Molecular Pathology

CHEMICAL COMPONENTS OF LIFE

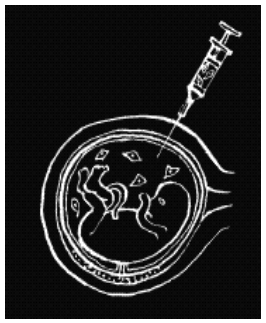
1. **PROTEINS**
2. **LIPIDS**
3. **NUCLEIC ACIDS:**
    - **DNA**
    - **RNA**

MOLECULAR PATHOLOGY

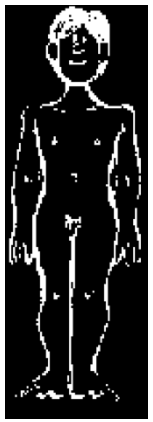
Molecular Pathology is a subspecialty of pathology that utilizes molecular biology techniques to:

- **Detect normal and disease states (diagnosis)**
- **Predict disease progression (prognosis)**

Molecular diagnostics: three levels



Prenatal
- hereditary disorders
- severe predispositions



Pre-symptomatic
- Predispositions to:
- cancer
- autoimmune d.
- degenerative d.
- behavioural d.
- pharmacogenetics

Real time
(monitoring)
- pathogens profile
- Immune status
- cancer status

What is a gene mutation?

A gene mutation is a permanent change in the DNA sequence that makes up a gene. Mutations range in size from a single DNA building block (DNA base) to a large segment of a chromosome.

Do all gene mutations affect Human health ?

Only a small percentage of mutations cause genetic disorders—most have no impact on health or development

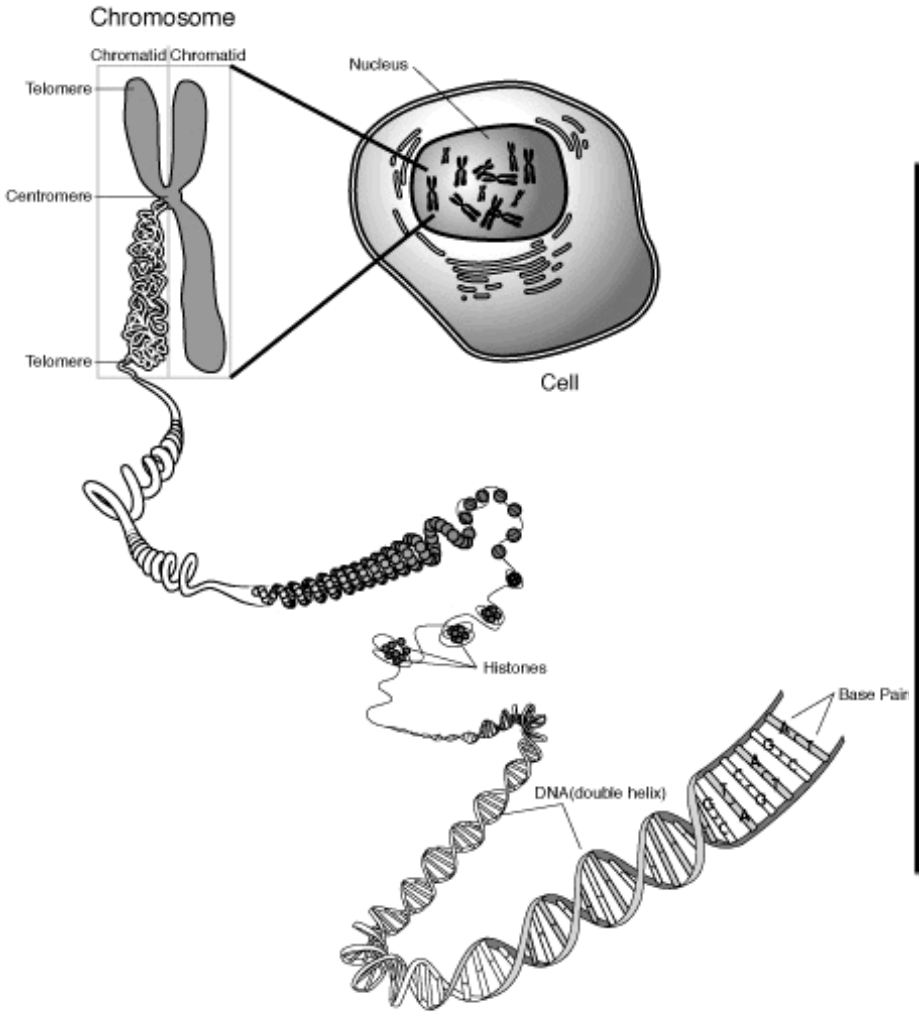
Gene mutations occur in two ways:

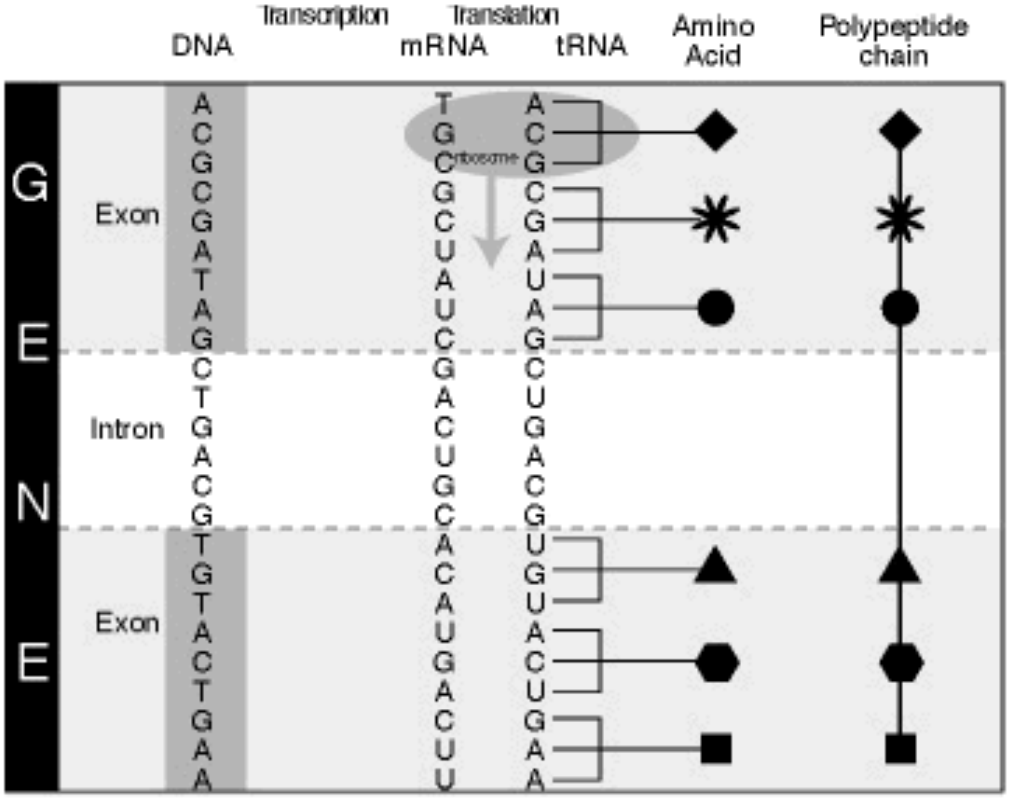
-inherited from a parent
(germ cells)

-acquired during a person's lifetime
(somatic cells)

From genotype to phenotype

Most cell functions are performed by proteins coded by genes, principal aim is to correlate normal and deregulated functions of the relative protein.





Molecular pathology of proteins

When a mutation alters a protein that plays a critical role in the body, it can disrupt normal development or cause a medical condition.

Qualitative alterations

Loss or gain of function

Quantitative alterations

Increase or decrease

Lack
accumulation
abnormal structure

Gene mutations
Transcriptional alterations
Processing e/o Splicing
Translational alterations
Post-Translational alterations

PROTEOMICA

Sinlge gene disorders may be classified into four categories:

a. Defects in membrane receptors and transport systems

b. Enzyme defects and their consequences

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

d. Mutation resulting in unusual reactions to drugs.

a. Defects in membrane receptors and transport systems

Mutations that decrease receptor function
"Loss of Function"
LDLR

Mutations that increase receptor function
"Gain of Function"
TSHR

What is hypercholesterolemia?
Hypercholesterolemia is a condition characterized by very high levels of cholesterol in the blood.

More than 34 million American adults have elevated blood cholesterol levels
(higher than 240 mg/dL)

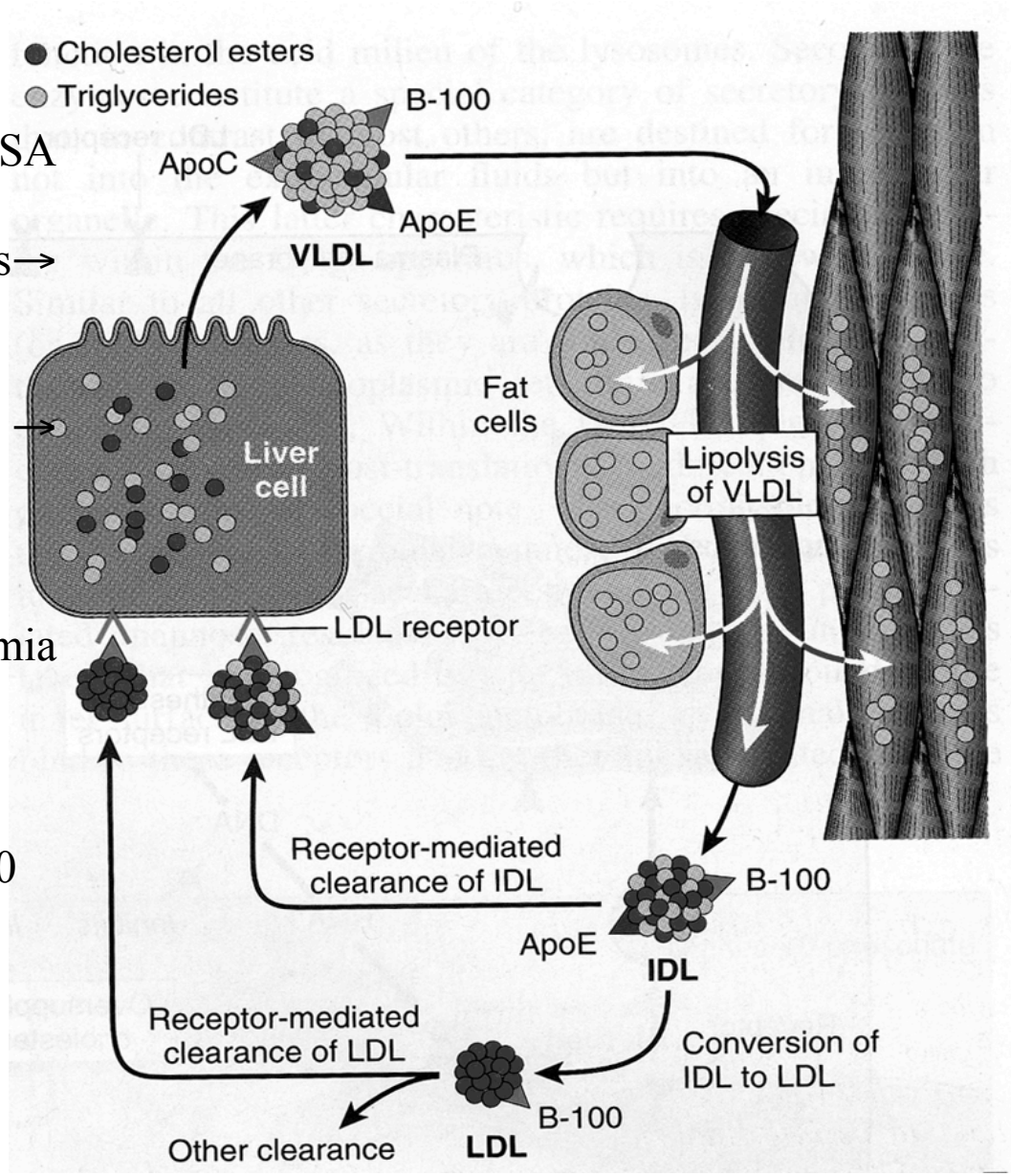
Mutations in the APOB, LDLR, LDLRAP1, and PCSK9 genes cause hypercholesterolemia

The Endogenous pathway

Familial Hypercholesterolemia

- Autosomal dominant disorder
- 1/500 individuals are affected in USA (Heterozygotes)
- Non functional Hepatic receptors → Hyperlipidemia (Hypercholesterolemia)
- Gene defect:

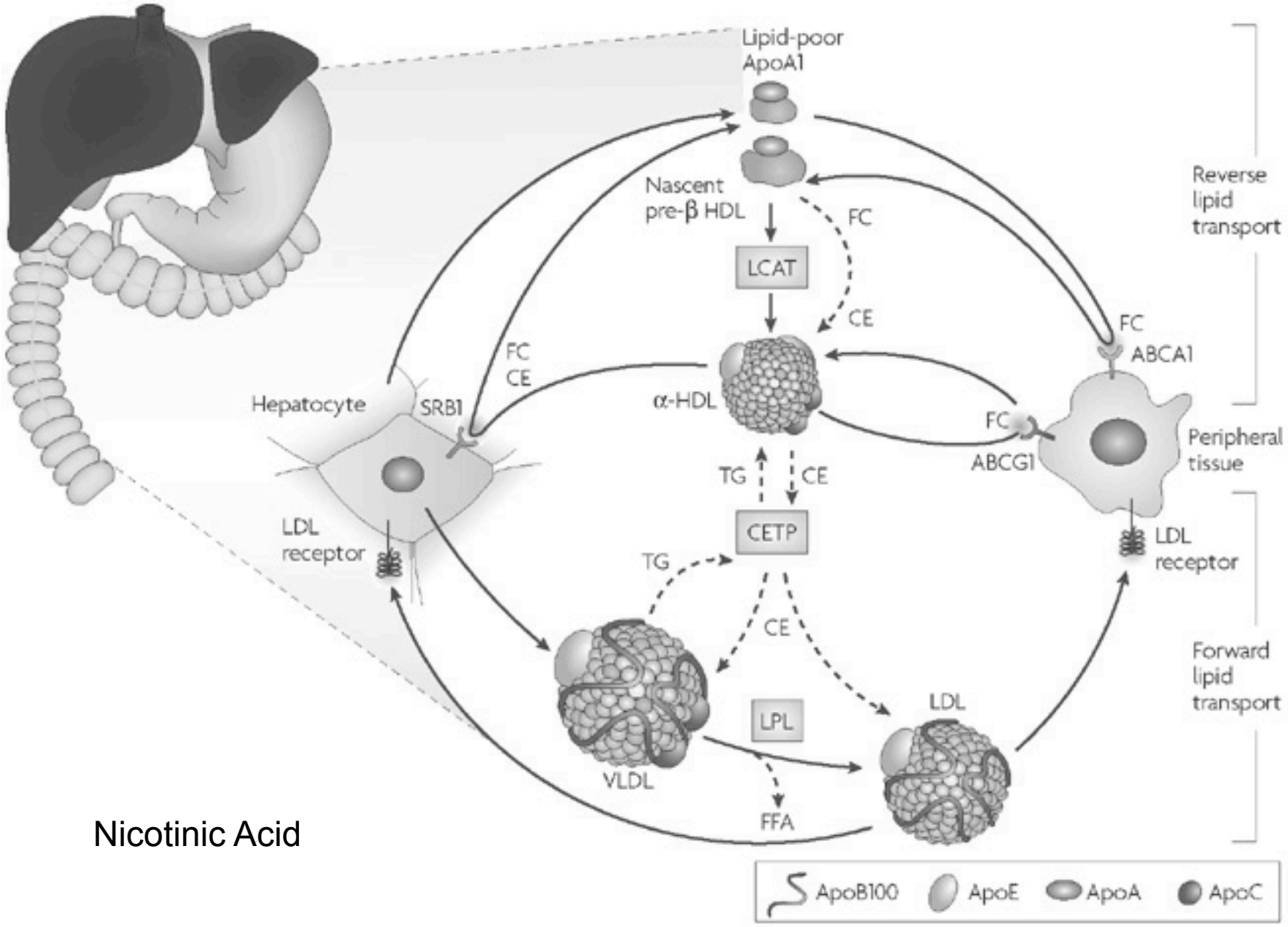
gene alteration of the LDL receptor → Familial Hypercholesterolemia



Patients affected by Hypercholesterolemia develop :

- Atherosclerosis
- Myocardial infaction
- Cerebral ictus
- Cardiovascular disease with peripheral occlusion

} Juvenile before age 20



Nicotinic Acid

Atherosclerosis

Reduced elasticity and hardening of the arterias occur with three different mechanisms:

1. Atherosclerosis, affect large and medium-sized arteries, starts in intima generating a plaque full of necrotic material.

2. Arteriosclerosis involve small vessels;

-Ialine when arterial walls are built up with extracellular material, such as macromolecules of hematic origin;

or

-Hyperplastic when muscular cells hyperplasia within the arterial walls, narrows arterial lumen and thickens the blood vessel wall.

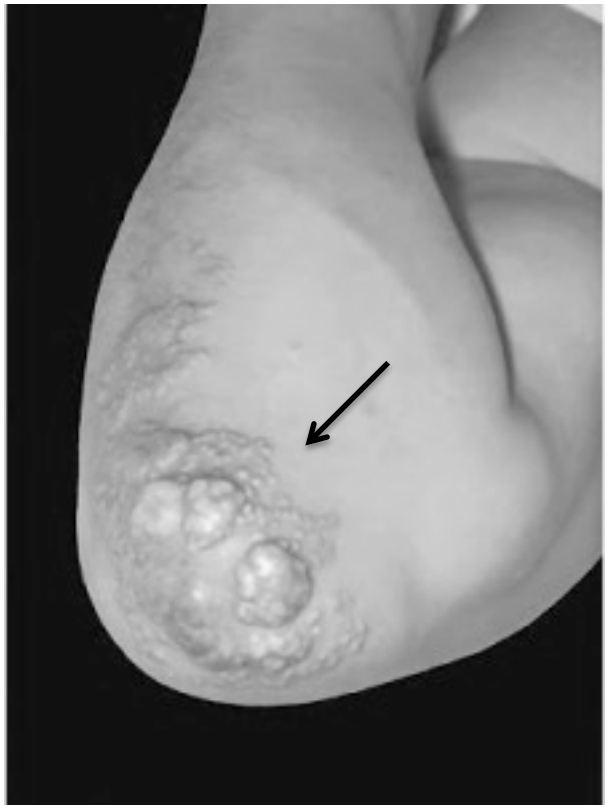
3. Monckeberg is a rare disease, a dystrophic calcification in the tunica media of large arteries.

Familial Hypercholesterolemia

- Autosomal dominant
- **Heterozygotes**
  - Prevalence 1/500, 5% of Hypercholemic, 5% of myocardial infarctions < age 60
  - Cholesterolemia 200-400mg/dl
  - Xhantomas: accumulation of cholesterol in tendos and skin
  - Arcus cornealis: accumulation of cholesterol at the edges of the clear, front surface of the eye (grey colored ring)
  - Atherosclerosis: coronary arteries disease, myocardial infarction, ictus.
- **Homozygotes**
  - Prevalence 1/1.000.000
  - Cholesterolemia >600mg/dl
  - Myocardial infarction < age 20
- **Genetic and environmental modifiers**
  - Sex (female<male)
  - the etnic group
  - Diet
  - Physical activity
  - Drug therapy for cholesterolemia

Xanthomas

A)



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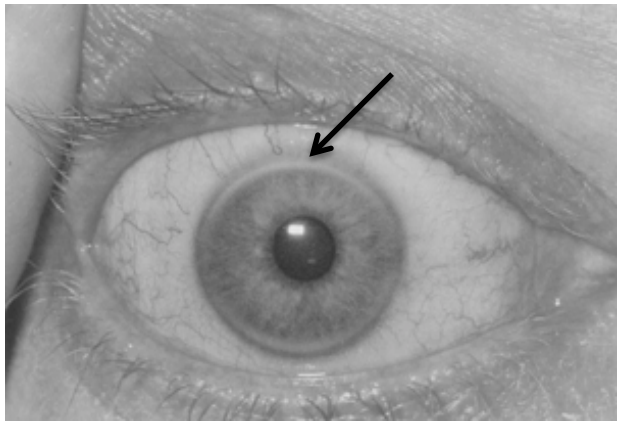

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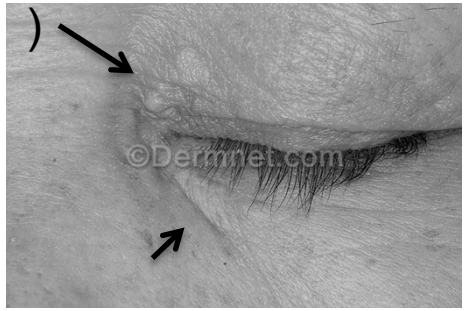

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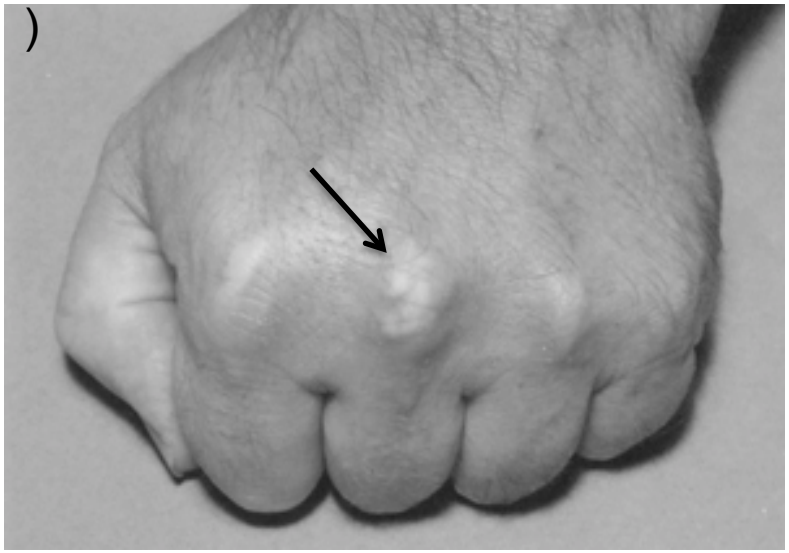


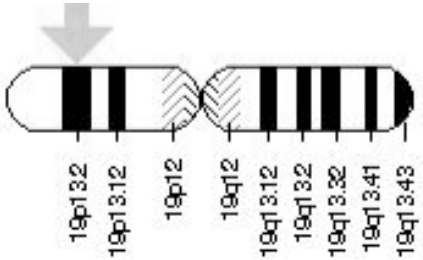
STRUCTURE AND FUNCTION OF THE RECEPTOR

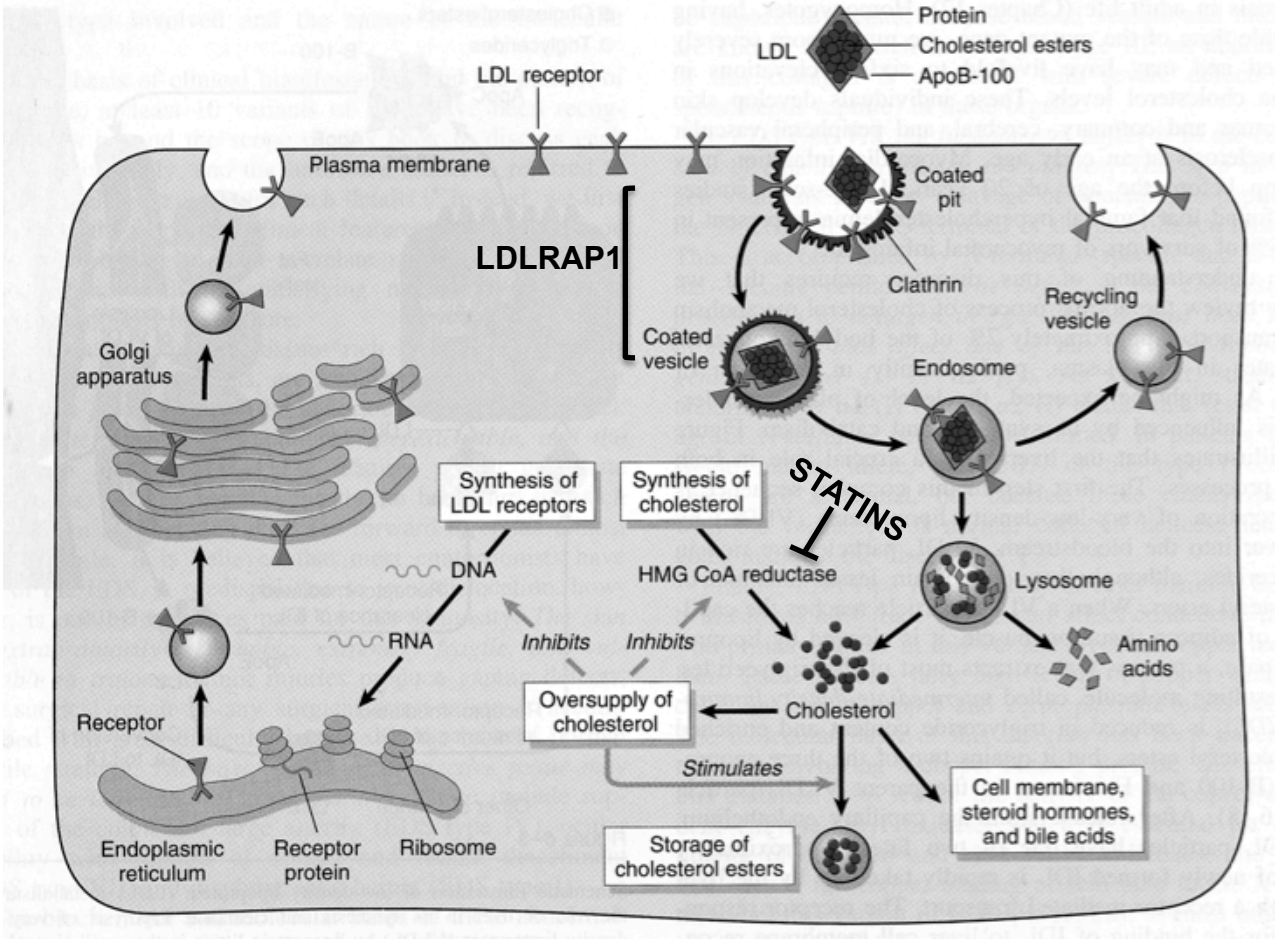
LDL receptor gene is located:

-Chromosome 9

-18 exons

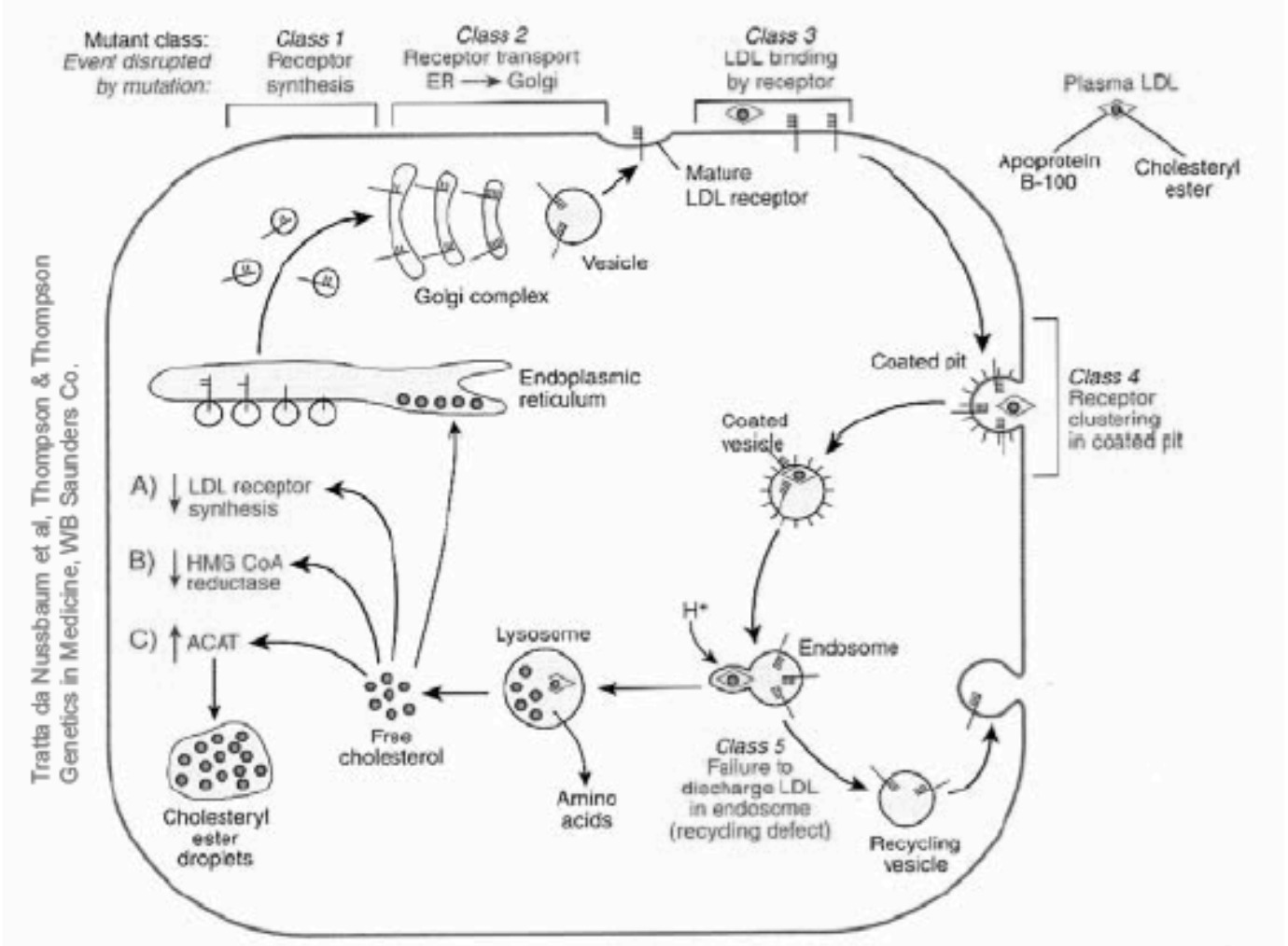
-Protein with 5 different functional domains





HEPATOCYTE

Classification of LDL receptor mutations based on abnormal function of the mutant protein.



Less commonly, hypercholesterolemia can be caused by mutations in the APOB, LDLRAP1, or PCSK9 gene.

These proteins are essential for the normal function of low-density Lipoprotein receptors.

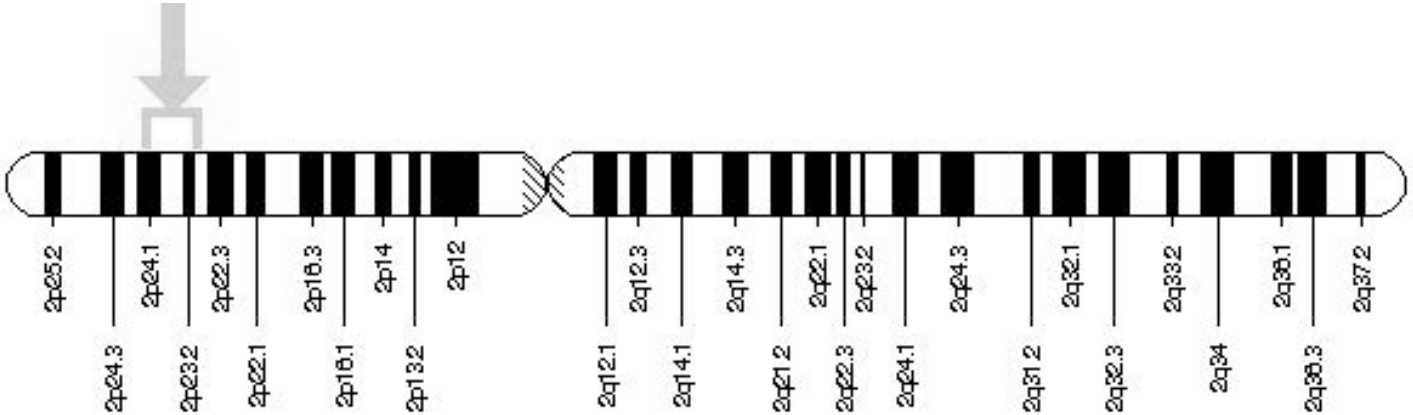
Mutations in any of these genes prevent the cell from making functional receptors or alter the receptors function.

APOB Mutations

APOB gene ➔inherited hypercholesterolemia

APOB → ApoB100, ApoB48 (short) } components of lipoprotein → VLDL,IDL,LDL; Chilomocroni

Cytogenetic Location: 2p24-p23

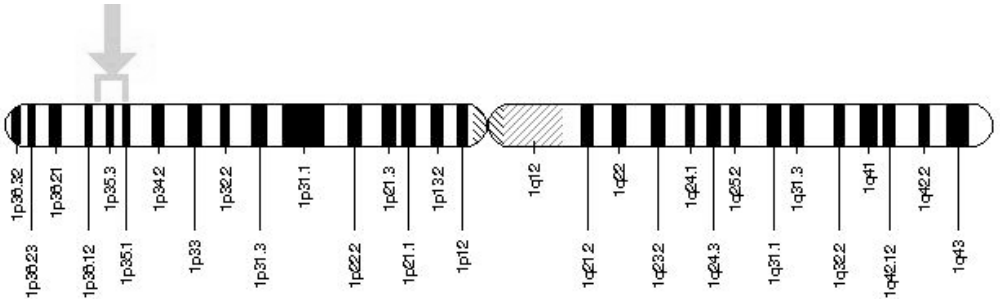


Any alteration prevents low-density lipoproteins from effectively binding to their receptors on the surface of cells.

LDLRAP1 mutations

LDLRAP1 mutations ➔are responsible for another type of inherited high cholesterol, autosomal recessive hypercholesterolemia (ARH).

More than 10 mutations



Abnormally small, nonfunctional version of the LDLRAP1 protein

Impaired transport into the cells (liver) of LDL receptor

Very high blood levels of cholesterol

PCSK9 gene

• The official name of this gene is “proprotein convertase subtilisin/kexin type 9.”
Function: control the number of low-density lipoprotein receptors

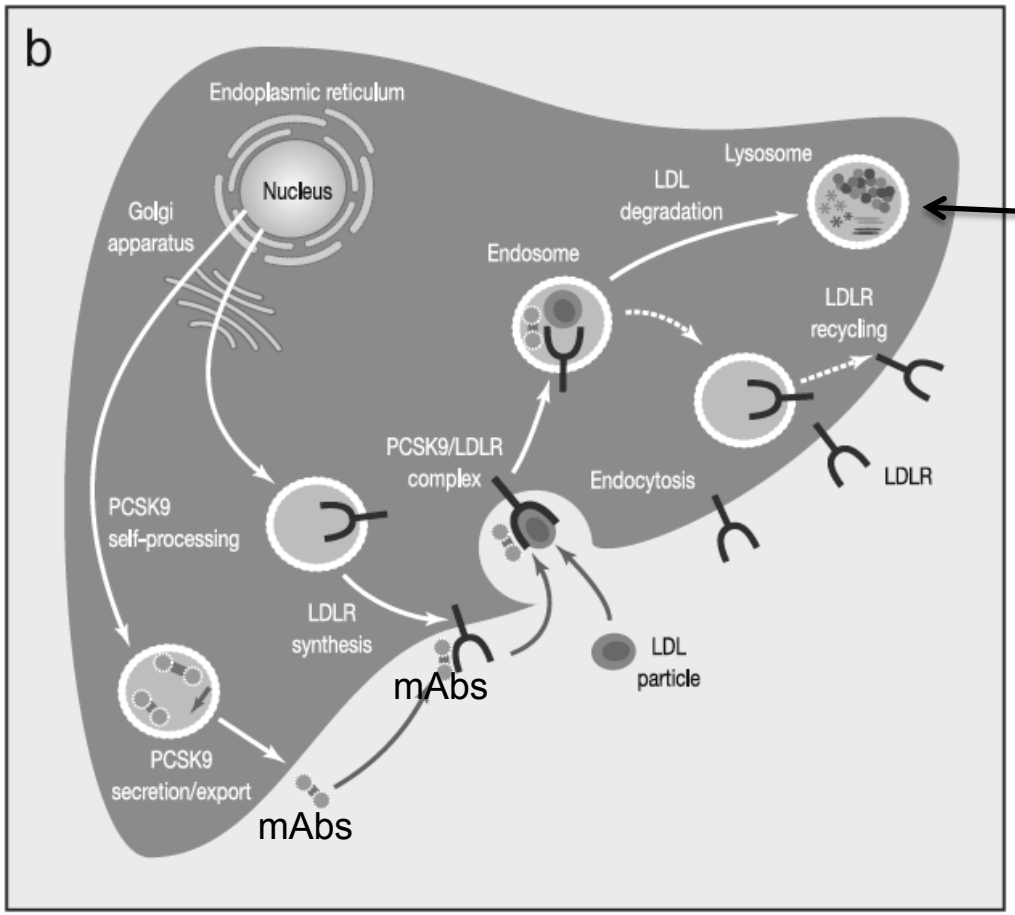
• several PCSK9 mutations➔ “gain of function”

•↑PCSK9 activity ↓ Number of LDL receptors ➔ Hypercholesterolemia

•PCSK9 mutations➔ “loss-of-function”

•↓PCSK9 activity ↑ Number of LDL receptors ➔Hypocholesterolemia

PCSK9 promotes LDLR degradation and induces conformational modification, avoiding normal recycling to the plasmamembrane.

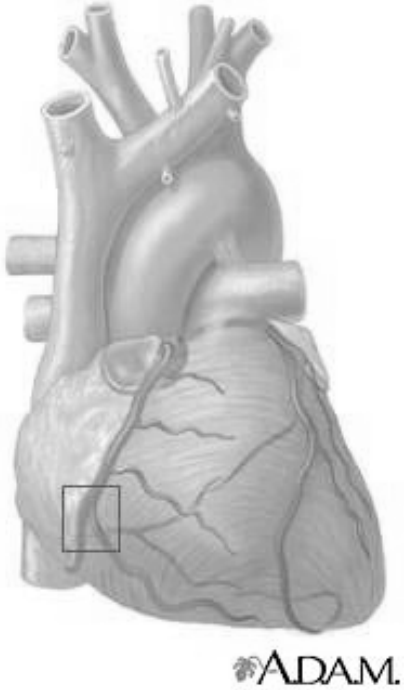


Enhances LDLR lysosomal degradation

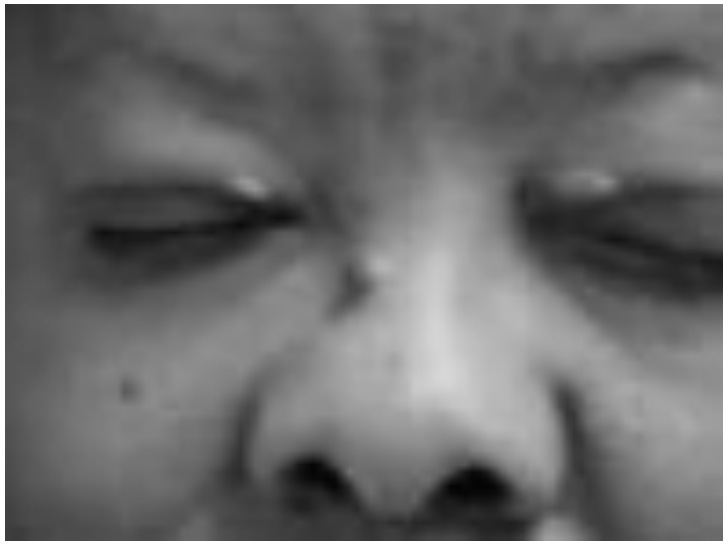
Statin administration to PCSK9 knock out mice enhanced LDL clearance from plasma.
PCSK9 inhibitor combined with a low statin dose seems an attractive strategy.

Atherogenesis and increased risk of cardiovascular diseases (CVD)

Blockage in right coronary artery



Skin Xanthomas



“Loss of Function” in Hormone receptors

Hormone resistance and molecular pathology of communication

- lack of protein receptor → Vitamin D-resistant rickets type II
- Inactivating mutations of the receptor → Diabetes mellitus
- Mutations or lack of signal transduction proteins → Hypothyroidism
- Mutations or absence of effectors → PLC, cAMP, cAMP-dependent kinase
- Mutations of final targets → Diabetes mellitus

RECEPTOR PATHOLOGY WITH GAIN OF FUNCTION

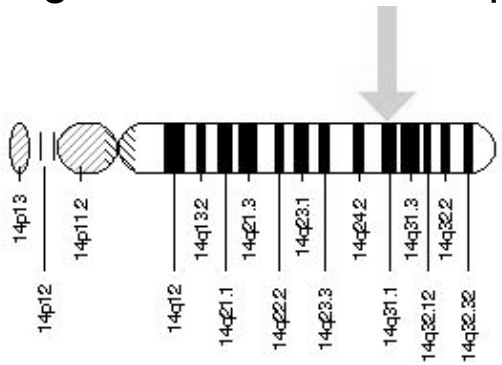
Mutations that constitutively activates the receptor determine an excess of ligand.

The “thyroid stimulating hormone receptor.” (TSHR)

TSHR

The TSHR gene provides instructions for making a receptor that serves as a customized binding site for a hormone called thyroid stimulating hormone (TSH).

Cytogenetic Location: 14q31



TSH binds to the extracellular portion of the receptor, activating a series of reactions that control development of the thyroid gland and its functions.

Thyroid gland produces: iodine-containing hormones (thyroid hormones), which help regulate growth, brain development, and the rate of chemical reactions in the body (metabolism).

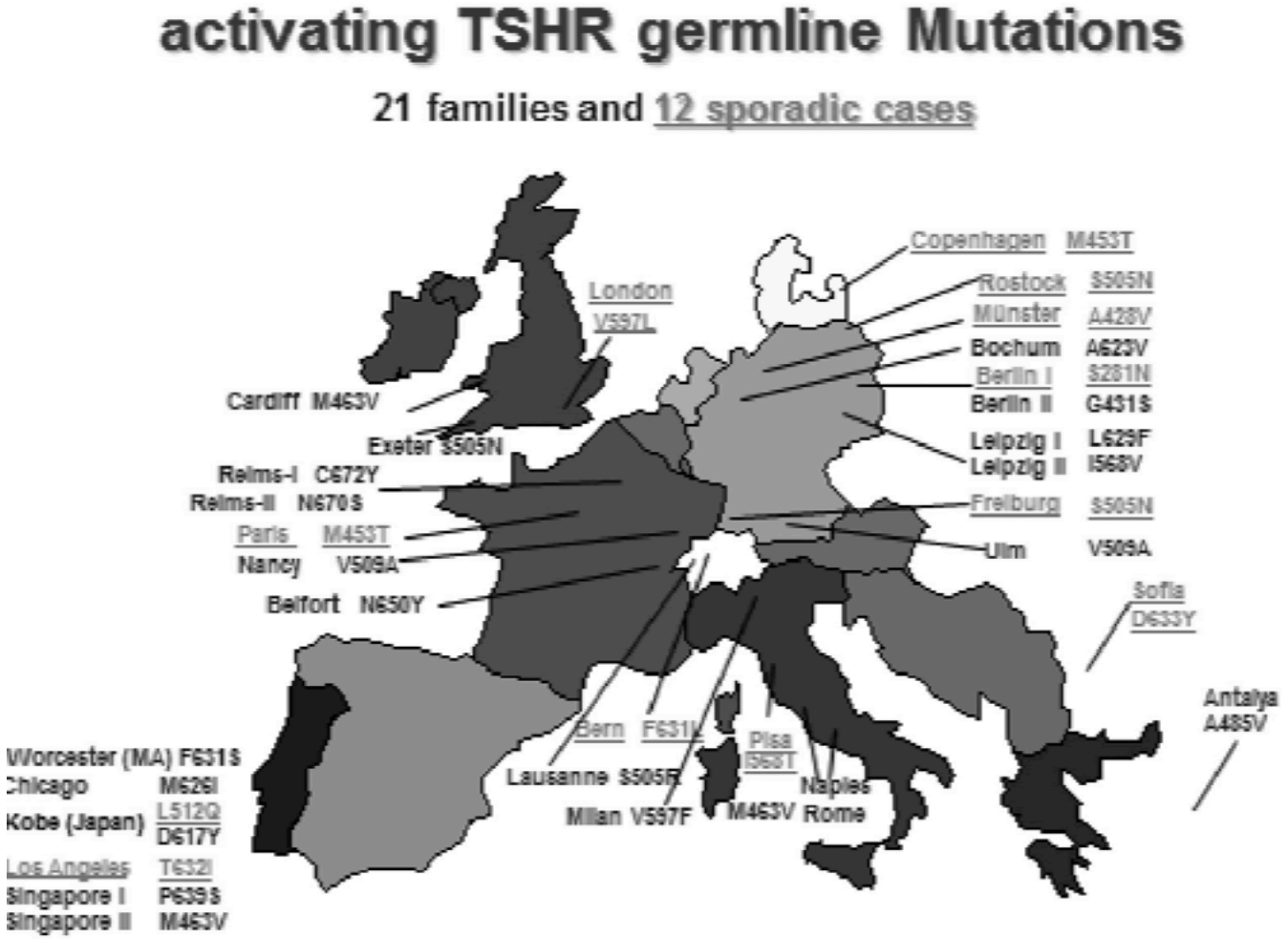
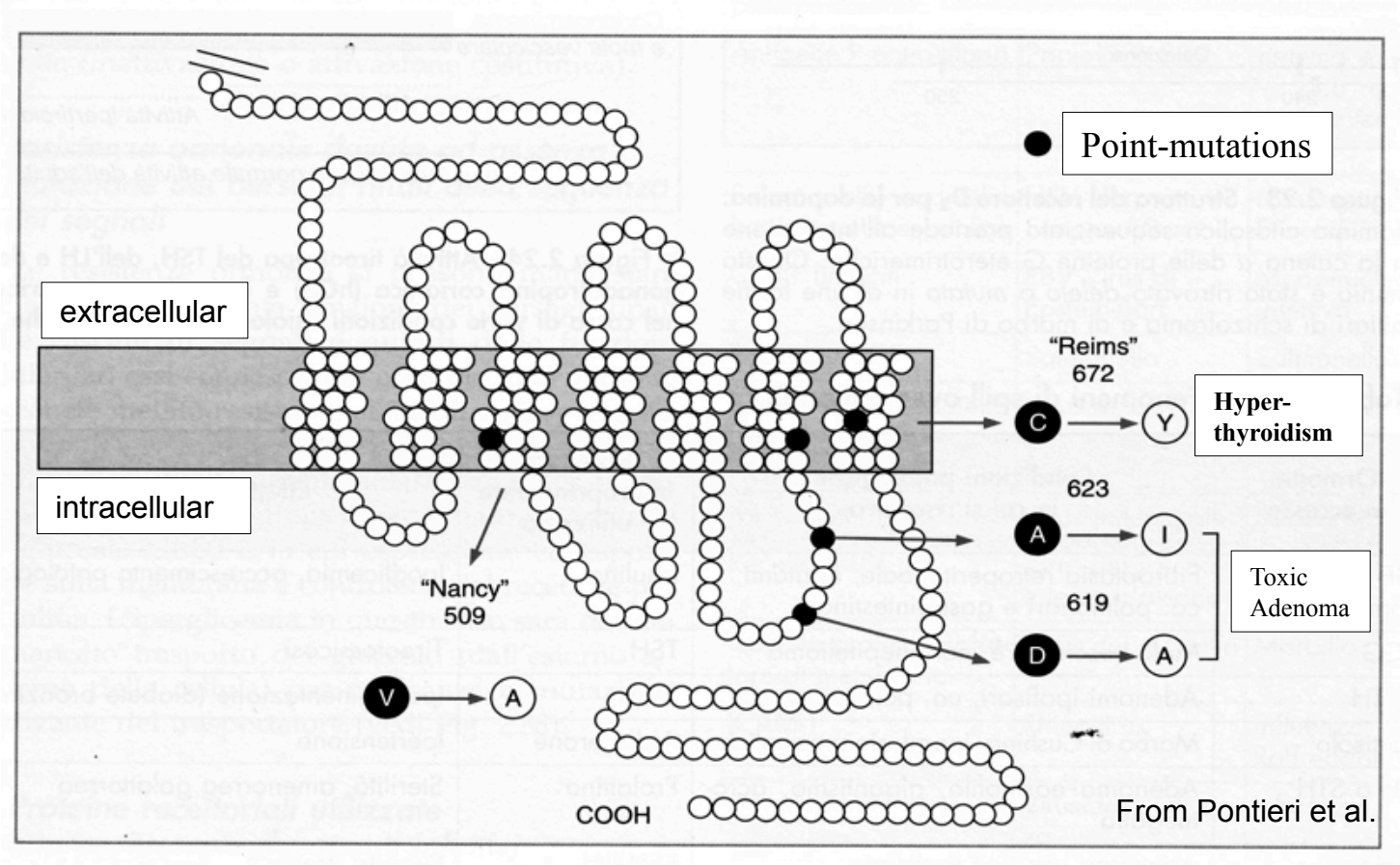


Fig. 2. Geographic distribution of TSHR germline mutation for 21 families and 12 sporadic cases in Europe.

The TSH receptor

This receptor spans the membrane of certain cells (called follicular cells) in the thyroid gland,



Mutations in the VI or VII helix of TSHR lead to constitutive acivation of the receptor ➔ Hyperthyroidism

Mutations in the cytosolic domain of TSHR lead to constitutive acivation of the receptor ➔ Adenoma