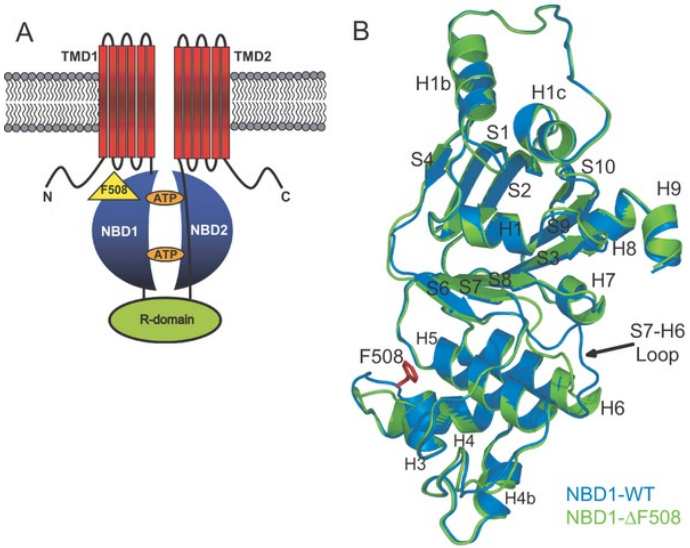
- More than **2200** CF variants associated to different mutations. Can be grouped into 4 classes:
  - **Class I**: altered **production** (*protein synthesis)*. complete CFTR loss (e.g. premature STOP codon, frameshift)
  - **Class II**: altered ***maturation*** (protein folding, processing and transport). The protein is not properly folded and is not glycosylated in the Endoplasmic Reticulum and Golgi. It is degraded before reaching the cell surface. Mutation ΔF508 (70% of CF)
  - **Class III**: altered ***regulation***. No ATP binding and hydrolysis. The protein does not function.
  - **Class IV**: Reduced ***conductance***. Mutations of the transmembrane domain (which forms the Cl^- channel with reduced function).



ΔF508 Mutation



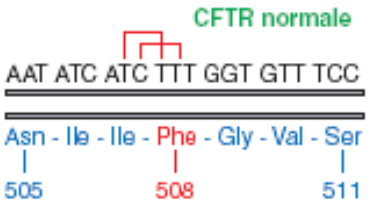
- Deletion of Phe 508
- Loss of three nt: TCT or CTT in the codons 507-508.
- Isoleucine (I) remains unaltered (ATT), while phenylalanine (F) is lost.
- Altered protein folding and consequent degradation.

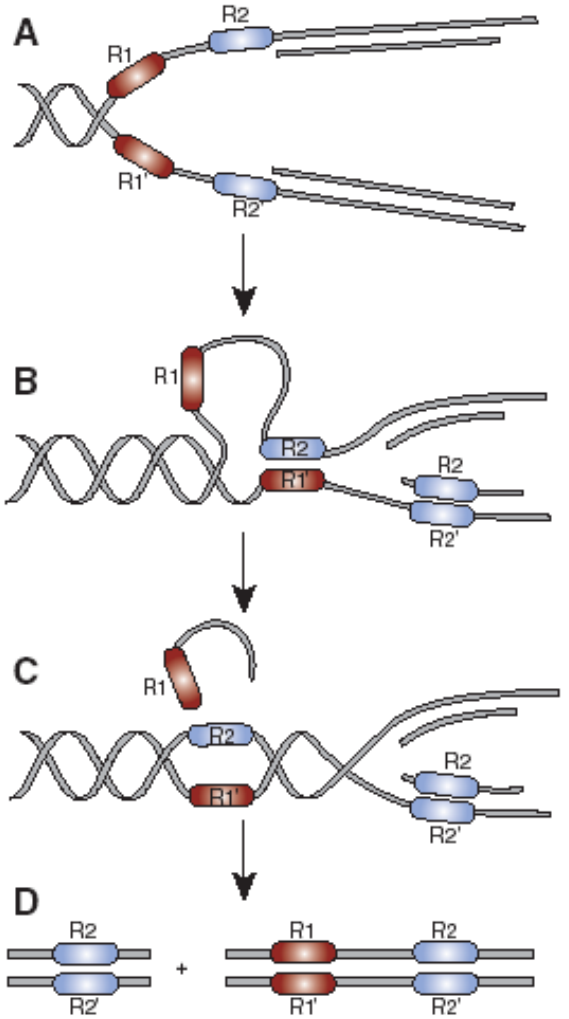


Why is F508 the most common mutation of CF?

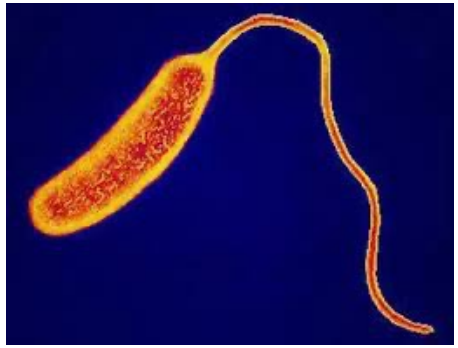
“Hot-spot” sites

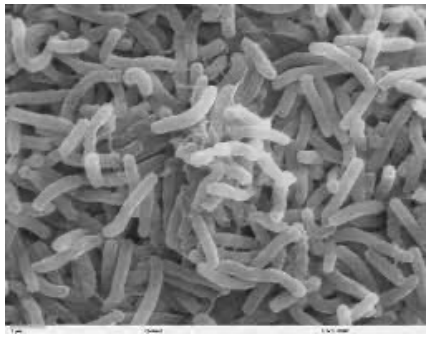
- Sites where mutations occur more frequently
- Direct or palindromic repeated sequences, <u>rich of A and T</u>
- During replication these regions may easily undergo **mismatch and sliding**
- *Stresenger Model*: mismatch originates in the replication fork.

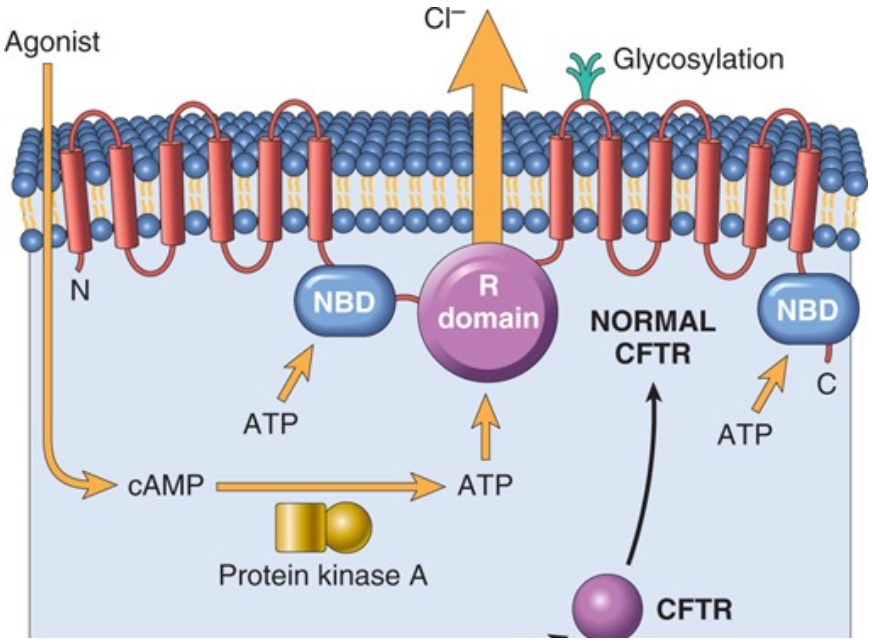


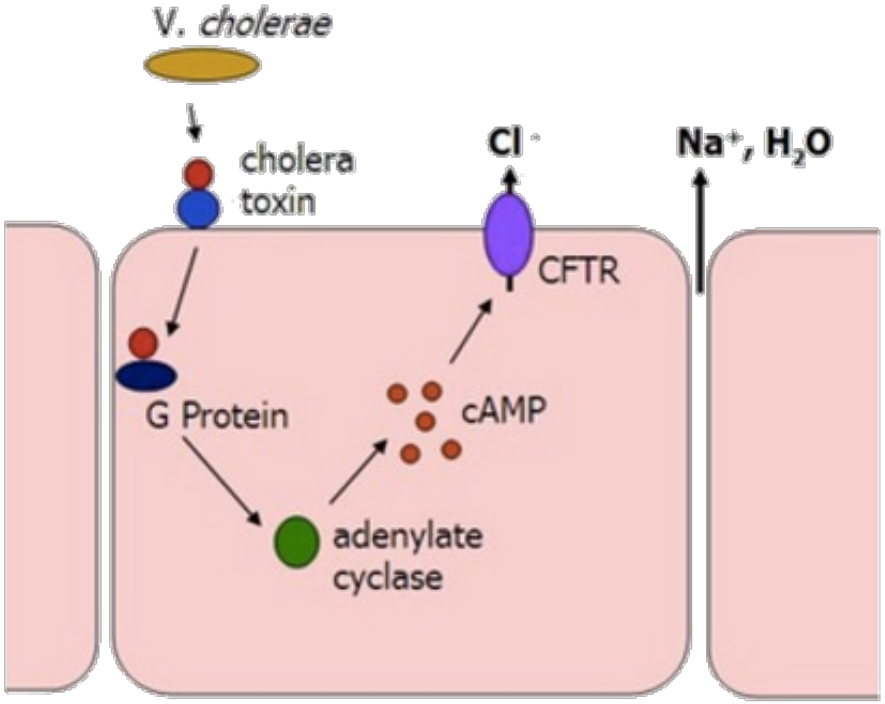


Cholera and CFTR



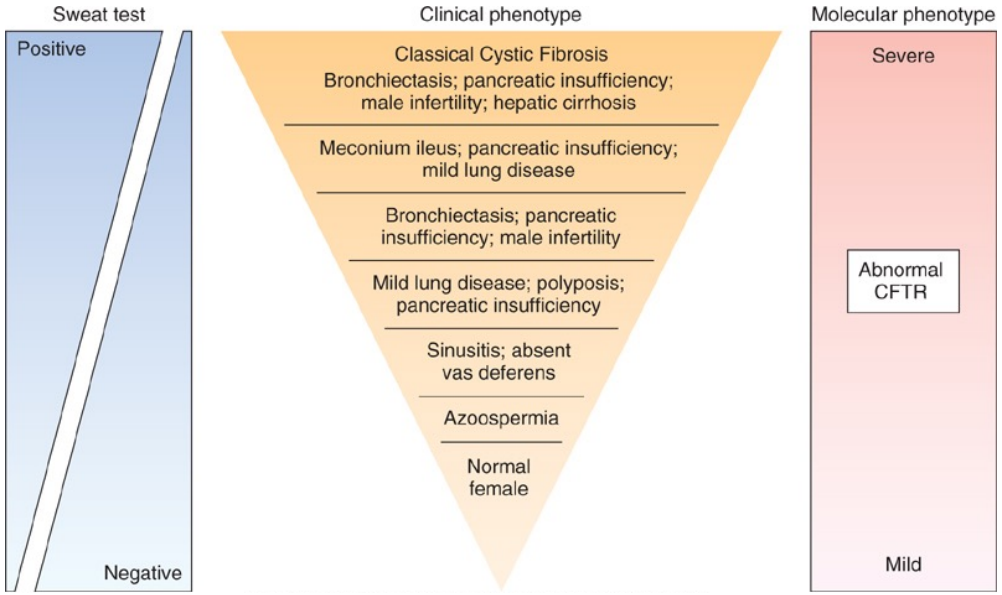






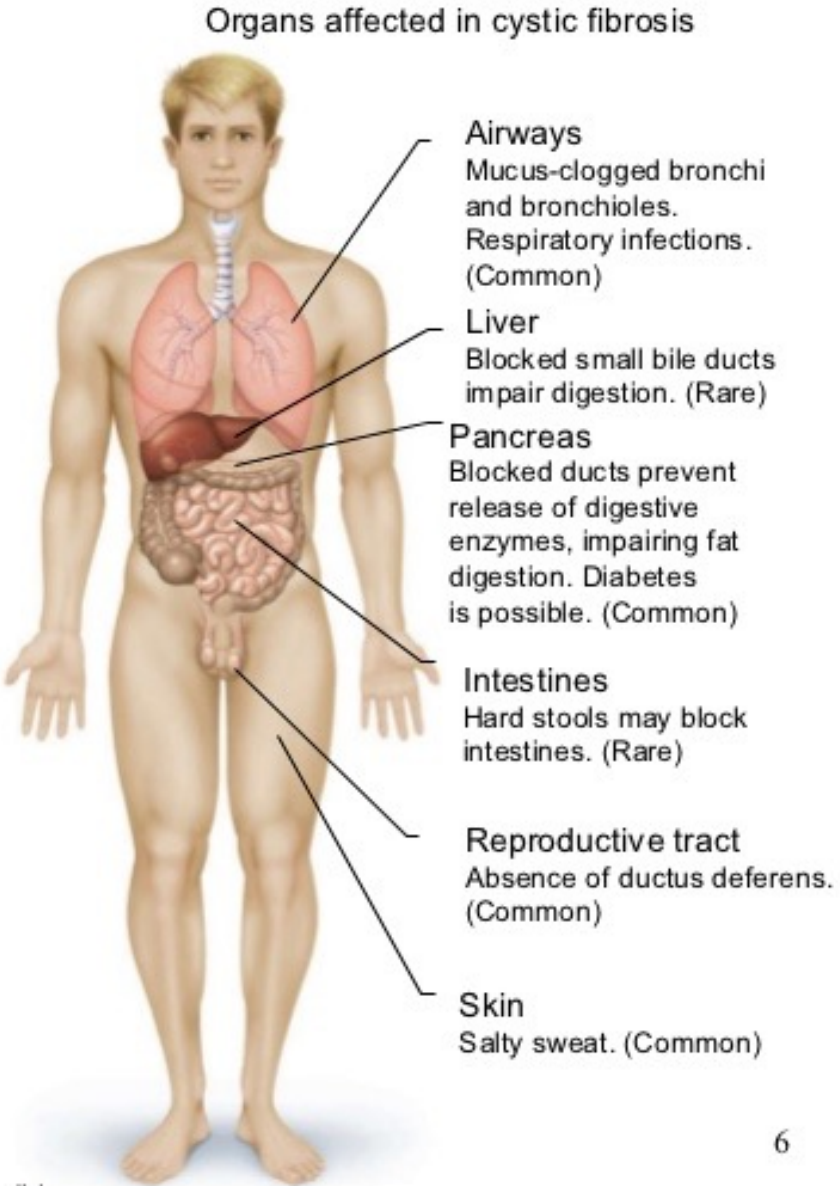
Phenotipic Variants

- CF is autosomal recessive. Both alleles must be mutated. The combination of different mutations of the two alleles originates a **wide spectrum of clinical variants**, affecting all organs or just some.
- Two variants, based on genotype/phenotype correlations:
  - **"Classical"** mutations leading to loss of CFTR (class I, II, III) and causing a severe phenotype. Airways and digestive tracts are affected.
  - **"Atypical"** mutations causing some residual CFTR function and a mild phenotype. *Pancreas is always affected.* The other organs may be affected or not.



Affected organs in the CF

- Airways and lung
- Pancreas
- Digestive tract
- Liver
- Gonads
- Salivary and sweat glands

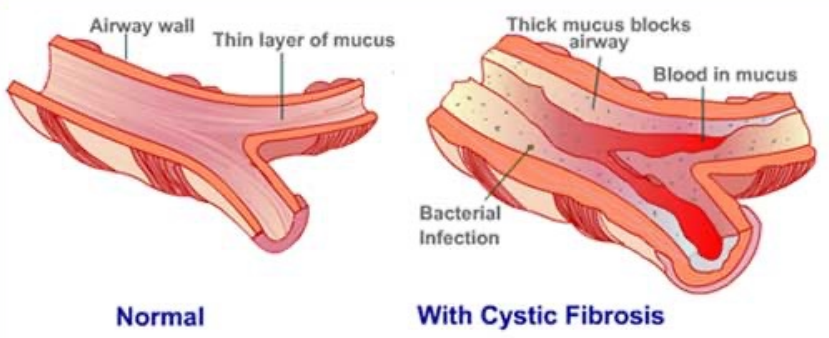


Pathogenesis of pulmonary alterations

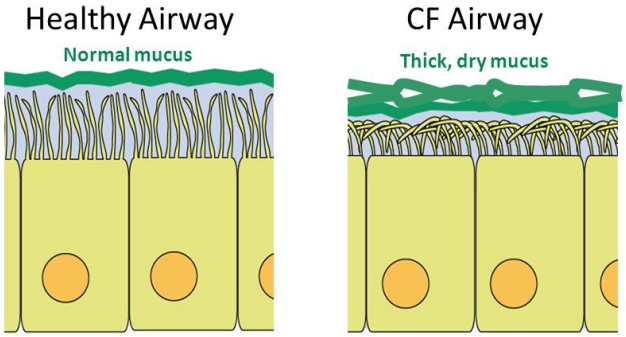
- The dehydrated mucus in the airways causes two consequences:

1. **Altered mucociliary movements (escalator) in the airways** and impaired clearance of microorganisms. Cilia in the apical cell surface can't move properly. This favors colonization of bacteria, in particular *P. aeruginosa*

2. **Formation of hypoxic environment** on the surface of the airways. This causes the production of **alginate** by *P. aeruginosa*, a mucopolysaccharide layer which forms a **biofilm** protecting bacteria from antibodies and antibiotics



Cystic Fibrosis patients' airways are dehydrated and cannot clear mucus.

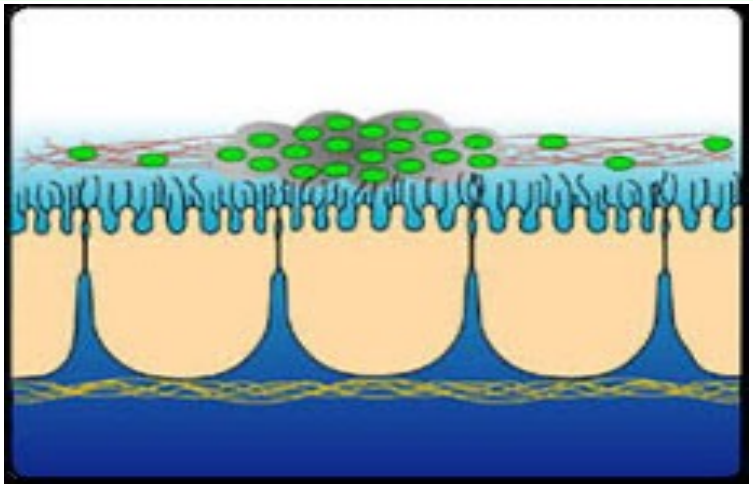


In the CF lungs are are more prone to infections

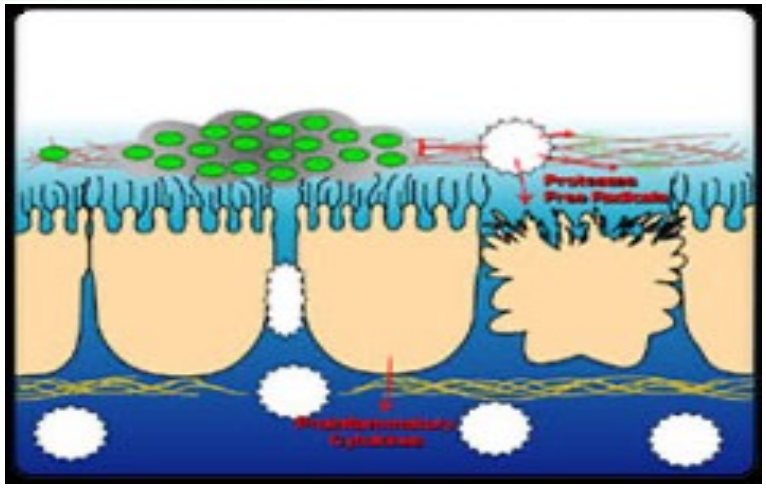
Pseudomonas aeruginosa
easily colonizes the mucus
in the airways and produces aliginate

Neutrophils are activated, then superactivated

The fight between neutrophils and bacteria always leads to **lung fibrosis and damage**



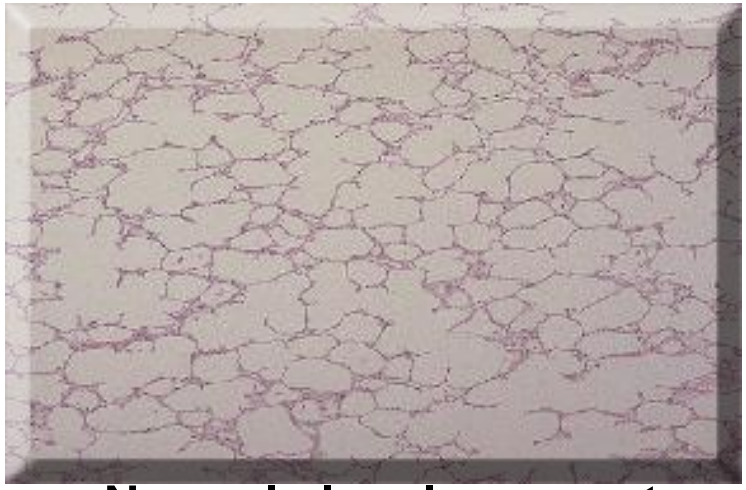
Mucus protects the bacteria and favors hypermutations



”Hyperinflammation" caused by neutrophils Unable to eliminate bacteria, but causing damage to the lung tissue

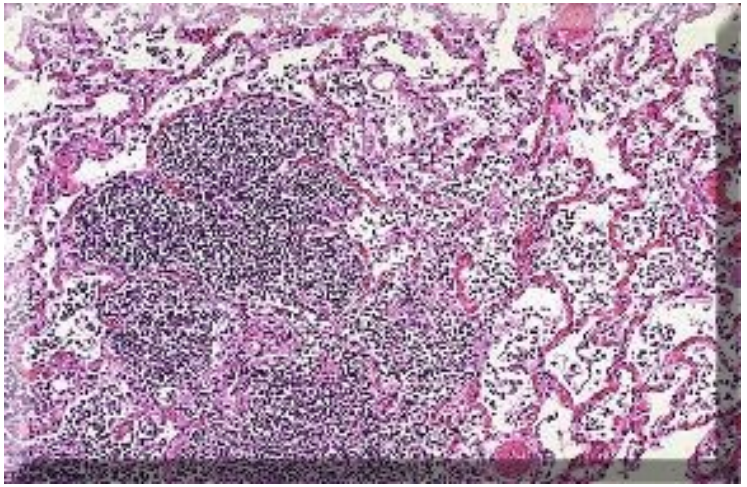
Lungs in the CF

Normal

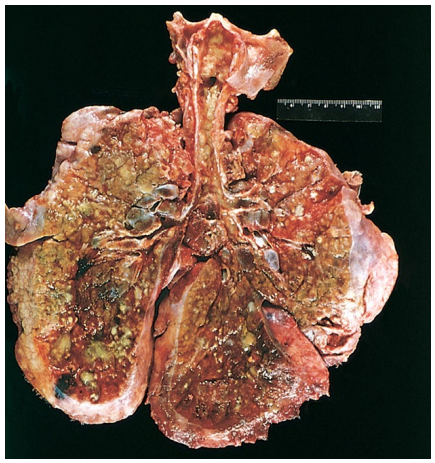


Normal alveolar aspect

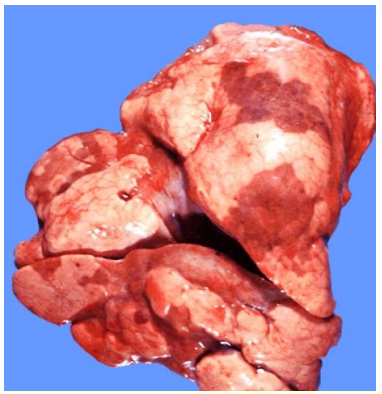
CF



Dilated Criptae with mucus and bacteria.

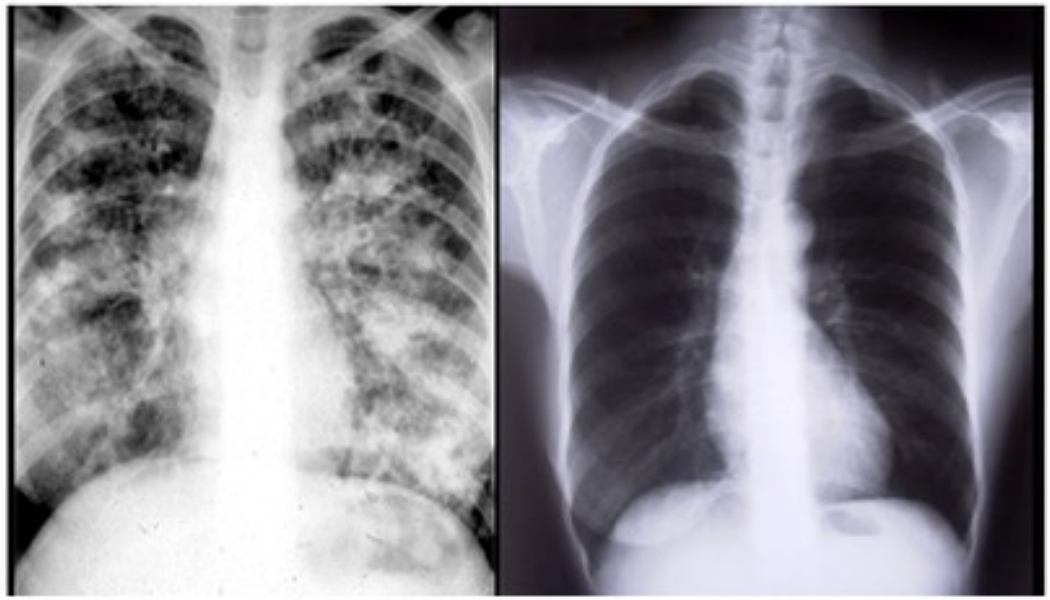


Lungs with mucus and pus

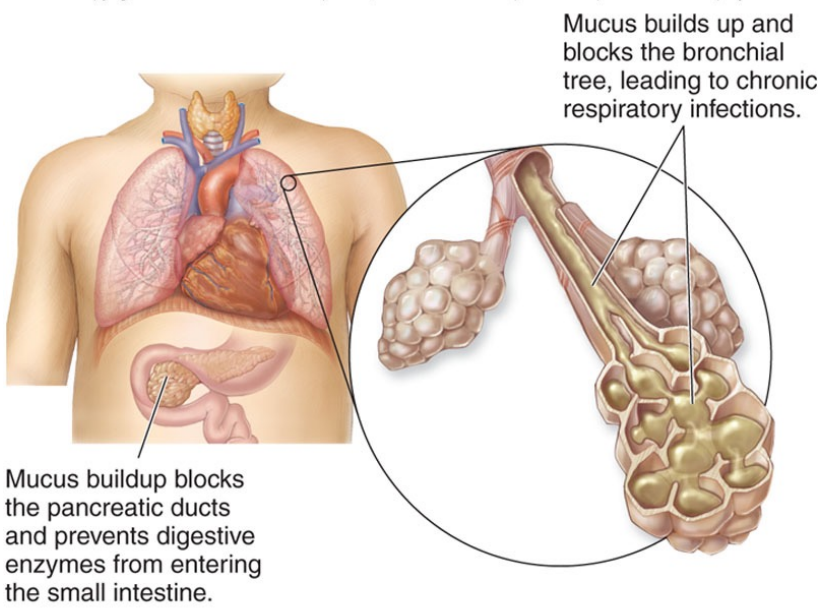


Absence of post-mortem lung collapse

Radiography

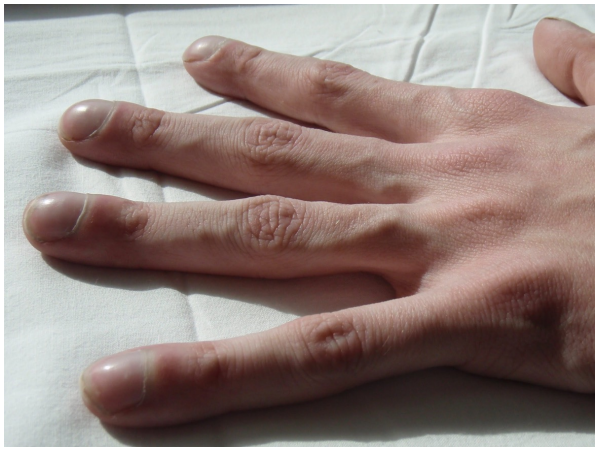


Cystic Fibrosis Lung Healthy Lung



Cystic fibrosis results in thickened mucus that obstructs both the respiratory passageways and ducts of glands such as the pancreatic ducts.

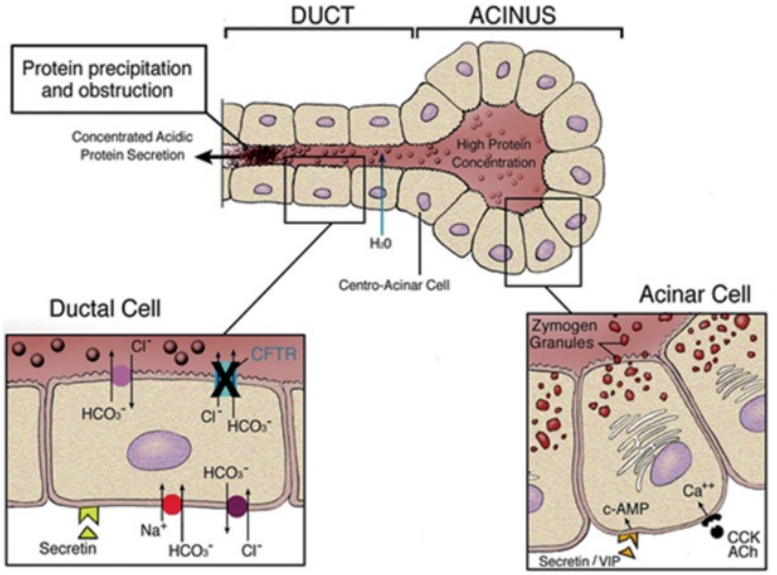
drumstick

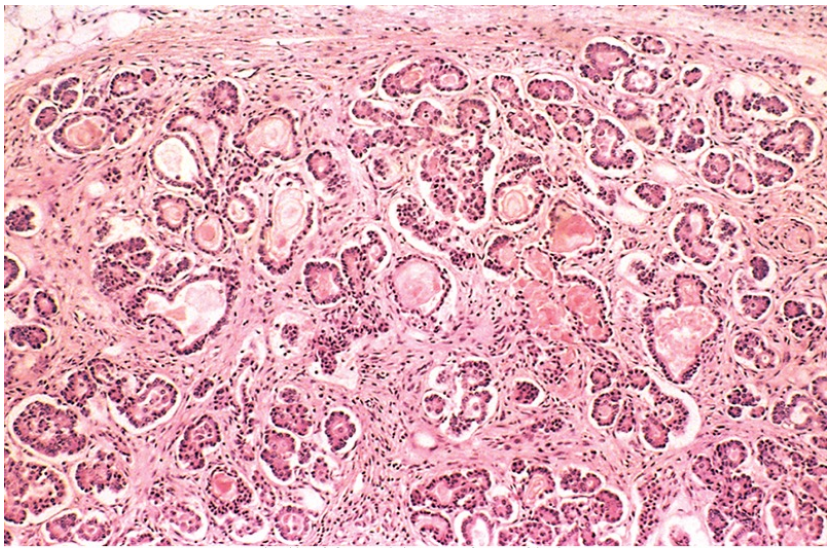


Nail clubbing
(chronic hypoxia, malabsorption)

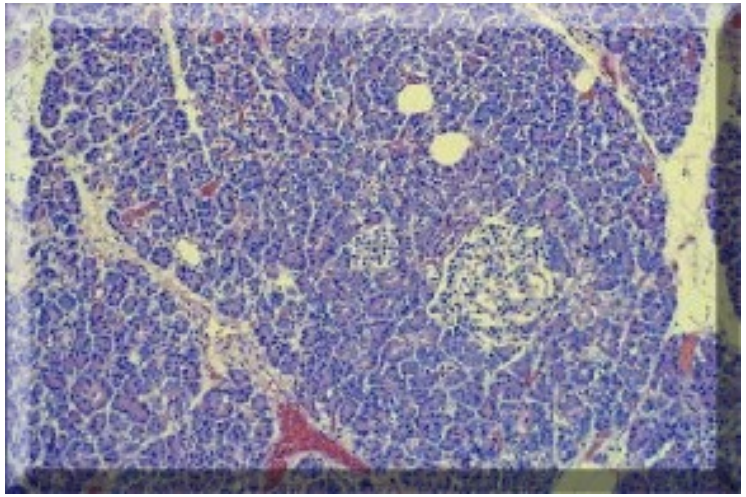
Pancreatic alterations

- 90% of patients have exocrine pancreatic insufficiency.
- Mucus buildup, protein precipitation blocks the excretory ducts with dilation (**cysts**) and then atrophy of the exocrine gland and progressive **fibrosis**.
- Inadequate amounts of digestive enzymes are secreted (lipases, amylases, proteases) with consequent **malabsorption, avitaminosis** and **malnutrition**
- **Growth retardation, diarrhea (Steatorrhea)**

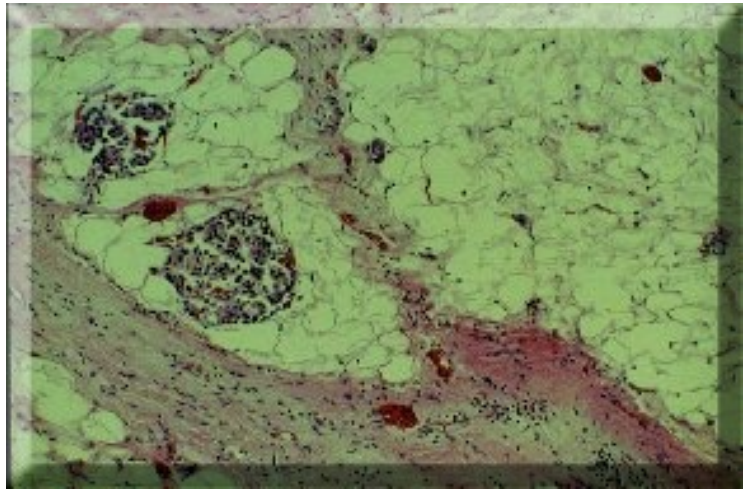




Diabetes in the CF



Normal Pancreas



Dilated criptae, full of mucus

Pancreatic enzymes cannot exit from the gland, causing damages

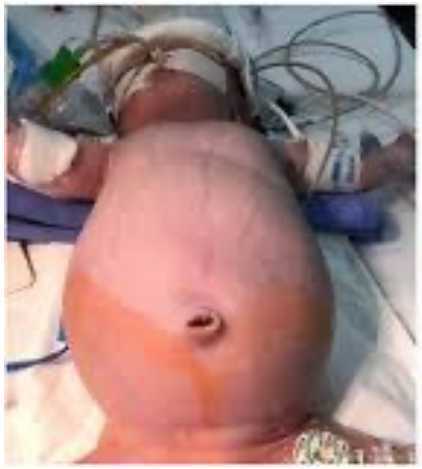
Reduction of insulin production

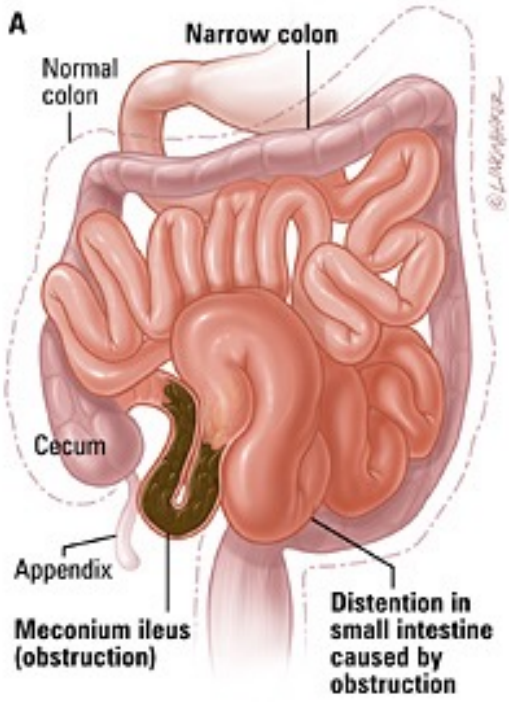
Glucose intolerance and diabetes

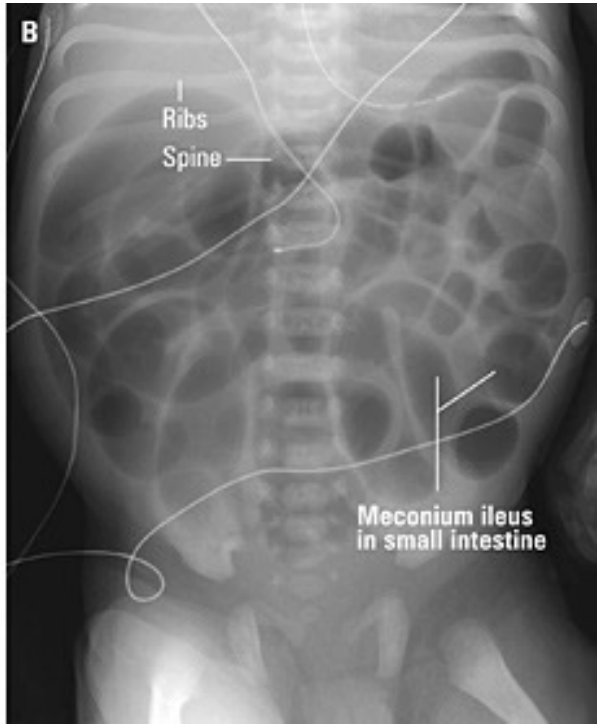
Survivors over 25 years of age:
1/3 with glucose intolerance and 1/3 with diabetes

Intestinal alterations

- ***Meconium ileus***: The content of the baby's bowel (meconium, composed of the material ingested/swallowed by the fetus from the amniotic fluid) is extremely sticky and causes intestinal obstruction at birth.



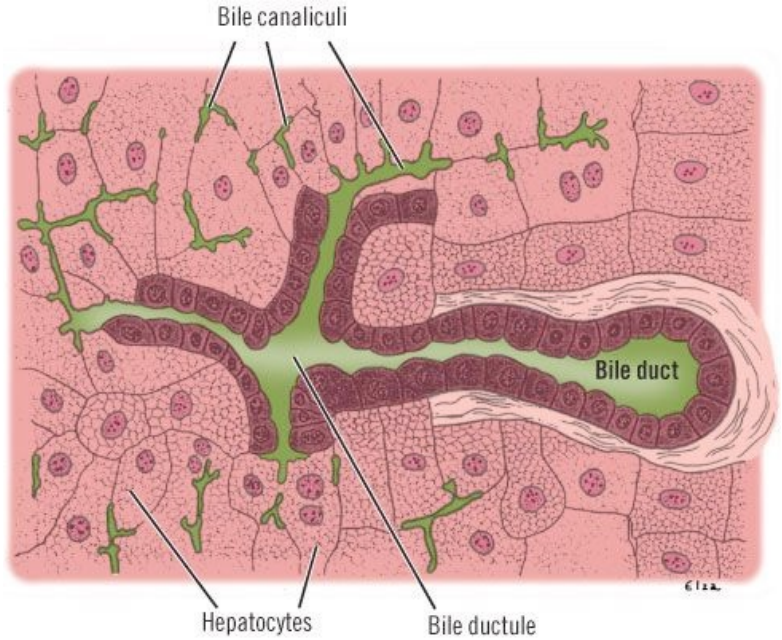


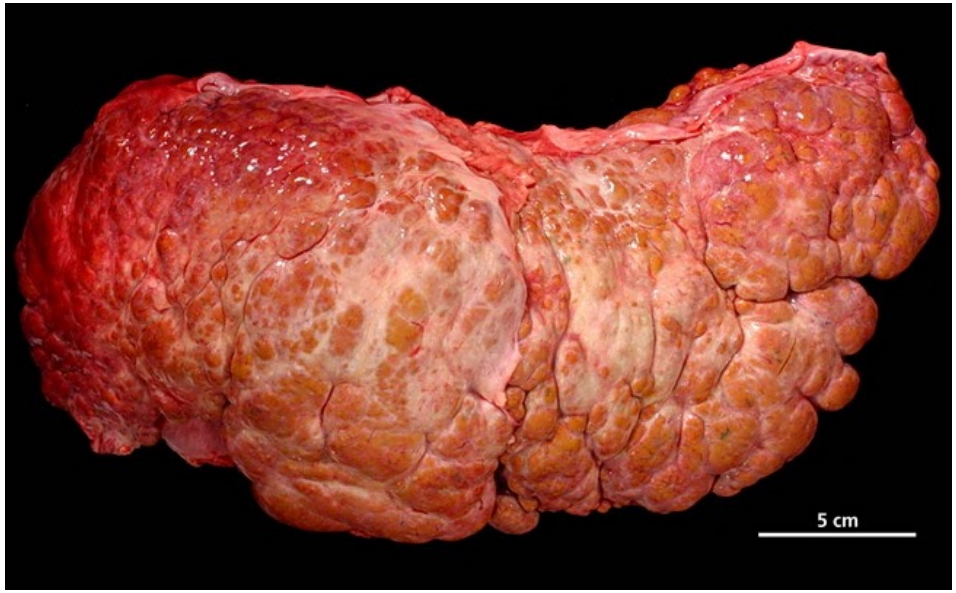


Intestinal loops filled with air

Liver

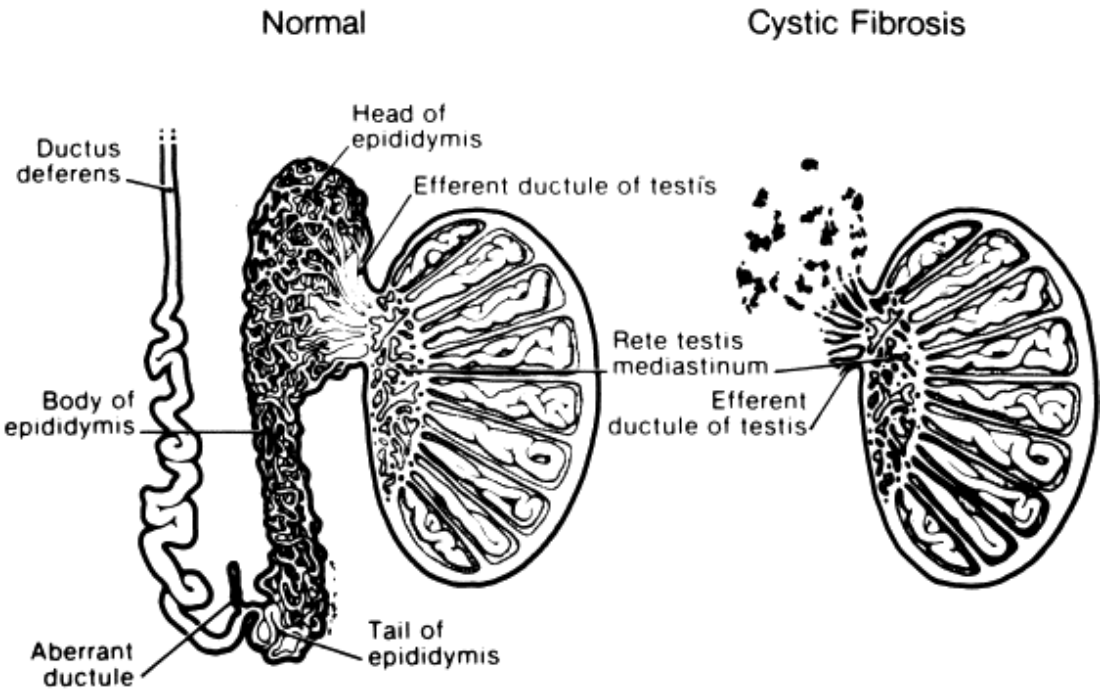
- ***Focal biliary cirrhosis*** : small biliary ducts are clogged by the mucus with biliary stasis leading to ***focal biliary cirrhosis*** (in 10% of patients). Possibility of *steatosis*.



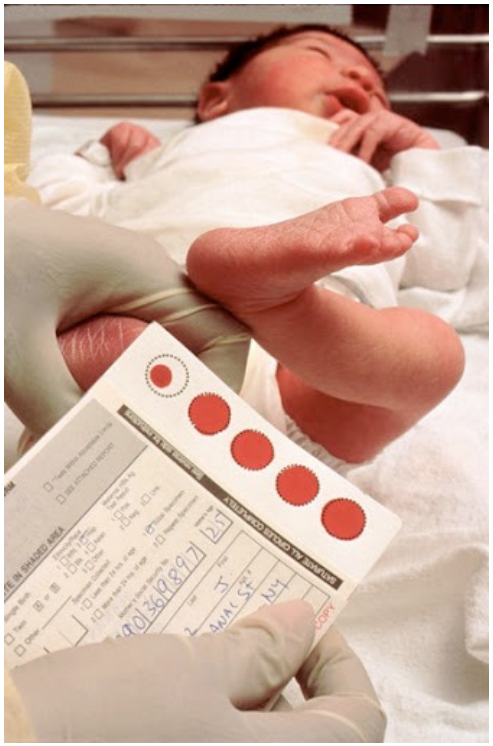


Gonads

- ***Obstructive azoospermia and infertility*** in 95% of adult males. Often associated to congenital bilateral **absence of deferent ducts**.



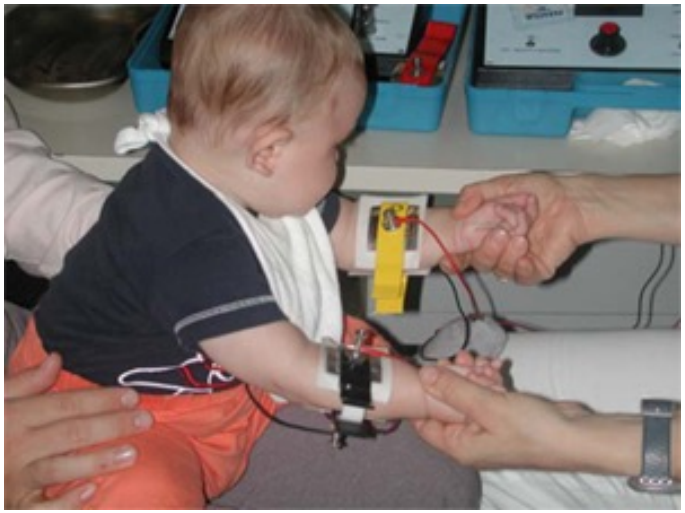
Neonatal Screening



- All newborns are screened for CF and other 50 frequent genetic diseases (PKU, congenital hypothyroidism, metabolic disorders, etc), within 72 hours from birth
- The heel of the baby is pricked with a needle and few drops of blood are spotted into a paper.
- Dosing of **immunoreactive trypsinogen (IRT)**, precursor of trypsin, which goes into the bloodstream because of blockage in the pancreatic ducts.

Chloride Sweat Test

- Measurement of the amount of NaCl in the sweat.
- **A disc with pilocarpine gel** (stimulating sweating – muscarinic receptor agonist) is applied to the arm, and then a little amount of electric current is applied with two electrodes.
- After 5 minutes the disc containing the sweat is removed and the NaCl content analyzed
- The **test is positive with values equal or higher than 60 mEq/L of NaCl**
- The test is **negative with values inferior to 40** mEq/L of NaCl (30 mEq/L in infants)
- For values in between the test must be repeated at least three times to ascertain whether it is CF.



Who gets the sweat test done?

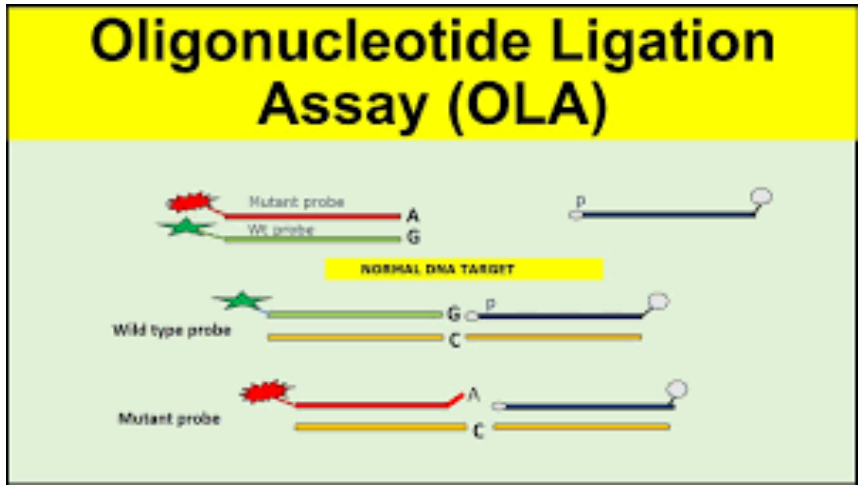
- **Infants** who tested **positive at newborn IRT** screening (elevated trypsin);
- In case of **meconium ileus** with negative screening
- **Children** with recurrent **respiratory infections, poor growth**, chronic diarrhea even if with negative neonatal screening
- **Adolescents and adults** with chronic or recurrent pancreatitis, male **infertility**, chronic sinusitis, recurrent **lung infections**, particularly in the presence of bronchiectasis.

Diagnosis of CF

- Elevated concentrations of NaCl in the sweat
- Clinical features → genetic test
- Familiar Anamnesis

Genetic testing

- It is performed with panels that analyze 34 or 139 or 152 mutations (depending on the type of analysis carried out), chosen among the most frequent in the geographical area; allows the identification of approximately 90% of carriers
- The geneticist can prescribe a genetic test to analyze the whole gene, covering all the mutations discovered so far



- The standard procedure is based on the Polymerase Chain Reaction (**PCR**) technique associated with **Oligonucleotide Ligation Assay** (OLA), and automatic sequencing

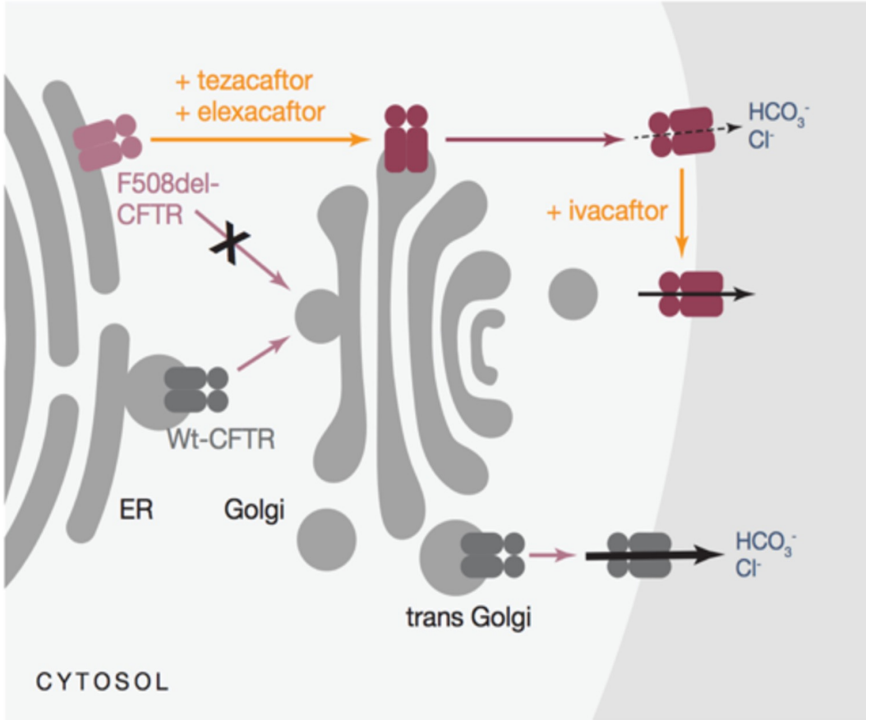
Therapy of CF

- Life expectancy of above 36 years.
- In the past, it was lethal in the infancy,
- The prognosis has then substantially improved thanks to:
  - Antibiotic therapy
  - Pancreatic enzymes
  - Lung transplantation

KAFTRIO

- **Trikafta** in the USA (Vertex, Boston, MA). Approved by EMA in 2020 and AIFA in 2021 for patients aged 6 and over with at least one ΔF508 mutation (estimated at over 90%).

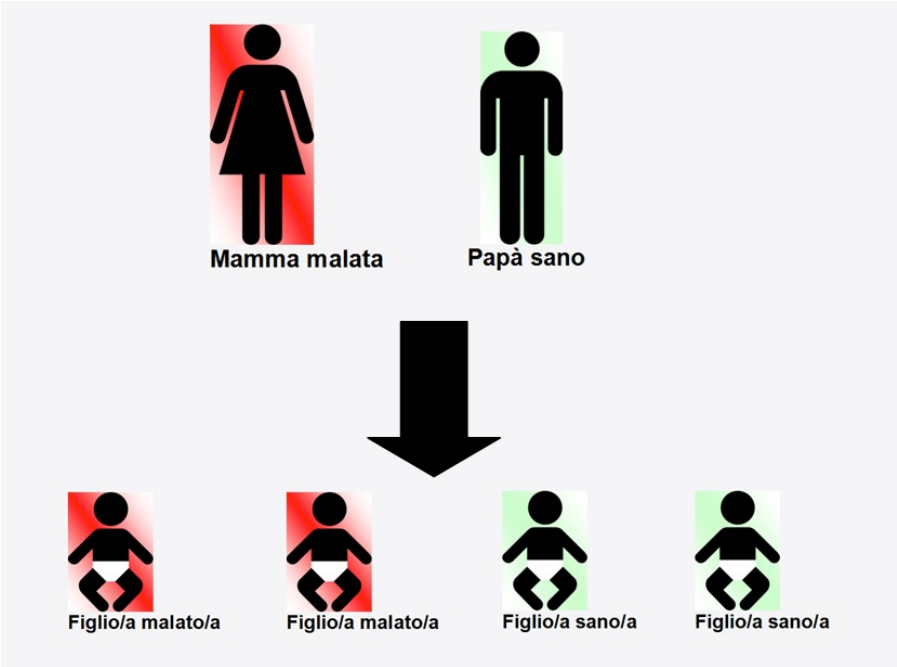
- Combination of three drugs:
  - **Ivacaftor**: *gating potentiator*. Increases the opening of the chloride channel
  - **Elexacaftor**: *folding corrector*. They bind CFTR D508 and increase availability at the cell membrane.
  - **Tezacaftor:** *folding corrector*, like elexacaftor



- **Dosage**: two tablets in the morning + one ivacaftor tablet in the evening, approximately 12 hours later.

- **Side effects**: headache, diarrhea, skin rashes
-
- **High costs**: 322 thousand dollars/year!

Autosomal Dominant Diseases



-Achondroplasia
-Marfan Syndrome
-Familiar Hypercholesterolemia
-Neurofibromatosis
-Familial colonic polyposis
-Polycystic kidney disease
-Myotonic dystrophy

Achondroplasia

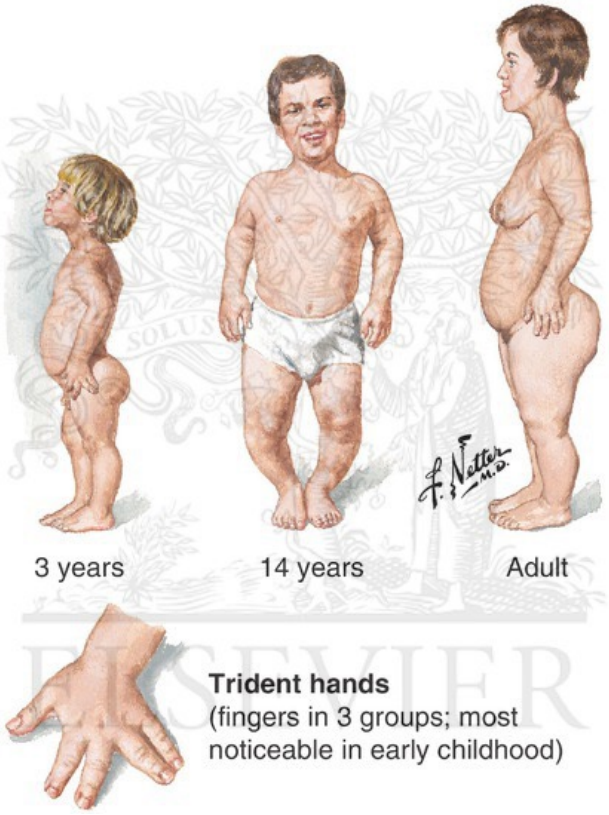
- Incidence: 1:25.000 newborns

- Dwarfism with disproportion between limbs (very short) and chest (almost normal). Big head with prominent forehead. Nonharmonic dwarfism.

- Lack of proper bone growth (proliferation of the chondrocytes in the epiphyseal plate is impaired)

- Gene mutated: **FGFR3** on chromosome 4. Member of a gene family, encoding fibroblast and chondrocyte growth factor receptor

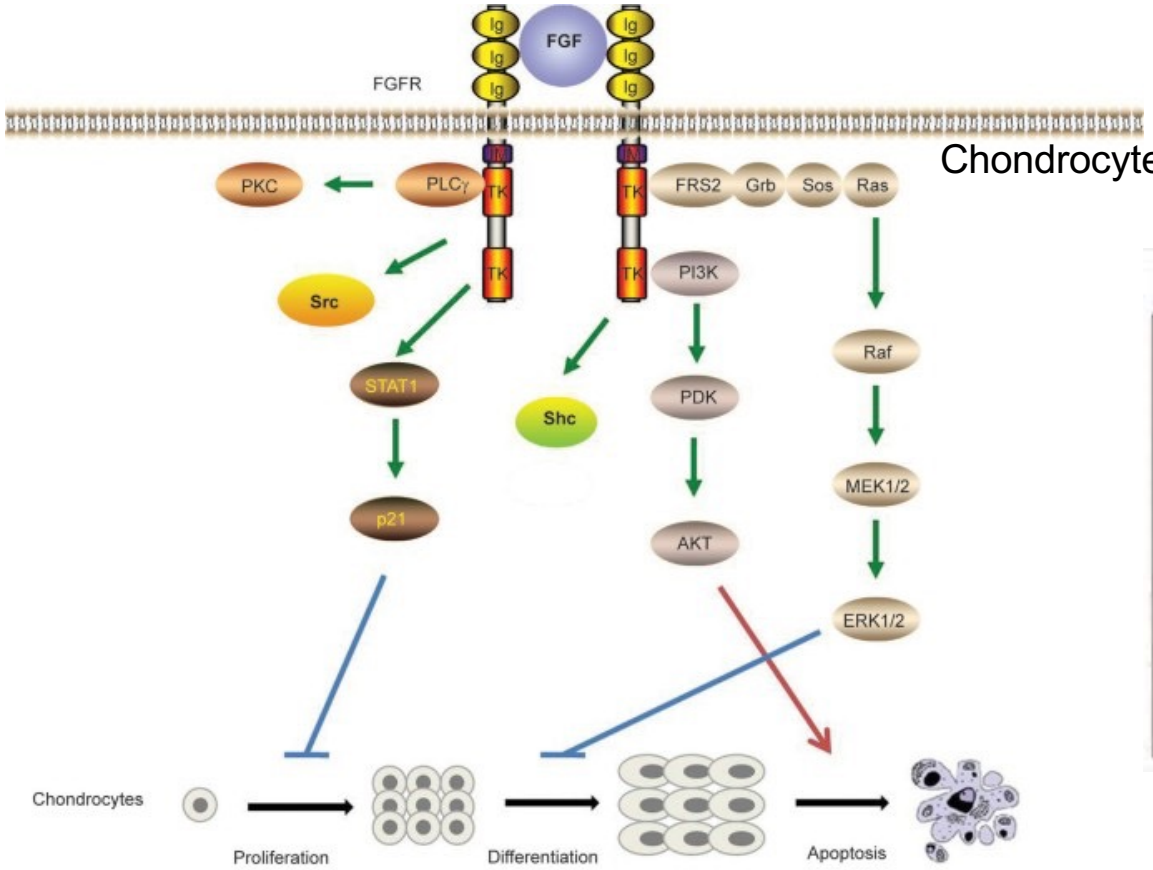
- Complete penetrance of the disease (all carriers have the disease)



Health problems associated to Achondroplasia

- Frequent **apneas**, especially at night
- **Hydrocephalus**
- **Motor delays**: affected individuals learn to walk later than other children
- **Difficulty speaking**, although this problem usually resolves by school age
- Difficulty bending the elbows
- **Spinal or vertebral stenosis**: can lead to compression of the cord and worsen with age. Sometimes children with achondroplasia may die suddenly in their sleep, due to **compression of the nerve centers of breathing**.
- Overweight/**obesity**
- Recurrent **ear infections** (due to anatomical abnormalities of the ear canal) which can lead to hearing loss

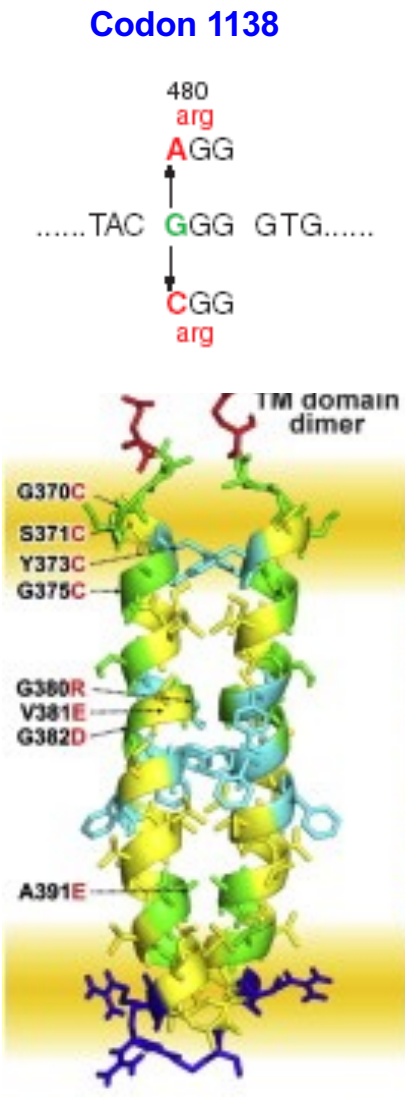
FGFR3 Pathway



Chondrocyte

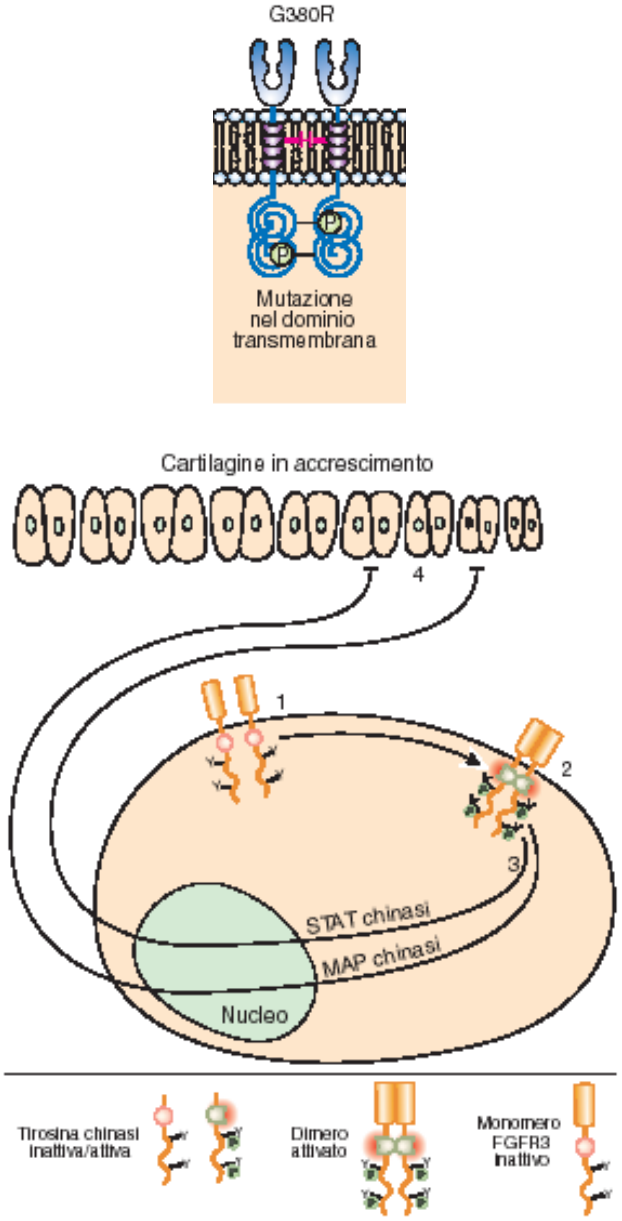
Mutation of the *FGFR3* gene

- In more than **90%** of cases is a sporadic disease**: healthy parents give birth to a patient with a new mutation**
- Novel mutations are of **paternal origin**, for replication errors during spermatogenesis. **The frequency increases with the age of the men**
- The mutation is always the same: **G380R**. Gly →Arg substitution in the receptor *transmembrane domain*
- The frequency of this mutation is very high (1.4 x 10^{-5}) and hits **nucleotide G in position 1138** of a C*pG dinucleotide.
- It is a **hot spot site for** mutations.



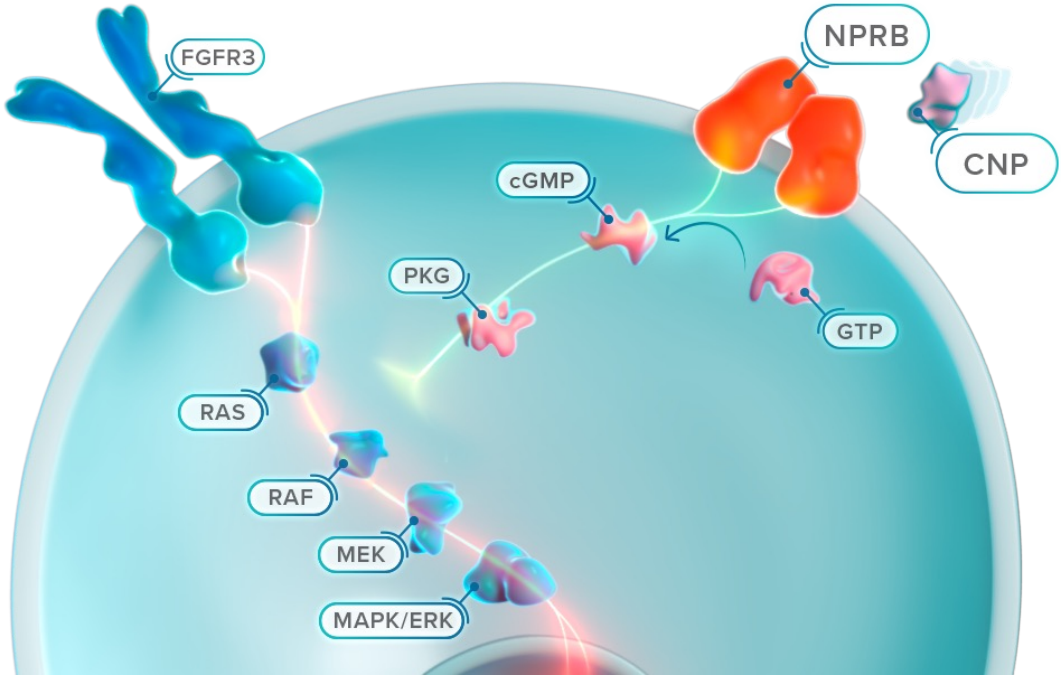
Effect of *FGFR3* mutation

- The mutated **G380R** receptor **forms stable and constitutively active dimers**
- This mutant precociously **blocks chondrocytes proliferation and the endochondral ossification of the epiphyseal plates**



VASORITIDE: BMN-111

(VOXZOGO™ *Biomarin*)

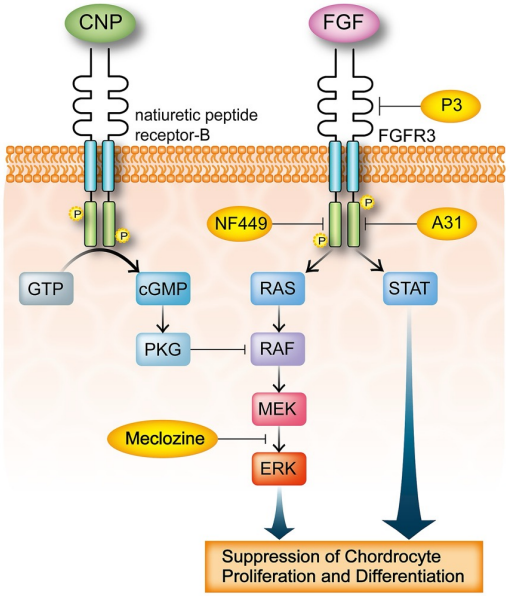


- **CNP** binds **NRPB** receptor and inhibits FGFR3 pathway
- **BMN-111 is a CNP analogue** (recombinant peptide)
- **Approved** by European Medicines Agencies (EMA) and Agenzia Italiana del Farmaco (AIFA) 28-07-2022!

CNP: C-type Natriuretic Peptide

NPRB: Natriuretic Peptide Receptor type B

VOXZOGO



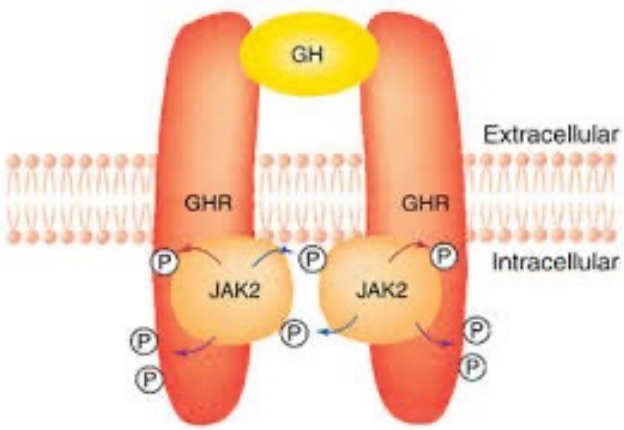
- In patients with a confirmed genetic diagnosis **aged 2 years and older** whose bones are still developing

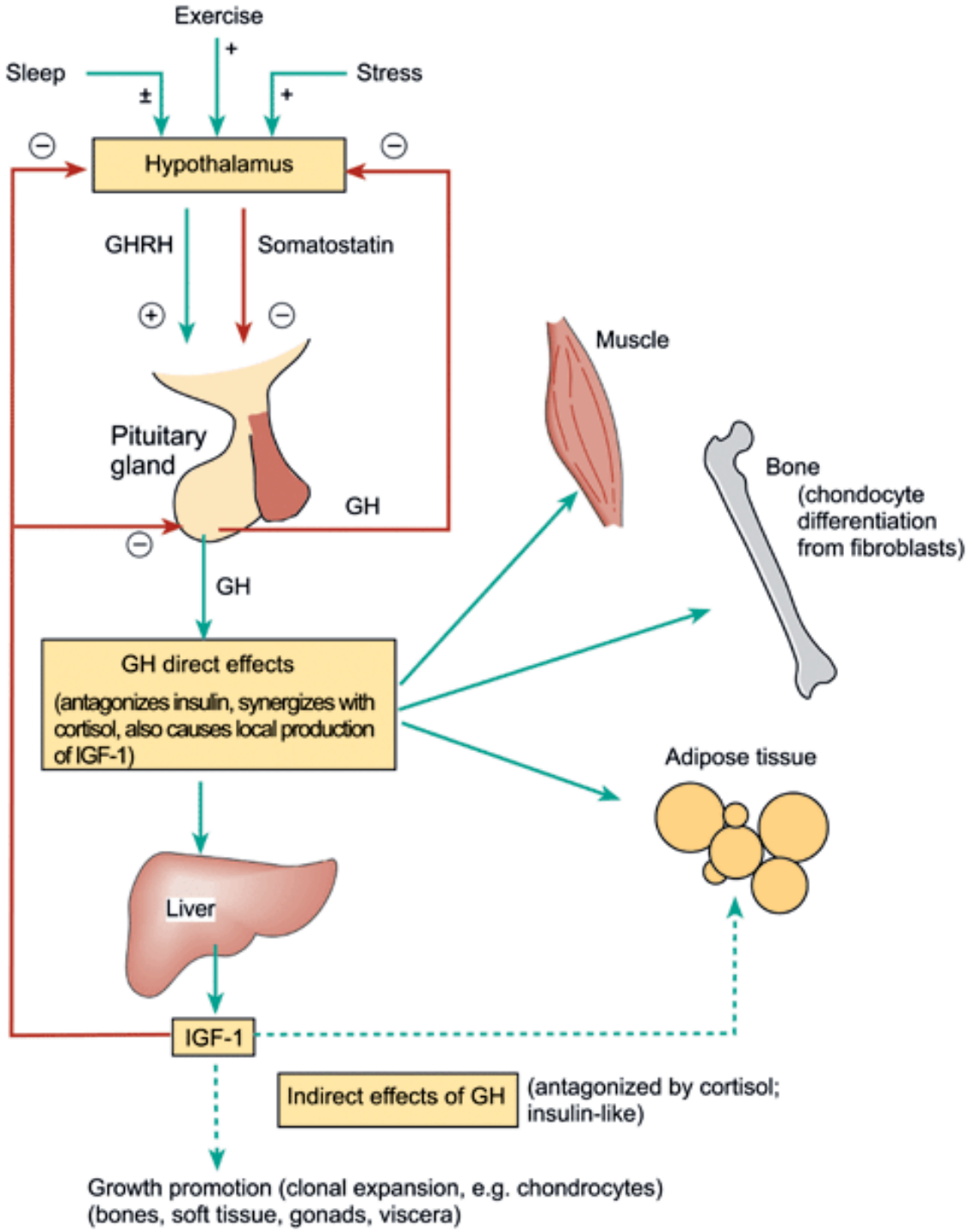
- **Subcutaneous injection** once daily: 15 micrograms per kg of body weight

- Children treated with Voxzogo grow approximately 1.57 cm/year more than those treated with placebo

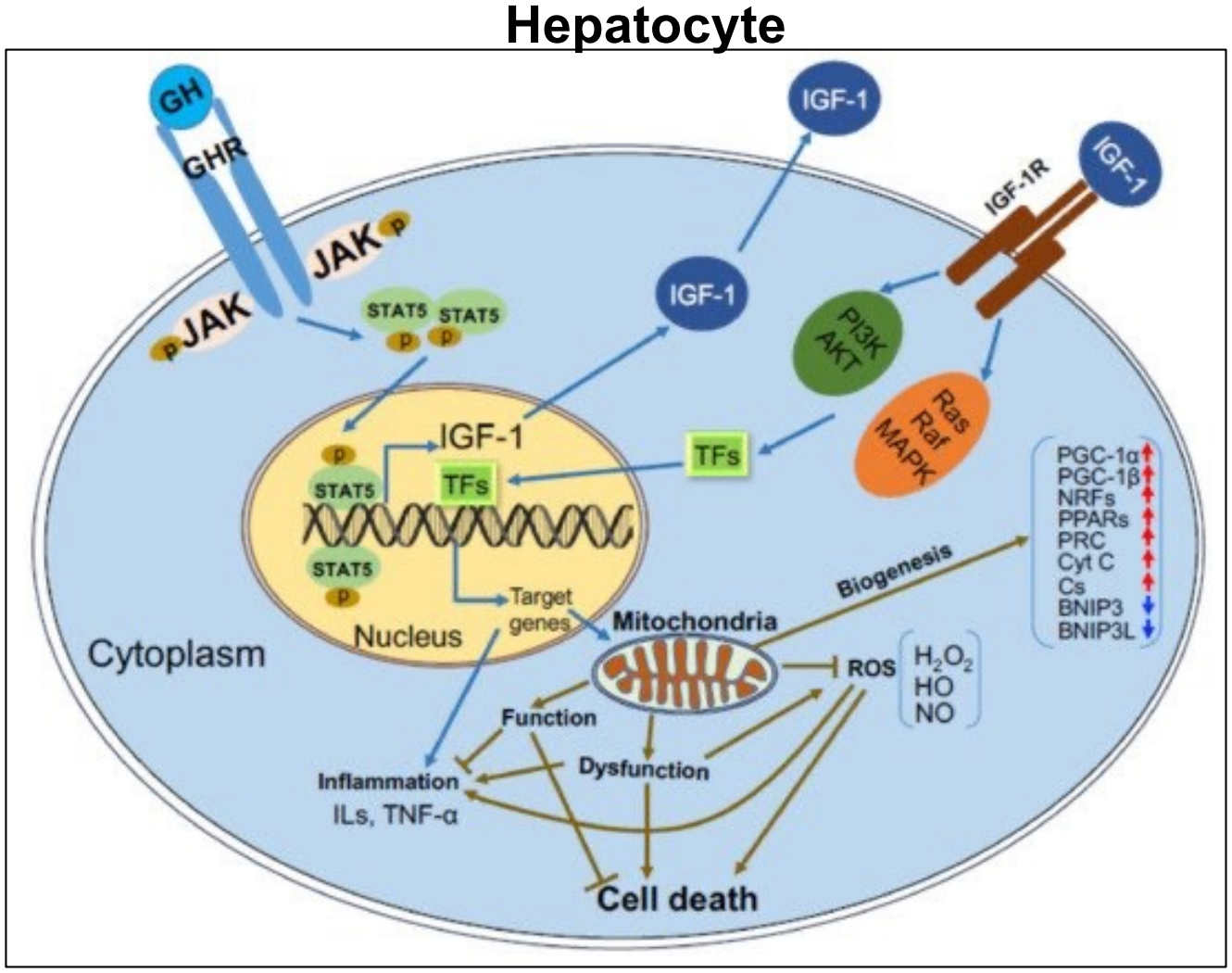
Laron Dwarfism

- **Autosomal Recessive disorder**
- **Pituitary dwarfism type 2**
- 1-9 cases/1.000.000
- Dwarfism, prominent forehead, small jaw, obesity, mental retardation
- Mutation of GH receptor gene (hormone binding domain): *GHR*



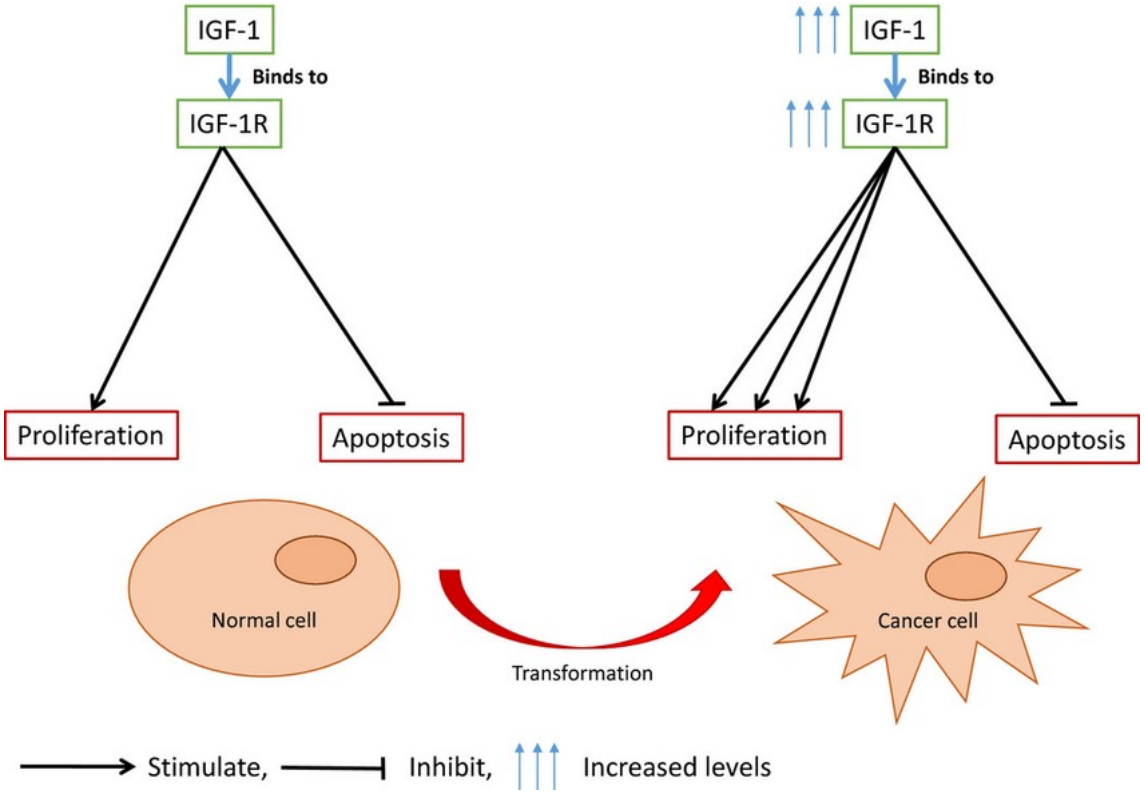

Exercise
+
Sleep
±
Stress
+
Hypothalamus
GHRH
Somatostatin
Pituitary gland
GH
GH
GH direct effects
(antagonizes insulin, synergizes with cortisol, also causes local production of IGF-1)
Muscle
Bone
(chondocyte differentiation from fibroblasts)
Adipose tissue
Liver
IGF-1
Indirect effects of GH
(antagonized by cortisol; insulin-like)
Growth promotion (clonal expansion, e.g. chondrocytes)
(bones, soft tissue, gonads, viscera)

IGF-1 regulation by GH

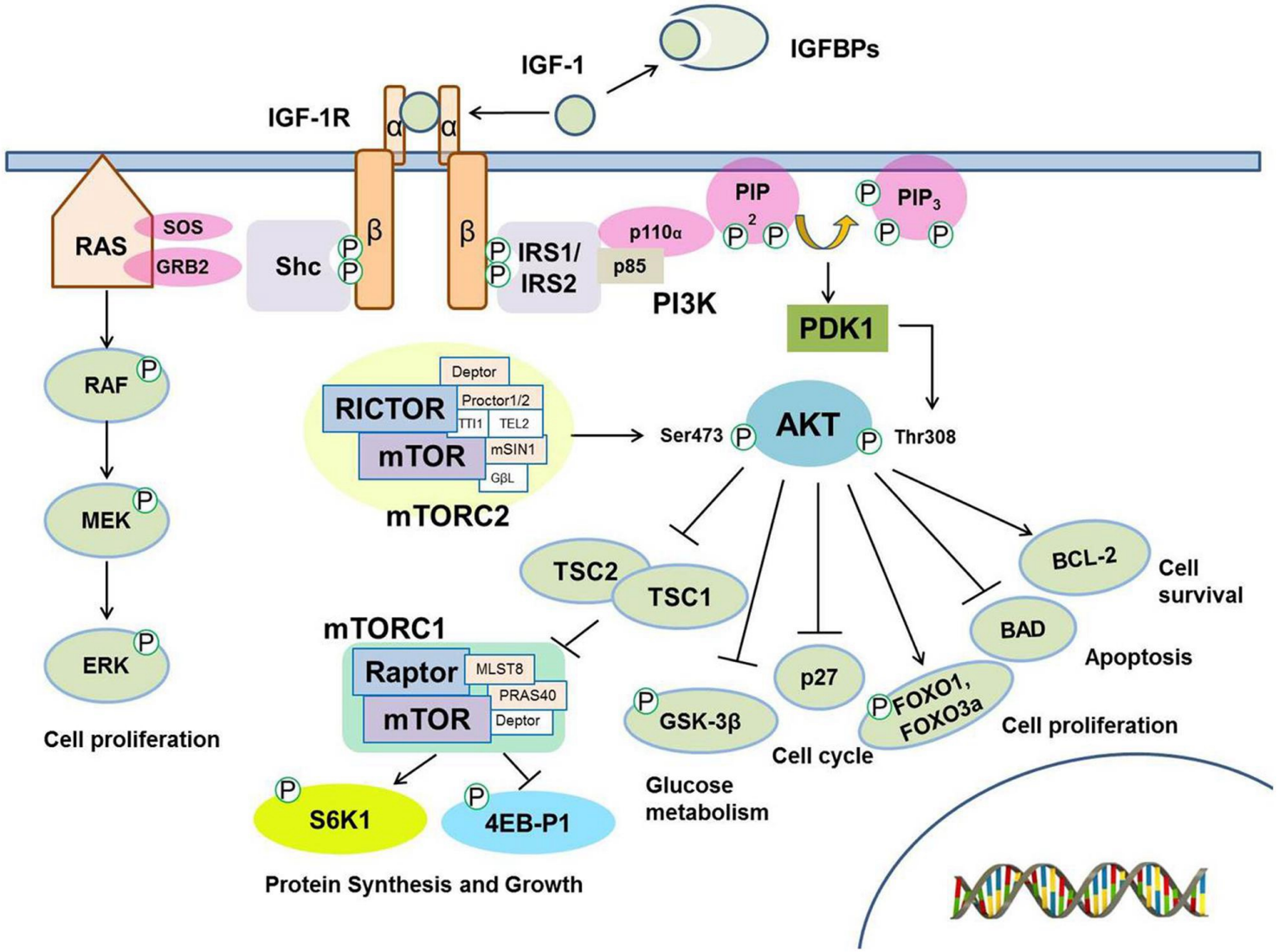


Laron and cancer

- **Low incidence of cancer, diabetes, delayed aging**
- Low levels of IGF-1



IGF-1 Signaling



Treatment

- **Mecasermin (Increlex): human IGF-1** recombinant. Produced in *E. Coli* from the human IGF-1 gene

- **Mecasermin rinfabate (IPLEX)** FDA approved. Recombinant IGF-1 + IGFBP-3 (IGF-1 transport protein). Longer half life than Increlex.