# GENETIC AND MOLECULAR DIAGNOSIS OF FH IN CHILDREN

**Livia Pisciotta**

**Department of Internal Medicine**

**University of Genoa – Italy**

**IRCCS AOU San Martino-IST**

# When genetic diagnosis can be useful ?

# In heterozygous phenotype

# HeFH: DIAGNOSTIC CRITERIA

**Simon Broome criteria**

| | | | |
|---|---|---|---|
| Total-cholesterol (LDL-C) in mg/dl >260 (155) in patients with age <18 years and >290 (190) in patients >18 years | AND | Family history of elevated total-cholesterol >290 mg/dl in first or second degree relative<br>or<br>Family history of coronary disease at age <60 years in first degree relative or <50 years in second degree relative | Possible FH |
| | | Tendon xanthomas in the patient or in first or second degree relative | Probable FH |
| | | DNA mutation consistent with FH | Definite FH |

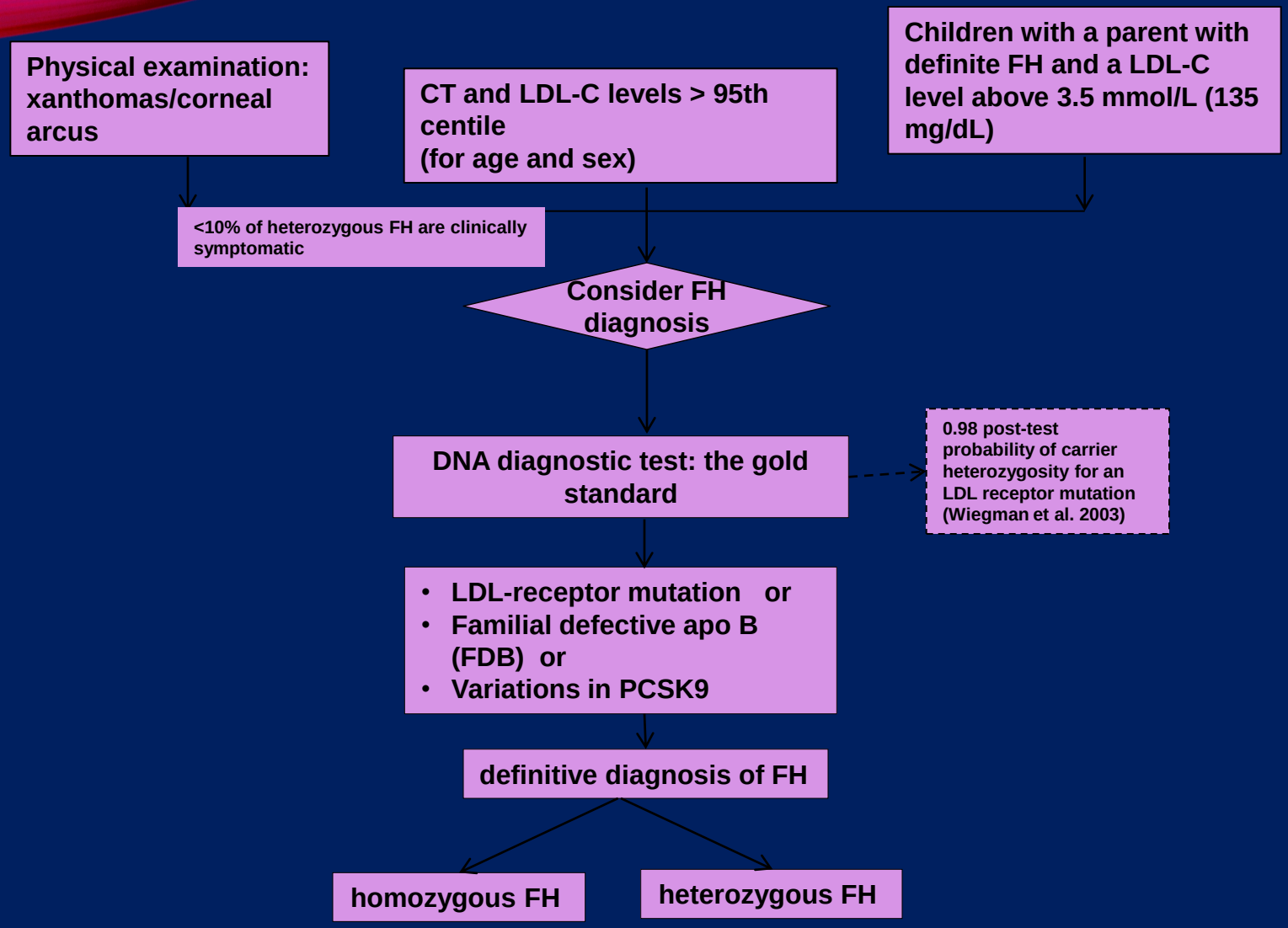
**MEDPED criteria (87% sensitivity and 98% specificity)**

| Age in years | Total-cholesterol (LDL-C) in mg/dl | | | |
|---|---|---|---|---|
| | General population | First degree relative | Second degree relative | Third degree relative |
| <18 | 270 (200) | 220 (155) | 230 (165) | 240 (170) |
| 18-29 | 290 (220) | 240 (170) | 250 (185) | 260 (185) |
| 30-39 | 340 (240) | 270 (190) | 280 (200) | 290 (210) |
| ≥40 | 360 (260) | 290 (205) | 300 (215) | 310 (225) |

**Dutch Lipid Clinic criteria**

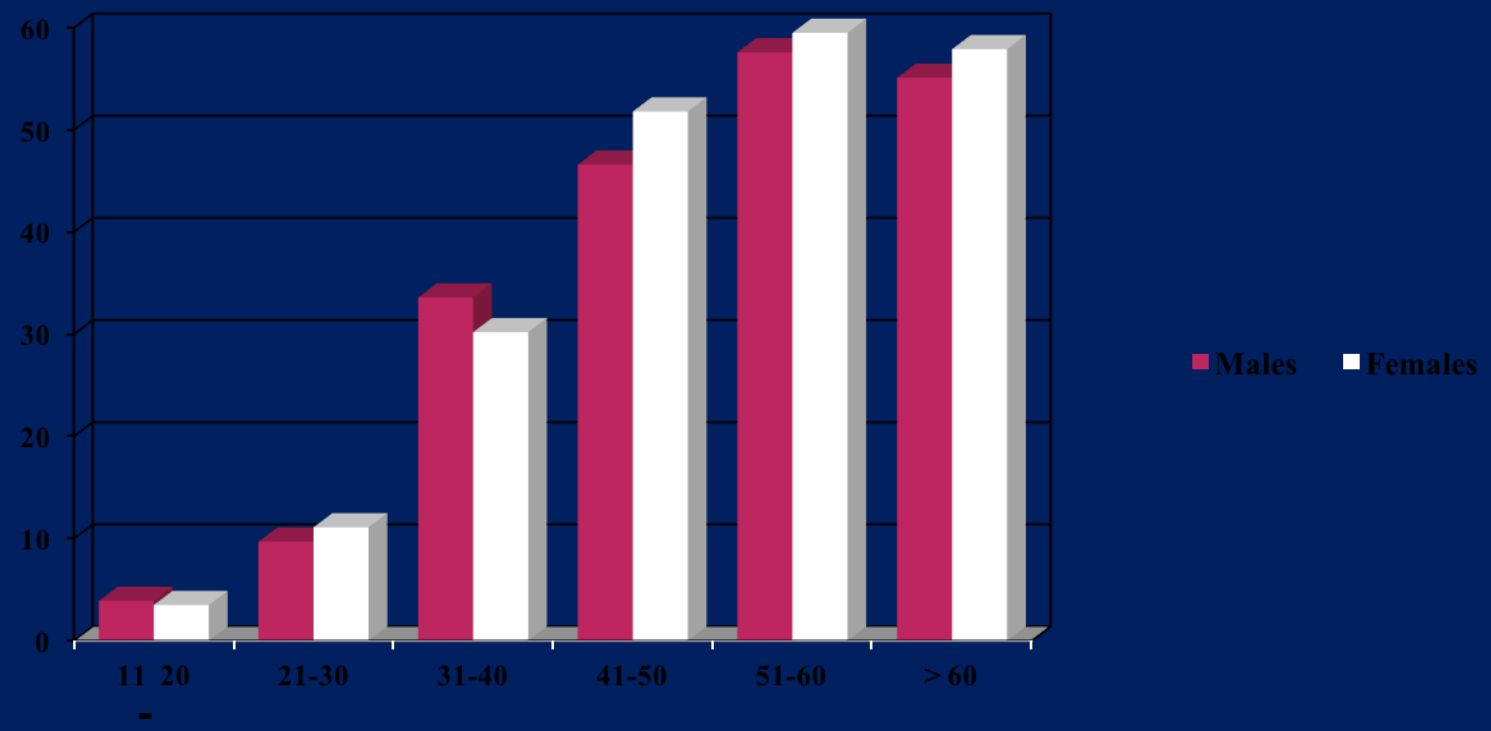
| | | |
|---|---|---|
| LDLR gene functional mutation or LDL-cholesterol >330 mg/dl | 8 points | Possible FH 3-5 points |
| Presence of tendon xanthoma | 6 points | |
| LDL-C between 250 and 329 mg/dl | 5 points | Probable FH 6-7 points |
| Presence of arcus corneae at age <45 years | 4 points | |
| LDL-C between 190 and 249 mg/dl | 3 points | Definite FH ≥8 points |
| Personal history of CAD or<br>First degree relative age <18 years with LDL-C >95th percentile or<br>First degree relative with tendon xanthoma or arcus corneae | 2 points | |
| LDL-C between 190 and 249 mg/dl or<br>Personal history of premature cerebral or peripheral artery disease or<br>First degree adult relative with premature CAD or LDL-C >95th percentile | 1 points | |

LDL-C: Low-density lipoprotein-cholesterol, FH: Familial hypercholesterolemia, DNA: Deoxyribonucleic acid, CAD: Coronary artery disease, LDLR: LDL receptor




*(J Inherit Metab Dis (2009) 32:699–705)*
*Circulation 2006;114;2710-2738*

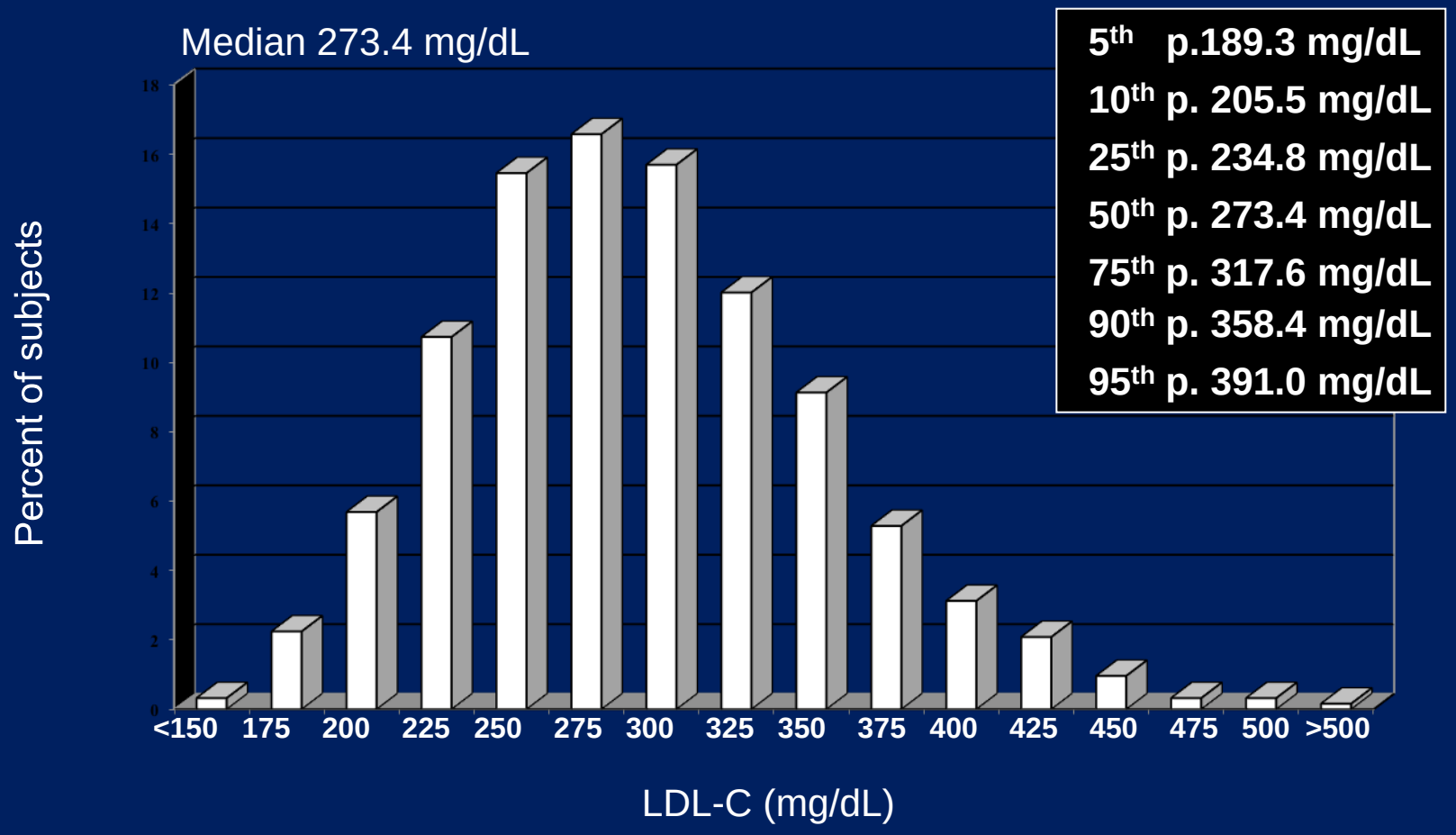
# Prevalence of Tendon Xanthomatosis (%) in 1116 FH patients in relation to age




Age (years)

# LDL-C levels in 1250 genetically characterized heterozygous FH patients

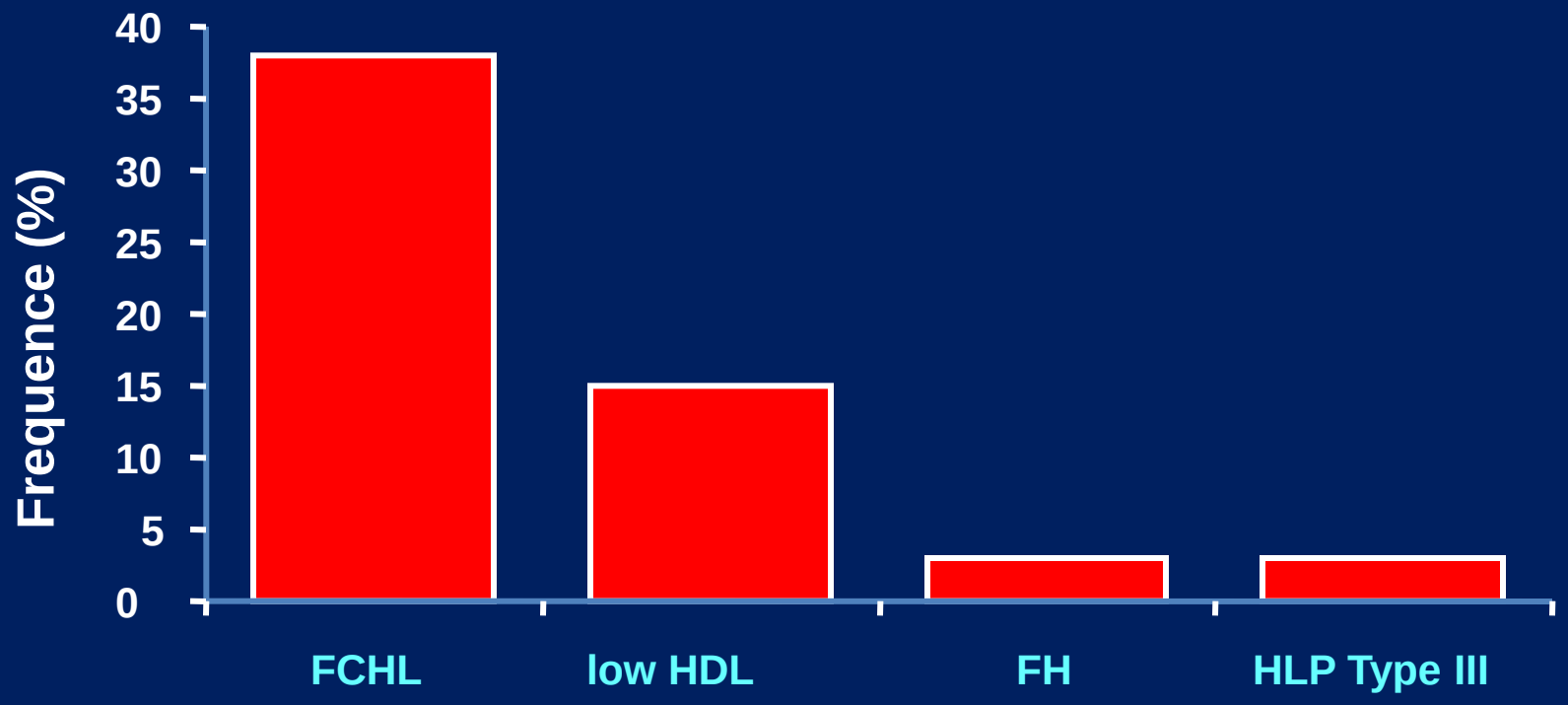
**(values are adj. for gender and age)**




***Bertolini S. 2011***

# INHERITED DYSLIPIDEMIA AND PREMATURE CHD (< 55 yrs)

**2/3 subjects with hyperlipidemia**




Williams RR, Arch Intern Med 1990;150:582-588

Abstract

Full text links

ELSEVIER OPEN ACCESS

J Am Coll Cardiol. **2008** Nov 4;52(19):1546-53. doi: 10.1016/j.jacc.**2008**.06.050.

**Frequency of low-density lipoprotein receptor gene mutations in patients with a clinical diagnosis of familial combined hyperlipidemia in a clinical setting.**

Civeira F[1], Jarauta E, Cenarro A, García-Otín AL, Tejedor D, Zambón D, Mallen M, Ros E, Pocoví M.

| | FCHL LDLR + |
|---|---|
| N. | 21/143 (14.7%) |
| M/F | 10/11 |
| Age (years) | 45.8±12.1 |
| BMI (kg/m$^2$) | 27.0±3.6 |
| Tc (mg/dl) | 365.0±55.4 |
| HDLc (mg/dl) | 46.6±19.6 |
| Tg (mg/dl, median, range) | 247 (147-810) |

Spanish FCHL subjects positive for mutations in LDLR gene

# Comparison between FH subjects over 30 y of age with pathogenic LDLR gene mutations according TG levels

| | TG ≤ 200 mg/dl | TG > 200 mg/dl | P |
|---|---|---|---|
| N. | 585 | 78 (11.7%) | |
| M/F | 260/325 | 41/37 | NS |
| Age (years) | 48.9±12.0 | 51.1±10.9 | NS |
| BMI (kg/m$^2$) | 24.2±3.4 | 26.7±3.4 | 0.0001 |
| Tx (%) | 47.2 | 53.2 | NS |
| CAD (%) | 30.6 | 39.7 | NS |
| TC (mg/dl) | 375.7±63.9 | 413.3±70.1 | 0.0001 |
| LDL-C (mg/dl) | 302.1±63.4 | 324.1±66.2 | 0.004 |
| HDL-C (mg/dl) | 51.3±13.9 | 42.4±10.6 | 0.0001 |
| TG (mg/dl, median, range) | 112 (31-200) | 244 (202-432) | 0.0001 |

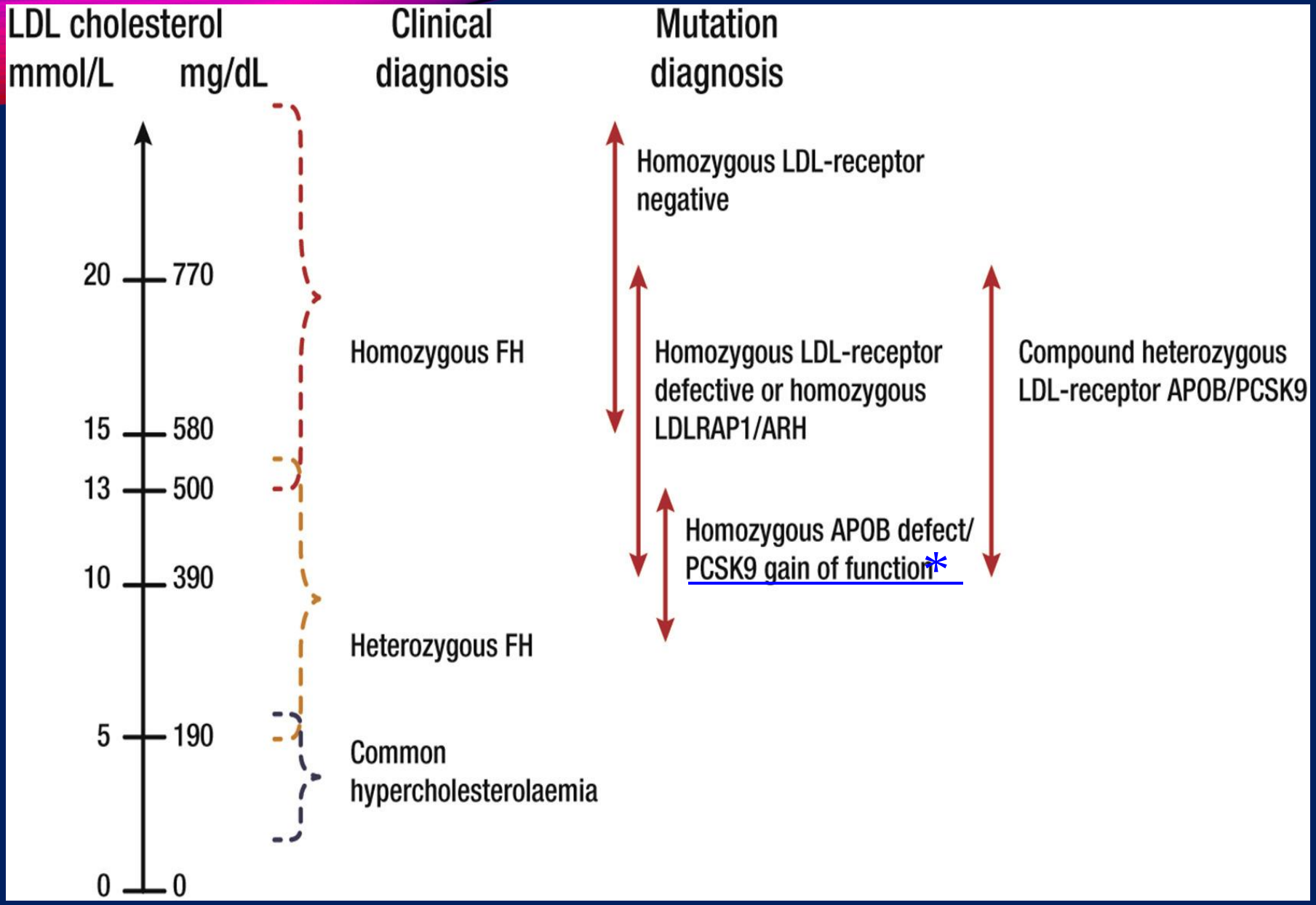
# Factors involved in TG increase

- Mutations in the LDLR repeat 5 (IDL binding)
- common variants in LPL, APOA5, APOC3 genes such as (for example D9N and N291S, or –1131 T>C and c.56 C>G (S19W)…
- APOE ε2 or ε4 allele
- Insulin resistance
- …

# In homozygous phenotype

# CLINICAL CRITERIA FOR HO-FH

1. Plasma LDL-C level >13 mmol/L (500 mg/dl)
2. Tendon and cutaneous xanthomas in infancy
3. Hystory of hypercholesterolemia in both parents



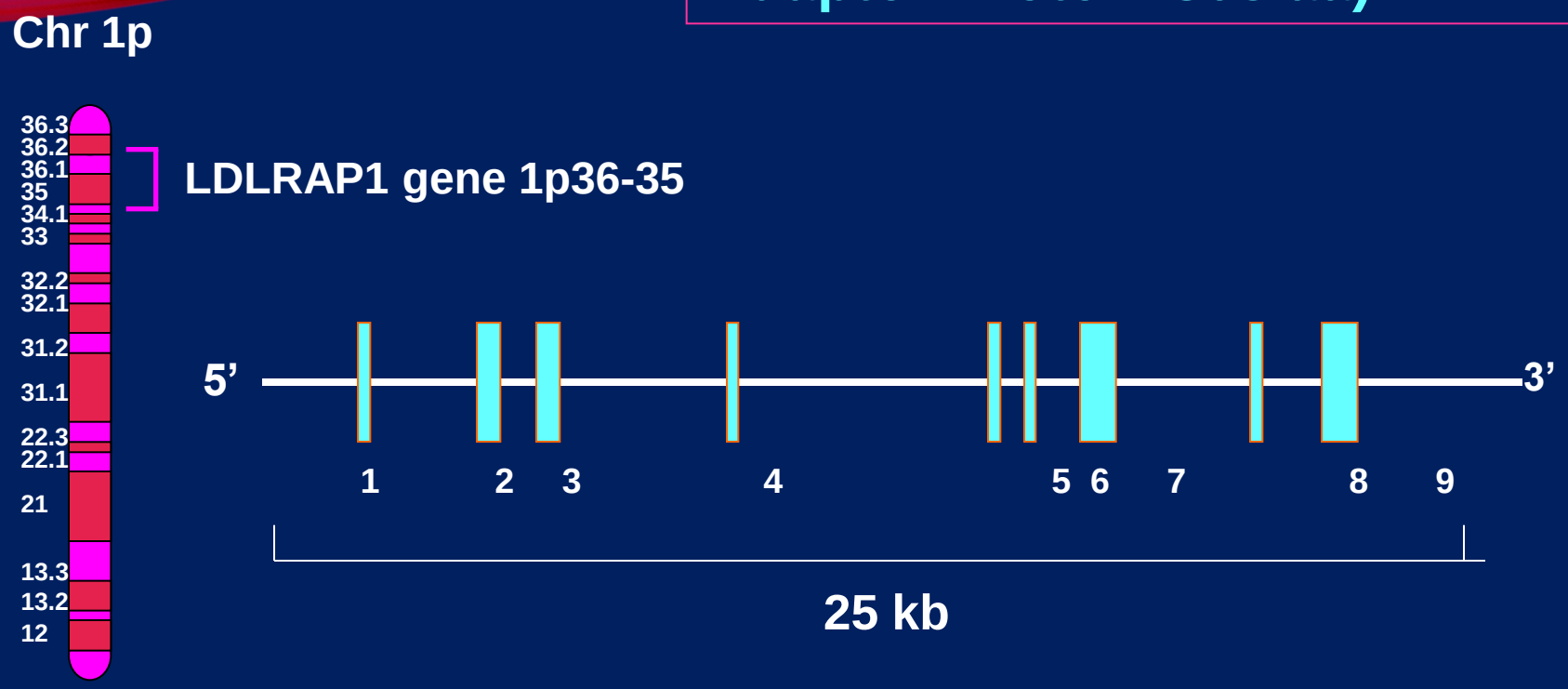

EAS Consensus Panel statement on homozygous FH Stock J. *Atherosclerosis* 2015; 242: 323-6

# In recessive phenotype

# Familial Hypercholesterolemia (Recessive forms)

| GENE | Disease |
|---|---|
| LDLRAP1 | ARH |
| ABCG5/G8 | Sitosterolemia |
| LIPA | LAL-D |

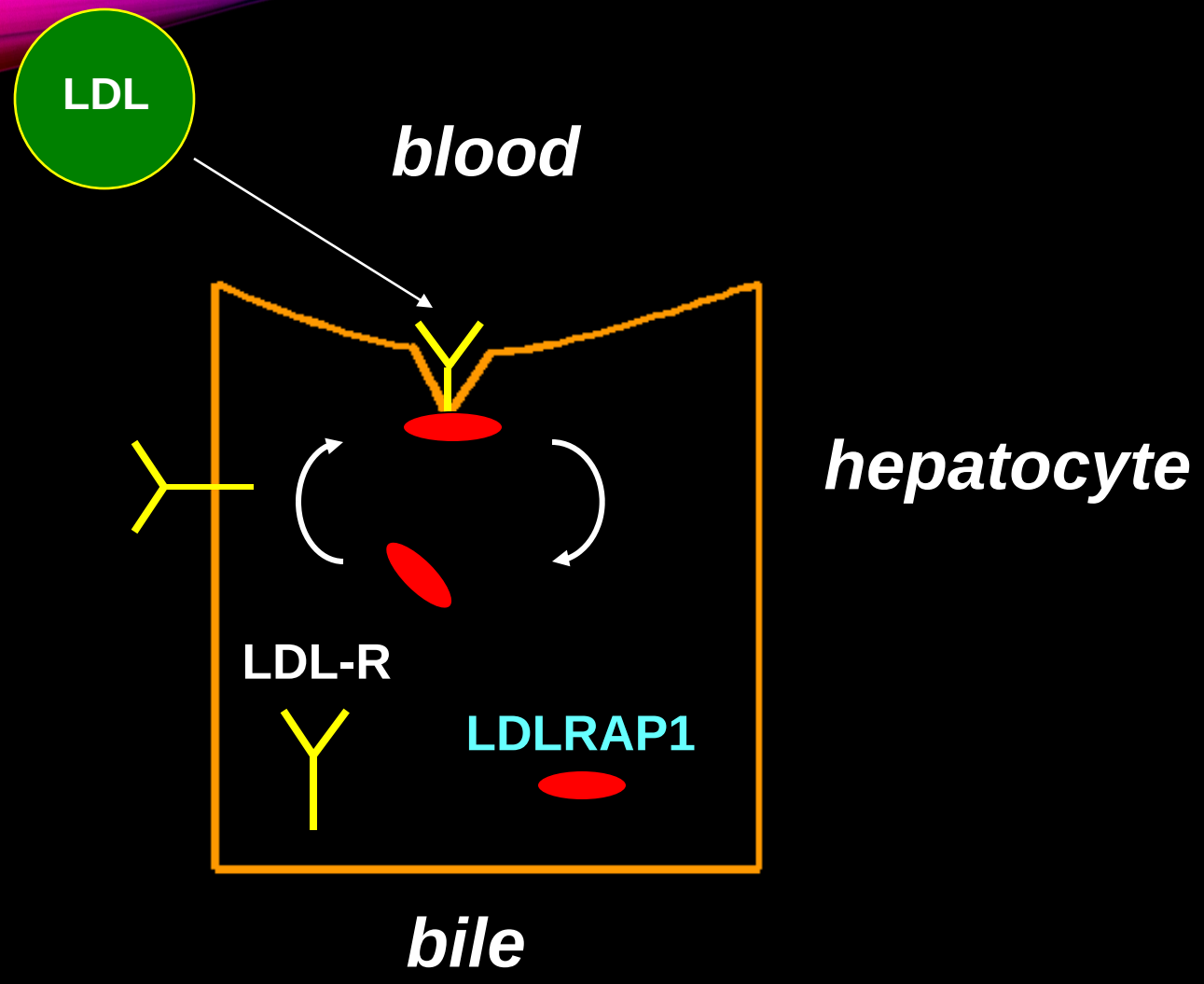
# LDLRAP1 gene (1p35)→ Putative Adaptor Protein 308 aa)




**Sequence similarity (170 aa) with the phosphotyrosine binding (PTB) domains of many adaptor proteins.**

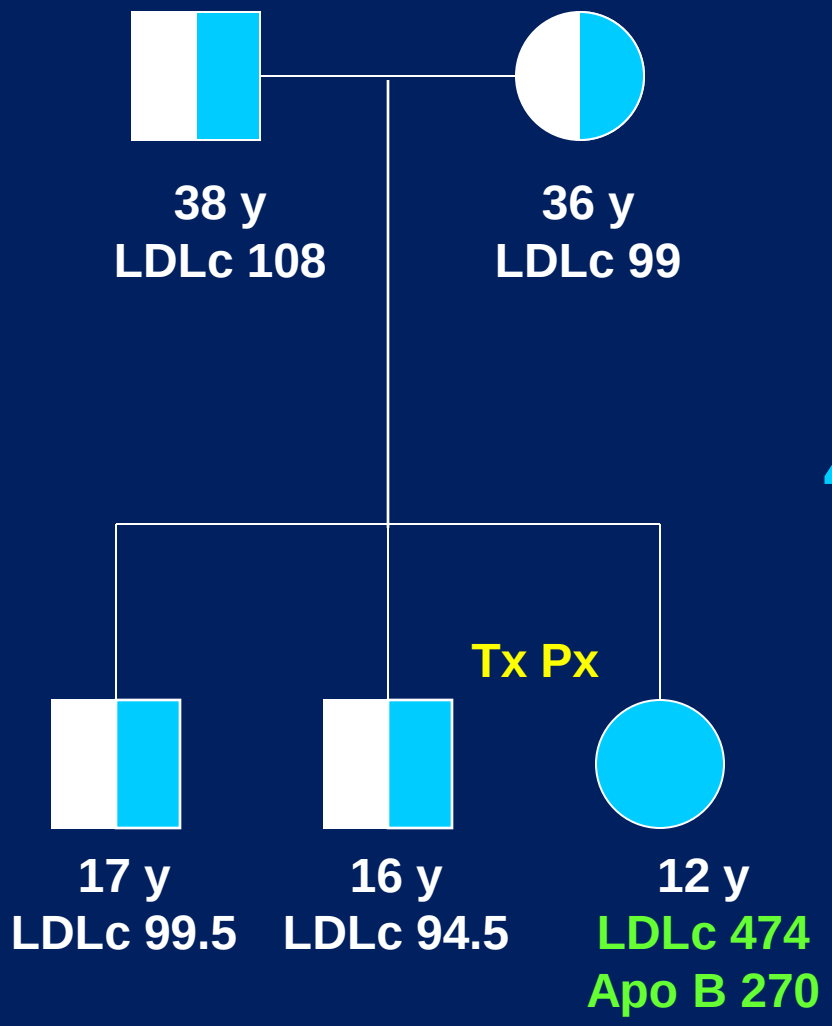
**PTB bind to consensus sequence NPXY of LDL-R in polarized cells, like HEPATOCYTES**

*Garcia et al. Science 2001; 292:1394-8*




**LDLRAP1: 1) stabilizes the association of LDL-R with LDL and 2) is required for the internalization of the LDL/LDL-R complex.**

## M. Family




**432insA**

c.65 G>A, p.(W22X)

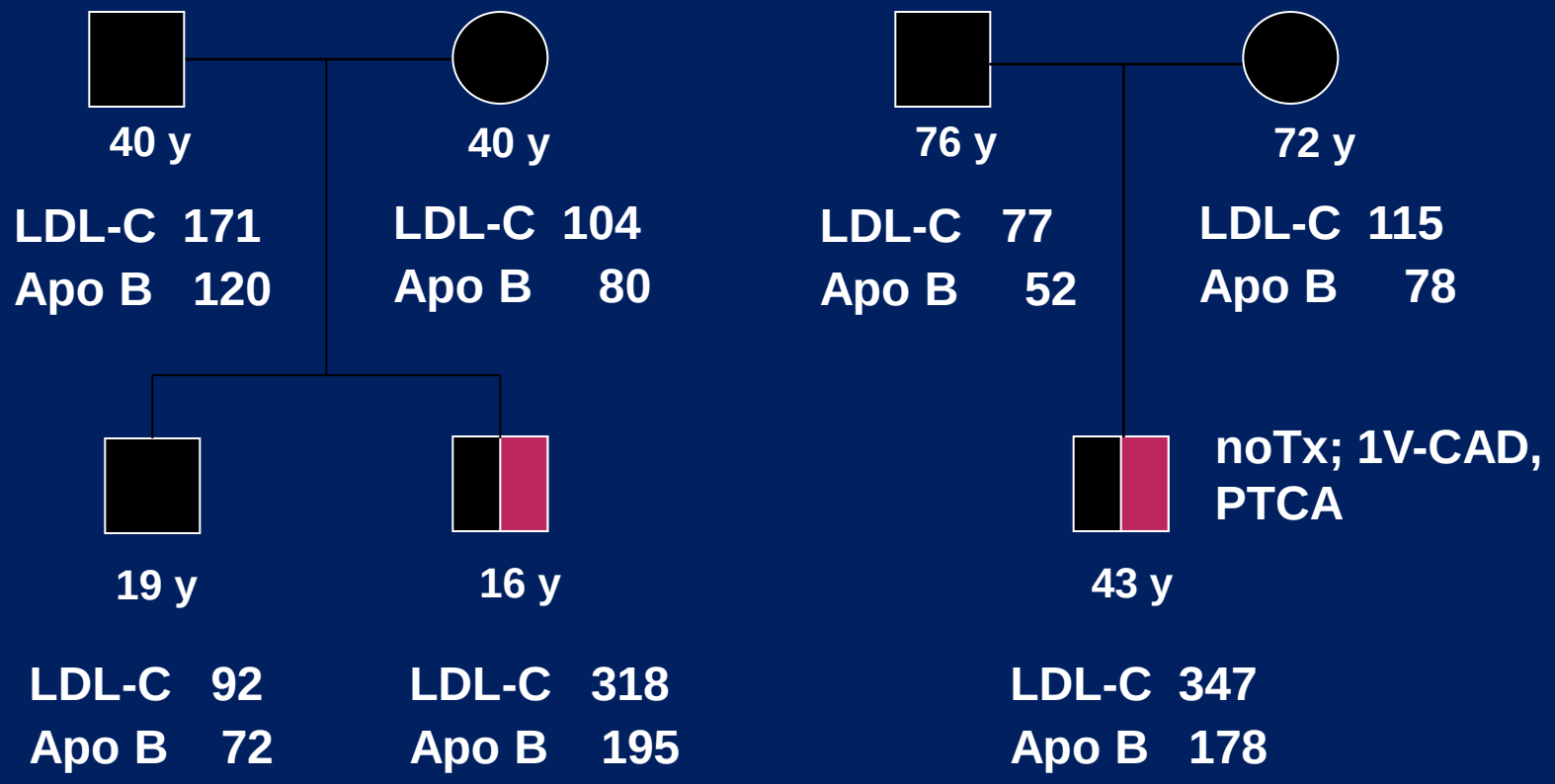
c.432insA, p.(A145Sfs*26)

**Frequency**

HO/CH 1:23.000
(31 unrelated/700.000 families)

HO/CH 1:33.000
(48/1.600.000 population)

# De novo LDLR gene mutations (differential diagnosis with ARH)




LDLR c.28 T>A (Ex 1)
p.Trp10Arg (SP)

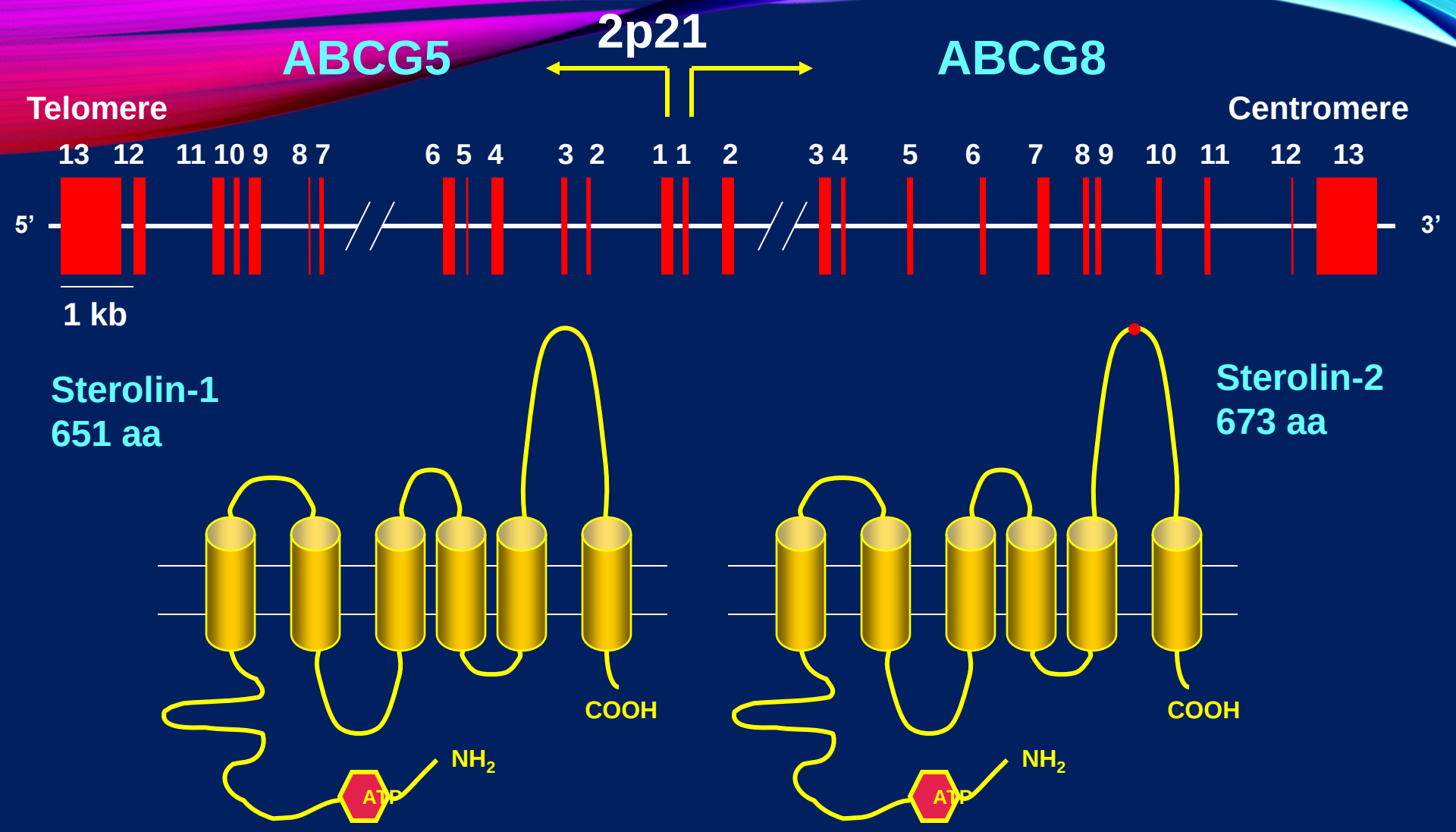
LDLR c.328 G>C (Ex 4)
p.Cys109Ser (repeat 3)

# β-SITOSTEROLEMIA/PHYTOSTEROLEMIA

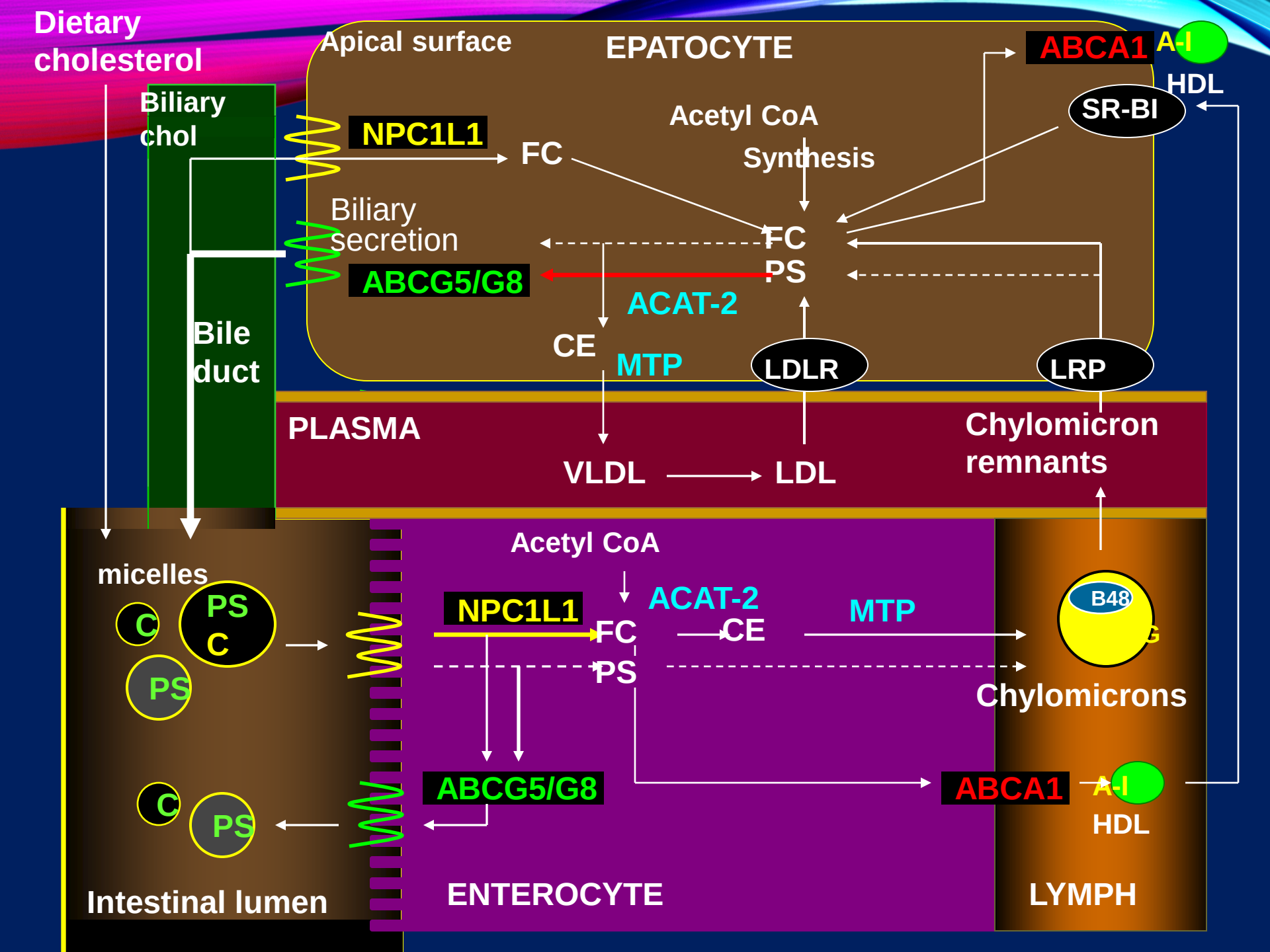
- Autosomal recessive (prevalence < 1: 1.000.000)
- Develops in childhood
- Generalized hyperabsorption of dietary sterols, including cholesterol
- Impaired biliary excretion of sterols
- >60% of ingesteed sitosterol is absorbed (<8% in normal individuals)
- Hypercholesterolemia: Total cholesterol 244.8±114.9; median 211; range 103-620 mg/dl
- From 30-fold to 100-fold elevation of plasma concentration of plant sterols: plasma sitosterol 33.2±22.0, median 27.0, range 2-97 mg/dl (n.v. 0.310 ± 0.117 mg/dl); plasma campesterol 18.7±17.6, median 12.9, range 2.3-78.5 mg/dl) (n.v. 0.484 ± 0.188 mg/dl)

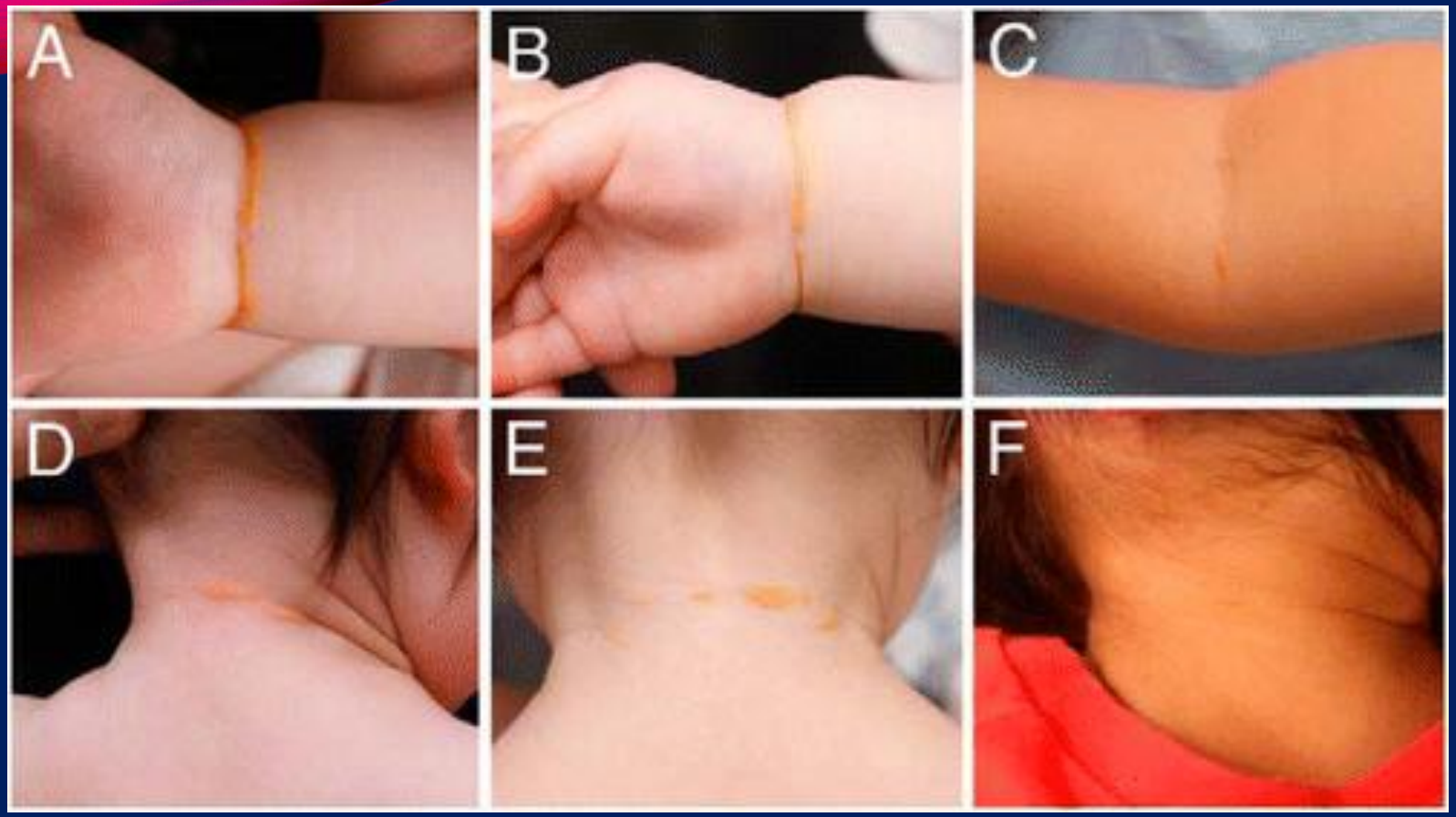
# β-SITOSTEROLEMIA/PHYTOSTEROLEMIA

- Phenotypic heterogeneity and variable age of diagnosis (0.3-75 y)
- Tendinous and/or tuberous xanthomas (some patients)
- Xanthelasma (some patients)
- Hemolytic anemia (incresed osmotic fragility of erythrocytes due to sitosterol accumulation in thr erithrocytes wall: stomatocytes, spherocutes, target cells)
- Macrothrombocytopenia, impaired hemostatic functions and splenomegaly
- Premature CAD and aortic ATS in some patients (plant sterol and stanols accumulation in monocyte-derived macrophages/ foam cells that deposit in the wall of the ascending aorta and coronary ostia)
- Arthritis and arthralgias (caused by sitosterol deposits)
- Possible adrenal and ovarian failure




Expression in jejunum and ileum (brush border membrane of enterocytes) and in the liver (canalicular membrane of hepatocytes)


Dietary cholesterol
Biliary chol
Bile duct
Apical surface
EPATOCYTE
NPC1L1
FC
Acetyl CoA
Synthesis
Biliary secretion
ABCG5/G8
FC
PS
ACAT-2
CE
MTP
LDLR
LRP
ABCA1
A-I
HDL
SR-BI
PLASMA
VLDL
LDL
Chylomicron remnants
micelles
C
PS
C
PS
C
PS
Intestinal lumen
Acetyl CoA
NPC1L1
FC
ACAT-2
CE
MTP
PS
ABCG5/G8
ENTEROCYTE
B48
Chylomicrons
ABCA1
A-I
HDL
LYMPH

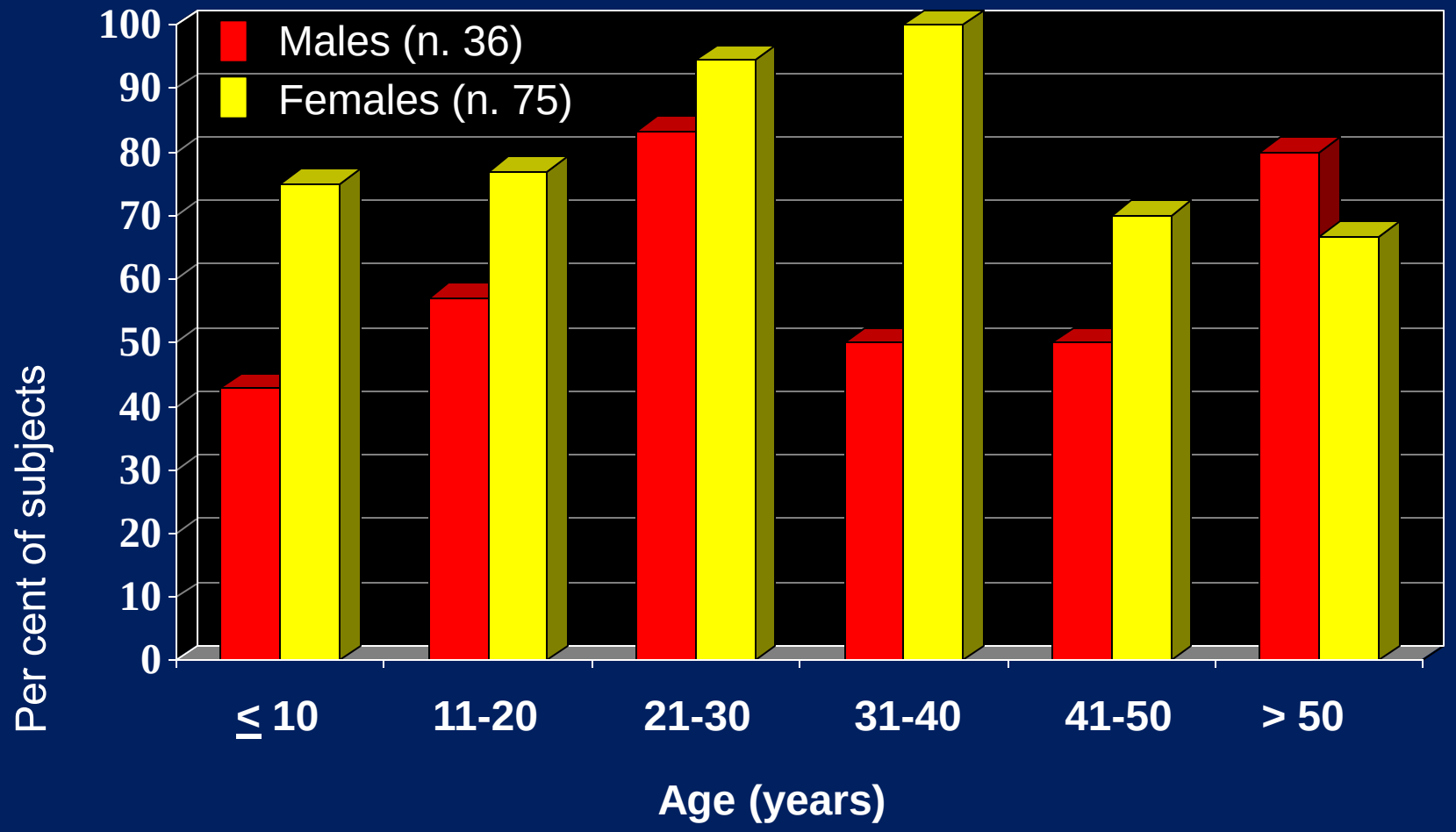



c.904+1G>A (p.Met 302Asnfs*82) and c.1336C>T (p.Arg446*) in *ABCG5* gene of a Korean girl.

Total cholesterol 675 mg/dl, LDL-C 540 mg/dL

*Park JH et al. J Clin Endocrinol Metab 2014; 99: 1512-18*

# Prevalence of Xanthomatosis in Sitosterolemic patients (83/111)



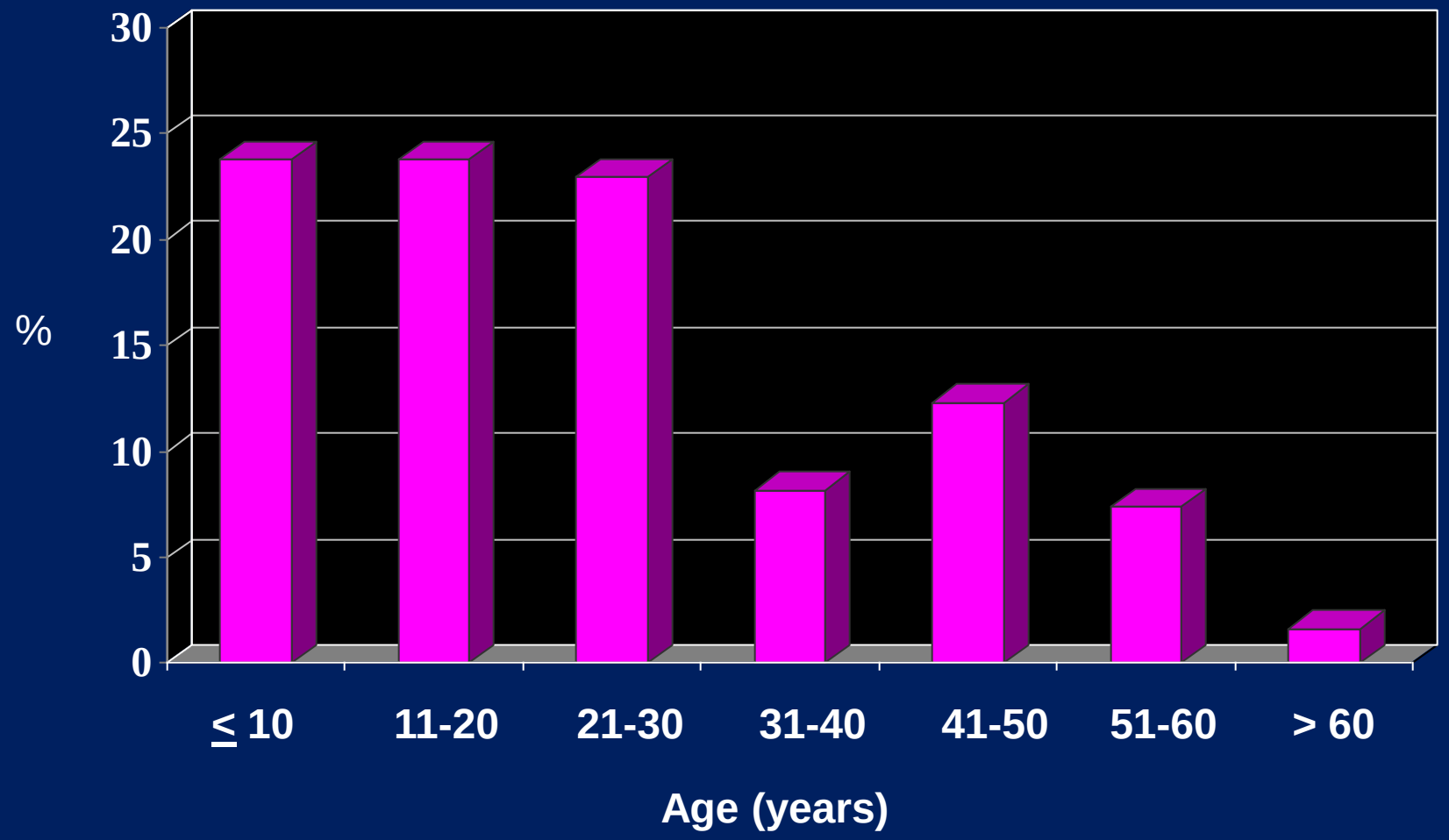
# DIAGNOSIS

**High Performance Liquid Chromatography**
**GAS Chromatography-Mass Spectrometry**

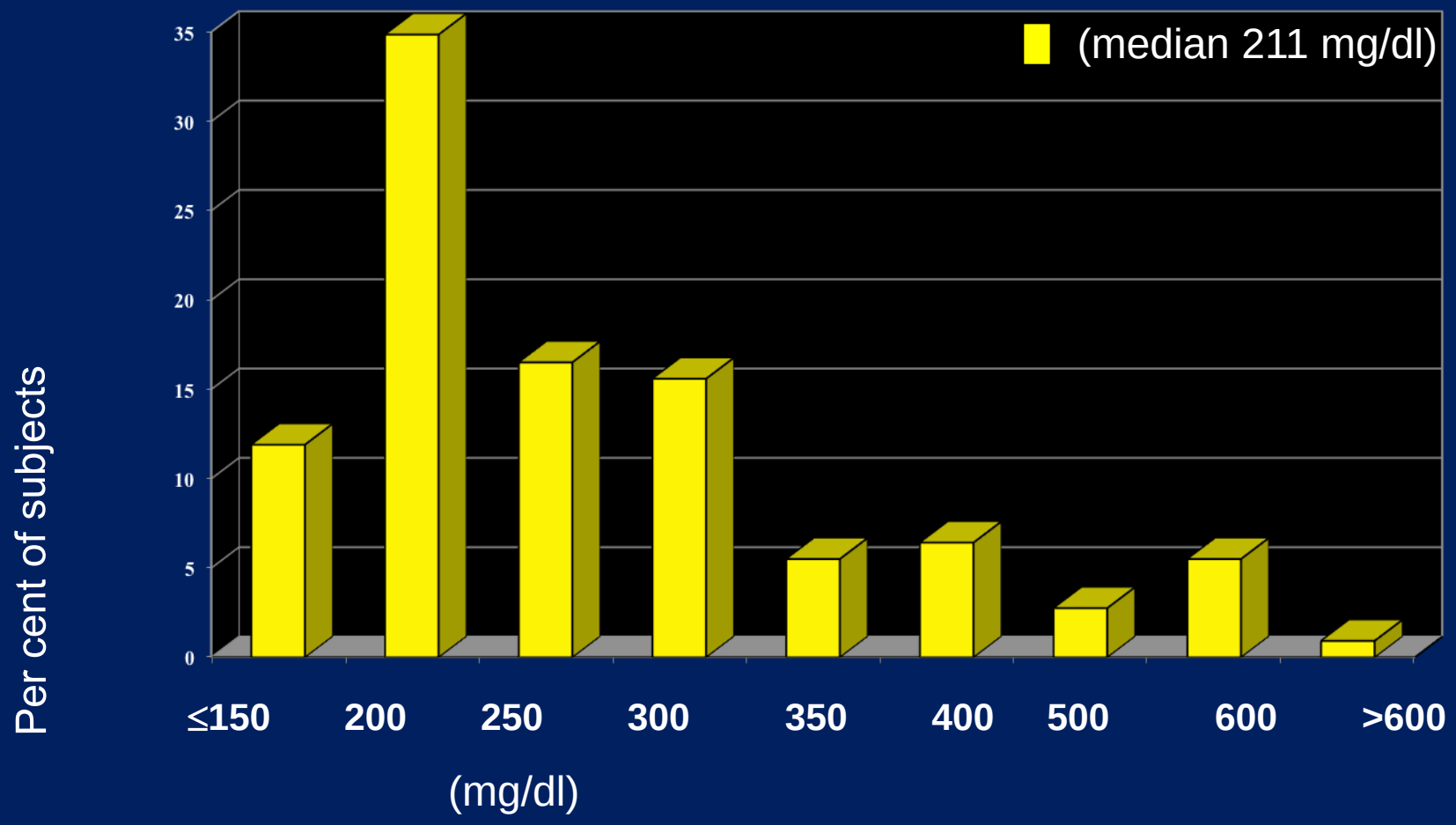
**Vs FH:** usually lower Cholesterol levels, rarely arcus cornea, recessive disease.

**Vs ARH**: usually lower Cholesterol levels, absence of massive Xanthomatosis.

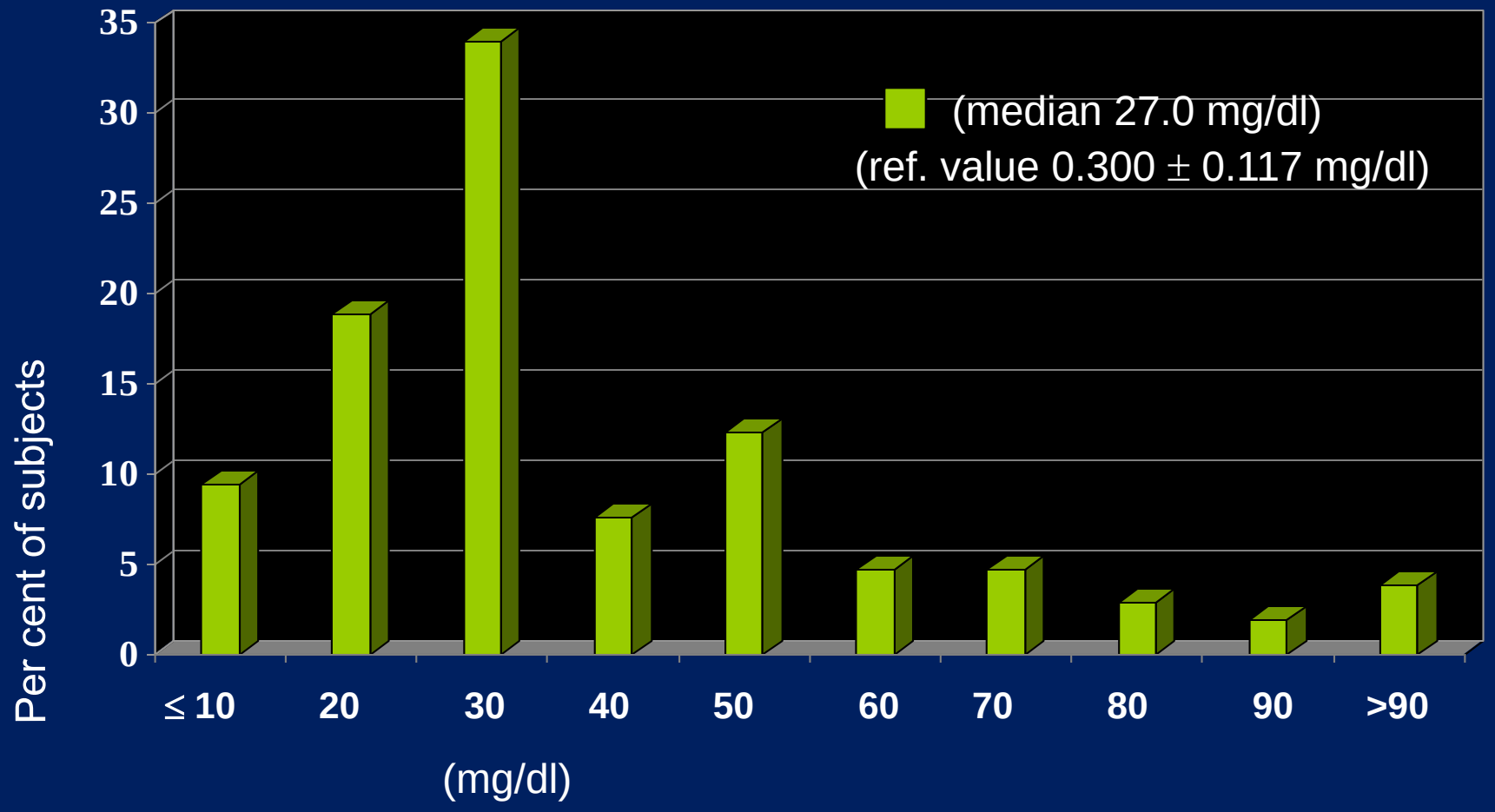
# Age distribution in 122 Sitosterolemic patients



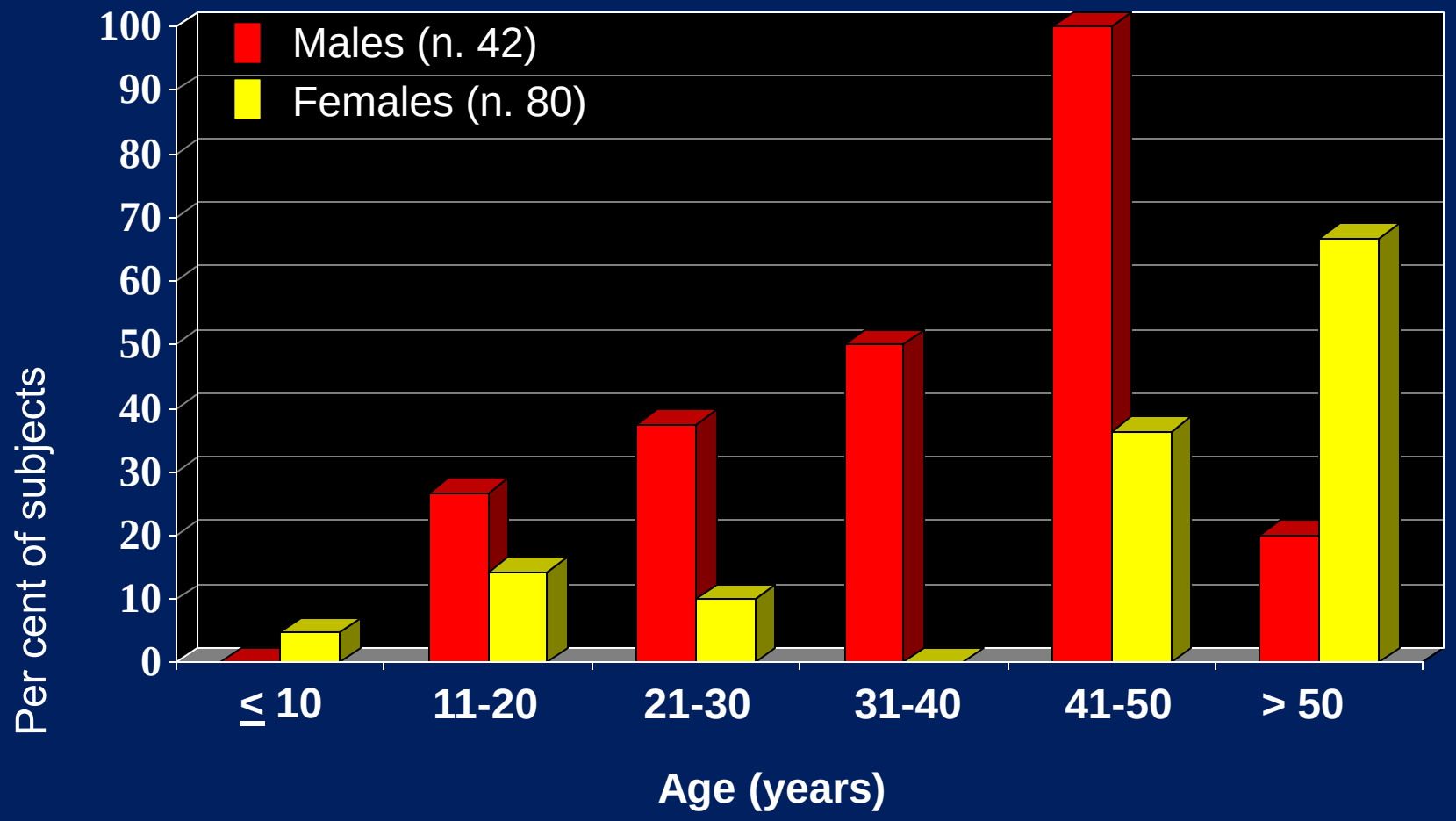
# Total plasma cholesterol distribution in 109 Sitosterolemic patients



# Plasma Sitosterol distribution in 106 Sitosterolemic patients



# Prevalence of CHD in Sitosterolemic patients (26/122)




OR (95% CI) for CHD

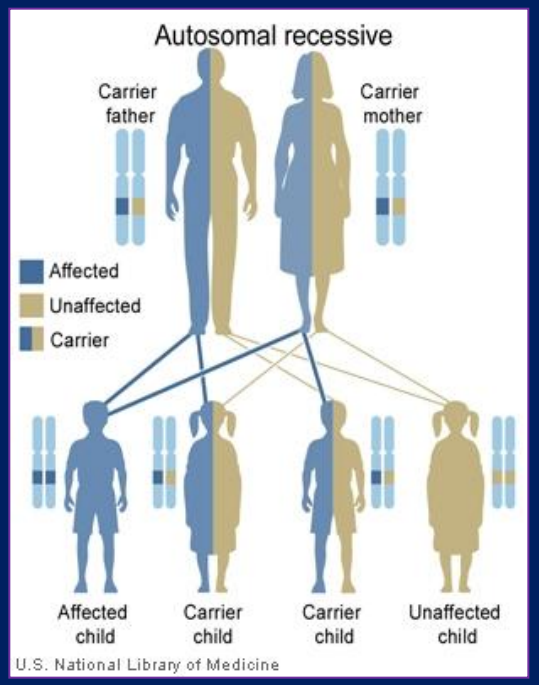
- 10 years increase in age: 1.679 (1.259-2.238), P = 0.0001
- Males vs Females: 2.666 (1.018-6.982), P < 0.05

## TREATMENT

- Low sterol diet
- Bile acids binding resins
- Ileal bypass surgery
- Plasmapheresis
- Ezetimibe

# LAL-D GENETICS

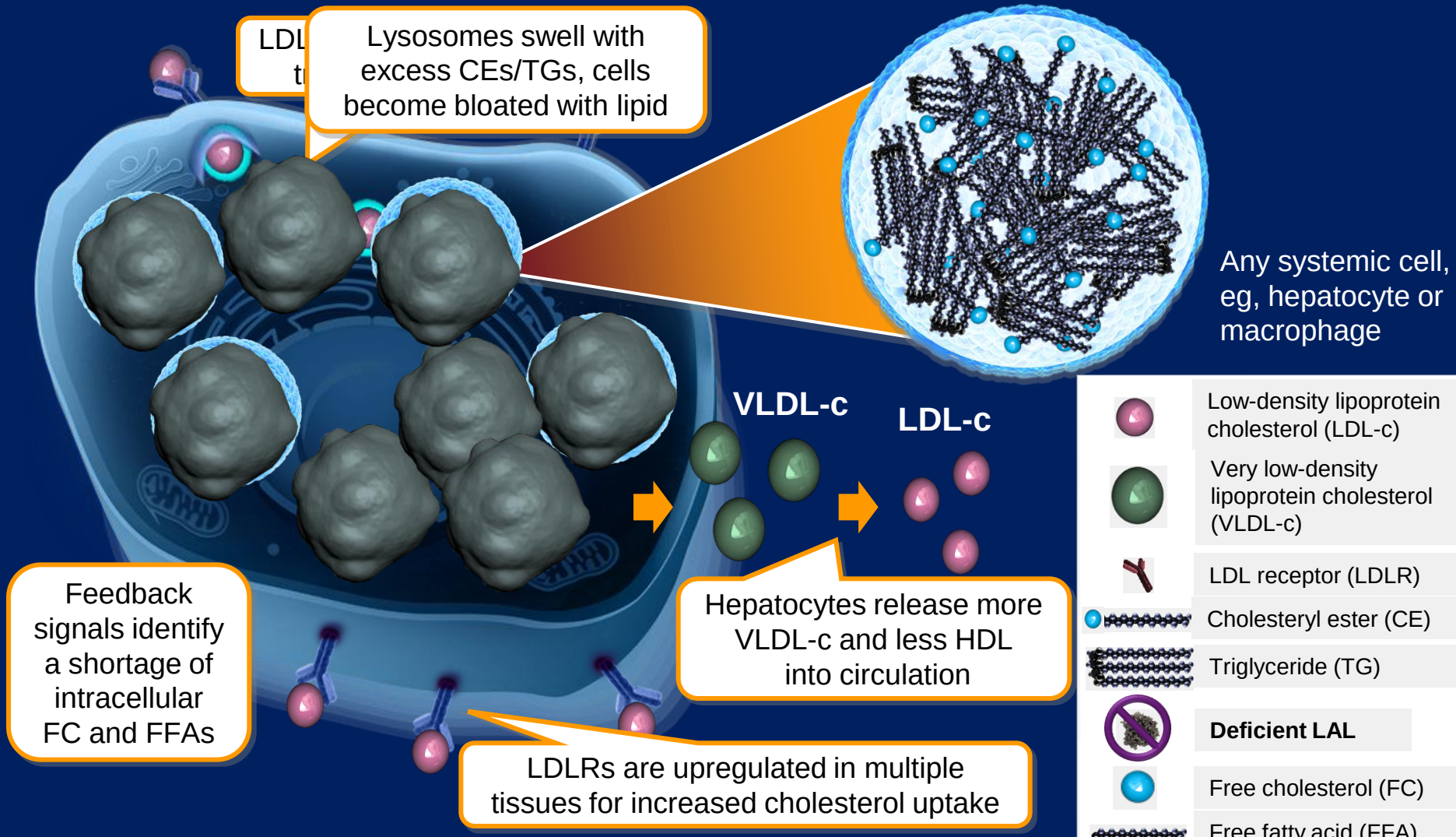
- Autosomal recessive disease[1]
- Due to homozygous or compound heterozygous mutations on the *LIPA* gene (chromosome 10)[1]
- Mutations may result in little to no LAL enzyme activity[2]




## *LIPA* gene structure

**1.** Reiner Ž, et al. *Atherosclerosis*. 2014;235(1):21-30. **2.** Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243.

# DEFICIENT LAL ENZYME DOES NOT PROCESS $CE_S$ AND $TG_S$[1,2]





**1.** Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243. **2.** Reiner Ž, et al. *Atherosclerosis*. 2014;235(1):21-30.
**3.** Grabowski GA, et al. In: Valle D, et al, eds. *The Online Metabolic & Molecular Bases of Inherited Disease*. New York, NY: McGraw-Hill; 2012.

# Patients of Any Age Are at Risk of Progressive LAL-D Complications[1]

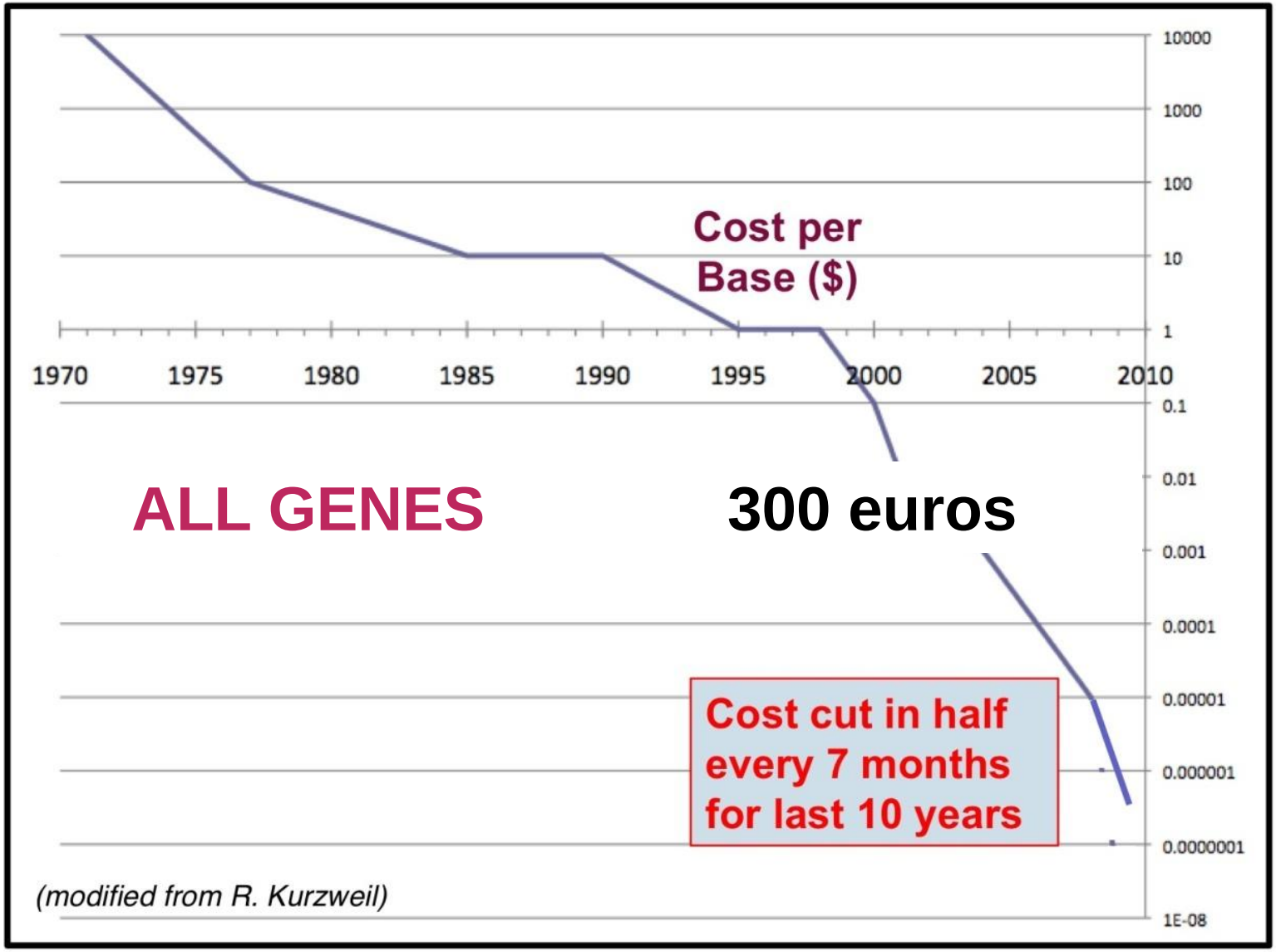
- LAL-D presents on a spectrum, with varying age of onset and severity[1,2]
- Regardless of age of onset, manifestations of LAL-D lead to liver failure, atherosclerotic progression, and premature death[1,2]

**Sebelipase alpha (recombinant-LAL) is available for these patients**

| **Infants[1,2]** (0-2) | **Children[1]** (2-21) | **Adults[2-4]** (>21) |
|---|---|---|
| **Medical emergency** | **Rapid progression to fibrosis, cirrhosis, and liver failure** | **Insidious development of liver fibrosis/cirrhosis** |
| • Rapidly progressive disease<br>• Liver failure and premature mortality (<6 months) | • Hepatosplenomegaly<br>• Dyslipidemia<br>• Slowed growth, short stature | • Variable transaminases<br>• Marked dyslipidemia<br>• Progressive atherosclerosis |

**1.** Reiner Ž, et al. *Atherosclerosis*. 2014;235(1):21-30. **2.** Bernstein DL, et al. *J Hepatol*. 2013;58(6):1230-1243. **3.** Elleder M, et al. *Journal of hepatology*. 2000; 32(3):528-534. 4**.** vom Dahl S, et al. *Journal of hepatology*. 1999; 31(4):741-746.

# Decreasing Cost of DNA Sequencing



# CONCLUSIONS

- DIAGNOSIS OF FH IS A CLINICAL DIAGNOSIS
- THE PHENOTYPE IS DETERMINANT IN THERAPEUTICAL DECISIONs
- GENETIC DIAGNOSIS, when available, is a GOLD STANDARD IN CHILDREN in particular in HOMOZYGOUS PHENOTYPE, for DIFFERENTIAL DIAGNOSIS with FCHL, for DIFFERENTIAL DIAGNOSIS in RECESSIVE PHENOTYPE or in ABSENCE OF FAMILY'S DATA

**Thank you for your attention**