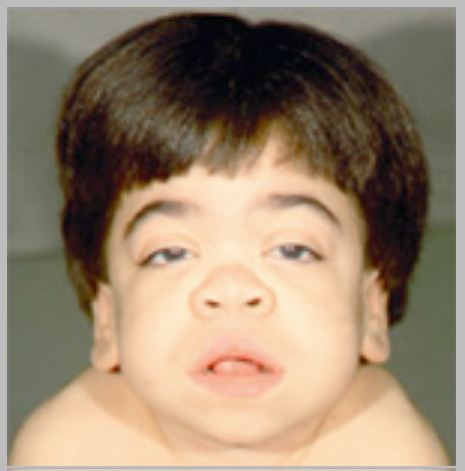
Molecular, biochemical and cellular basis of genetic disease

Defects in receptor proteins and transport defects



Prof. Dr. O.M. Vanakker

Center for Medical Genetics

Ghent University Hospital

Classes of proteins

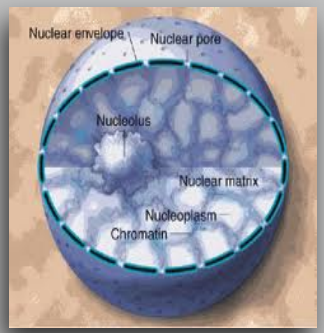
- Two general classes based on pattern of expression

Housekeeping proteins

- **Present in virtually all cells**
- **Fundamental roles in maintenance of cell structure and function**

Speciality proteins

- **Tissue specific**
- **Functions contribute to the individuality of the cell**



Eukaryotic cells: 10.000-15.000 genes expressed

90% of mRNA encode HK proteins

SP

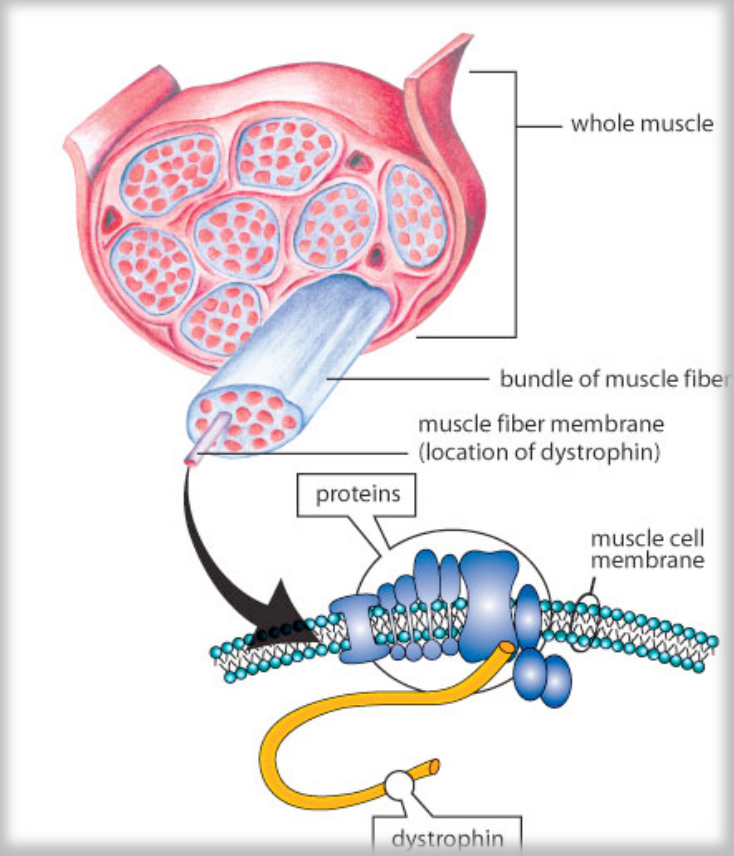
Protein expression and disease

- Knowledge of the tissues where a protein is (highly) expressed can help to understand pathogenesis of disease

Mutation in **tissue specific protein** most often leads to disease restricted to that tissue

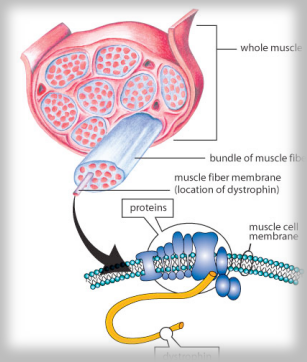
Aberrant **housekeeping proteins** rarely cause pathological changes in all tissues

Mutation in **tissue specific protein** most often leads to disease restricted to that tissue



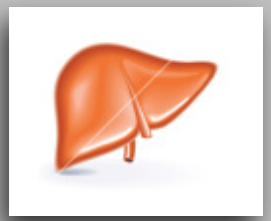
Dystrophin → **Duchenne muscular dystrophy**

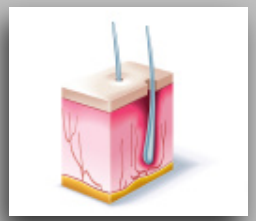
Mutation in tissue specific protein most often leads to disease restricted to that tissue



HOWEVER:

- There may be secondary effects on other tissues





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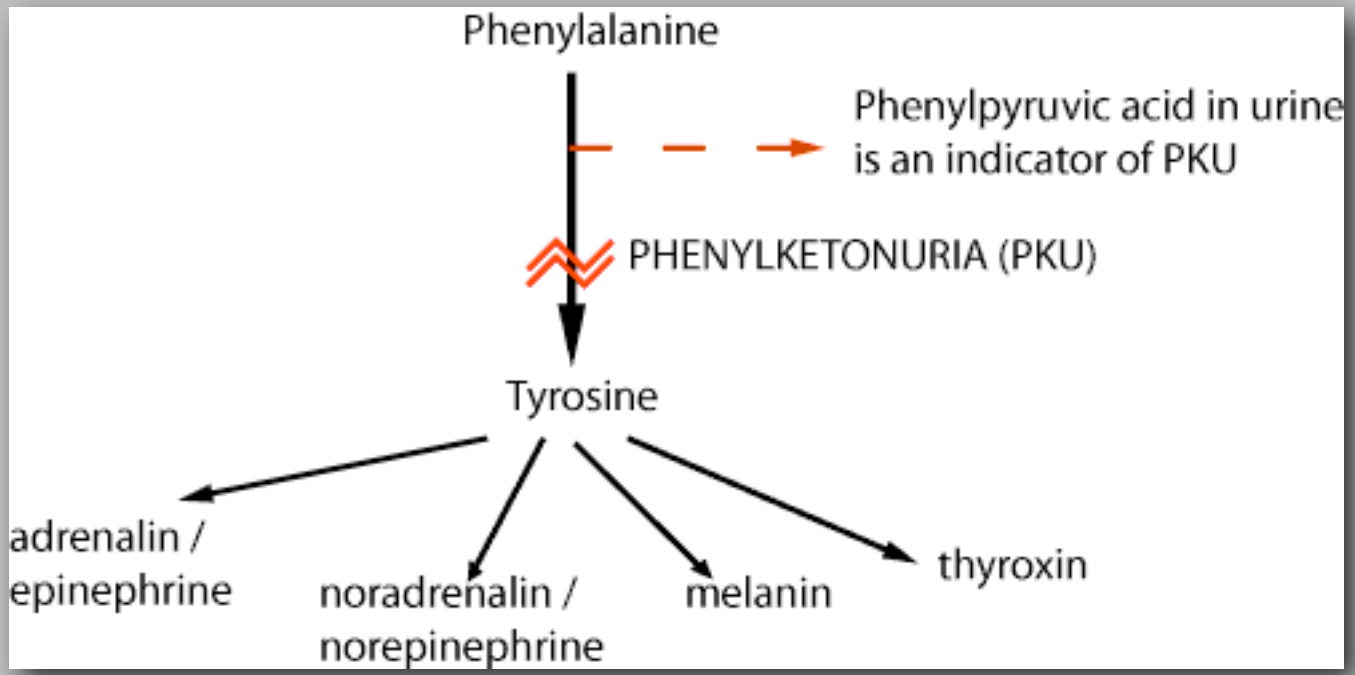
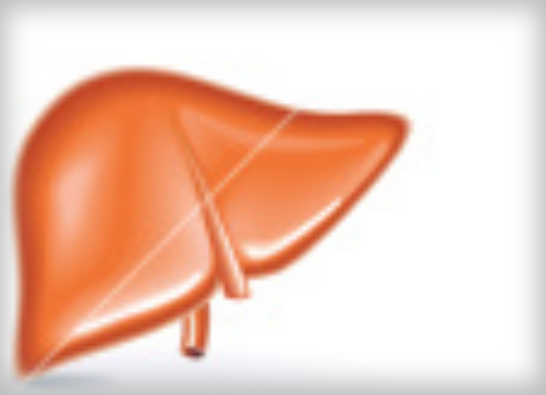
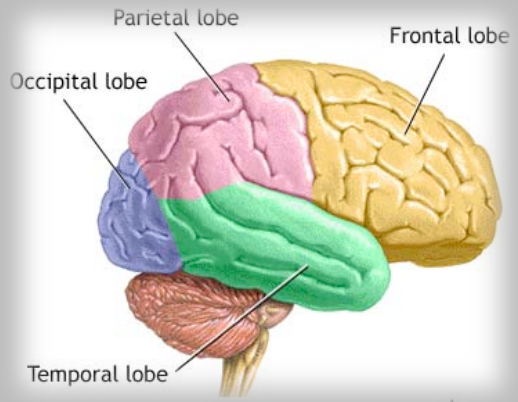
- In some cases, the site of disease may be unpredictable
  - **Mutation in TS protein may lead to abnormalities in cells & organs that do not normally express protein**
  - **The tissue expressing the protein may be entirely unaffected**

Phenylketonuria

Phenylketonuria



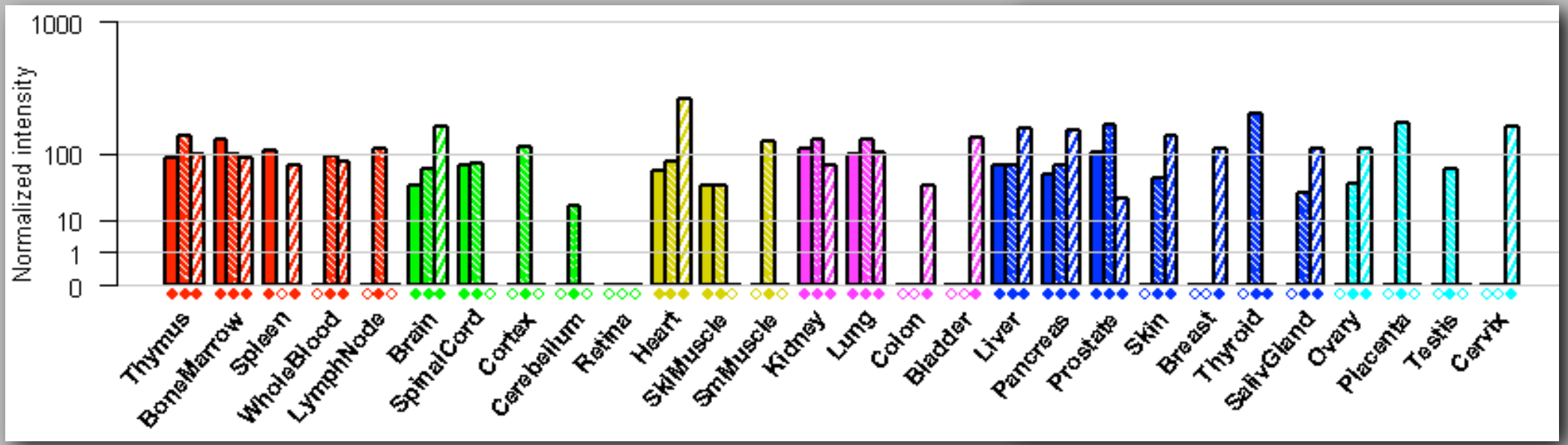




Mutation in housekeeping protein rarely affects all tissues

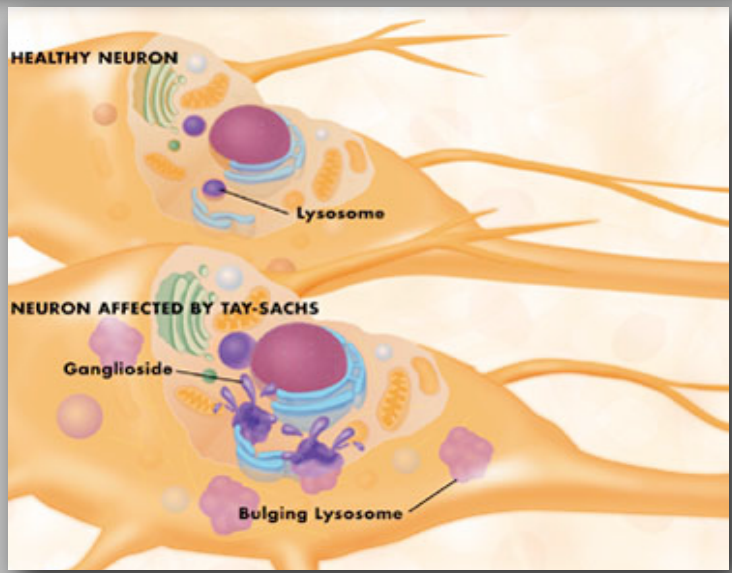
- Often not compatibel with life (actin, DNA polymerase, ...)

  → Limited clinical effects in few tissues



Speciality function

- **One specific tissue is affected**
- **The affected protein serves a speciality function**
- **E.g. Tay-Sachs disease due to ↓ hexosaminidase A activity**
  - **ubiquitously expressed**
  - **absence leads to neurodegeneration**
  - **other cell types are not harmed**



Relationship between genotype & phenotype

Genotype-phenotype correlation

Variation in clinical phenotype

Allelic heterogeneity

Locus heterogeneity

Effect of modifier genes

Allelic heterogeneity

- The occurence of more than 1 allele at a locus

  → Different mutations cause the same disease

- Most common form of genetic heterogeneity

  - **Alleles which confer more residual function are associated with a milder form of the disease**

    OR: associated with only a partial phenotype (subset of one or more clinical features of the whole)

    → Certain *CFTR* variants only give congenital absence of the vas deferens but no other symptoms of cystic fibrosis

Locus heterogeneity

- Association of more than one locus with a certain disease
- Numerous examples of polygenic diseases
- E.g. Hyperphenylalaninemia

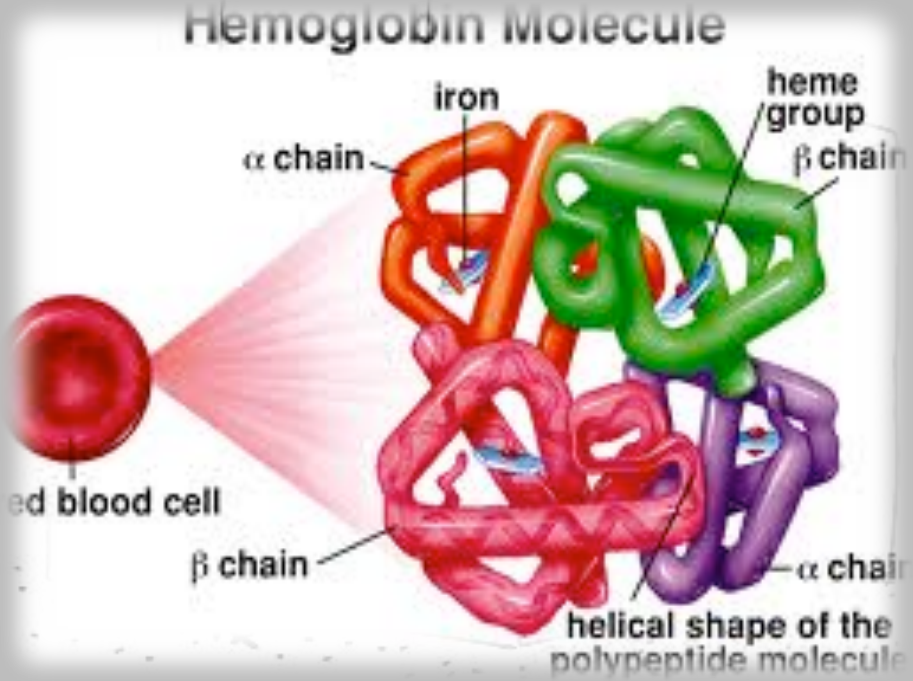
Biochemical Defect	Incidence/ 10^6 Births	Enzyme Affected	Gene Location	Inheritance	Treatment
Mutations in the Gene Encoding Phenylalanine Hydroxylase					
Classic PKU	5-350	PAH	12q24.1	AR	Low-phenylalanine diet*
Variant PKU	Less than classic PKU	PAH	12q24.1	AR	Low-phenylalanine diet (less restrictive than that required to treat PKU*)
Non-PKU hyperphenylalaninemia	15-75	PAH	12q24.1	AR	None, or less restrictive low-phenylalanine diet*
Mutations in Genes Encoding Enzymes of Tetrahydrobiopterin Metabolism					
Impaired BH_4 recycling	1-2	PCD	10q22	AR	Low-phenylalanine diet + L-dopa, 5-HT, carbidopa
		DHPR	4p15.31	AR	Low-phenylalanine diet + L-dopa, 5-HT, carbidopa + folinic acid
Impaired BH_4 synthesis	Rare	GTP-CH	14q22	AR	Low-phenylalanine diet + L-dopa, 5-HT, carbidopa + folinic acid, and pharmacologic doses of BH4
		6-PTS	11q22.3-23.3	AR	As with GTP-CH deficiency

*BH_4 supplementation may increase the PAH activity of some patients in each of these three groups.
AR, autosomal recessive; BH_4, tetrahydrobiopterin; DHPR, dihydropteridine reductase; GTP-CH, guanosine triphosphate cyclohydrolase; 5-HT, 5-hydroxytryptophan; PAH, phenylalanine hydroxylase; PCD, pterin 4α-carbinolamine dehydratase; PKU, phenylketonuria; 6-PTS, 6-pyruvoyltetrahydropterin synthase.

- **Careful comparison of the phenotypes commonly (??) reveals that the phenotype is not as homogeneous as initially believed**

Modifier genes

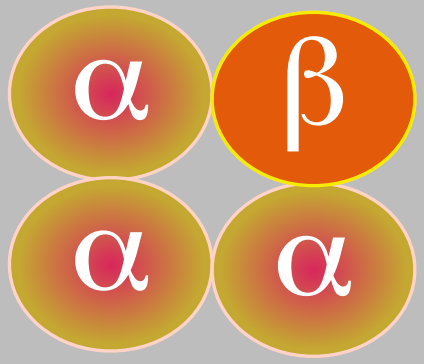
- No genotype/phenotype correlation in a specific patient group
  → environmental factors
  → modifier genes



- **Modifier genes are difficult to identify**
- **Few clinically relevant modifiers have been discovered**

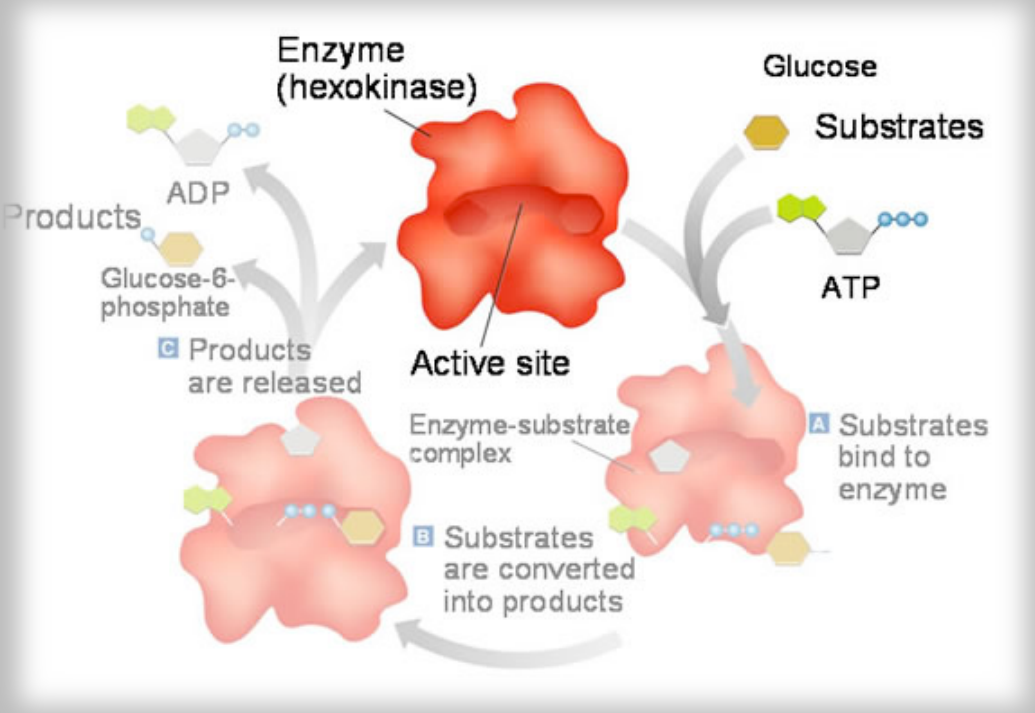
- **ß-thalassemia homozygotes**
- **Co-inherited α-thalassemia allele**
- **Sometimes less severe clinical picture**

- **Cystic fibrosis**
- **Patients homozygous for ΔF508**
- **Highly variable lung disease**



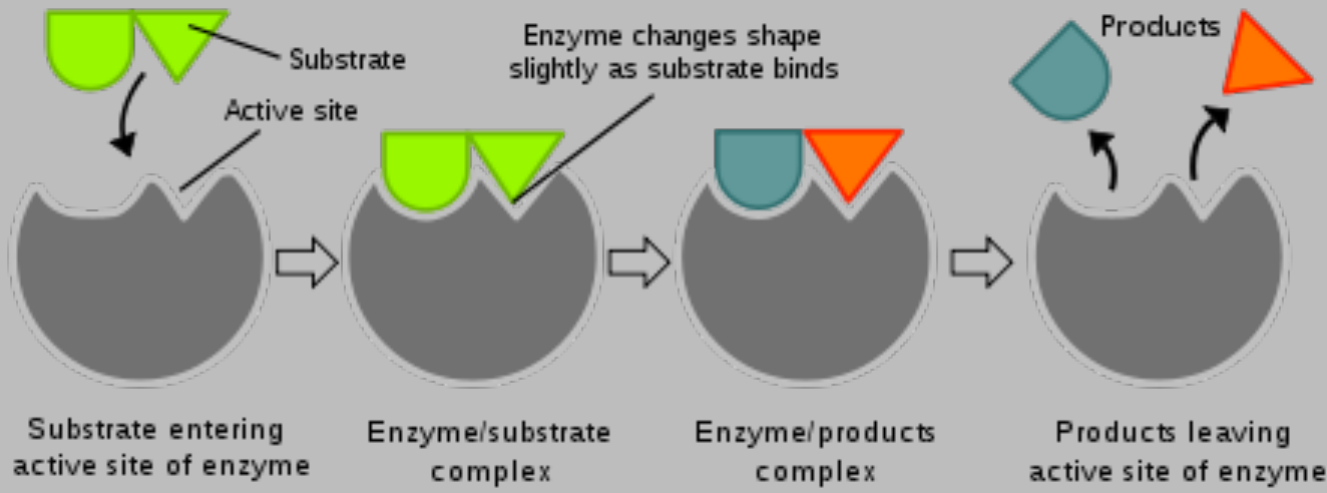
α-thalassemia

Diseases involving enzymes



Enzymes

- Biological catalysts which mediate conversion of a substrate to a product



- Huge diversity of substrates ⇒ many enzymes needed
  - **5000 genes encode enzymes in the human genome**
  - **Any of these enzymes can cause disease when mutated**

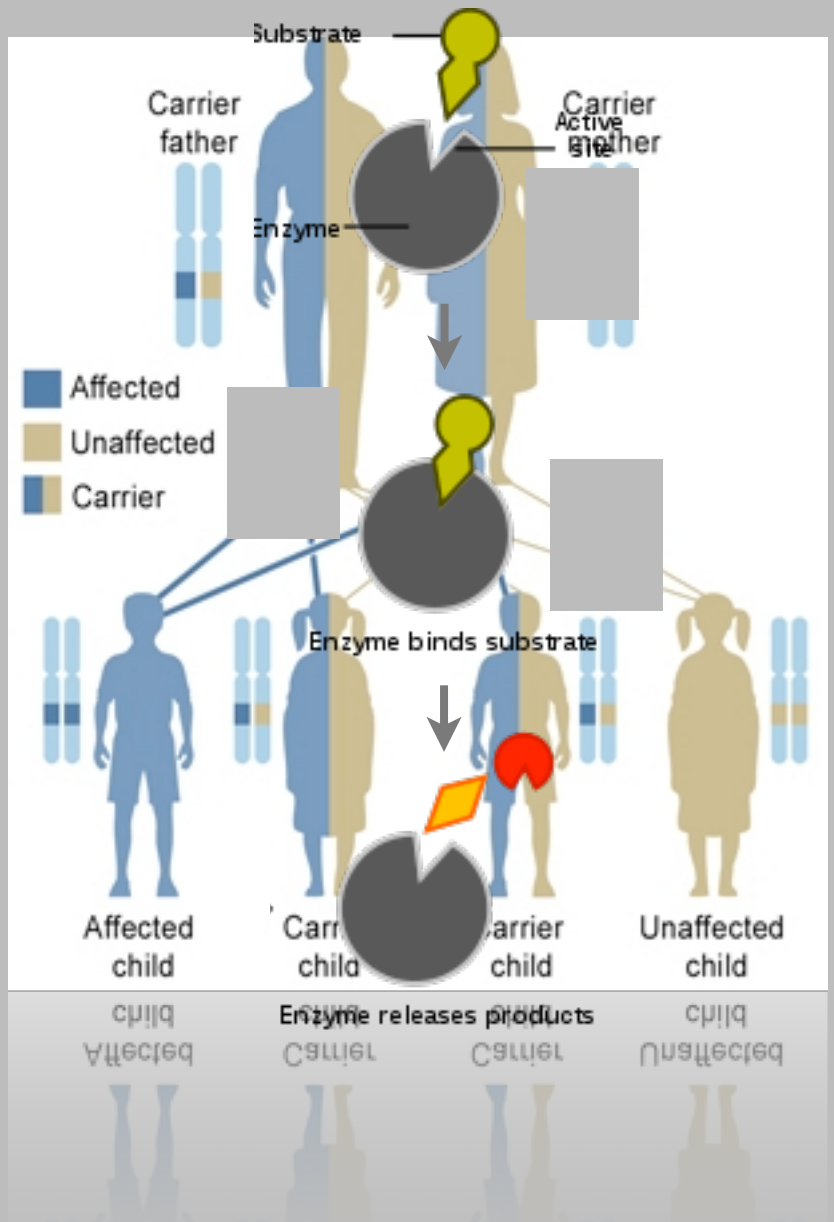
Enzymopathies

Enzymopathies

- Aminoacidopathies
- Lysosomal storage diseases
- Posttranslational modification abnormalities
- Co-factor diseases
- Alpha1-antitrypsin deficiency
- Acute intermittent porphyria

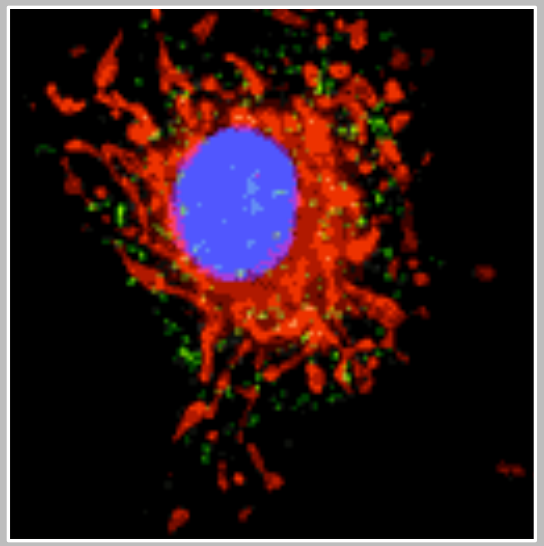
Concepts of enzyme deficiencies and diseases

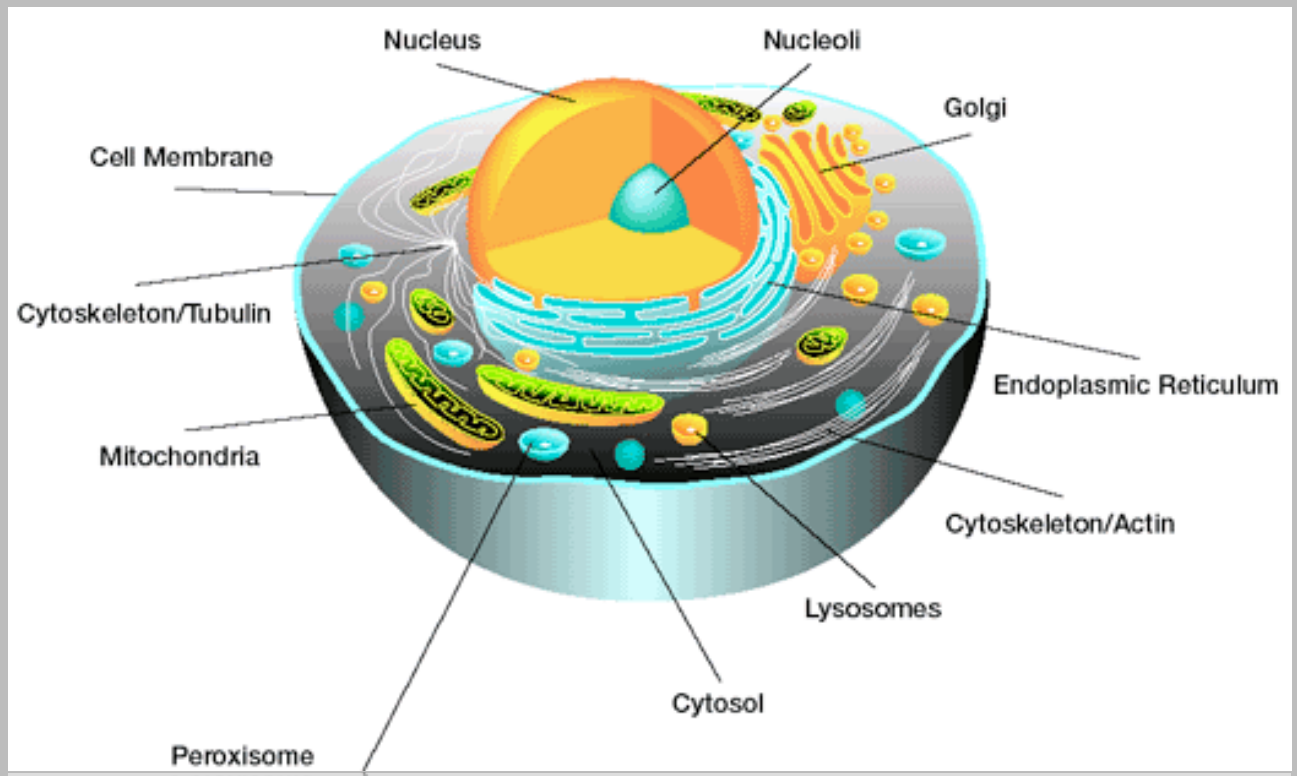
- Enzymopathies are almost always autosomal recessive
  - **Most enzymes are produced in excess quantities**
  - **Minimal enzyme activity may be up to 10%**
  - **Heterozygotes: 50% activity = normal**
- Substrate accumulation or product deficiency
  - **Or a combination of both**
- Diffusible versus macromolecular substrates
  - **Substrate = small molecule**
    - **distributed by diffusion or transport**
    - **effect unpredictable: substrate/metabolites can move freely through the body**
  - **Substrate = macromolecule**
    - **remains trapped inside organelle/cell**
    - **effect confined to tissues of accumulation**



Concepts of enzyme deficiencies and diseases

- Loss of multiple enzyme activities
  - **Single gene defect may result in loss of function of more than one enzyme**
    - **gene may encode co-factor**
    - **gene may encode something that multiple enzymes have in common**
      - **→ subunit, activating protein, processing protein, stabilizing protein**
    - **enzymes may be processed by a common modifying enzyme**
    - **abnormal formation of the organelle in which the enzymes are normally active**





Concepts of enzyme deficiencies and diseases

- Phenotypic homology
  - **Pathological and clinical features of an enzymopathy are often shared**
    - **by diseases due to a defect in enzymes in the same area of metabolism / metabolic cycle**
    - **by phenotypes that result from partial versus complete defects of one enzyme**

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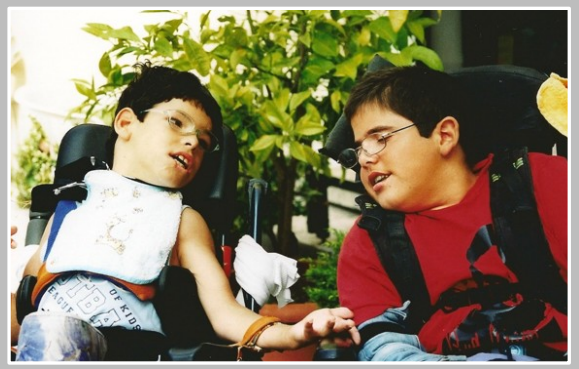
Partial defects often present as a subset of clinical symptoms of the total deficiency

Hypoxanthine guanine phosphoribosyltransferase

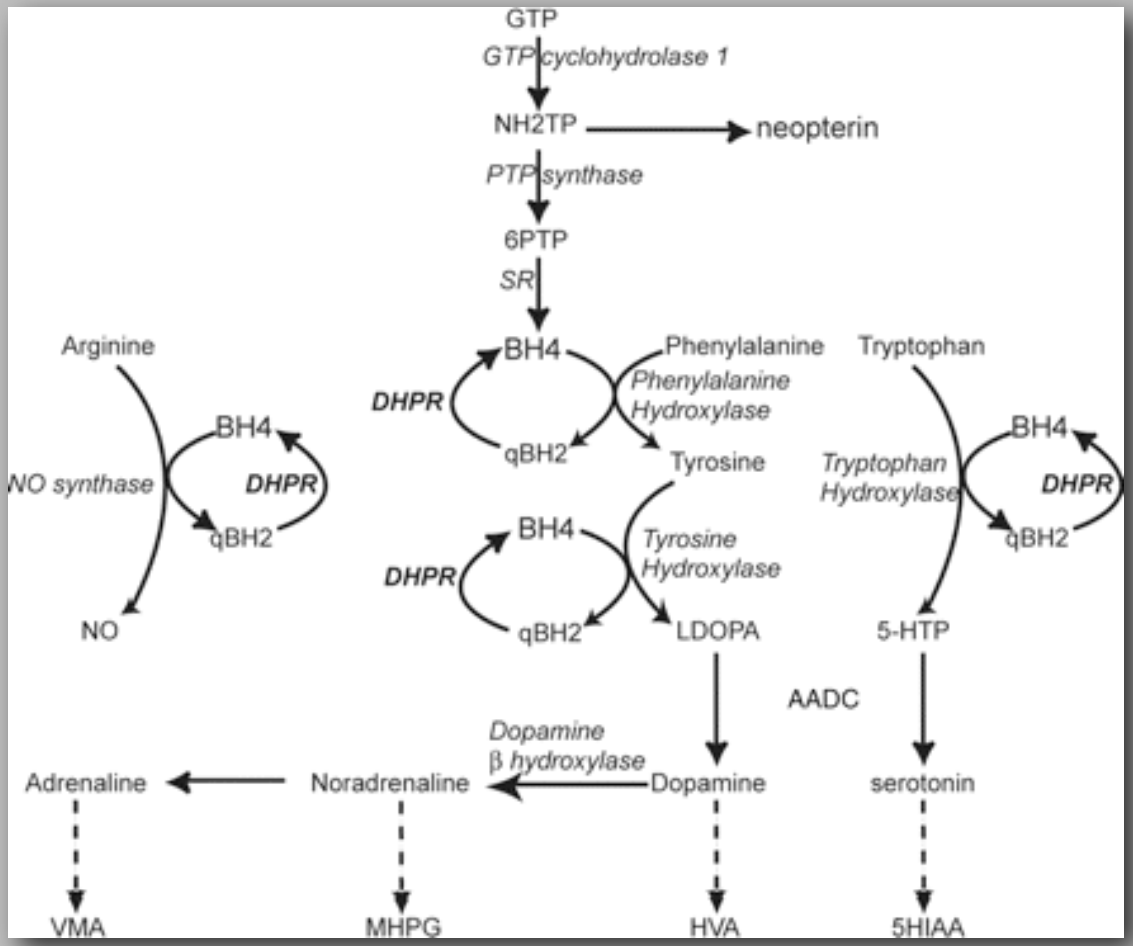
Isolated Hyperuricemia



Lesch-Nyhan syndrome

Aminoacidopathies: hyperphenylananinemias

- Result in increased blood concentration of phenylalanine
- Phenylketonuria is most common (phenylalanine hydroxylase deficiency)



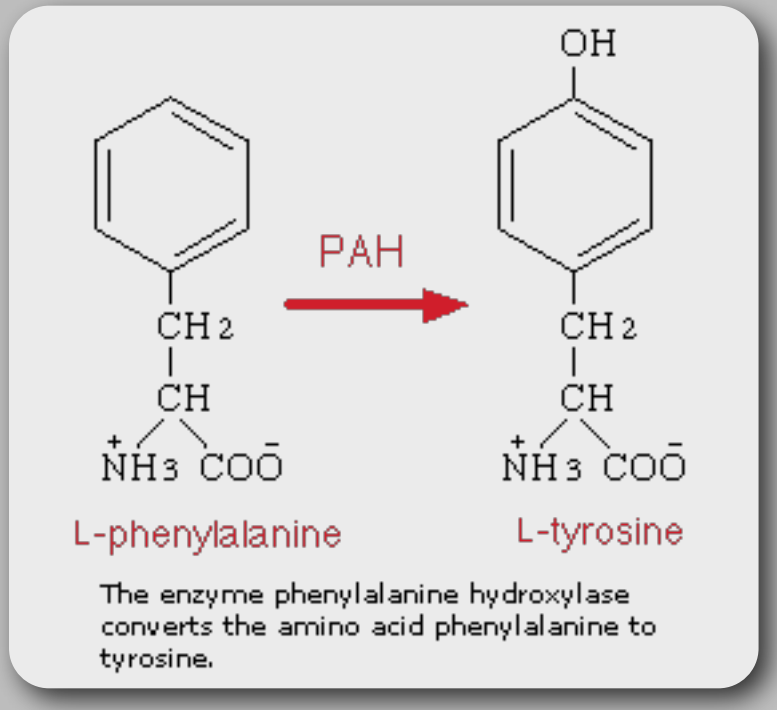
- **Loss-of-function of phenylalanine hydroxylase or co-factor (tetrahydrobiopterin) synthesis**

Phenylketonuria

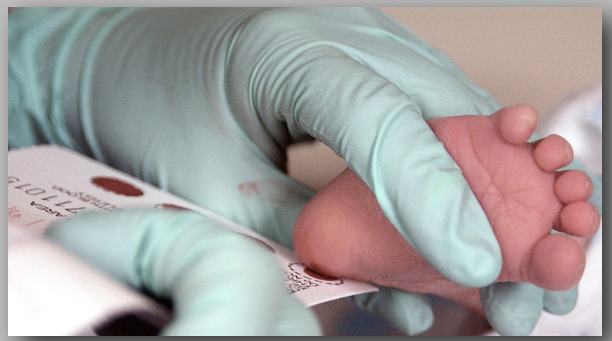
- AR, mutations in the *PAH* gene
- Accumulation of Phe in body fluids

↓

Negative influence on CNS development
Mechanism is not known



- Treatment: phenylalanine-poor diet + high dosis of co-factor Tetrahydrobiopterin
- Prevents the neurological damage
- Importance of early detection and hence newborn screening



Allelic heterogeneity

- More than 400 *PAH* mutations described
- 6 different mutations account for 2/3 of known mutants in Europeans
- Significant enzymatic and phenotypic variability

  - **No good genotype-phenotype correlations**
  - **Sometimes a link with level of reduction of the activity can be found**

  - **Some variants can give classic PKU or any of the milder associated phenotypes:**

    - Non-PKU hyperphenylalaninemia: plasma concentrations below 1mM (10x normal)

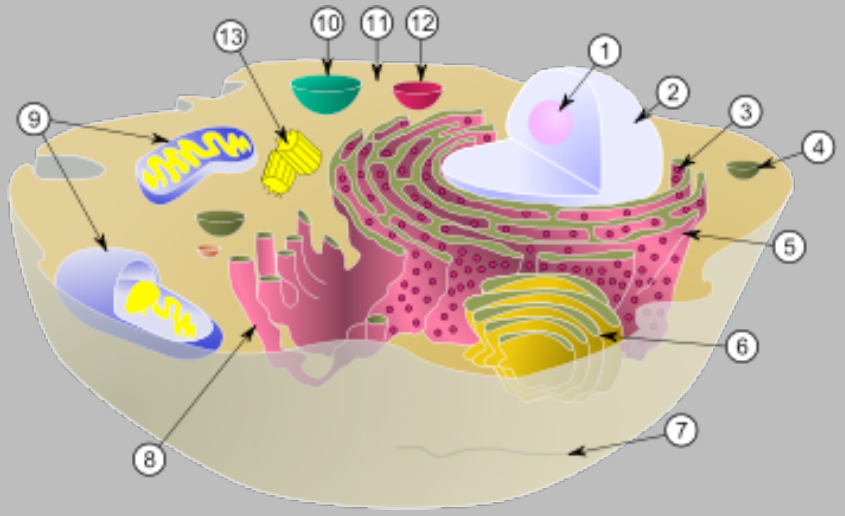
      -Normal phenotype
      -Identified because of newborn screening

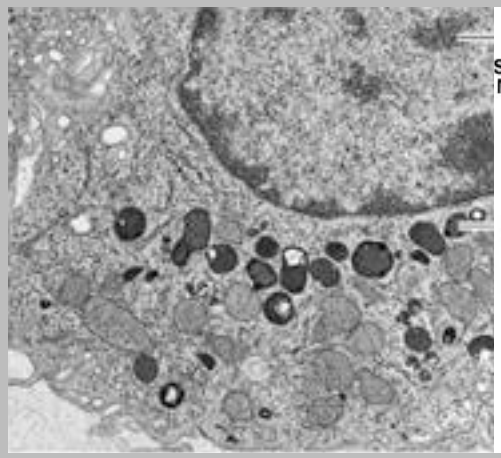
    - Variant PKU: plasma concentrations between classic and non-PKU

Clinical heterogeneity

Lysosomal storage diseases

- Membrane-bound organelle
- Hydrolytic enzymes: degradation of macromolecules





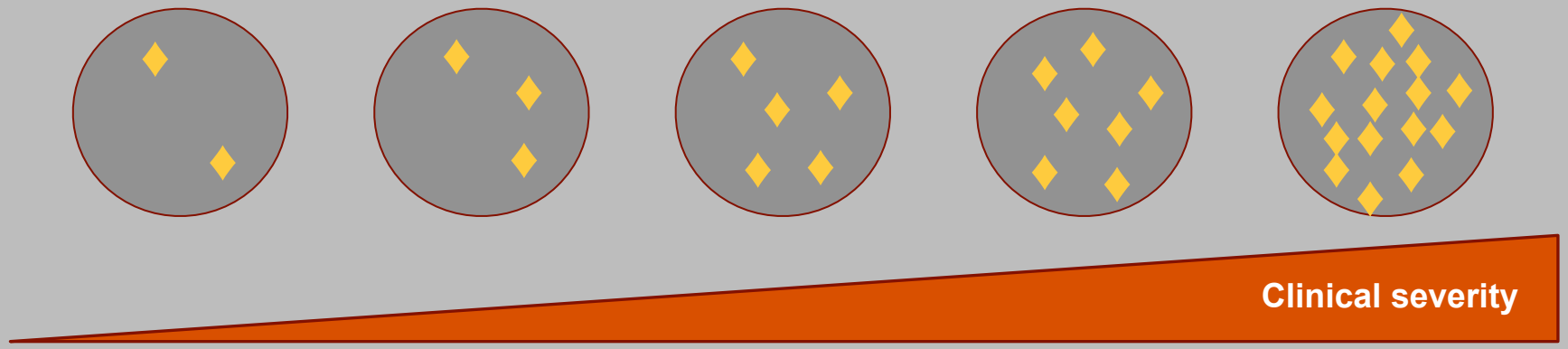
- Defect = accumulation of these molecules in the lysosome

  ⇒ Cellular dysfunction

  ⇒ Cell death

Lysosomal storage diseases

- **Gradual accumulation ⇒ relentless progression**



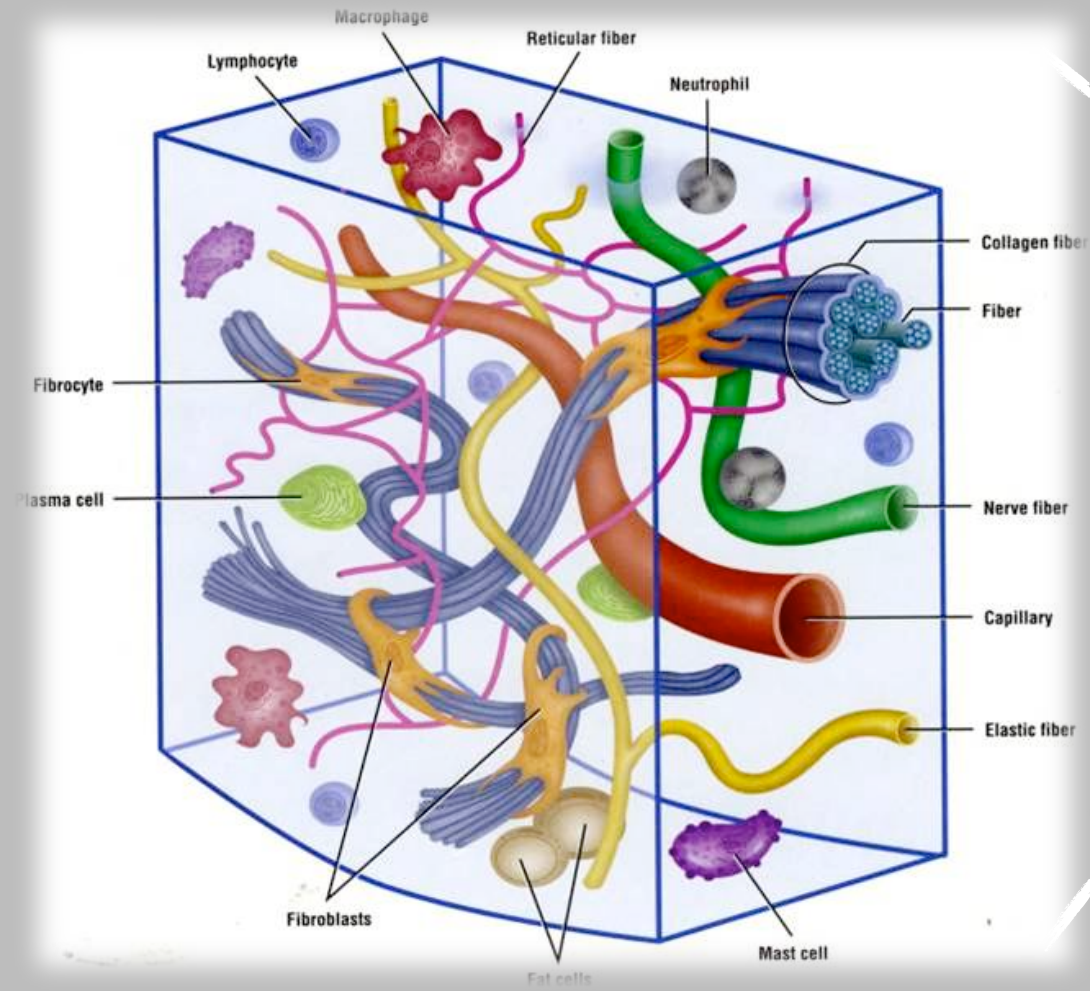
- **Substrate storage manifests as enlargement of affected organs**
- **Brain affliction causes neurodegeneration**

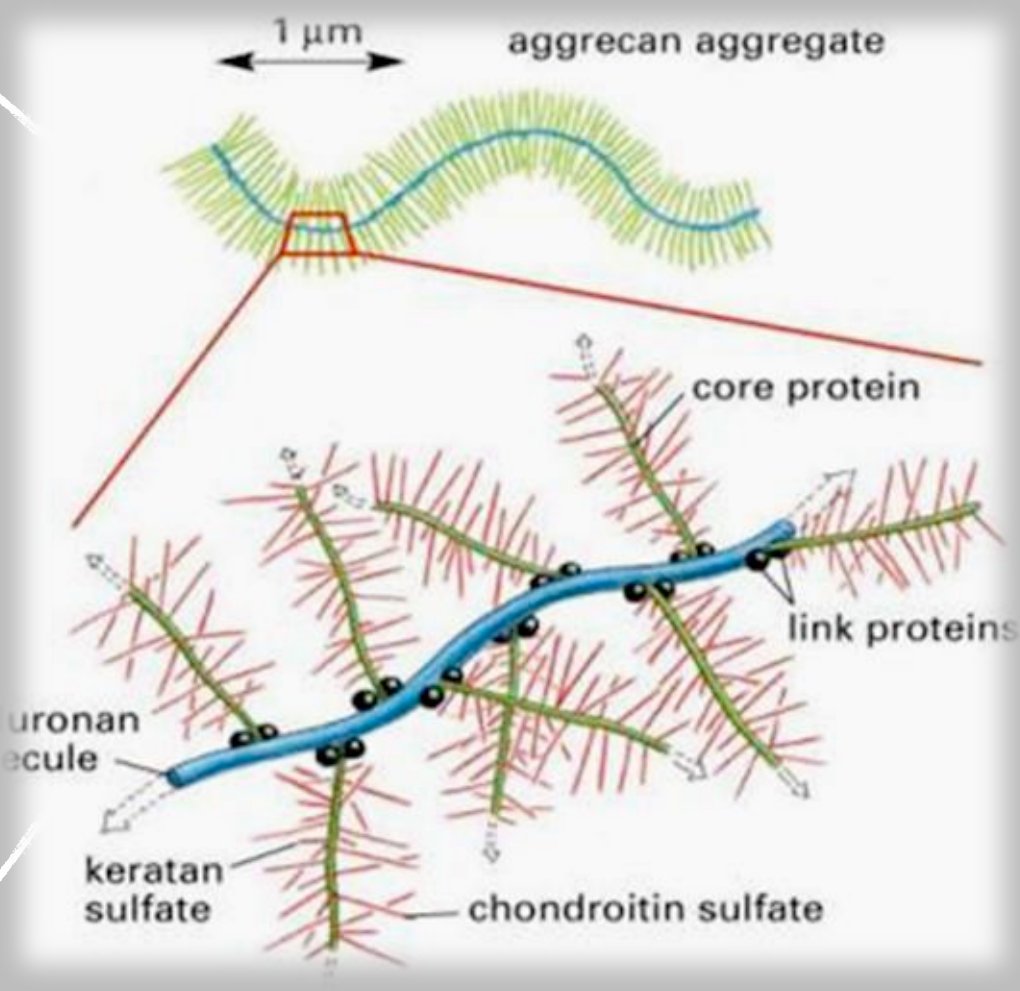
- **Clinical phenotype suggests the class of storage disease, not the disease itself**
- **More than 50 lysosomal disorders described**
  - **lysosomal hydrolase deficiency**
  - **lysosomal membrane transport deficiency**

- Until recently: no treatment available
- **Now**: enzyme replacement therapy for some

Mucopolysaccharidosis

- = glycosaminoglycans (GAGs)
- Polysaccharide chains synthetized by connective tissue cells



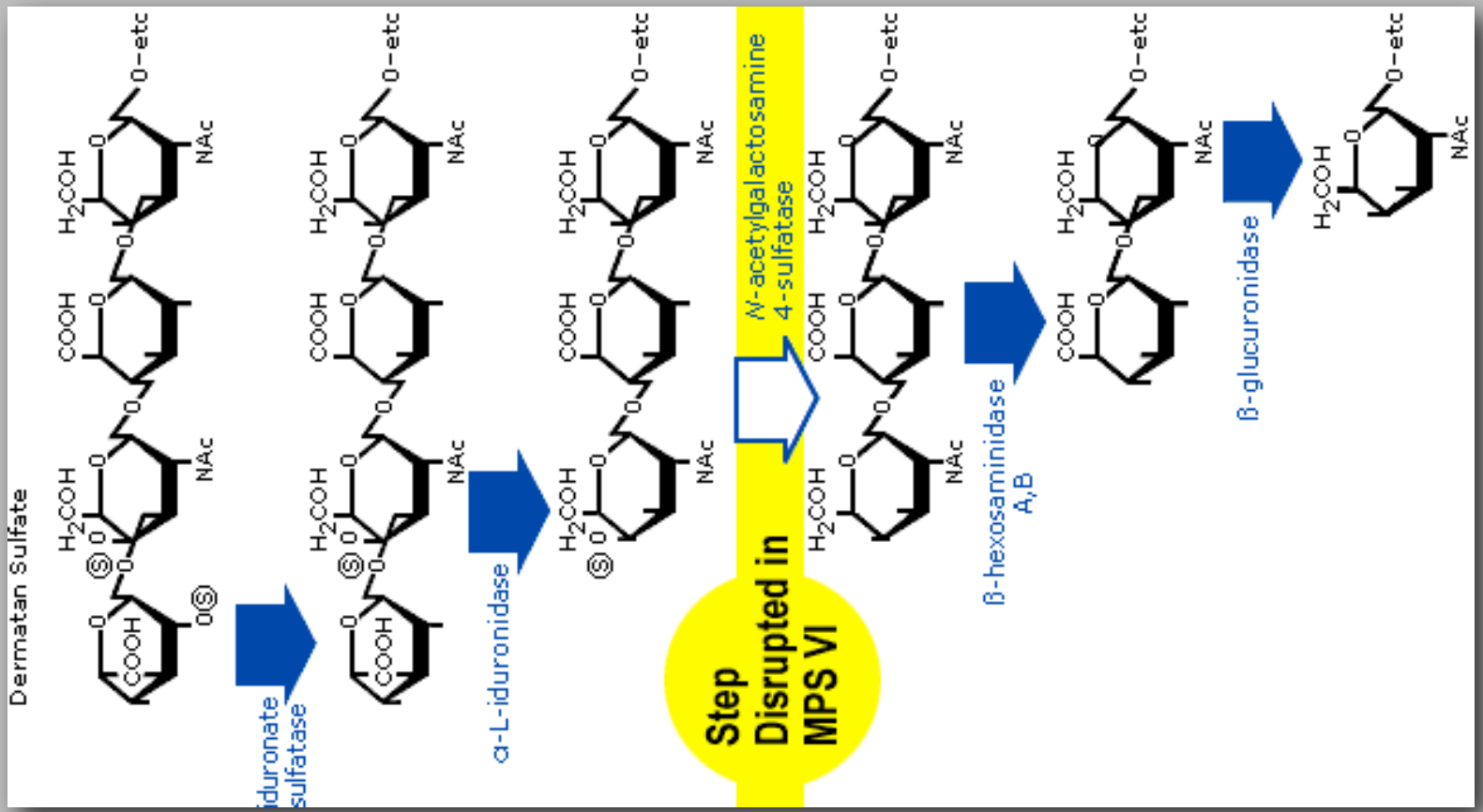


Degradation of GAGs

- Degradation occurs in lysosome
- Removal of monosaccharide at the end of the chain
- Specific enzyme for the monosaccharide and its bond

  ⇒ a series of enzymes is needed to degrade any GAG

  ⇒ an enzyme often participates in the degradation of more than one GAG



Mucopolysaccharidosis

- Heterogeneous group of diseases

Table 1 - Mucopolysaccharidosis classification[2]

Type	Eponym	Enzyme deficiency	Glycosaminoglycans excreted in urine	Inheritance
MPS I	Hurler Hurler/Scheie Scheie	α-L-iduronidase	ds/hs	AR
MPS II	Hunter	Iduronate sulfatase	ds/hs	XLR
MPS III	Sanfilippo A Sanfilippo B Sanfilippo C Sanfilippo D	Heparan N-sulfatase α-N-acetylglucosaminidase Acetyl-coa- α-glucosaminide Acetyltransferase N-acetylglucosamine -6-sulfatase	hs	AR
MPS IV	Morquio A Morquio B	Galactosamine-6-sulfatase β-galactosidase	ks/ chondroitin 6-sulphate ks	AR AR
MPS VI	Maroteaux-Lamy	N- acetylgalactosamine 4-sulfatase	ds	AR
MPS VII	Sly	β-Glucuronidase	ds/hs/chondroitin 4-,6-sulphate	AR
MPS IX*	Natowicz	Hyaluronidase	Hyaluronic acid	AR

AR = autosomal recessive; ds = dermatan sulphate; hs = heparan sulphate; ks = keratan sulphate; MPS = mucopolysaccharidoses; XLR = X-linked recessive.
* Just one patient has been described in the literature.

- **Depending on the defective enzyme, one or more GAGs can accumulate in MPS**
- **Urine detection of GAGs is used as screening test**

Hunter and Hurler syndrome

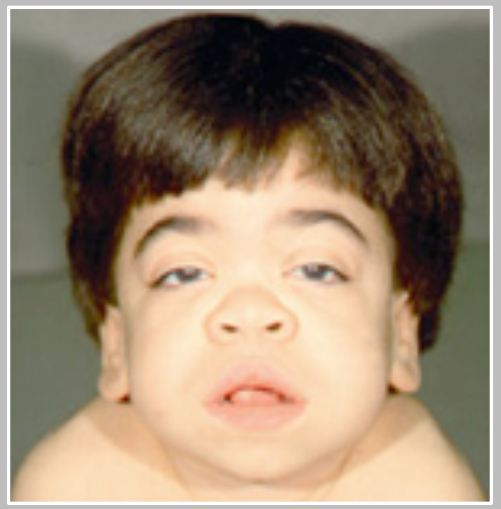
Hunter

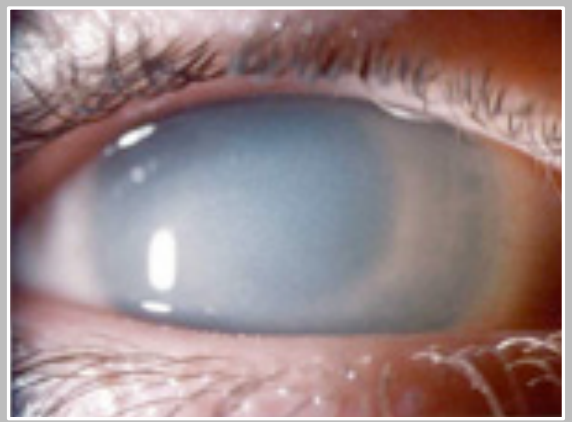
- X-linked recessive
- Slower progression

Hurler

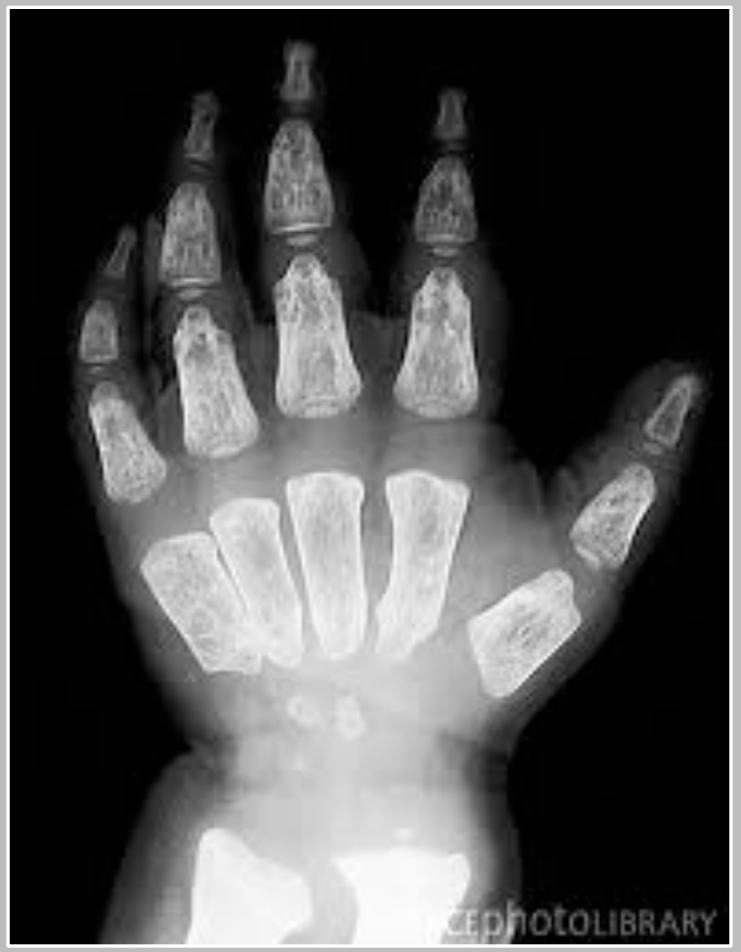
- Autosomal recessive
- More severe phenotype

- Mental retardation
- Coarse facies
- Corneal clouding
- Skeletal changes
- Short stature
- HSM





Hurler disease



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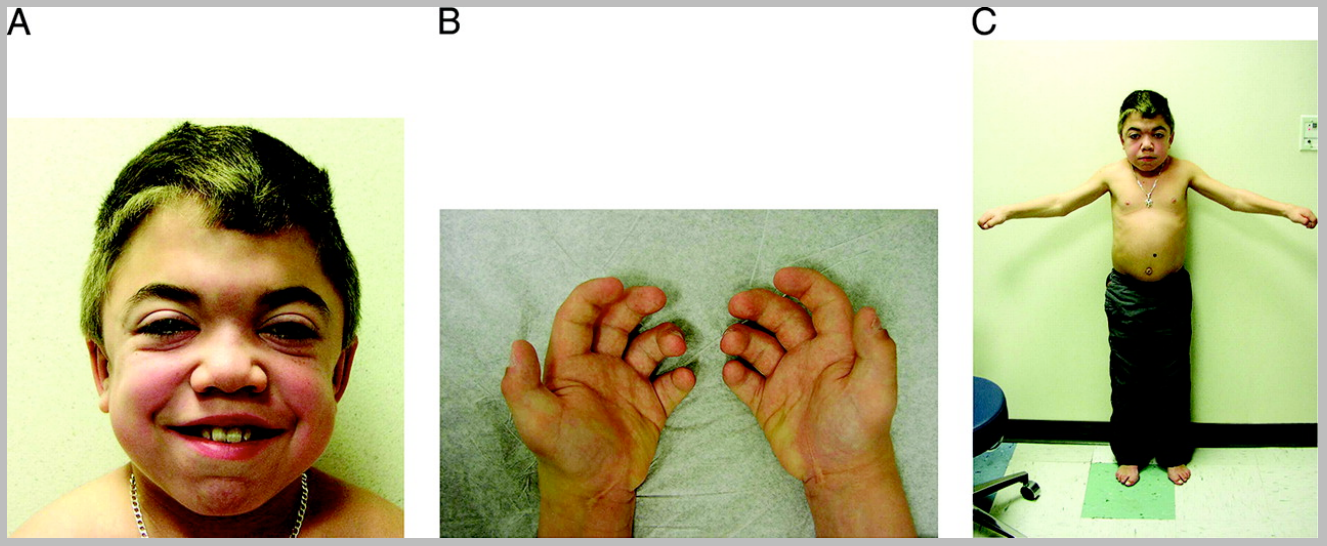


Hunter disease

Genetic etiology: Hurler versus Hunter syndrome

- Different mode of inheritance indicated different gene involvement
- Difference is also observed in fibroblasts of patients
  - **Both accumulate MPS in culture medium**
  - **This accumulation is corrected by co-cultivation of both cell types in the same culture dish**
    → due to uptake of normal L-alpha-iduronidase released by Hunter fibro's by the Hurler fibro's

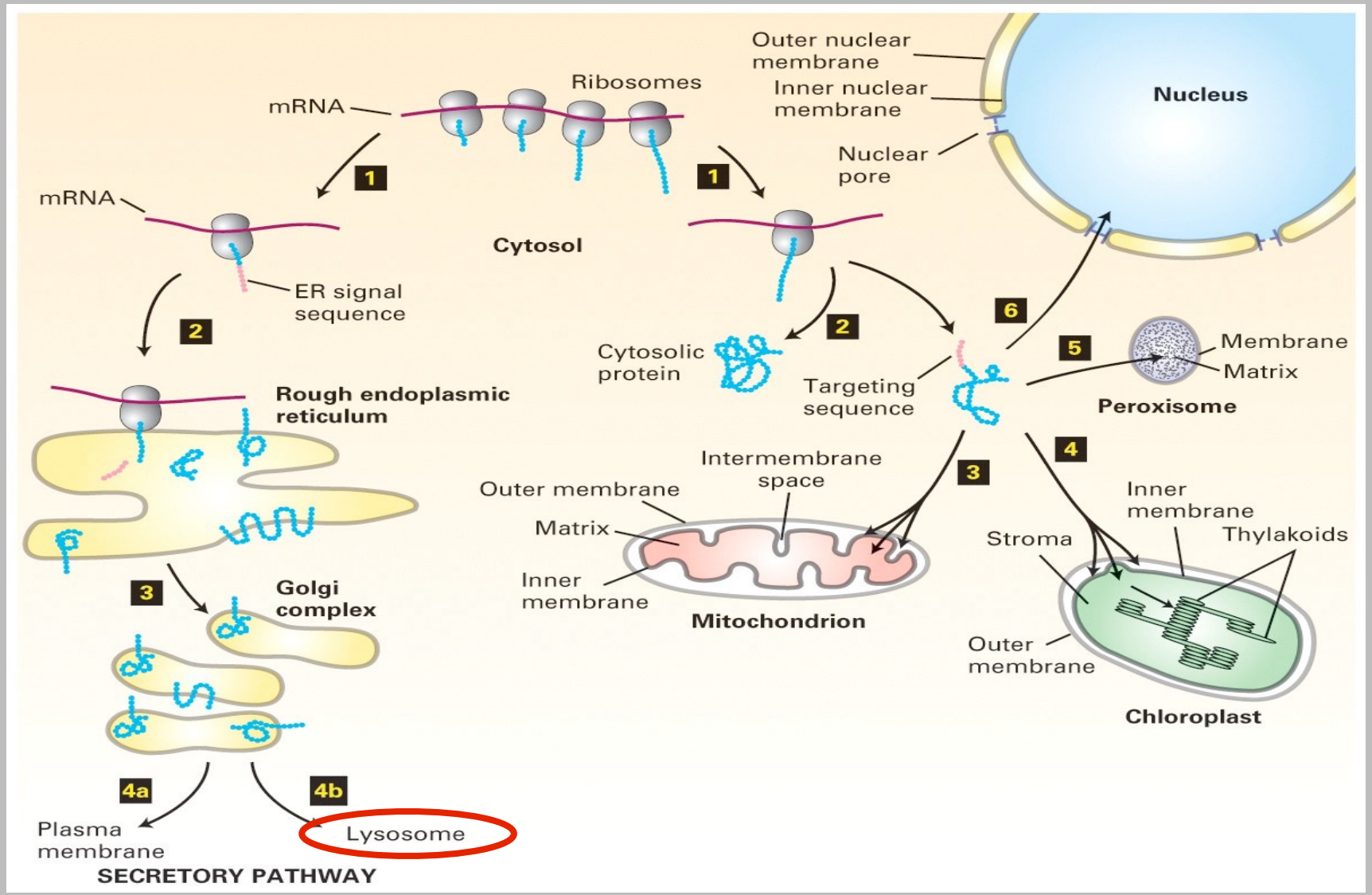
- Ability of cell to take up the lysosomal enzyme it needs from the EC fluid is mechanism by which transplantation of normal cells may correct defect
  - ⇒ Bone marrow transplantation
  - ⇒ Enzyme replacement therapy

Genetic complementation/ complementation analysis

Demonstration that a product of the genome of one mutant is able to correct the biochemical effect in another mutant

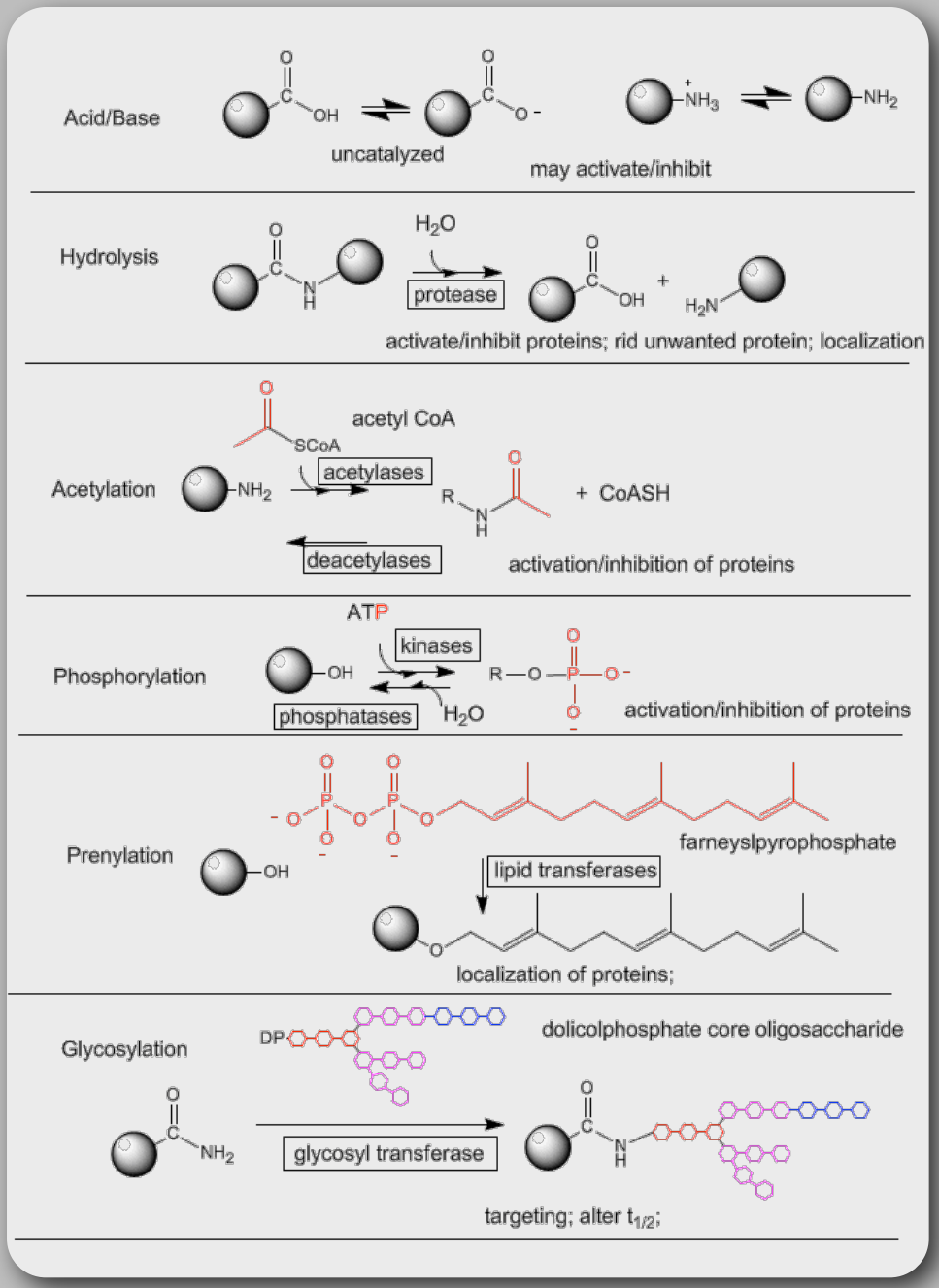
Abnormal posttranslational modification

How to proteins get to their correct locations inside the cell?



- **Info in primary AA sequence directing them**
- **Post-translational modifications**

Post-translational modification



Loss of glycosylation

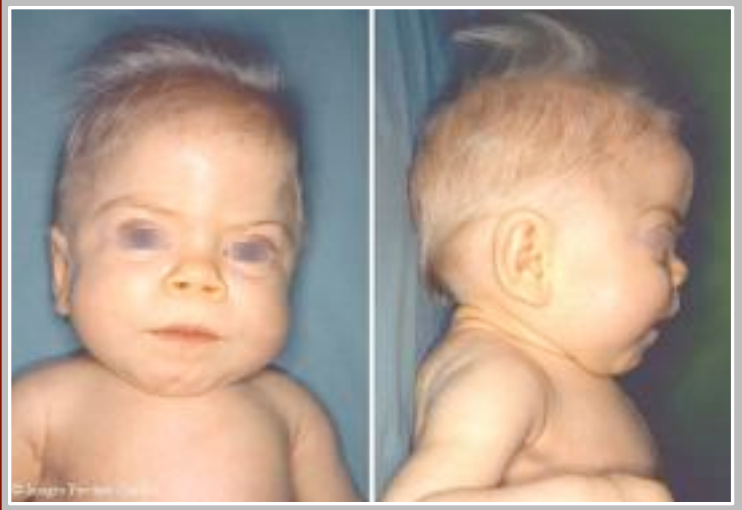
- **I-cell disease**

Gain of glycosylation

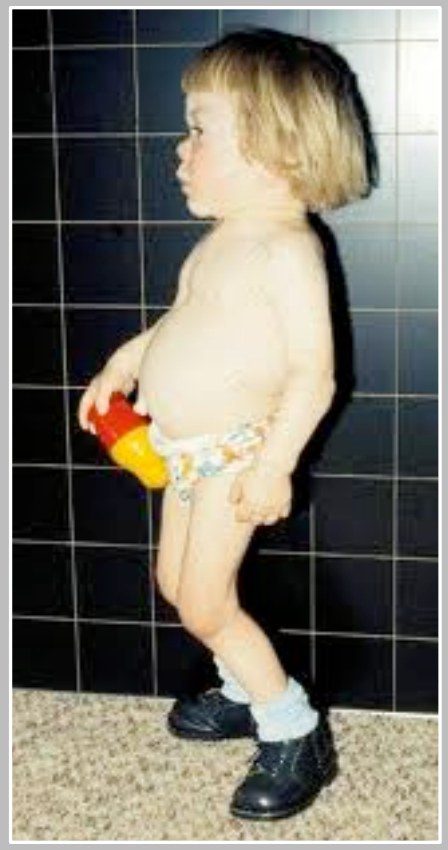
- **Mendelian Susceptibility to Myobacterial Disease**

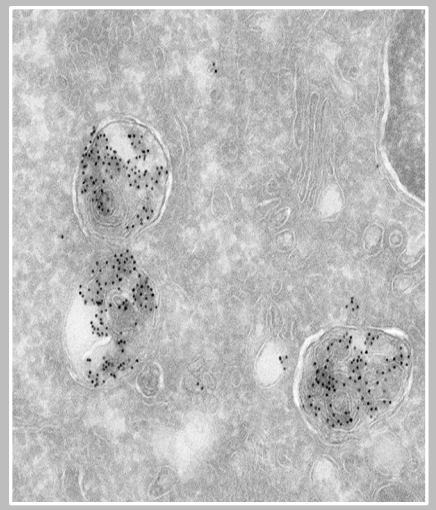
I-cell disease

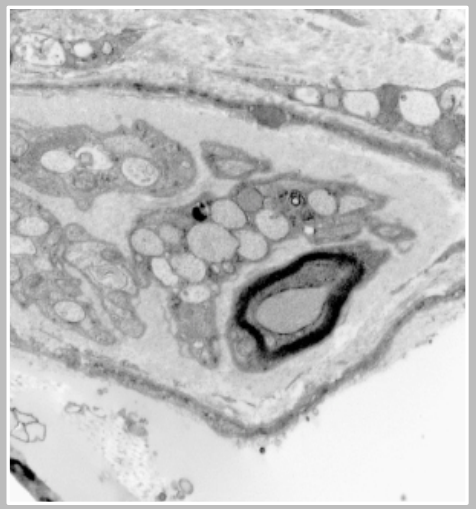
- Severe AR lysosomal storage disorder



- **Mental retardation**
- **Facial features**
- **Skeletal changes**
- **Short stature**
- **HSM**
- **Mean age: 5-7 years**





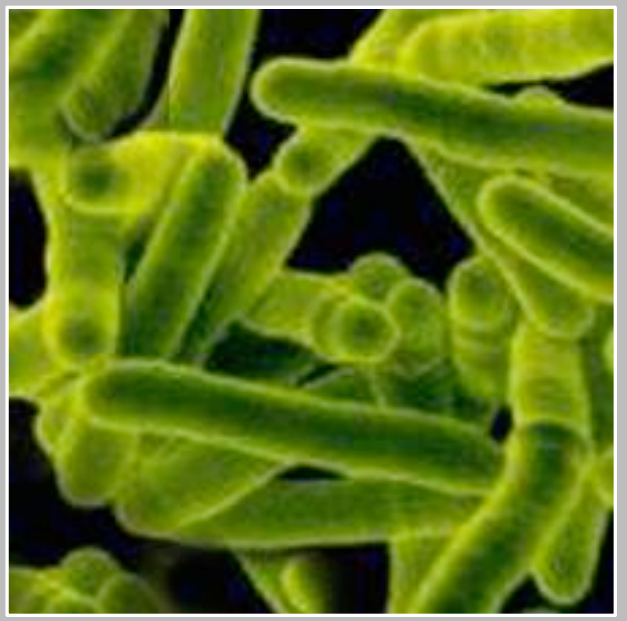


- **Fibroblasts contain abnormal lysosomes**
- **Inclusions in cytoplasm**

- Disturbed trafficking of acid hydrolases
- Acid hydrolases found in excess in body fluids and diminished cellularly
- Due to failure of post-translational modification

Creation of novel glycosylation sites

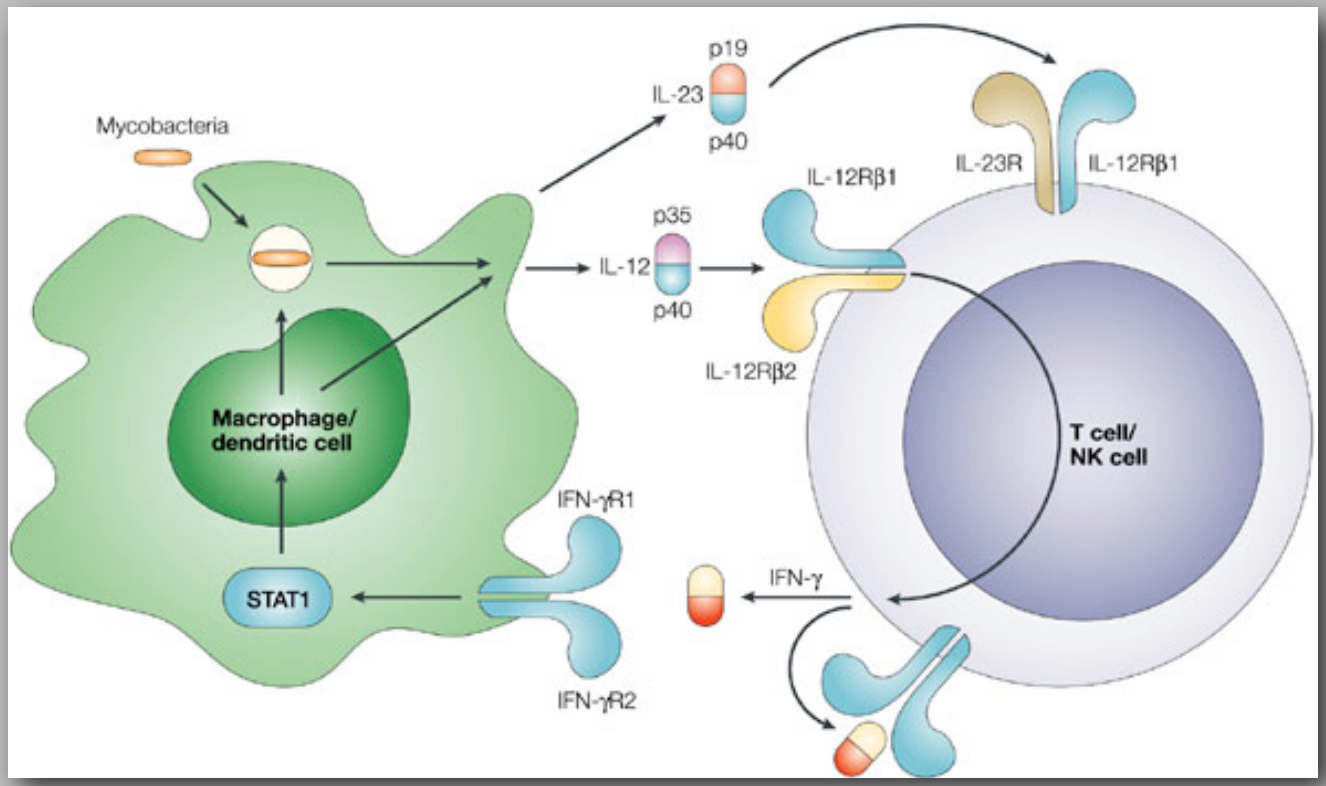
- 1.5% of missense mutations causing human disease may be associated with abnormal gains of N-glycosylation
- Studied in MSMD (Mendelian Susceptibility to Myobacterial Disease)
  - **Autosomal recessive**
  - **Defect in gene(s) that regulate defense against infection**
  - **Susceptible to dissiminated infections when exposed to mycobacterial species**
    - **BCG (tuberculosis) vaccin (Bacillus Calmette-Guerrin)**
    - **innocent non-tuberculous environmental bacteria**



Creation of novel glycosylation sites

- 1.5% of missense mutations causing human disease may be associated with abnormal gains of N-glycosylation
- Studied in MSMD (Mendelian Susceptibility to Myobacterial Disease)
  - **Can result from mutations in the *IFNGR2* gene (interferon gamma receptor 2)**
  - **Mutation generates novel glycosylation sites in the mutant protein**
  - **Leads to an abnormally glycolysed (and hence large) receptor**

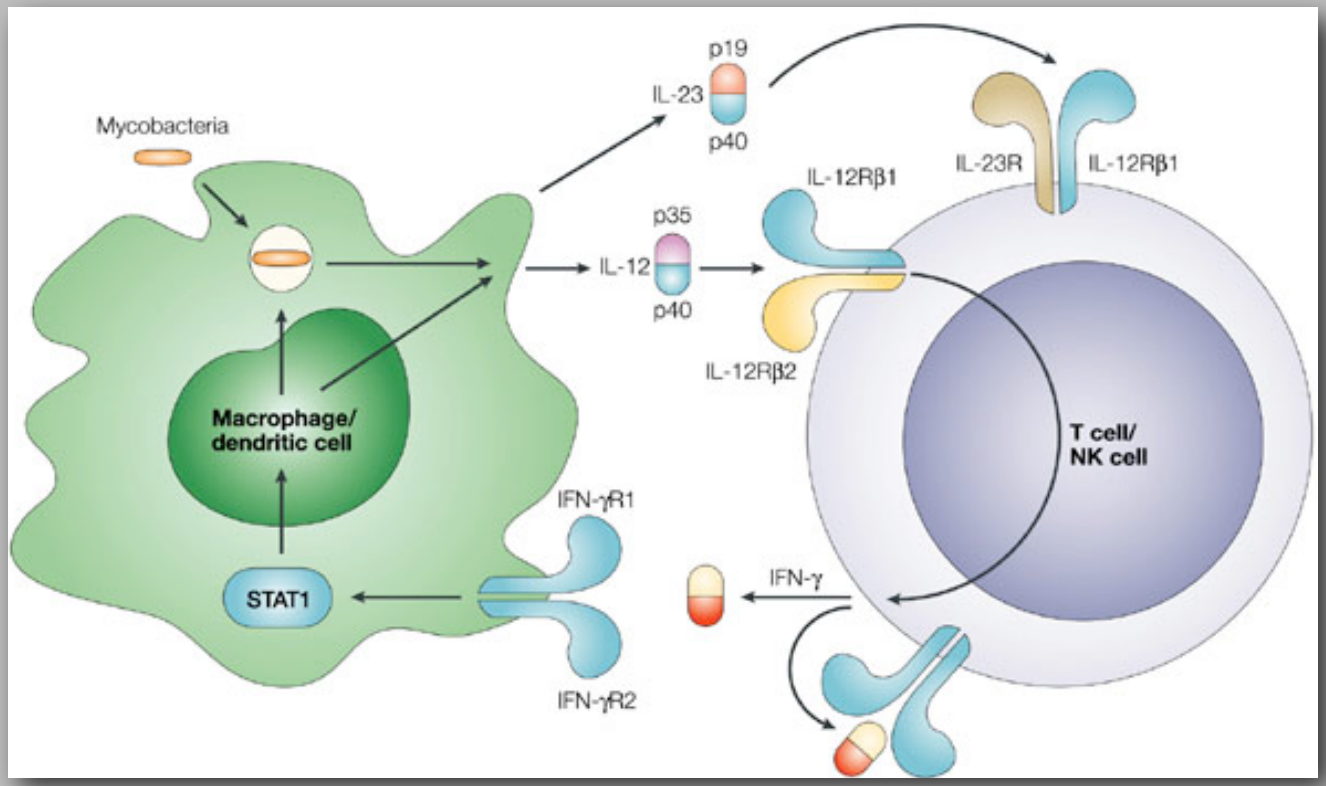
    → does not respond to interferon gamma



Creation of novel glycosylation sites

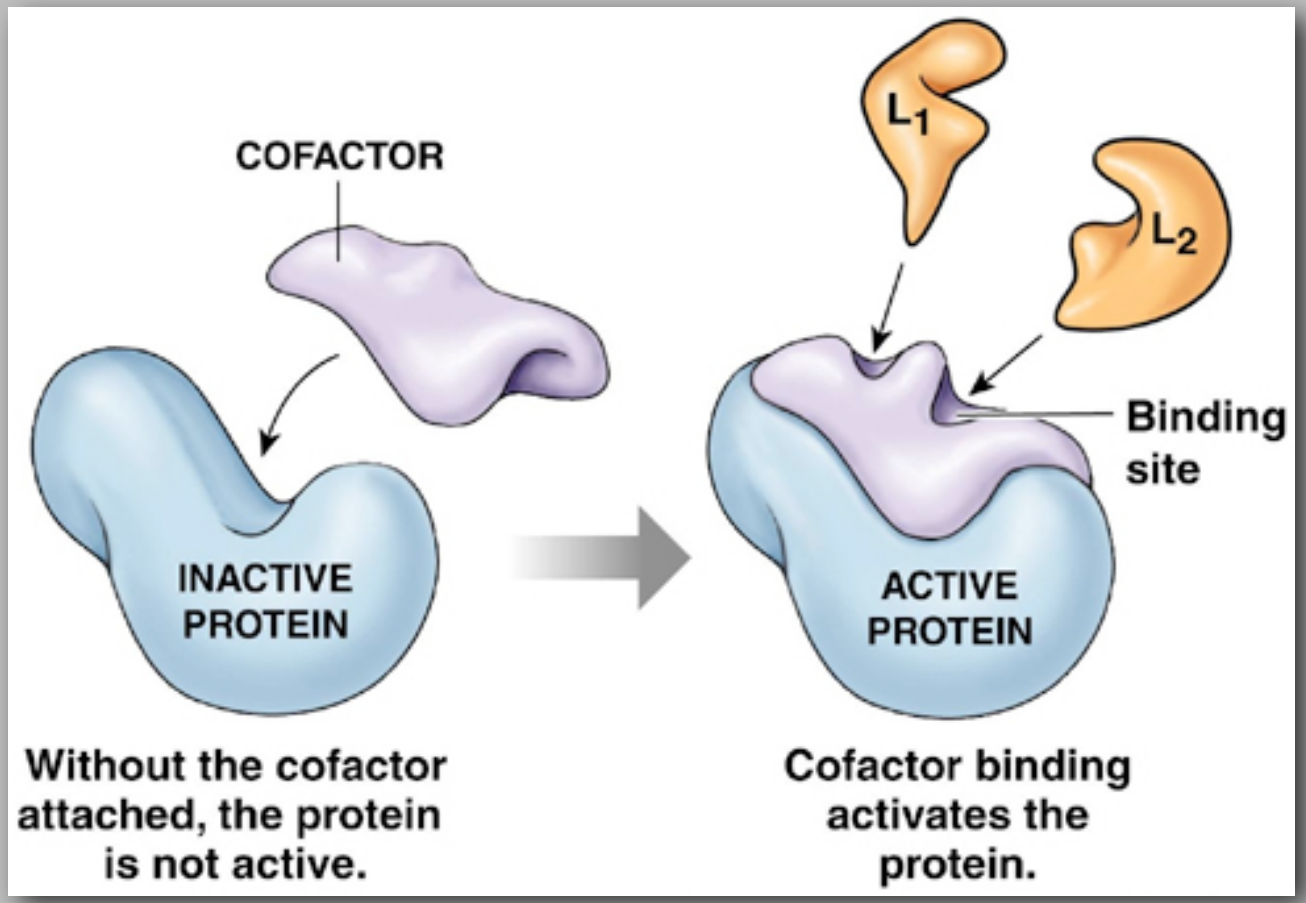
- 1.5% of missense mutations causing human disease may be associated with abnormal gains of N-glycosylation
- Studied in MSMD (Mendelian Susceptibility to Myobacterial Disease)

  - **Upon removal of the carbohydrate chains: normal responsiveness to IFN-γ**
  - **Prospect of chemical therapies?**



Impaired binding/metabolism of co-factors

- Association with cofactor may be required for biological activity



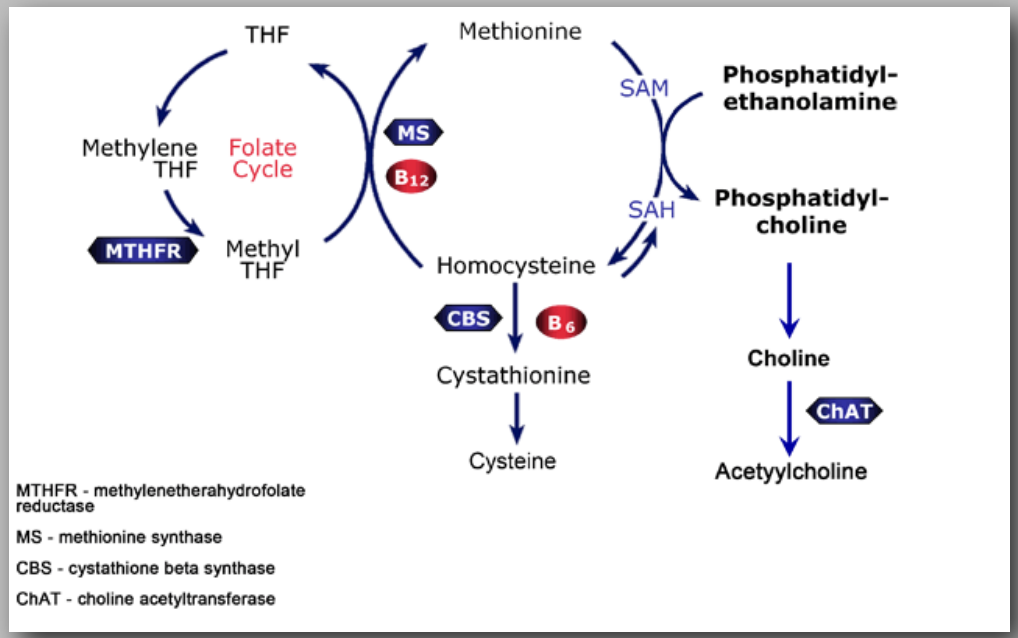
- **Cofactor synthesis**
- **Cofactor transport**
- **Cofactor binding**
- **Cofactor removal**

- Increasing the IC concentration of cofactor may restore some residual activity
- Responsiveness to therapy (cofactor is often water-soluble vitamin)

Disorders due to cofactor metabolism

- Decreased availability of cofactor
- Dietary vitamin deficiencies (acquired - e.g. vegetarians)
  - **Vitamin B_{12} deficiency**
    - anaemia; neurological disease
  - **Vitamin D-deficient rickets**

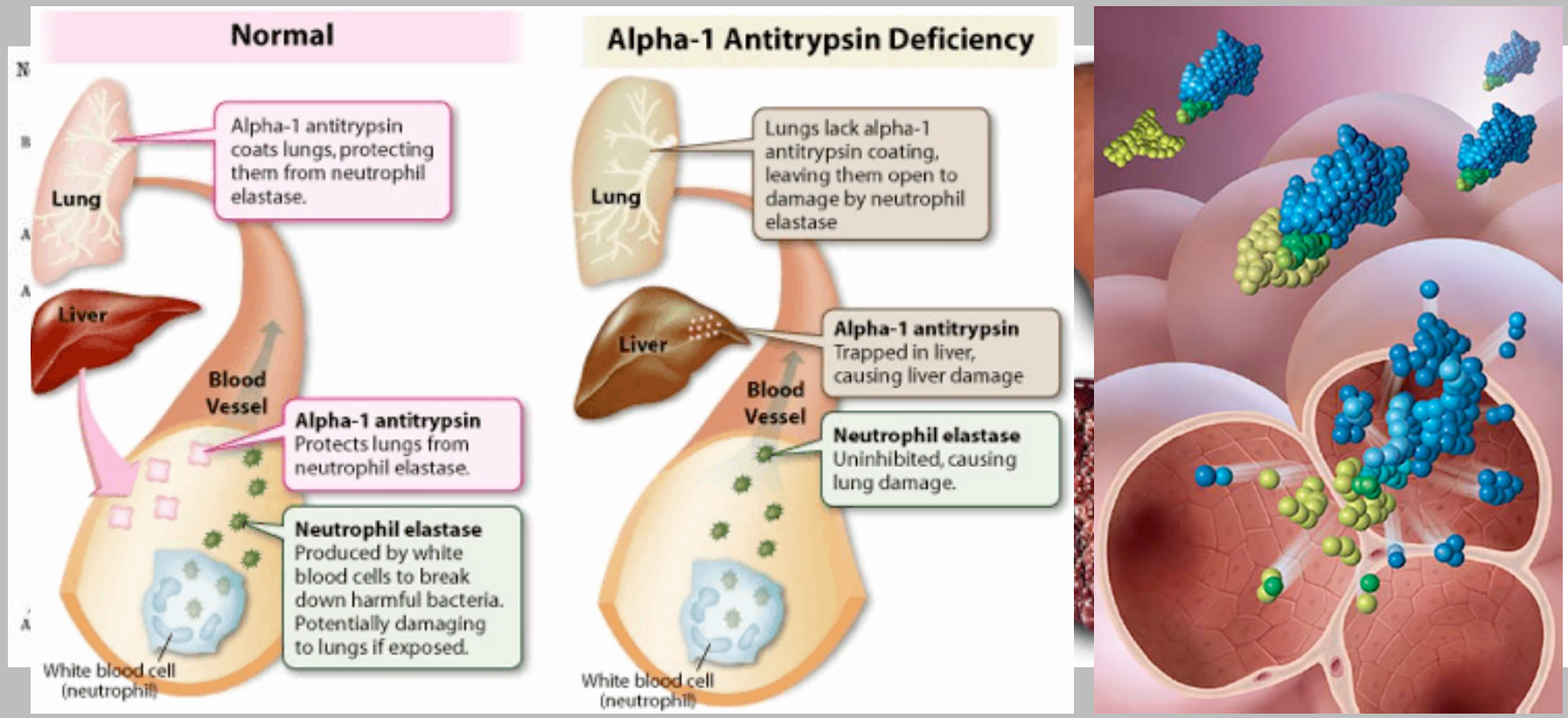
- Hereditary: mutation in genes that impair provision of vitamin B_{12}
  - **Cobalamin transport (intestinal absorption), metabolism**
  - **Methionine synthetase deficiency leads to homocystinuria**



  - **Often treated with high doses of vitamin B_{12}**

α-1 antitrypsin deficiency

- AR, leads to pulmonary emphysema and liver cirrhosis



- α-1 antitrypsin = serine protease inhibitor (serpin)
- Inhibits wide spectrum of proteases, but principle role is to inhibit (neutrophil) elastase

α-1 antitrypsin deficiency

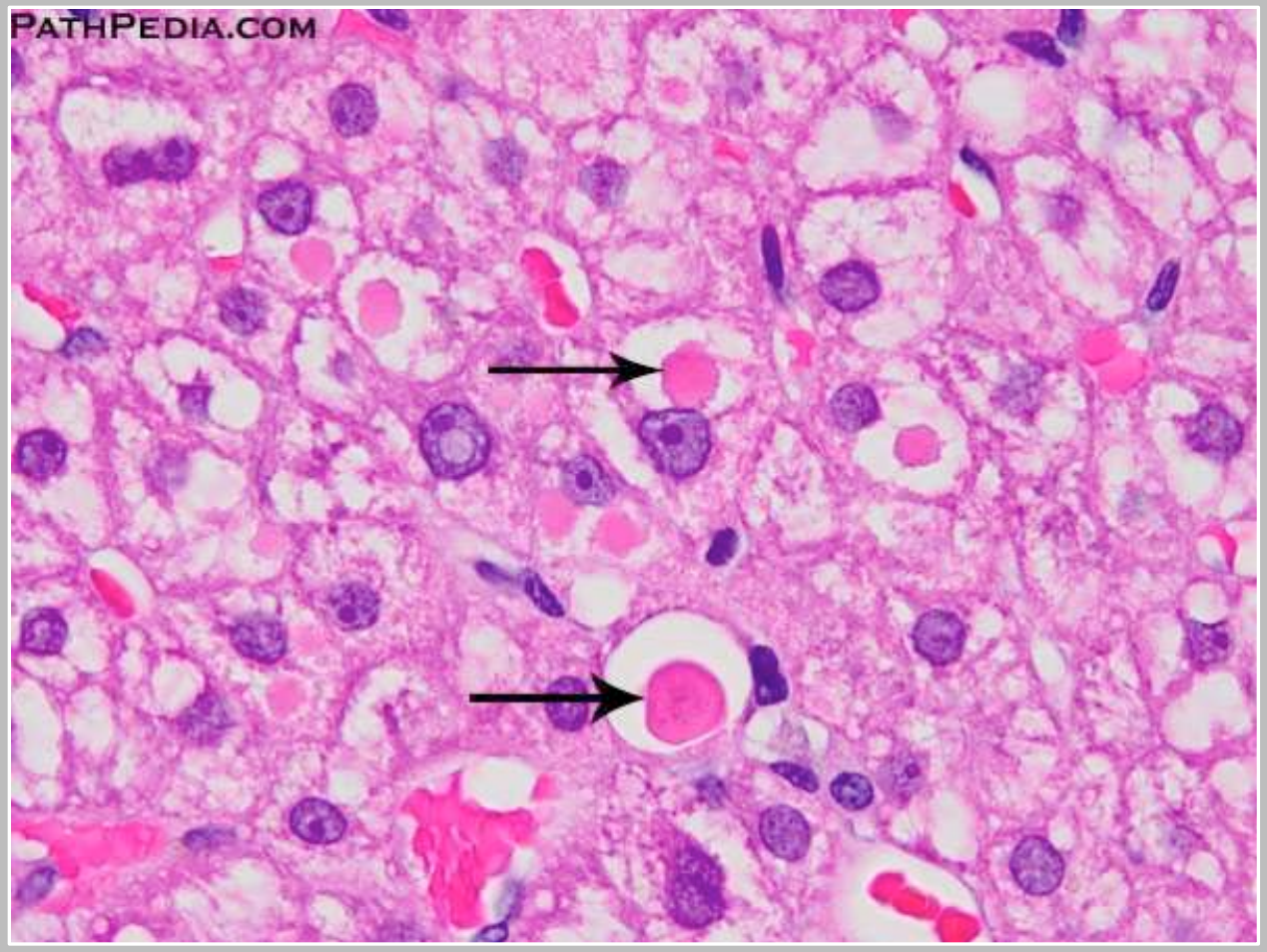
- 1/5000, carrier frequency of 2%
- 60.000 patients in US -> significant health impact
- Several associated alleles; Z-allele is most frequent (founder effect?)

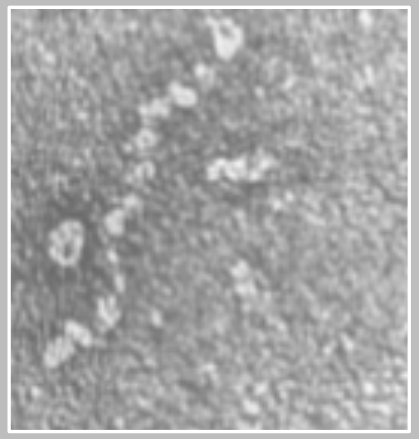
TABLE 1 Common pathogenic variants of α_1-antitrypsin (α_1-AT)

Variant, mutation, polymerisation tendency	Circulating deficiency in homozygotes	Association with clinically significant liver disease	Epidemiology
Z, Glu342Lys, +++	Severe (10–15% of normal levels)	Yes in homozygotes. Lower burden of hepatocyte inclusion bodies seen in heterozygotes, not associated with clinical disease	1 out of 27 of North European populations' heterozygotes. Most common severe deficiency variant. Allele frequency decreases from North-West to South-East Europe
Siiyama, Ser53Phe, +++	Severe	Yes	Most common severe deficiency variant in Japanese populations
Mmalton Δ52Phe, +++	Severe	Yes	Most common severe deficiency variant in Sardinian populations
S, Glu264Val, +	Moderate (60% of normal levels in homozygotes, equivalent to MZ α_1-AT heterozygotes)	Reported in SZ α_1-AT compound heterozygotes	Most common deficiency allele; 1 out of 5 Europeans are heterozygotes. Frequency decreases from South-West to North-East Europe
I, Arg39Cys, +	Mild (extrapolation from levels in heterozygote)	Case report in IZ α_1-AT heterozygote	Only reported in compound heterozygotes

α-1 antitrypsin deficiency

- *α-1AT* gene mostly expressed in liver (secretion into plasma)
  - **Z/Z homozygotes:**
    - **17% presents with neonatal jaundice**
    - **20% develops cirrhosis**





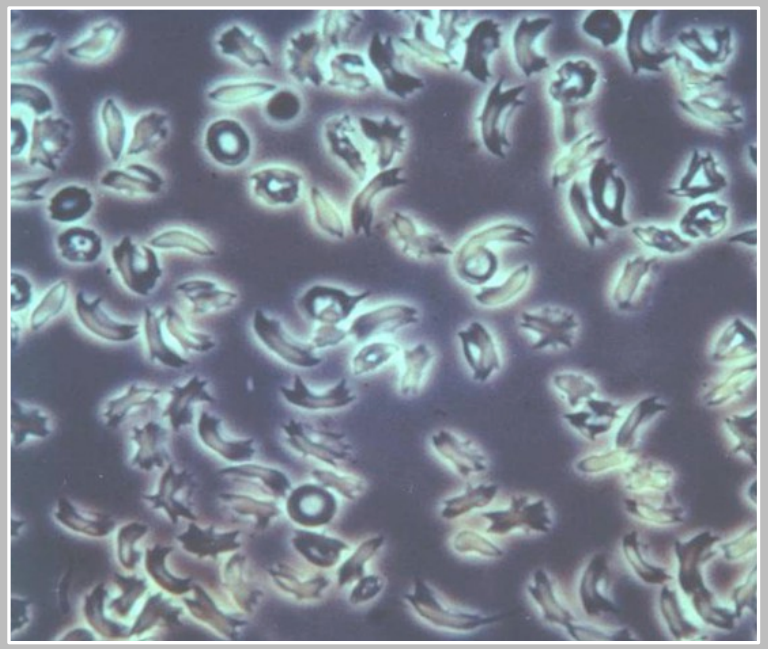
- **Aggregation/ trapping of the mutant in the ER**
- **Structural change in protein: formation of bead-like polymers**

↓

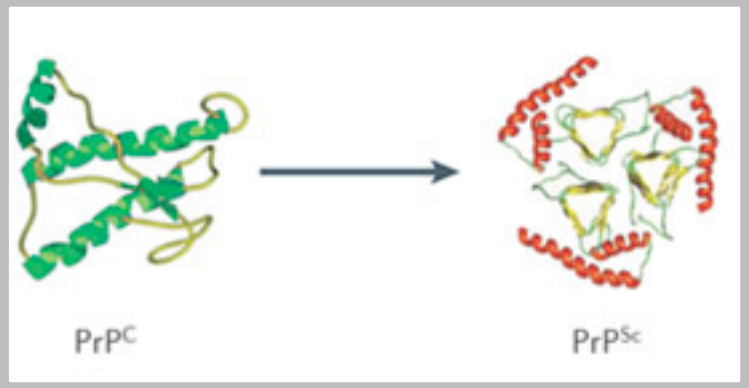
Conformational disease

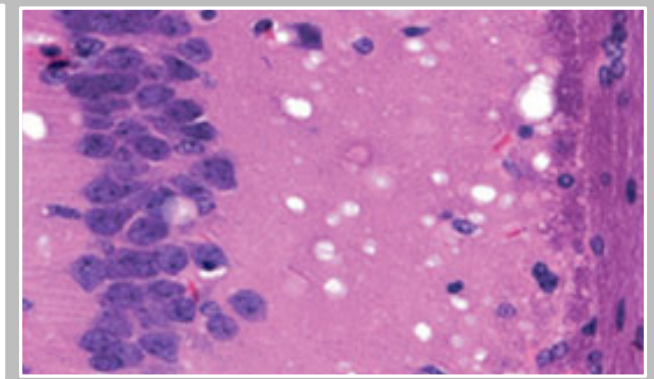
Conformational diseases

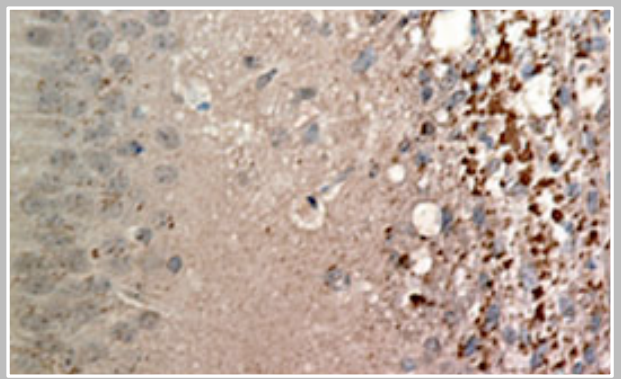
- Mutation causes the shape or size of the protein to change
- Predisposition to self-association and tissue deposition



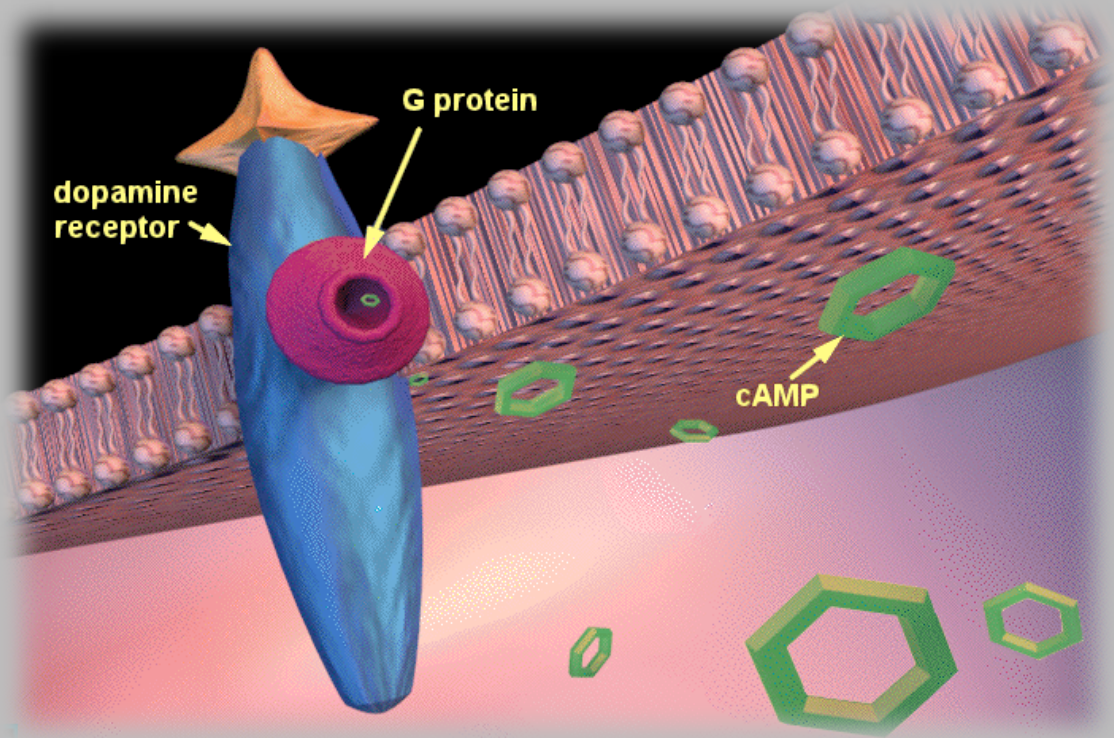
- Fraction of the mutant protein is correctly folded
- Not always single gene disorders (e.g. prion diseases)







Defects in receptor proteins

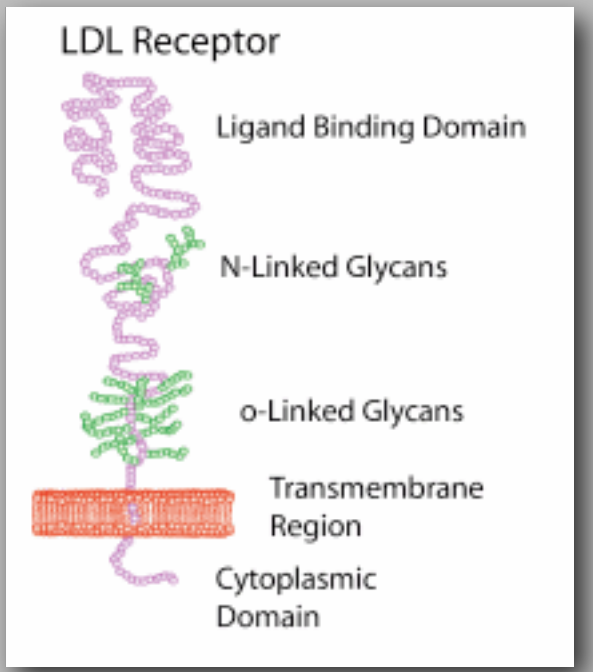


Receptor protein diseases: how it began...

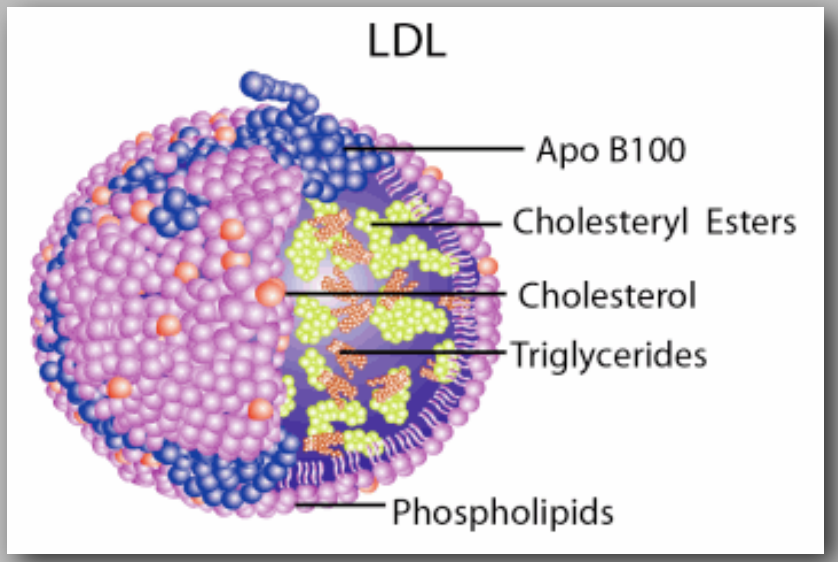
- 1974: identification of the LDL-receptor being implicated in familial hypercholesterolaemia

Joseph Goldstein

Michael Brown



- Elevated plasma cholesterol
- Increased risk for AMI



Cholesterol metabolism

Cell surface receptors

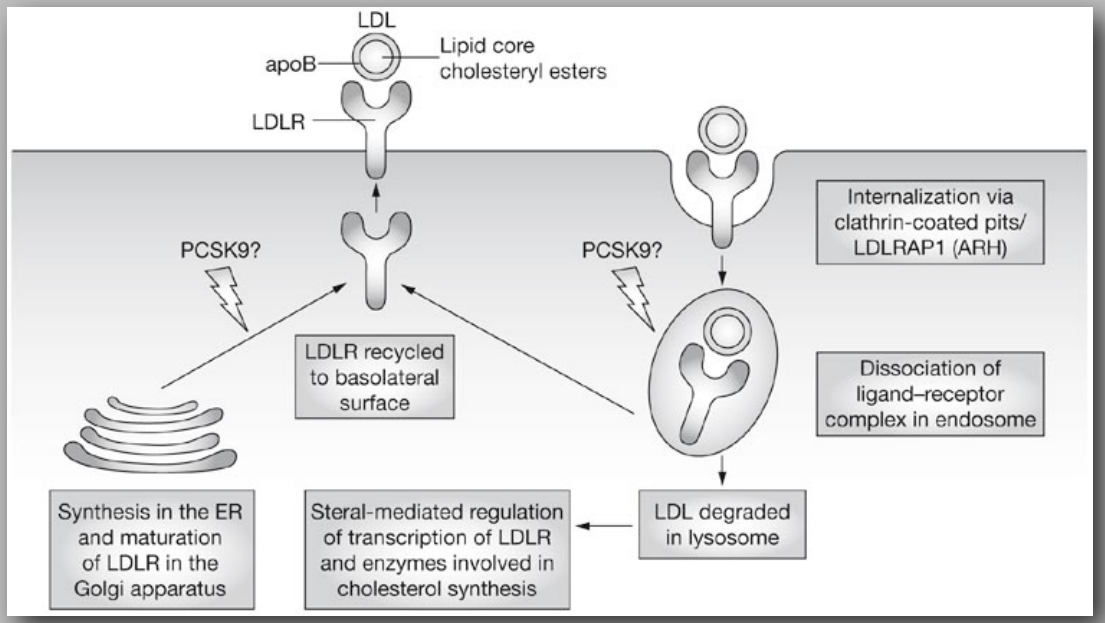
Familial hypercholesterolaemia

- Example of hyperlipoproteinaemia: increased plasma concentration of:
  - **Lipids (cholesterol, triglycerids, both)**
  - **Specific plasma lipoproteins**
  - **Several monogenic hyperlipoproteinaemias have been identified**

- Due to mutations in one of 4 genes
  - **LDL receptor**
  - **ARH adaptor protein (coated pit)**
  - **ApoB**
  - **PCSK9 (receptor degradation)**

Closely related

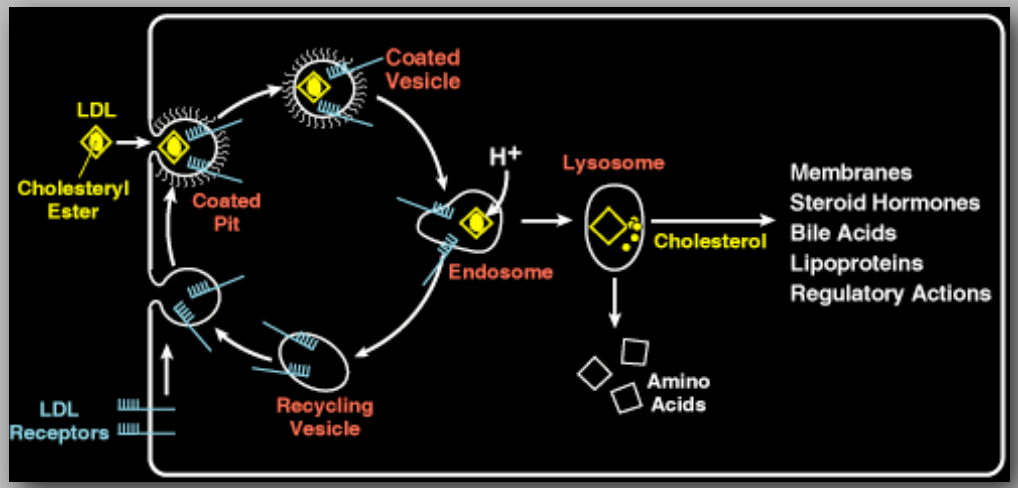
Phenotypically difficult to distinguish



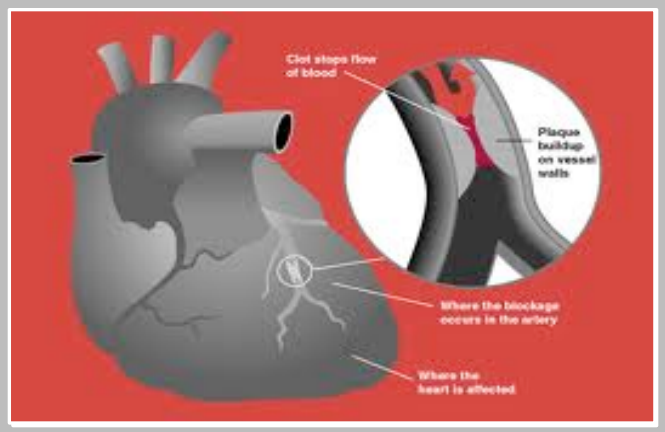
- Sometimes dual effect: harmful vs. protective (e.g. *PCSK9* mutations)

LDL receptor mutations

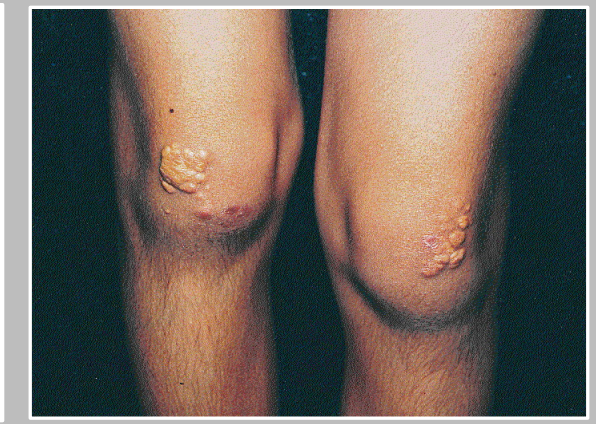
- Most common cause of familial hypercholesterlaemia
  - **Membrane bound receptor**
  - **Binds LDL and delivers to cell interior**



- Both homozygotes and heterozygotes develop premature heart disease



Atheroma



Xanthoma

Corneal arcus

LDL receptor mutations

- Autosomal semi-dominant trait
  - **Both homozygous and heterozygous phenotypes are known; gene dosage effect is evident**

Homozygous

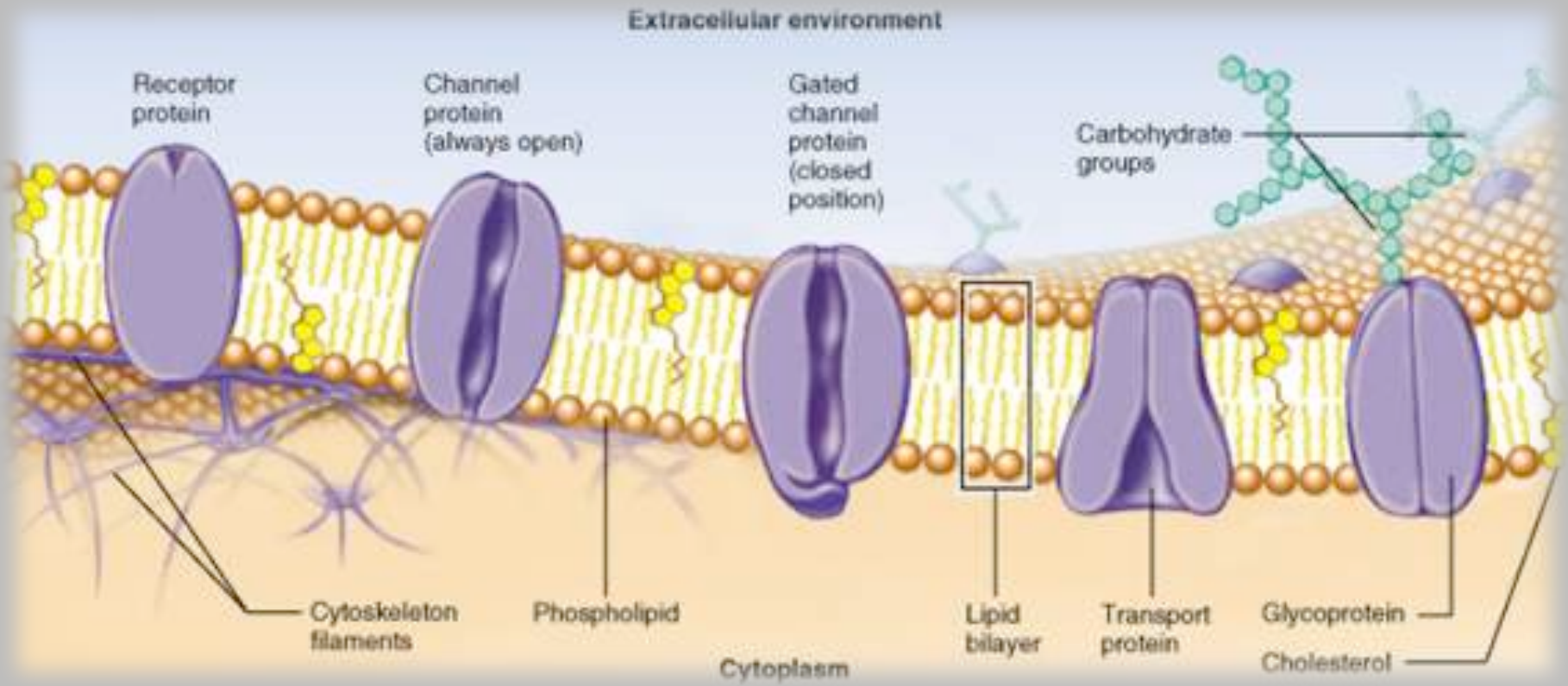
- **Manifests earlier**
  - **clinically significant CHD in childhood**
  - **often demise before the third decade**
- **More severely**
- **Greater reduction in n LDL receptors**
- **Greater LDL cholesterol elevation in plasma**

Heterozygous

- **1:500**
- **Cholesterol levels x2 compared to controls**

- Important to screen for in AMI survivors (5% heterozygotes)
- Only 1:20 hypercholesterolaemia patients suffer from familial HCH.

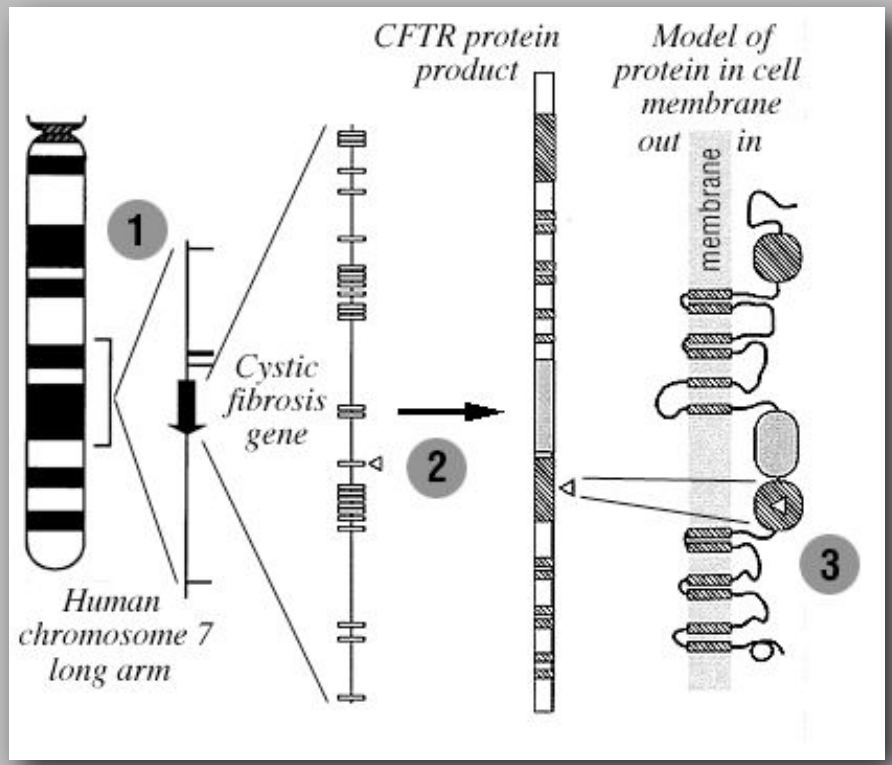
Transporter defects

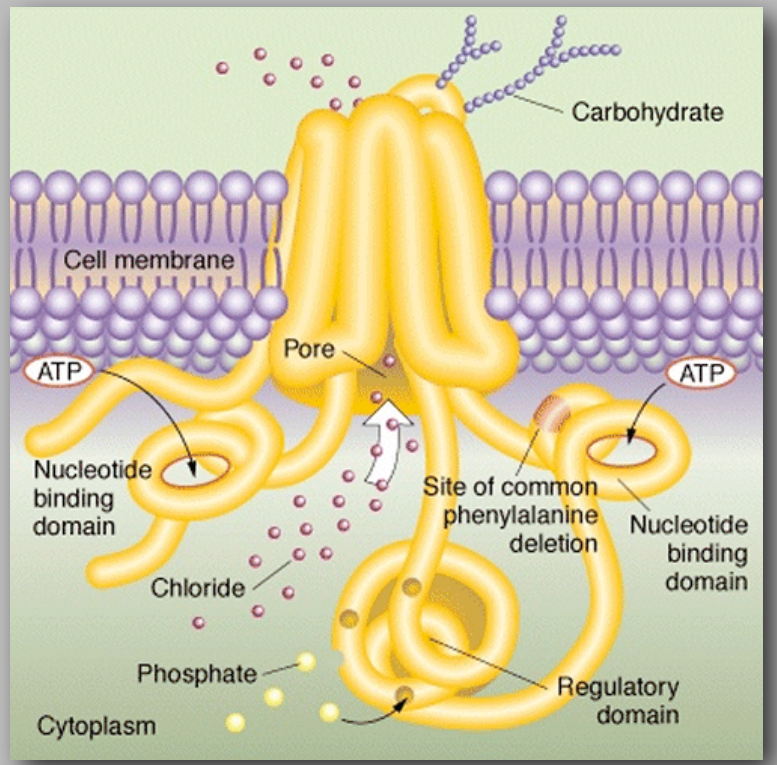


Cystic fibrosis

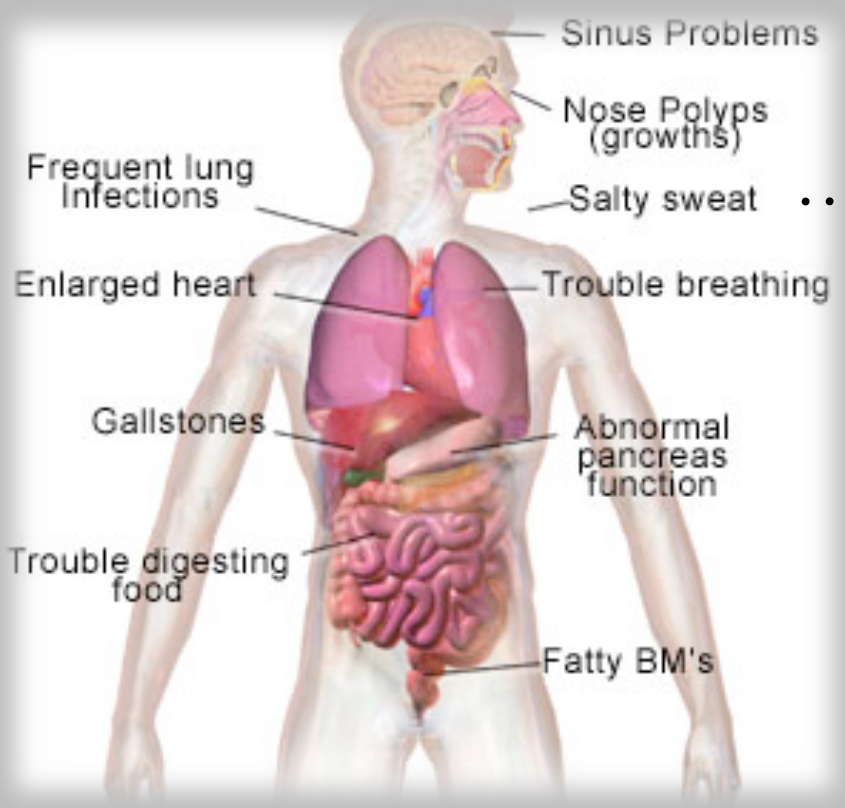
- Most common fatal autosomal recessive disorder in Caucasians
- Incidence 1:2500; carrier frequency 1:25

- 1989: positional cloning of the *CFTR* gene
- Shortly after: encodes a regulated chloride chanel in the apical membrane of epithelial cells





Phenotypes of cystic fibrosis



Increased sodiumchloride concentrations in sweat

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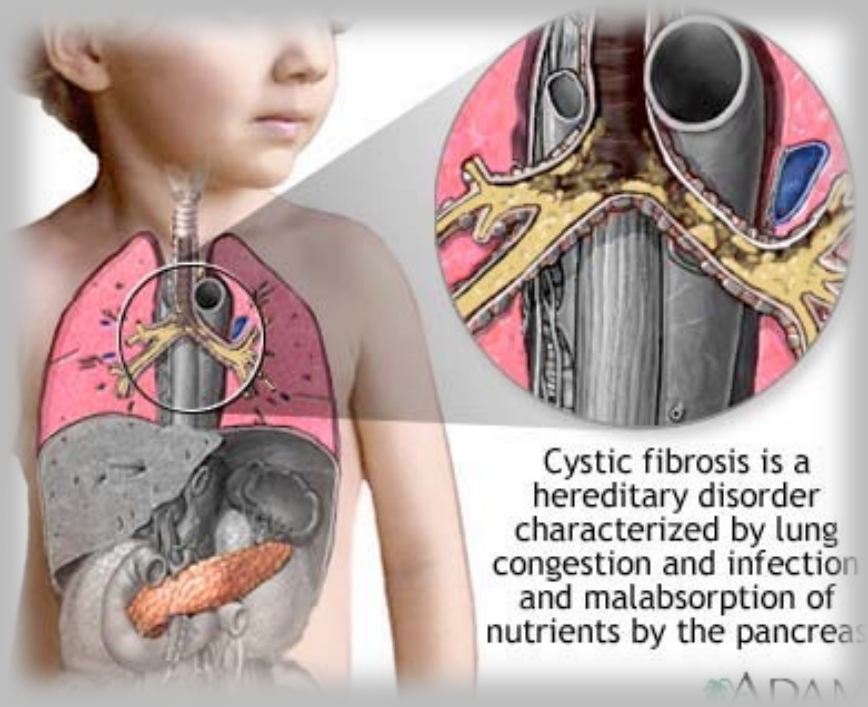
Diagnostic test

<2% of patients has normal sweat Cl^- concentration

↓

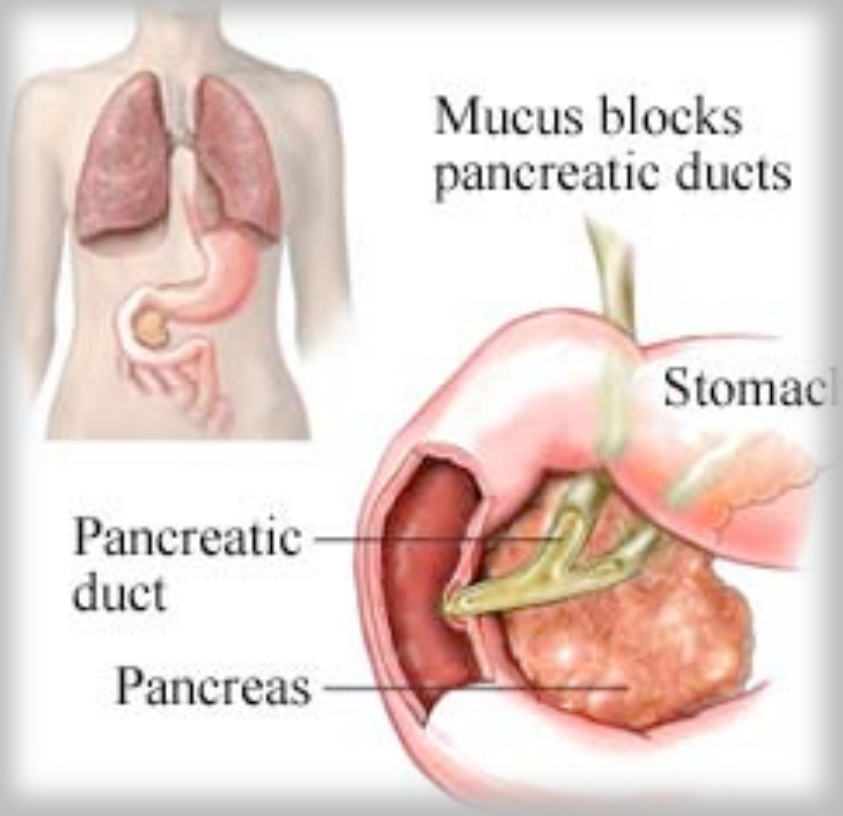
Molecular test

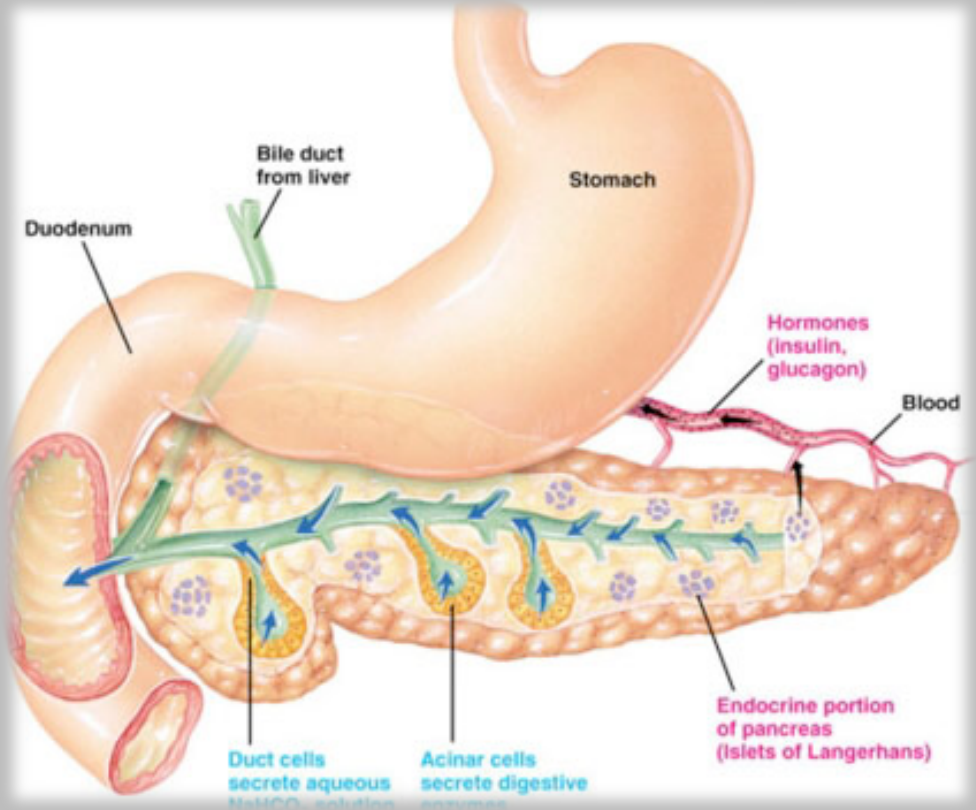


Pulmonary disease

- **Thickened secretions**
- **Recurrent infections**
- **Obstructive lung disease**
- **Bronchiectasias**
- **Often cause of death**

Phenotypes of cystic fibrosis



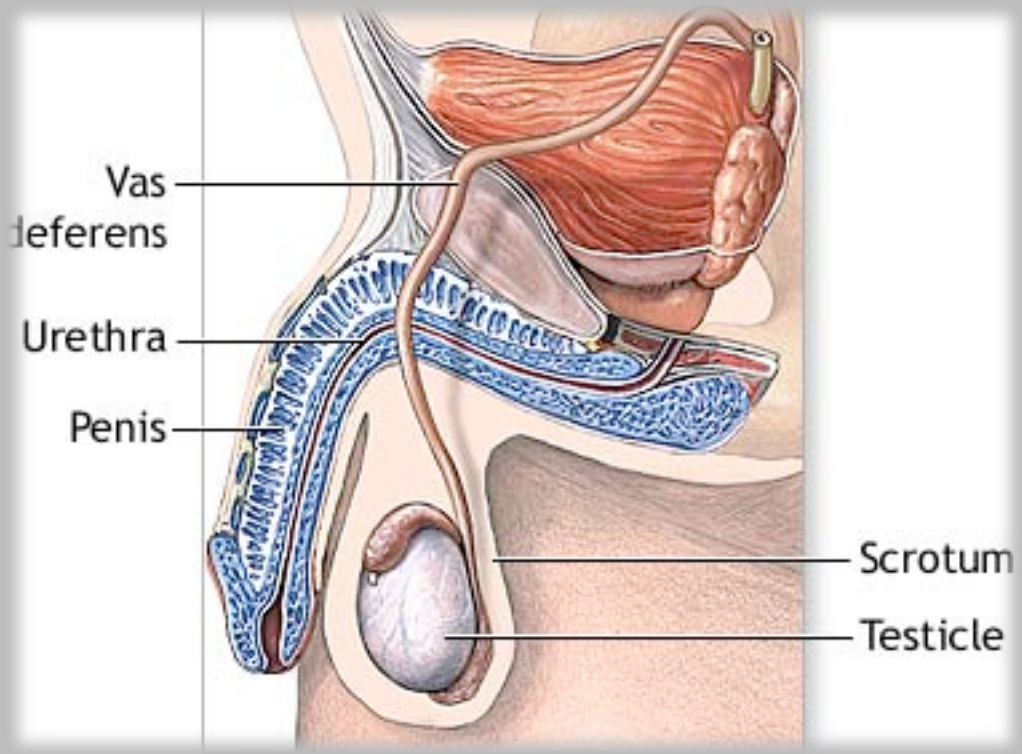


Pancreatic disease

- Maldigestion due to deficient secretion of enzymes (lipase, trypsin, chymotrypsin)
- Can be restored by enzyme supplements
- 5-10% of patients are pancreatic sufficient: enough residual function
- Overall prognosis of the latter is better
- DIFFERENCE: allelic heterogeneity

Phenotypes of cystic fibrosis

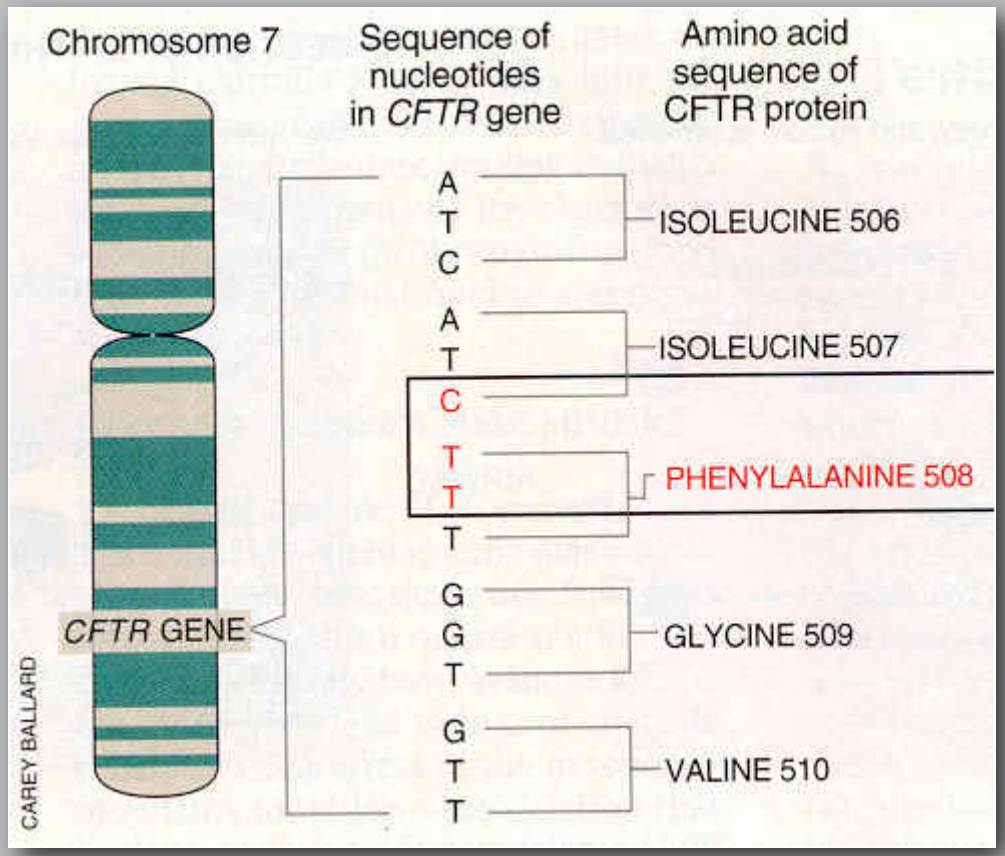
- Meconium ileus: postnatal lower intestinal tract obstruction
  - **10-20% of newborns with CF**
- Fertility problems
  - **Females may have reduced fertility**
  - **Males: 95% are infertile because of congenital bilateral absence of the vas deferens (CABVD)**

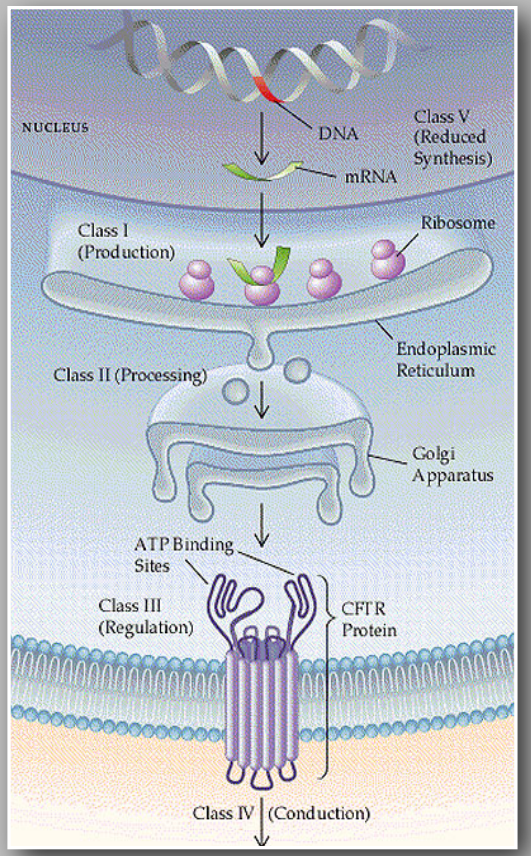


  - **Form of allelic heterogeneity: some CABVD infertile mails have specific CFTR variants and no other systemic manifestations (cf. idiopatic chronic pancreatitis)**

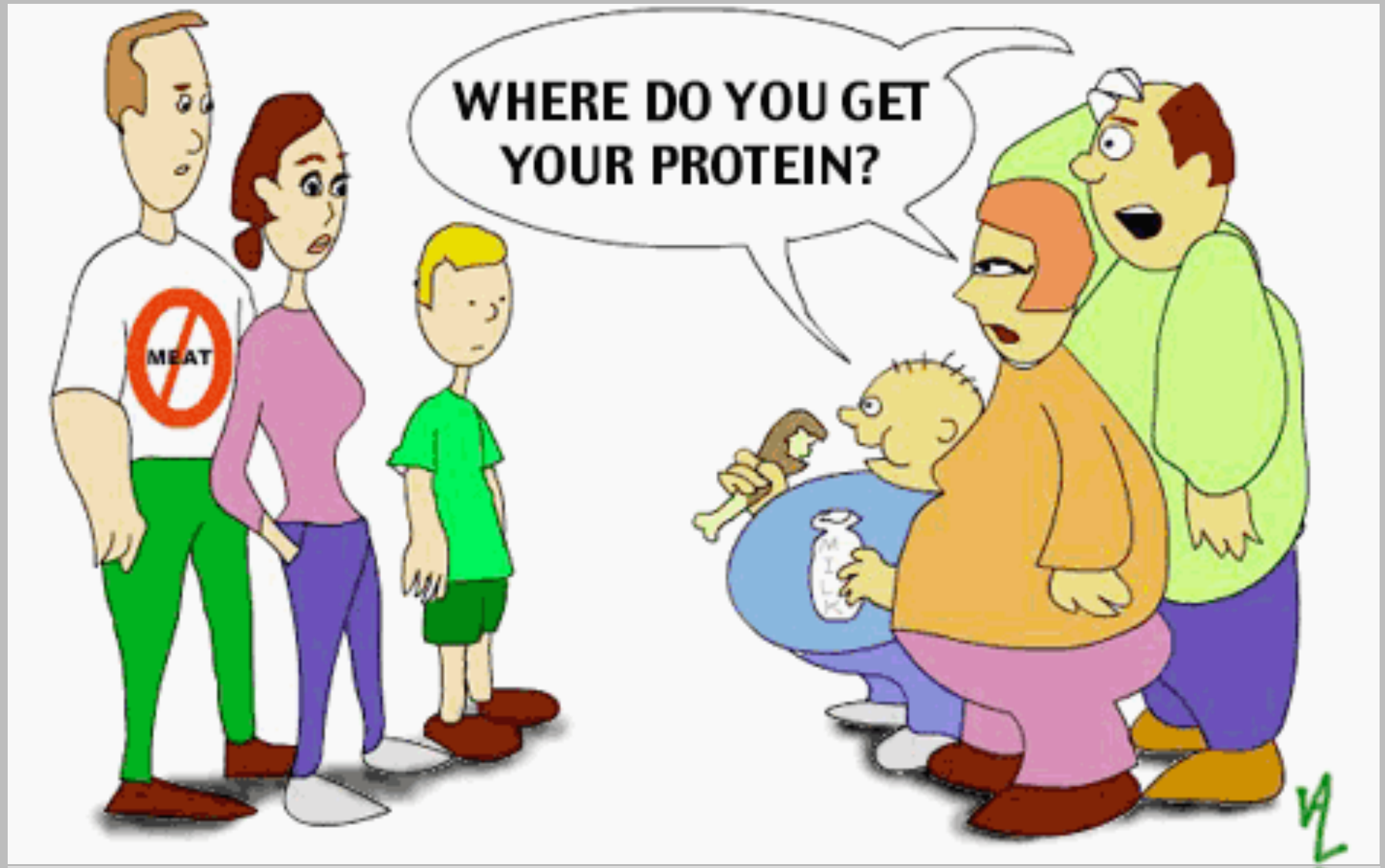
Genetics of cystic fibrosis

- Over 1200 variants described
- Most common is deletion of Phenylalanine (70% in Causasians) in NBD1





- 6 classes of mutant dysfunction


WHERE DO YOU GET YOUR PROTEIN?
MEAT
MILK