Single gene disorders may be classified into four categories:

a. Defects in membrane receptors and transport systems

a. Enzyme defects and their consequences

a. Alterations in the structure, function, or quantity of nonenzyme proteins

a. Mutation resulting in unusual reactions to drugs.

Molecular Pathology

The enzyme defects
and
Their consequences

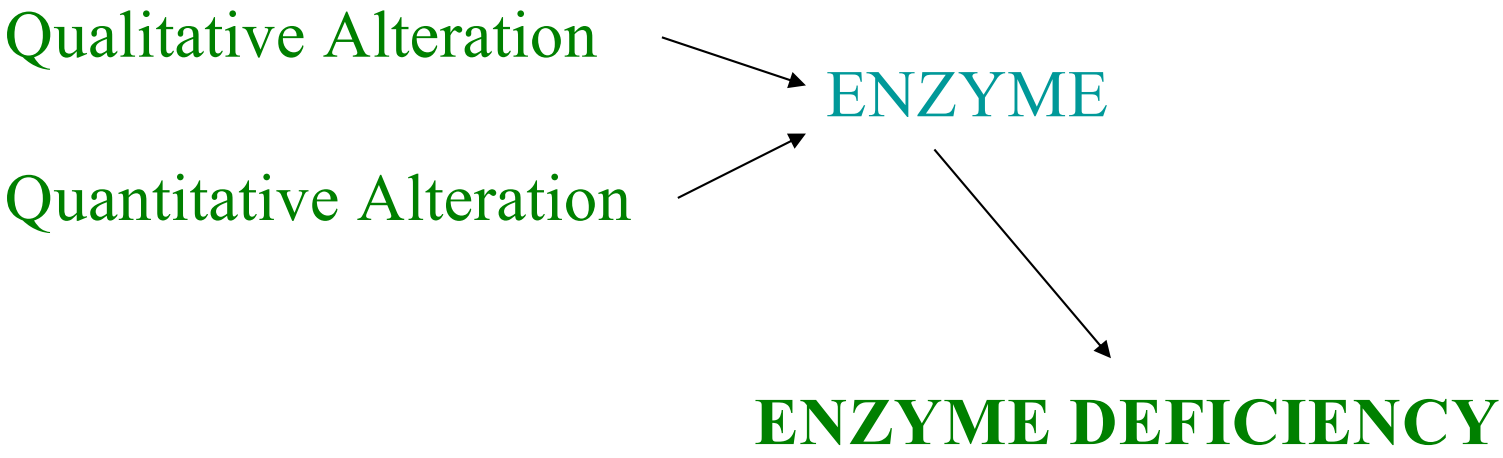
DEFECTS OF ENZYMATIC PROTEINS

Catalysts that increase (accelerate) the rate of chemical reactions

A specific substrate

Small quantities

Active Site



Biochemical mechanisms in inborn errors of metabolism

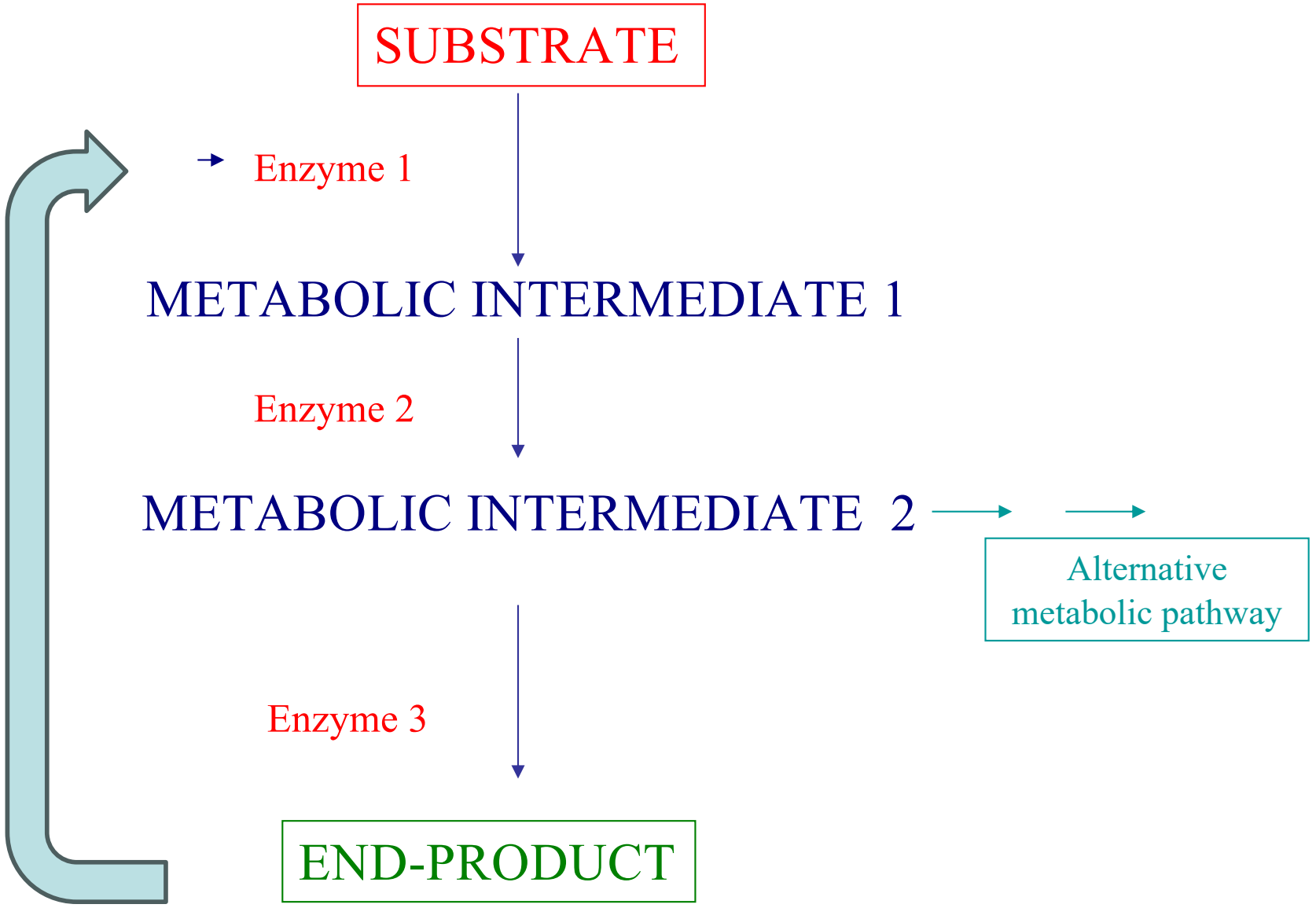
A single gene defect can have several impact on metabolic pathways that can lead to four main biochemical consequences:

1. **Failure to complete a metabolic pathway→ metabolic block:** the end-product is not formed because the required enzyme to complete the metabolic sequence is missing. (Albinism)

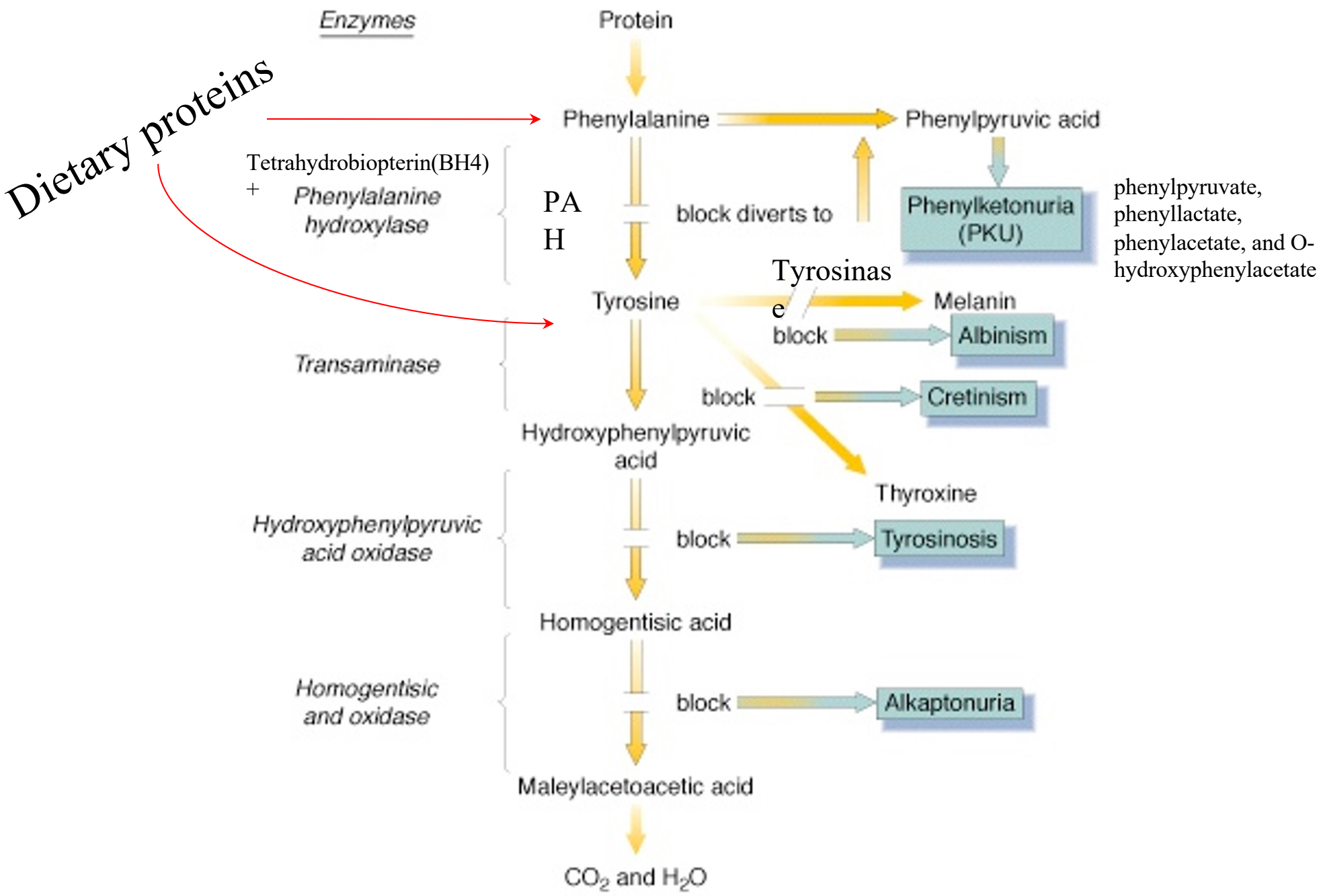
2. **Accumulation of unmetabolized substrate:** the enzyme that converts the initial substrate into the first metabolic intermediates may be missing, in that case the initial substrate accumulates in excess. (Phenylketonuria, lysosomal storage diseases)

3. **Storage of a metabolic intermediate:** a metabolic intermediate, which is normally quickly processed into the final product and so is usually present only in minute amounts, but accumulates in large quantities if the enzyme for its metabolism is lacking. (von Gierke)

3. **Failure to inactivate a tissue-damaging substrate.** (α1-antitrypsin)


SUBSTRATE
Enzyme 1
METABOLIC INTERMEDIATE 1
Enzyme 2
METABOLIC INTERMEDIATE 2
Alternative metabolic pathway
Enzyme 3
END-PRODUCT

Diseases by disturbance of Phenylalanine and Tyrosine metabolism



Biochemical mechanisms in inborn errors of metabolism

A single gene defect can have several impact on metabolic pathways that can lead to four main biochemical consequences:

1. **Failure to complete a metabolic pathway→ metabolic block:** the end-product is not formed because the required enzyme to complete the metabolic sequence is missing.

2. **Accumulation of unmetabolized substrate:** the enzyme that converts the initial substrate into the first metabolic intermediates may be missing, in that case the initial substrate accumulates in excess.

3. **Storage of an intermediary metabolite:** a metabolic intermediates, which is normally quickly processed into the final product and so is usually present only in minute amounts, accumulates in large quantities if the enzyme for its metabolism is lacking. (von Gierke)

- **Failure to inactivate a tissue-damaging substrate.** (α1-antitrypsin)

GLYCOGENOSES

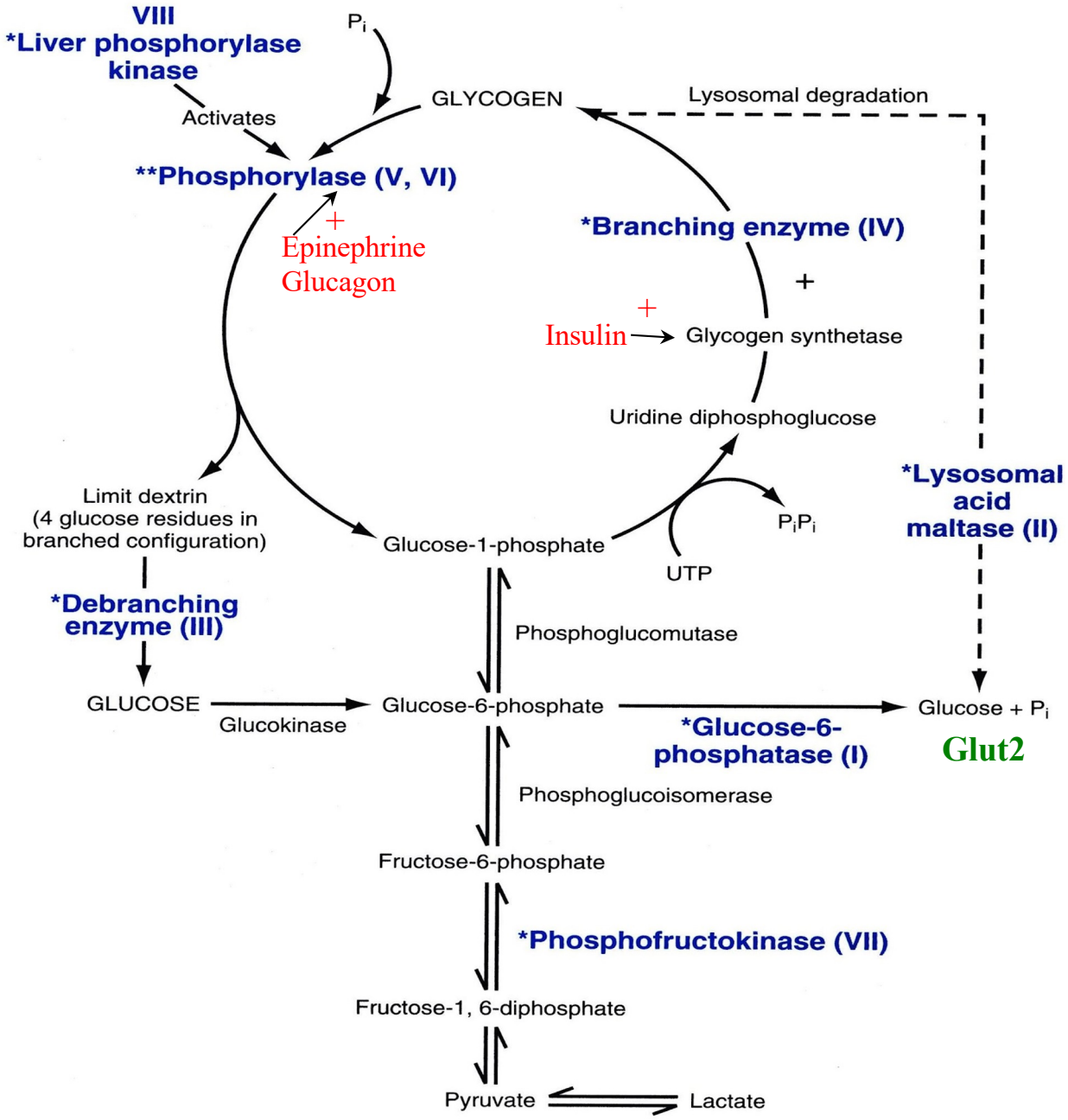
Glycogen storage diseases

Glycogenoses

- Glycogen storage diseases:
- Characterized by pathologic accumulation of glycogen
- Structurally normal or abnormal,
- Due to genetic enzymatic deficiency in one of the enzymes involved in glycogen metabolism
- Hereditary diseases
  - autosomal recessive
  - X-linked
- Target organs: liver, muscle tissue, kidney, myocardium

Glycogen metabolism

Pathway of Glycogen Synthesis and Glycogenolysis in liver



Glycogenoses

Autosomal recessive

Two main causes of damage

- Cell damage by accumulation of glycogen
- Energy deficiency for nearly absent glycolysis

Hepatic forms - Types I,III,VI,VIII

-Hepatomegaly by accumulation of glycogen in liver (and other organs)

-Hypoglycemia by low glucose

Muscle Forms – Types (II), V, VII

- low glycolysis in muscles ⇒ lack of energy ⇒ Muscle weakness, cramps

Others:

- Type II Pompe – Lysosomal acid maltase, accumulation of glycogen in lysosomes, prevalent heart damage

- Type IV (Anderson) – branching enzyme, ubiquitous deposition of abnormal glycogen, damages in nervous system, heart, muscles, hepatocytes.

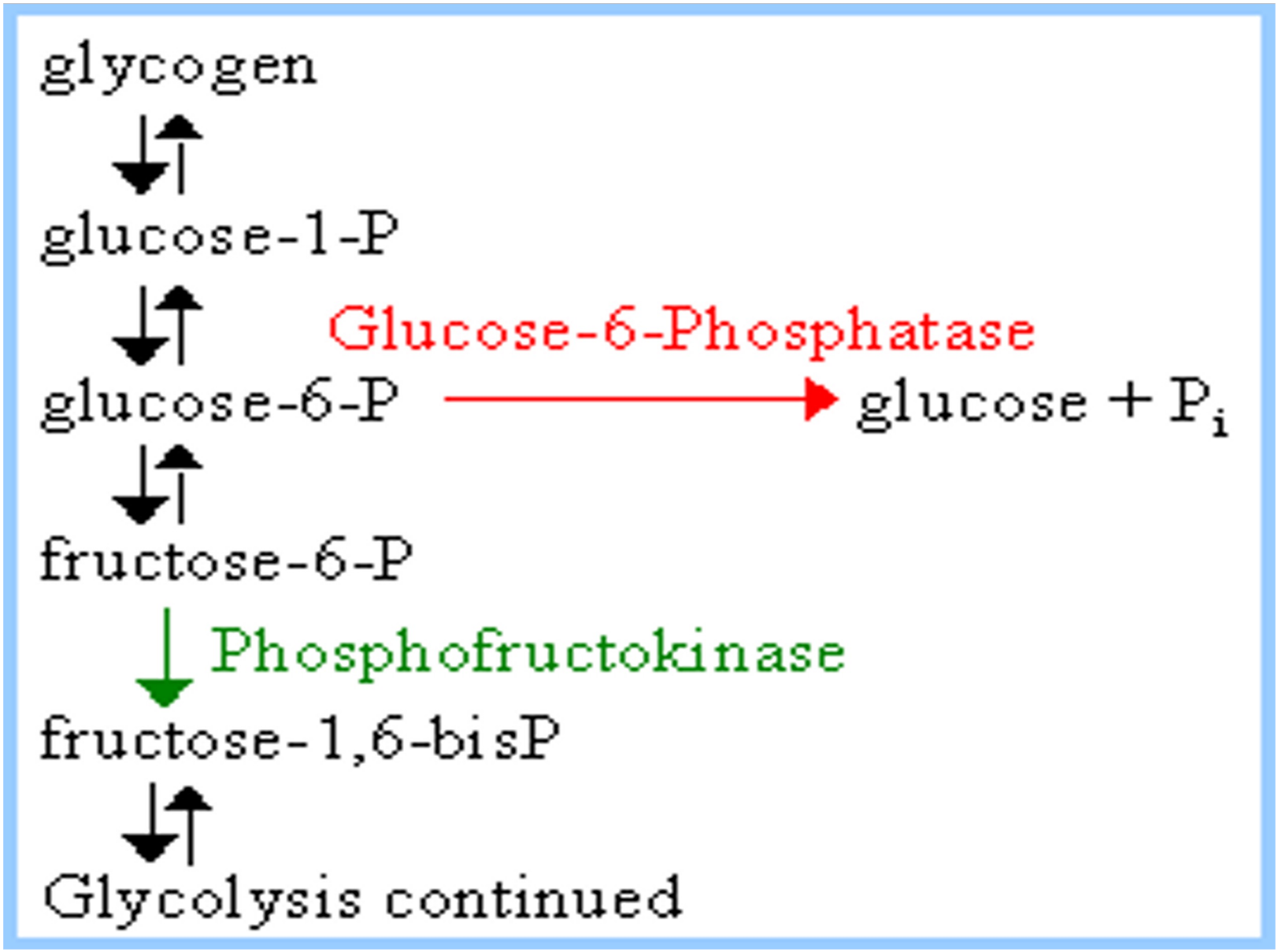
TABLE 2 Diagnosis of glycogen storage disease by molecular sequencing.

	Gene sequencing	Prenatal diagnosis available
Type 0		No
Liver isoform	*GYS2*	
Muscle isoform	*GYS1*	
Type I b, c, d	*SLC37A4*	Yes
Type I a	*G6PC*	Yes
Type II	*GAA*	Yes
Type III	*AGL*	Yes
Type IV	*GBE1*	Yes
Type V	*PYGM*	Yes
Type VI	*PYGL*	Yes
Type VII	*PFKM*	Yes
Type IX a	*PHKA2*	Yes
Type IX b	*PHKB*	Yes
Type IX c	*PHKG2*	Yes
Type IX d	*PHKA1*	Yes
Type X	*PGAM2*	Yes

TABLE 5–7 Principal Subgroups of Glycogenoses

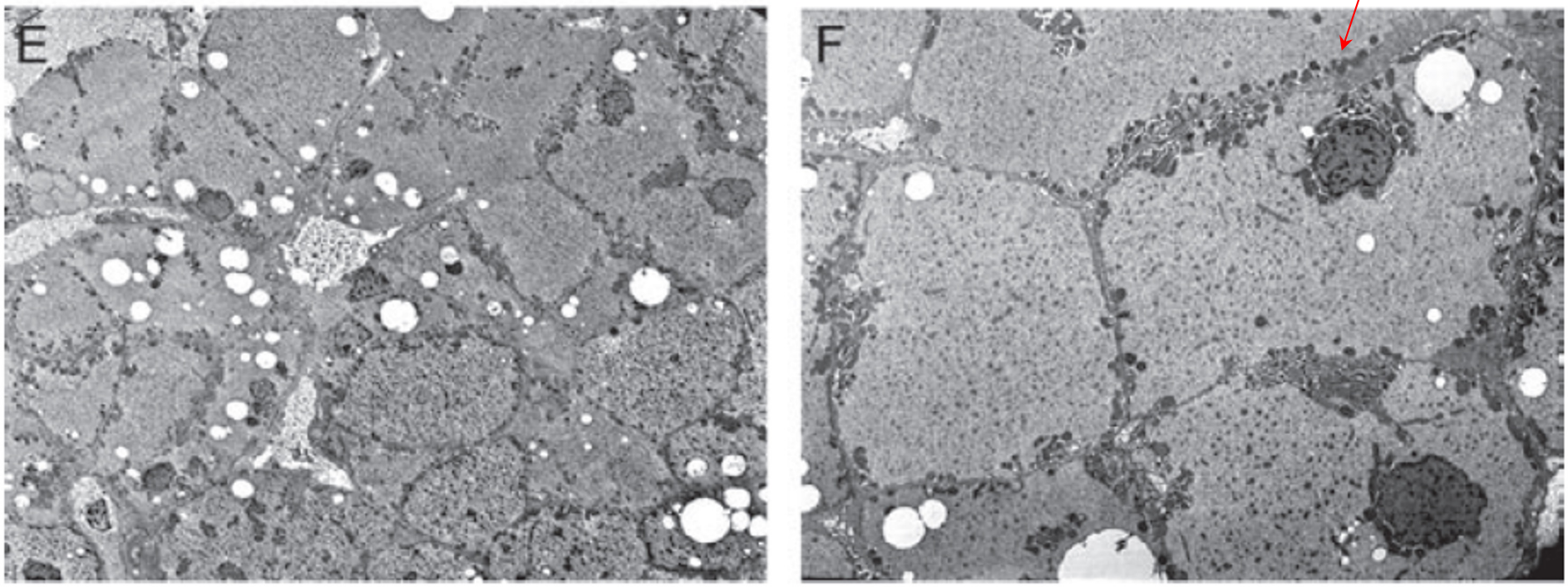
Clinicopathologic Category	Specific Type	Enzyme Deficiency	Morphologic Changes	Clinical Features
Hepatic type	Hepatorenal—von Gierke disease (type I)	Glucose-6-phosphatase	Hepatomegaly—intracytoplasmic accumulations of glycogen and small amounts of lipid; intranuclear glycogen Renomegaly—intracytoplasmic accumulations of glycogen in cortical tubular epithelial cells	*In untreated patients*: Failure to thrive, stunted growth, hepatomegaly, and renomegaly Hypoglycemia due to failure of glucose mobilization, often leading to convulsions Hyperlipidemia and hyperuricemia resulting from deranged glucose metabolism; many patients develop gout and skin xanthomas Bleeding tendency due to platelet dysfunction *With treatment:* Most survive and develop late complications (e.g., hepatic adenomas)
Myopathic type	McArdle syndrome (type V)	Muscle phosphorylase	Skeletal muscle only—accumulations of glycogen predominant in subsarcolemmal location	Painful cramps associated with strenuous exercise; myoglobinuria occurs in 50% of cases; onset in adulthood (>20 years); muscular exercise fails to raise lactate level in venous blood; serum creatine kinase always elevated; compatible with normal longevity
Miscellaneous types	Generalized glycogenosis—Pompe disease (type II)	Lysosomal glucosidase (acid maltase)	Mild hepatomegaly—ballooning of lysosomes with glycogen, creating lacy cytoplasmic pattern Cardiomegaly—glycogen within sarcoplasm as well as membrane-bound Skeletal muscle—similar to changes in heart	Massive cardiomegaly, muscle hypotonia, and cardiorespiratory failure within 2 years; a milder adult form with only skeletal muscle involvement, presenting with chronic myopathy

Glycogenoses type 1˚
von Gierke Disease



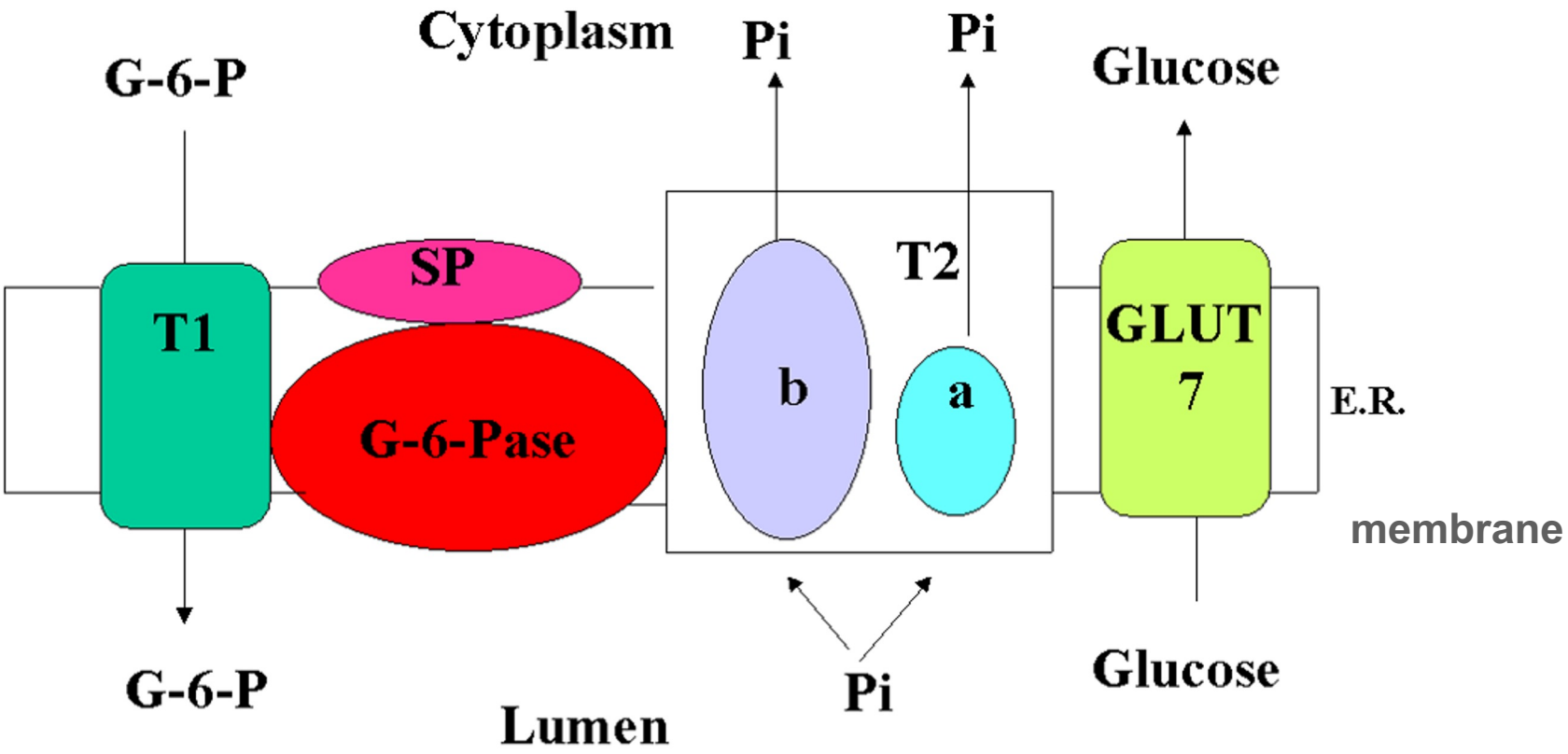
Glycogenoses Type I
vonGierke disease

Thickened plant-like membrane



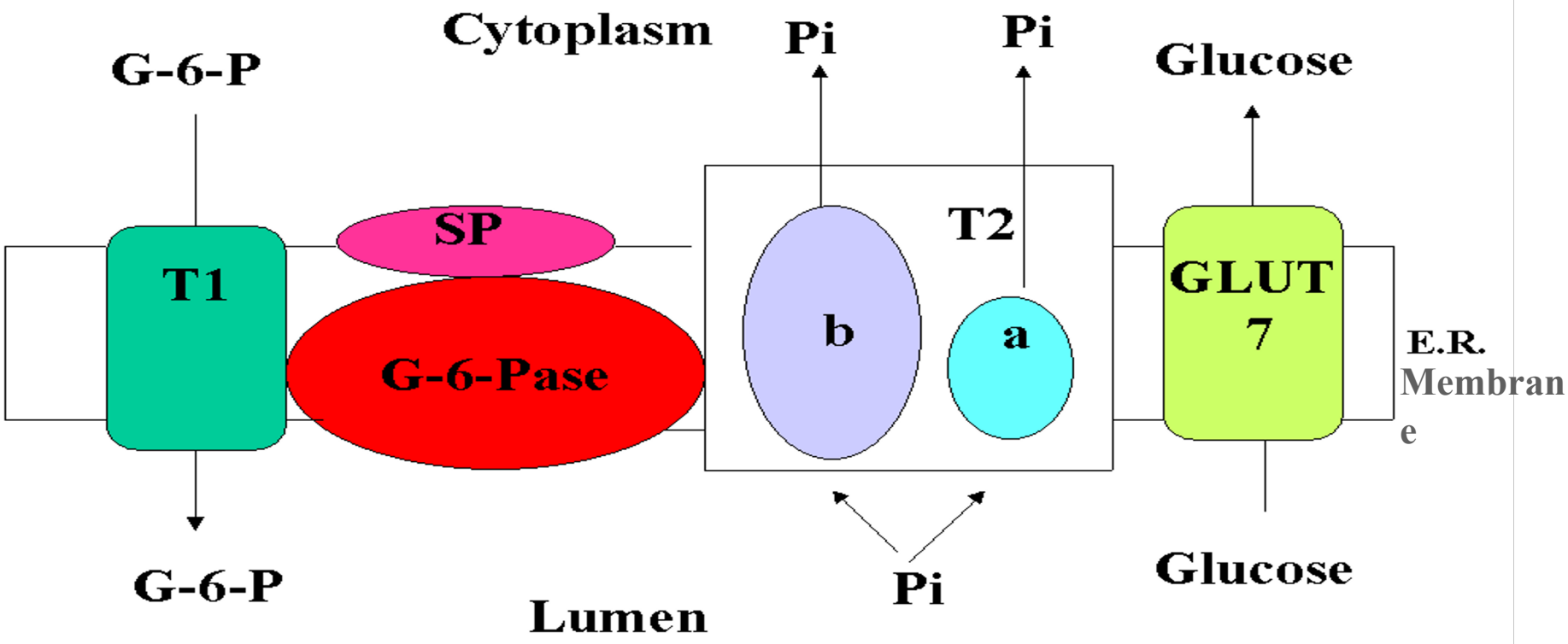
Hepatocytes

Glucose-6-posphatase



- Glucose-6-phosphatase is a complex enzymatic system in liver, kidney and present in small amount in platelets.
- Located in Endoplasmic Reticulum membrane (active site in lumen)
- Normal enzymatic activity: catalytic activity of G-6-Pase and regulatory protein (SP)
- 3 proteins to transport G-6P, Pi and glucose

Glucose-6-posphatase



- **G-6-Pase Deficiency** — **Syndrome type 1°**
- **SP Deficiency** — **Syndrome type 1a SP**
- **T1 Deficiency** — **Syndrome type 1b**
- **T2 Deficiency** — **Syndrome type 1c**
- **GLUT7 Deficiency** — **Syndrome type 1d**

Glycogenoses type 1°
von Gierke disease

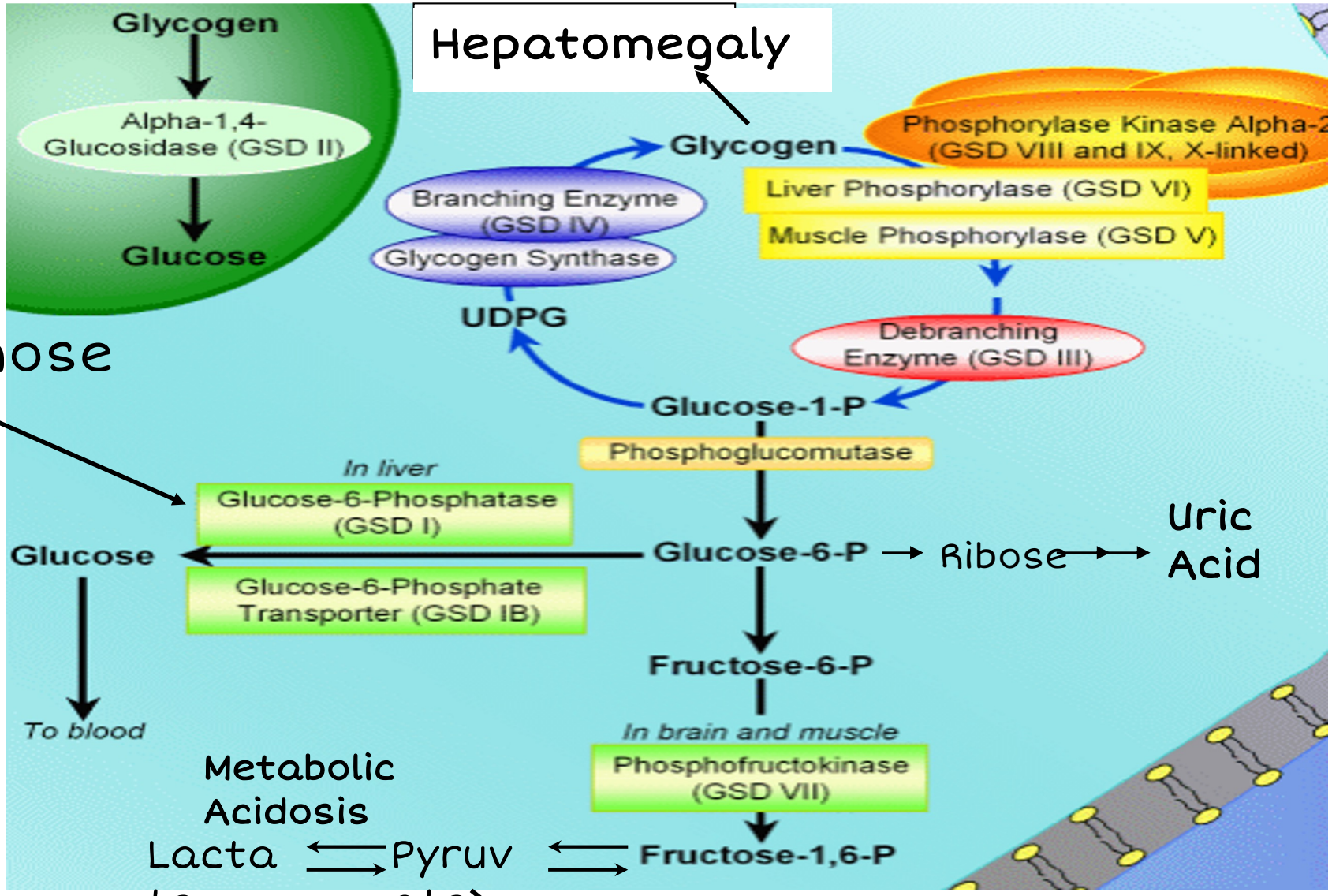
- **Deficit of glucose-6-phosphatase**

- **Accumulation of *normal glycogen* in liver and kidney**
  clinical manifestations start at age 1:
- **HYPOGLYCEMIA especially**
- **HYPERLIPIDEMIA lipolysis in adipose tissue**
- **HEPATIC STEATOSIS and KETOSIS β-oxidation of fatty acids**
- **HYPER-PIRUVICEMIA increase of pyruvic acid** } **metabolic**
- **HYPERLACTACIDEMIA increase of lactic acid** } **acidosis**

- **HEPATOMEGALY due to accumulation of glycogen and fatty acids**
- **GROWTH FAILURE**
- **OSTEOPOROSIS (metabolic acidosis)**
- **HIGH RISK OF HEMORRHAGE abnormal platelet function**
- **ADIPOSITY localized to face and bottom**
- **CUTANEOUS XANTHOMAS**

- **SEVERE PROGNOSIS**

Glycogenose s tipo I



Insulin ⊤ HYPO-GLICEMIA

Metabolic Acidosis

Lactate ⇌ Pyruvate ⇌

Pyruvate → Acetil-Co-A synthesis → Cholesterol

Mobilization of Fatty Acids and Block of protein synthesis Steatosis

Glycogenoses type 1a

Important hypoglycemic crisis in the first year

Later low of glycemia are tolerated

Brain start using Ketone bodies

HYPOGLYCEMIA:

-Block insulin secretion

-Stimulates glucagon resulting in Hepatocytes proliferation

-Accelerates lipolysis in adipose tissue

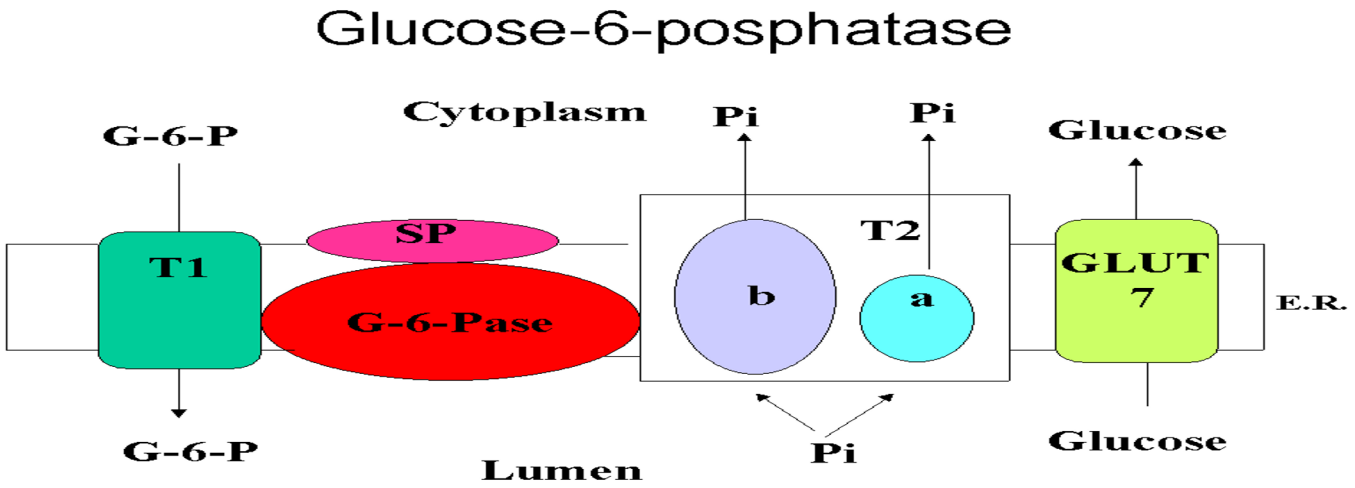
-Stimulates β-oxidation of Fatty Acids and gluconeogenesis in liver

(Hyperlipidemia, hepatic steatosis, Ketosis)

-Accumulation of glucose-6-phosphate increases glycolysis

Hyperpiruvicemia, Hyperlactacidemia

Glycogenoses type 1°
von Gierke Disease



Molecular basis

- ***Gene of G-6-Pase*** Chr. 17 5 exons 12.5 Kb

Different mutations:

- EXON 2 Arg 83 Cys catalytic activity
- EXON 3 insertion 2 bp TA 452 stop codon truncated protein
- EXON 5 Gln 347 Stop codon truncated protein

GSD1a Treatment Strategies

Primary Objective

Maintain normoglycemia or avoid hypoglycemia in GSD1a.

Surgical Approach

Liver Transplantation

Benefit

Corrects glucose homeostasis effectively.

Limitation

Does not prevent renal dysfunction long-term.

Novel Clinical Trial

Gene Therapy (AAV8)

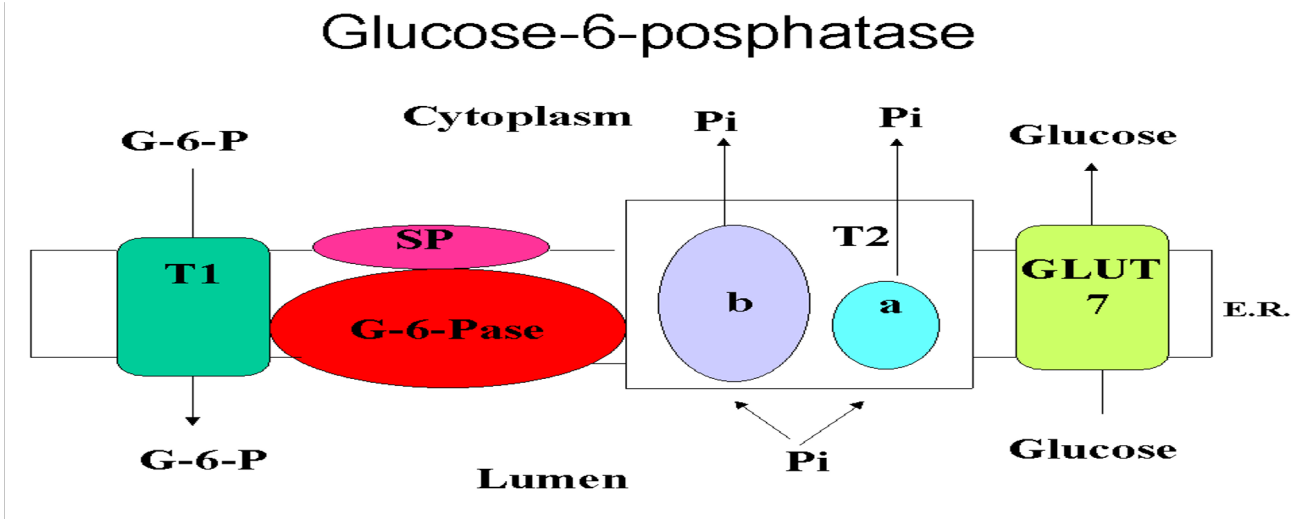
Trial Reference

Phase I/II Trial (NCT03517085)

Uses adeno-associated virus 8 (AAV8) to deliver the G6Pase-α gene directly to the liver to correct defective G6PC (27).

May establish gene therapy as a promising future corrective approach.

Glycogenoses type 1b



- Hereditary transmission (autosomal recessive)
- Clinical symptoms similar to G. type 1a

Additional hallmark features:

- Neutropenia and impaired neutrophil function

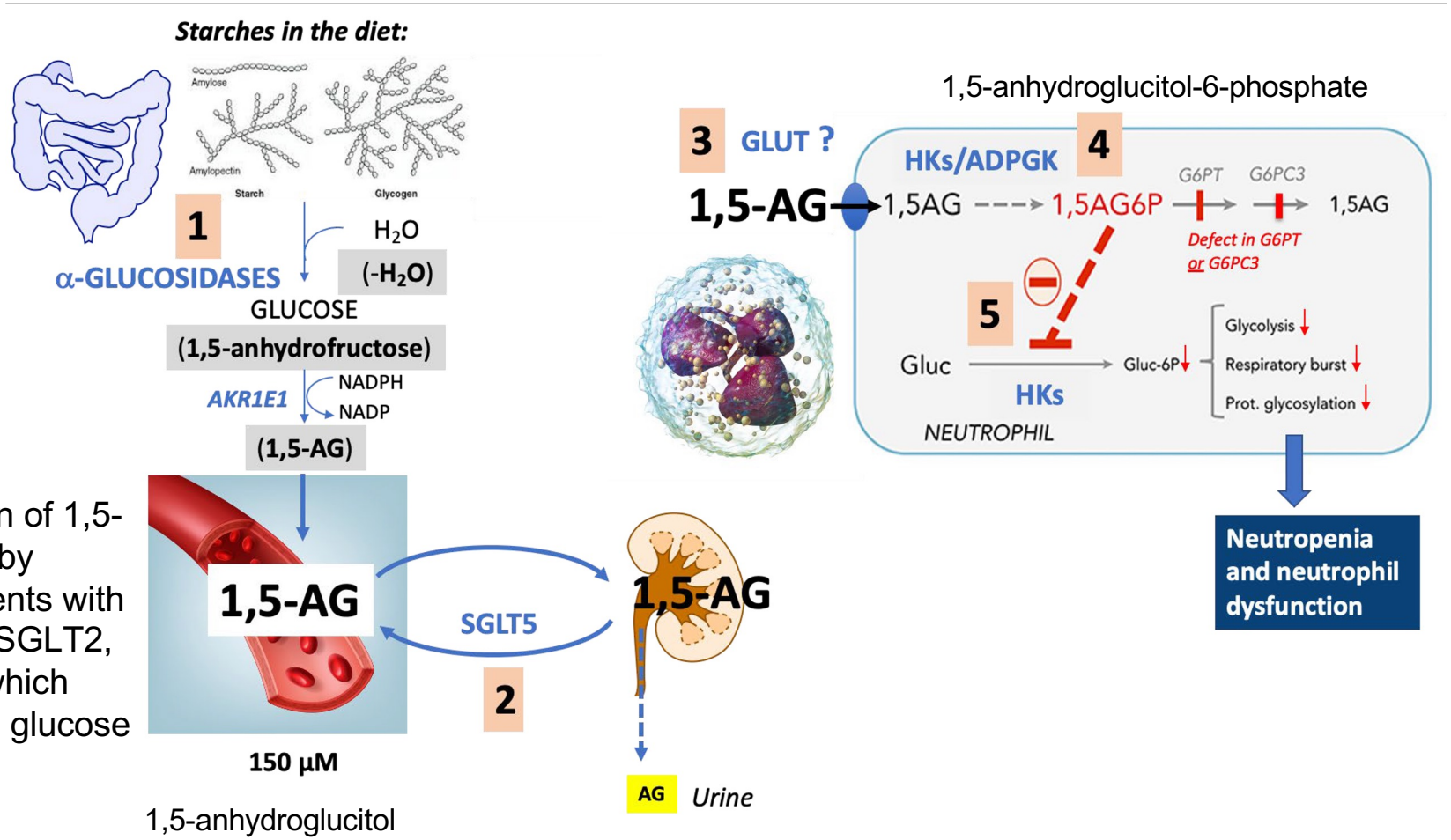
Molecular basis

Gene of transport **(T1)**

Chr. 11q23 5,3 Kb 9 exons

Different mutations, that impair the enzyme ability to transport

Mechanism of immune dysfunction in GSD1b



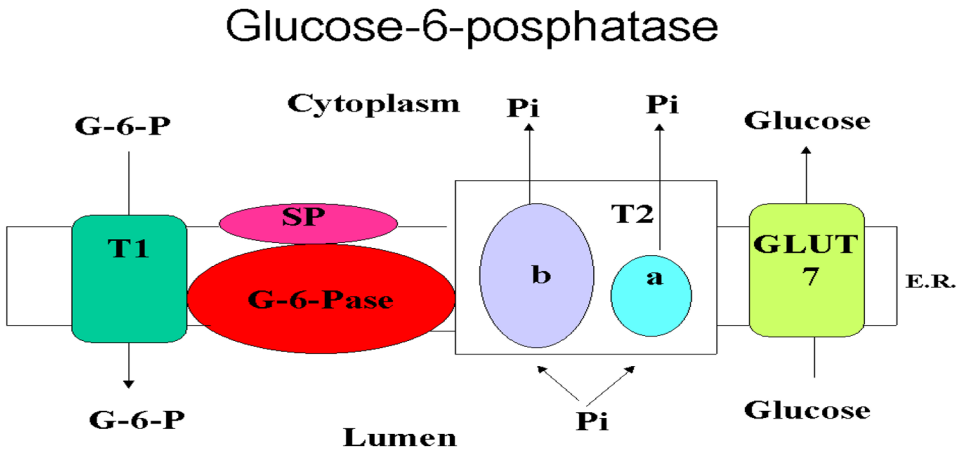
lowering the concentration of 1,5-AG in blood by treating patients with inhibitors of SGLT2, (Gliflozins) which inhibits renal glucose reabsorption

Key differences between GSD1a and GSD1b

Feature	GSD 1a (von Gierke Disease)	GSD 1b
Mutated Gene	`G6PC` (Deficient catalytic α-subunit)	`SLC37A4` (Deficient G6PT transporter)
Neutropenia	❌ **Absent** (Normal white blood cell count)	🩸 **Present & Chronic** (Severe reduction of neutrophils)
Immune Function	**Normal** (Standard resistance to pathogens)	⚠️ **Severely Impaired** (Defective chemotaxis and bacterial killing)
Intestinal Pathology	None	🩺 **IBD-like Phenotype** (Crohn's-like mucosal ulcerations)
Recurrent Infections	Same risk as the general population	😘 **High Risk** (Frequent otitis, severe gingivitis, perianal abscesses)
Gold Standard Diagnosis	**Genetic testing** for `G6PC` mutations	**Genetic testing** for `SLC37A4` mutations

- **GSD 1a** is a **purely metabolic** disorder.
- **GSD 1b** is a **metabolic-immunological syndrome**, where the loss of G6PT induces energy failure and cellular toxicity specifically within neutrophils.

Glycogenoses type 1c



- ***Deficiency of the enzyme transporting Pi***
- Clinical mild symptoms similar to G. 1b

Molecular Basis

- Gene of Pi transport
- Chr. 11q23-24.2

Mutations

- Mutations of splicing site exon 8/intron 8

GLYCOGEN STORAGE DISEASES & AUTOIMMUNITY

Different immune phenotypes — different levels of evidence

GSD TYPE Ib

- Neutropenia and impaired neutrophil function
- Recurrent infections and intestinal inflammation
- Autoimmune manifestations reported in small cohorts
- Altered T-cell metabolism and reduced regulatory T-cell (Treg) activity
- Lower FOXP3 expression (a key Treg transcription factor)

↔

GSD TYPE IX

- Mainly metabolic, liver and/or muscle manifestations
- Immune abnormalities only sporadically reported
- No established link with autoimmunity
- Impact of altered glycogen breakdown on immune cells remains unclear

POTENTIAL LINK: IMMUNOMETABOLISM

Glucose and glycogen metabolism influence immune-cell activation, survival and function

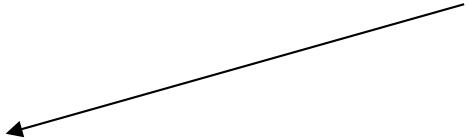
TAKE-HOME MESSAGE | Metabolic dysfunction can alter immune regulation, but immune dysregulation does not necessarily lead to autoimmunity.

Glycogenoses : myopathic forms

Glycogenoses causing constant weakness

- Weakness related to the amount of glycogen storage in muscle cells
- Dependent on specific enzyme defects

example: Acid Maltase deficiency (17q23)

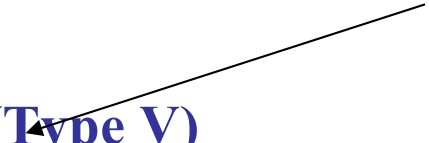


Pompe Disease (Type II)

Glycogenoses that cause a reduced exercise tolerance, cramps and myoglobinuria

- Generally after intense exercise
- Dependent on specific enzyme defects

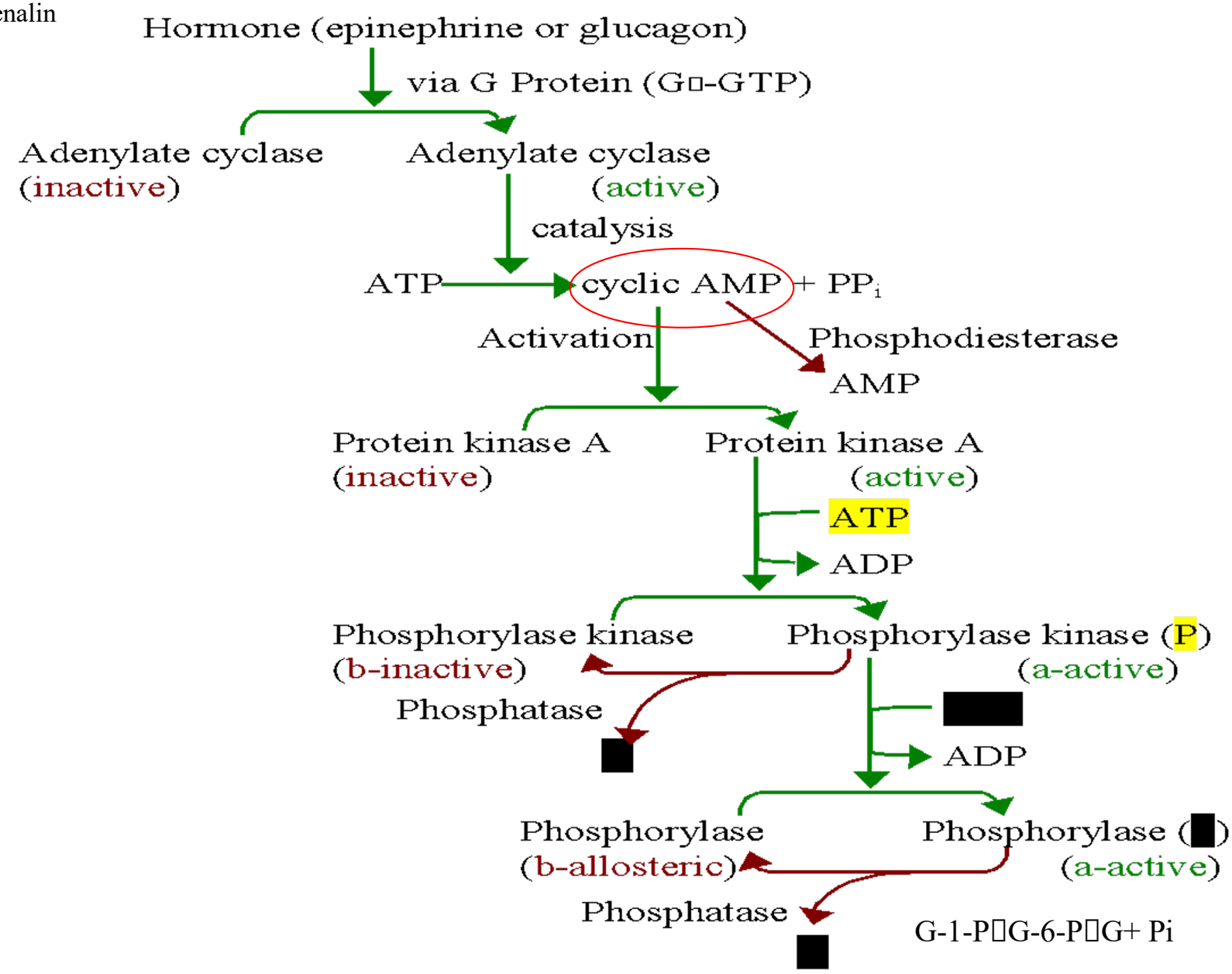
example: Phosphorylase deficiency (11q13)



McArdle Disease (Type V)

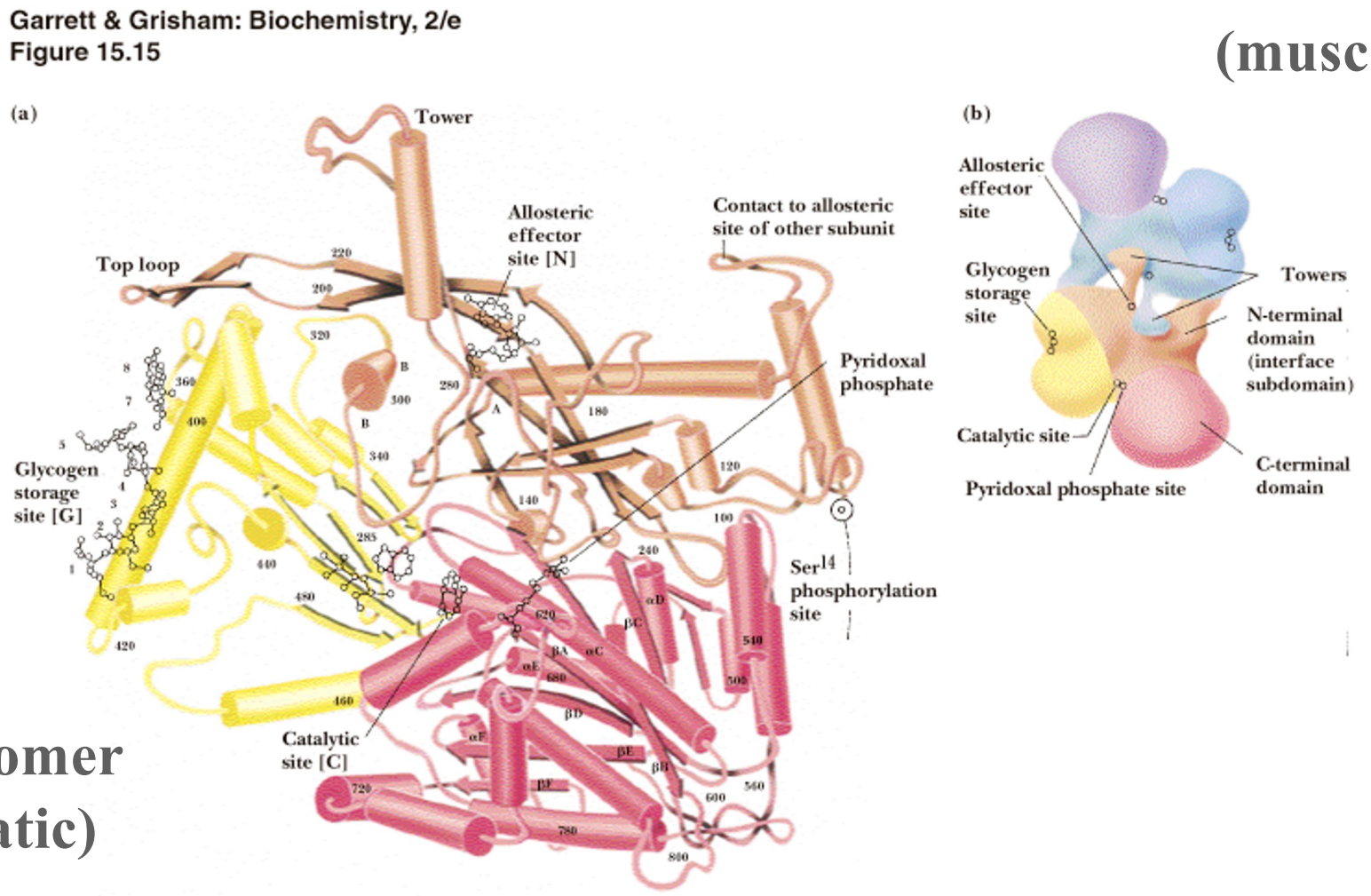
Regulation of glycogen phosphorylase

Muscle in contraction
Adrenalin



Active forms of glycogen phosphorylase

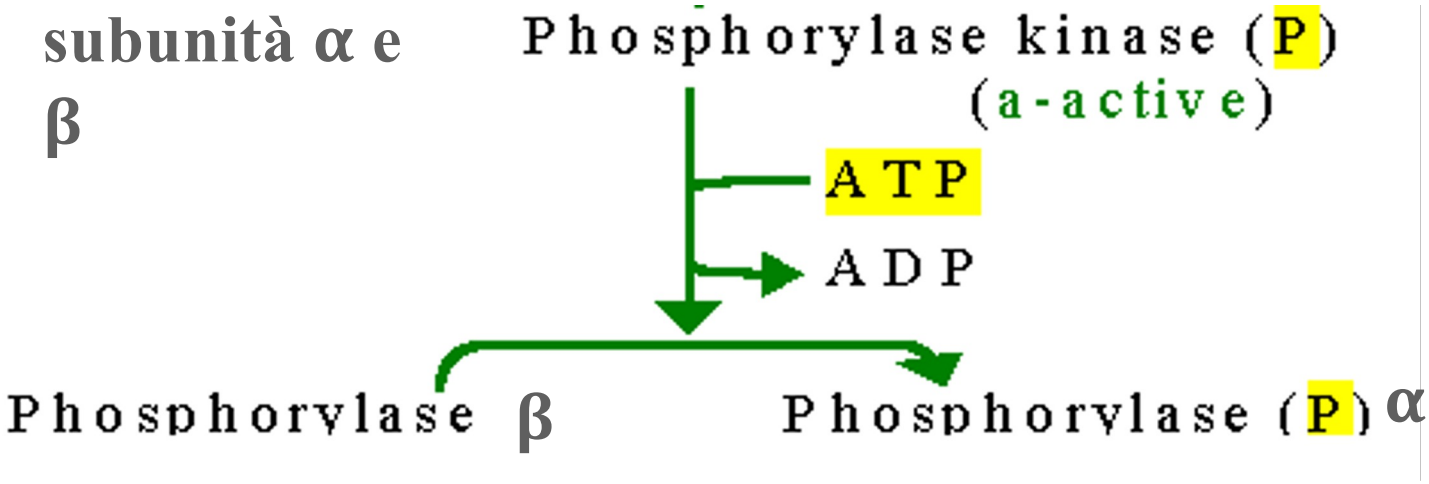
Dimer (muscolar)



Monomer (hepatic)

Saunders College Publishing

Alterations of glycogen phosphorilase

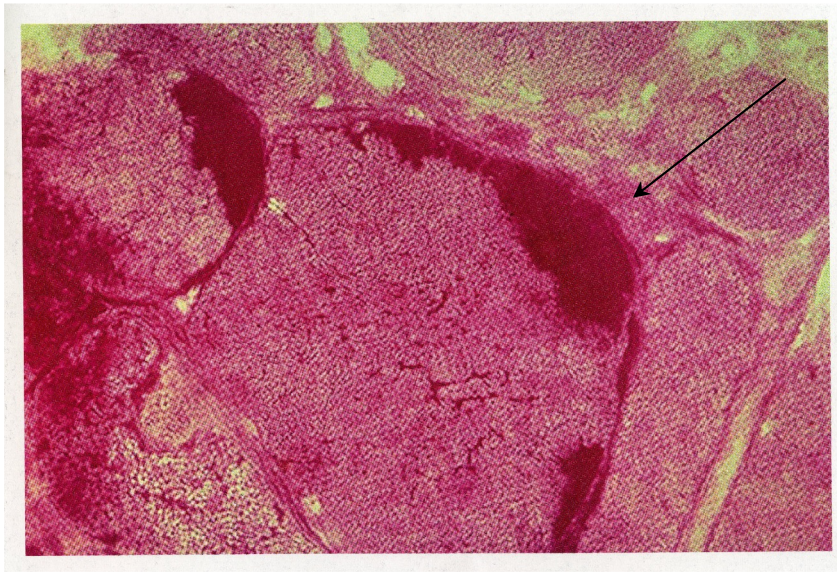


- Deficiency of muscle glycogen phosphorylase: GSD V
- Deficiency hepatic glycogen phosphorylase: GSD VI
- Deficiency phosphorylase kinase: GSD IX

Glycogenosis type V
McArdle Disease

- Deficiency of **Muscle Phosphorylase**
- Accumulation of normal Glycogen in muscles

Clinical features:

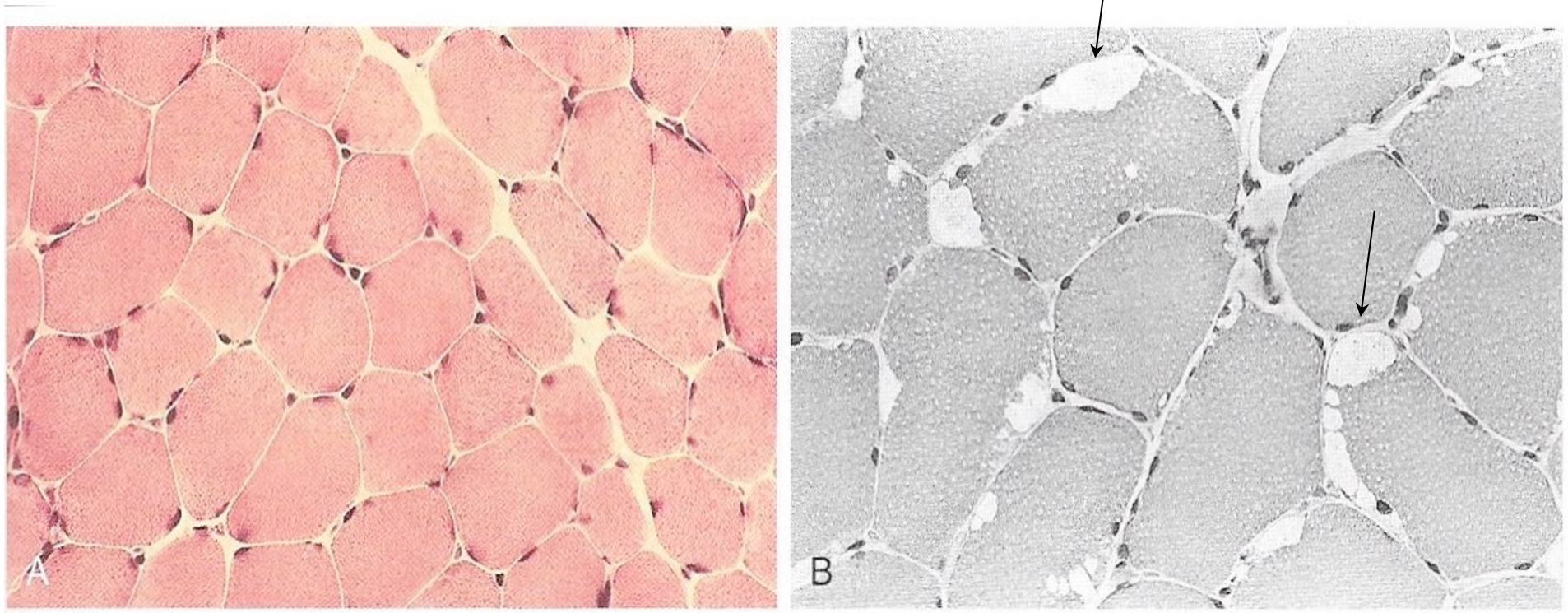


- Myalgia
- Cramps
- Muscle hardening after intense exercise
- Myoglobinuria causing Renal failure
- No increase in lactacidemia after muscle exercises (altered glycogenolysis)
- Normal Glicemia

McArdle Disease

muscle fibers

Large peripheral vacuoles



Normal

McArdle

Rubin - Pathology

Glycogenosis type V

Molecular basis

Gene of muscle glycogen phosphorylase (PYGM)

Chr. 11 14 Kb 20 exons

5' region multiple promoters

Region –592 CTCCAAAAGG necessary for efficient transcription

Nonsense: Exon 1 CGA TGA Stop codon (frequent)

Missense: Exon 1 frameshift: rapidly degraded peptide

Esone 5	G 204 S	GGC AGC	altered protein
Esone 8	L291P	CGT CCG	less active
Esone 14	K452 T	AAG ACG	no stabilized

Deletions: Exon 14 1844 deletion di 67 basi

Exone 17 D TTC Deletion of AA: altered protein folding

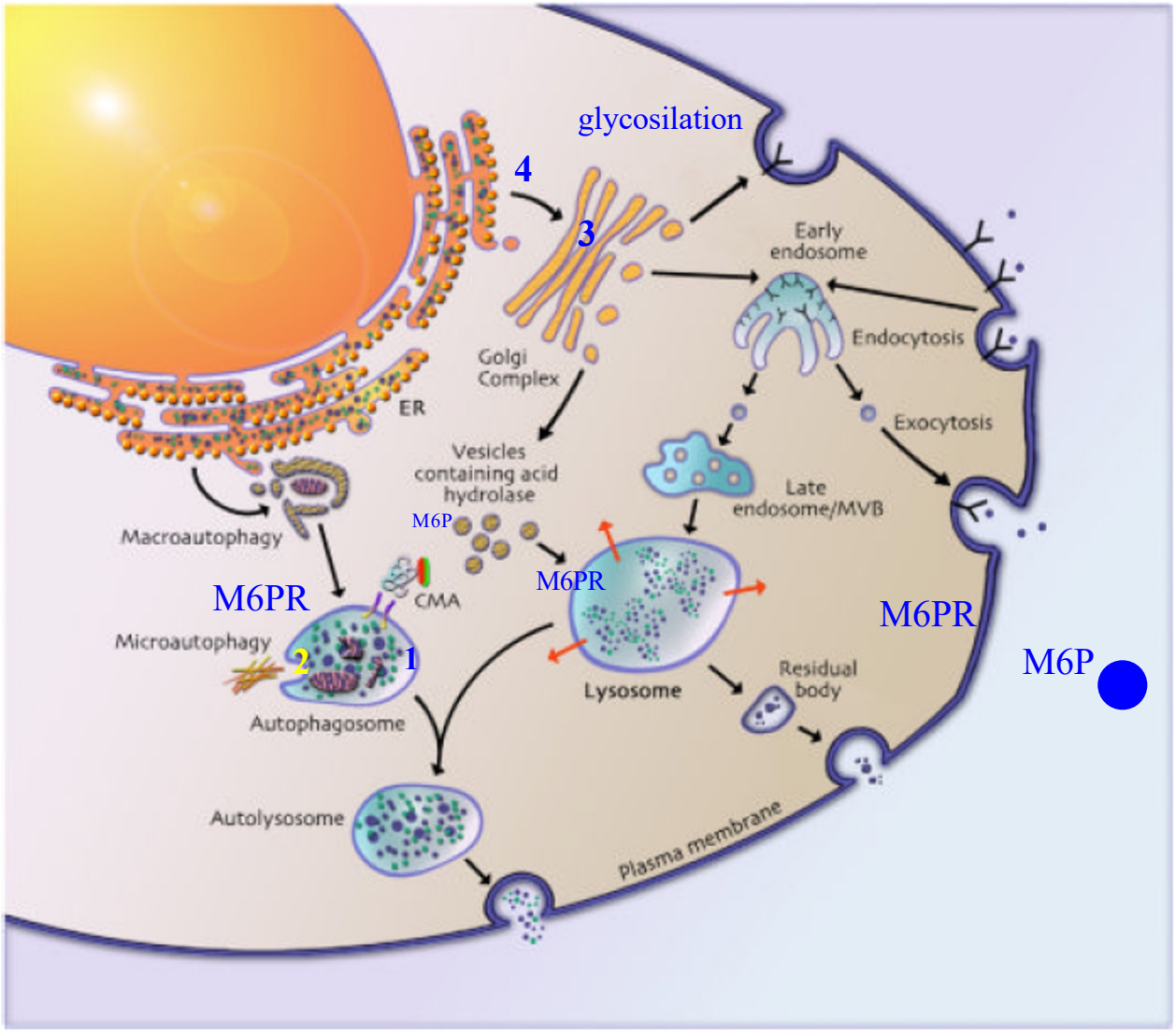
Many variants are catalogued in the Leiden Open Variation Database (LOVD) (http:databases.lovd.nl/shared/variants/PYGM).

Lysosomal storage disorders

Disease	Affected protein	Storage material	Mechanism	Affected organs	Pathology
Niemann-Pick disease type C	NPC1, NPC2	Cholesterol, Sphingolipids	Lysosomal cholesterol and lipid export, Foam cells in visceral organs and neuronal storage	Liver, CNS	Ataxia, Dysarthria, Dysphagia, Dystonia, Dementia, Seizures, Hepatosplenomegaly, Thrombocytopenia
Fabry disease	α-Galactosidase A	Globotriaosylceramide, Galabiosylceramide, Globotriaosylsphingosine, blood-group-B glycolipids	Lipid storage in endothelial and smooth muscle cells of blood vessels	Kidney, Heart	Acroparesthesias, Angiokeratoma, Renal failure, Cardiomyopathy, Stroke, Gastrointestinal symptoms
Gaucher disease	β-Glucosidase/ Glucocerebrosidase	Glucosylceramide, GM1, GM2, GM3, GD3, Glucosylsphingosine	Lipid storing macrophages	Spleen, Liver, Bone marrow, CNS (not type 1)	Hepatosplenomegaly, Thrombocytopenia, Anemia, Skeletal deformations, Bone fractures
Pompe disease	α-Glucosidase	Glycogen	(Autophagic) accumulation in type II muscle fibers	Skeletal muscle, Cardiac muscle	Cardiomegaly, Hypotonia, Cardiorespiratory failure, Hepatomegaly, Muscle weakness

Summarised from Ballabio et al. [4], Parkinson-Lawrence et al. [5], Platt et al. [6].

Protein trafficking as a basis of lysosomal storage disorders



Mutations in:

1. lysosomal protein (inactive)
2. integral lysosomal proteins, NPC1for export of lysosomal products

1. Defective folding

1. Inability to exit the ER

1. Lysosomal uptake (Mannose-6-phosphate receptor)

Lysosomal Storage disease
Pathologic consequences

- ➔ Accumulation of the partially degraded insoluble metabolite within the lysosome. (*Primary accumulation*).
- ➔ Lysosomes become large and numerous and interfere with normal cell functions ⟹ lysosomal storage disease.

- ➔ Impaired autophagy resulting in *secondary accumulation* of autophagic substrates (polyubiquitinated proteins, old and exhausted mitochondria).
- ➔ Decreased ability to buffer Ca++ and altered membrane ⟹ ROS ⟹ Apoptosis.
- ➔ Autophagic defects are frequent in lysosomal disorders and damage tissues.

Glycogenosis type II
Pompe Disease

- **Deficiency of lysosomal *acid Maltase***
- **Accumulation of normal glycogen in all organs, in vacuoles.**

- ***Infantile Phenotype*: in the first trimester**

Lead quickly to die (failure c.c., pulmonitis, etc)

Important muscle hypotonia, Cardiomegaly, normal Glicemia

- ***Juvenile Phenotype* : onset in the first decade of life**

Muscle hypotonia, pulmonary infections, respiratory failure

Glycogen mainly increased in skeletal muscles

- ***Adult Phenotype*: reduced morbidity**

Onset in the second decade of life

respiratory failure (diaphragm muscles)

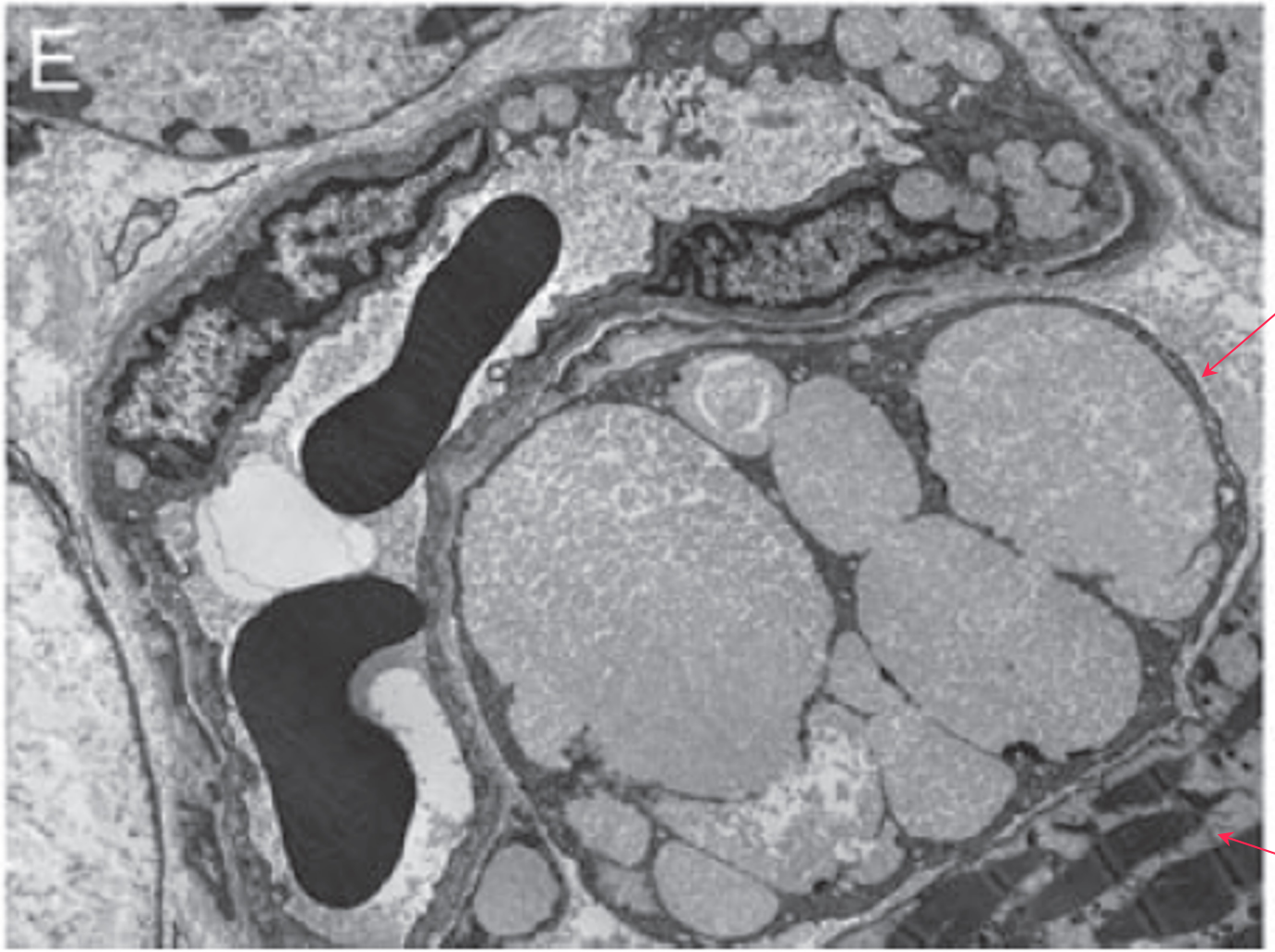
Glycogenosis Type II
Pompe disease

Storage of glycogen in autophagic vacuoles, where other molecules are digested

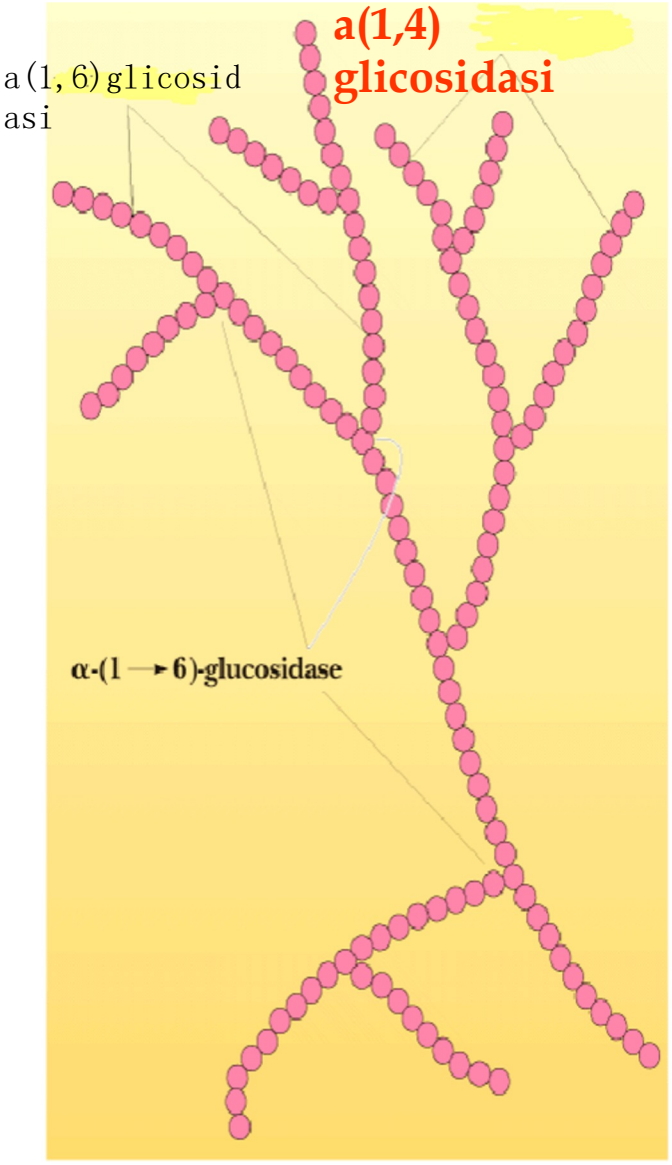
In autophagic vacuoles glycogen is not metabolized by cytoplasmic glycogenolytic enzymes

Lysosomal degradation of lysosomal glycogen is necessary to mobilize glycogen in neonatal liver

Glycogenosis Type II
Pompe disease



Glycogenosis type II
Pompe Disease



Molecular Basis

Gene of α(1,4) glucosidase

Chr. 17 20 exons

The enzyme is synthesized as an inactive precursor form 110 Kd

carried in

lysosomes

Lysosomal form	Lysosomal form
70 Kd	76 Kd

Glycogenosis type II

genotype-phenotype correlation

Infantile phenotype

- **Missense** Exon 5: Met 318 Thr
  Exon 11: Glu 521 Lys catalytic site catalytic activity
  Exon 14: Cys 647 Trp also in adult phenotype
  Exon 5: Leu 299 Arg
- **Deletion:** Exon 10 Δ13 nt (1456-1468) Stop codon truncated protein
  Exon 18 Δ18 lacking catalytic domain

Adult Phenotype

- **Missense:** Exon 14: Asp 645 Glu **residual catalytic activity**
  Exon 14: Gly 643 Arg **10-12%**
  Exon 15: Arg 725 Trp
- **Nonsense:** Exon 18 Arg 854 Stop codon truncated protein
- **Deletion:** Exon 10 Δ10 important mutation
  Exon 18 Δ18 loss of proteolytic cleavage site
  Exon 2 Δ2 for enzyme maturation

Chorionic villi and skin fibroblasts

Pompe disease

Clinical-genetic correlations:

- Level of residual enzyme activity correlates with:

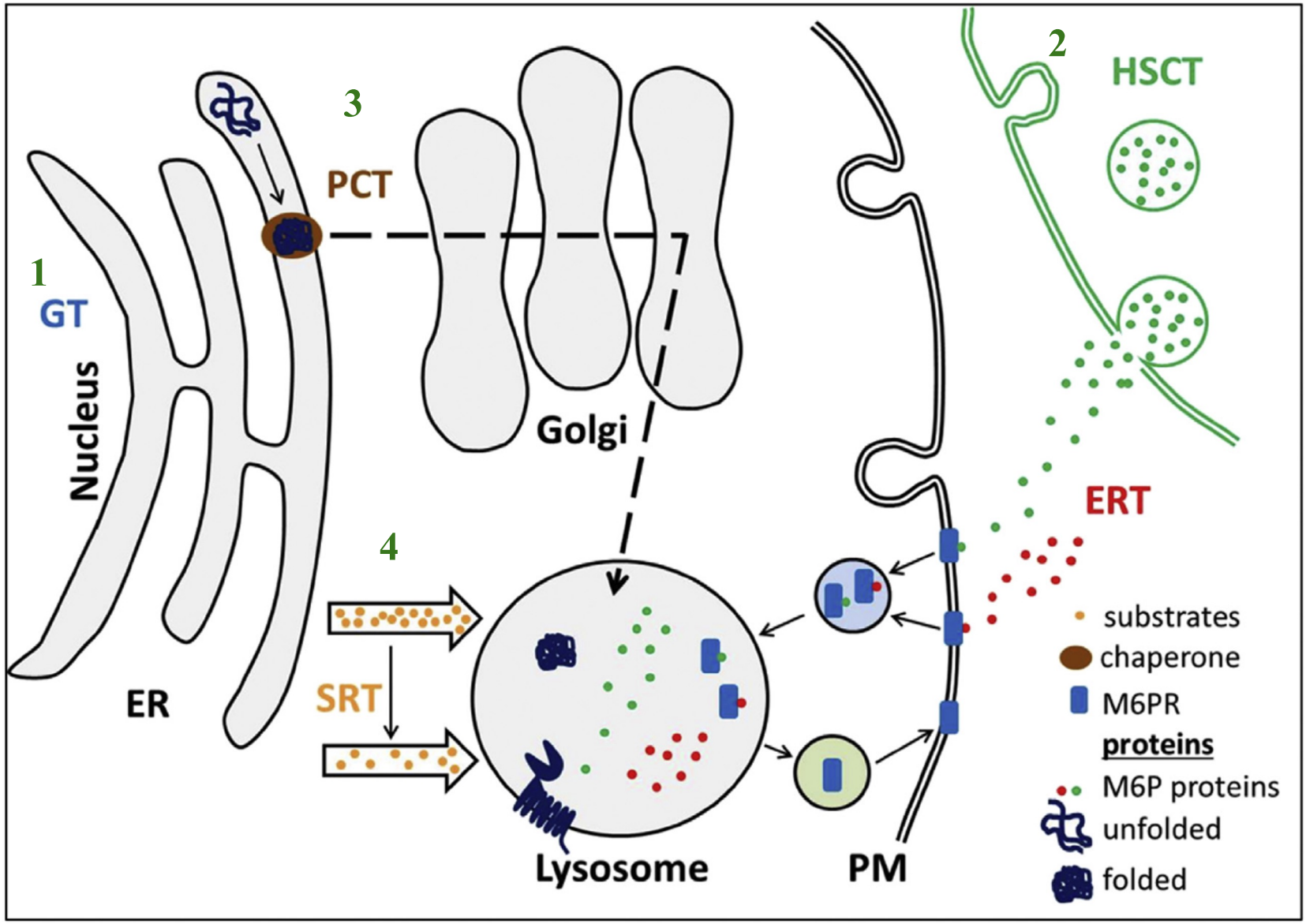
Severity of disease - Age of disease onset - Location of mutations

There is the need to:

➔ Increase the amount or activity of functional enzyme

➔ Reduce the amount of substrates accumulating in the lysosome

General strategies for the treatment of lysosomal storage disorders



Enzyme replacement therapy (ERT)

Molecular Genetics and Metabolism 122 (2017) 80–85

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journal homepage: www.elsevier.com/locate/ymgme



Effect of enzyme replacement therapy with alglucosidase alfa (Myozyme®) in 12 patients with advanced late-onset Pompe disease

- Recombinant human GAA
- Long term effect
- Increased time of autonomous ventilation
- Enlarged distance in assisted walk

Single gene disorders may be classified into four categories:

a. Defects in membrane receptors and transport systems

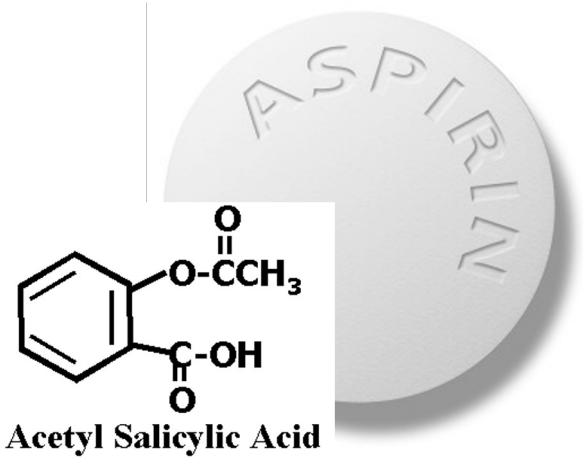
a. Enzyme defects and their consequences

a. Alterations in the structure, function, or quantity of nonenzyme proteins

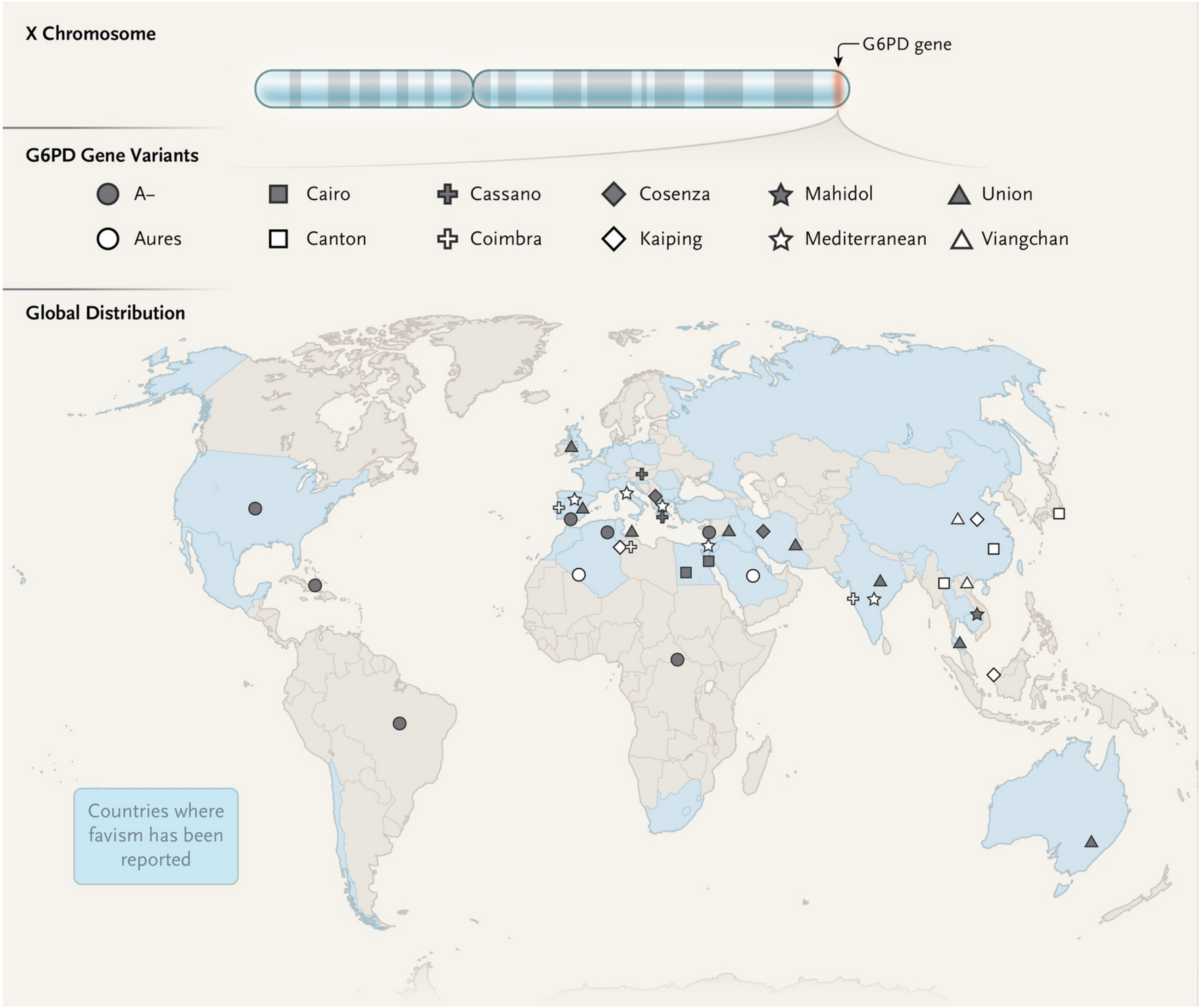
a. Mutation resulting in unusual reactions to drugs.

Genetically determined adverse reactions to drugs



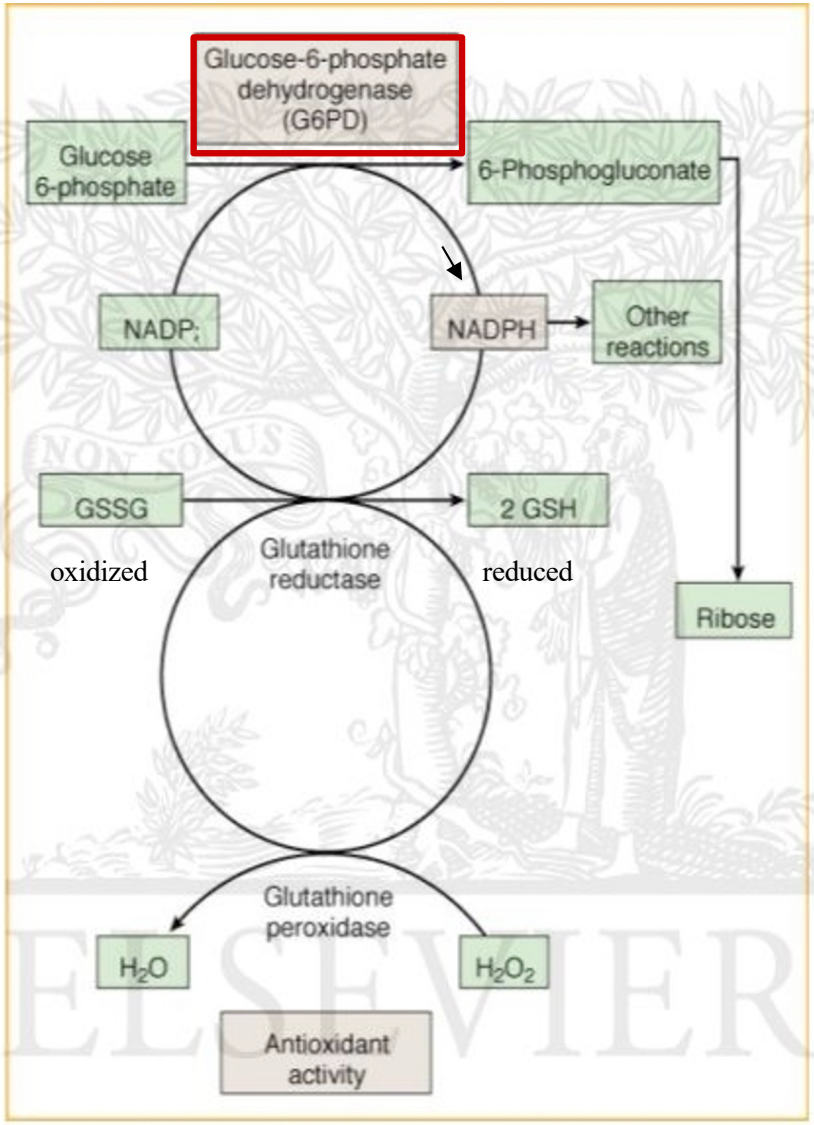


Acetyl Salicylic Acid


X Chromosome
G6PD gene
G6PD Gene Variants
A–
Aures
Cairo
Canton
Cassano
Coimbra
Cosenza
Kaiping
Mahidol
Mediterranean
Union
Viangchan
Global Distribution
Countries where favism has been reported

GLUCOSE-6-PHOSPHATE DEHYDROGENASE (G-6-PD)

G-6-PD deficiency reduces the ability of red blood cells to protect themselves against oxidative injuries and lead to hemolysis



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-More than 150 molecular variant of this enzyme (misfolded protein)
-Frequent in mediterranean
-Gene located on chr. X (Xq28)
-Male ➡ all erythrocytes are affected

-Heterozygous female
- ↗ defective erythrocytes
- ↘ normal erythrocytes

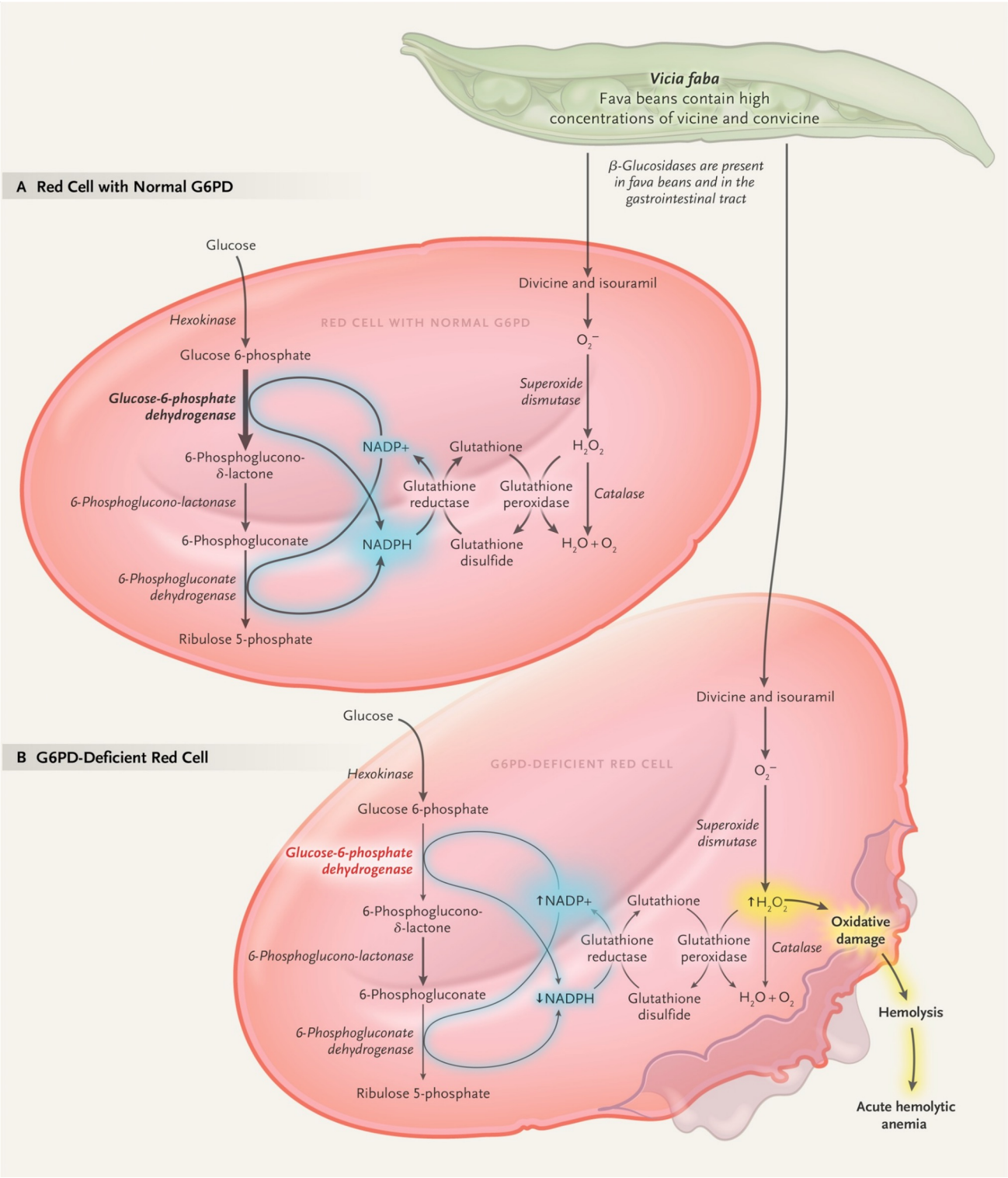
Role of G6PD in Protection against Oxidative Damage.

Potential oxidant:
-infections
-drugs
-foods

http://www.g6pd.org/it

http://www.g6pd.org/it/G6PDDefieciency-it/SafeUnsafe-it/DaEvitare_IS-it.aspx

intravascular and extravascular hemolysis



Two variants cause clinically significant Hemolytic Anemias:

Misfolding of the protein more susceptible to proteolytic degradation

G6PD^{-}

G6PD mediterranean

10% American black

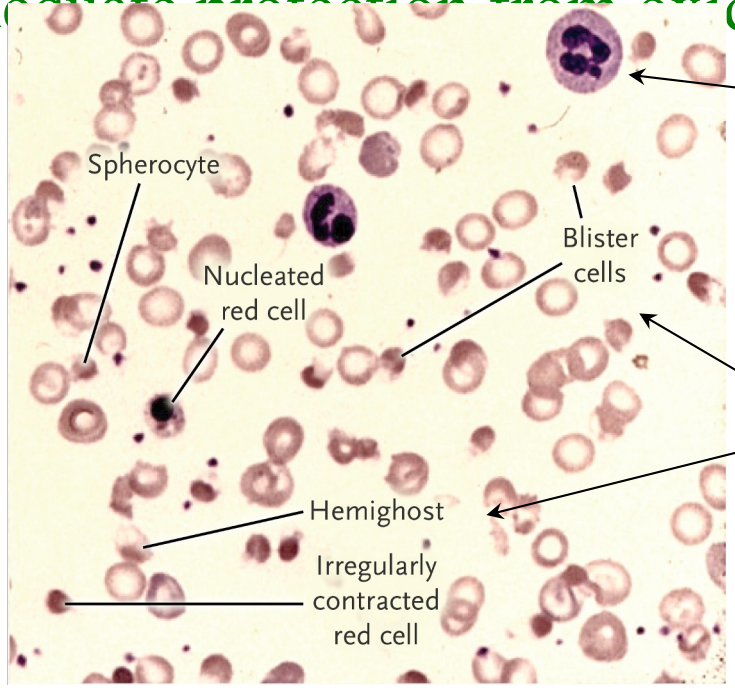
Half-life moderately reduced

Middle East

markedly abnormal

inadequate protection from oxidants

anisocytosis
poikilocytosis



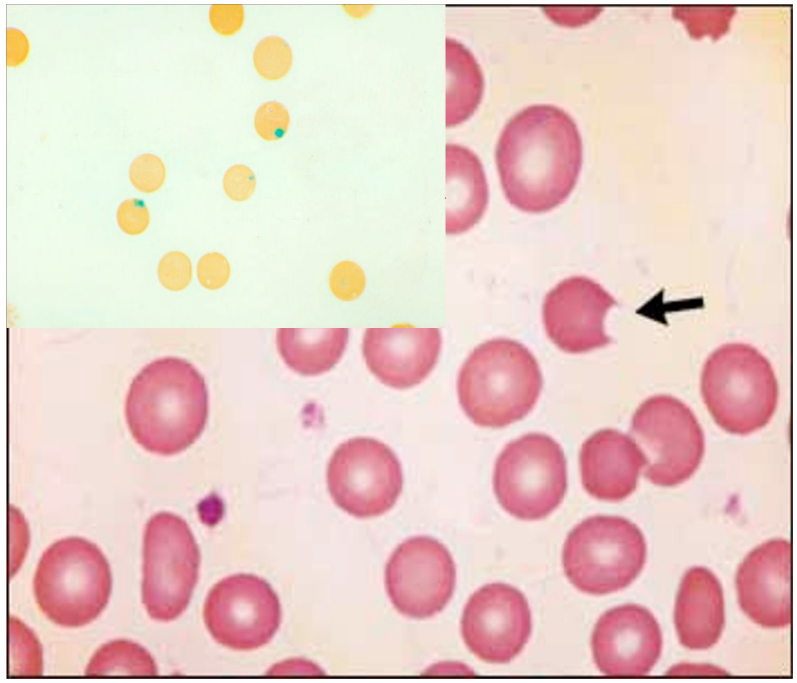
inflammation

characteristic of oxidative hemolysis

Infections and drugs and certain foods can trigger hemolysis

The oxidant denatures Hb → Heinz precipitates

(membrane-bound precipitates)



Macrophages pluck out the Heinz bodies → membrane damage (trapped in spleen)

Clinical manifestations:

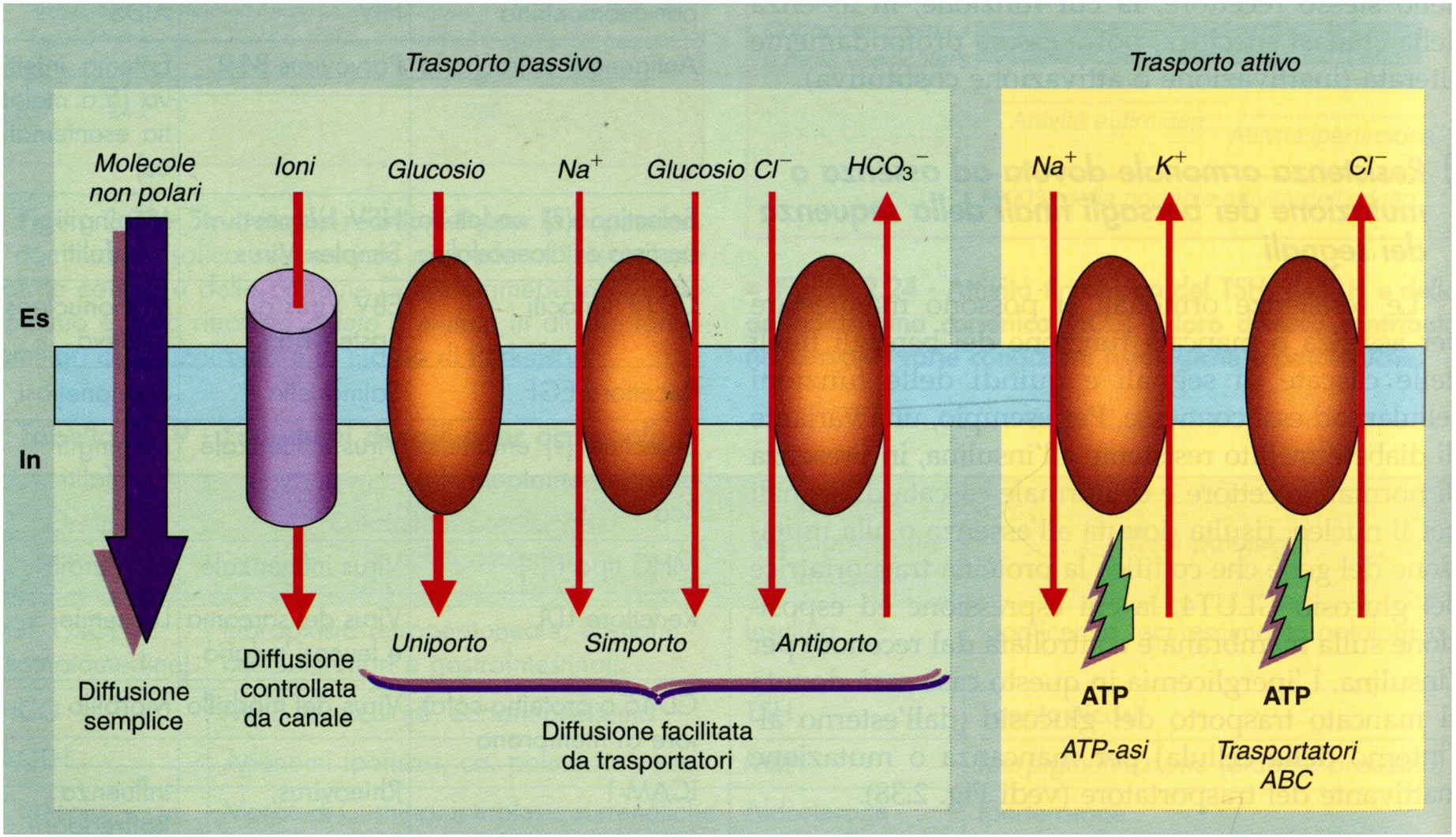
After exposure to the oxidant → Acute intravascular hemolysis

(2-3 days later)

- Marked Anemia
- Hemoglobinemia
- Hemoglobinuria
- Hematocrit
- Self-limited

Through Membranes

Polar molecules and ions are transported between intra- and extra-cell and in intra-cellular compartments according to or against to a gradient. Most of these transports have a vital role in our physiology



Multidrug Resistance Proteins

A family of ATP-dependent efflux pump

12 members of of the MRP/CTFR subfamily belonging to the 48 human ATP-binding cassette (ABC) transporters

Expression of Multidrug Resistance–Associated Proteins Predicts Prognosis in Childhood and Adult Acute Lymphoblastic Leukemia

Sabine L.A. Plasschaert,[1] Eveline S.J.M. de Bont,[1] Marike Boezen,[2] Dorina M. vander Kolk,[3] Simon M.J.G. Daenen,[3] Klaas Nico Faber,[4] Willem A. Kamps,[1] Elisabeth G.E. de Vries,[5] and Edo Vellenga[3]

Conclusions: The present study shows that a subset of ALL patients with high MRP expression has an unfavorable prognosis independently of age.

Single gene disorders may be classified into four categories:

a. Defects in membrane receptors and transport systems

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