Genetic counselling.Inborn errors of metabolism

Dr. György Fekete

Semmelweis Egyetem,
II. Sz. Gyermekgyógyászati Klinika

Who needs counselling?

- Congenital malformation (s)
- Mental retardation
- Immune deficiency
- Advanced maternal age
- Consanguinity
- Infection, irradiation, mutagen effect during pregnancy
- Hereditary disease in the family
- Infertility
- Recurrent miscarriages
- Stillbirth

History

- USA / Davenport 1910
- UK 1946
- More than 40 counselling centres in UK and more than 450 in the USA
- Hungary 1960
- Genetic amniocentesis 1966
- Prenatal genetic diagnosis of inborn error of metabolism (IEM)/ Lesch-Nyhan sy. 1968
  Neonatal screening of IEM 1961

Practicing physician and genetics

- Assessment of the risks in a family
- Reproductive options
- Prenatal genetic diagnosis
- Precision of clinical diagnosis is fundamental
- Family studies

Suggestive signs / symptoms of IEM in newborns

- Maternal conditions
- - Maternal phenylketonuria
- - Acute fatty liver in pregnancy
- - Very strict vegetarian diet (veganic) : methylmalonic acidemia due to B12 deficiency

Presenting symptoms of IEM after infancy

- Multisystemic disease
- Failure to thrive
- Recurrent, severe infections
- Myopathy, cardiomyopathy
- Dysmorphic features
- Skeletal anomalies
- Hepatosplenomegaly
- Anemia, myeloproliferative syndrome

Laboratory results suggestive of IEM

- Metabolic acidosis
- Increased anion gap
- Hypoglycemia
- Ketosis
- Ketonuria
- Lactic acidosis
- Hyperammoniemia
- Hyperuricemia
- Hypertriglyceridemia
- Granulocytopenia, thrombocytopenia

Rare diseases

- Incidence: less than 1: 2000
- In Hungary: 90000 newborns/ year
- Phenylketonuria (inborn error of amino acid metabolism) 1: 10000
- Some lipid storage disorders (lysosomal metabolic diseases): 1: 30000- 40000
- Down- syndrome: 1:700, cystic fibrosis: 1: 3000

- **This is the main problem of the affected family!!!**

Praising rarity

Something rare is precious,

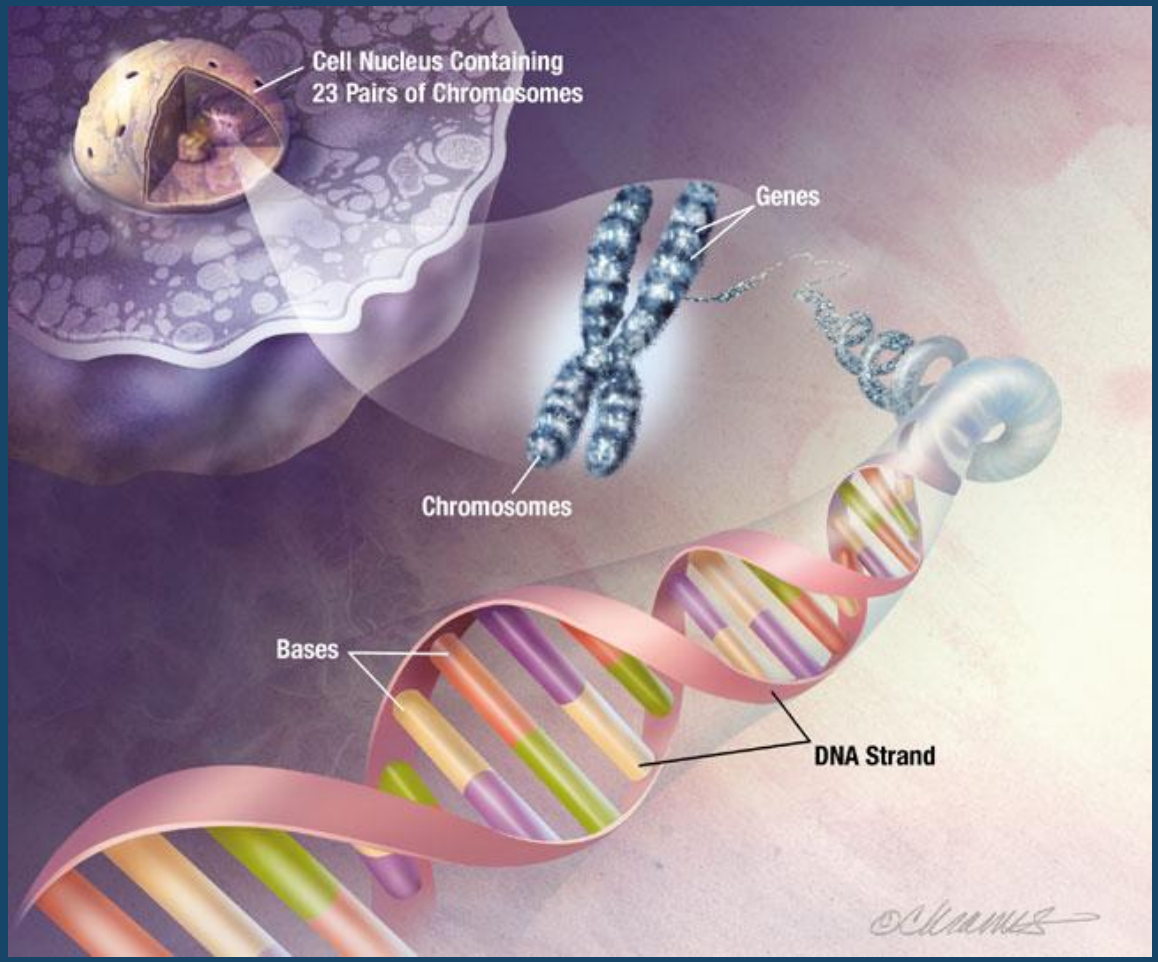
Study it, do not let it go.

Understand it and make it your own.

(David Attenborough)

Special methods

- Laboratory data
- Imaging techniques (CT, MRI)
- Cytogenetics
- DNA analysis
- Biochemical studies


Cell Nucleus Containing
23 Pairs of Chromosomes
Genes
Chromosomes
Bases
DNA Strand

Autosomal recessive inheritance: Mucopolysaccharidoses- „gargoyle”

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M. Hurler (MPS type I)

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MPS I, clinical symptoms

- Glycosaminoglycans (GAG):heparan- and dermatan-sulphate accumulation in lysosomes of the cells
- 3 forms: severe, moderately severe, mild
- CNS: mental retardation to normal intellectual capacity, hydrocephalus, compression of peripheral nerves
- Eye: cornea clouding, glaucoma, retina degeneration
- Ear: hearing loss
- Bones, joints: multiple dysostoses , contractures
- Respiratory involvement
- Heart and vessels
- Hepatosplenomegaly

MPS I, gene locus: 4p16.3, AR

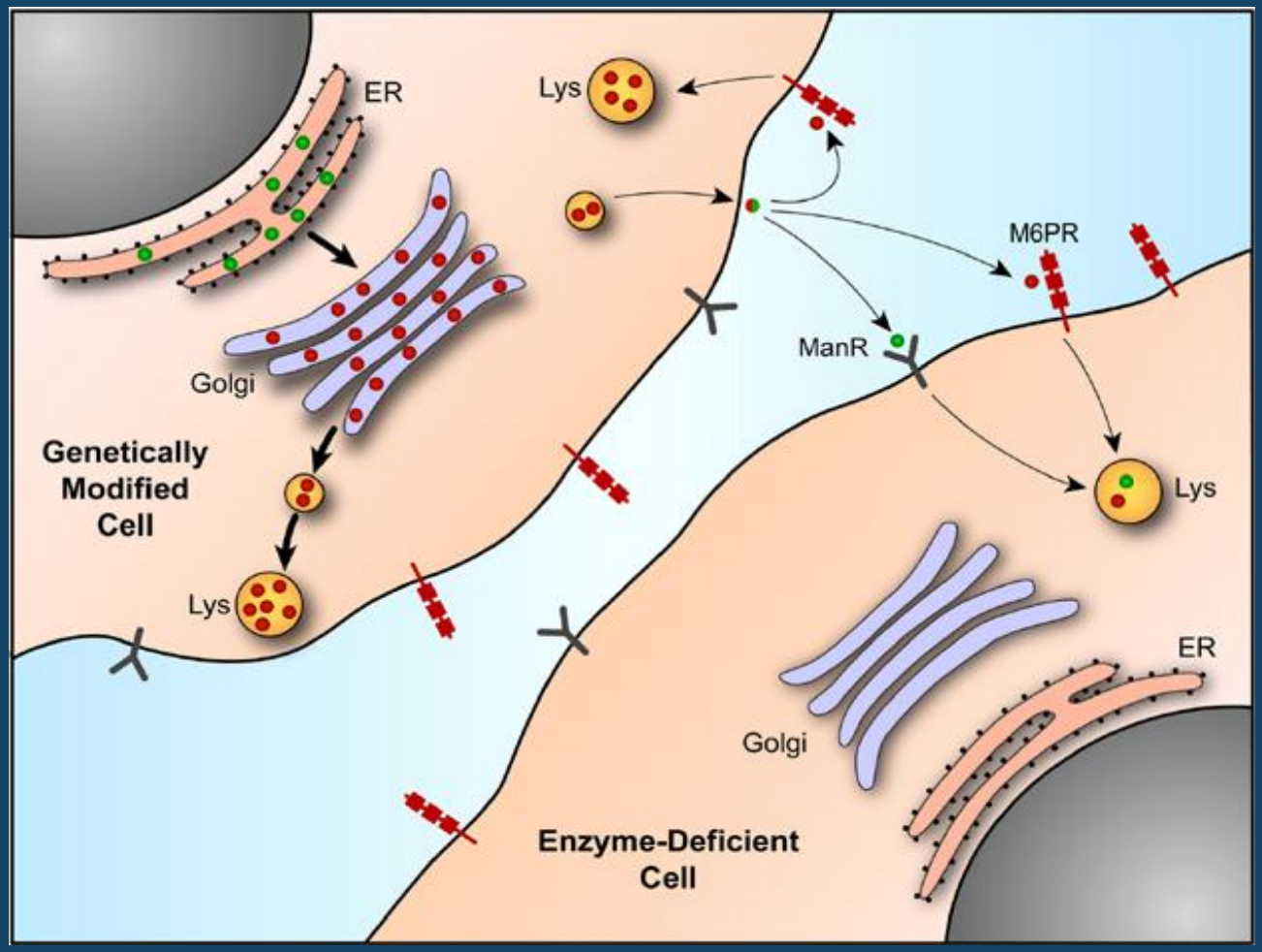
- Gertrud Hurler, German paediatrician (1889- 1965), published in 1919
- Harold Glendon Scheie, American ophthalmologist (1909- 1990), published in 1962
- Charles A. Hunter, Scottish-Canadian internist (1873- 1955) /MPS II /
- 1: 100000- 500000

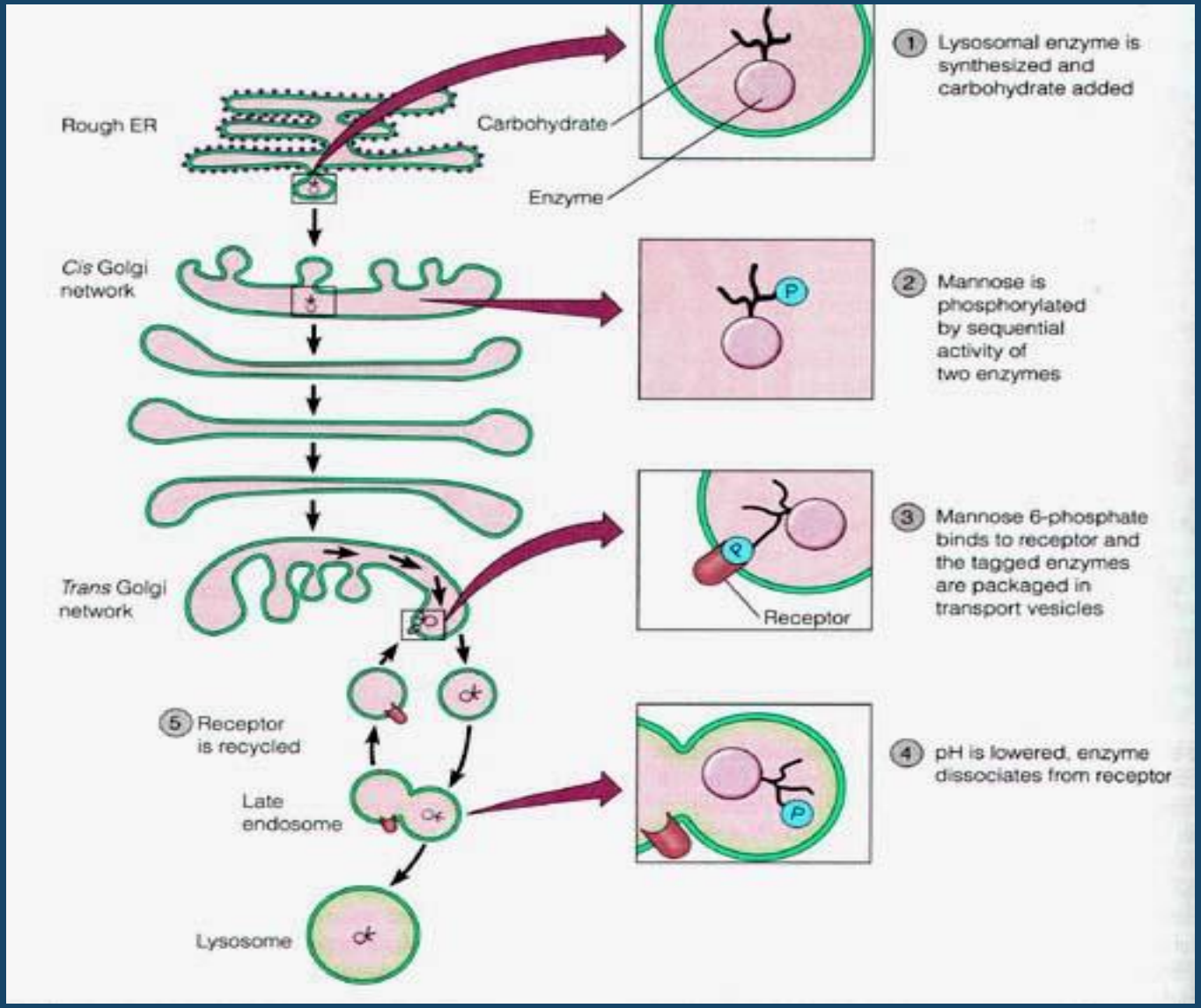
MPS I

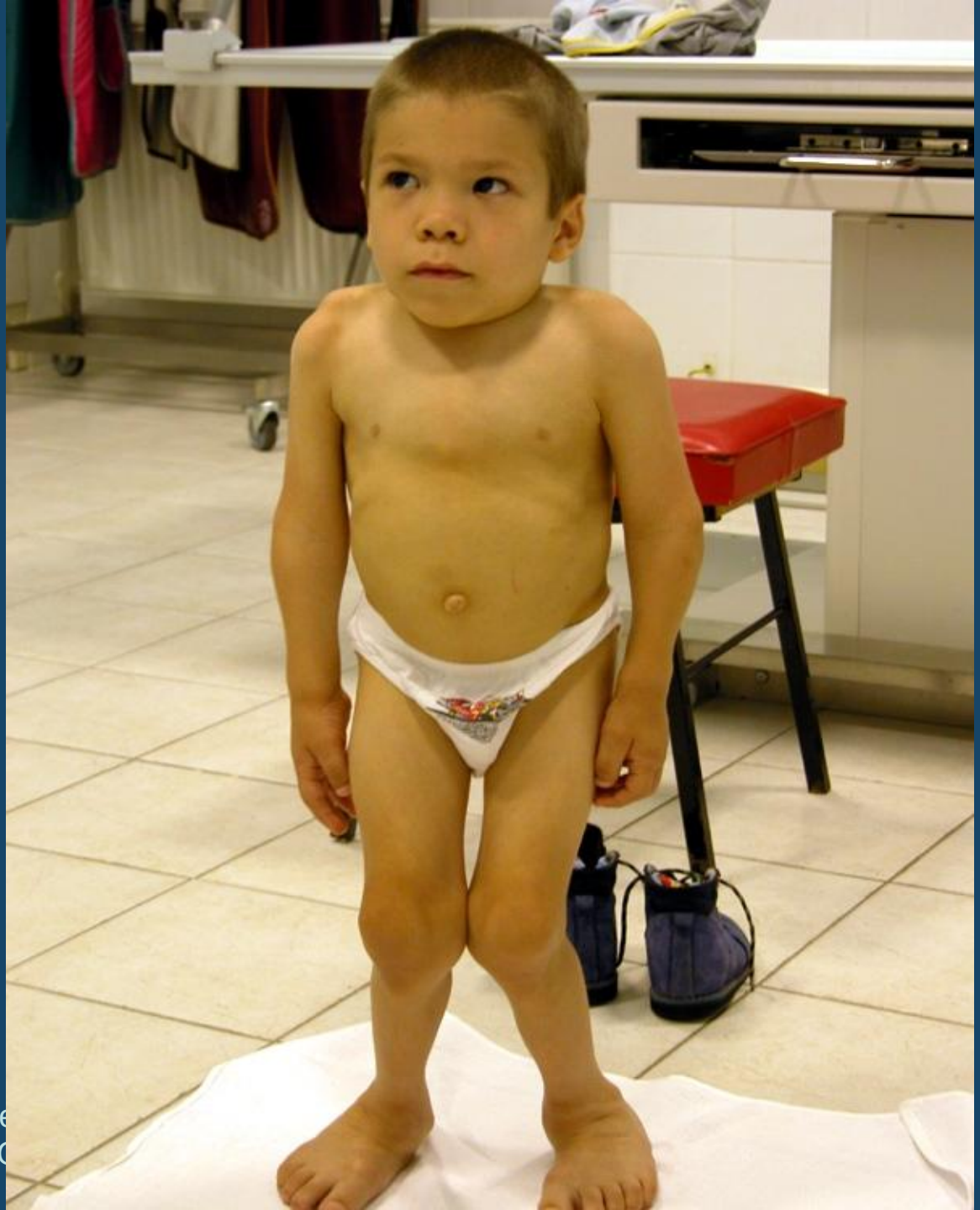
- Progressive
- High mortality, in severe form before the age of 10 years
  - Obstructive pulmonary insufficiency, recurrent infections
  - Cardiovascular complications

MPS I, treatment

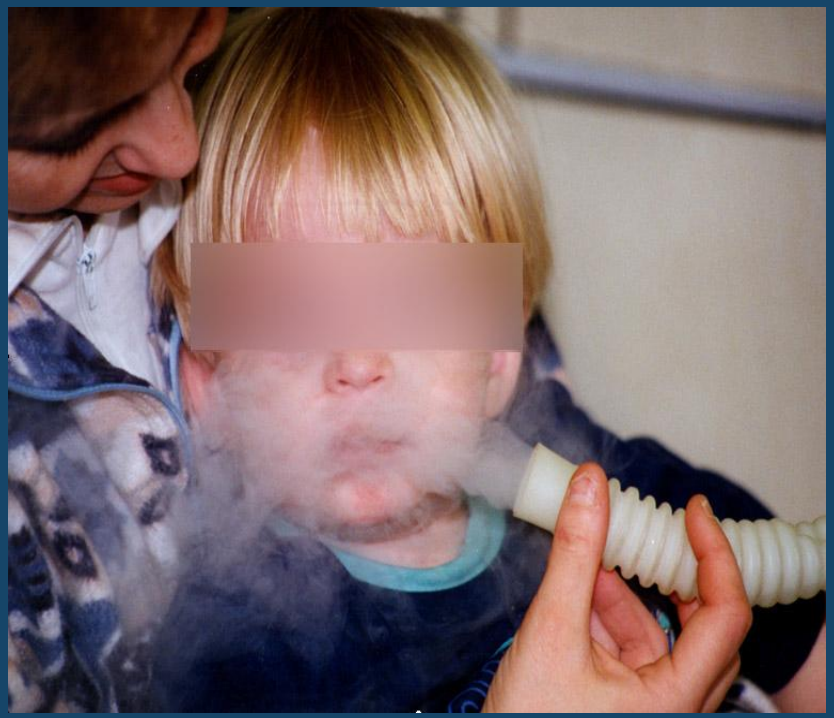
- Symptomatic
- Bone marrow (stem- cell) transplantation
- Enzyme replacement
- Multidisciplinary approach


ER
Lys
M6PR
ManR
Golgi
Genetically
Modified
Cell
Lys
Lys
ER
Golgi
Enzyme-Deficient
Cell

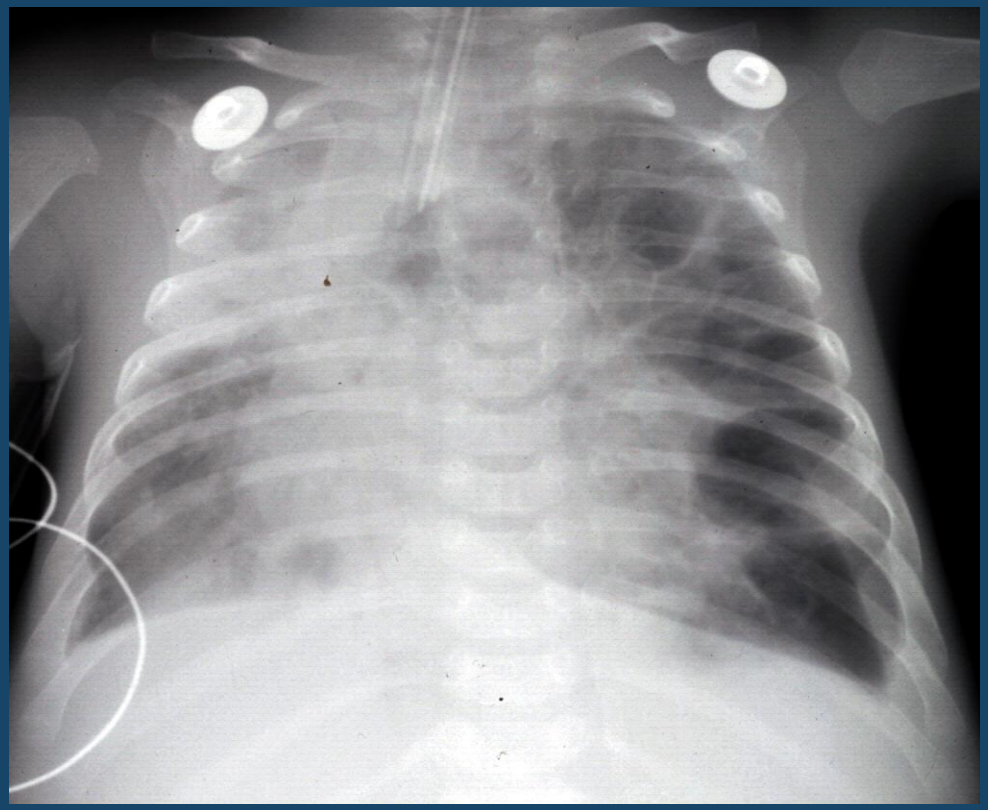

Rough ER
Carbohydrate
Enzyme
1 Lysosomal enzyme is synthesized and carbohydrate added
Cis Golgi network
2 Mannose is phosphorylated by sequential activity of two enzymes
Trans Golgi network
3 Mannose 6-phosphate binds to receptor and the tagged enzymes are packaged in transport vesicles
Receptor
5 Receptor is recycled
4 pH is lowered, enzyme dissociates from receptor
Late endosome
Lysosome



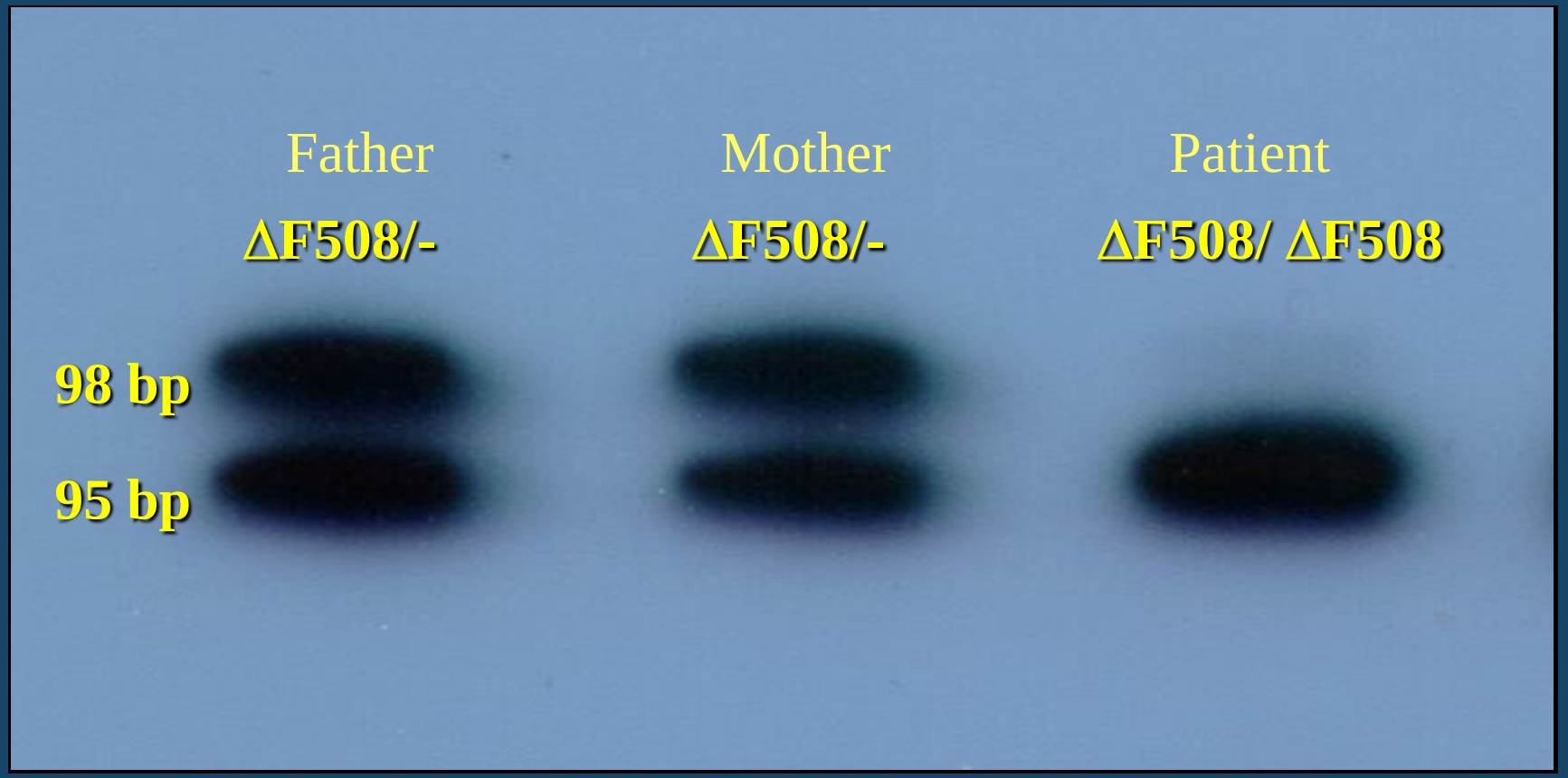
- Cystic fibrosis:
  differencial-diagnosis, more than 1500 known mutations, prenatal diagnosis feasible



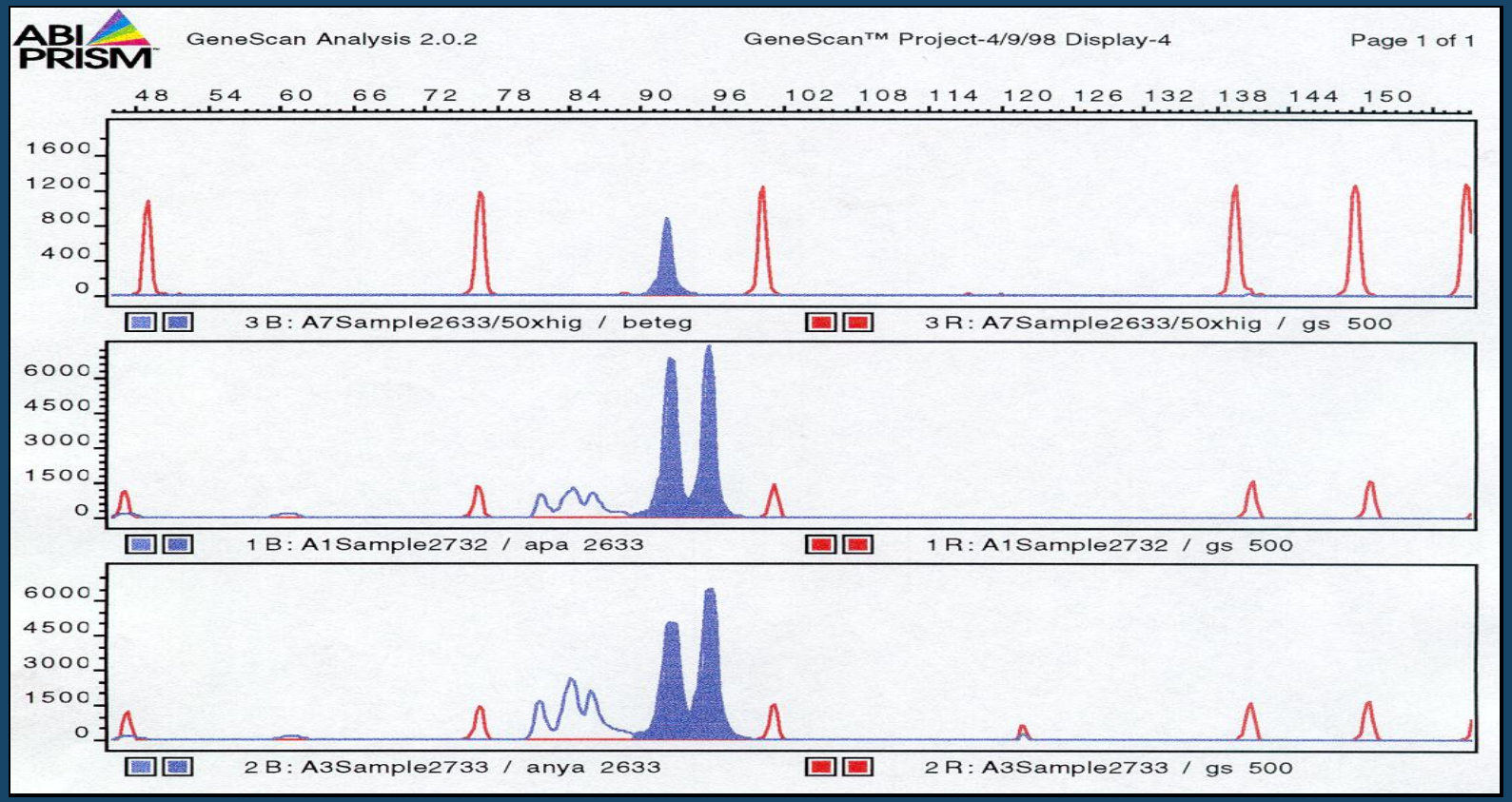
Cystic fibrosis



ΔF508 mutation



CFTR gene: deltaF508 mutation



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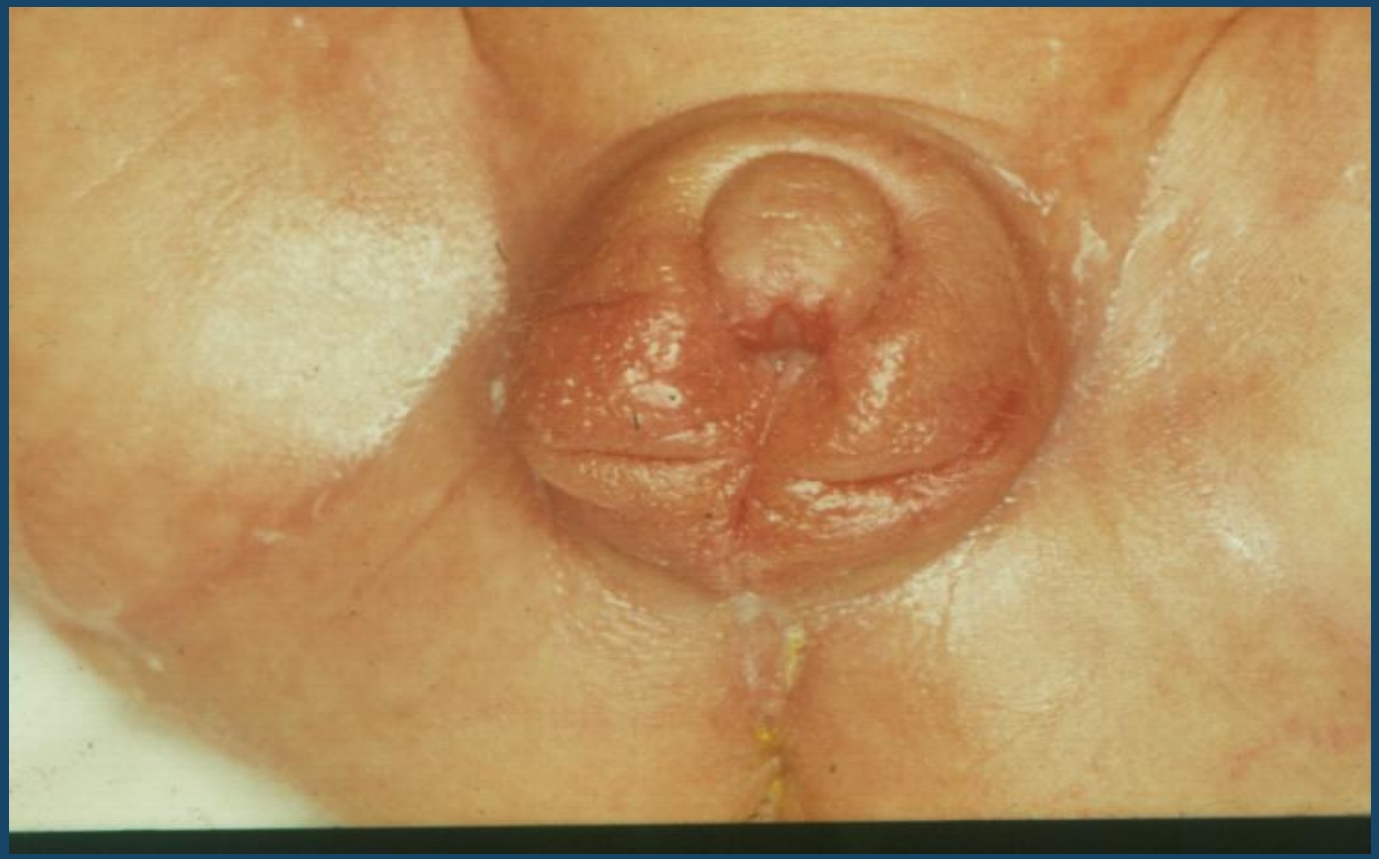
Congenital adrenal hyperplasia (CAH)



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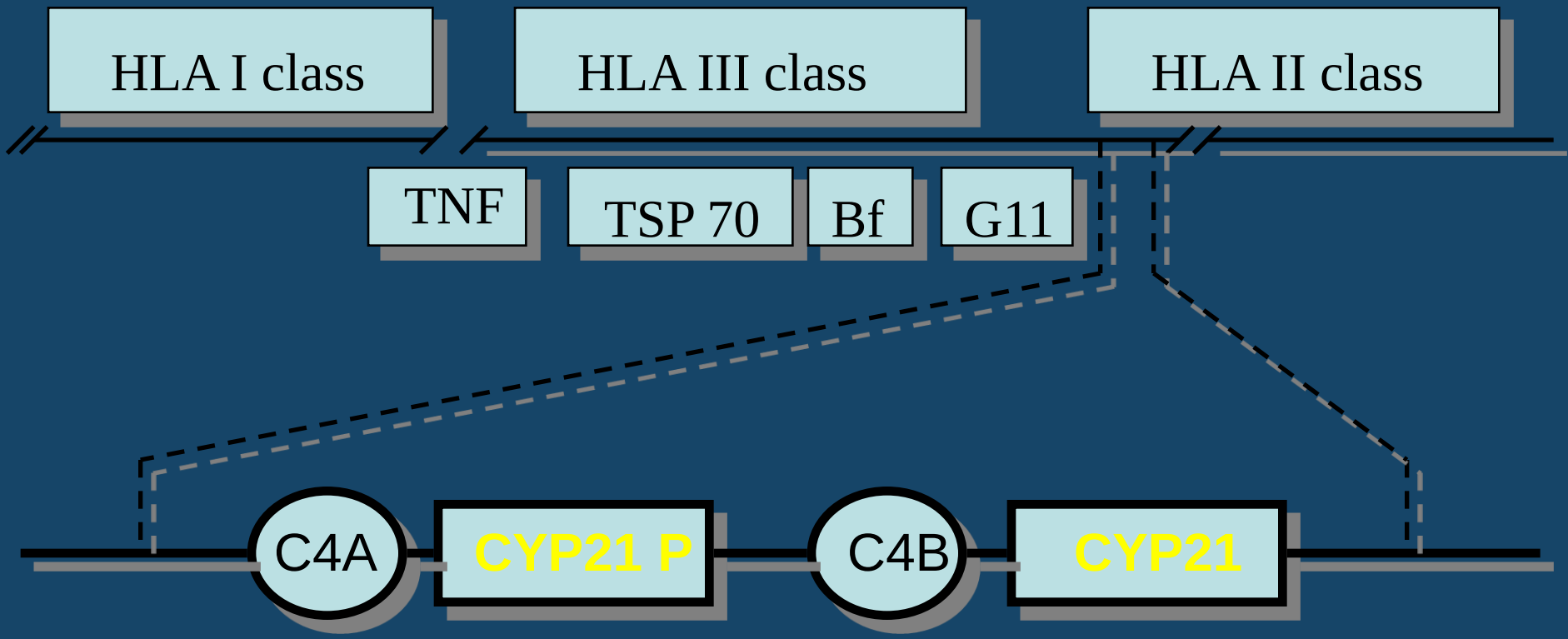
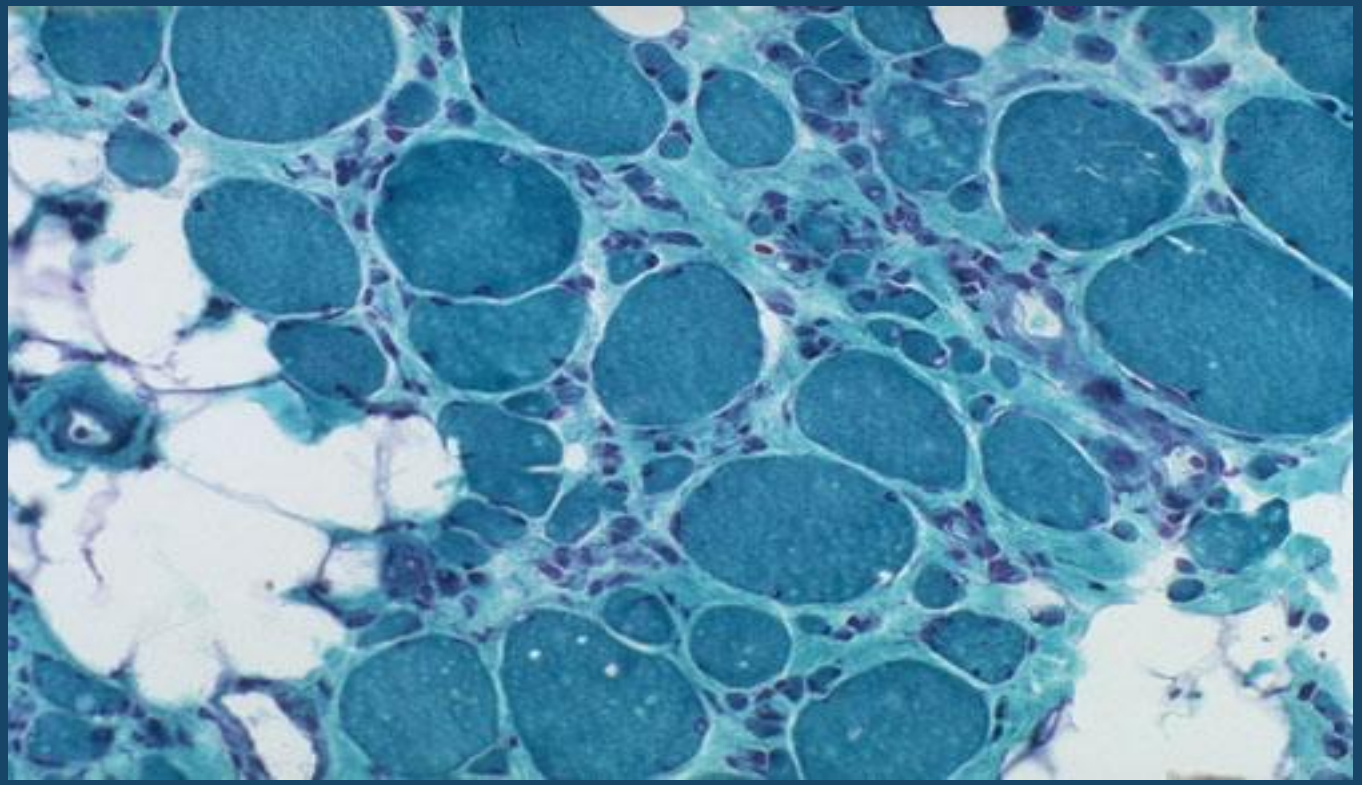
CYP21 gene


HLA I class
HLA III class
HLA II class
TNF
TSP 70
Bf
G11
C4A
CYP21 P
C4B
CYP21

X – linked recessive inheritance

- Dystrophia musculorum progressiva (Duchenne), among boys 1:4000, dystrophin gene mutations, Xp21.2 , Becker type muscle dystrophy is milder
- Haemophilia A and B, among boys 1:10000-15000, A: Xq28; B: Xq27.1-27.2
- Adrenoleukodystrophy (ALD), ABCD1 gene mutations,Xq28
- Fabry - disease

Dystrophia musculorum progressiva



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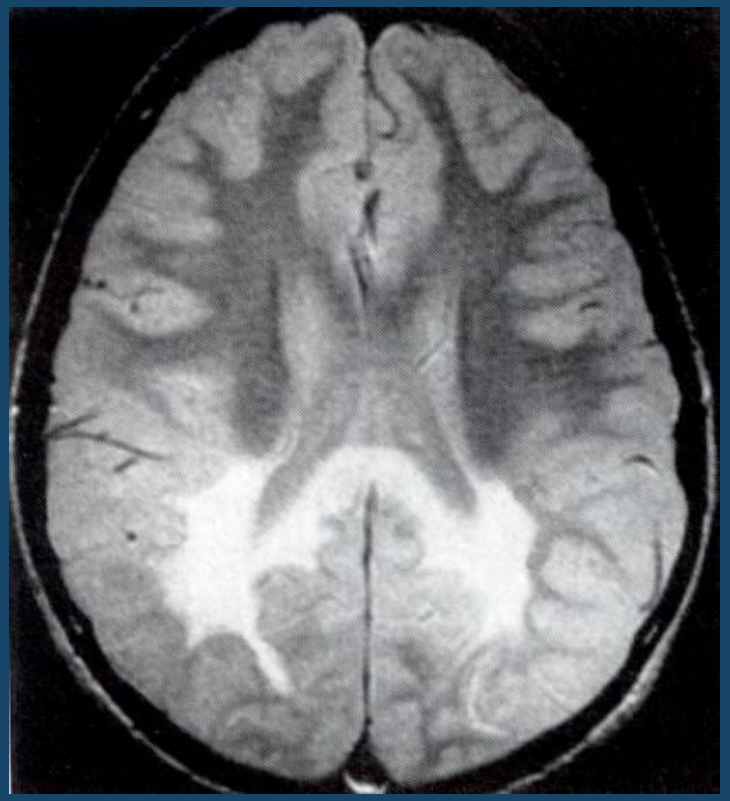
X- linked inheritance: Adrenoleukodystrophy (XR)



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Fabry disease

- lysosomal storage disorder
- α-galactosidase (ceramide trihexosidase) deficiency
- glycophospholipid (glycosphingolipid, globotriaosylceramide, galabiosylceramide)- accumulation in endothelial cells
- X – linked recessive mode of inheritance
- Girl/ female hemizygote carriers may also present symptoms

Fabry-disease
Alfa galactosidase A gene: Xq22

- Mutation analysis: 8 patients
- 4 different mutations
- Enzyme replacement therapy is feasible
- Early diagnosis and treatment would be necessary
- Failure to thrive in infancy and in young children

Fabry disease, symptoms

- Early onset in boys, late onset in girls/women
- Skin

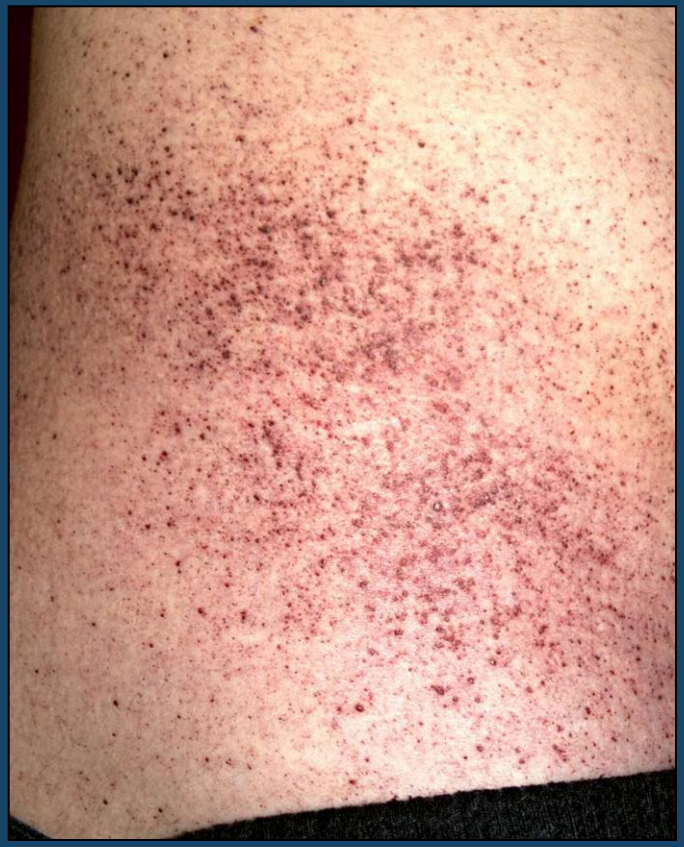
  - Angiokeratoma

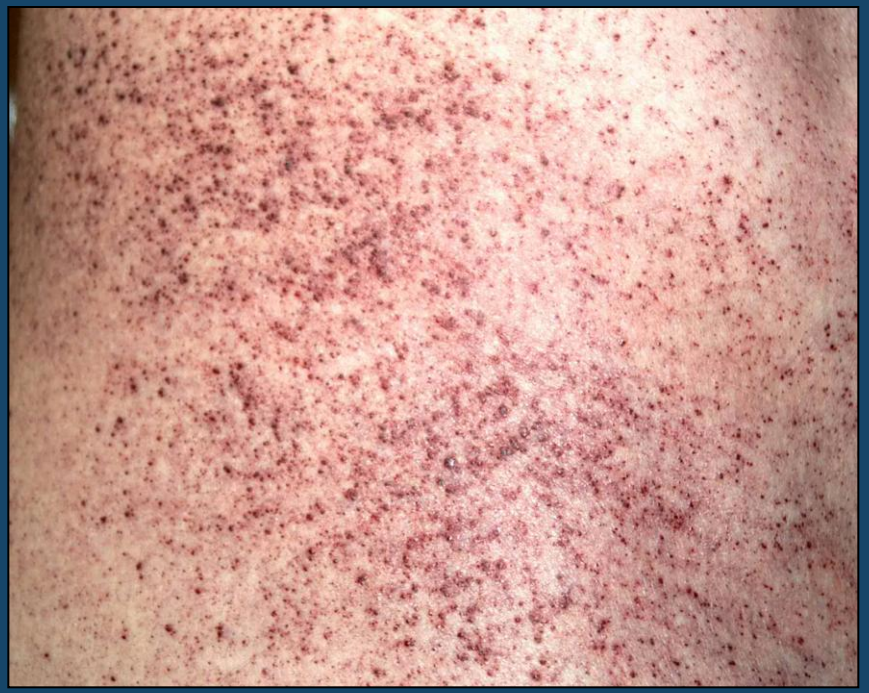
  - Hypohidrosis
- Eye

  cornea dystrophy (cornea verticillata)

  cataract

Fabry-disease, angiokeratoma





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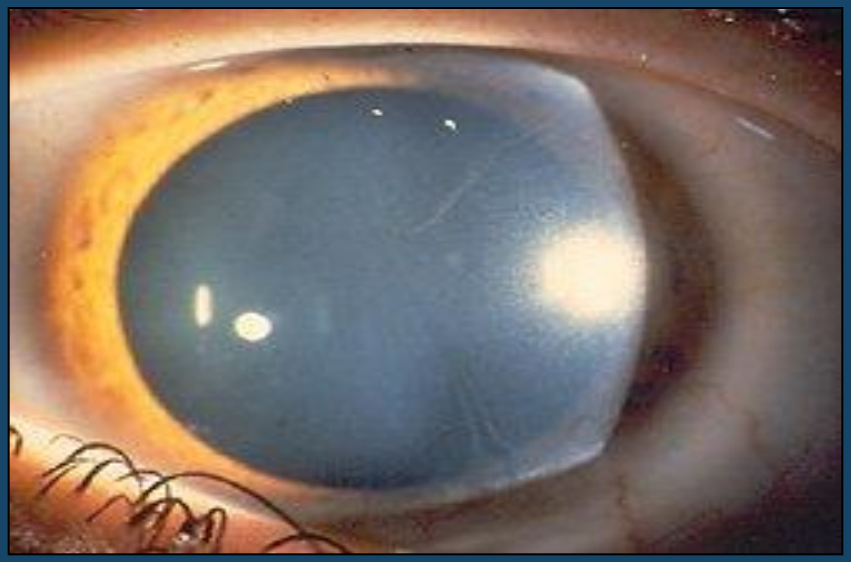
Fabry-disease, cornea dystrophy



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- **Gastrointestinal:**
  - **Pain, paroxismal**
  - **Diarrhoea**
  - **Nausea, vomiting**
- **Kidney:**
  - **Proteinuria**
  - **Uraemia**
- **Haematology:**
  - **Anaemia**
  - **Lipid-storing macrophages in bone marrow**

Gaucher disease, AR, 1q21

- Philippe Charles Ernest Gaucher, French dermatologist (1854-1918)
- 1882: type I was published
- Not yet recognized the multiorgan involvement
- Glucocerebrosidase enzyme deficiency
- Macrophages: glucocerebroside accumulation

Gaucher disease, clinical symptoms

- Type I, adults, children, chronic, non-neuropathic form
- Hepatosplenomegalia
- Anaemia, thrombocytopenia
- Bone – and joint pain, bone fragility, deformity of femur (Erlenmeyer-flask-like deformity)
- Skin: yellow-brown patchy pigmentation
- Eye: pinguecula at the border of sclera and cornea

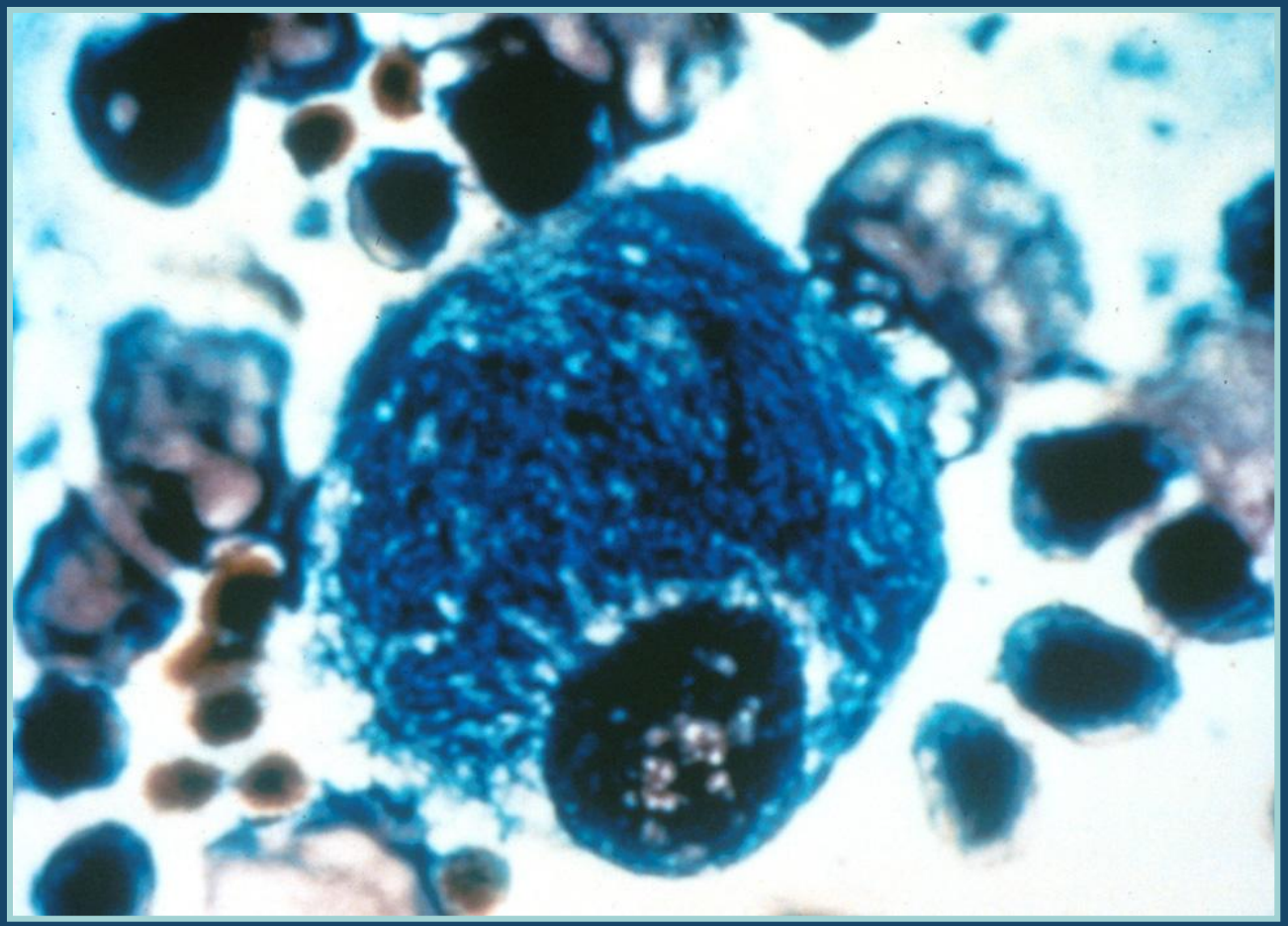
Gaucher disease, clinical symptoms

- Type II: infantile, acute neuropathic form
- First pathological neurological signs at the age of 3-6 months
- Hepatosplenomegalia
- Recurrent respiratory infections
- Failure to thrive
- Death at the age of 2-3 years
- Congenital form: ichthyosis, hydrops fetalis

Gaucher disease, clinical symptoms

- Type III, juvenile, subacute, neuropathic form
- Symptoms start before the age of 14 years
- Symptoms are transition between types I and II
- More frequent in Sweden

Gaucher cell in the bone marrow



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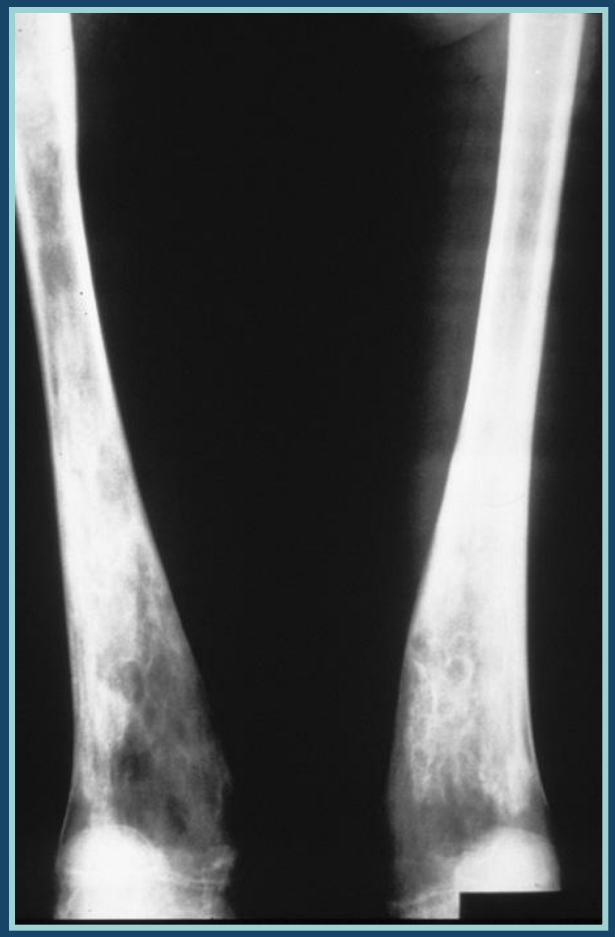
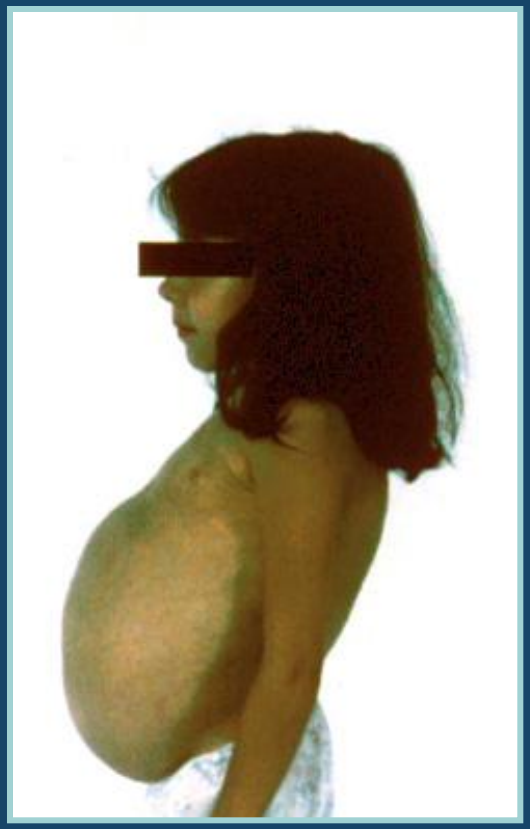
Gaucher disease, femur deformity

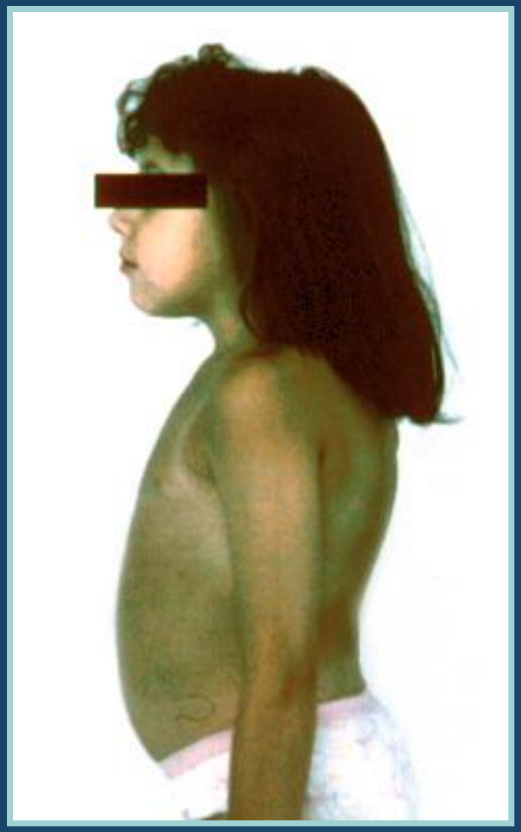


Gaucher disease, therapy

- Since 1991: enzyme replacement
- Imiglucerase (Cerezyme) produced by recombinant molecular technique
- Alglucerase (Ceredase) produced from human placenta

Gaucher disease, before and after treatment





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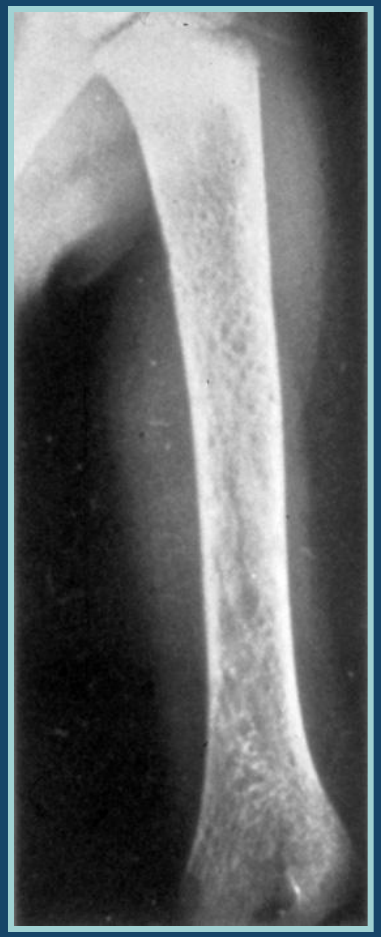
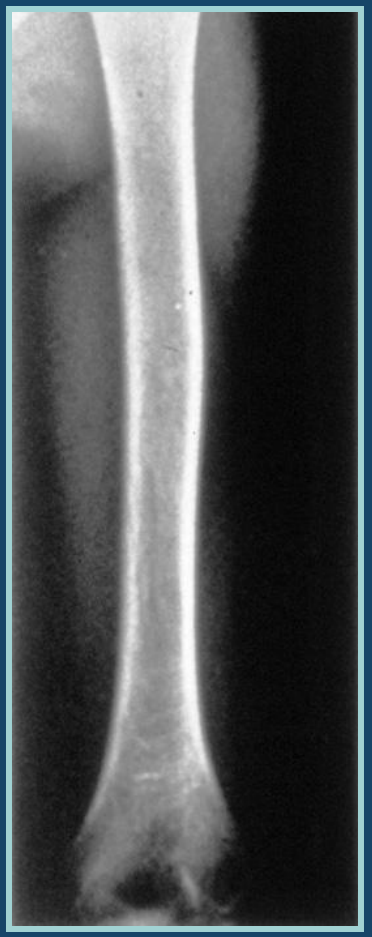
Gaucher disease, before and after treatment





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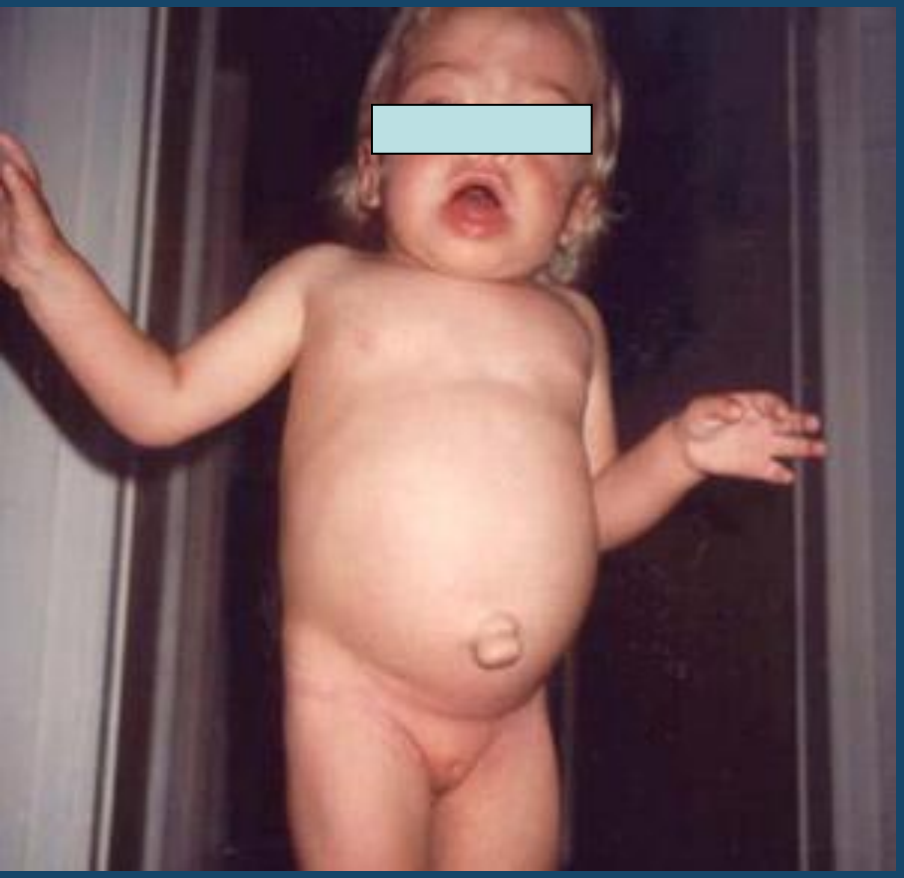
Pompe disease, AR, 17q25, GAA gene

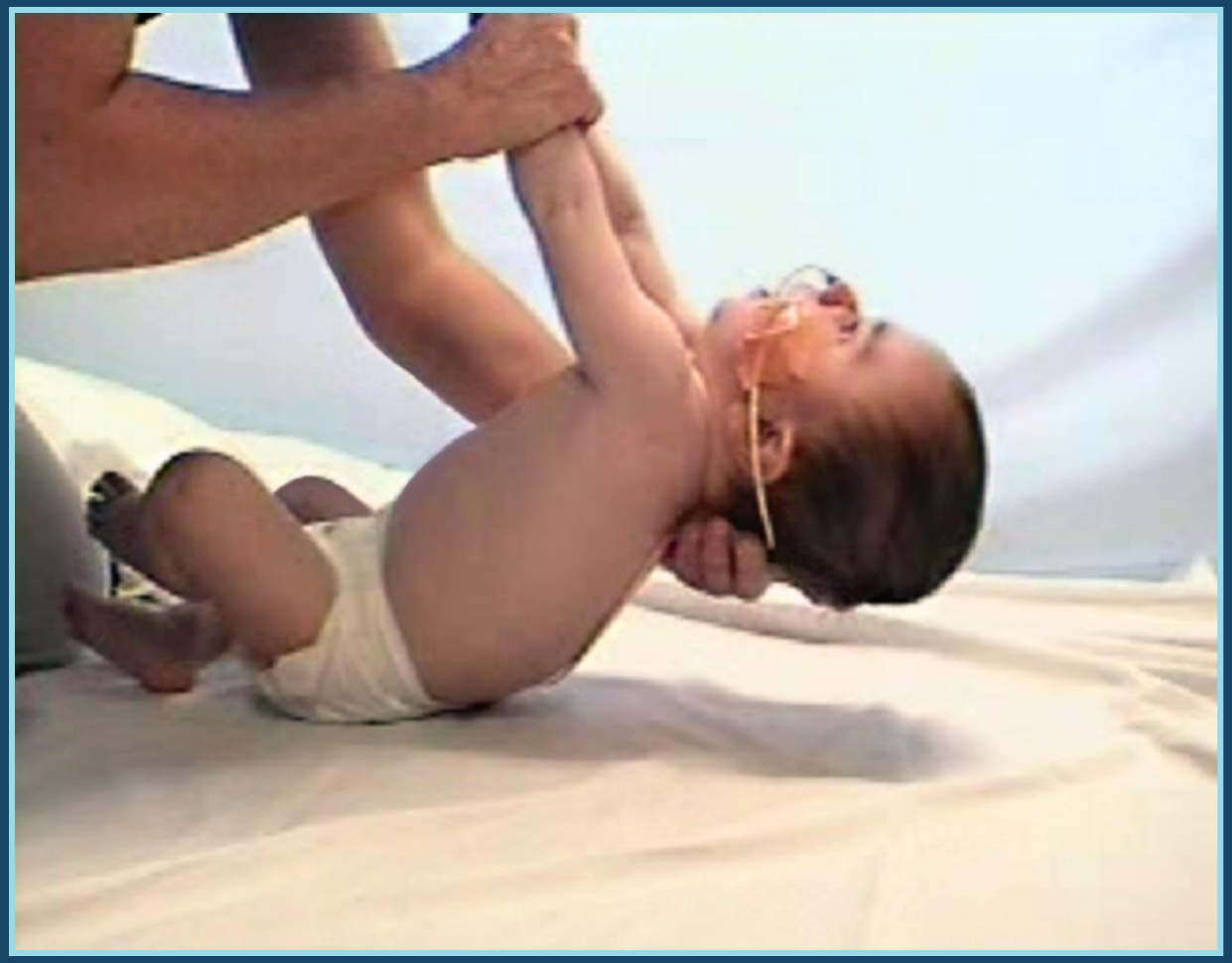
- Joann Cassianius Pompe, Dutch physician
- Published in 1932
- Glycogenosis types II a and II b , deficiency of lysosomal acid alfa-glykosidase (maltase)
- Glycogen accumulation in lysosomes
- Type II a: classical, infantile, lethal form

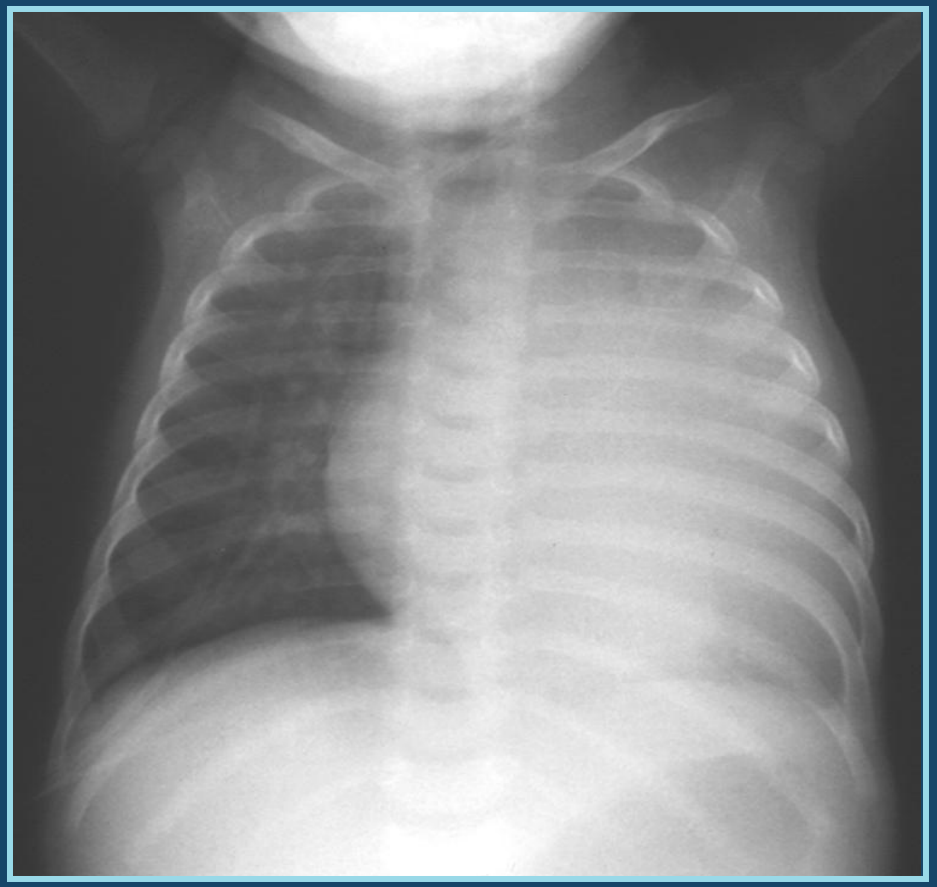


Pompe disease (infantile)

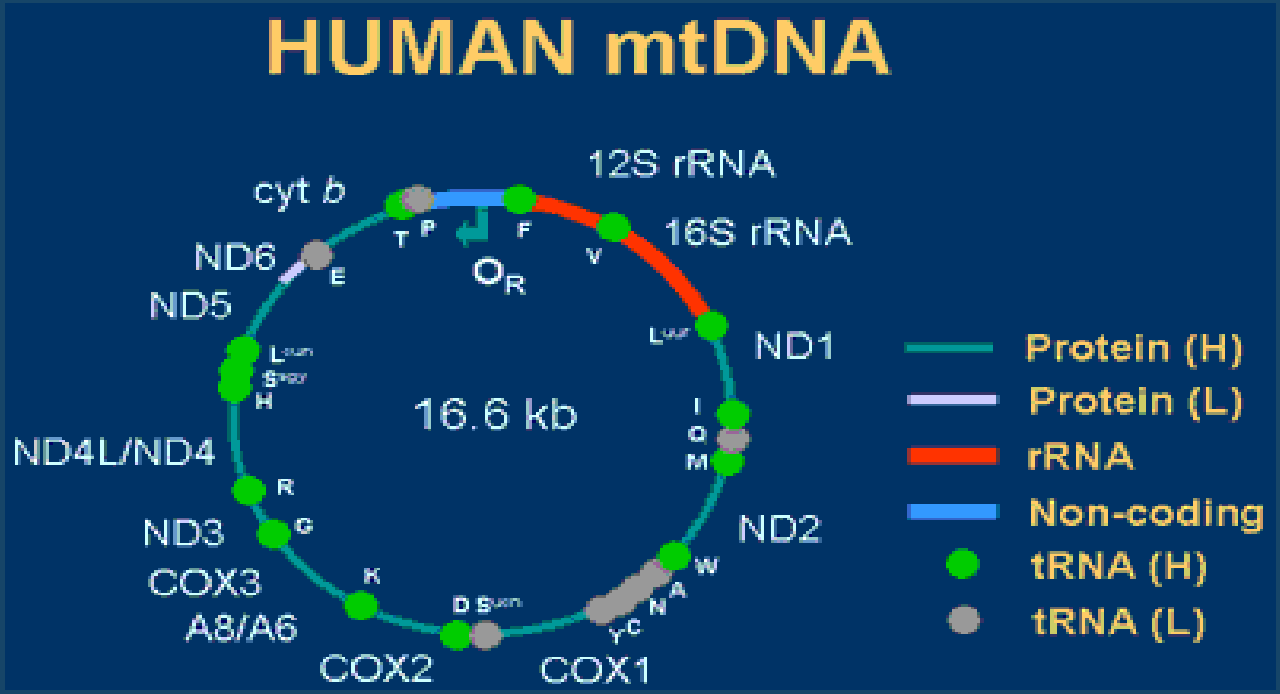
- **No symptom at birth**
- Early muscle hypotony
- Macroglossia
- Severe cardiomyopathy
- Mild hepatomegaly
- Dystrophy
- Cardiovascular insuff.
- Elevated se- CK, LDH
- No mental retardation!
- Death before the age of 2 years

Treatment: Enzyme substitution as soon as possible!





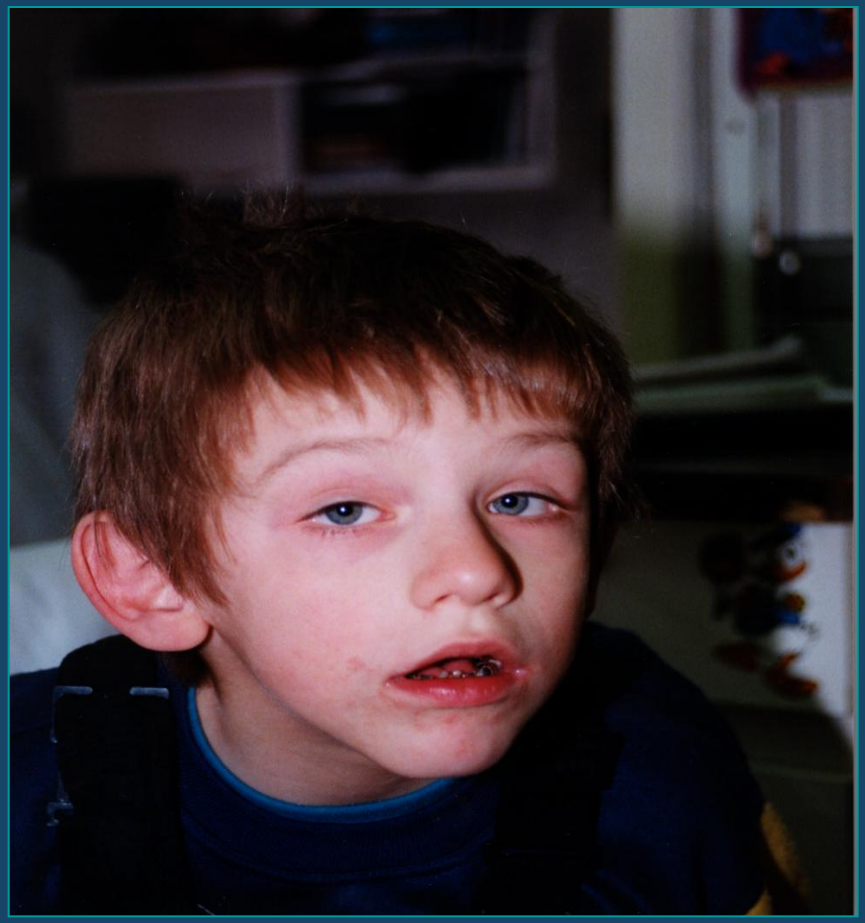
Mitochondrial DNA



Trinucleotide (triplet) repeats (expansions)

- Fragile X syndrome, among boys 1:4000, FMR1 gene mutations, Xq27.3
- Huntington disease, 1:5000-20000, CAG repeats, 4p16.3, huntingtin protein
- Myotonic dystrophy (Steinert disease), 1 :25000, CTG repeats, 19q13.2
- Friedreich ataxia, 1:25000, GAA repeats, 9q13-21, frataxin protein

Fragile X syndrome



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DNA diagnosis, mutation analysis

- Blood taking
- DNA extraction, isolation
- Analyis of the involved gene region (polimerase chain reaction= PCR, DNA sequencing)

Screening programs

- Identifying newborns with genetic disorders
- Clearly defined condition
- Treatable
- Reasonable frequency
- Rapid test
- Inexpensive
- Specificity, sensitivity
- Prompt initiation of treatment

Screening programs /Hungary

- PKU
- Galactosemia
- Hypothyroidism
- Biotinidase deficiency
- Hip dyslocation
- Vision, hearing

Screening programs – other countries

- Tay- Sachs
- Sickle cell disease
- Beta thalassemia
- CF
- CAH

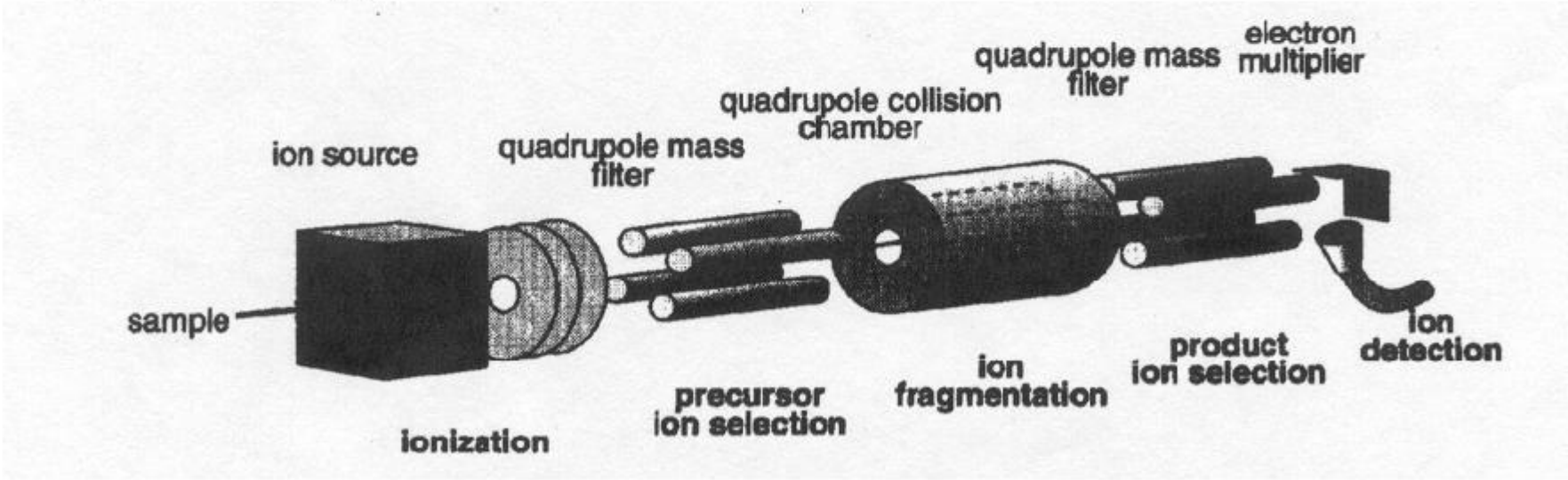
Genetic screening methods

- Neonatal screening
  - congenital (inborn) metabolic disorders
  - congenital hip dyslocation
  - visual, hearing test

  - Screening is no diagnosis! Diagnostic methods are the second step in evaluation.

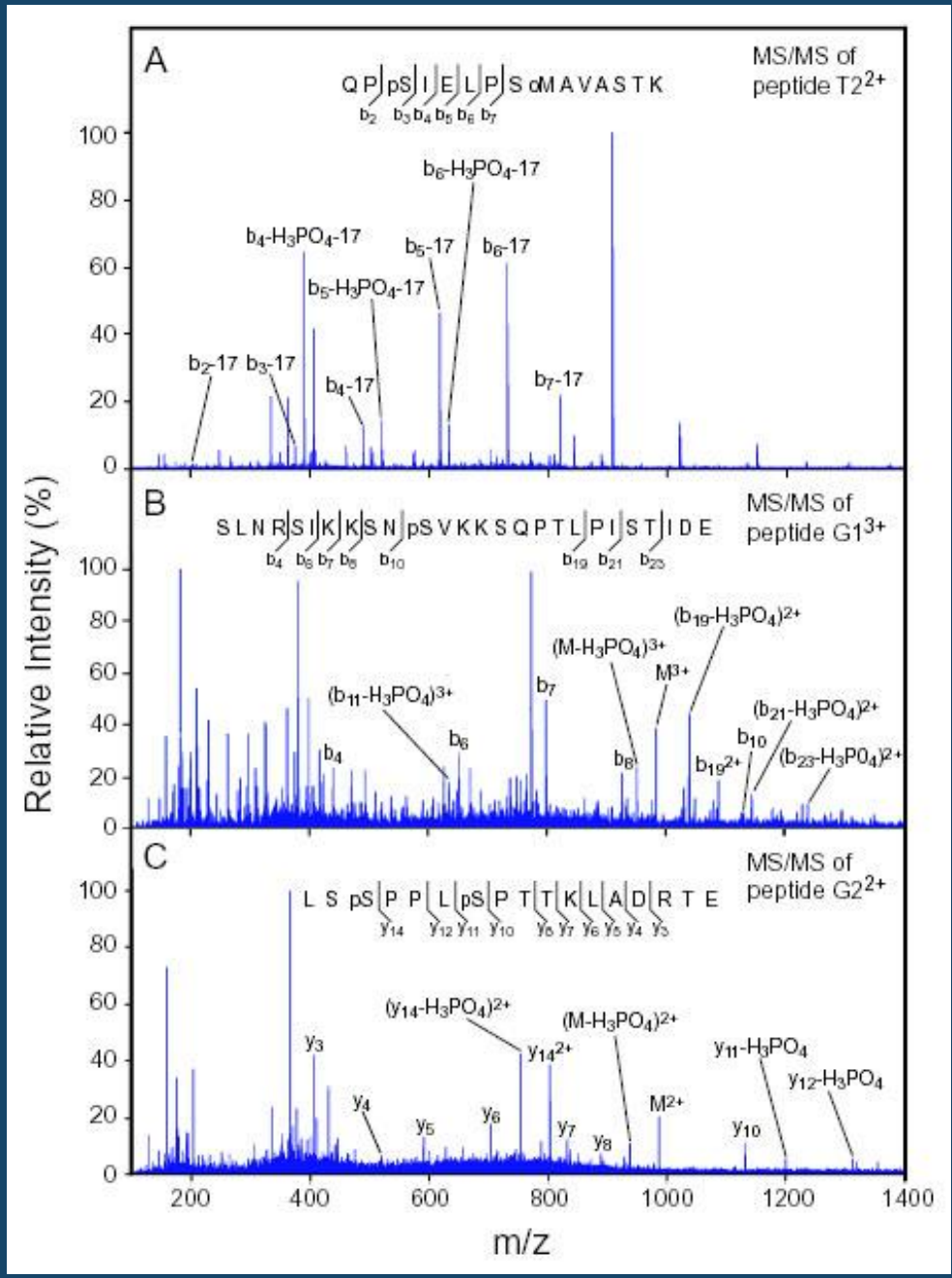
TANDEM Mass Spectrometry

- Tandem mass spectrometry uses two stages of mass analysis, one to preselect an ion and the second to analyze fragments induced by collision with an inert gas like argon or helium.



Triple Quadrupole Mass Spectrometer

Taken fromHoffman, Edmond, "Tandem Mass Spectrometry: A Primer", Journal of Mass Psectrometry, Vol 31, 129-137, 1996.


A
MS/MS of peptide T2 2+
Q P pS I E L P S oM A V A S T K
b2 b3 b4 b5 b6 b7
b6-H3PO4-17
b4-H3PO4-17
b5-17
b6-17
b5-H3PO4-17
b2-17 b3-17
b4-17
b7-17
B
MS/MS of peptide G1 3+
S L N R S I K K S N pS V K K S Q P T L P I S T I D E
b4 b5 b7 b8 b10 b19 b21 b23
(b19-H3PO4)2+
(M-H3PO4)3+
M3+
(b11-H3PO4)3+
b7
(b21-H3PO4)2+
b10
b6
b4
b8
b19 2+
(b23-H3PO4)2+
C
MS/MS of peptide G2 2+
L S pS P P L pS P T T K L A D R T E
y14 y12 y11 y10 y9 y7 y6 y5 y4 y3
(y14-H3PO4)2+
(M-H3PO4)2+
y3
y14 2+
y11-H3PO4
y12-H3PO4
M2+
y4
y5
y6
y7
y8
y10
Relative Intensity (%)
m/z
0
20
40
60
80
100
200
400
600
800
1000
1200
1400

Neonatal metabolic screening in Hungary

- Phenylketonuria (PKU)
- Galactosemia
- Biotinidase deficiency
- Cong. athyreosis, hypothyreosis

- Selective screening: congenital adrenal hyperplasia (CAH)

Centers of IEM are necessary

- Screening
- Diagnosis
- Treatment
- Longitudinal follow-up, care
- Improvement of quality of life
- Support in transition to adulthood

Conclusions

- Thousands of genes regulate physiologic functions, metabolic processes
- Even one defect at a gene locus may cause severe developmental failures
- Novel therapy methods: individual optimal feeding, special diets, enzyme replacement, surgery, etc.
- Children are also different as regards their genomic predisposition to malnutrition, malabsorption, and to impairments from environmental factors…

- …but
  - independently of age, gender, and genes…

…everybody needs about the same quantity of love!

A smile costs nothing, but gives much.

It enriches those who receive, without making poorer those who give.

It takes but a moment, but the memory of it sometimes lasts forever.

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