Dyslipidémies

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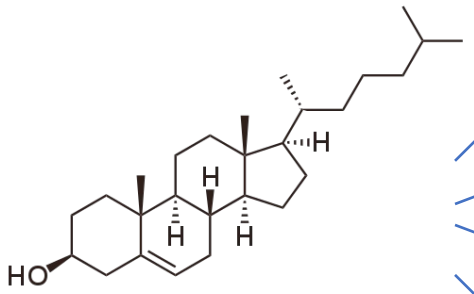
Master 1 Thorax, 24 octobre 2024

Le cholestérol: une molécule essentielle à la vie…



Le cholestérol

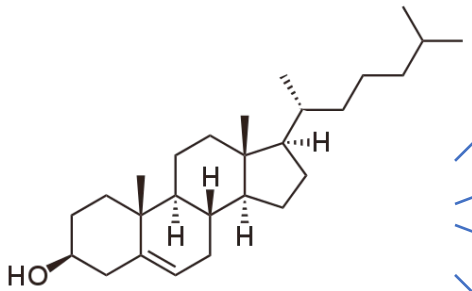
Nécessaire à la structure membranaire des cellules

Hormones stéroïdiennes
(Cortisol, aldostérone, oestrogènes, testostérone, etc…)

Vitamine D3

Acides biliaires

Le cholestérol: une molécule essentielle à la vie… dont l'homéostasie est indispensable à la santé



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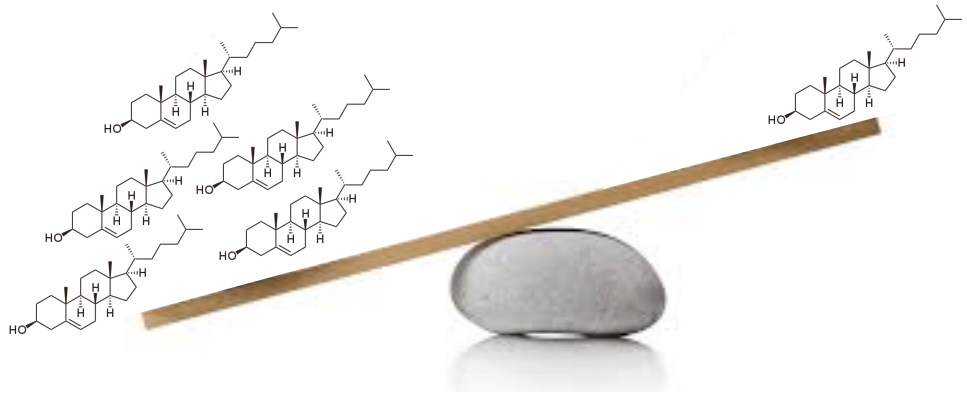

Le métabolisme du cholestérol est étroitement régulé chez les patients « sains »

THE NEW ENGLAND JOURNAL OF MEDICINE

BRIEF REPORT

NORMAL PLASMA CHOLESTEROL IN AN 88-YEAR-OLD MAN WHO EATS 25 EGGS A DAY

Mechanisms of Adaptation

FRED KERN, JR., M.D.

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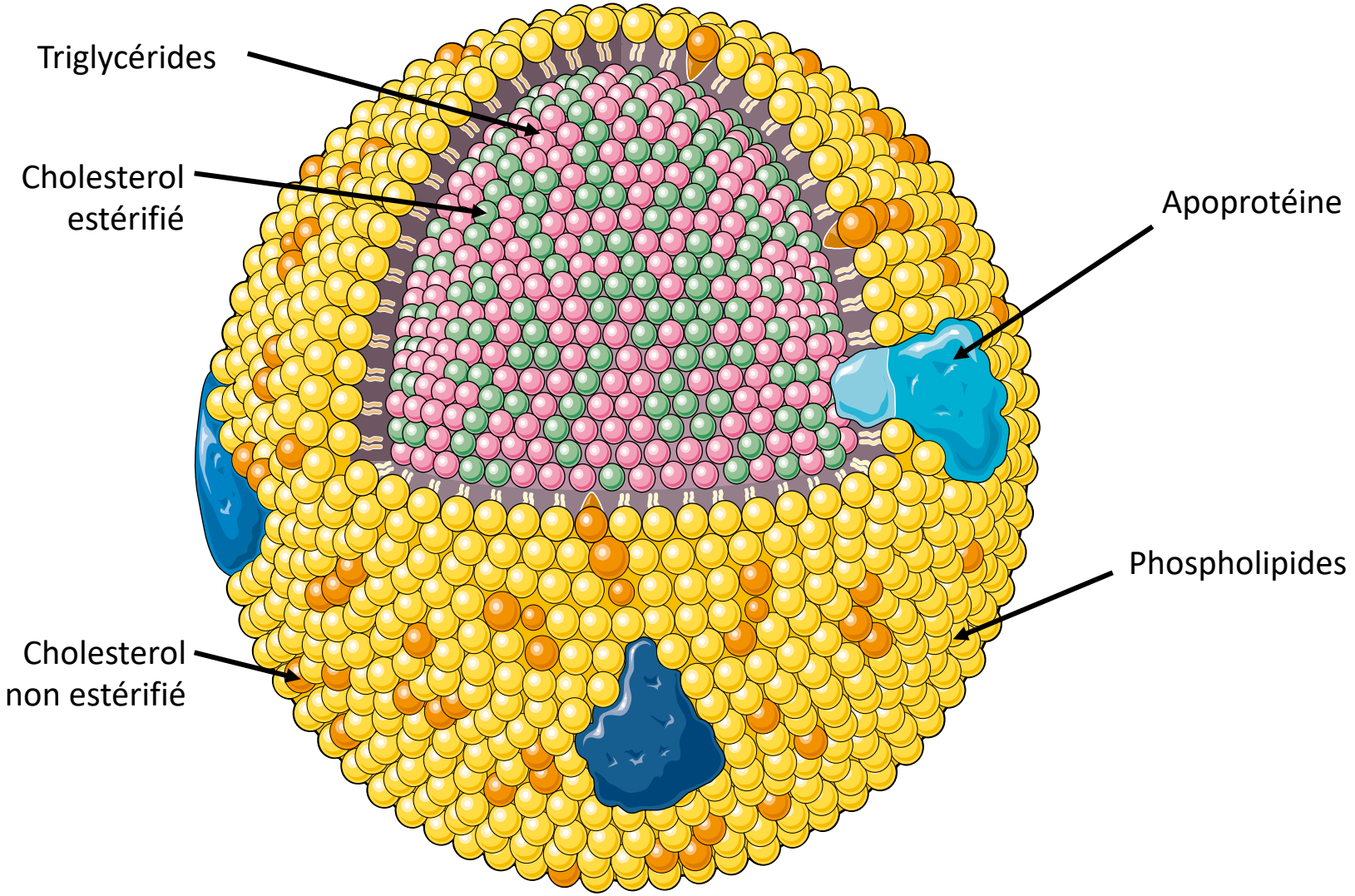
Cholestérolémie normale

1,42g/L LDL cholestérol
0,45g/L HDL cholestérol

Pas d'accidents cardiovasculaires

→ Voies impliqués dans la régulation du métabolisme du cholestérol

Le cholestérol et les triglycérides sont véhiculés dans le sang sous forme de lipoprotéines

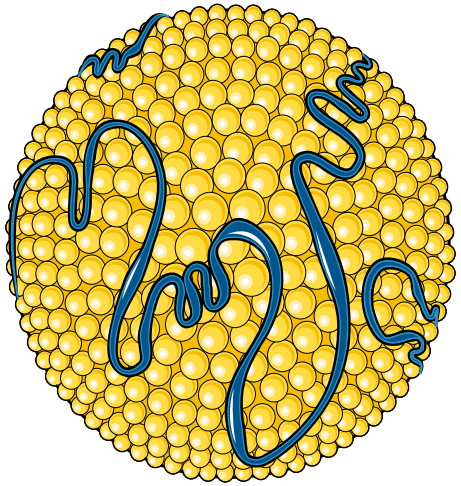


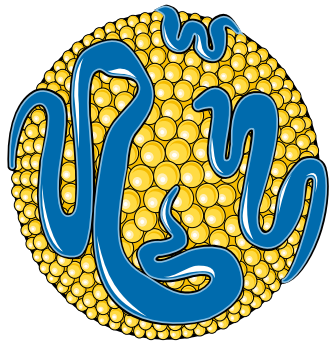
Les lipoprotéines à apolipoprotéines B et A1

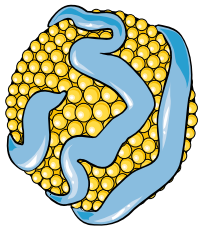
L'apolipoprotéine B est un reflet des particules athérogènes: principalement des LDL

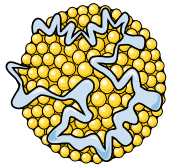
L'apolipoprotéine A1 est un reflet des particules anti-athérogènes: les HDL

	Chylomicrons	VLDL	LDL	HDL
Taille:	75-1200nm	30-80nm	18-27nm	>12nm
Ratio Chol/TG:	1/19	1/3,3	1/0,23	1/0,22
Densité:	0,93	0,93-1,006	1,019-1,063	>1,063

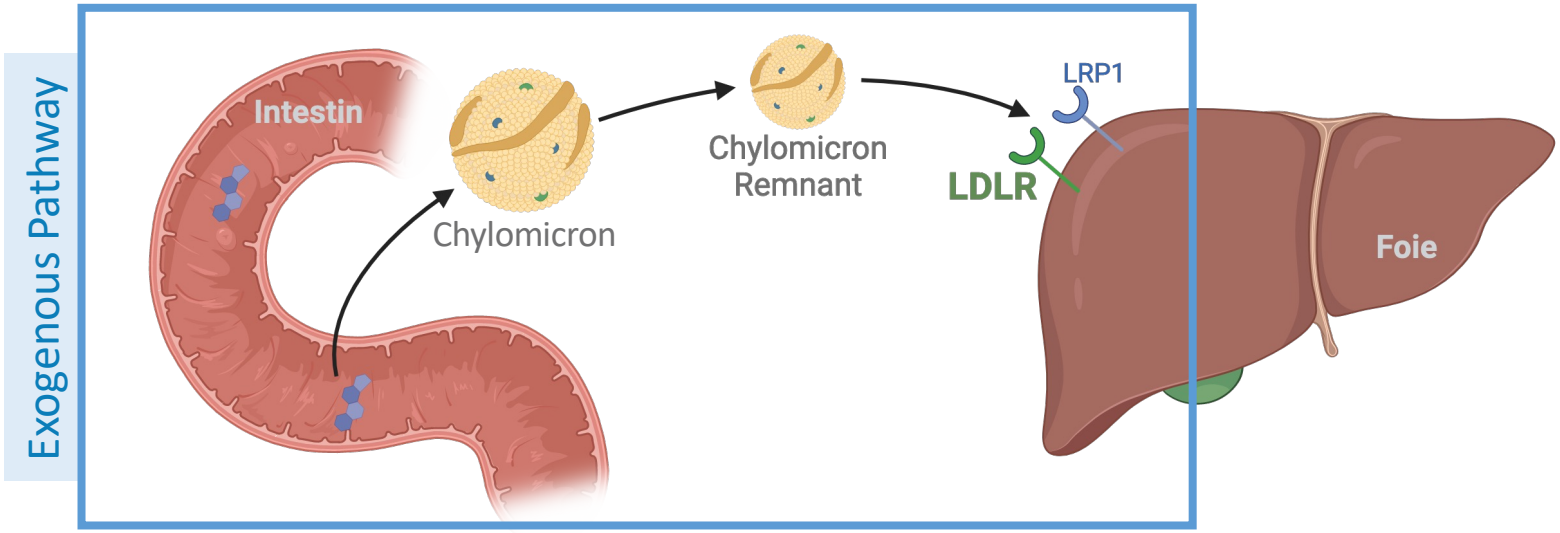






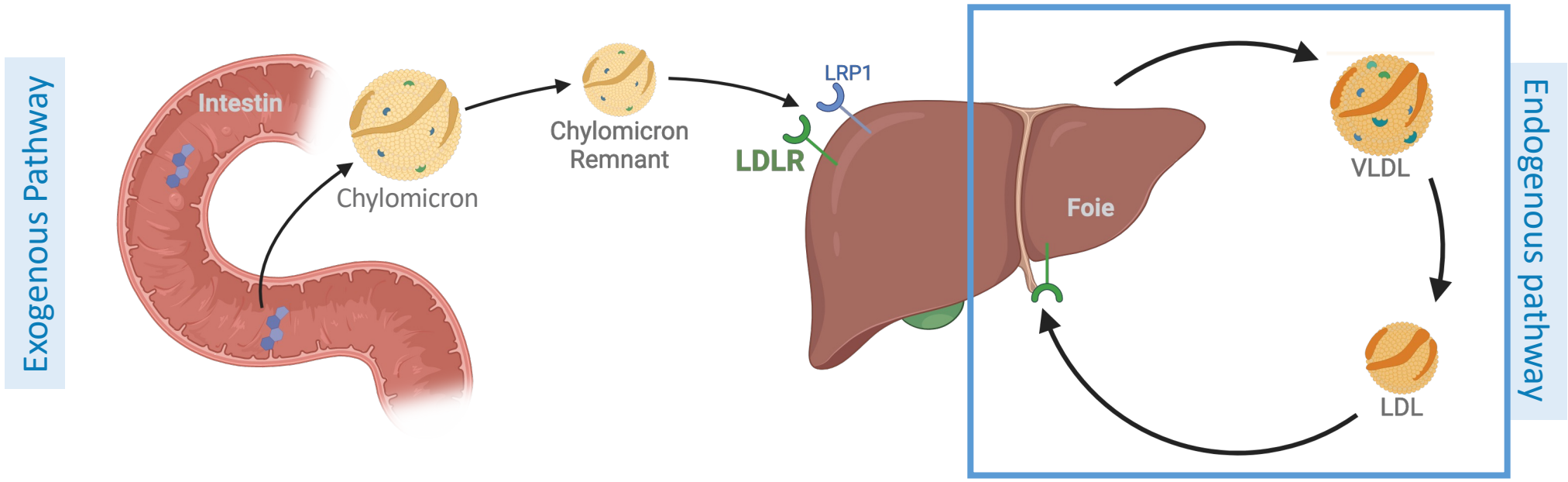


Cholesterol homeostasis



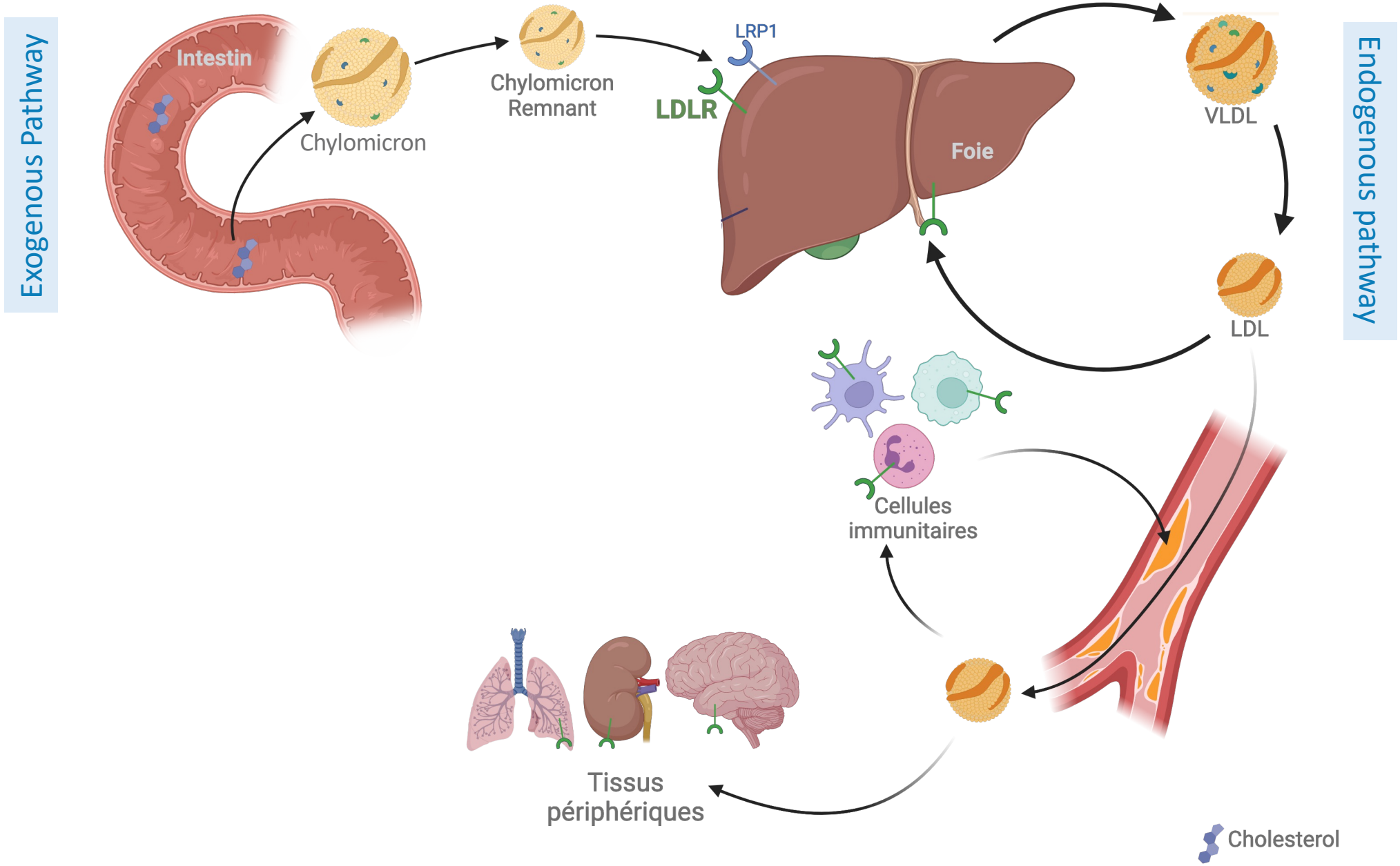
LDLR = Low Density Lipoprotein Receptor

Cholesterol homeostasis



Cholesterol

Cholesterol homeostasis



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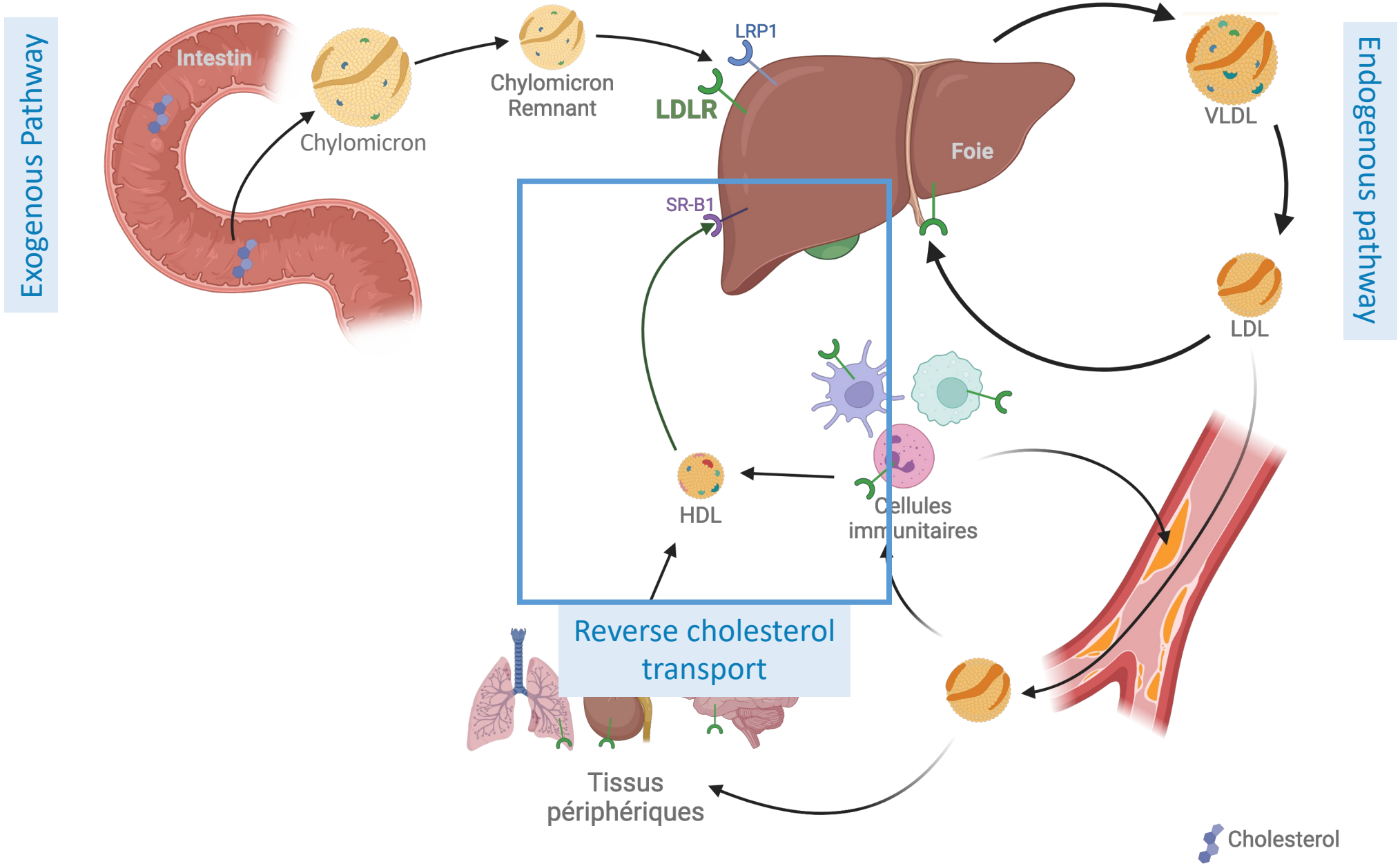
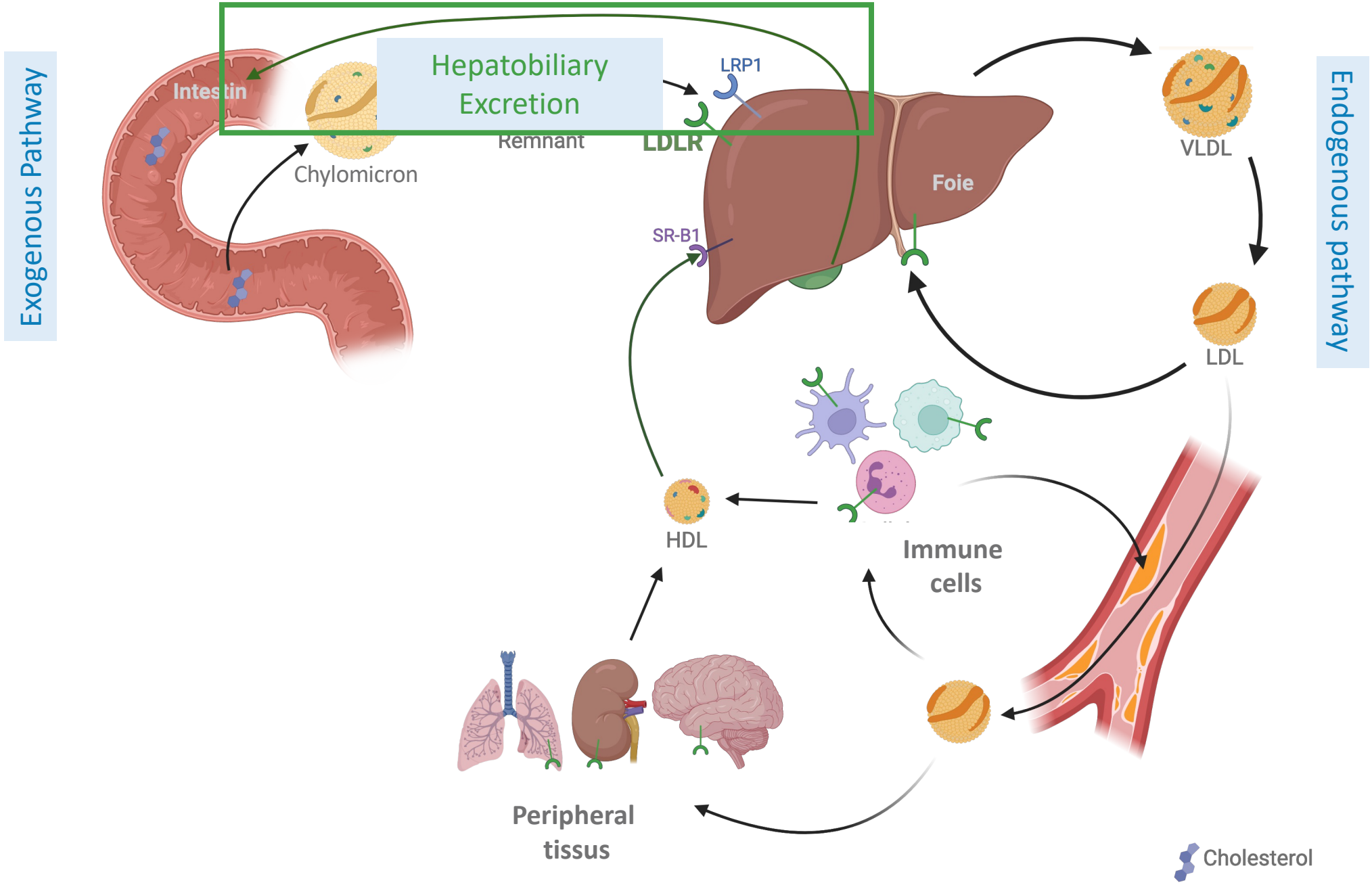
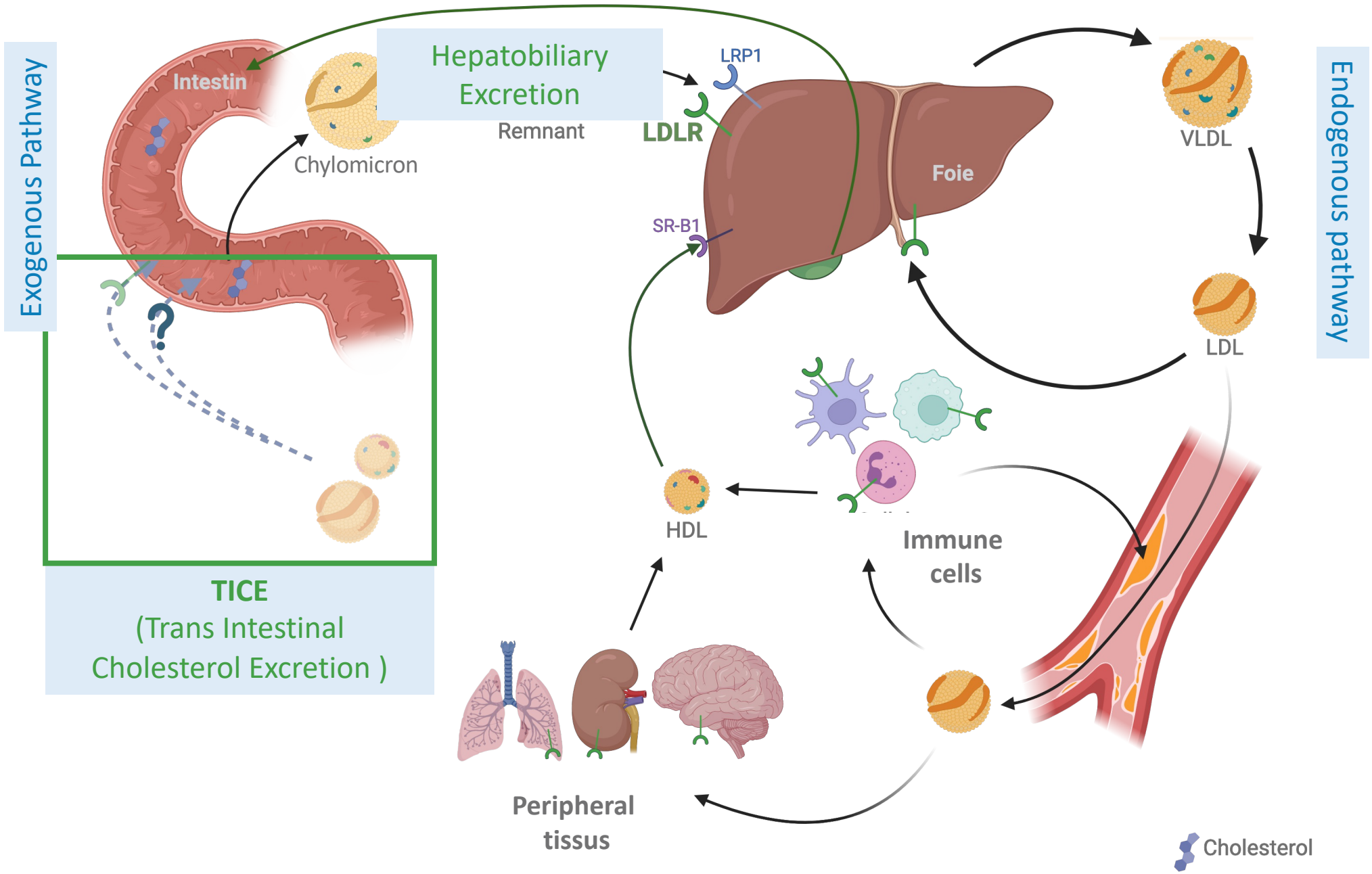


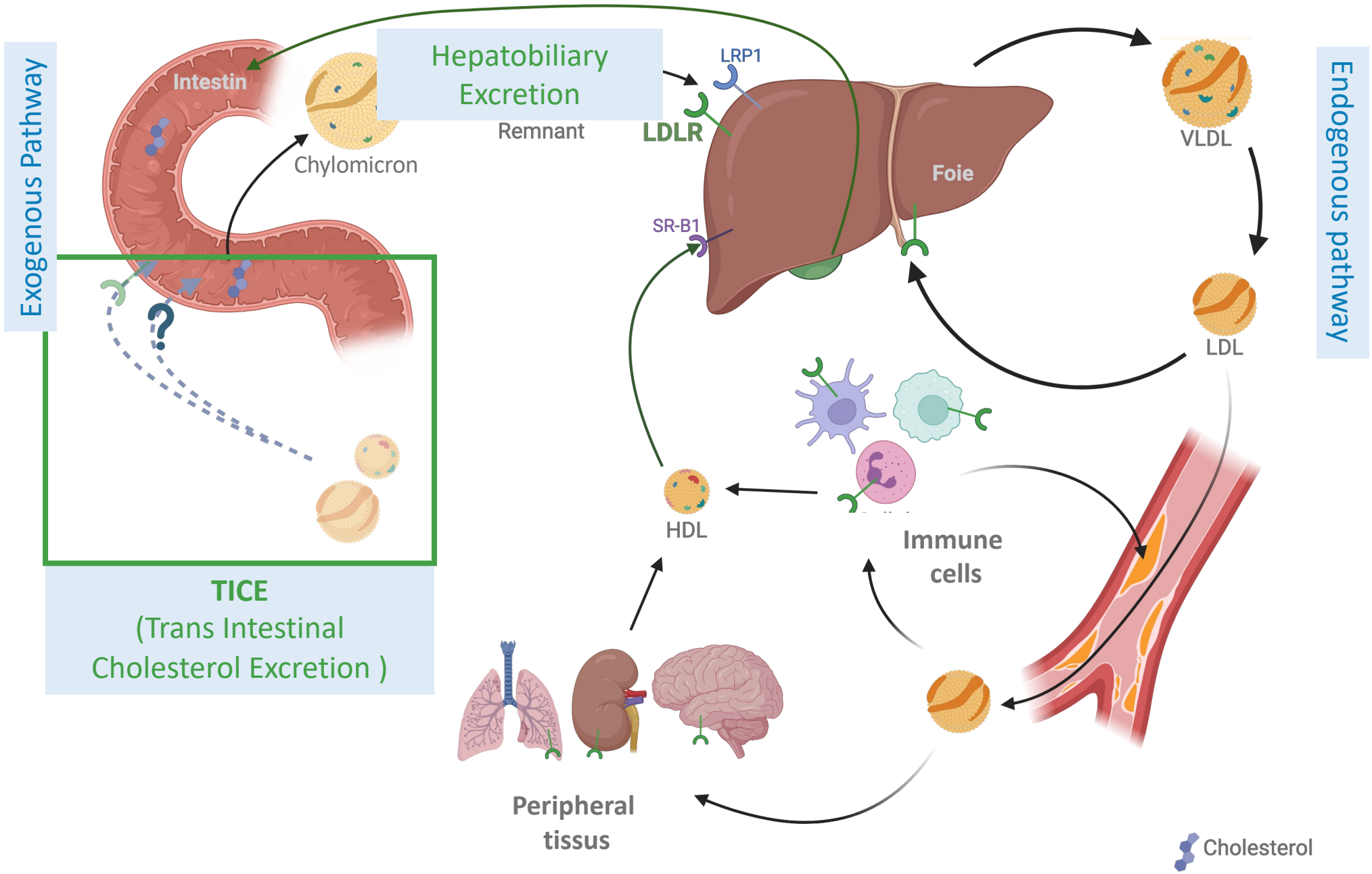
Cholesterol homeostasis



Cholesterol homeostasis



Cholesterol homeostasis



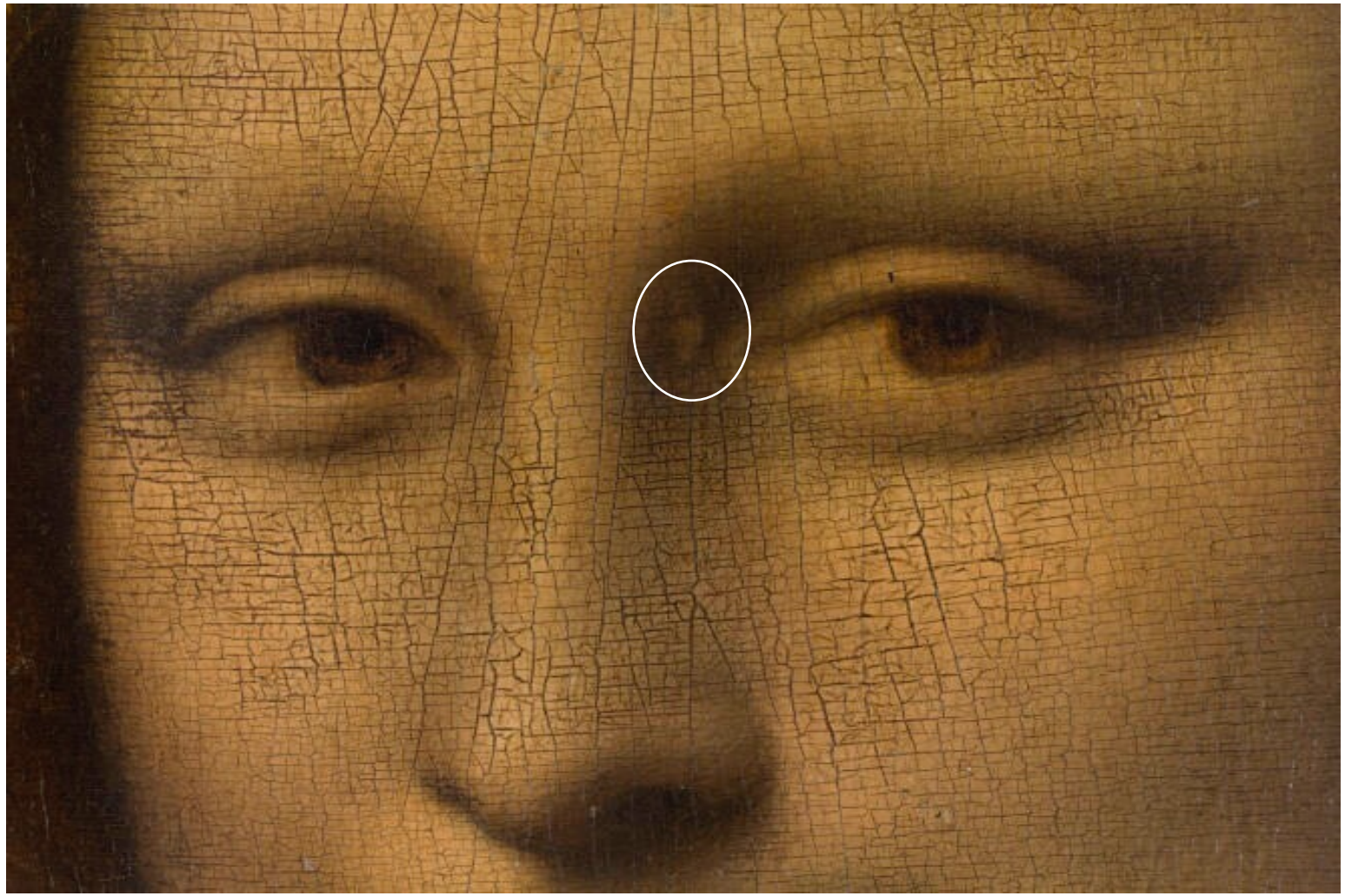
Dyslipidémies

Table 11 Genetic disorders of lipoprotein metabolism

Disorder	Prevalence	Gene(s)	Effect on lipoproteins
HeFH	1 in 200–250	*LDLR* *APO B* *PCSK9*	↑LDL-C
HoFH	1 in 160 000–320 000	*LDLR* *APO B* *PCSK9*	↑↑LDL-C
FCH	1 in 100/200	*USF1* + modifying genes	↑LDL-C ↑VLDL-C ↑ApoB
Familial dysbetalipoproteinaemia	1 in 5000	*APO E*	↑↑IDL and chylomicron remnants (βVLDL)
Familial lipoprotein lipase deficiency (familial chylomicron syndrome)	2 in 10^6	*LPL* *APO C2* *ApoAV, GPIHBP1* *LMF1*	↑↑chylomicrons and VLDL-C
Tangier disease (analphalipoproteinaemia)	1 in 10^6	*ABCA1*	↓↓HDL-C
Familial LCAT deficiency	1 in 10^6	*LCAT*	↓HDL-C

European Heart Journal (2020) 41, 111-188

FAMILIAL HYPERCHOLESTEROLEMIA (FH)



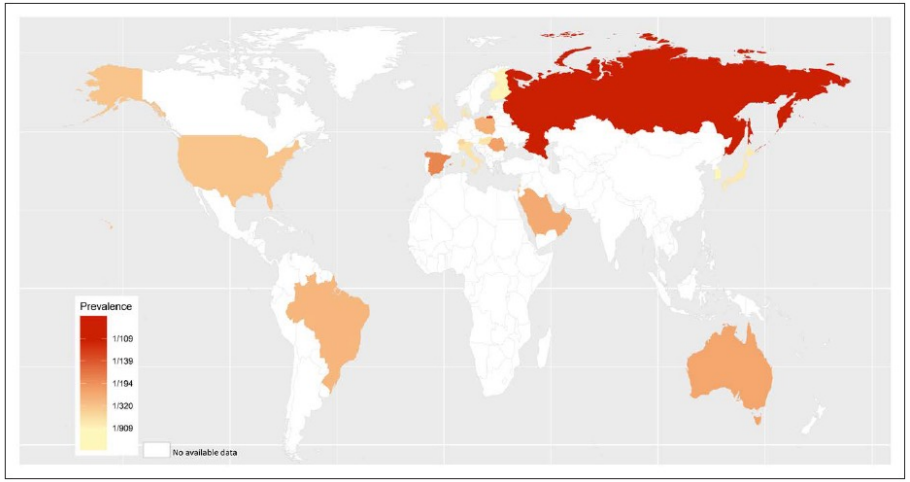
FH: the most common genetic disease

Circulation

ORIGINAL RESEARCH ARTICLE

Prevalence of Familial Hypercholesterolemia Among the General Population and Patients With Atherosclerotic Cardiovascular Disease

A Systematic Review and Meta-Analysis



Prevalence:
1/311-313

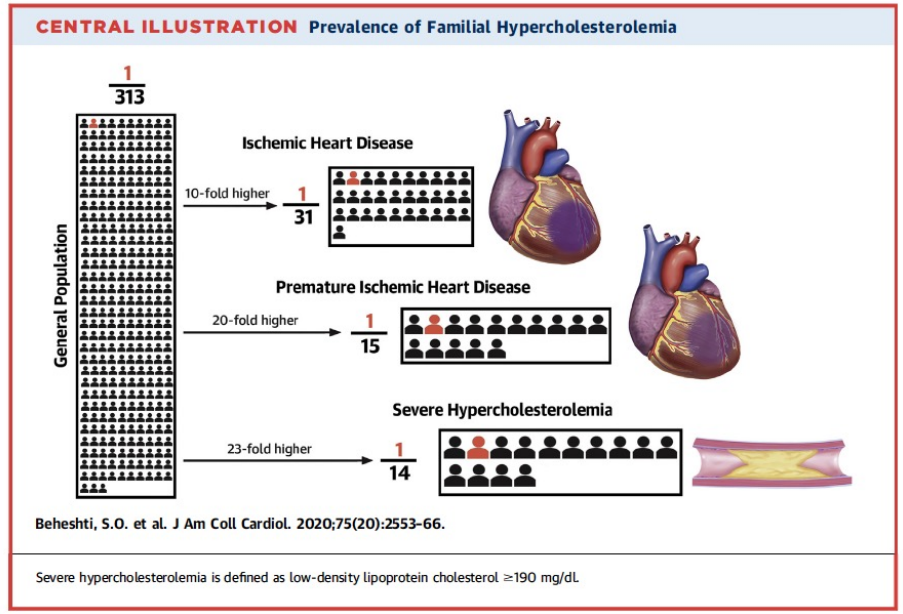
JOURNAL OF THE AMERICAN COLLEGE OF CARDIOLOGY

PUBLISHED BY ELSEVIER

Worldwide Prevalence of Familial Hypercholesterolemia

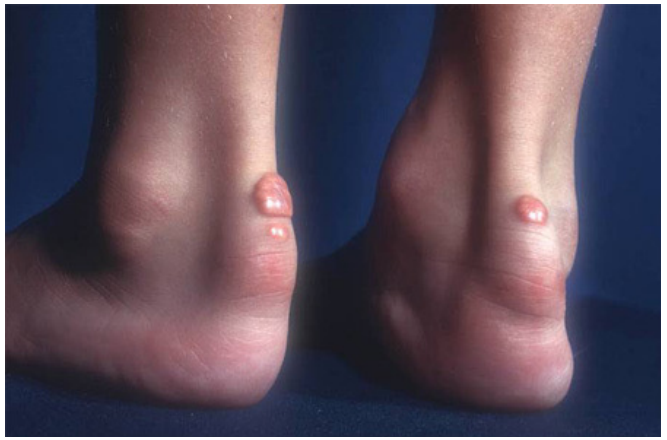
Meta-Analyses of 11 Million Subjects

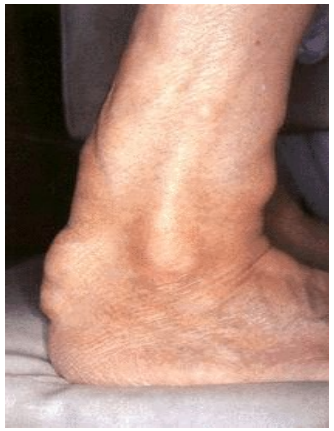
Sabina O. Beheshti, BSc, Christian M. Madsen, MD, Anette Varbo, MD, PhD, Børge G. Nordestgaard,

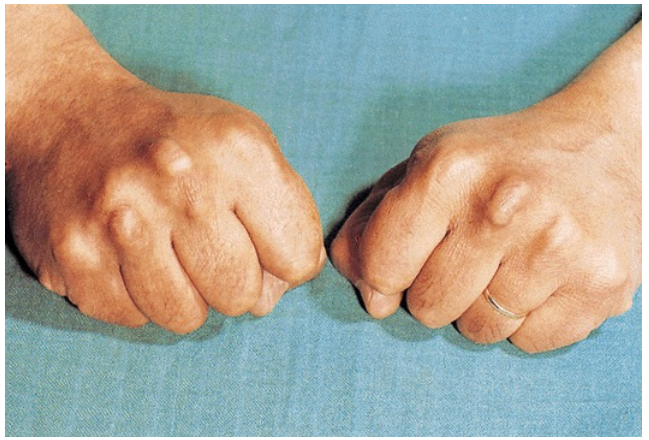


213 000 subjects in France

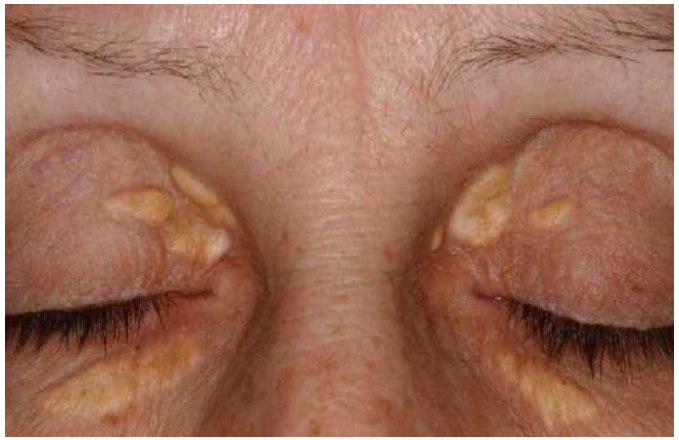
Extra-vascular depots of cholesterol in FH



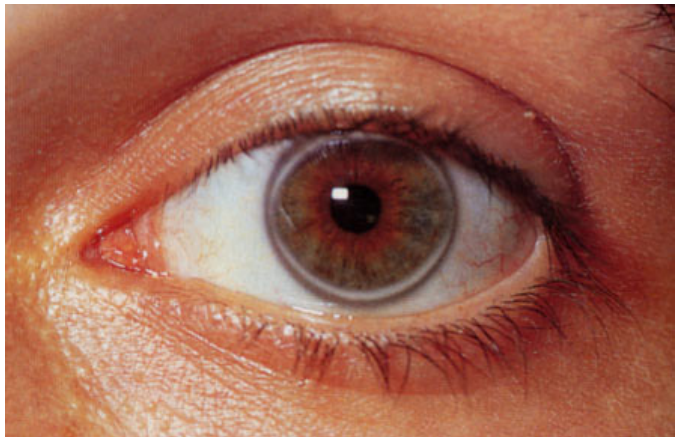




tendinous xanthomata



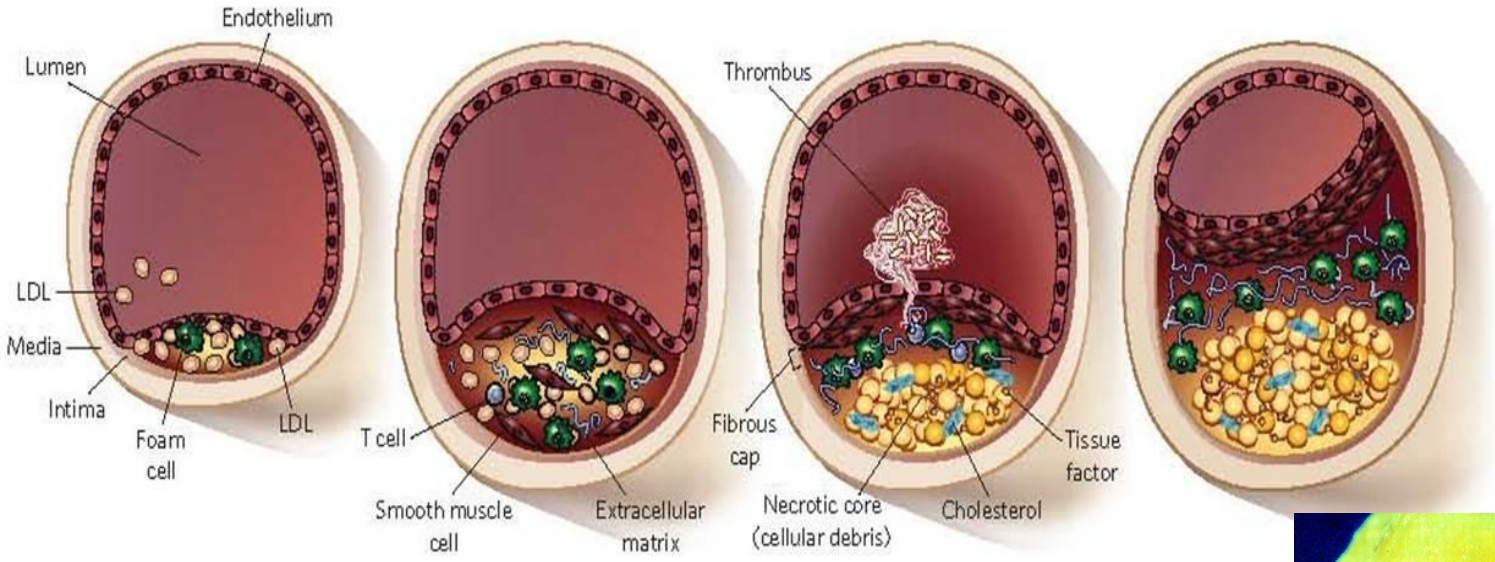
xanthelasma

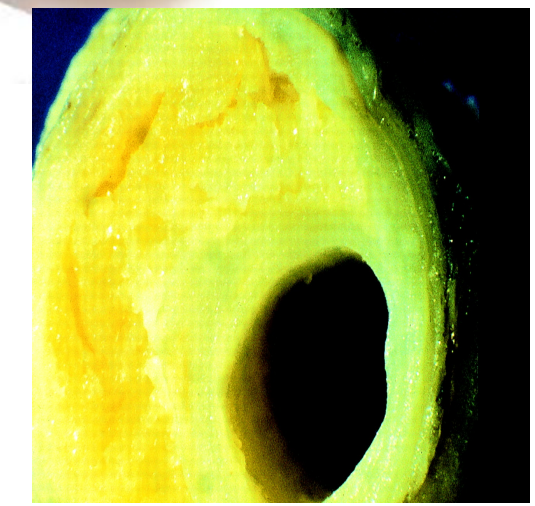


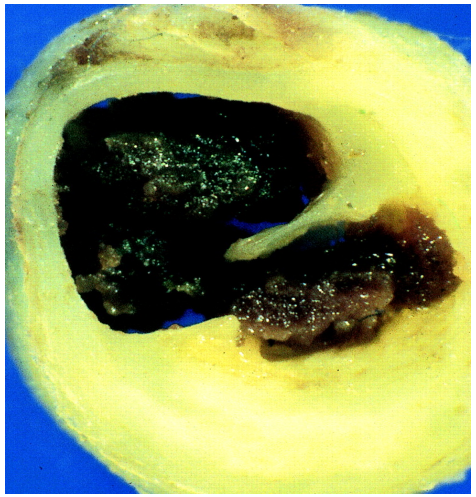
Arcus cornealis (< 45 yrs)

Vascular depots of cholesterol in FH

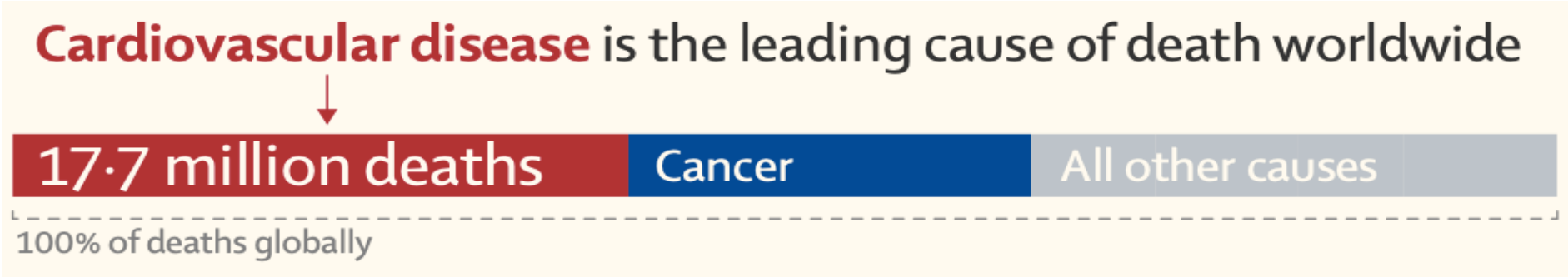
Atherosclerosis progresion

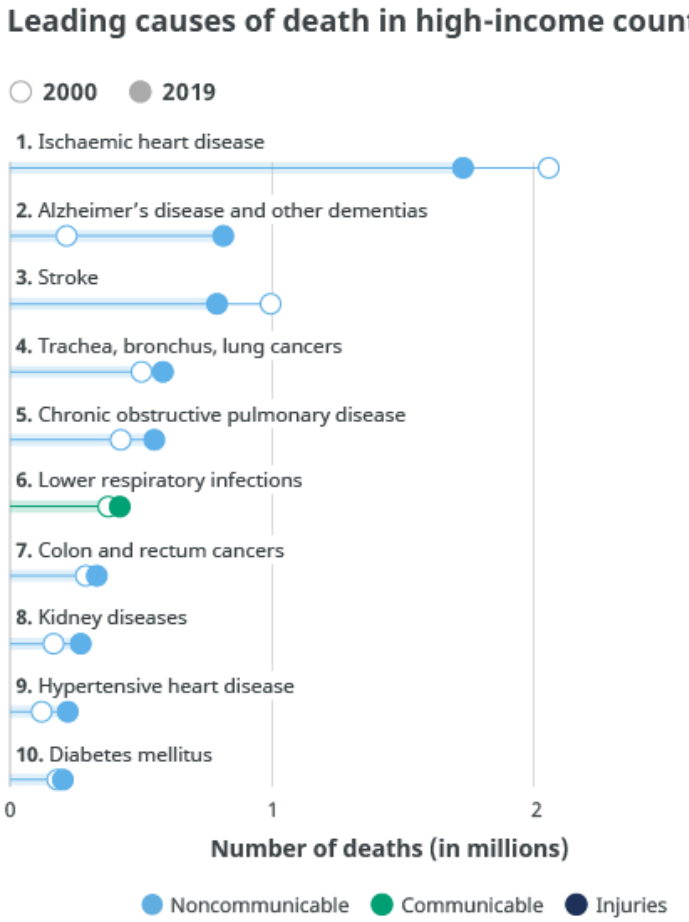






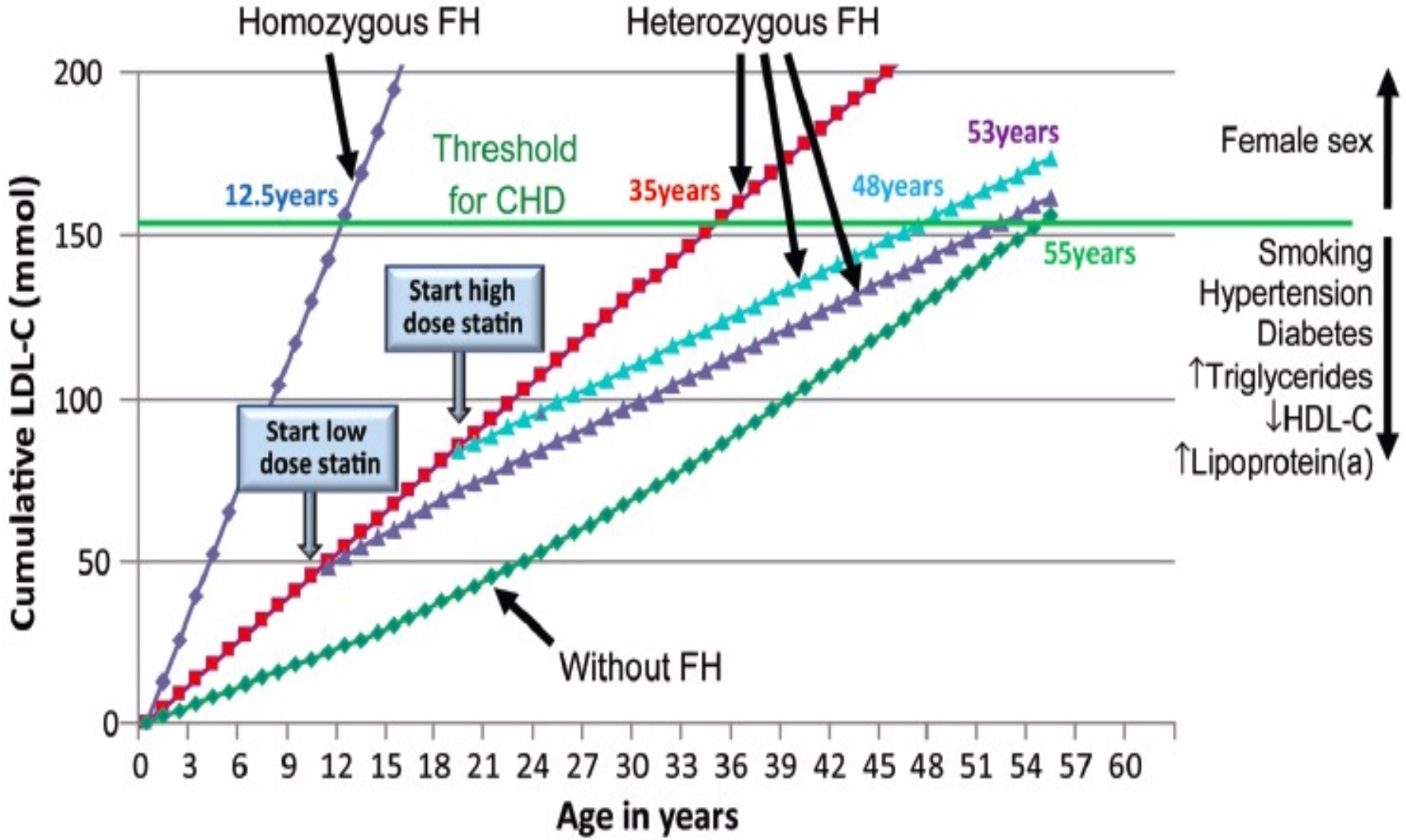
Context





- Cardiovascular diseases remain the first killer worldwide
- **LDL-C causality**: lowering LDL-C prevents CVD ("lower is better")

Goal of FH management = avoid the cholesterol burden



European Heart Journal (2013) 34, 3478–3490

Lower LDLc sufficiently to prevent cardiovascular risk

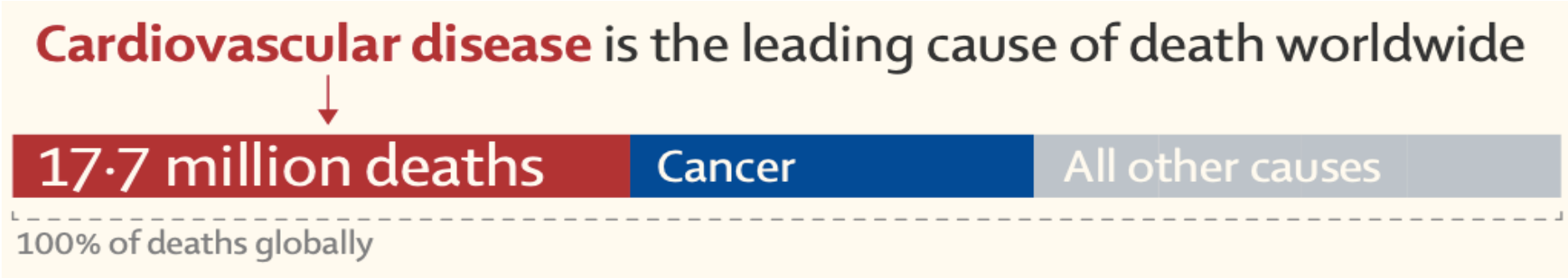
ESC
European Society of Cardiology

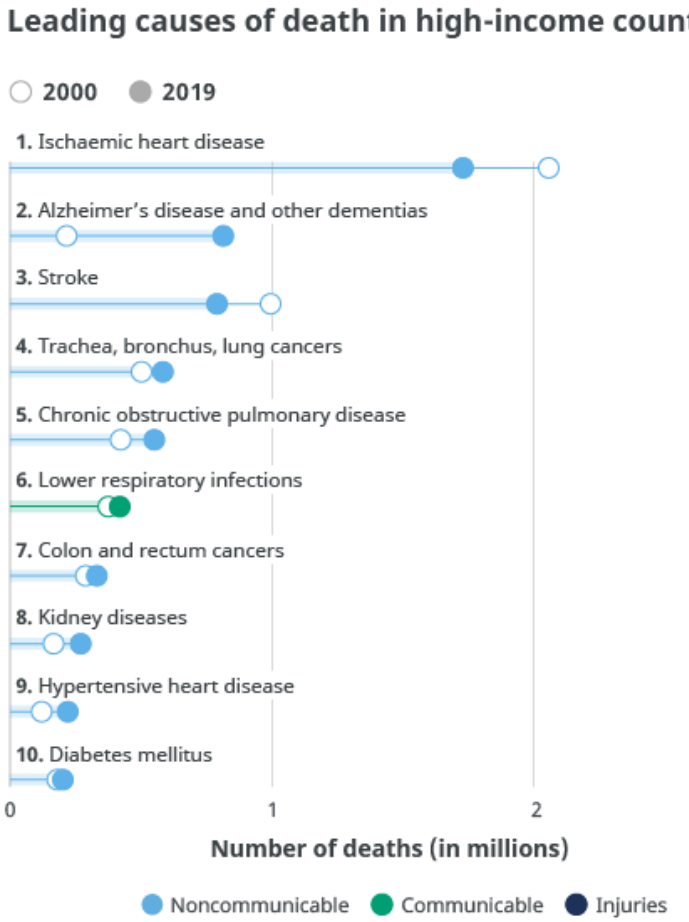
European Heart Journal (2020) **41**, 111–188
doi:10.1093/eurheartj/ehz455

ESC/EAS GUIDELINES

2019 ESC/EAS Guidelines for the management of dyslipidaemias: *lipid modification to reduce cardiovascular risk*

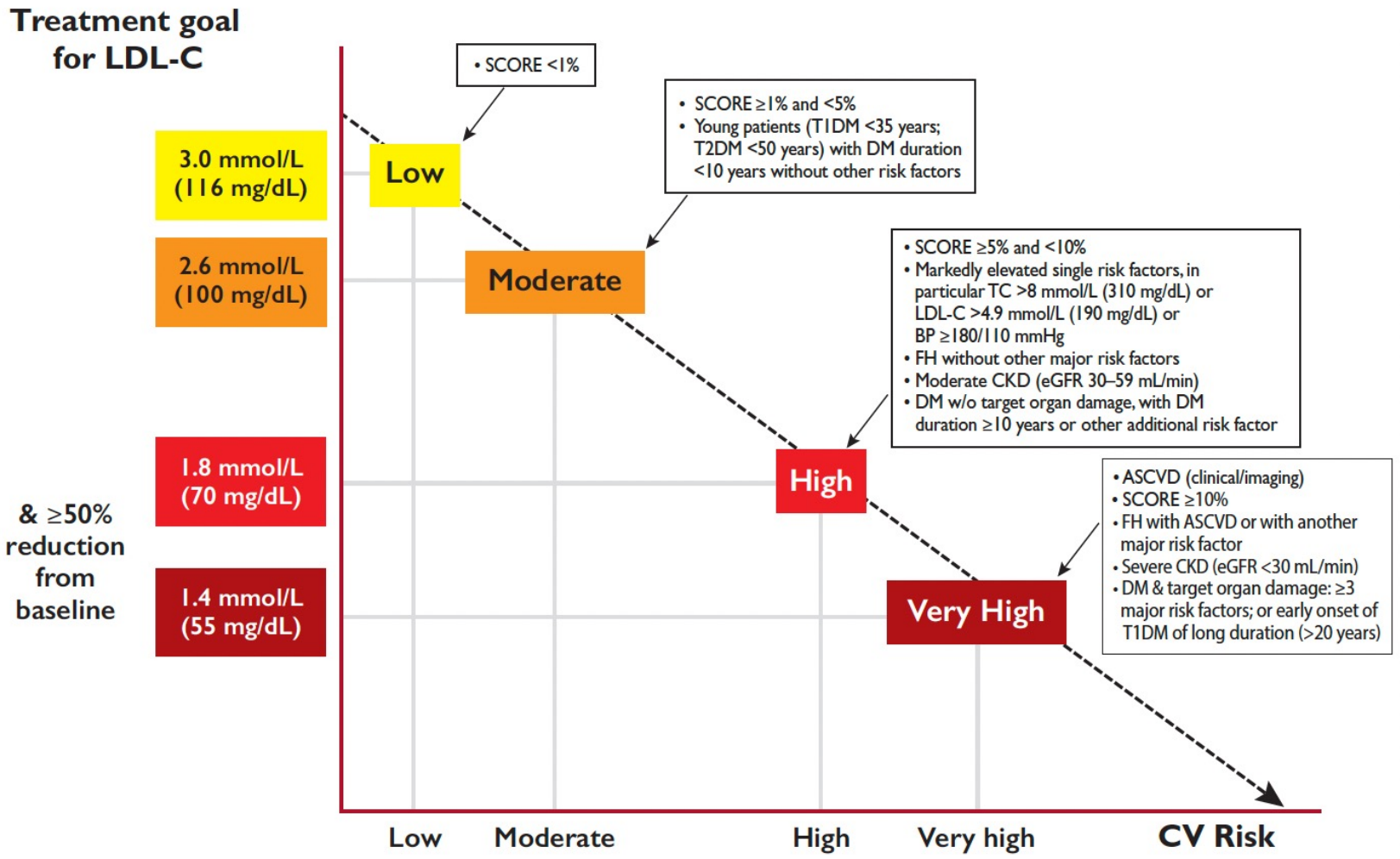
Context





- Cardiovascular diseases remain the first killer worldwide
- **LDL-C causality**: lowering LDL-C prevents CVD ("lower is better")
- LDL-C goal was further lowered in novel European 2019 guidelines

Lower LDLc sufficiently to prevent cardiovascular risk



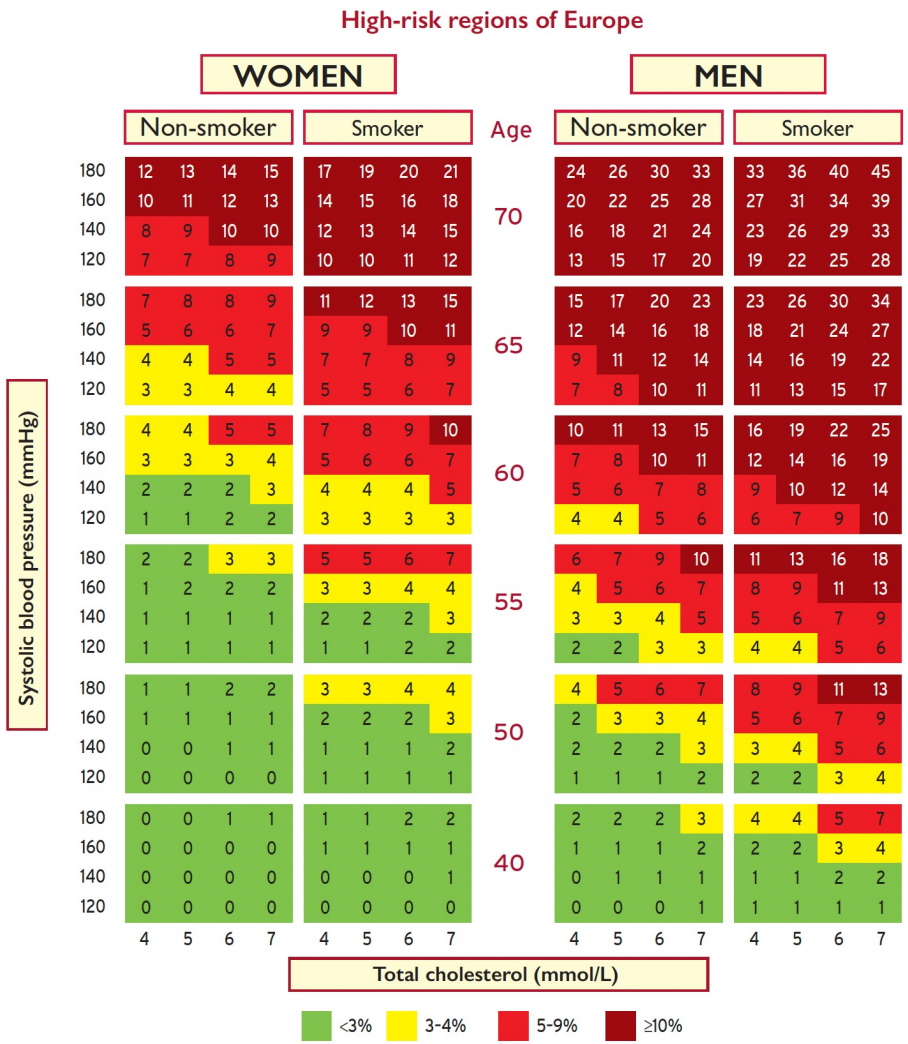
European Heart Journal (2020) 41, 111-188

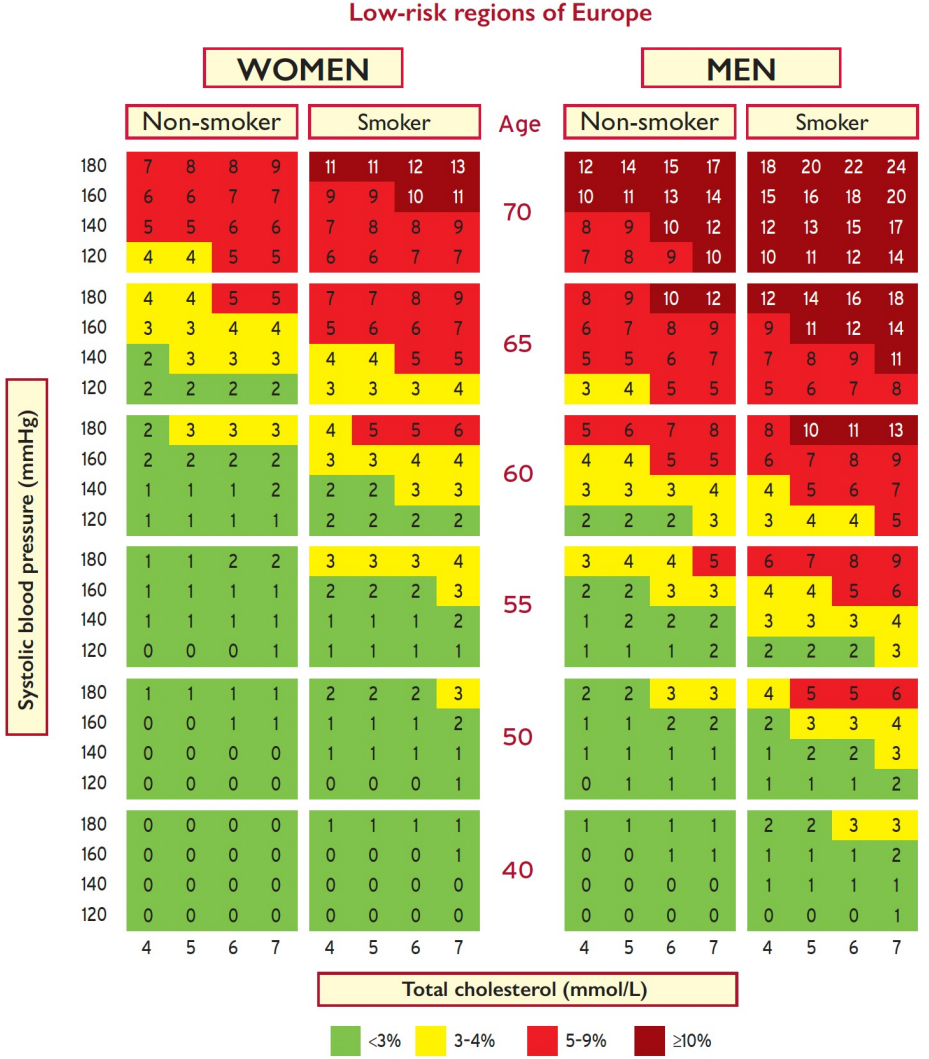
Define the cardiovascular Risk SCORE

The **high-risk charts** should be considered for use in Albania, Algeria, Armenia, Bosnia and Herzegovina, Croatia, Czech Republic, Estonia, Hungary, Latvia, Lebanon, Libya, Lithuania, Montenegro, Morocco, Poland, Romania, Serbia, Slovakia, Tunisia, and Turkey.

SCORE Cardiovascular Risk Chart
10-year risk of fatal CVD

The **low-risk charts** should be considered for use in Austria, Belgium, Cyprus, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Israel, Italy, Luxembourg, Netherlands, Norway, Malta, Portugal, Slovenia, Spain, Sweden, Switzerland, and the UK.





L'hypercholestérolémie familiale: les mutations du LDLr

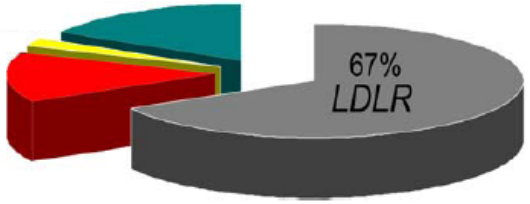
	FH1
chromosome	19p13
defect	LDL ⇧
gene	*LDLR*

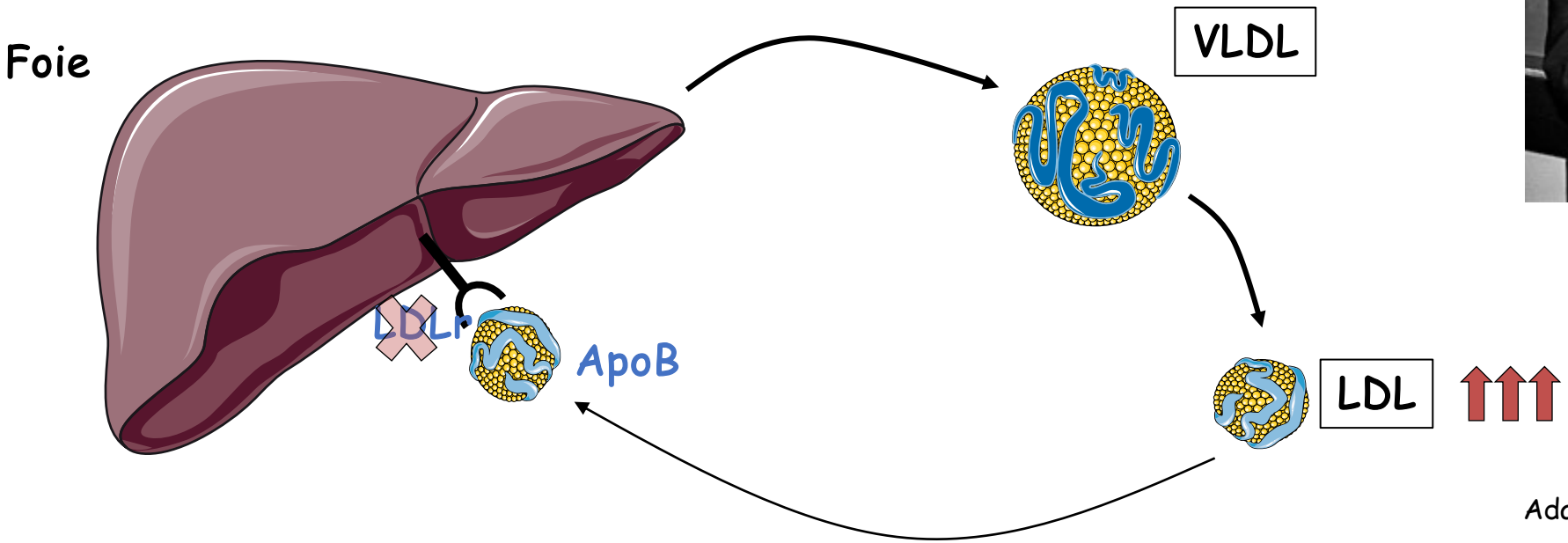
LDLR

Prévalence

+/- 1/300

-/- 1/ 9x10^5





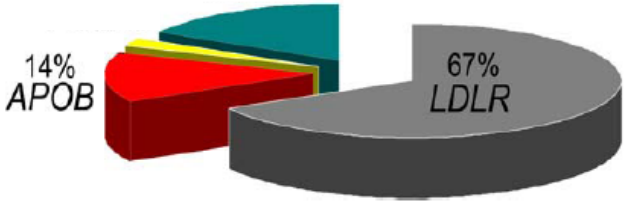
Brown m. **Goldstein J.**

Nobel Prize 1985

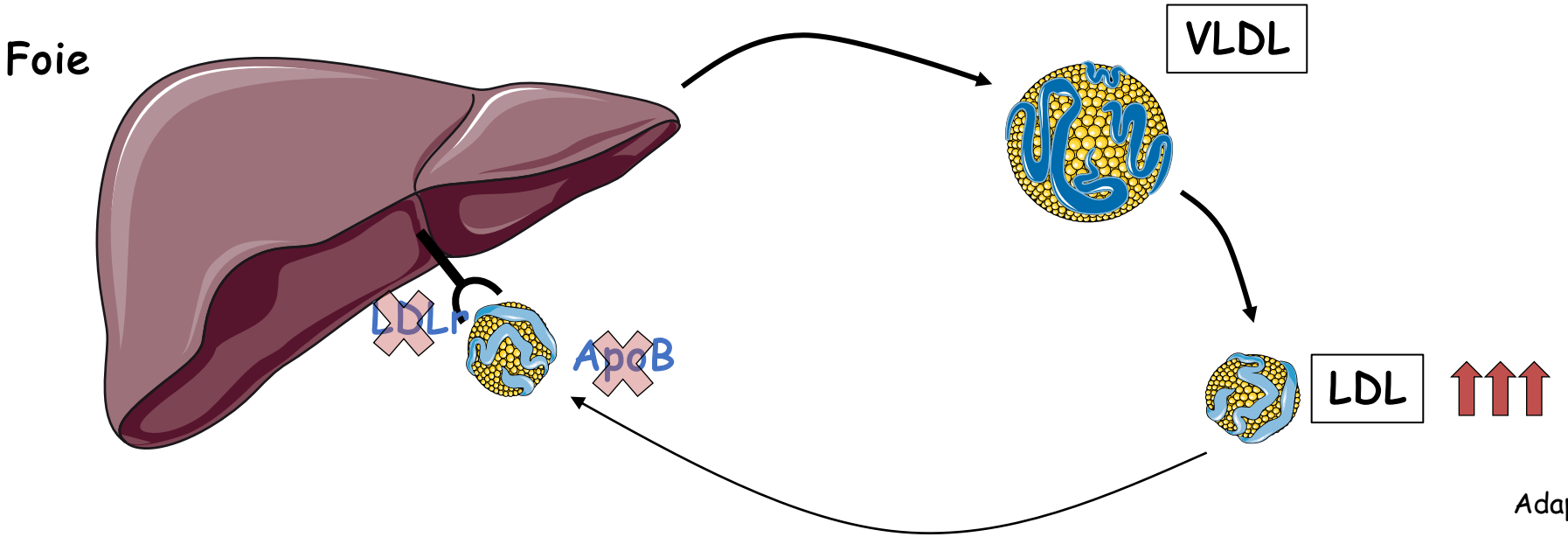
Adapté de NG Seidah et al. J. Mol. Med. 2007

L'hypercholestérolémie familiale: les mutations de l'ApoB

	FH1	FH2
chromosome	19p13	2p23-24
defect	LDL ⇧	LDL ⇧
gene	*LDLR*	*APOB*



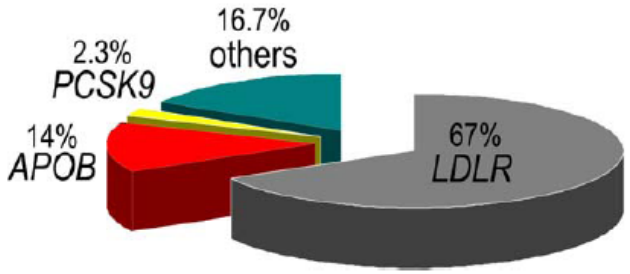
	Prévalence	
LDLR	+/- 1/300	-/- 1/ 9x10^5
ApoB	+/- 1/1000	-/- 1/ 4x10^6



Adapté de NG Seidah et al. J. Mol. Med. 2007

L'hypercholestérolémie familiale: les mutations de PCSK9

	FH1	FH2	FH3...
chromosome	19p13	2p23-24	1p32
defect	LDL ↑	LDL ↑	LDL ↑
gene	*LDLR*	*APOB*	*PCSK9*

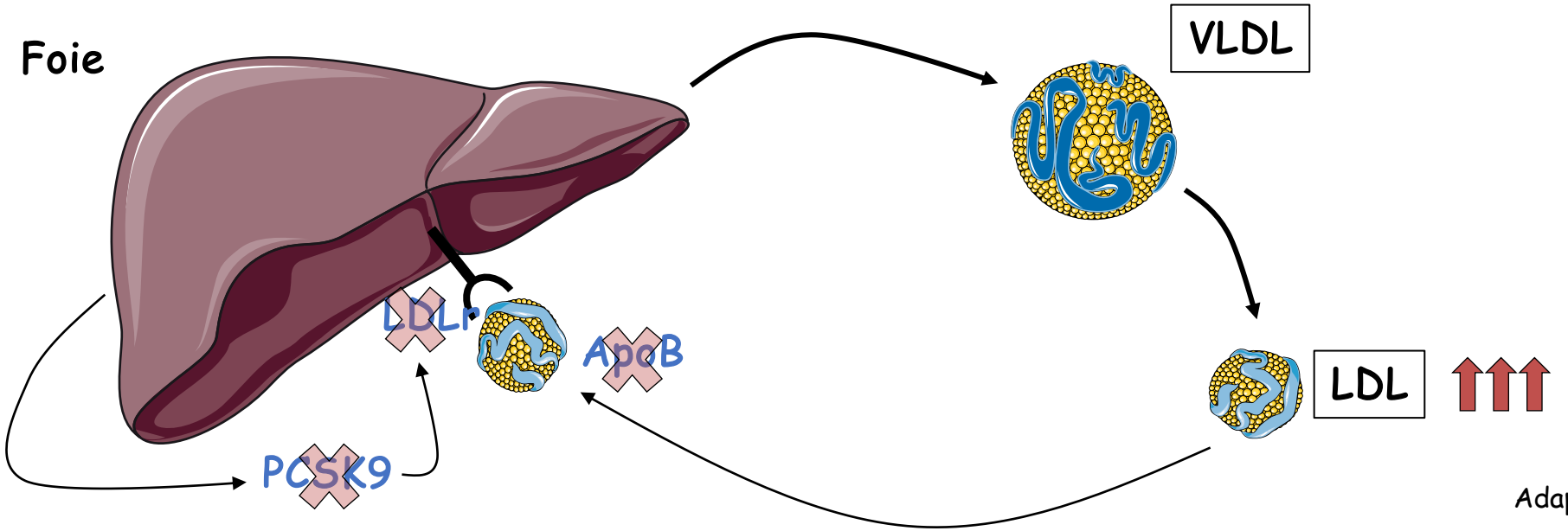


Prévalence

LDLR	+/- 1/300	-/- 1/ 9x10^5
ApoB	+/- 1/1000	-/- 1/ 4x10^6
PCSK9	+/- <1/2500	

Seidah N.

Boileau C.



Adapté de NG Seidah et al. J. Mol. Med. 2007

PCSK9: an example of fast-track research

Mutations in *PCSK9* cause autosomal dominant hypercholesterolemia

Marianne Abifadel[1,2], Mathilde Varret[1], Jean-Pierre Rabès[1,3], Delphine Allard[1], Khadija Ouguerram[4], Martine Devillers[1], Corinne Cruaud[5], Suzanne Benjannet[6], Louise Wickham[6], Danièle Erlich[1], Aurélie Derré[1], Ludovic Villéger[1], Michel Farnier[7], Isabel Beucler[8], Eric Bruckert[9], Jean Chambaz[10], Bernard Chanu[11], Jean-Michel Lecerf[12], Gerald Luc[12], Philippe Moulin[13], Jean Weissenbach[5], Annick Prat[6], Michel Krempf[4], Claudine Junien[1,3], Nabil G Seidah[6] & Catherine Boileau[1,3]

Nature Genetics 2003

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The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Sequence Variations in *PCSK9*, Low LDL, and Protection against Coronary Heart Disease

Jonathan C. Cohen, Ph.D., Eric Boerwinkle, Ph.D., Thomas H. Mosley, Jr., Ph.D., and Helen H. Hobbs, M.D.

Nature Genetics 2003 → **NEJM 2006**

The genetic proof of concept for PCSK9 inhibition

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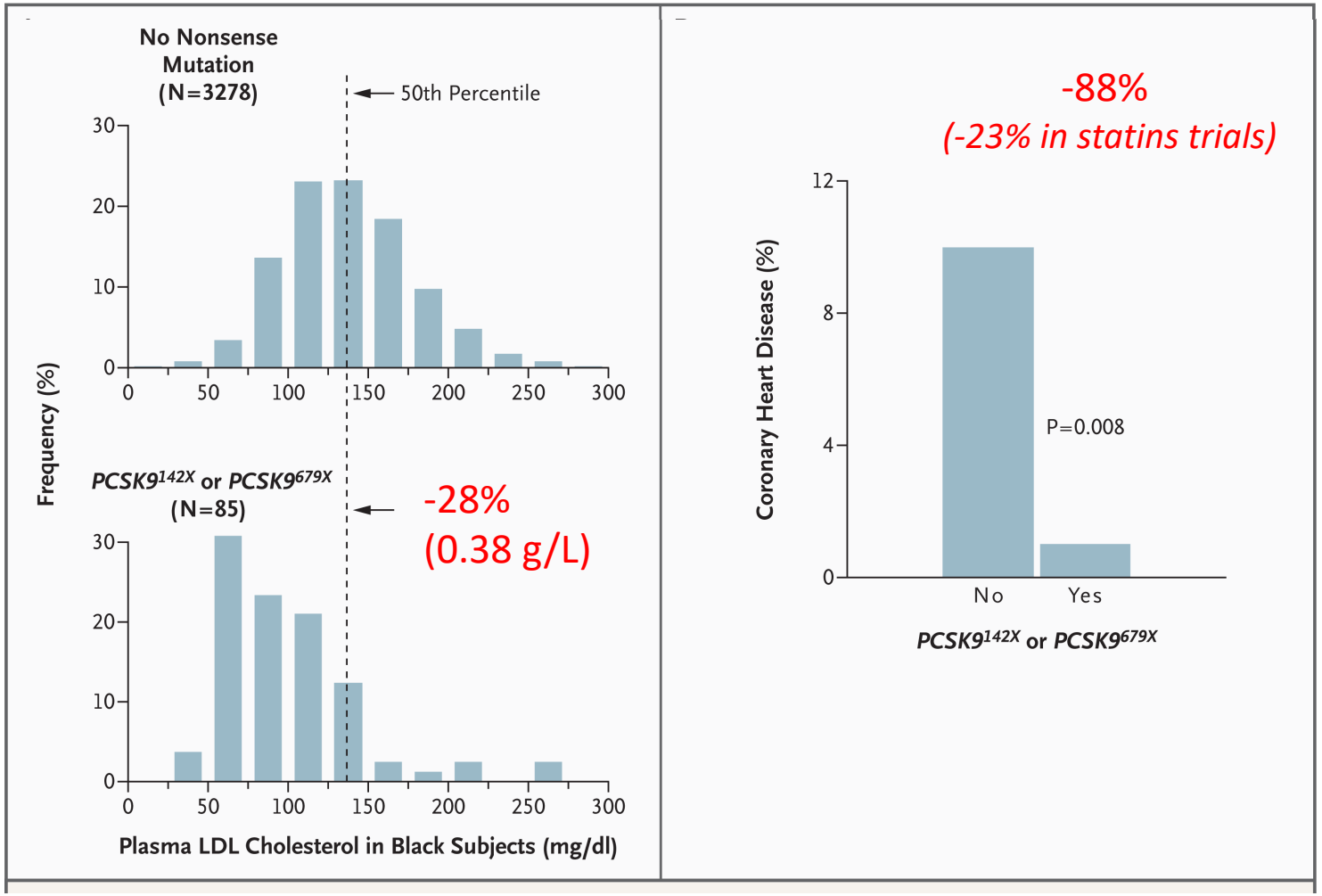
2006. NEJM Volume 354 (12):1264-1272



Of the 3363 black subjects examined, 2.6 percent had nonsense mutations in *PCSK9*; these mutations were associated with a 28 percent reduction in mean LDL cholesterol and an 88 percent reduction in the risk of CHD.

PCSK9: an example of fast-track research

Mutations in *PCSK9* cause autosomal dominant hypercholesterolemia

Marianne Abifadel[1,2], Mathilde Varret[1], Jean-Pierre Rabès[1,3], Delphine Allard[1], Khadija Ouguerram[4], Martine Devillers[1], Corinne Cruaud[5], Suzanne Benjannet[6], Louise Wickham[6], Danièle Erlich[1], Aurélie Derré[1], Ludovic Villéger[1], Michel Farnier[7], Isabel Beucler[8], Eric Bruckert[9], Jean Chambaz[10], Bernard Chanu[11], Jean-Michel Lecerf[12], Gerald Luc[12], Philippe Moulin[13], Jean Weissenbach[5], Annick Prat[6], Michel Krempf[4], Claudine Junien[1,3], Nabil G Seidah[6] & Catherine Boileau[1,3]

Nature Genetics 2003

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NEJM 2006

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Effect of a Monoclonal Antibody to PCSK9 on LDL Cholesterol

Evan A. Stein, M.D., Ph.D., Scott Mellis, M.D., Ph.D., George D. Yancopoulos, M.D., Ph.D., Neil Stahl, Ph.D., Douglas Logan, M.D., William B. Smith, M.D., Eleanor Lisbon, M.D., M.P.H., Maria Gutierrez, M.D., Cheryle Webb, M.D., Richard Wu, Ph.D., Yunling Du, Ph.D., Therese Kranz, R.N., M.B.A., Evelyn Gasparino, B.S., and Gary D. Swergold, M.D., Ph.D.

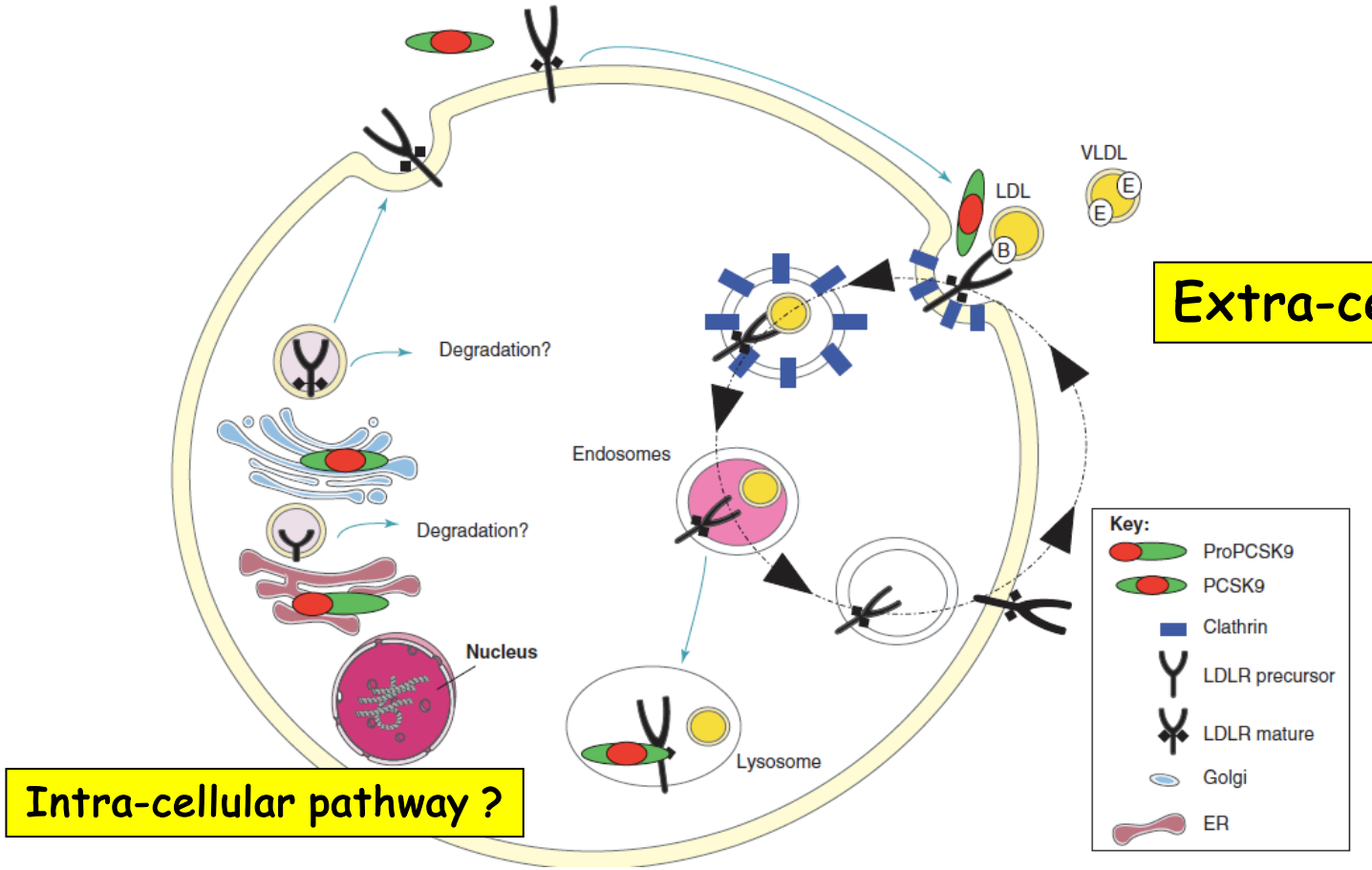
NEJM 2012

PCSK9 : mode of action

↑ PCSK9

↓ LDL-R

↑ LDL-C

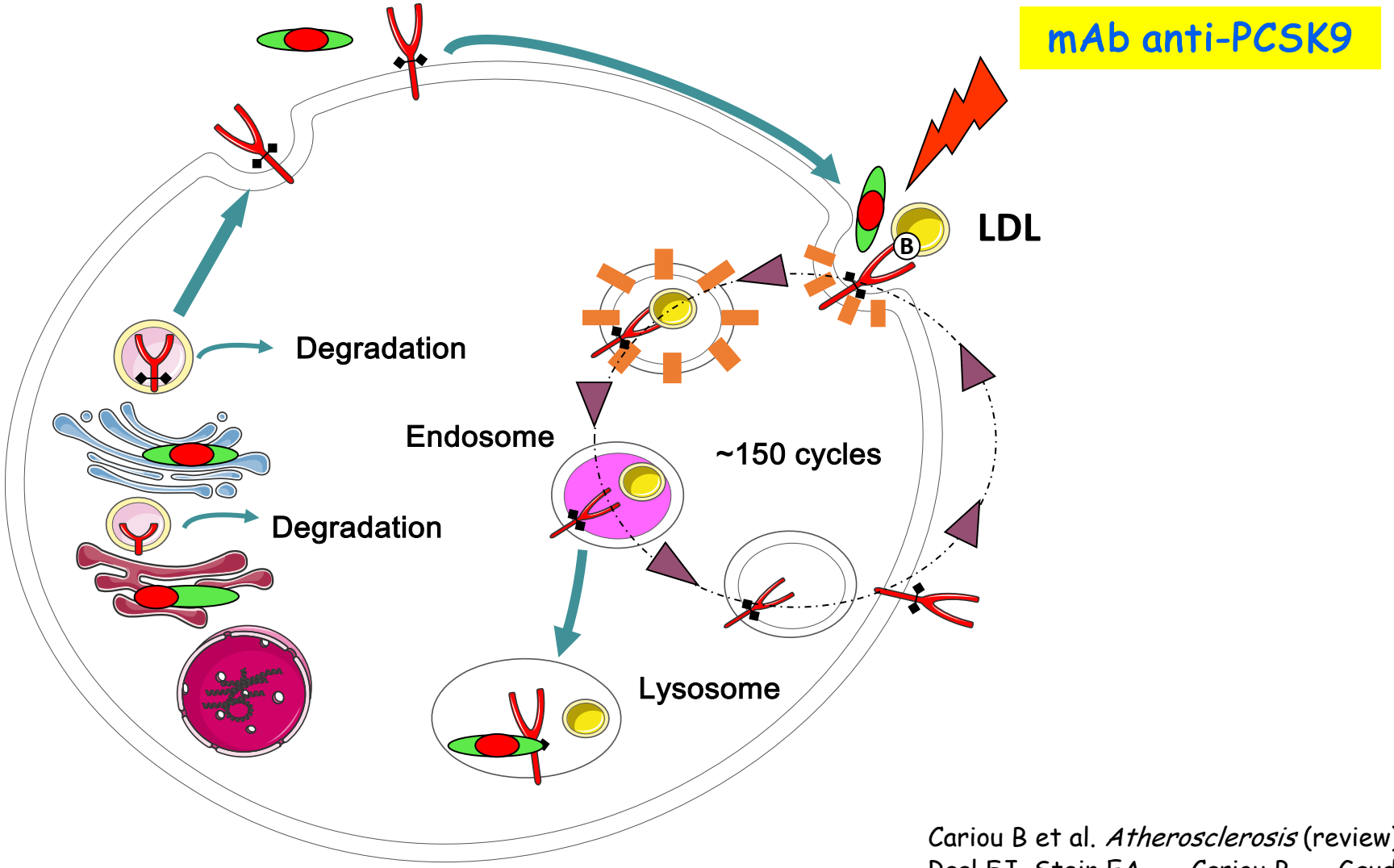


Extra-cellular pathway

Intra-cellular pathway ?

Costet P., Krempf M., Cariou B. *TIBS* 2008, 33: 426.

Strategies for PCSK9 inhibition



Cariou B et al. *Atherosclerosis* (review) 2011
Raal FJ, Stein EA, …, Cariou B, …, Gaudet D. *Lancet* 2015
Cannon CP, Cariou B, et al. *Eur Heart J* 2016
Colhoun HM, …., Cariou B,…Eckel RH. *Eur Heart J* 2016
Leiter LA, Cariou B, et al. *Diabetes Obes Metab 2017*
Leiter LA, …, Cariou B. *Diabet Med* 2018

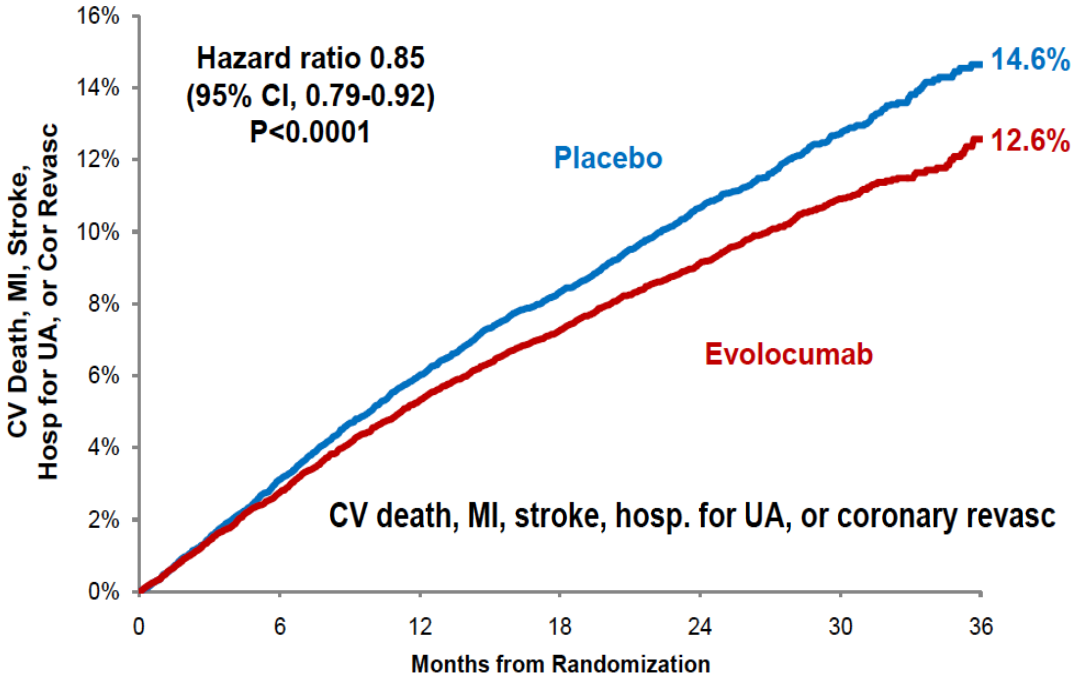
Efficacy of PCSK9 mAb on lipid parameters

PCSK9 Inhibitors	Doses	Other lipid-lowering treatment	% Change from baseline to end of follow-up beyond that with control*						
			Δ Total cholesterol	Δ LDL-C	Δ HDL-C	Δ Triglycerides	Δ ApoB	Δ ApoA-1	Δ Lipoprotein(a)
Alirocumab[57-59]	150 mg every 2 weeks	± Statins (± ezetimibe)	-35 to -44	-57 to -67	6 to 10	-6 to -29‡	-44 to -58	14 [1‡]	-19 [-29‡]
Evolocumab[510-514]	420 mg every 4 weeks and 140 mg every 2 weeks	± Statins (± ezetimibe)	-33 to -42	-50 to -66	4 to 9	-6 to -34	-42 to -56	0 to 4	-18 to -32

Ray KK & Vallejo-Vaz AJ. *Lancet* 2015 ; 386: 412-14

Efficacy of PCSK9 mAB on Cardiovascular (CV) events

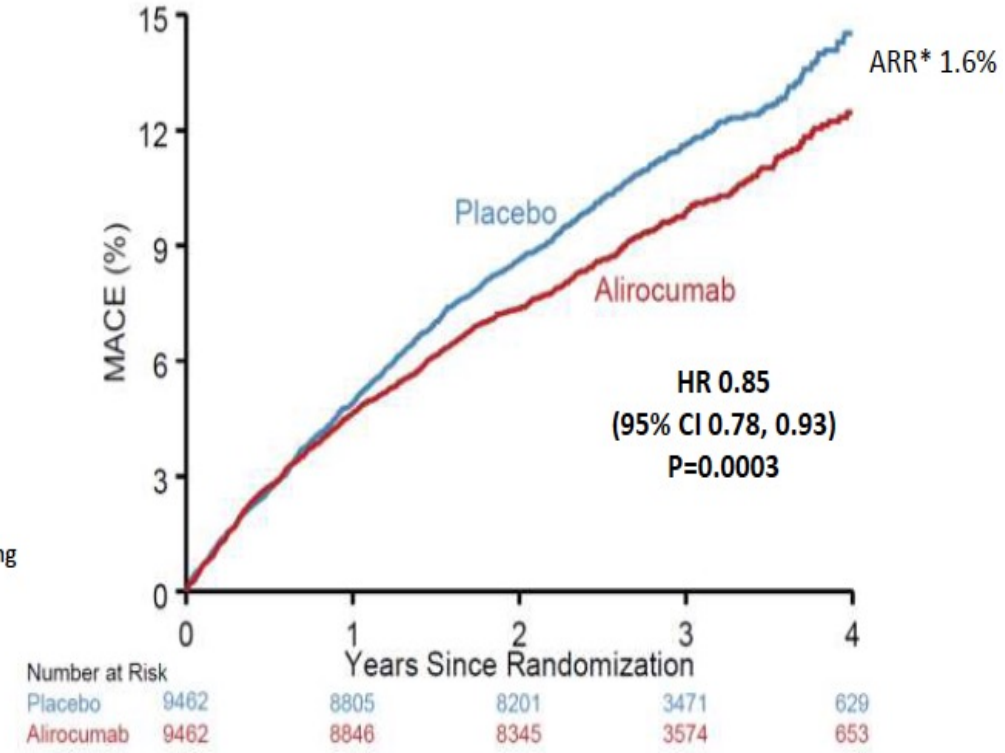
FOURIER
Primary outcome



Sabatine MS et al. *N Engl J Med* 2017, 376: 1713

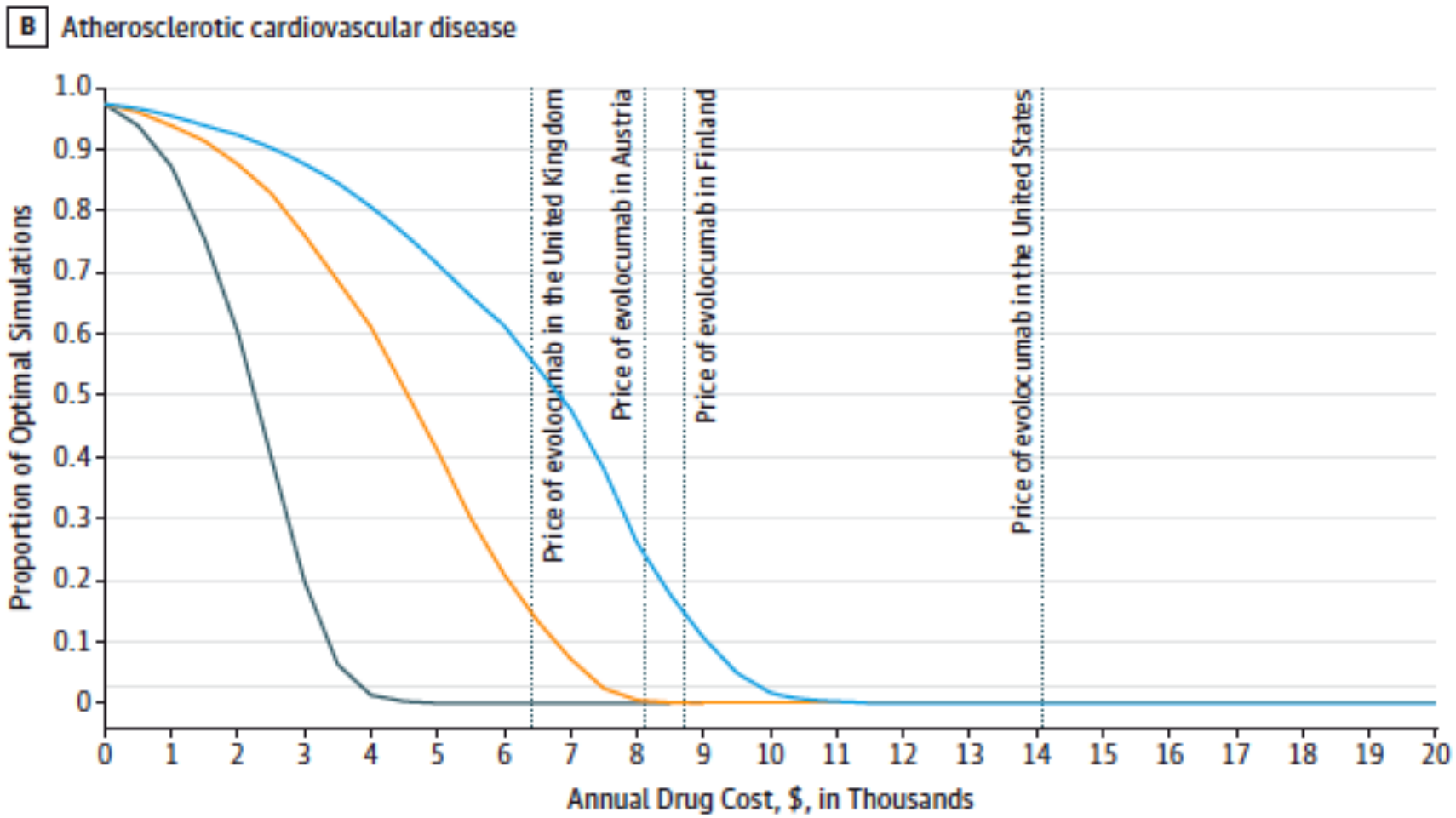
ODYSSEY OUTCOMES
Primary outcome

MACE: CHD death, non-fatal MI, ischemic stroke, or unstable angina requiring hospitalization



Steg PG. Communication orale ACC 2018

The cost-efficacy of PCSK9 inhibitors is questionnable

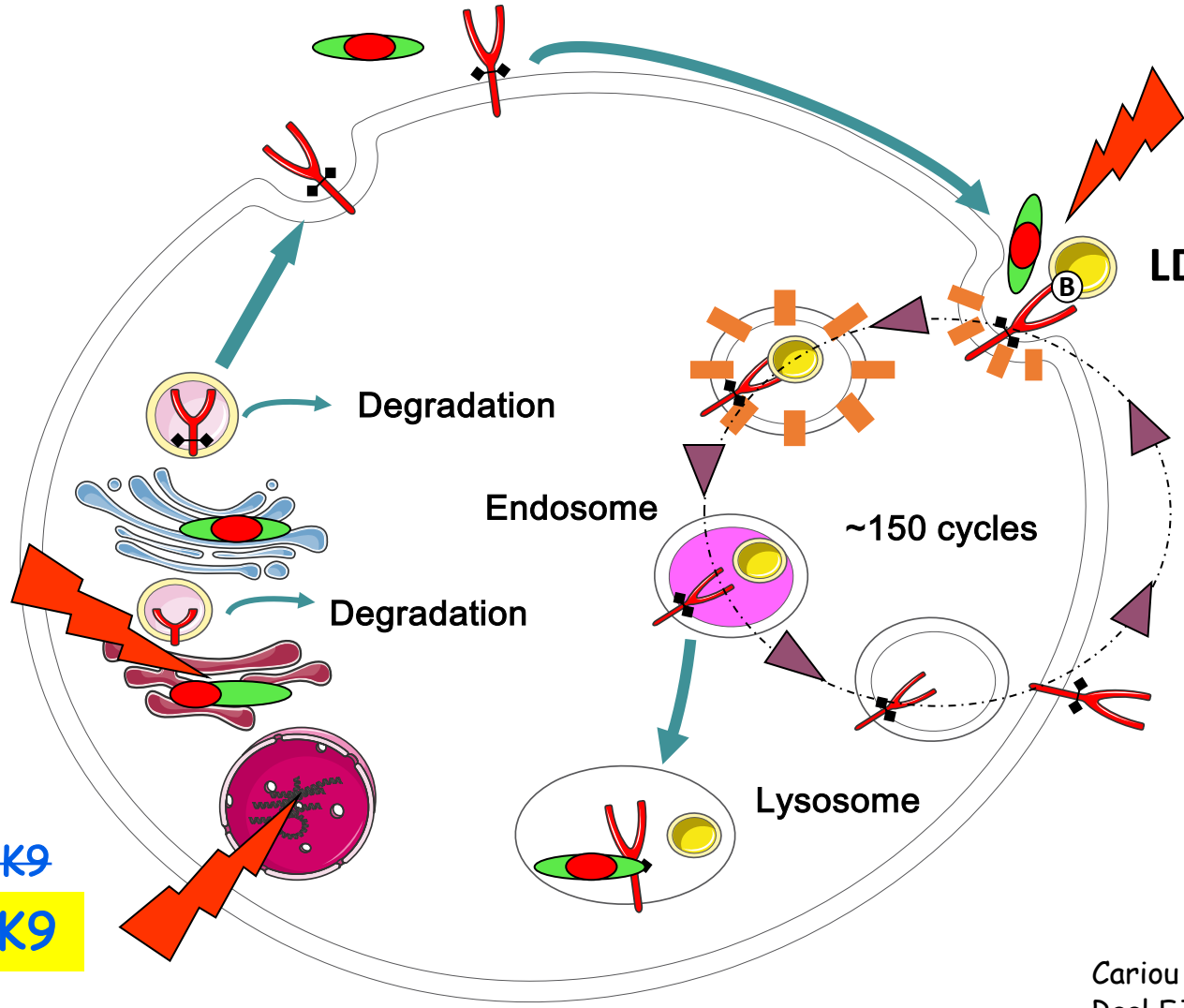


⇒ **Reduce the prize to $14 000 /yr to ≈ $4500 yr** in US to meet $100 000/QALY

⇒ Need for a PERSONALIZED approach +++

Khazi D. et al. JAMA 2016; 316: 743-53

Strategies for PCSK9 inhibition



mAb anti-PCSK9

Adnectins (lerodalcibep)
Vaccines
Oral PCSK9 inhibitor

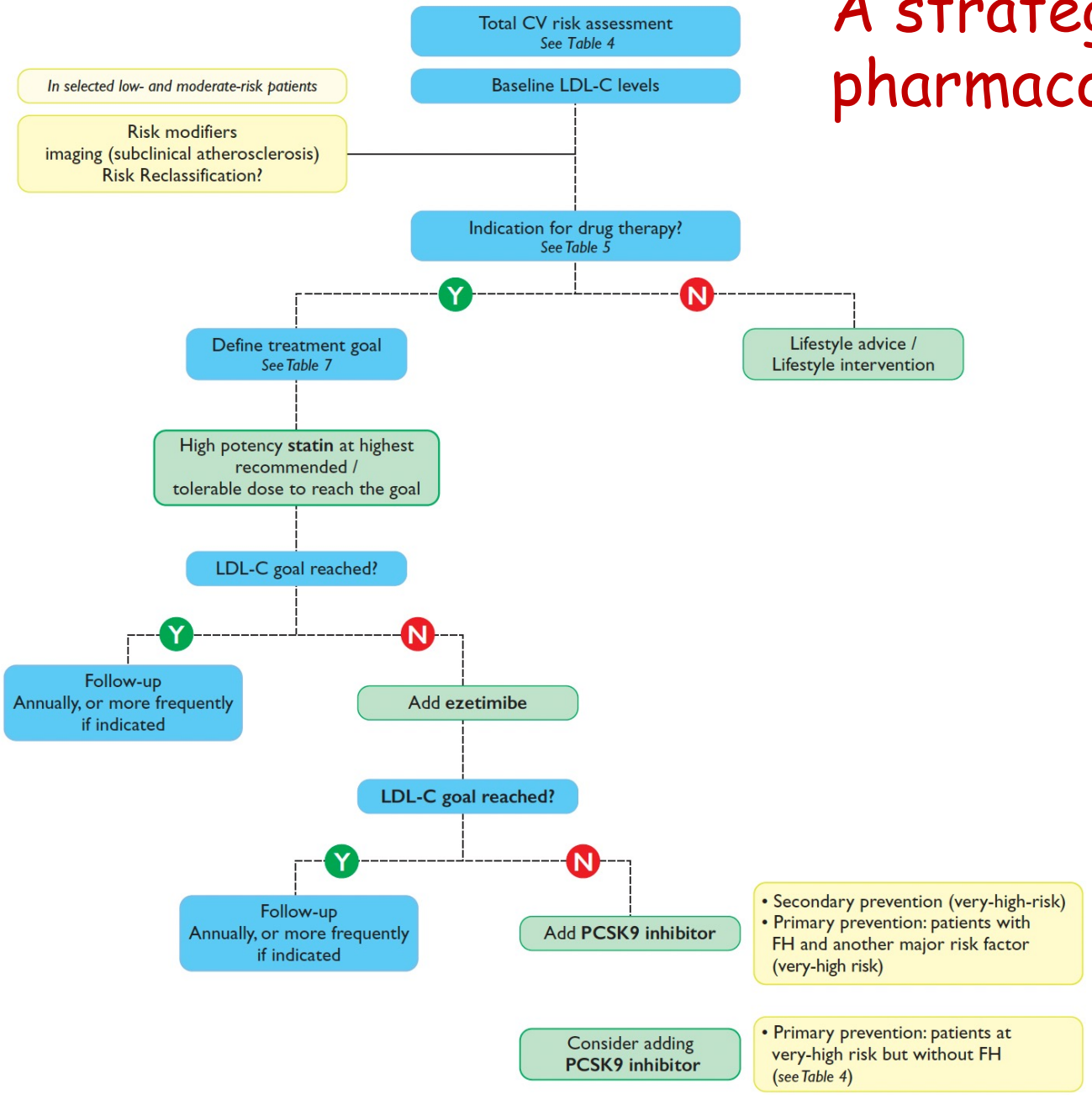
~~Anti-sense anti-PCSK9~~

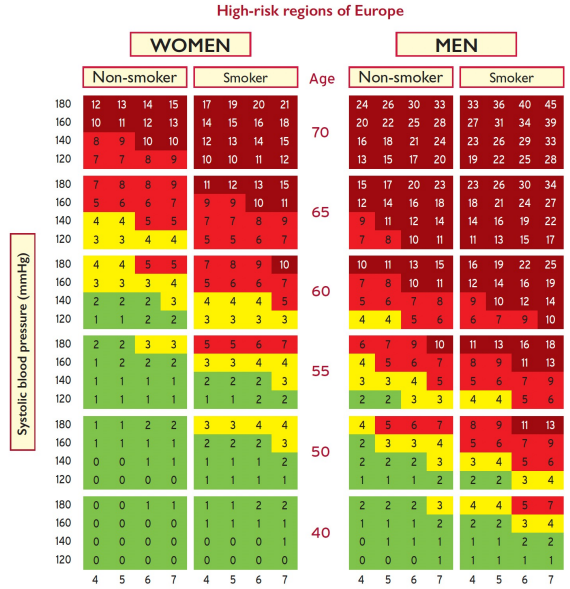
siRNA anti-PCSK9

CRISPR-Cas9: in vivo genomic edition

Cariou B et al. *Atherosclerosis* (review) 2011
Raal FJ, Stein EA, ..., Cariou B, ..., Gaudet D. *Lancet* 2015
Cannon CP, Cariou B, et al. *Eur Heart J* 2016
Colhoun HM, ..., Cariou B,...Eckel RH. *Eur Heart J* 2016
Leiter LA, Cariou B, et al. *Diabetes Obes Metab 2017*
Leiter LA, ..., Cariou B. *Diabet Med* 2018

A strategy for achieving optimal pharmacological reduction in LDLc levels.

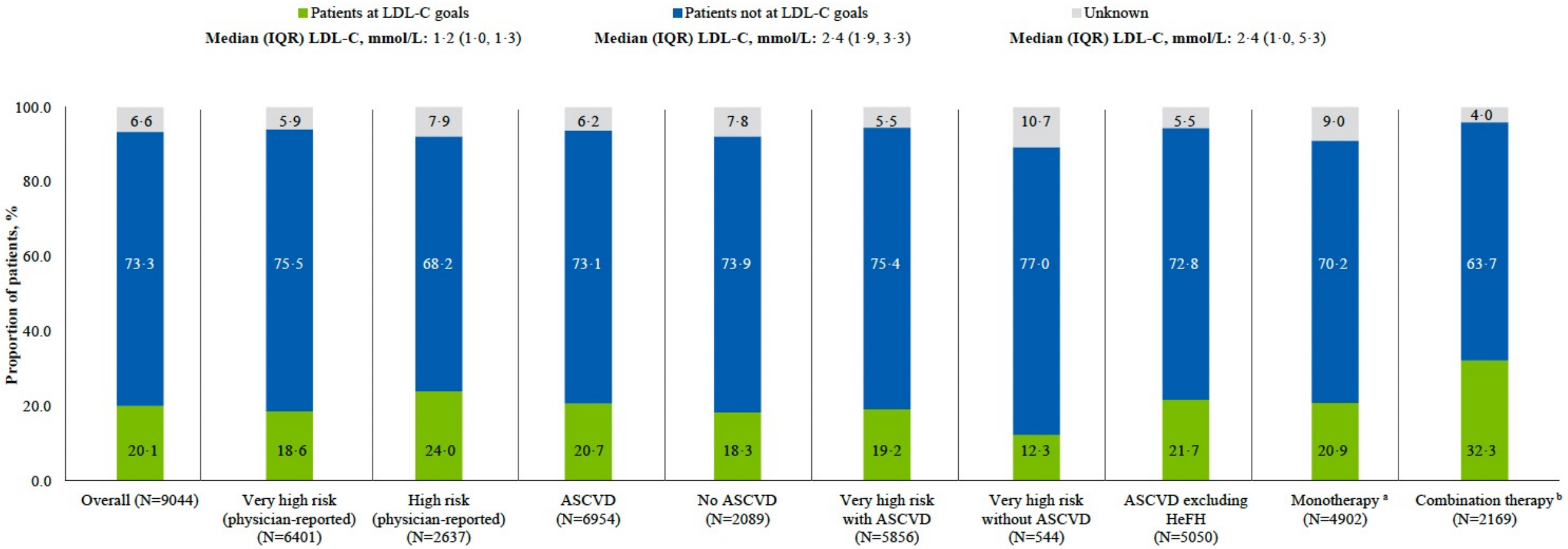




Intensity of lipid lowering treatment

Treatment	Average LDL-C reduction
Moderate intensity statin	≈ 30%
High intensity statin	≈ 50%
High intensity statin plus ezetimibe	≈ 65%
PCSK9 inhibitor	≈ 60%
PCSK9 inhibitor plus high intensity statin	≈ 75%
PCSK9 inhibitor plus high intensity statin plus ezetimibe	≈ 85%

Despite the availability of effective new lipid-lowering drugs, numerous patients remain unable to attain the recommended LDL-C target.



The Lancet Regional Health – Europe - 2023;29: 100624

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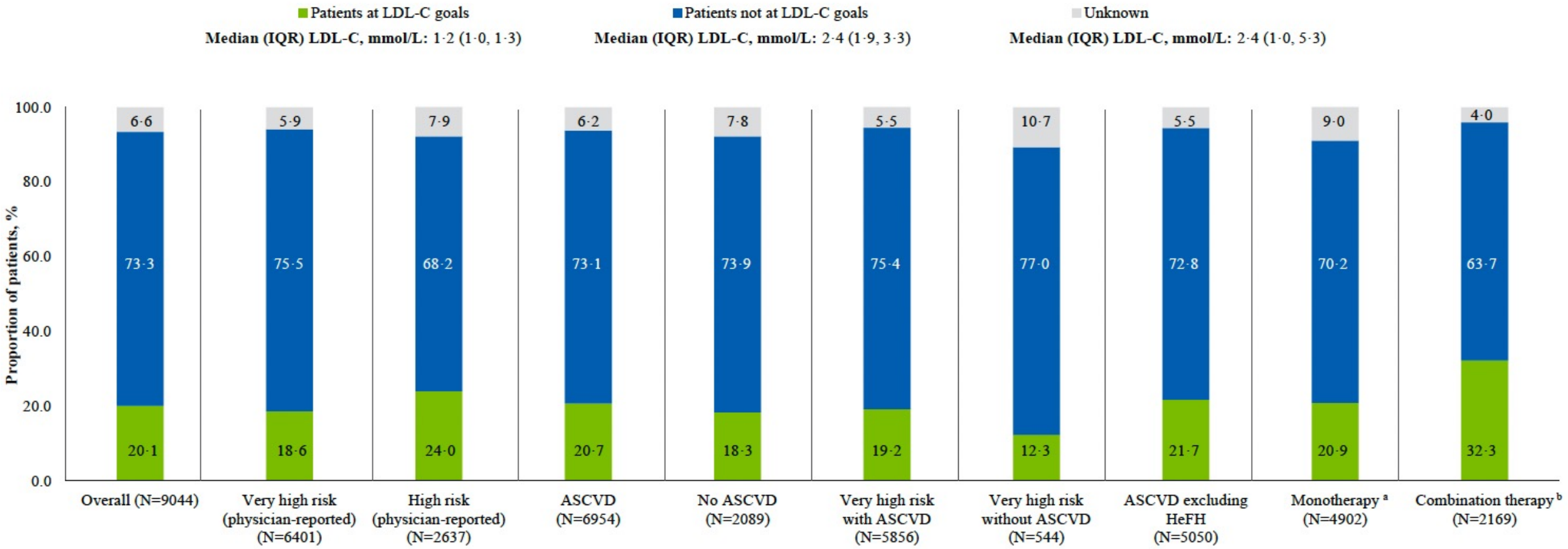
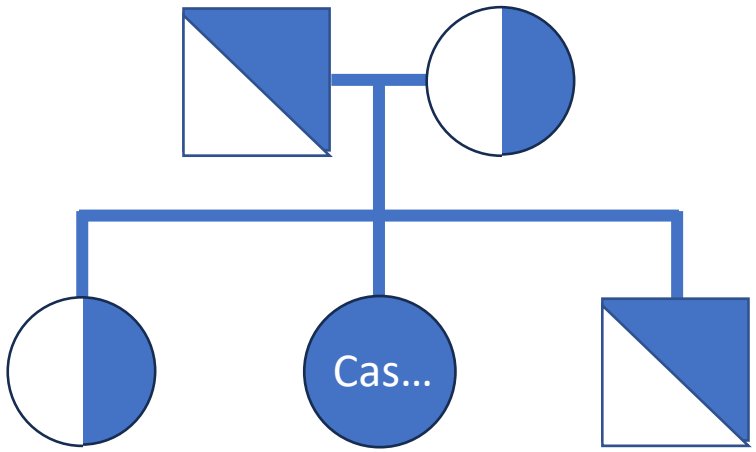


Significant proportion of patients fail to achieve LDL-c targets

Homozygous FH patients with no functional LDL Receptor

The Lancet Regional Health – Europe - 2023;29: 100624

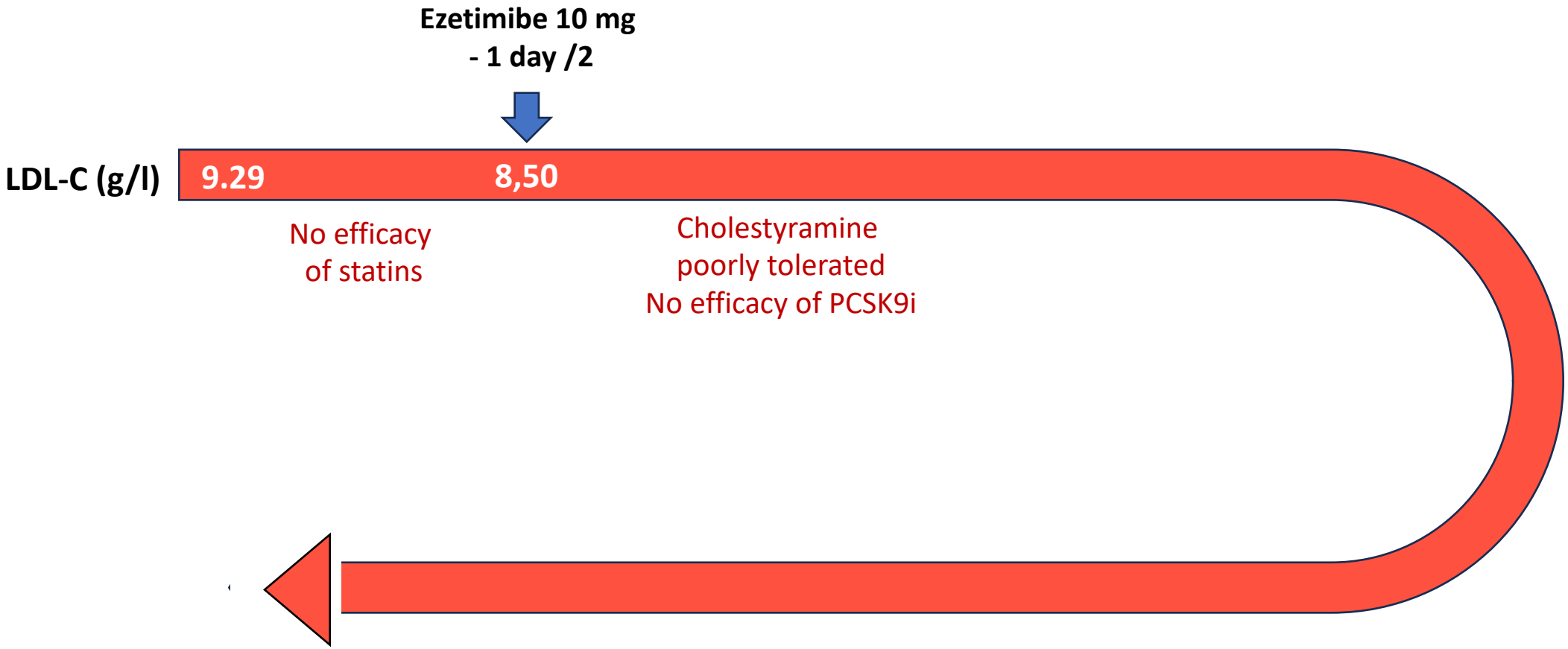
Illustrating the difficulty of treating Homozygous FH (HoFH) patients effectively



An extremely severe case of homozygous familial hypercholesterolemia

- Discovery of HoFH in a young girl at the age of 8 as part of a familial screening
- TC: 9,70 g/L, **LDL-C: 9,29 g/L**, TG: 0, 88 g/L, HDL-C: 0,23 g/l, Lp(a): 0,66 g/L
- Homozygous null mutation in exon 3 of the LDLR gene = STOP codon
- Coronary artery bypass (anterior & posterior ventricula arteries + marginal)
- Clinical **angina => occlusion of 2/3 bypasses**
- **50% stenosis of the external left carotid artery and 50% stenosis of the superior mesenteric artery**

Illustrating the difficulty of treating Homozygous FH (HoFH) patients effectively



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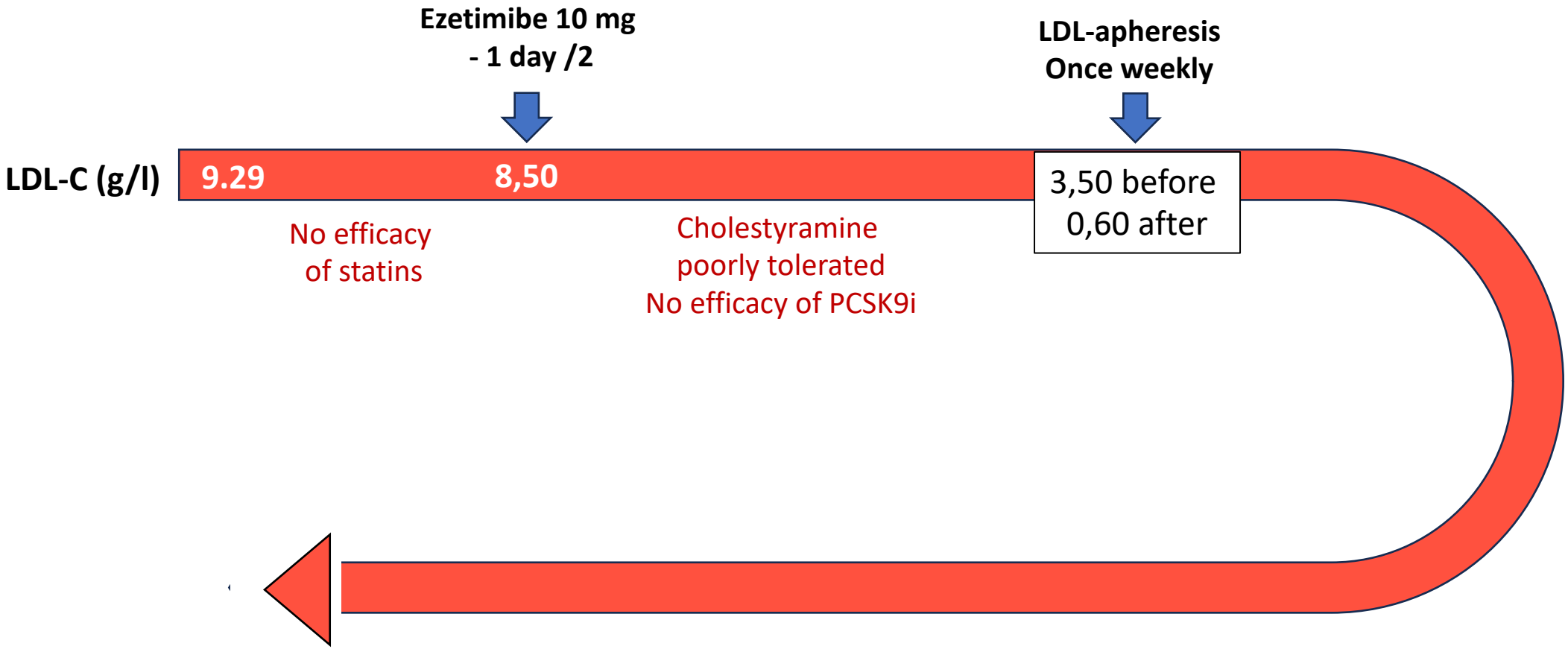

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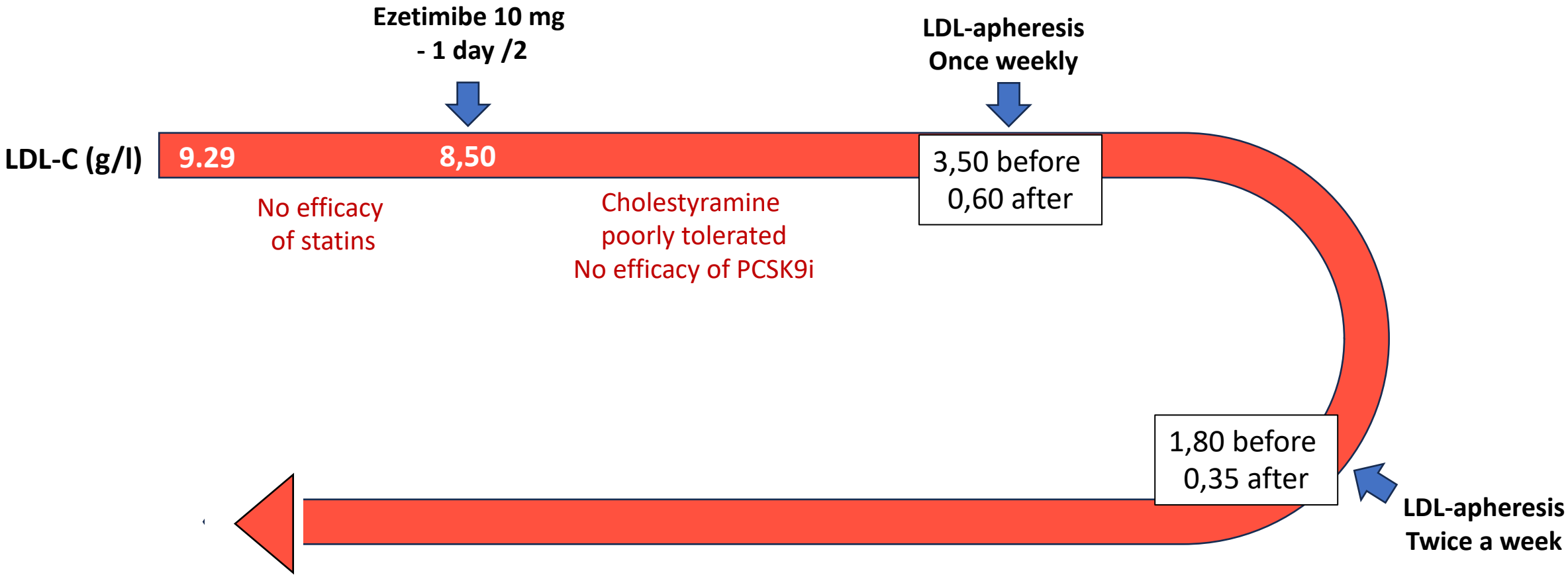

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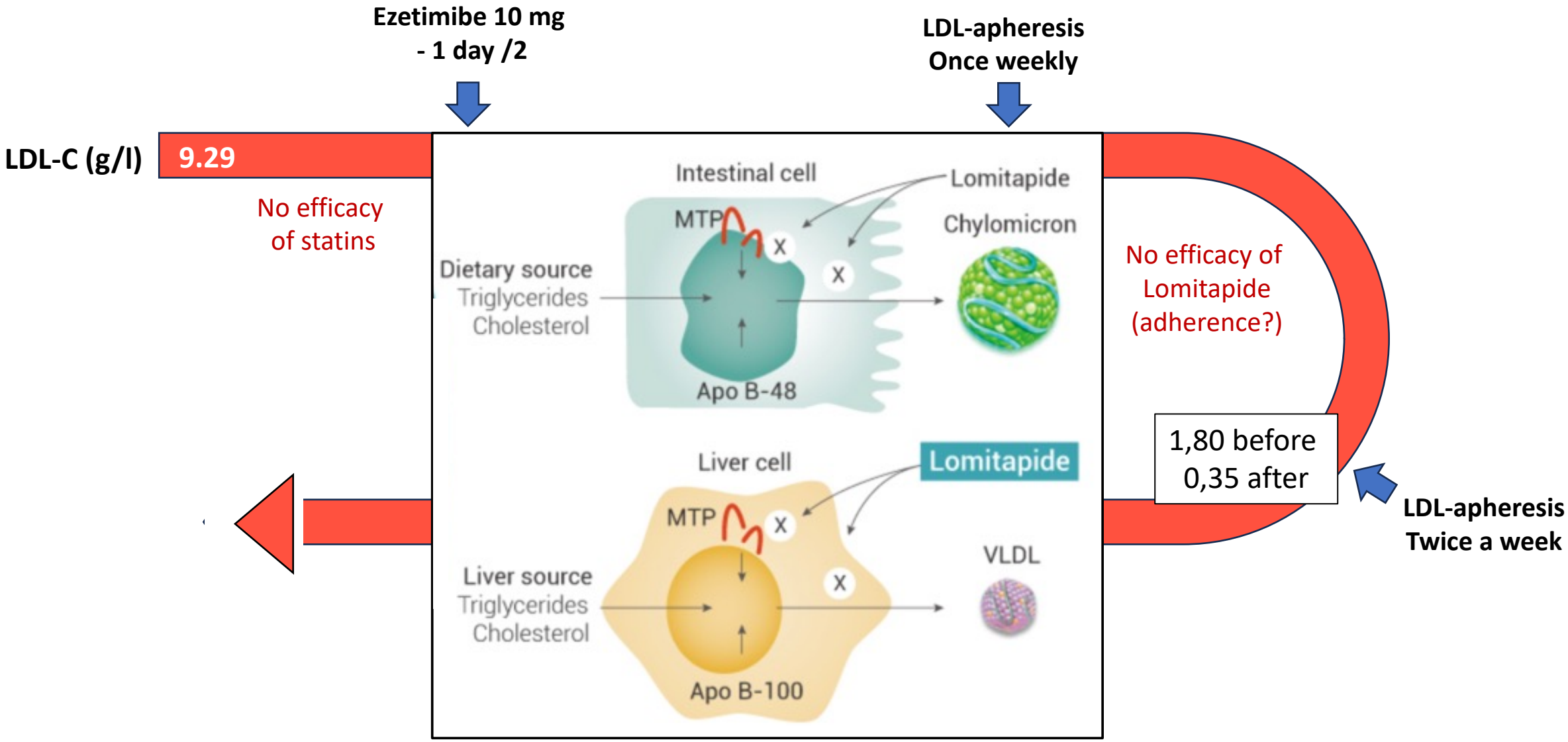
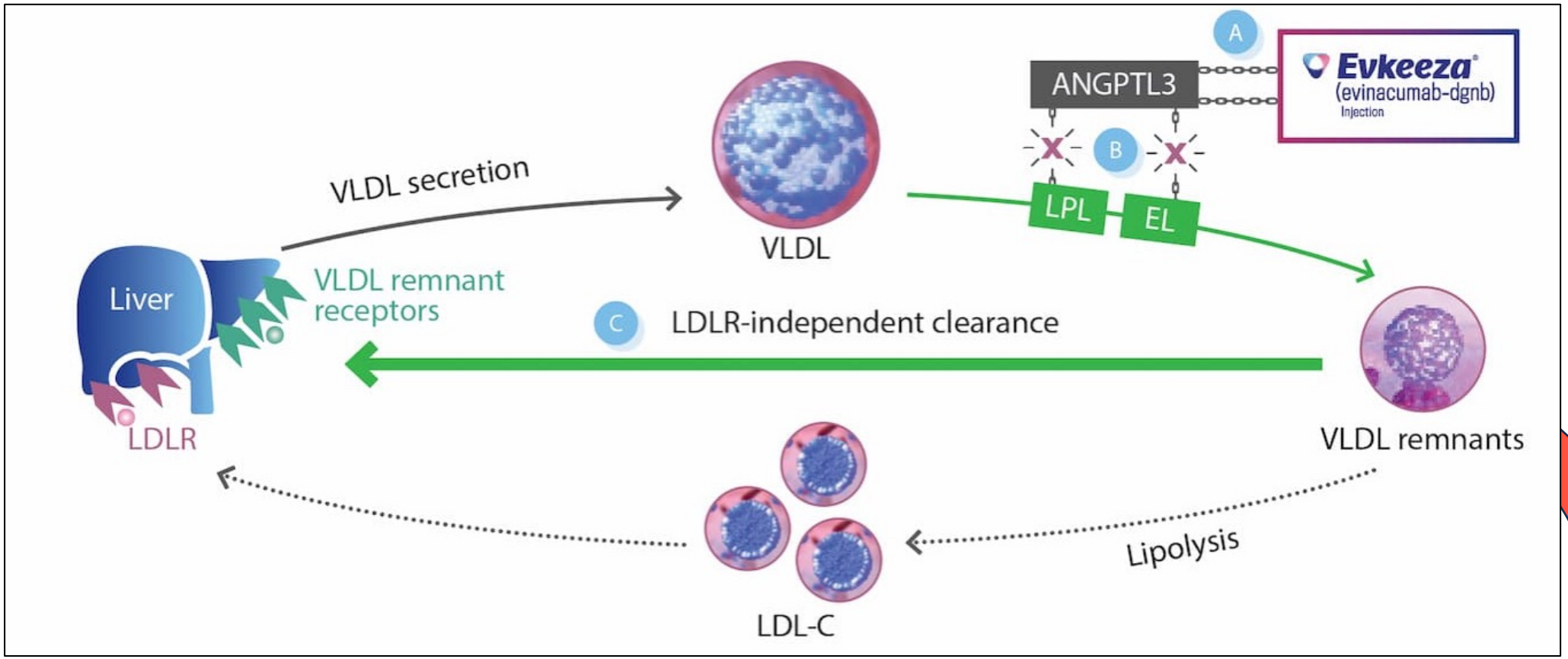




Evinacumab
1 infusion/month

1,80 before
0,35 after

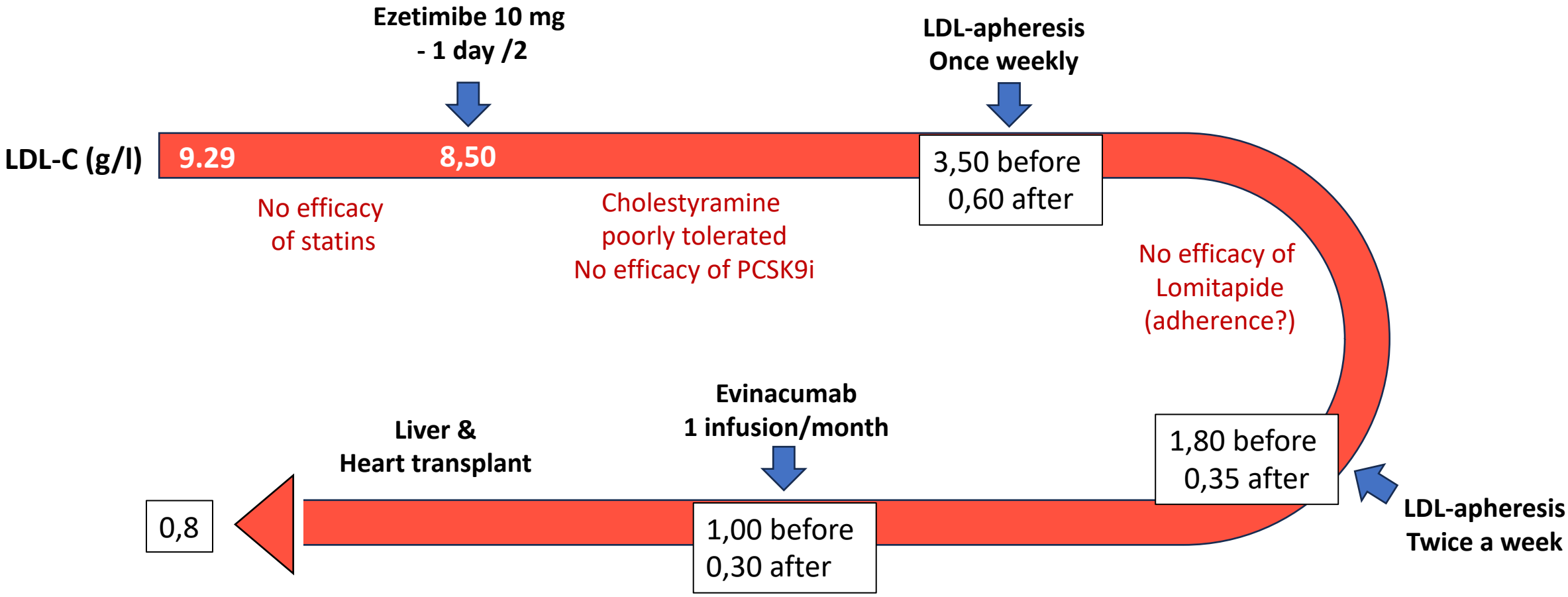
1,00 before
0,30 after

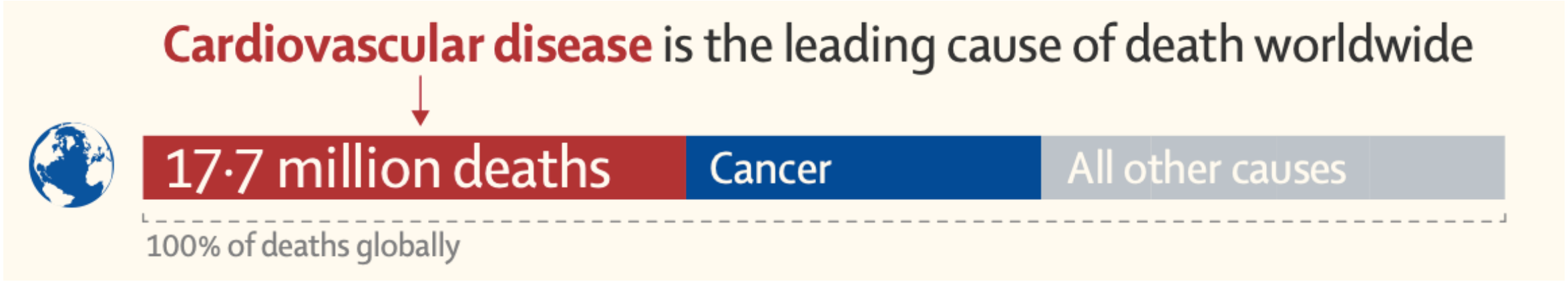
LDL-apheresis
Twice a week

Illustrating the difficulty of treating Homozygous FH (HoFH) patients effectively

- **Last treatment combination**: LDL apheresis twice a week + ezetimibe + evinacumab + lomitapide
- Very poor quality of life
- Very high cardiovascular risk with clinical angina when heart beats > 100-120 /min
- Collegial decision of **combined Heart +Liver transplantation** (AP-HP Necker, PARIS)

Illustrating the difficulty of treating Homozygous FH (HoFH) patients effectively





Dyslipidemia, including elevated LDL-C & TGRLs, is a major and modifiable risk factor

Significant proportion of patients fail to achieve LDL targets

Homozygous FH patients with no functional LDL Receptor

Need for new efficient therapeutic target

Identify new targets

GLOBAL SCIENTIFIC STRATEGY

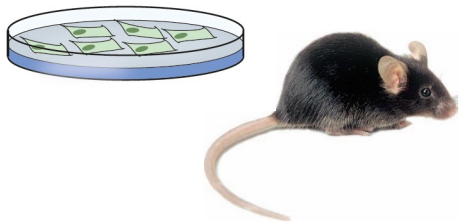
LIPID RELATED CARDIOMETABOLIC DISEASES

PATIENTS & POPULATION BASED COHORTS

GENETICS & FUNCTIONAL GENOMICS

FUNCTIONAL CARACTERIZATION

Advanced in-vitro / vivo models



BIOLOGICAL INSIGHTS & POTENTIAL DRUG TARGETS

chopin

Identify new targets

GLOBAL SCIENTIFIC STRATEGY

LIPID RELATED CARDIOMETABOLIC DISEASES

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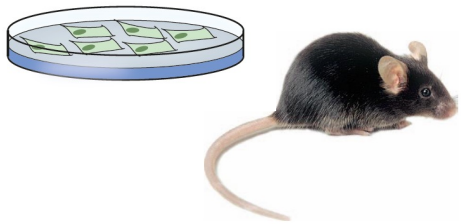

BIOLOGICAL INSIGHTS & POTENTIAL DRUG TARGETS

HYPOCHOL Cohort

Patients with very low plasma LDL-c levels

chopin

Identification of a french patient with combined hypocholesterolemia

Ph. Moulin
M. Di Filippo

Hospices Civils de Lyon

- **A1 - propositus**

Man, 61 years
Acute coronary syndrom with atherosclerotic plaques; 3 stents
Type 2 diabetes

	A1
	73 y
	Propositus
TG (0.35-1.50 g/L)	1.32
Chol (1.35-2.70 g/L)	1.06
LDL (0.75-1.60 g/L)	0.58
HDL (>0.40 g/L)	0.21
ApoA1 (1.05-2.05 g/L)	0.56
ApoB (0.55-1.15 g/L)	0.78
Lpa (<0.300g/L)	NA
Phospholipids (1.8 - 2.3 mmol/L)	1.35
Cholesterol (mmol/L)	2.74
Ratio PL/Chol	0.49
*Esterification (60-80%) **	0.63

Identification of a french family with combined hypocholesterolemia


Ph. Moulin
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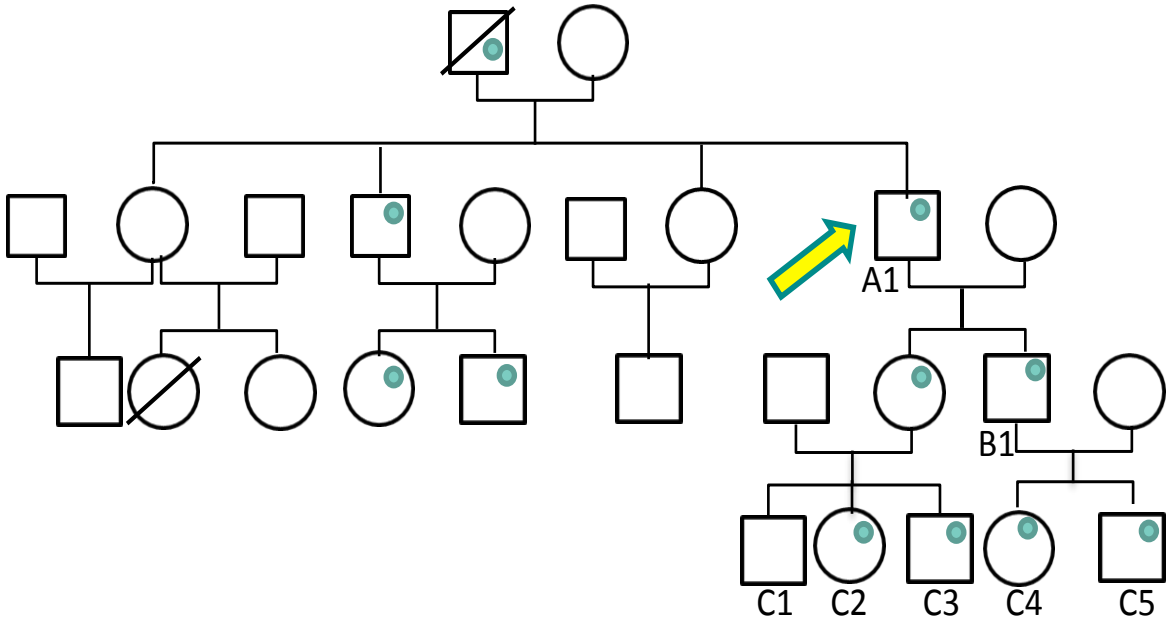


- **A1 - propositus**

Man, 72 years
Acute coronary syndrom with atherosclerotic plaques; 3 stents
Type 2 diabetes

	A1	A2	B1	C1	C2	C3	C4	C5
	73 y	75y	39 y	20 y	16 y	11 y	8 y	5 y
	Propositus		*		*	*	*	*
TG (0.35-1.50 g/L)	1.32	0.84	0.84	1.02	0.54	0.84	0.48	0.54
Chol (1.35-2.70 g/L)	1.06	1.92	0.80	1.39	0.46	0.82	0.53	0.53
LDL (0.75-1.60 g/L)	0.58	1.30	0.48	0.84	0.20	0.53	0.31	0.29
HDL (>0.40 g/L)	0.21	0.45	0.15	0.35	0.14	0.12	0.12	0.13
ApoA1 (1.05-2.05 g/L)	0.56		0.54		0.48	0.42	0.41	0.45
ApoB (0.55-1.15 g/L)	0.78	NA	0.61	0.78	0.35	0.61	0.40	0.41
Lpa (<0.300g/L)	NA	NA	NA	0.23	0.10	0.03	NA	NA
Phospholipids (1.8 - 2.3 mmol/L)	1.35		1.02		0.72	1.01	0.67	0.80
Cholesterol (mmol/L)	2.74		2.07		1.19	2.12	1.37	1.37
Ratio PL/Chol	0.49		0.49		0.60	0.48	0.49	0.58
*Esterification (60-80%) **	0.63		0.65		0.60	0.66	0.66	0.64

LIPC-E97G is a rare GOF variant involved in familial combined hypocholesterolemia

Circulation

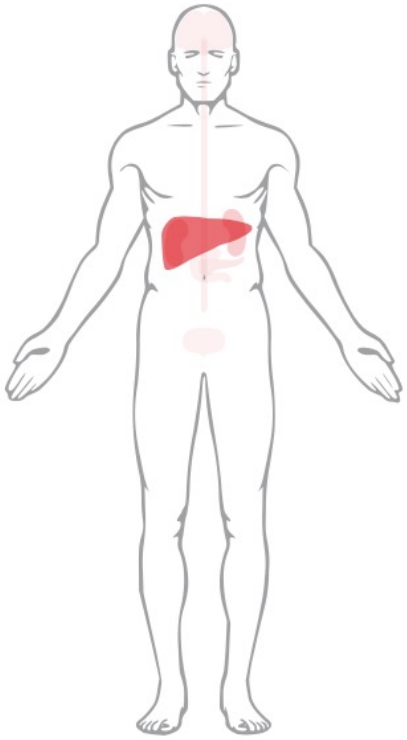
ORIGINAL RESEARCH ARTICLE

Identification of a Gain-of-Function *LIPC* Variant as a Novel Cause of Familial Combined Hypocholesterolemia

Wieneke Dijk, PhD*; Mathilde Di Filippo, PharmD, PhD*; Sander Kooijman, PhD; Robin van Eenige, MSc; Antoine Rimbert, PhD; Amandine Caillaud, PhD; Aurélie Thedrez, PhD; Lucie Arnaud; Amanda Pronk; Damien Garçon, PhD; Thibaud Sotin, MSc; Pierre Lindenbaum, PhD; Enrique Ozcariz Garcia; Jean-Paul Pais de Barros, PhD; Laurence Duvillard, PhD; Karim Si-Tayeb, PhD; Nuria Amigo, PhD; Jean-Yves Le Questel, PhD; Patrick C.N. Rensen, PhD; Cédric Le May, PhD†; Philippe Moulin, MD, PhD†; Bertrand Cariou, MD, PhD†

Dijk, Di Filippo et al., Circulation 2022

LIPC gene encodes hepatic lipase

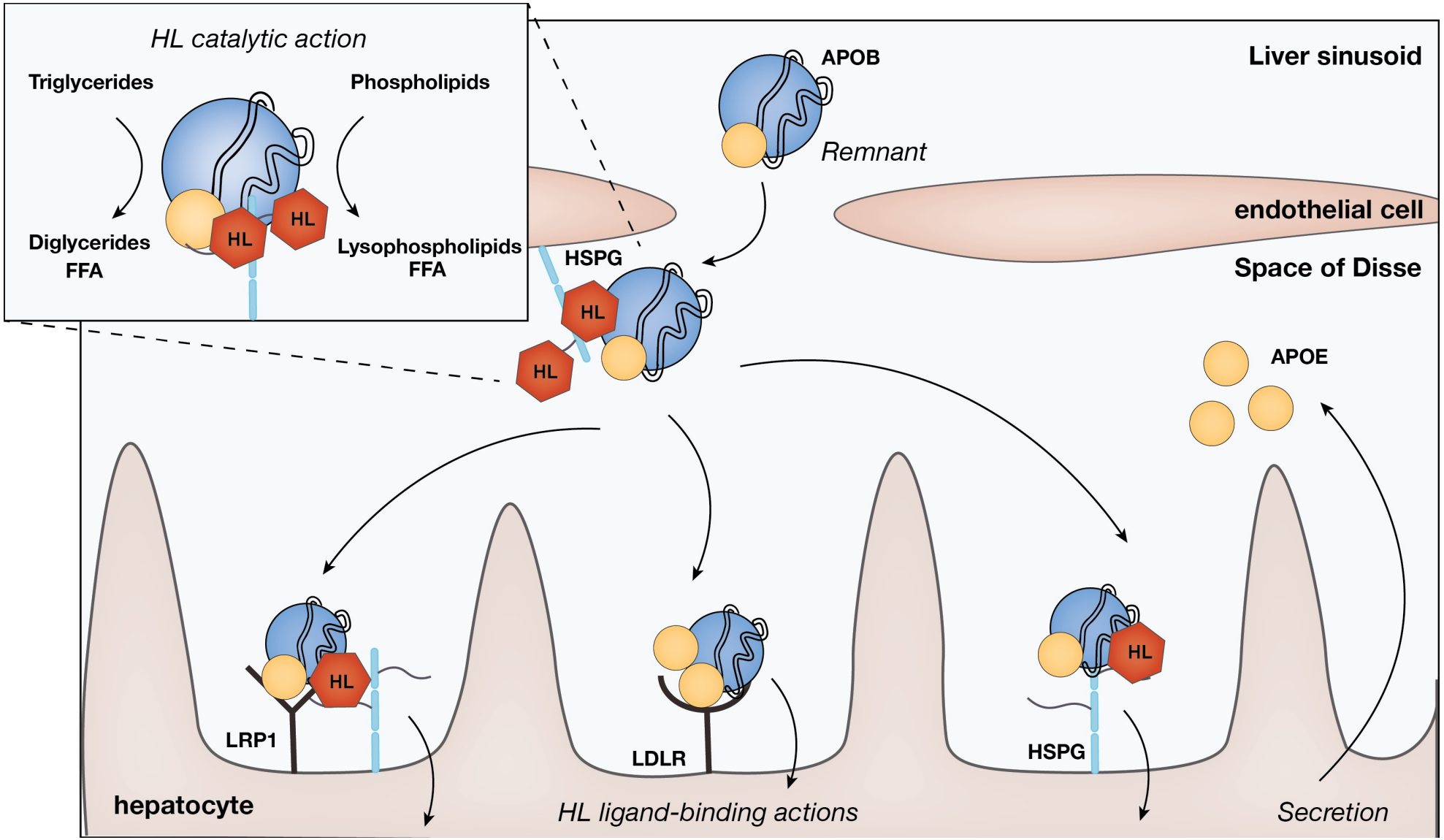


- ✓ 477 amino acids
- ✓ 65 kDa
- ✓ Mainly found in hepatocytes but also in adrenals and ovaries
- ✓ Localized at the surface of parenchymal cells in the space of Disse
- ✓ 90% of the WT form of the hepatic lipase activity in the liver

- ✓ At the surface cell in human ≠ circulating in mice

- ✓ Members of the Lipase family : Lipoprotein lipase (LPL), Endothelial Lipase (EL)

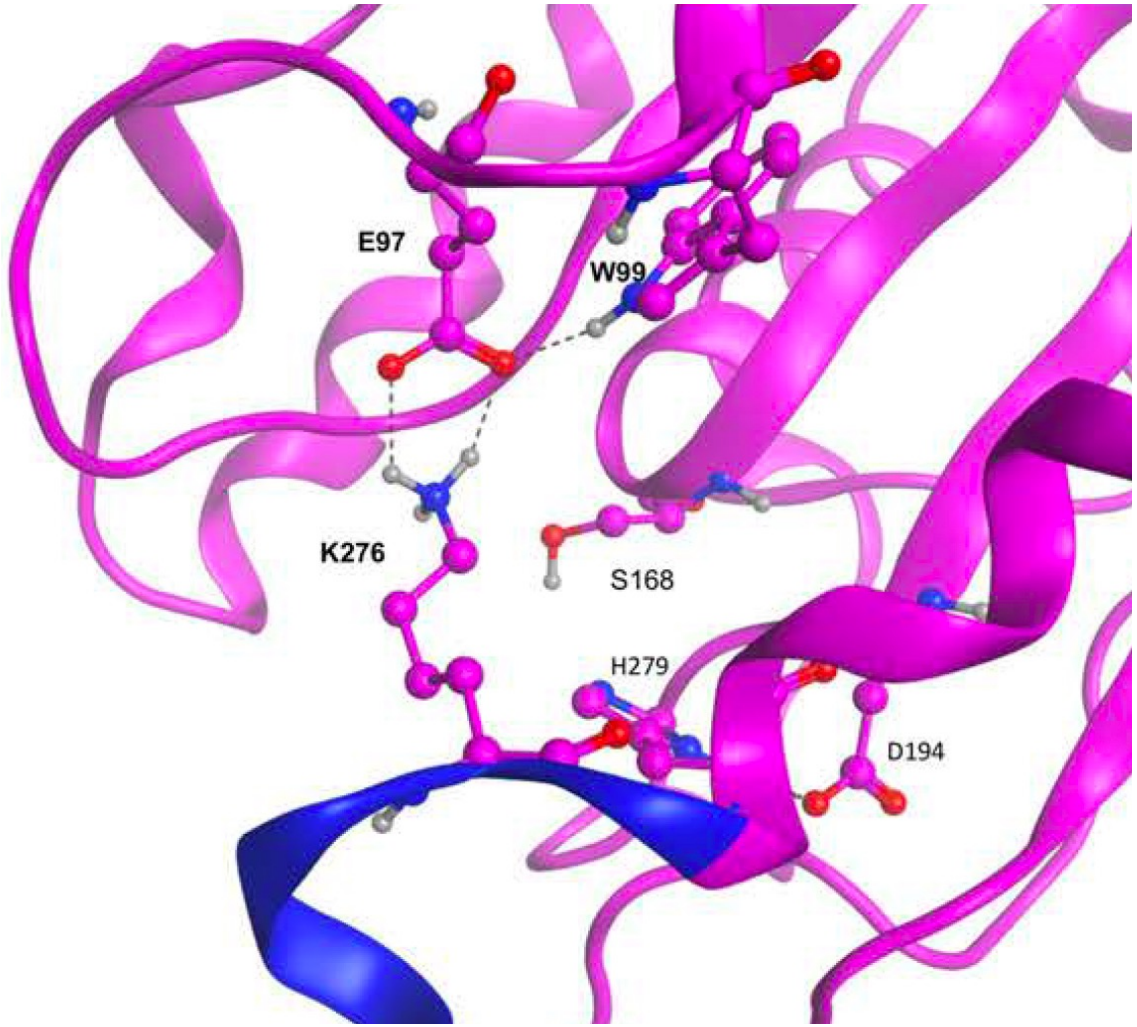
Hepatic lipase has a dual lipase activity and a ligand-binding function



How LIPC-E97G variant affects HL function ?

Structural consequences analysis

- Homology-based modelling based on crystallized LPL
- 45.6% sequence homology between HL and LPL



Jean-Yves LE QUESTEL, Ceisam, Nantes

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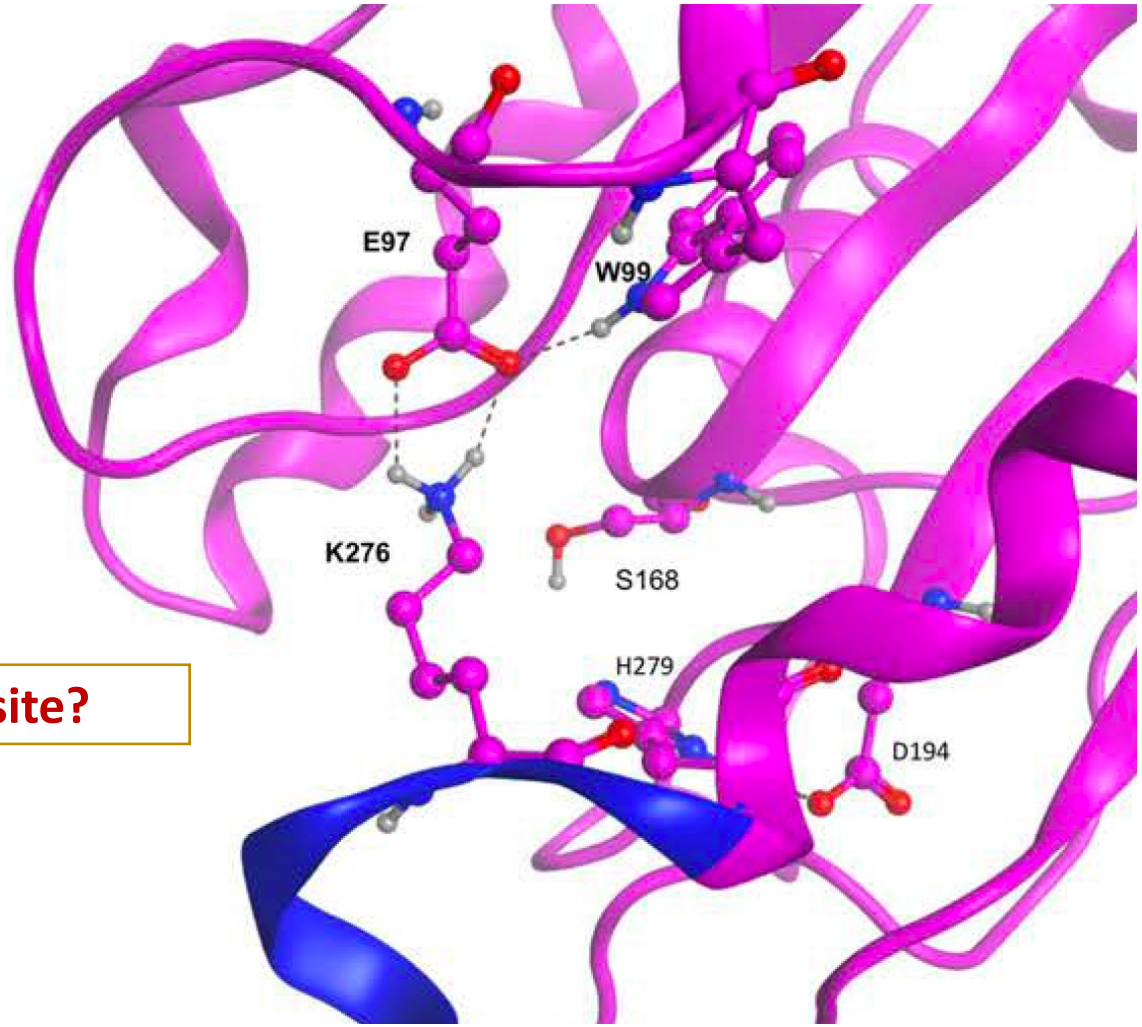
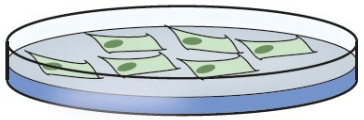


Glu97 is not part of catalytic triad
Glu97 is part of the Lid domain

Impact on substrate access to the catalytic site?

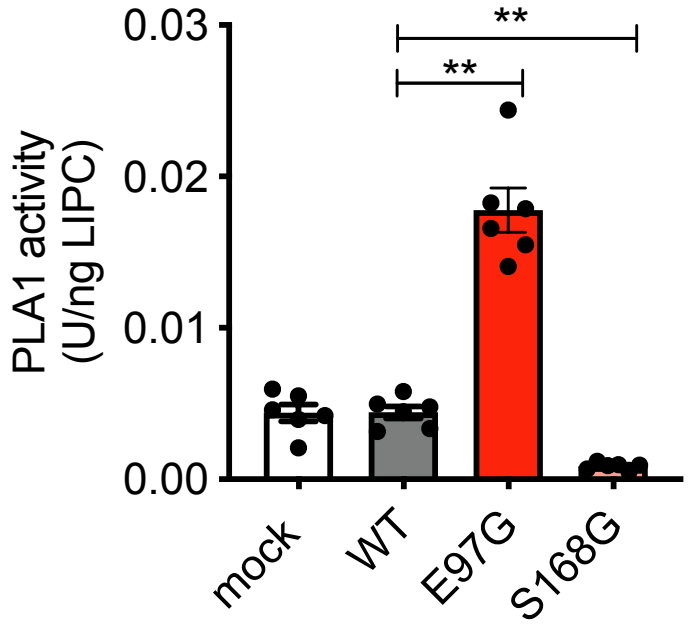
Jean-Yves LE QUESTEL, Ceisam, Nantes

HL-E97G increases phospholipase activity without affecting TG lipase activity

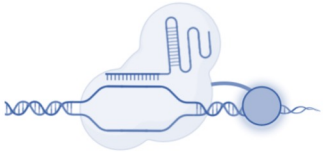


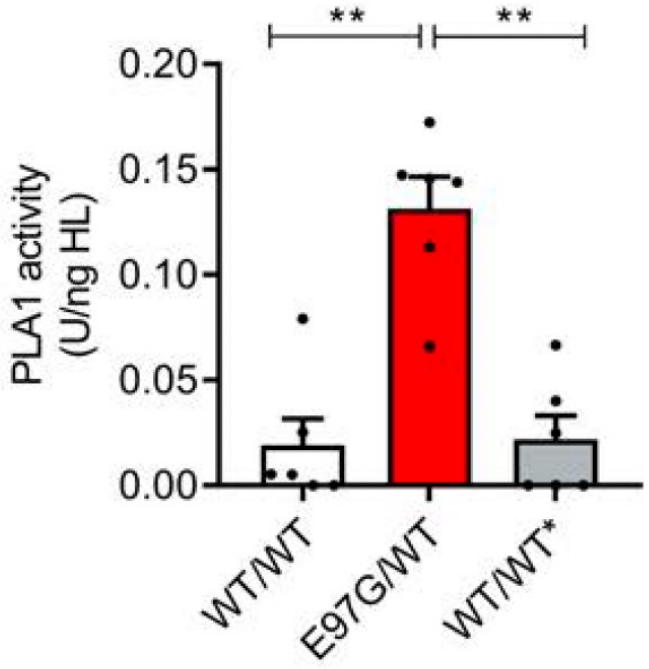
Immortalized Human Hepatocytes Cells

Overexpression



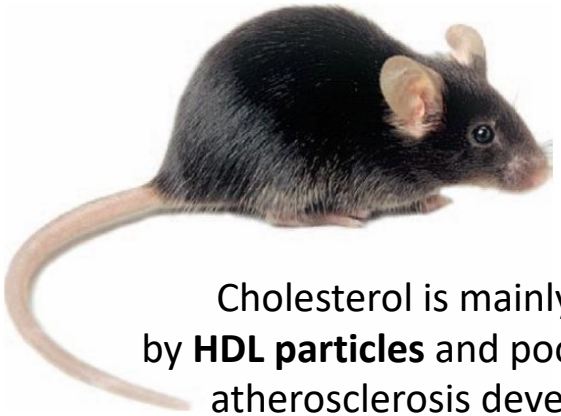
Genome editing by CRISPR-Cas9



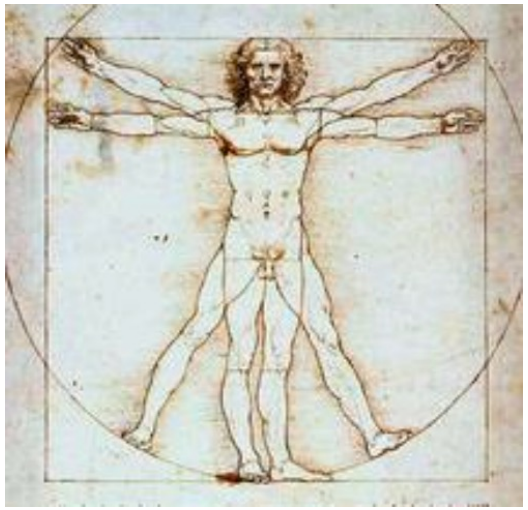


Wieneke Dijk

Use of a humanized mouse model for lipoprotein metabolism

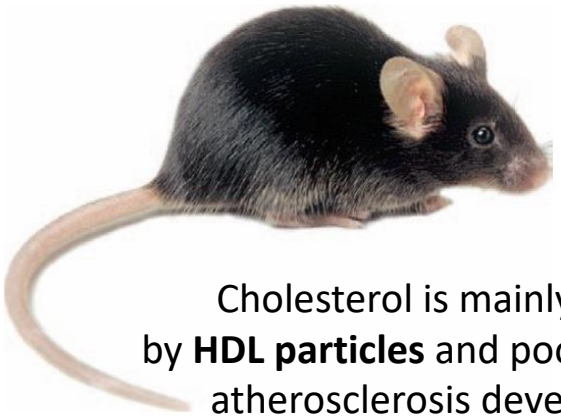


Cholesterol is mainly carried by **HDL particles** and poorly prone to atherosclerosis development



Cholesterol is mainly carried by **LDL particles**

Use of a humanized mouse model for lipoprotein metabolism



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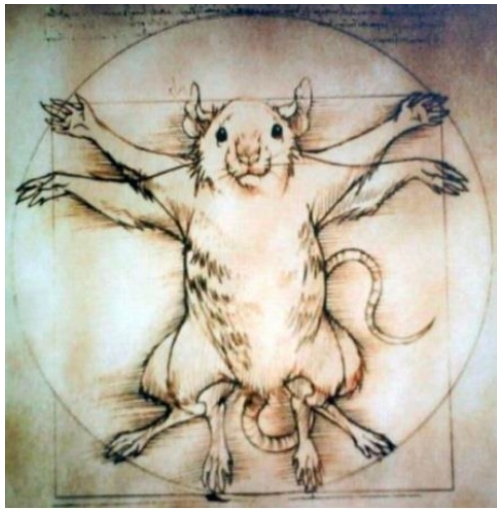
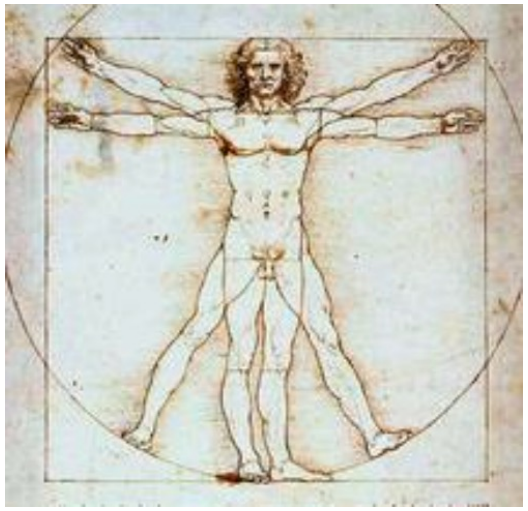


APOE*3-Leiden.CETP mice
Cholesterol is mainly carried
by **(V)LDL particles**



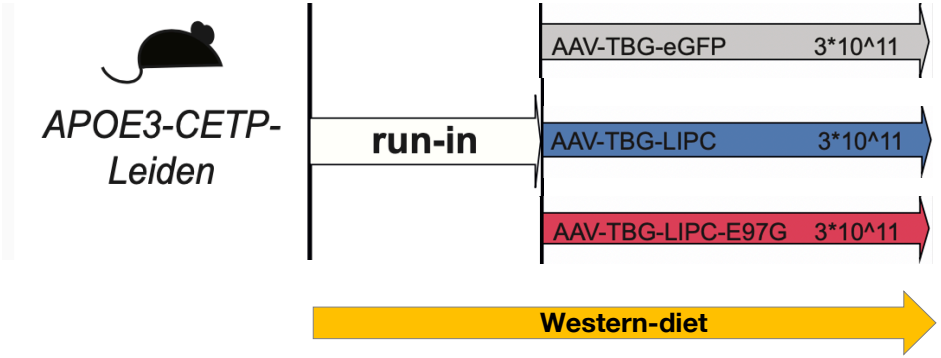
Cholesterol is mainly carried
by **LDL particles**

- ✓ develop diet-induced hyperlipidemia and atherosclerosis
- ✓ intact apoE-LDLr clearance pathway (unlike *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice)
- ✓ respond in human-like manner to lipid-lowering interventions

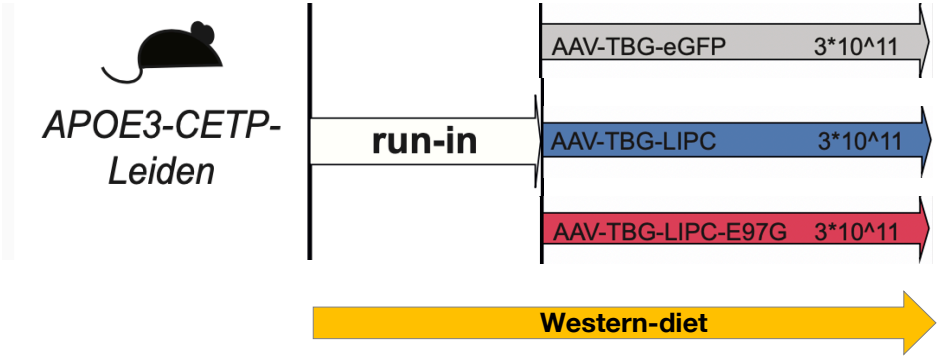
ATVB 2004; Circulation 2008; Eur Heart J 2014; Circulation 2021, Circulation 2022; JCI Insight 2023; eLife 2023

Impact of hepatic overexpression of human HL E97G on APOE*3 Leiden CETP mice

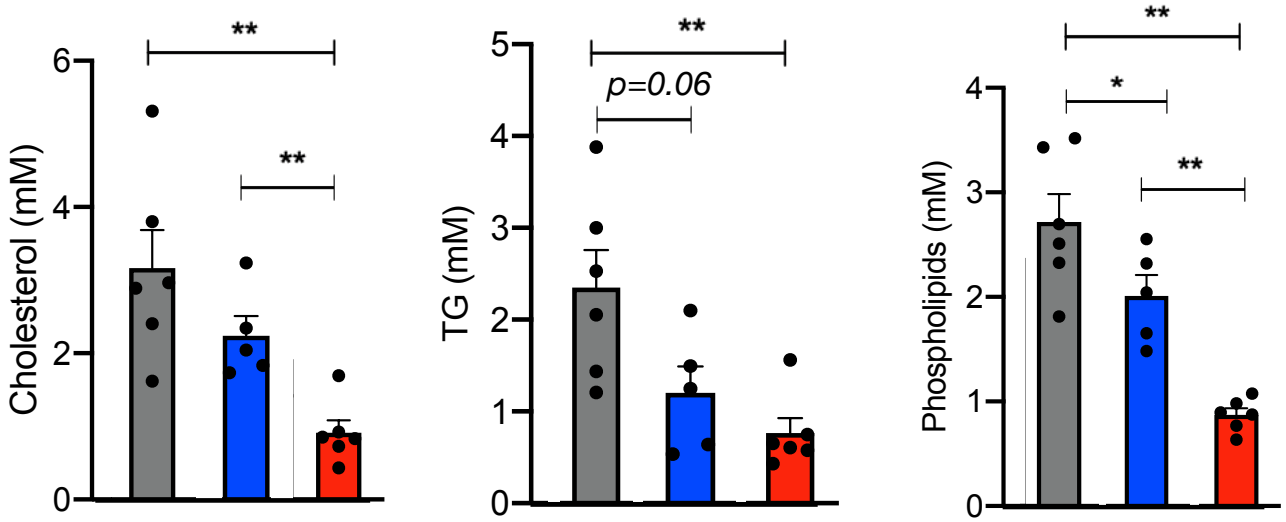
P. Rensen
Leiden, Pays-bas



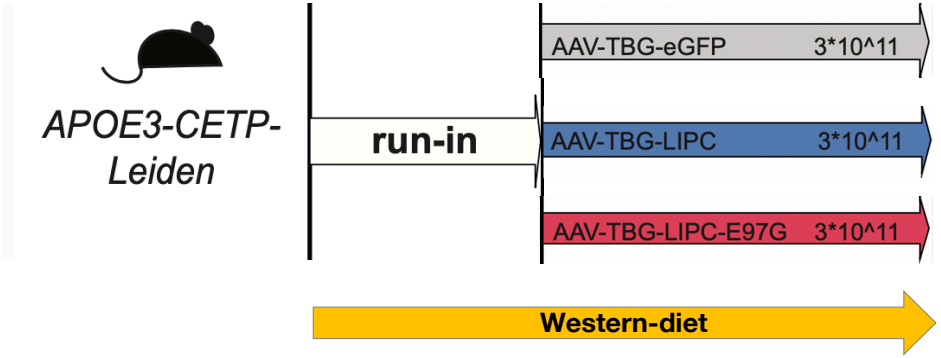
Mice expressing the E97G variant form of HL develop combined hypolipidemia



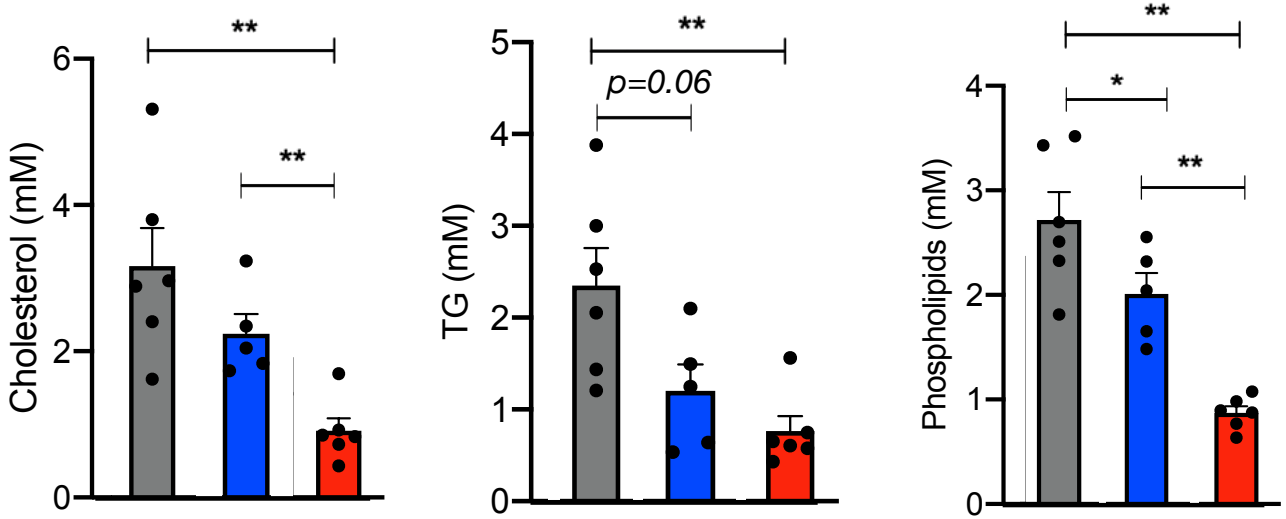
Plasma lipids



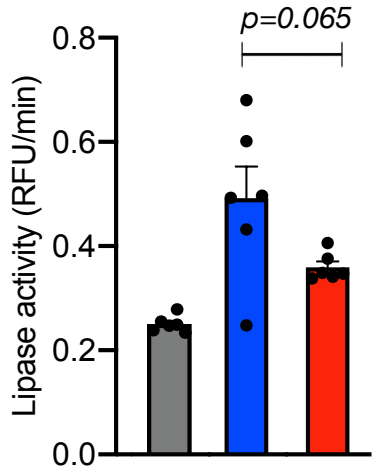
Mice expressing the E97G variant form of HL develop combined hypolipidemia and increased phospholipase activity



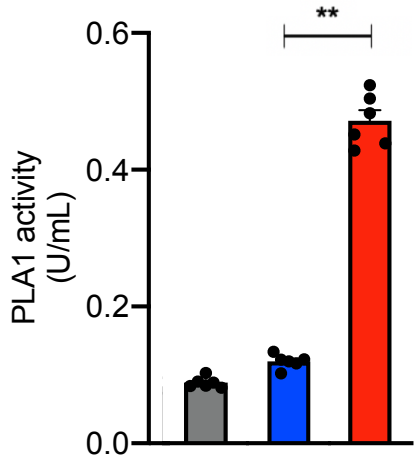
Plasma lipids



TG lipase activity



Phospholipase activity



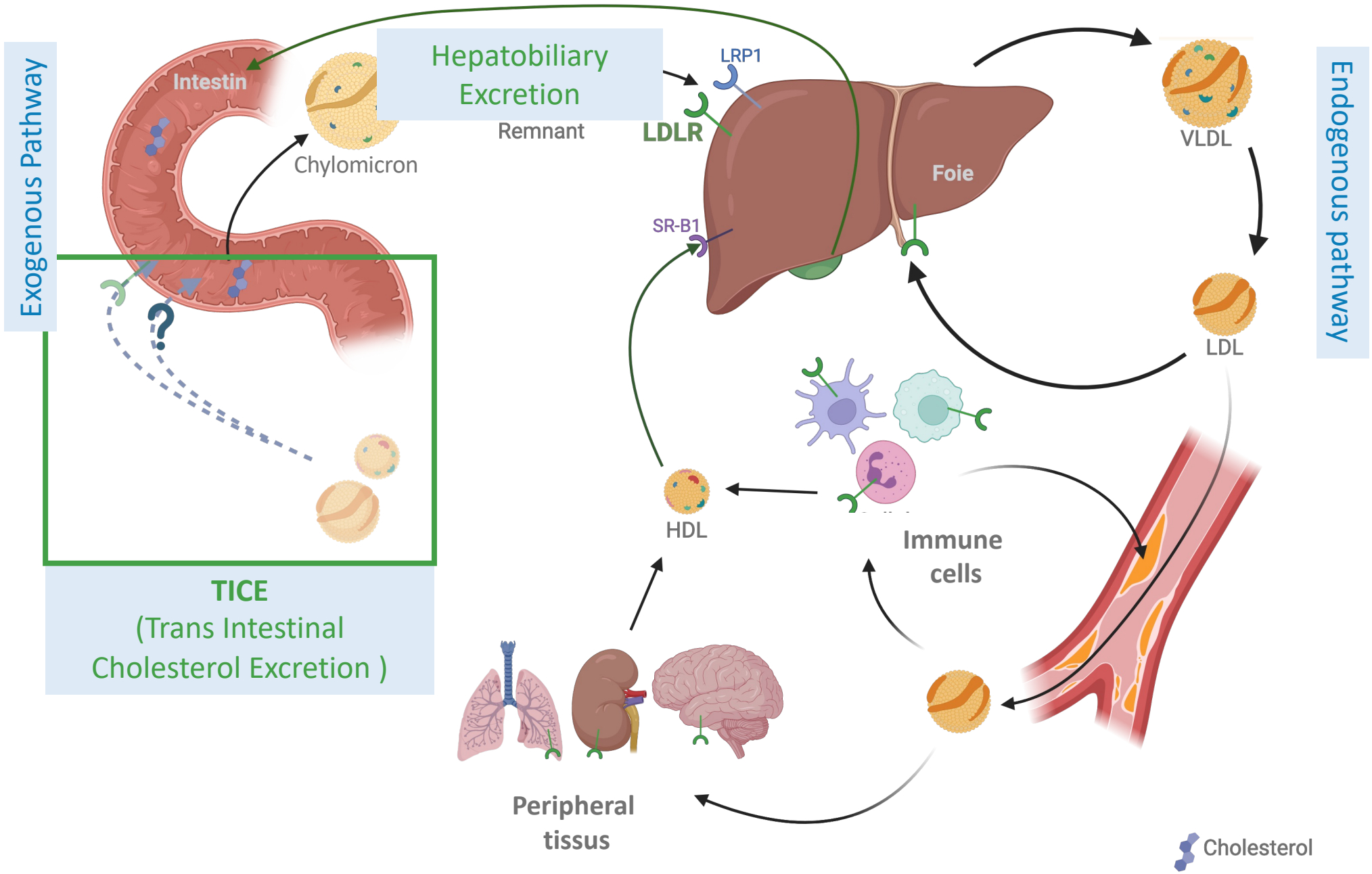
Conclusion 1

Variant *HL*-E97G :

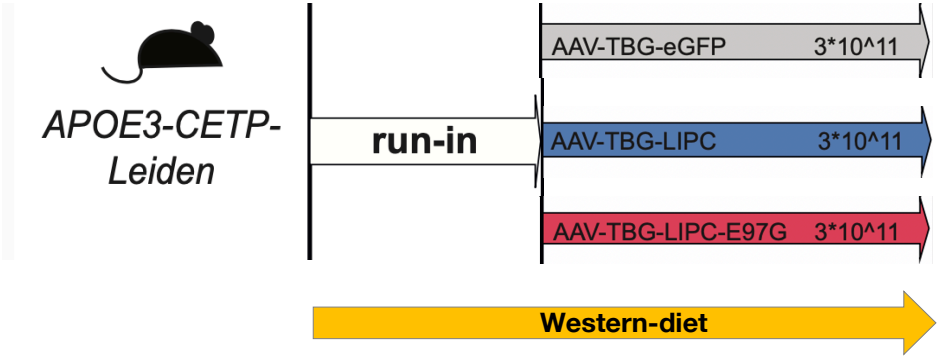
- Alters the Lid region that regulates the accessibility of the substrate to the catalytic site
- Does not impact the triglyceride lipase activity
- Strongly increases the phospholipase activity (confirmed in humans, mice and cells)
- Induces a strong combined hypolipemia

Mode of action ?

Cholesterol homeostasis

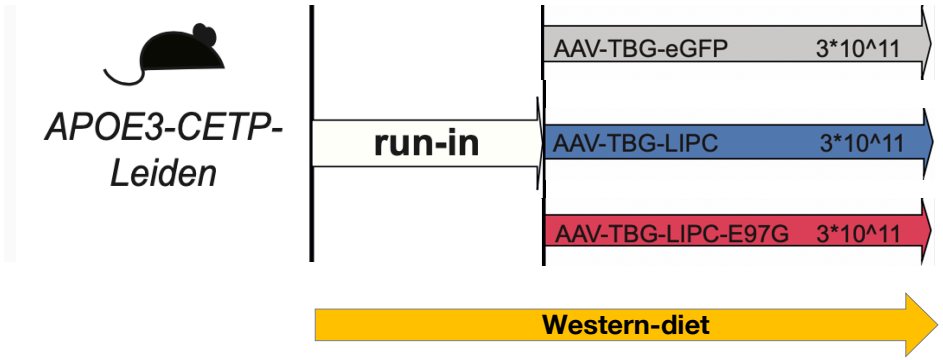


E97G variant does not affect the hepatic release of VLDL particles



Intravenous injection of triton (LPL inhibitor)

E97G variant does not affect the hepatic release of VLDL particles



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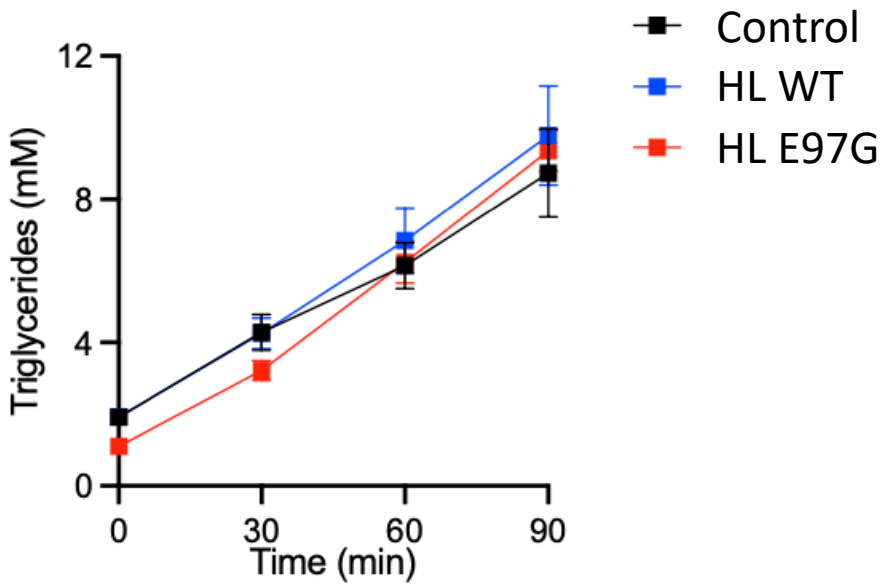
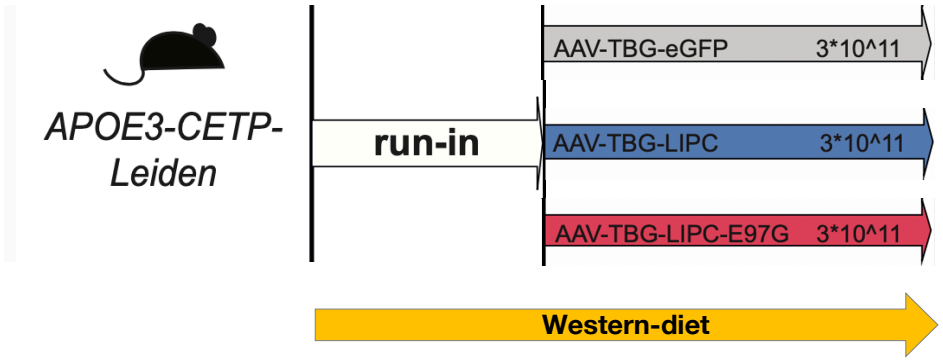
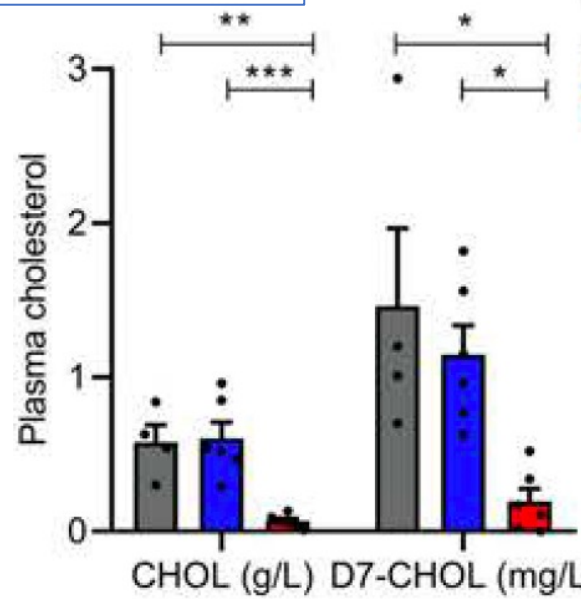


E97G variant promotes a rapid clearance of plasma cholesterol

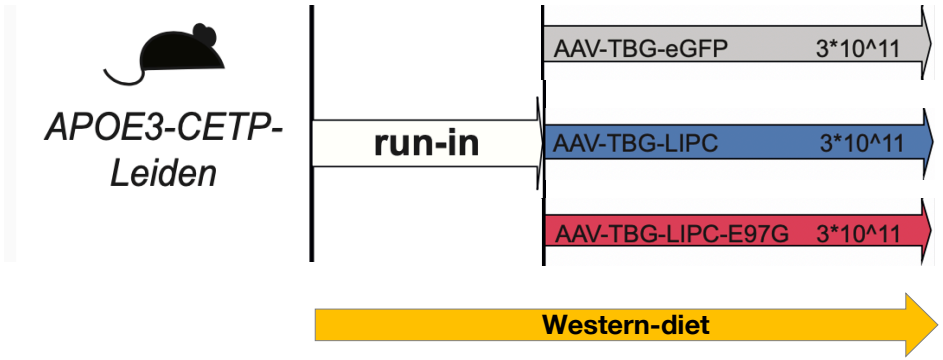


Intravenous injection of deuterated cholesterol (D7)

Plasma Clearance



E97G variant promotes a rapid clearance of plasma cholesterol with no impact on hepatic or fecal cholesterol content



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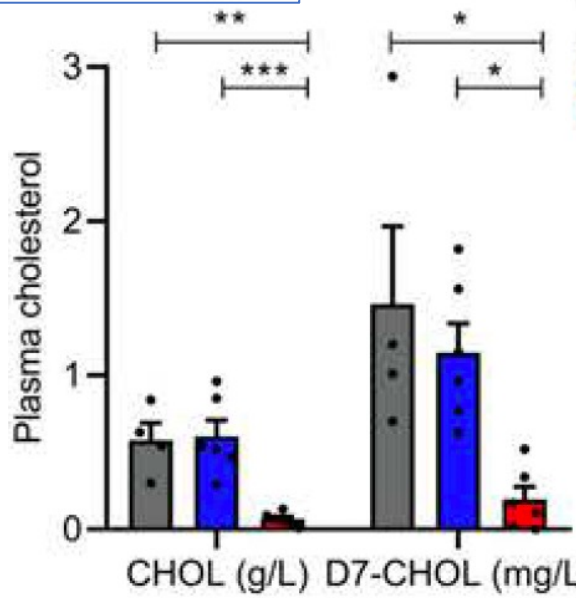
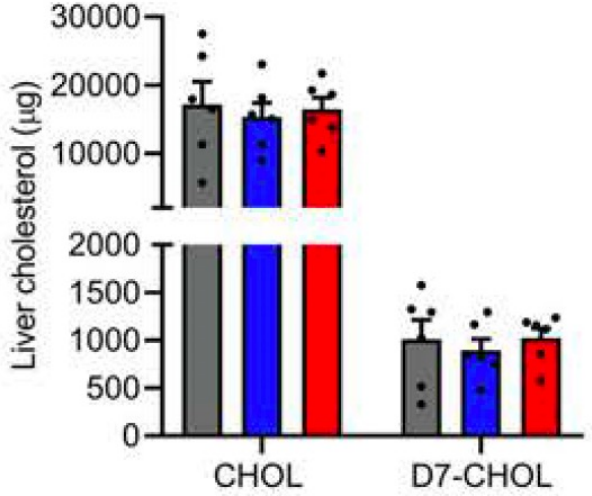
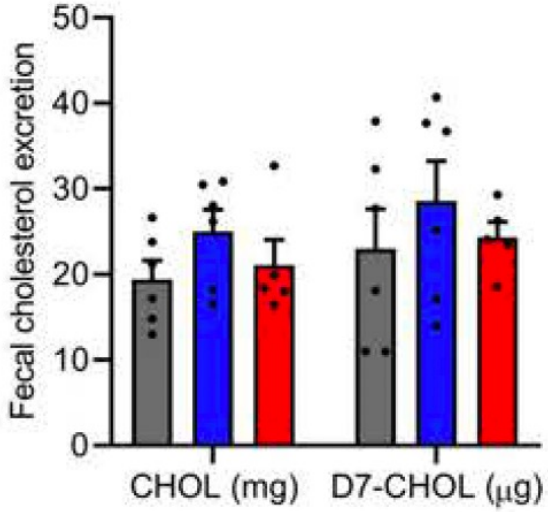


Hepatic uptake

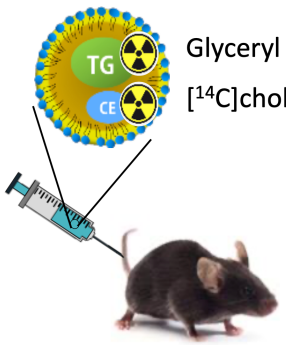


Fecal cholesterol elimination



E97G variant induces VLDL clearance by an extrahepatic pathway?

VLDL-like particles
(n=8/group)

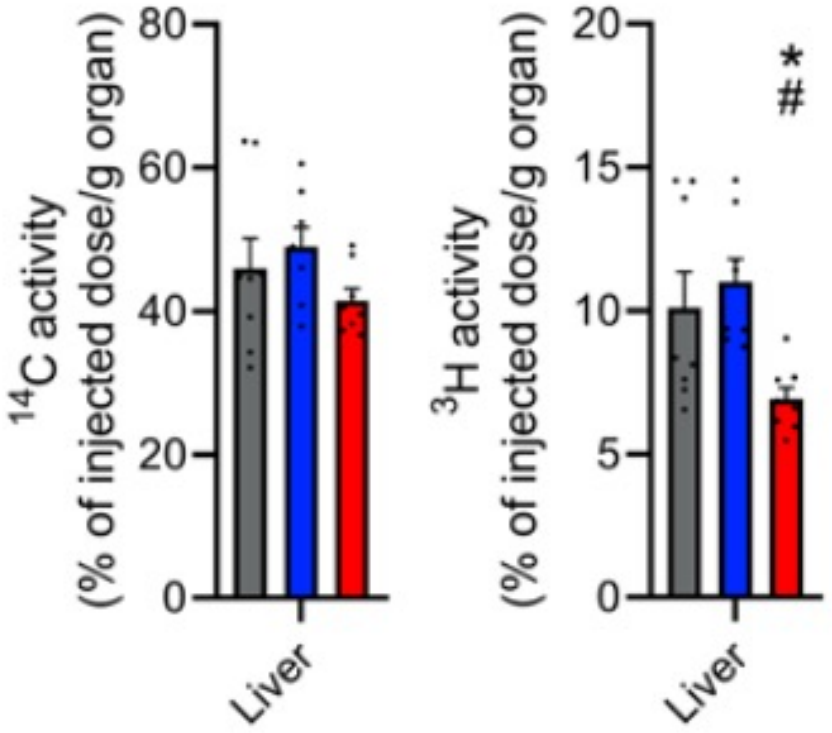


Glyceryl tri[^{3}H]oleate

[^{14}C]cholesteryl oleate

Control
HL WT
HL E97G

$
*
#



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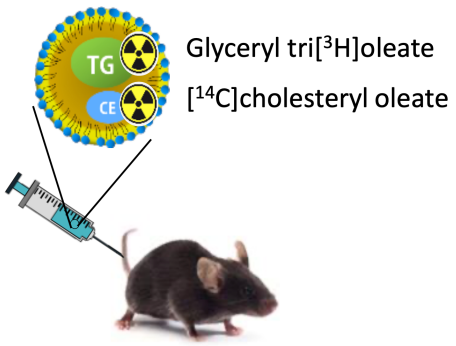

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HL WT
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$
*
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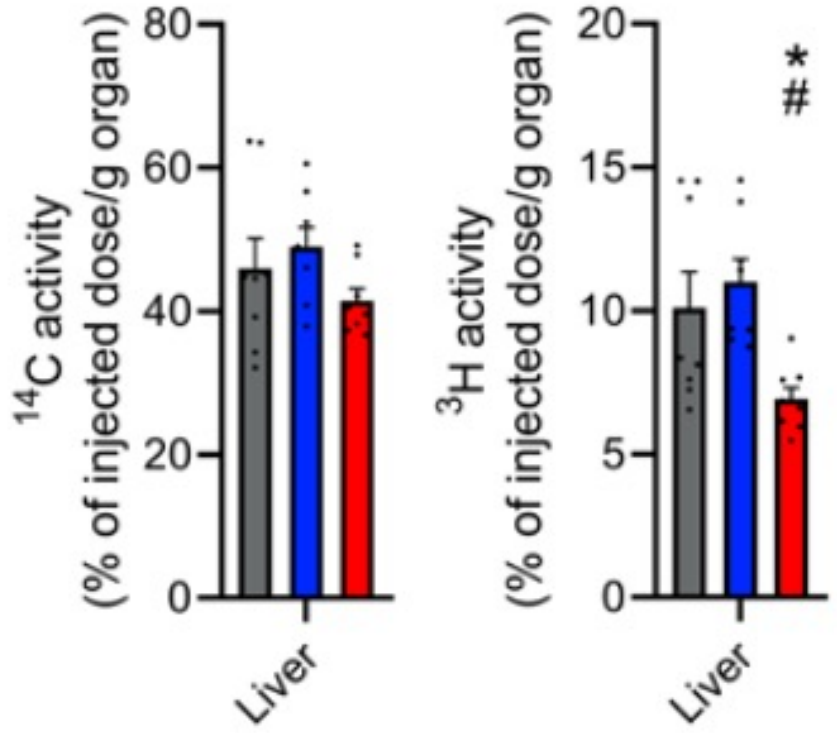
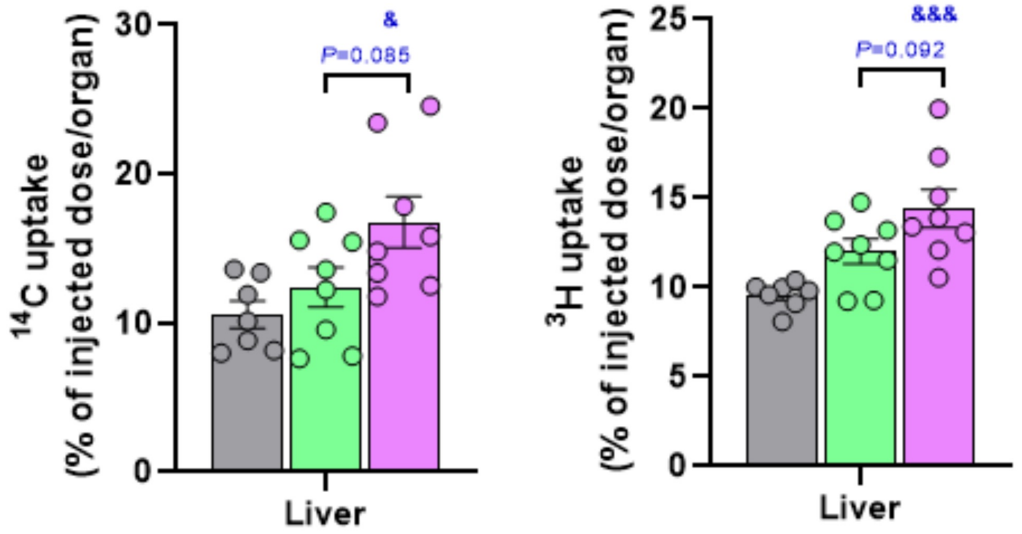
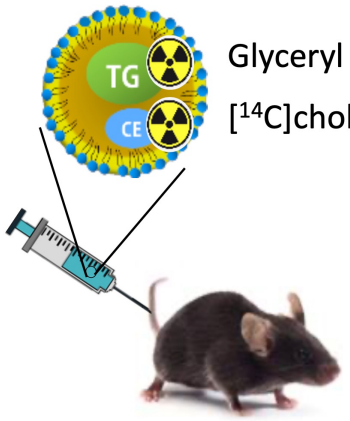


Control
HL WT
HL E97G



E97G variant significantly increases LDL clearance by an hepatic pathway

Human LDL
(n=8/group)



Glyceryl tri[^{3}H]oleate

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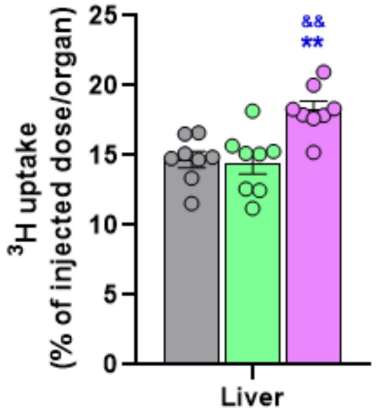
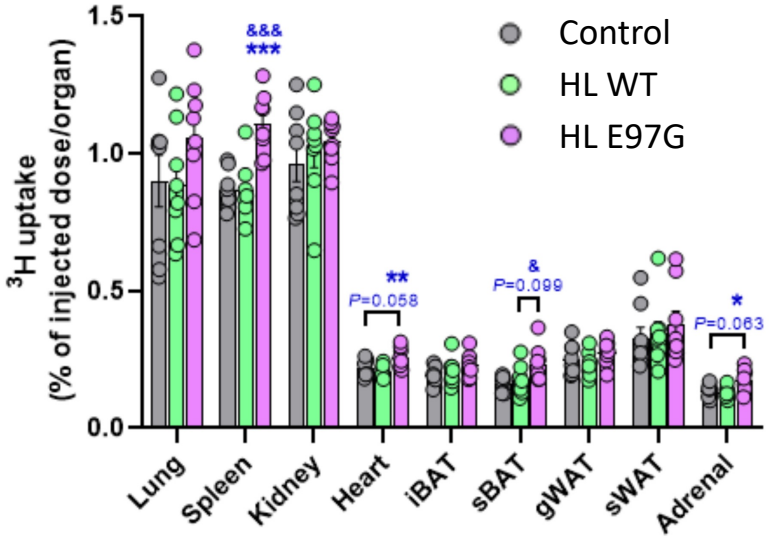
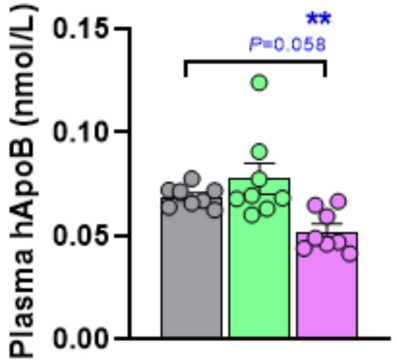
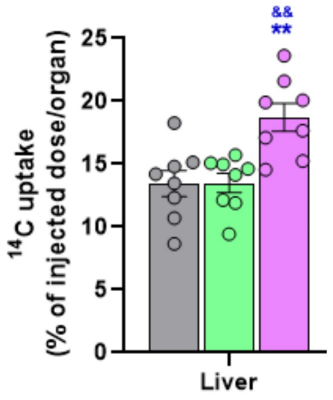
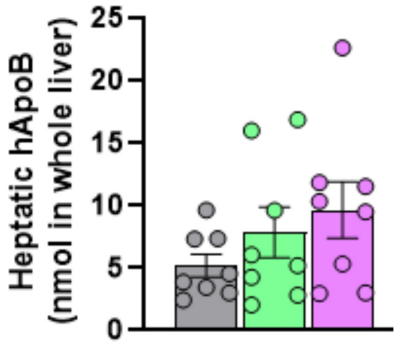
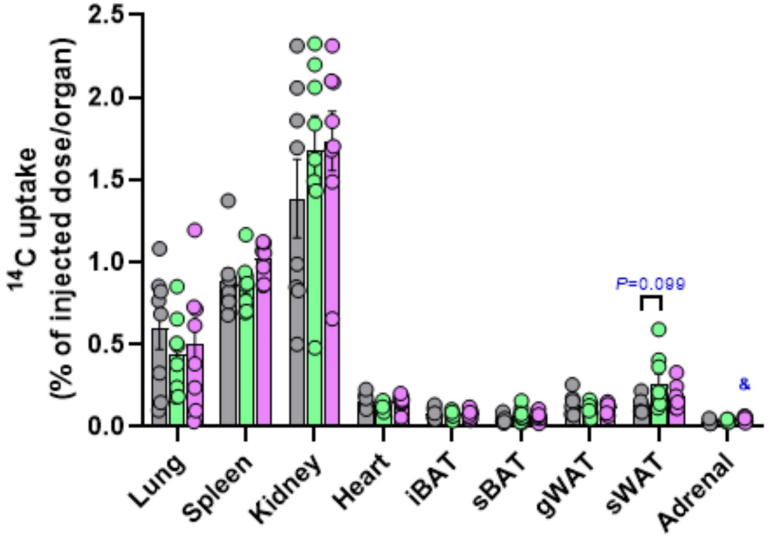




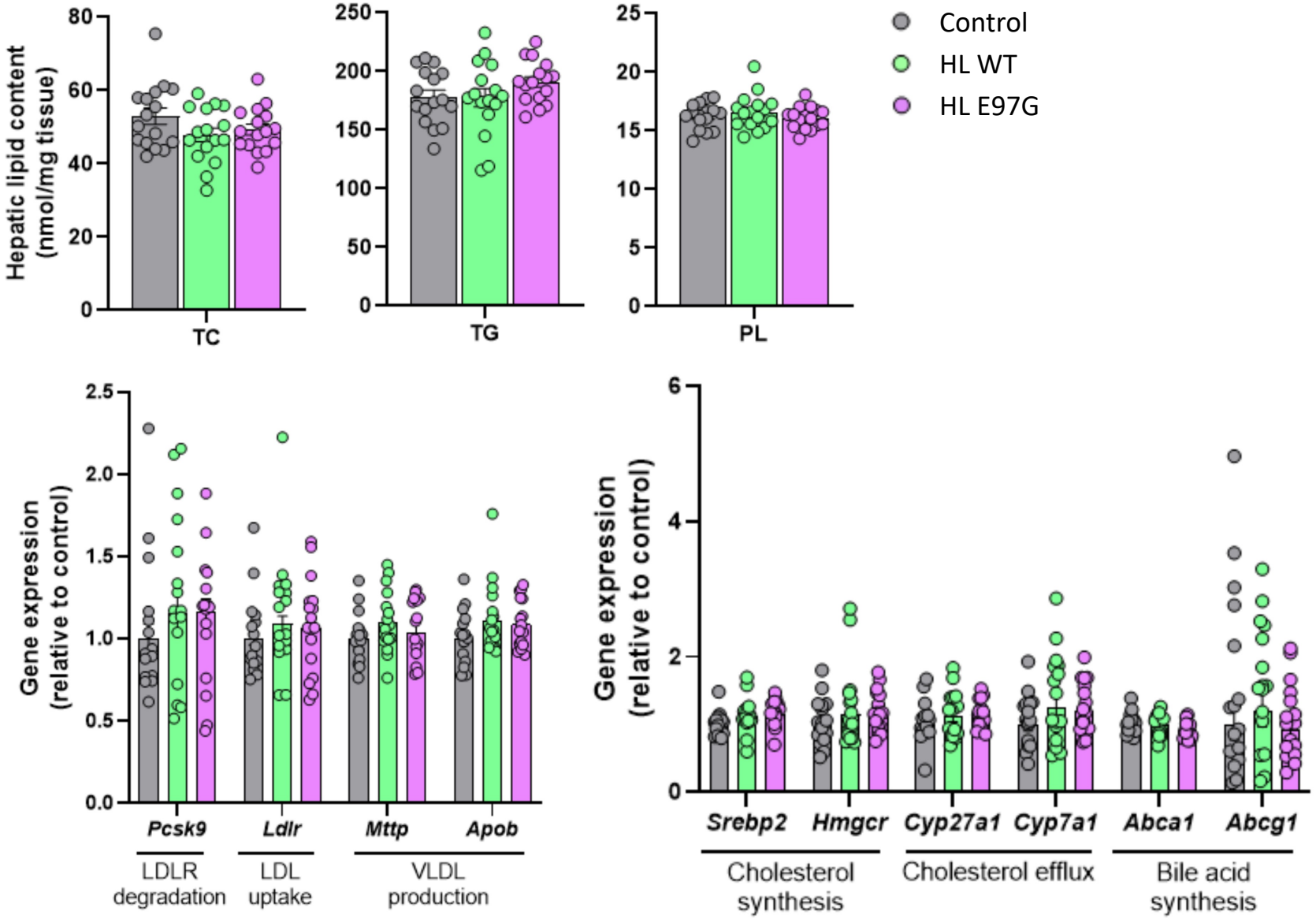








HL E97G expression has no impact on liver lipid content or hepatic gene expression.



Conclusion 2

Variant *HL*-E97G :

- Alters the Lid region that regulates the accessibility of the substrate to the catalytic site
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- Strongly increases the phospholipase activity (confirmed in humans, mice and cells)
- Induces a strong combined hypolipemia
- Strongly increase the plasma cholesterol clearance
- Does not affect the fecal excretion of neutral sterol
- Does not impact the VLDL production
- Effect on the hepatic uptake of VLDL is not so clear
- May promote the hepatic uptake of LDL…?

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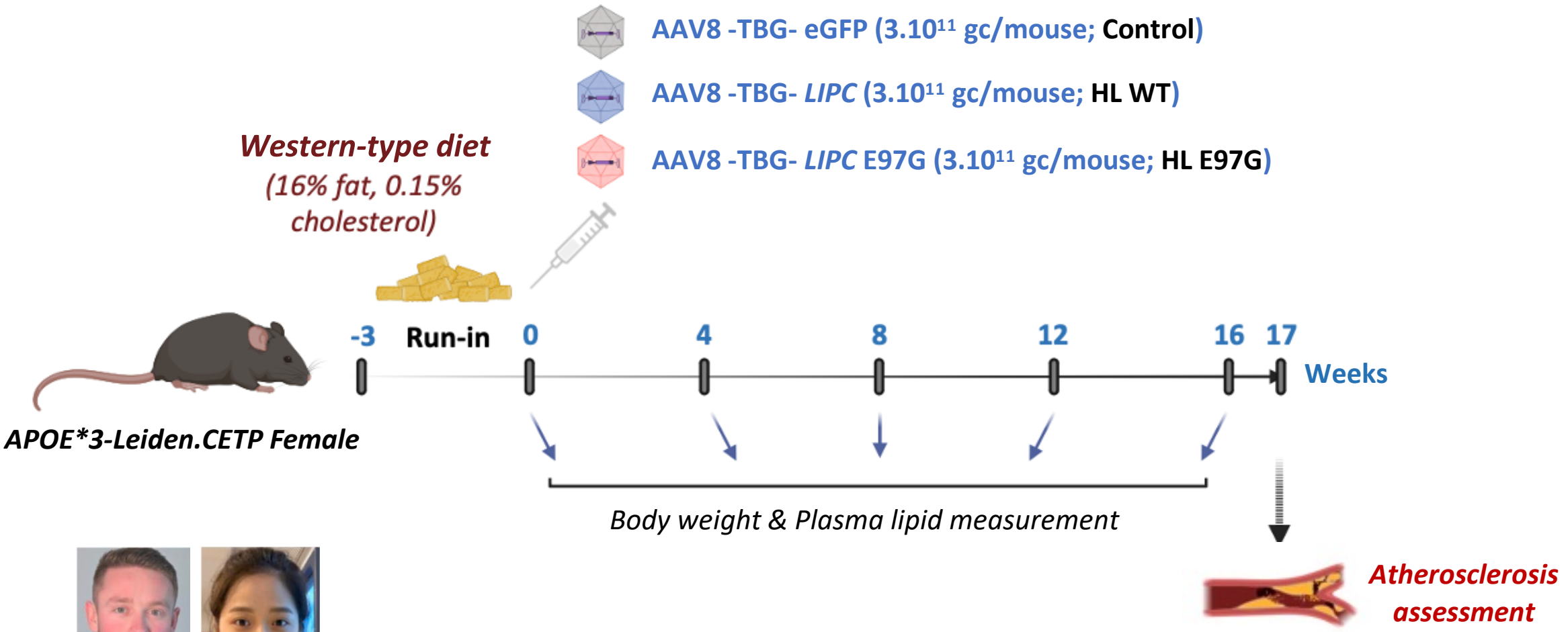
- May promote the hepatic uptake of LDL…? What about HDL particles ?

Does HL-E97G affects atherosclerosis progression ?

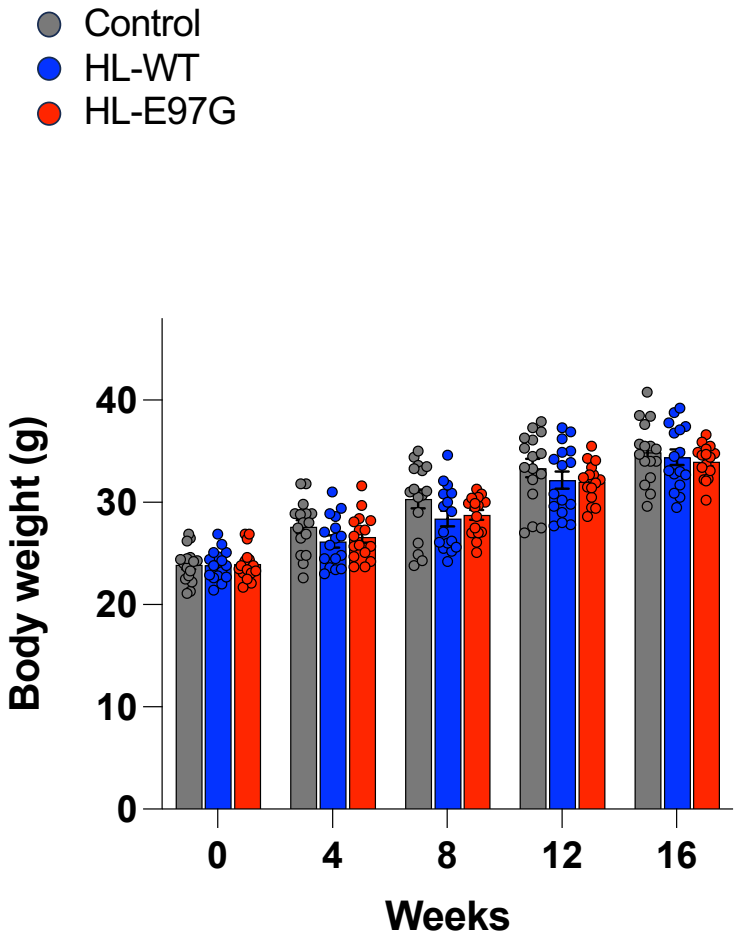
To test the effect of hepatic overexpression of human HL E97G on atherosclerosis

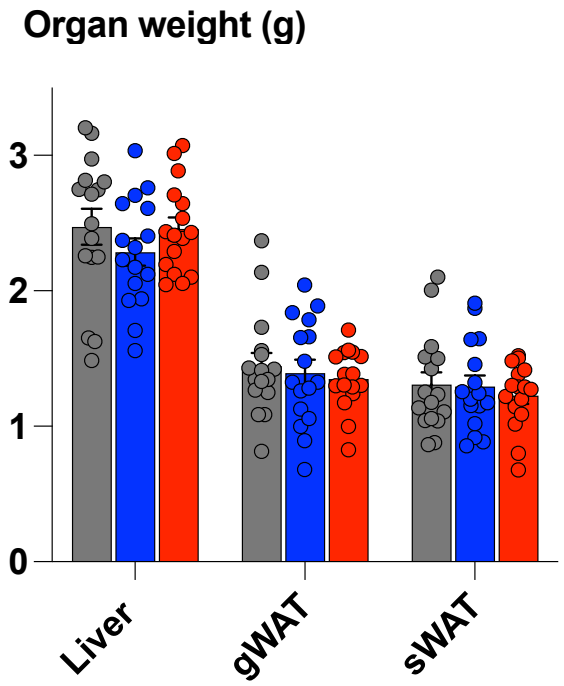


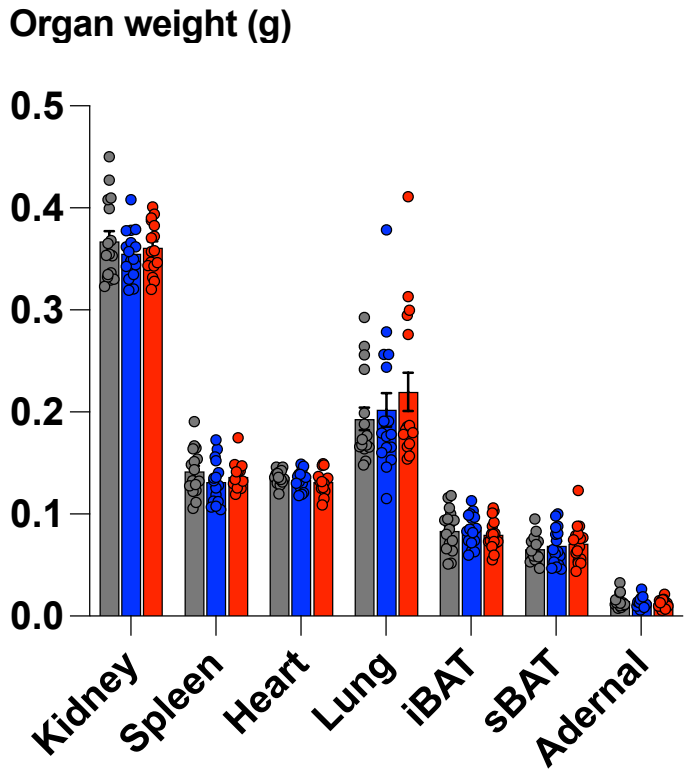
Thibaud Sotin

Xiaoke Ge

HL E97G does not affect body weight and organ weight



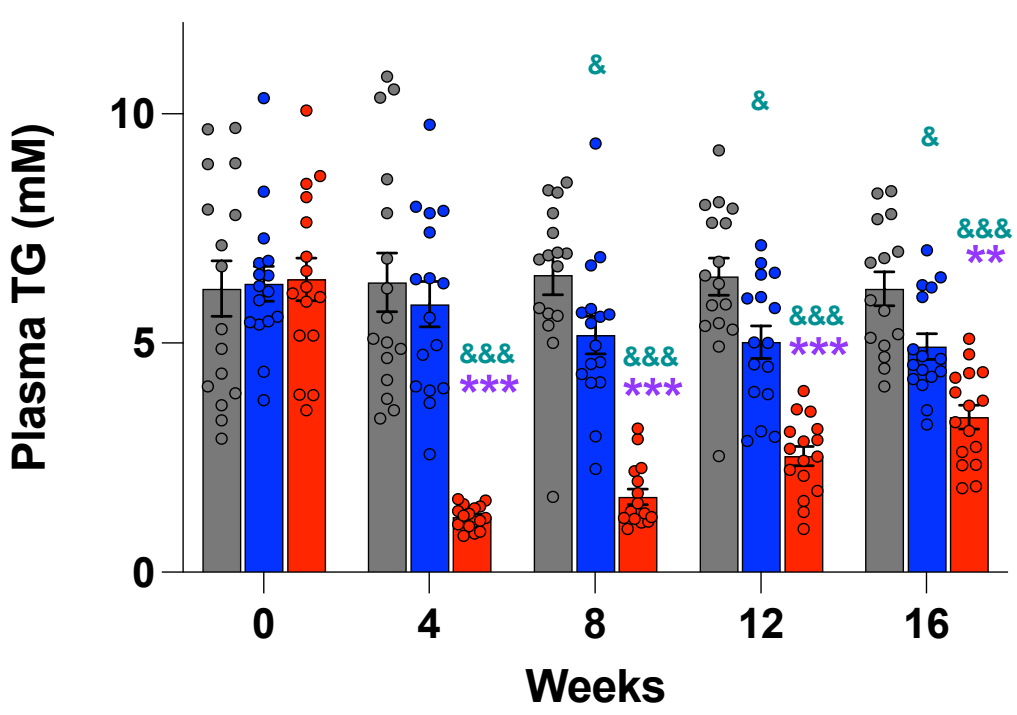


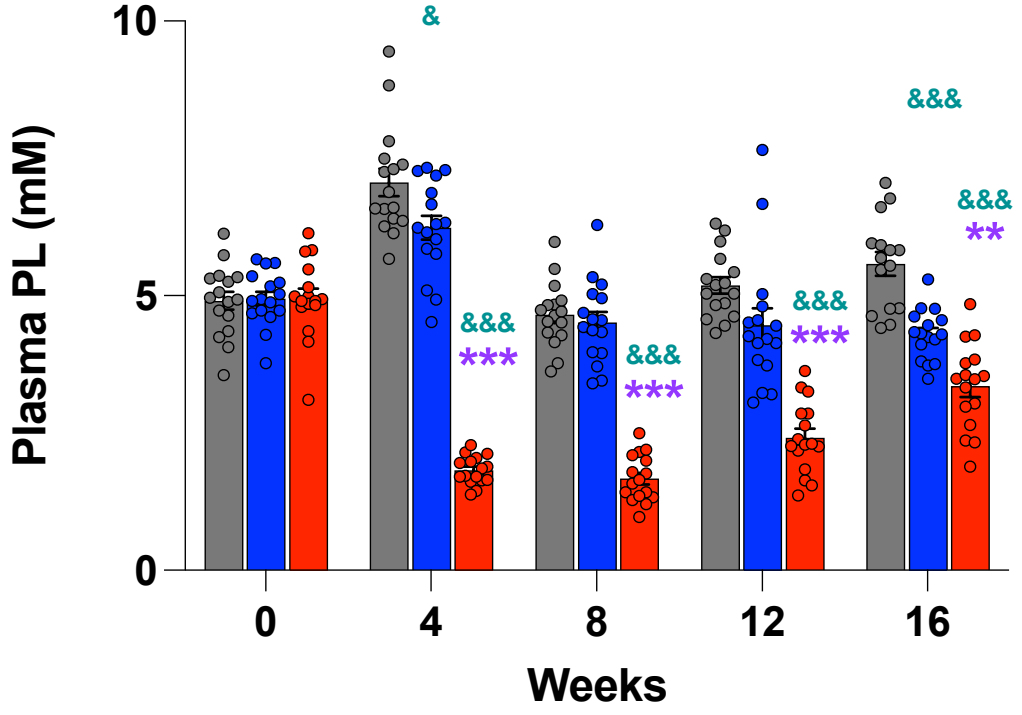


HL E97G strongly reduces plasma triglycerides and phospholipids levels

Control
HL-WT
HL-E97G

& vs control eGFP
* vs HL-WT

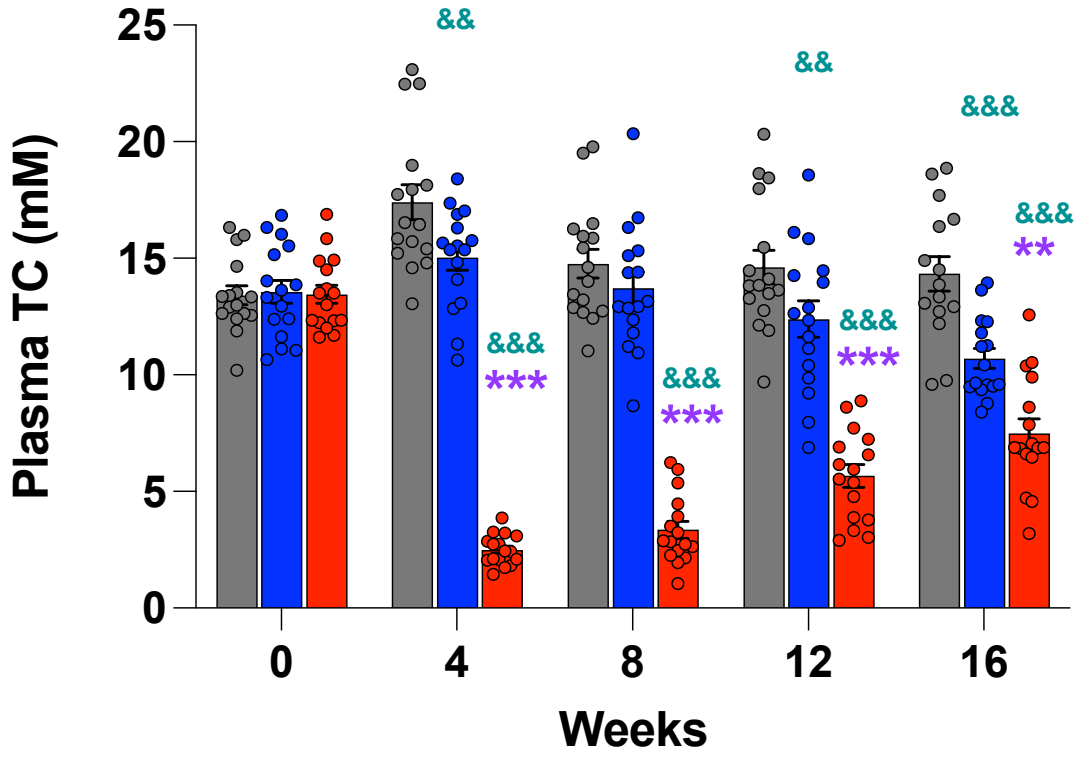


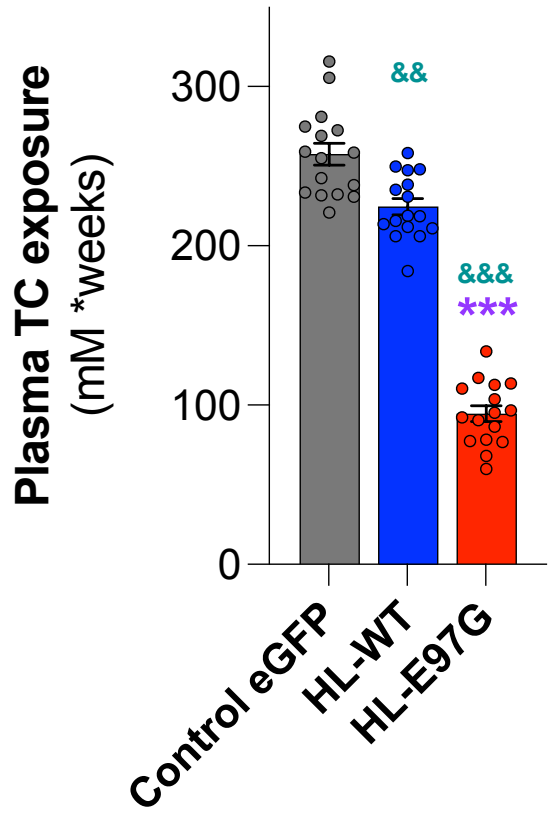


HL E97G strongly decreases plasma TC and long term TC exposure

Control
HL-WT
HL-E97G

& vs control eGFP
* vs HL-WT

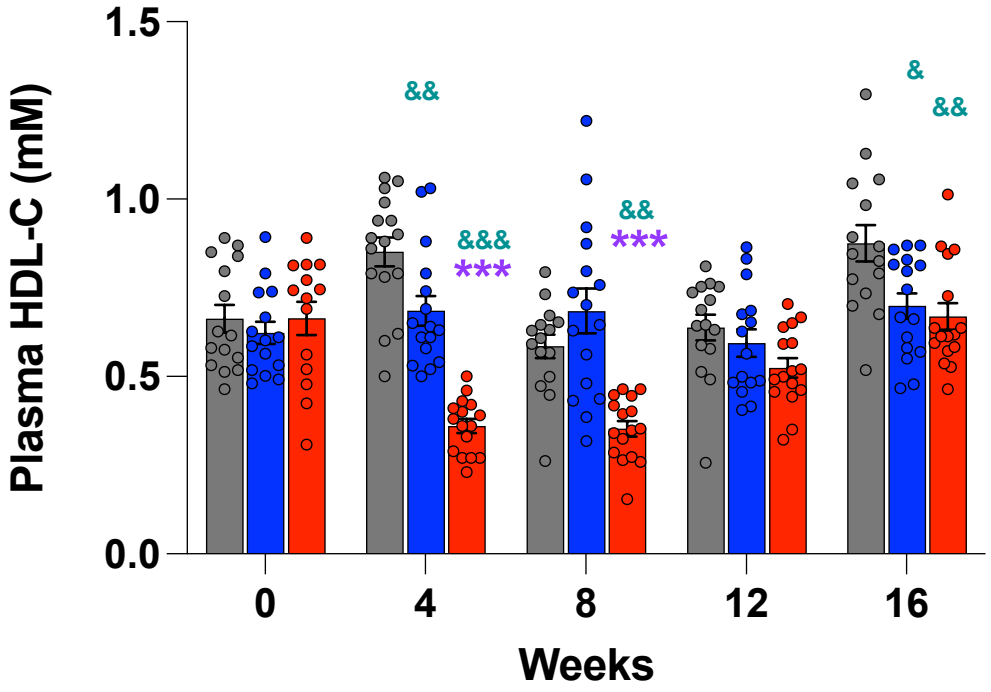


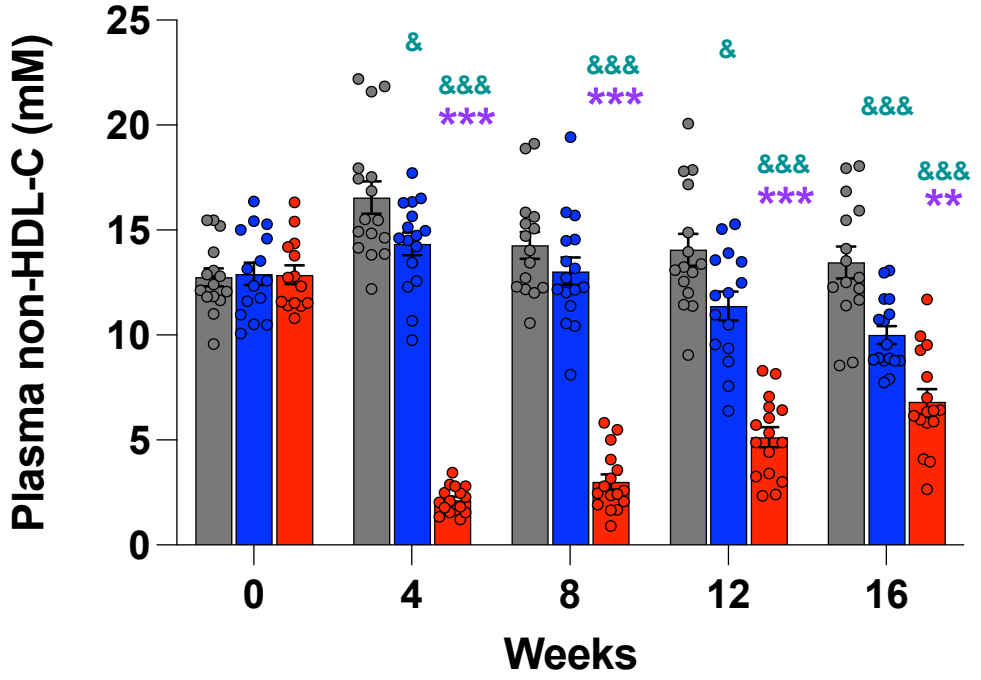


HL E97G affects both plasma HDL and non-HDL cholesterol levels

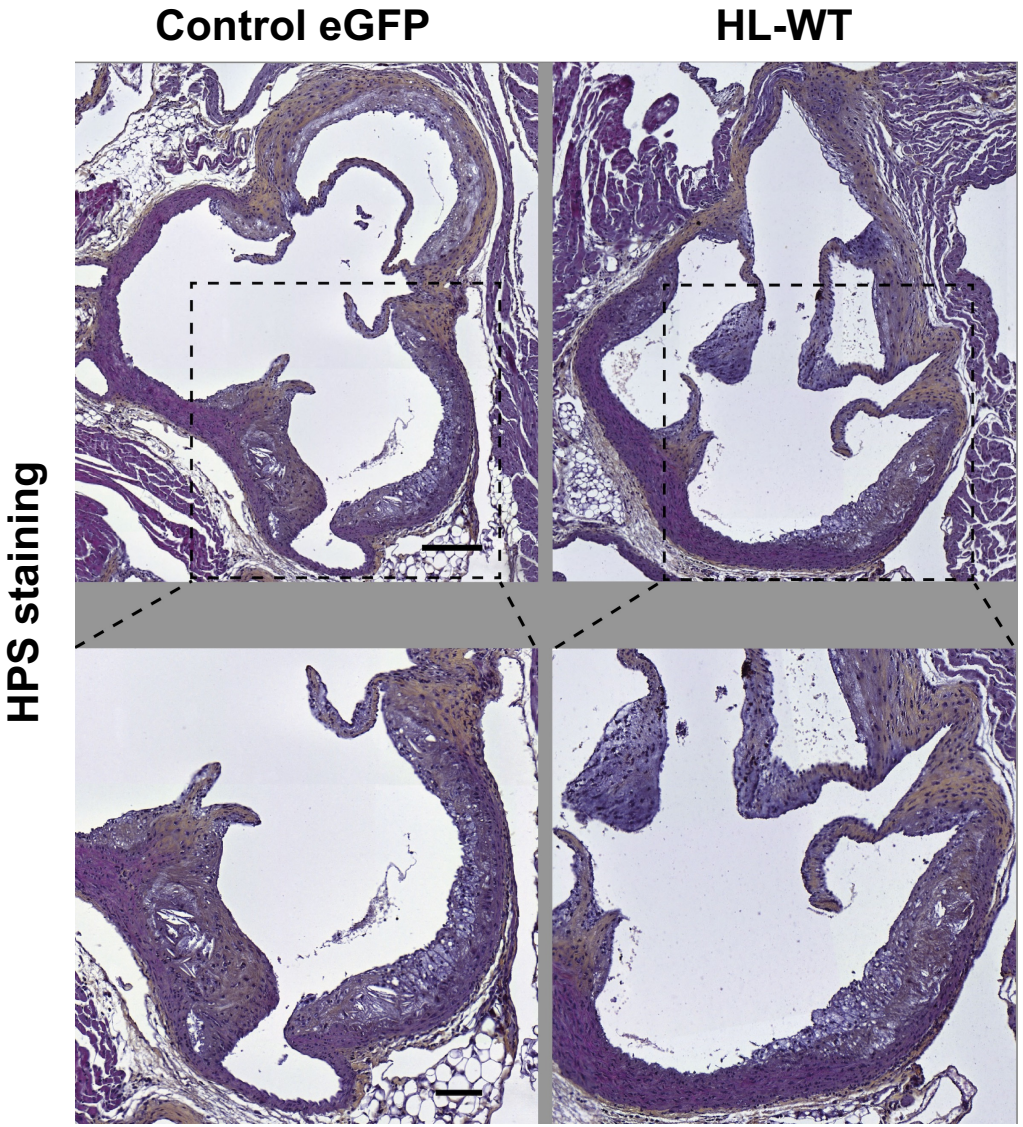
Control
HL-WT
HL-E97G

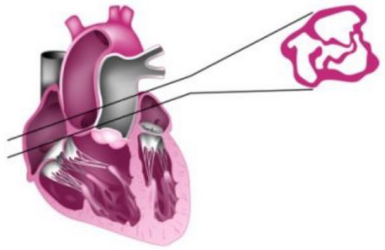
& vs control eGFP
* vs HL-WT

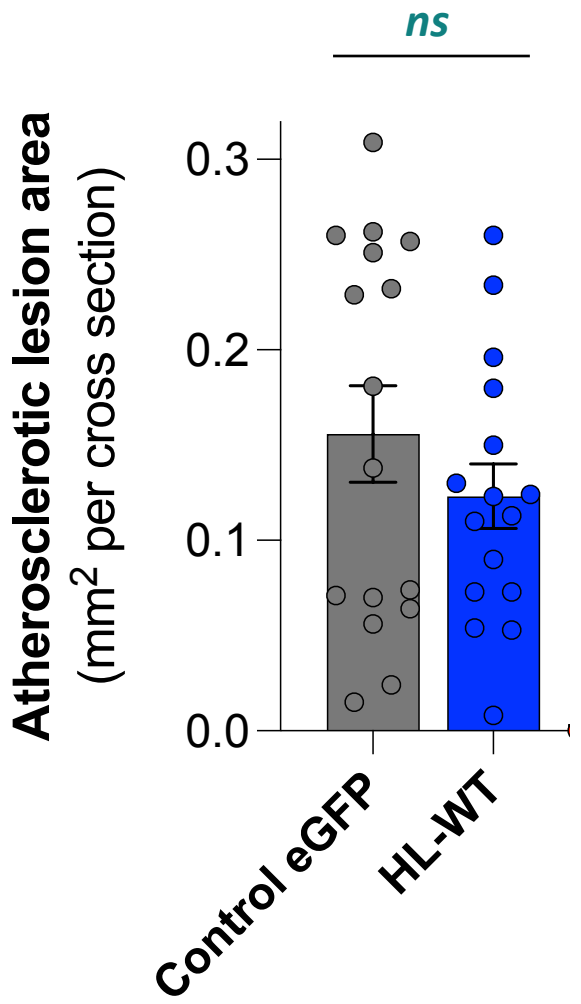




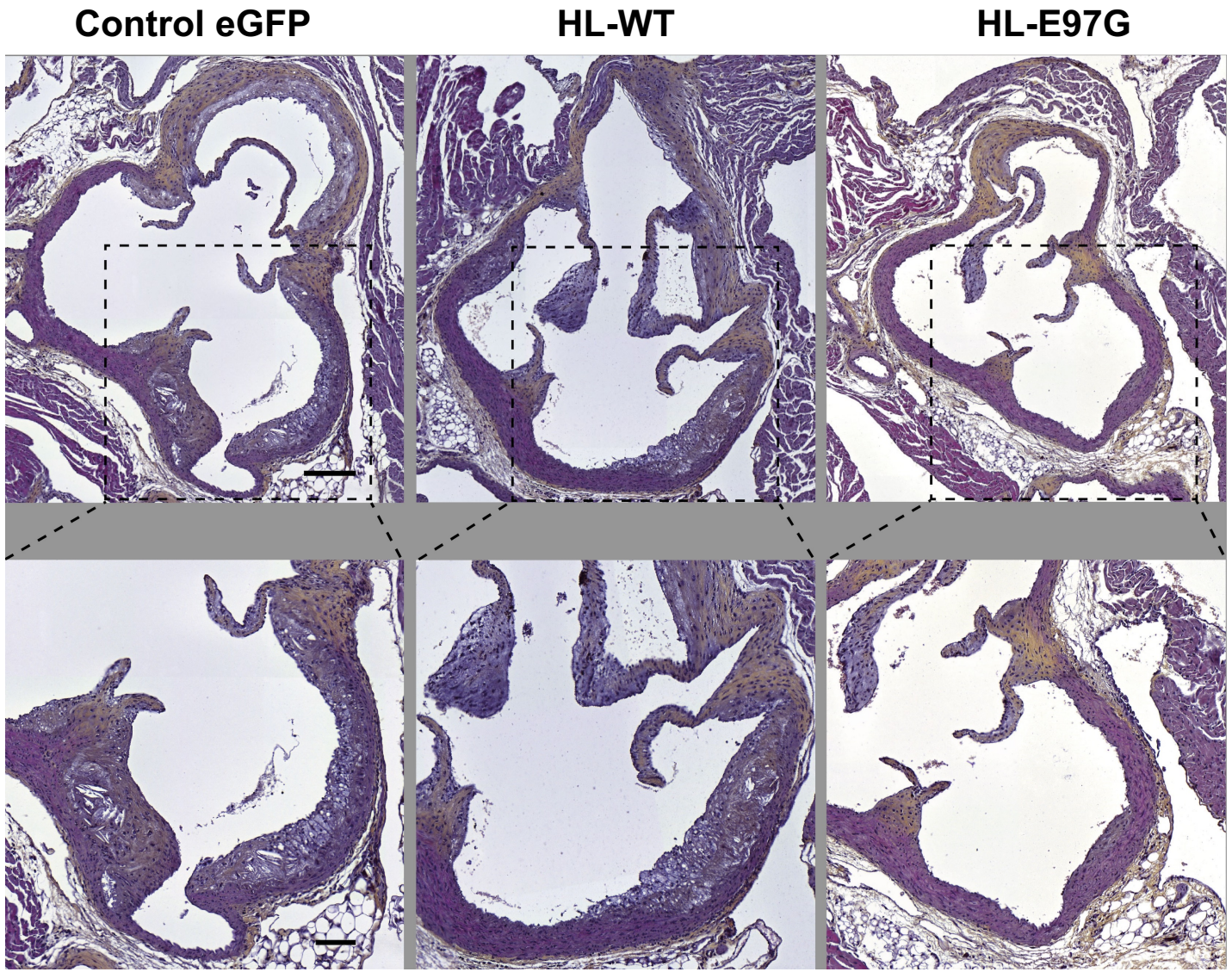
Control & HL WT mice show atherosclerotic lesions in the aortic root

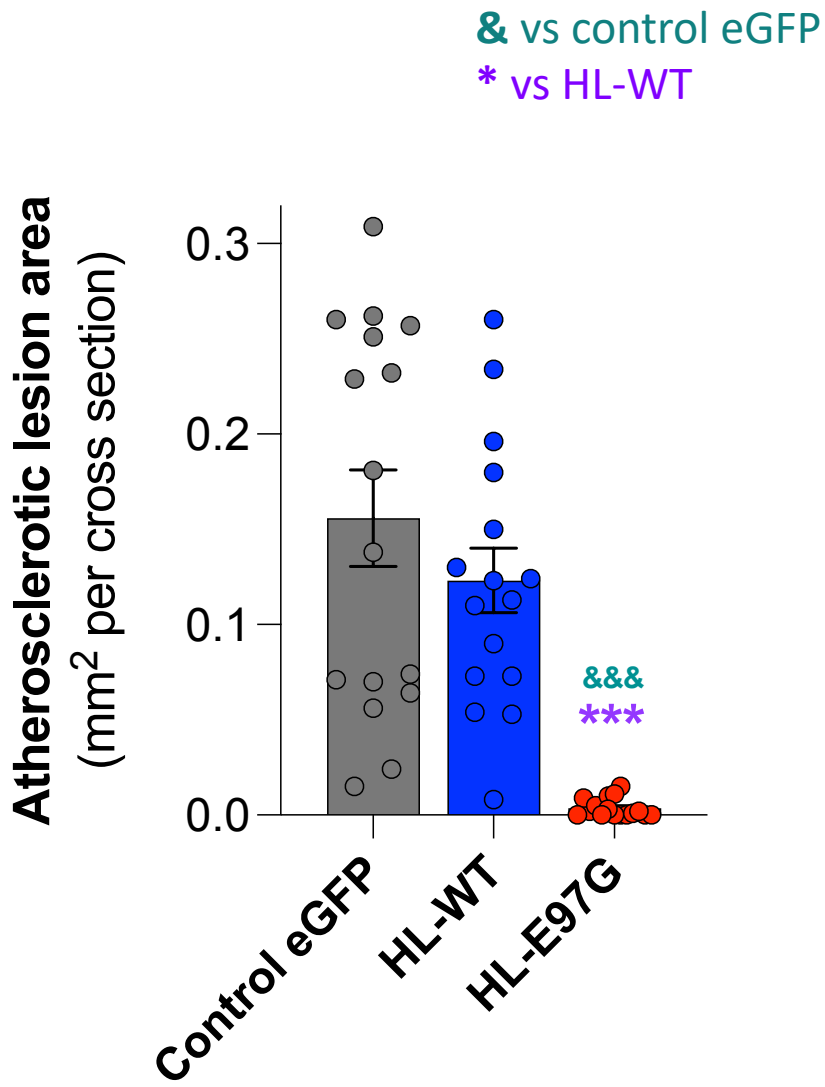




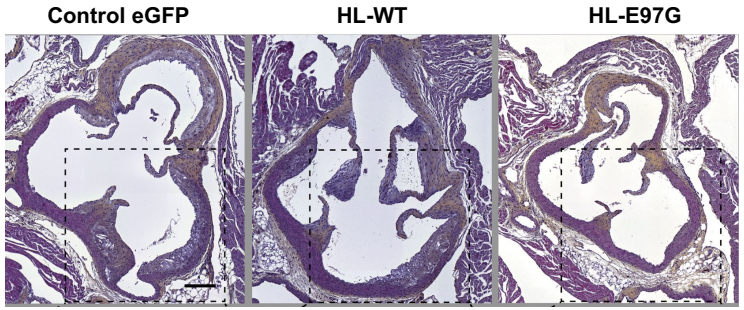


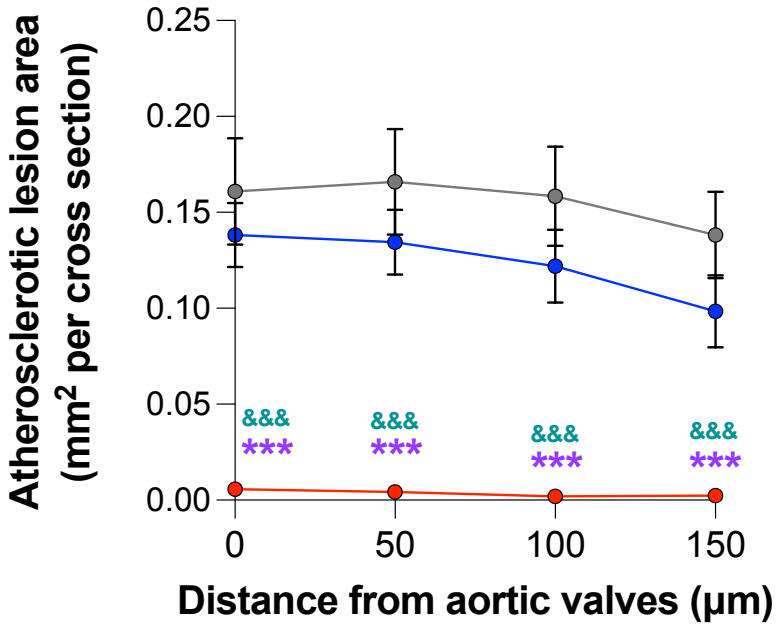
HL E97G largely reduces atherosclerotic lesions area

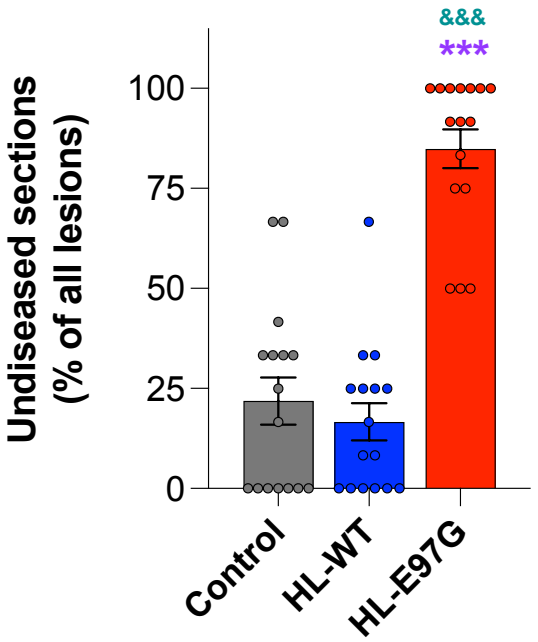


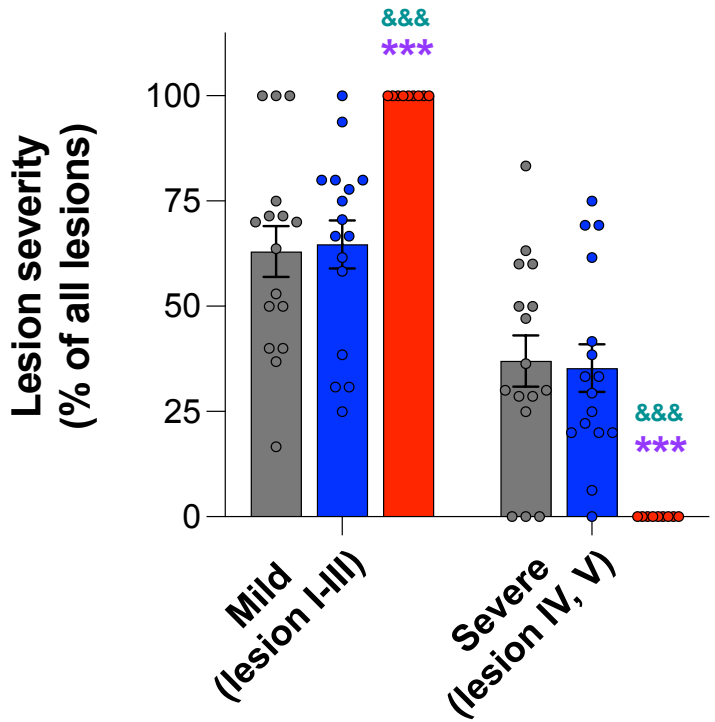


HL E97G largely reduce atherosclerotic lesions area and severity









Do the effects of HL-E97G on lipid and atherosclerosis depend on the presence of the LDLR?

Description of the experimental protocol

AAV8 -TBG- eGFP (3.10^{11} gc/mouse; **Control**)

AAV8 -TBG- *LIPC* (3.10^{11} gc/mouse; **HL WT**)

AAV8 -TBG- *LIPC* E97G (3.10^{11} gc/mouse; **HL E97G**)

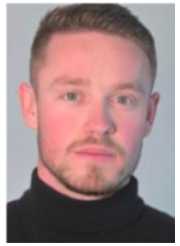
Pro-atherogenic diet *(1.25% Cholesterol and 0.5 Cholate)*

***Ldlr$^{-/-}$ M*ale & Female**

0 4 8 14 **Weeks**

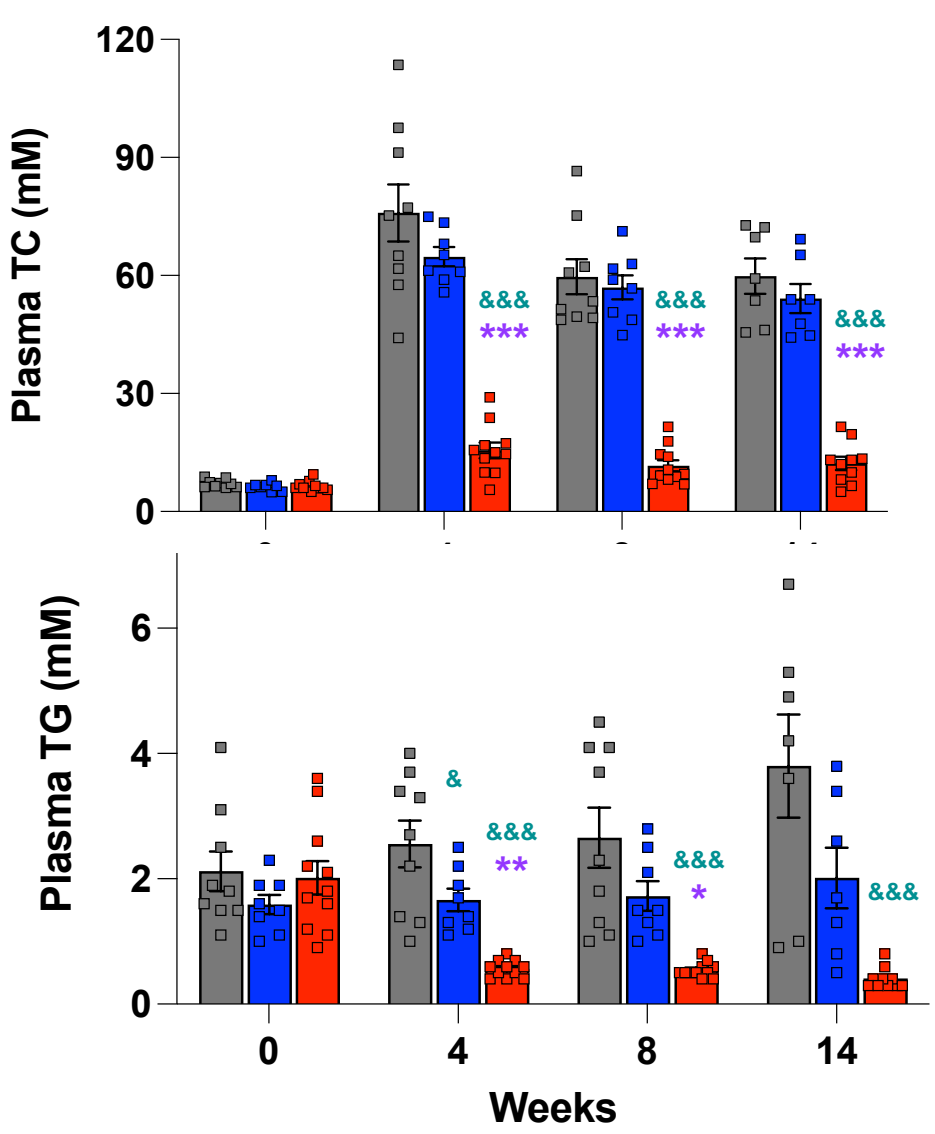
Body weight & Plasma lipid measurement

Atherosclerosis assessment



Thibaud Sotin

The combined lipid-lowering effect of HL E97G is unaffected by LDLR deficiency.

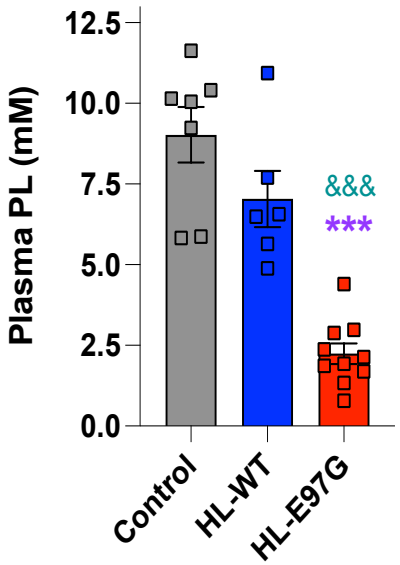


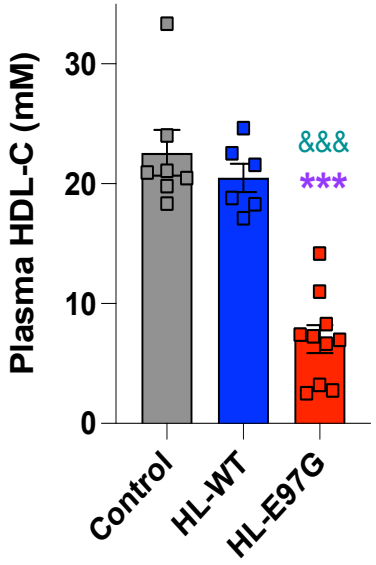
Control
HL-WT
HL-E97G

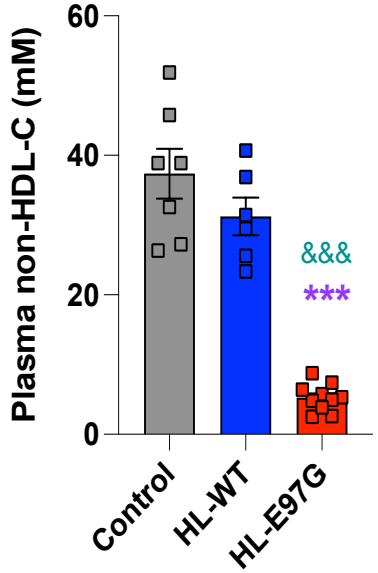
& vs control eGFP

***** vs HL-WT

Week 14



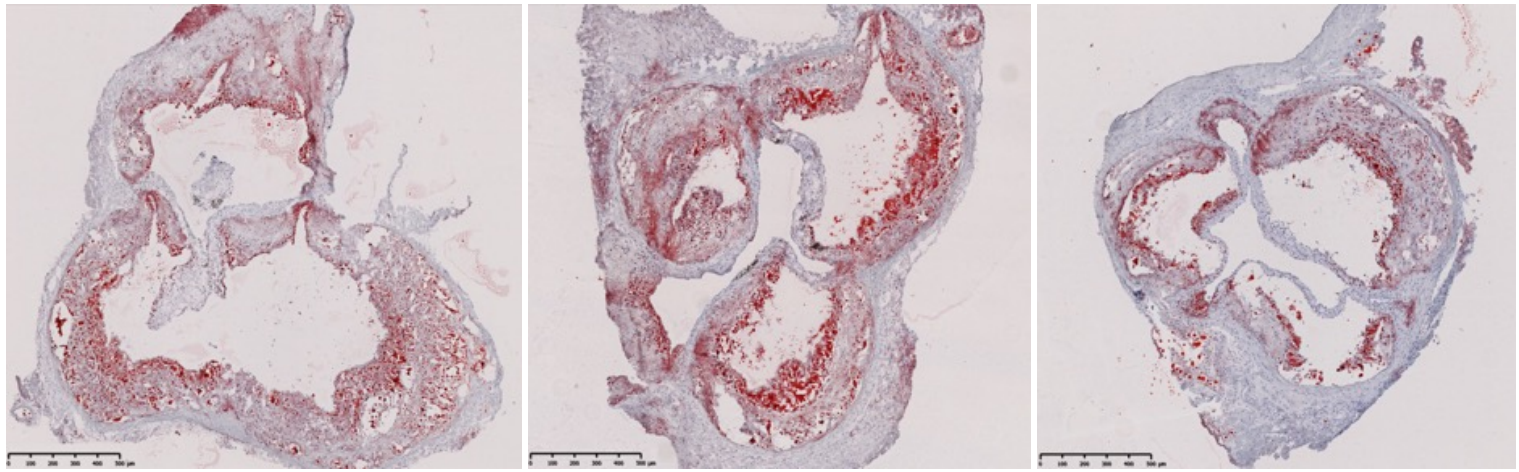


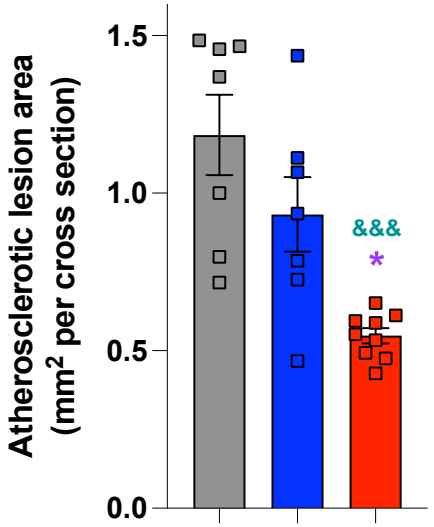


HL-E97G strongly reduces atherosclerotic lesions in *Ldlr*$^{-/-}$ mice

Aortic root

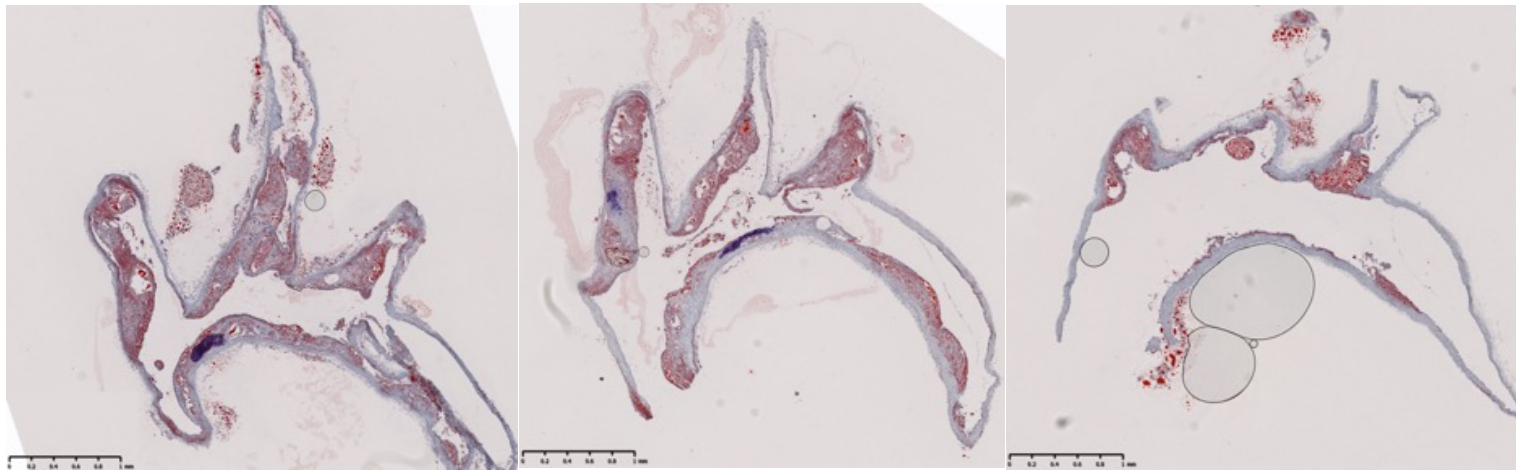
Control | HL-WT | HL-E97G

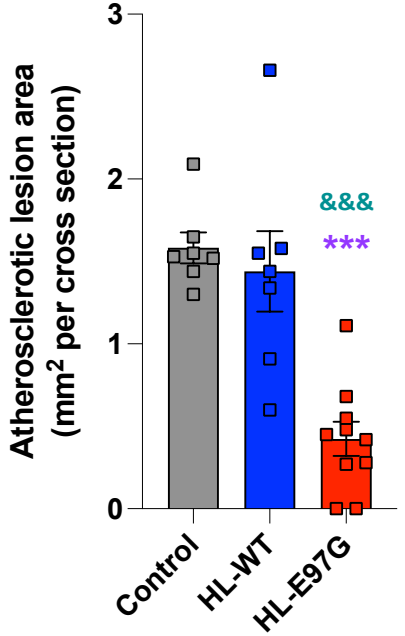




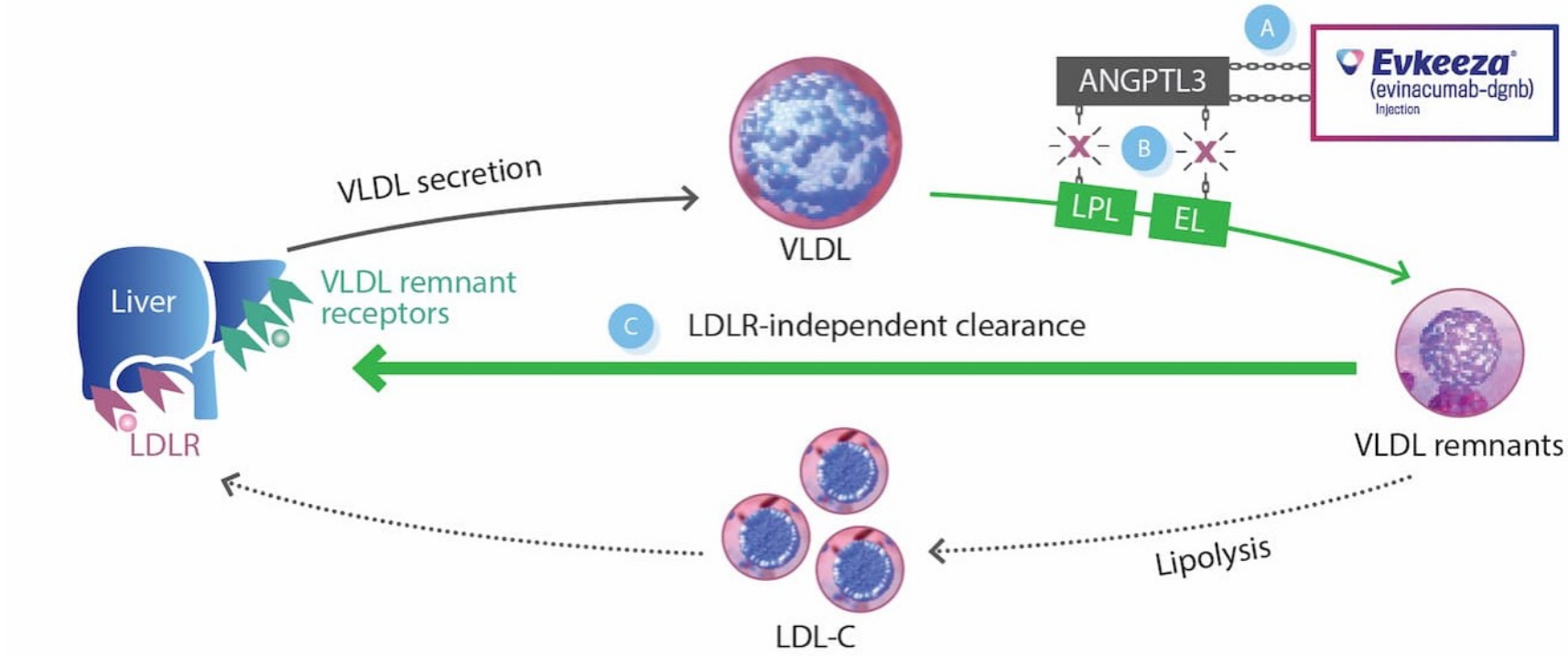
Aortic arch

Control | HL-WT | HL-E97G





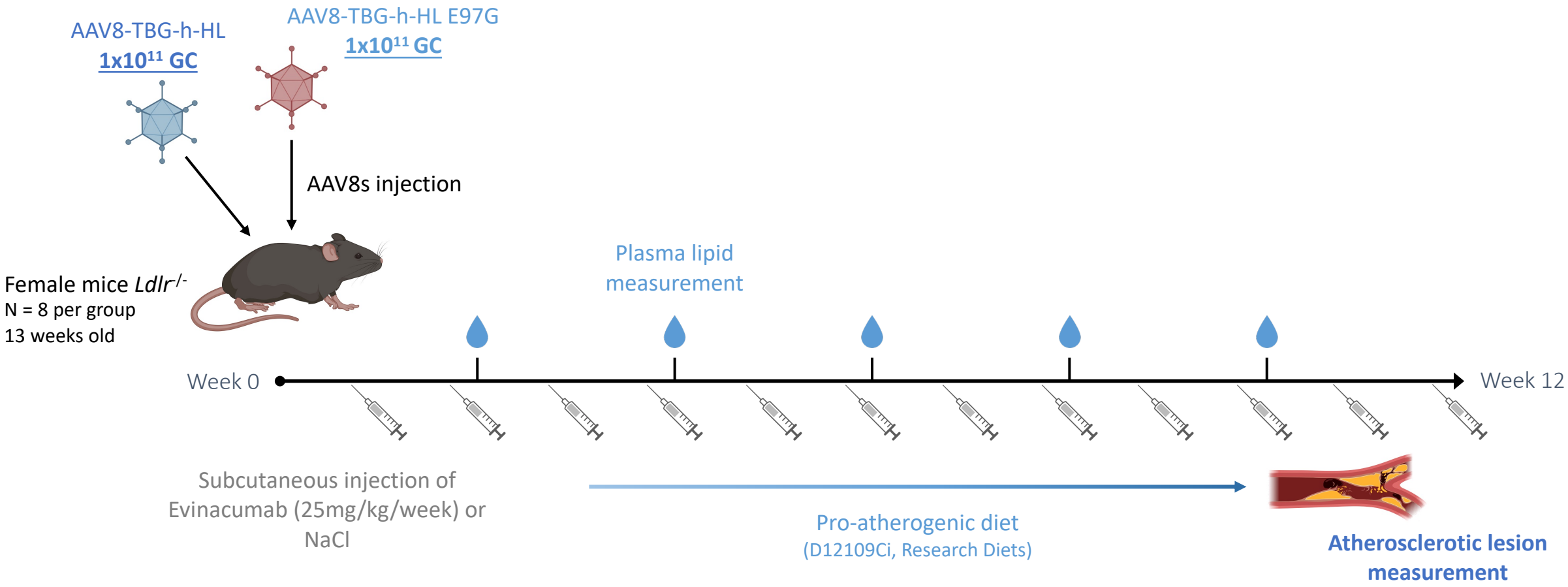
Can the effects of HL-E97G be combined with ANGPTL3 inhibitors ?



Similarities in the mode of action:
- Associated with familial combined hypocholesterolemia
- LDLR independent pathway

By inhibiting ANGPTL3, Evinacumab stimulates LPL & EL but not the WT form of HL (**quid of HL E97G ?**)

Description of the experimental protocol



Description of the experimental protocol

Lucie Vince

AAV8-TBG-h-HL
1x10^{11} GC

AAV8-TBG-h-HL E97G
1x10^{11} GC

AAV8s injection

Female mice *Ldlr*$^{-/-}$
N = 8 per group
13 weeks old

Plasma lipid measurement

Week 0

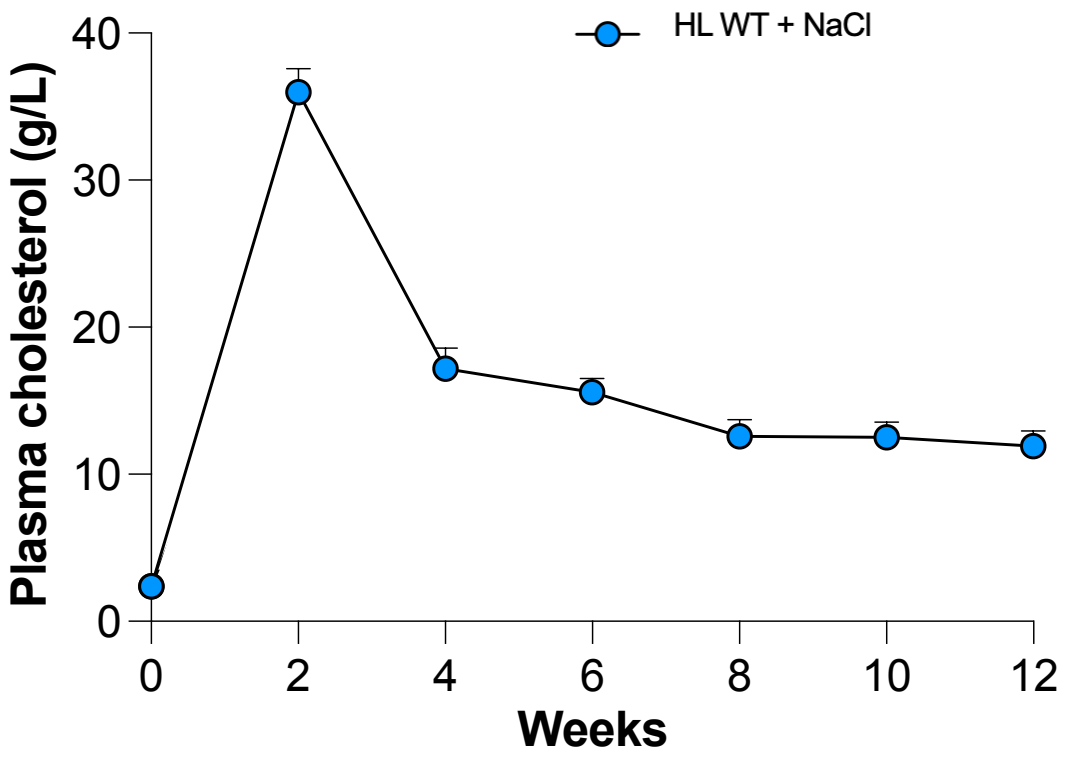
Week 12

Subcutaneous injection of Evinacumab (25mg/kg/week) or NaCl

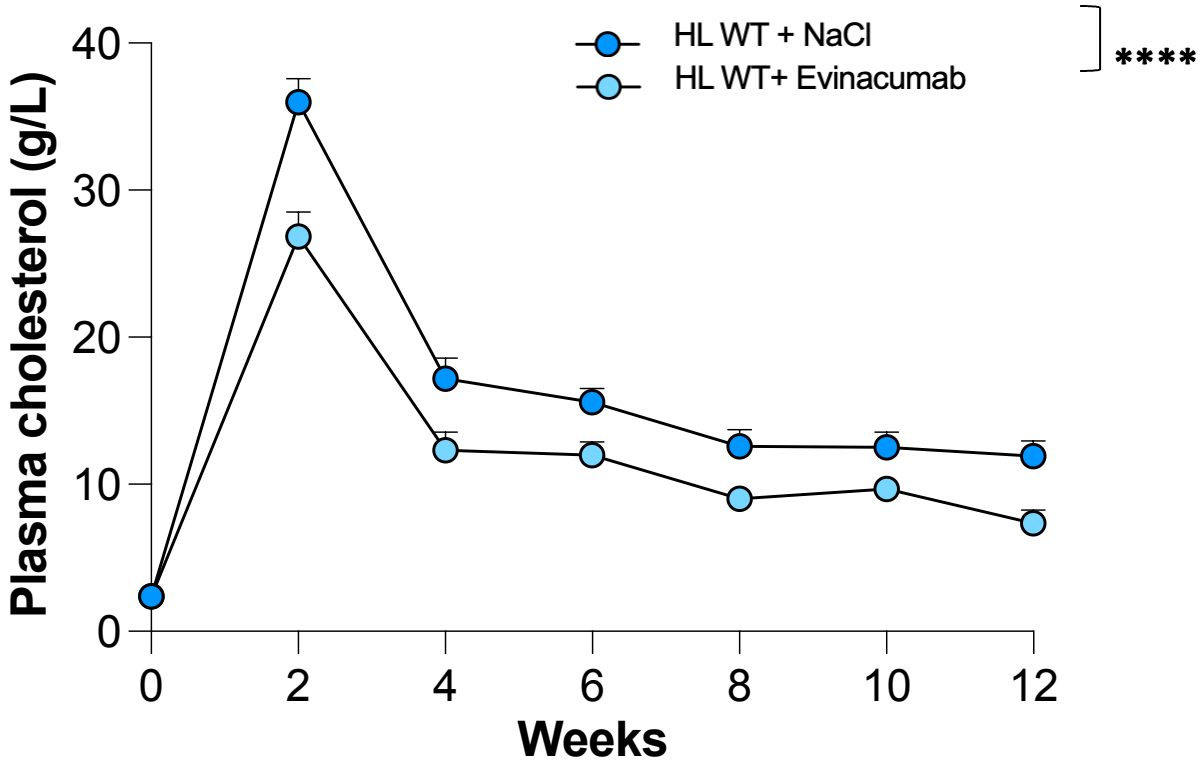
Pro-atherogenic diet
(D12109Ci, Research Diets)

Atherosclerotic lesion measurement

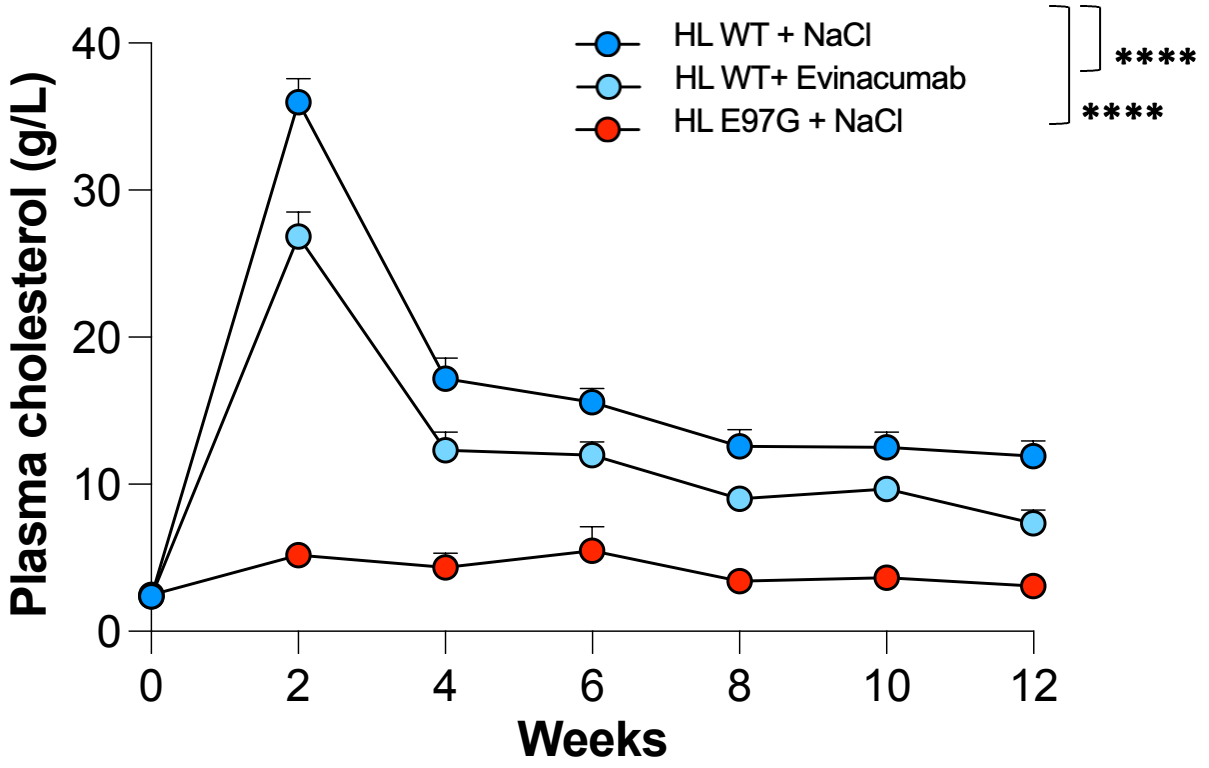
Ldlr$^{-/-}$ mice fed a pro-atherogenic diet develop severe hypercholesterolaemia



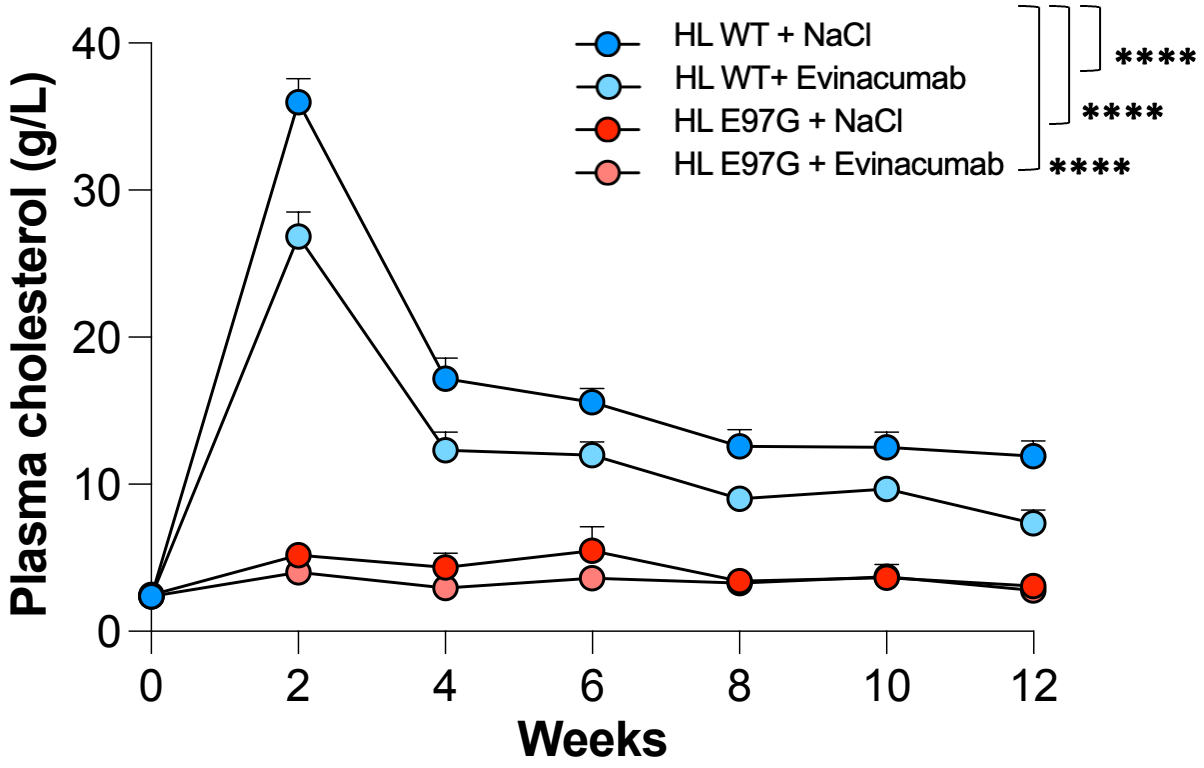
Evinacumab significantly decreases plasma cholesterol levels, independently of LDLR



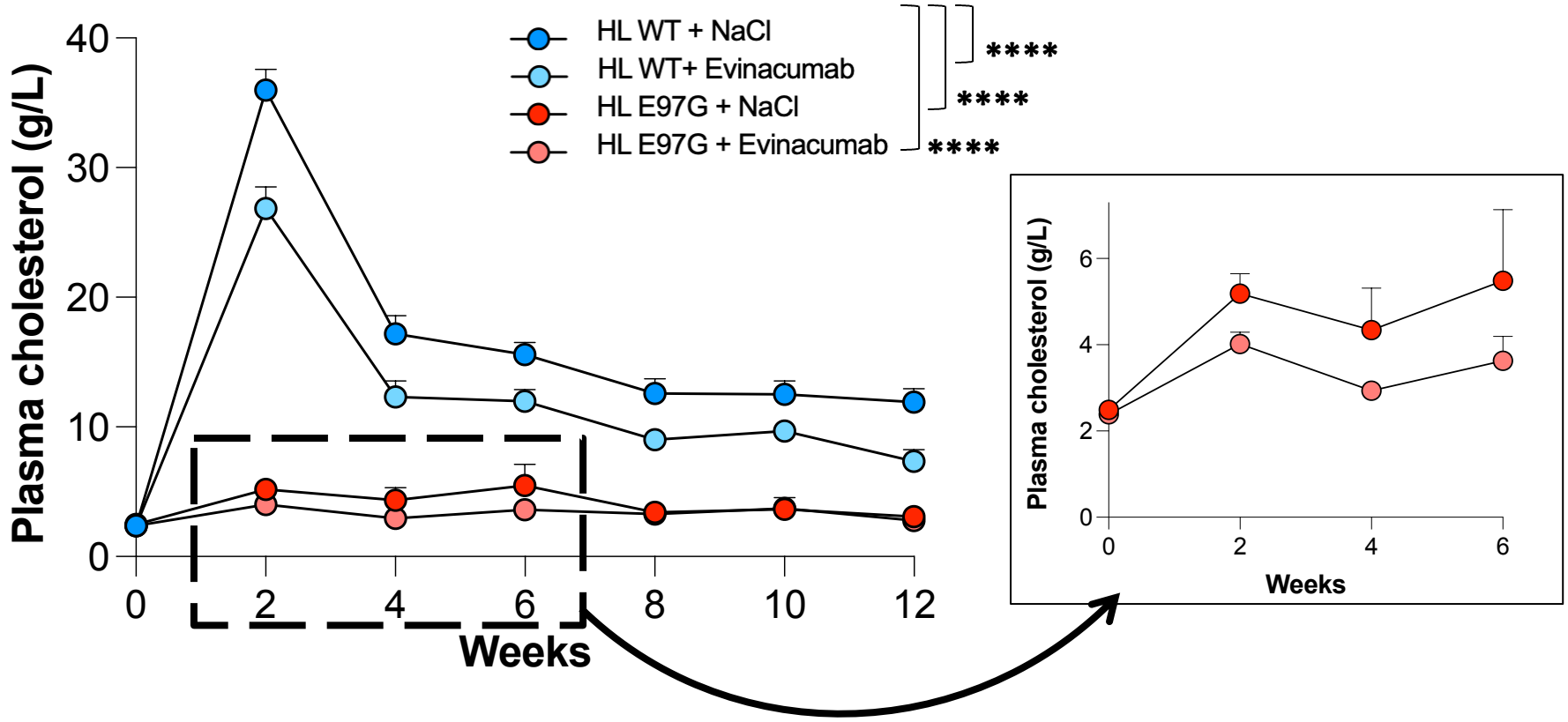
HL E97G drastically reduces plasma cholesterol levels



The cholesterol-lowering effect of combined treatments is difficult to see because of the lipid-lowering efficacy of HL E97G alone



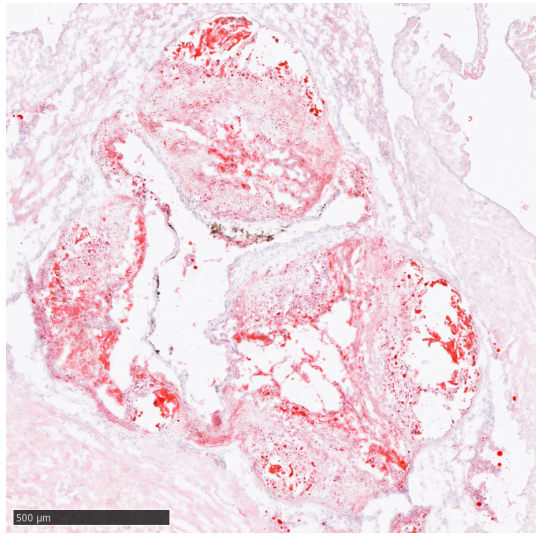
The cholesterol-lowering effect of combined treatments is difficult to see because of the lipid-lowering efficacy of HL E97G alone

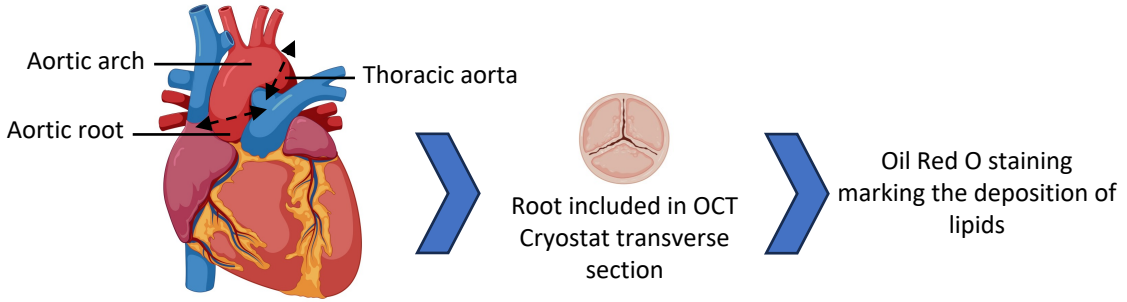


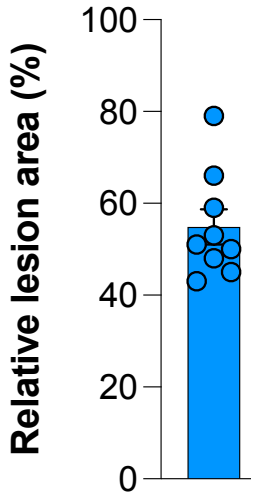
Atherosclerotic lesions are highly developed in the aortic root.

NaCl

HL wt





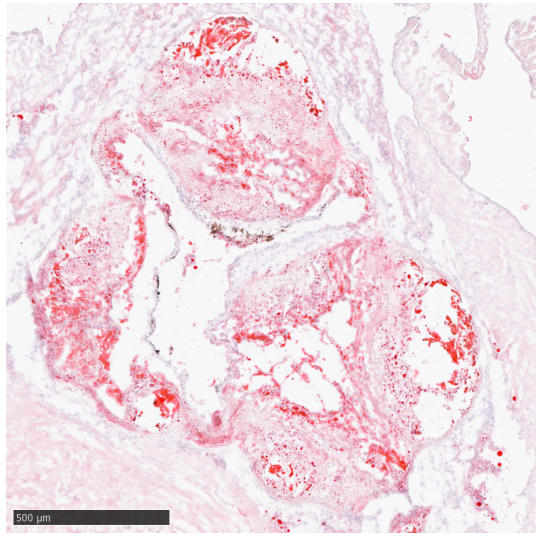


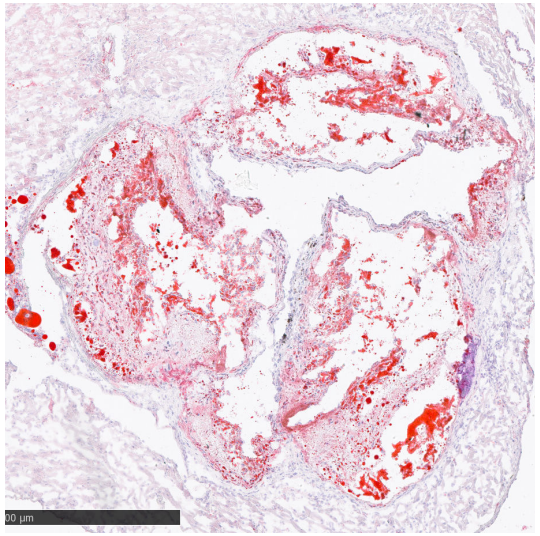
Both Evinacumab and HL E97G tend to limit plaque development in the aortic root

NaCl

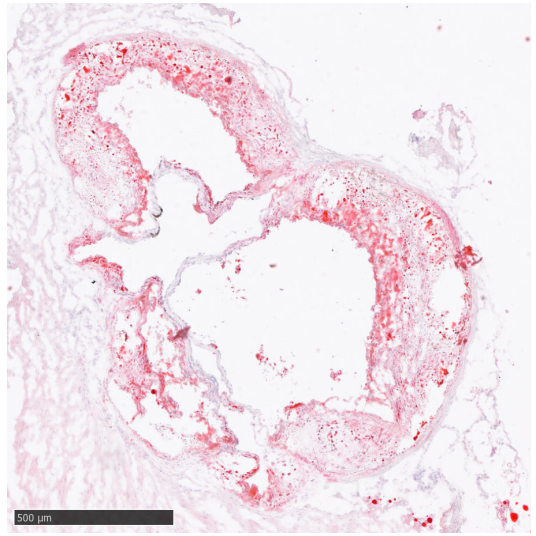
Evinacumab

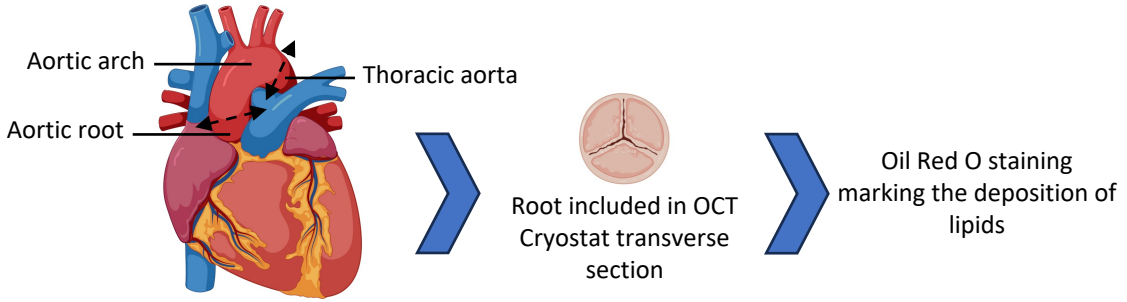
HL wt

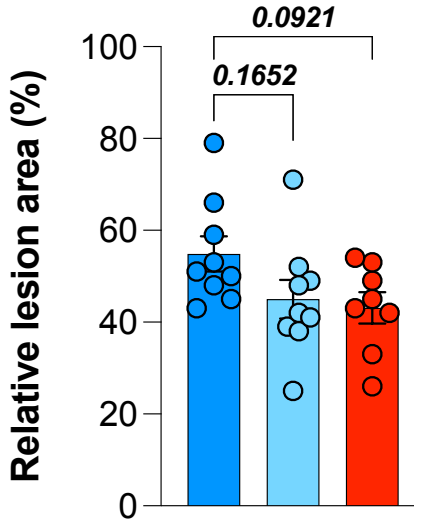




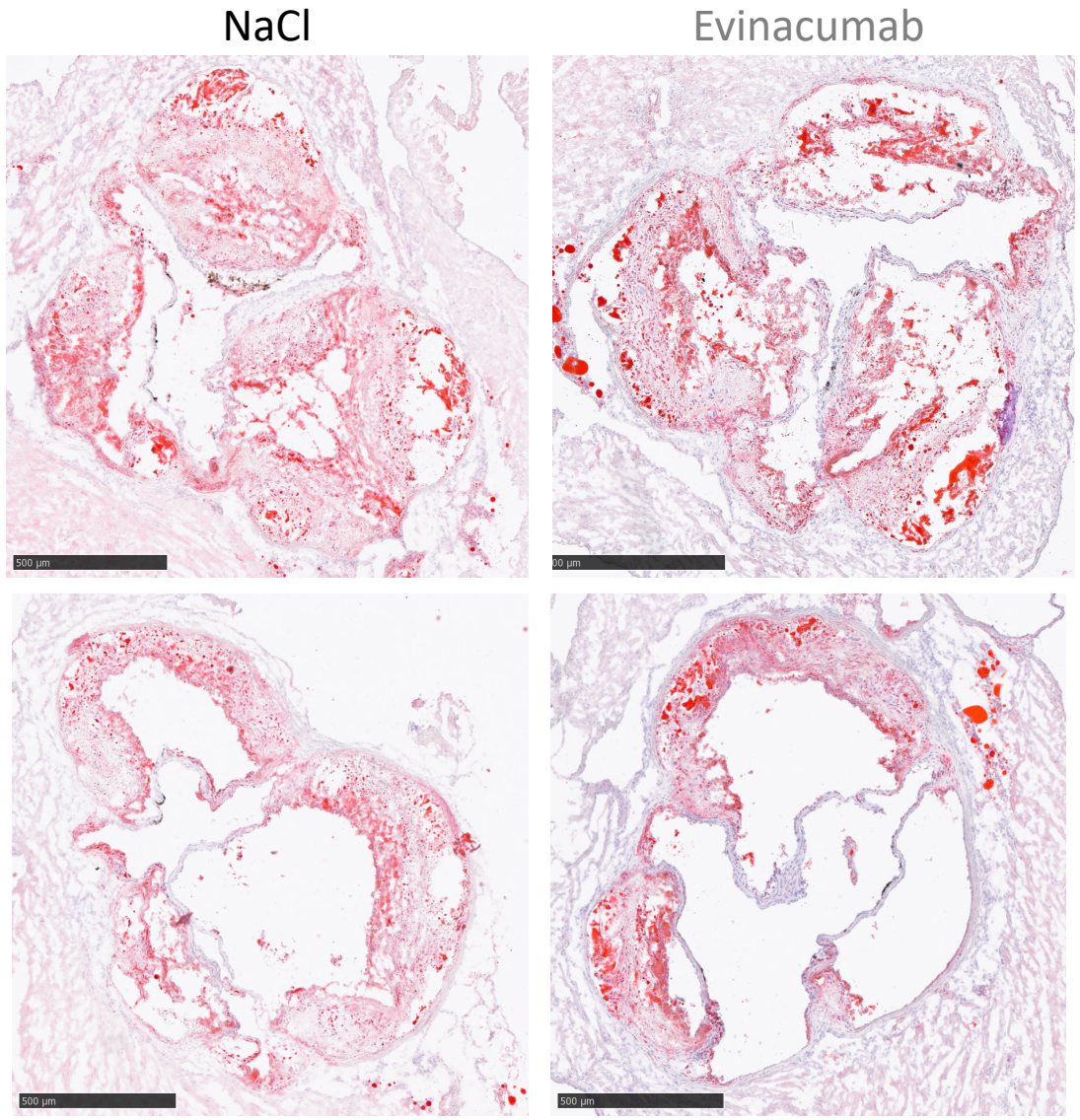
HL E97G

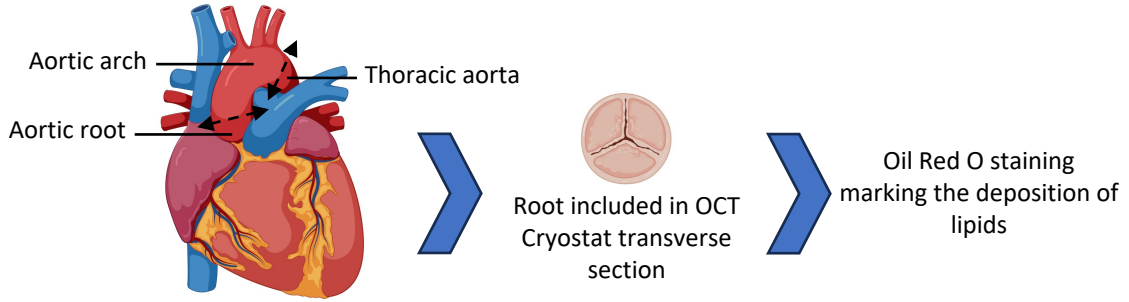


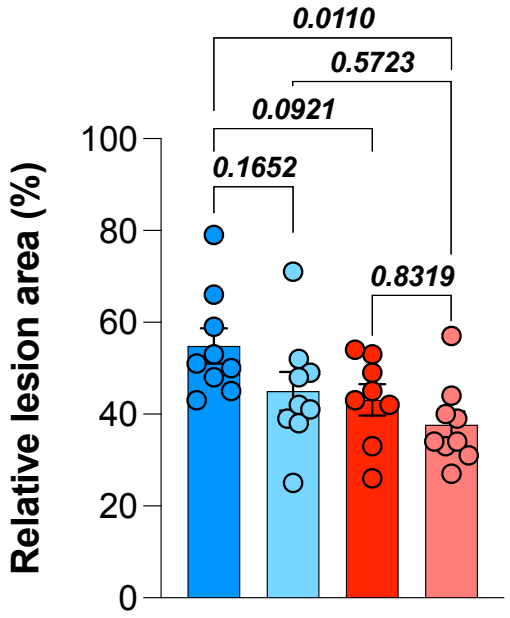




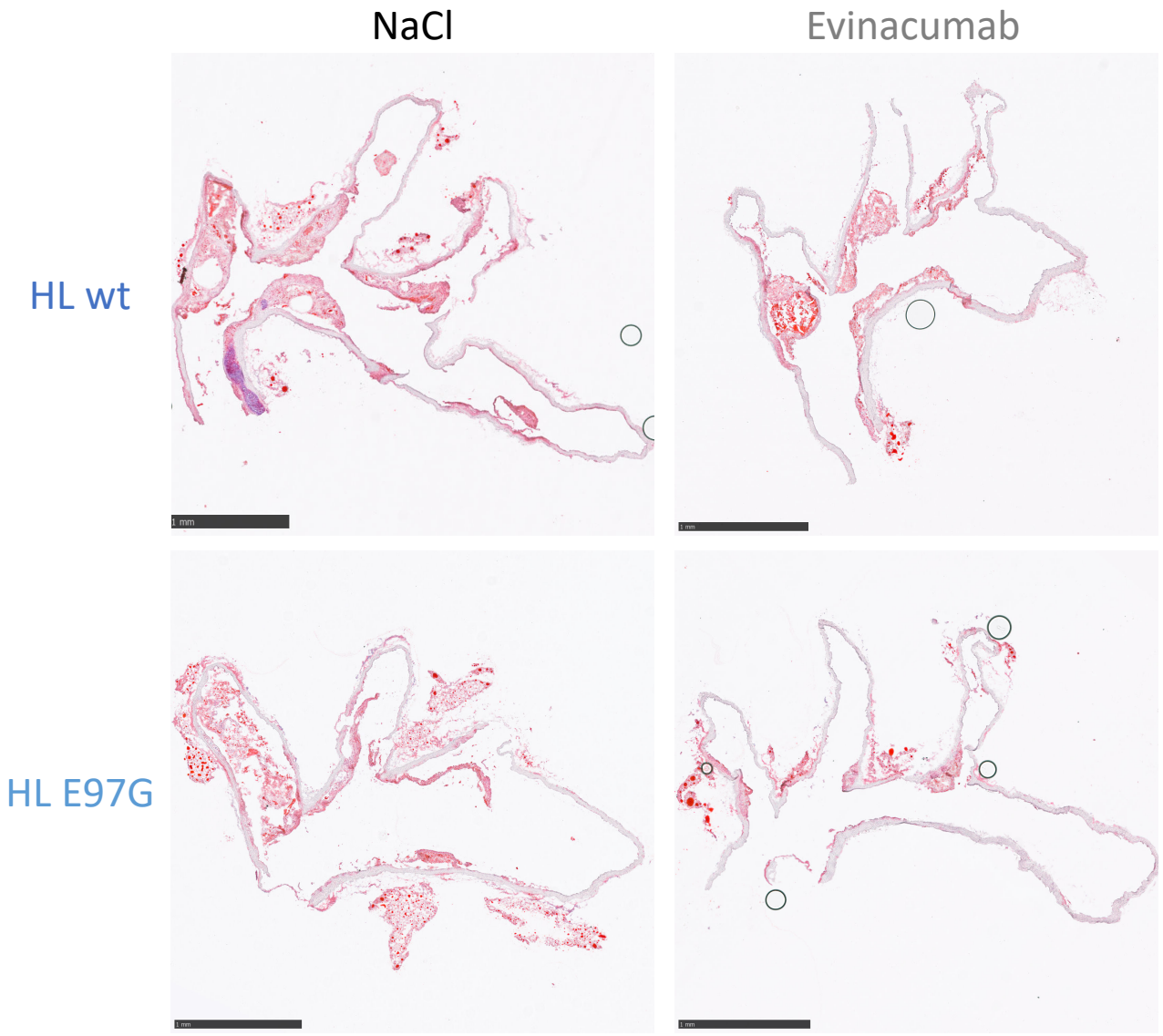
Co-treatment significantly reduces atherosclerotic lesions in the aortic root

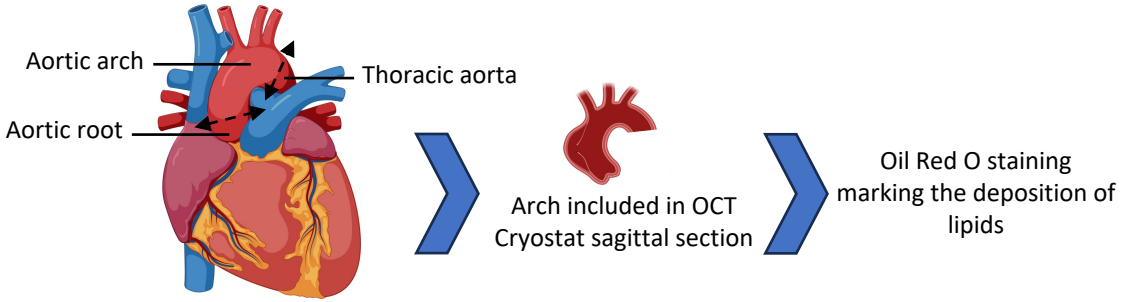


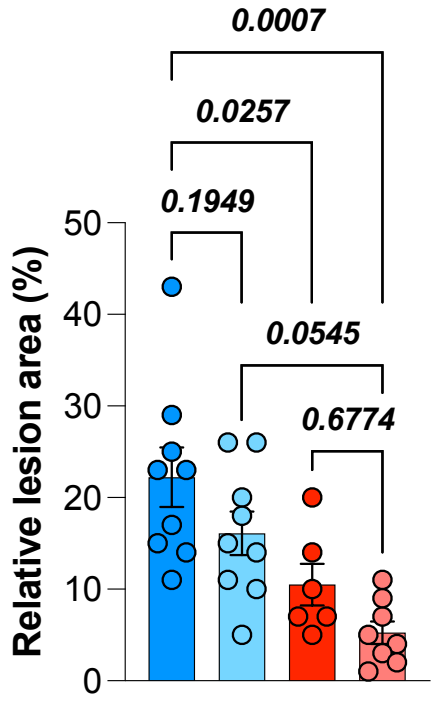




The HL E97G/Evinacumab combination drastically limits atherosclerosis progression







Conclusion 3

Variant *HL*-E97G :

- Alters the Lid region that regulates the accessibility of the substrate to the catalytic site
- Does not impact the triglyceride lipase activity
- Strongly increases the phospholipase activity (confirmed in humans, mice and cells)
- Induces a strong combined hypolipemia
- Strongly increase the plasma cholesterol clearance
- Does not affect the fecal excretion of neutral sterol
- Does not impact the VLDL production
- Effect on the hepatic uptake of VLDL is not so clear
- May promote the hepatic uptake of LDL… ? What about HDL particles ?
- Strongly attenuates atherosclerosis development, with respect to lesion size and severity
- Acts independently of the LDLR and ANGPTL3
- Relevance in humans ?

Conclusion 3

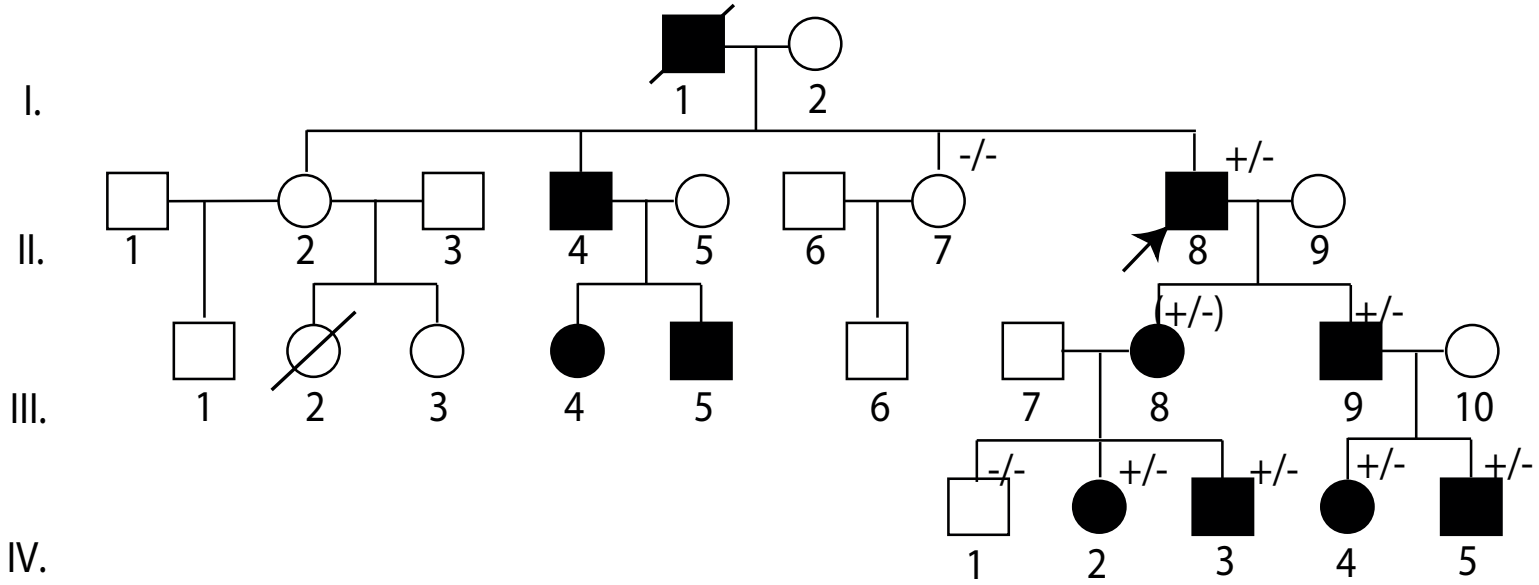
Variant *HL*-E97G :

- Relevance in humans ?


Ph. Moulin
M. Di Filippo

Hospices Civils de Lyon

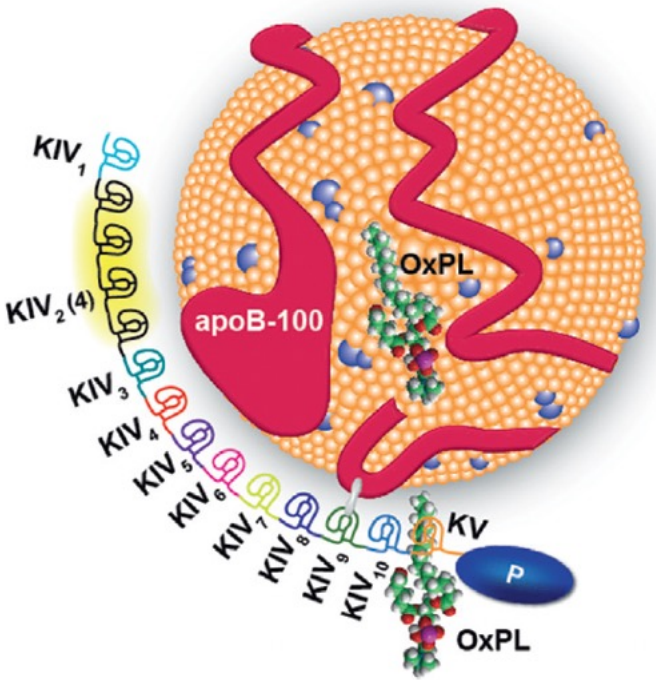
Do E97G carriers have atherosclerotic lesions ?



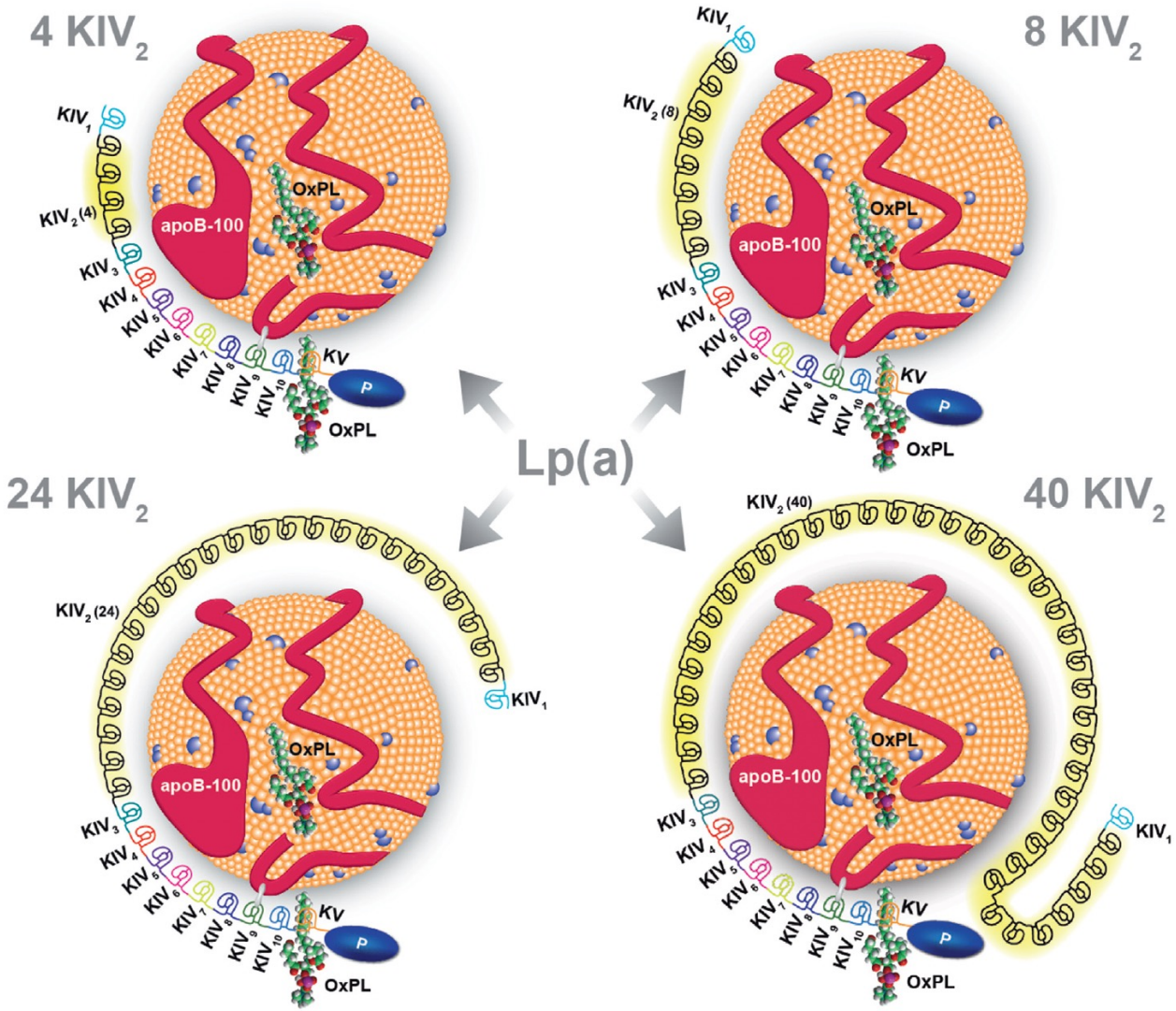
Assess atherosclerosis using
carotid ultrasonography

La Dernière Cible ?

La Lipoprotéine(a) ou Lp(a)



Les lipoprotéines (a) – une large famille



<u>Homologie de structure avec le plasminogène</u>

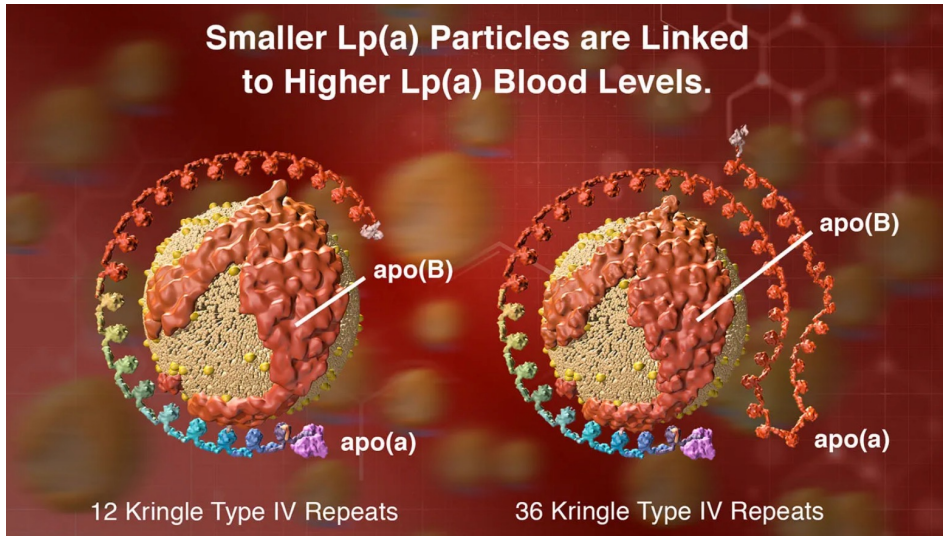
Ne contient pas de Kringle I, II, III

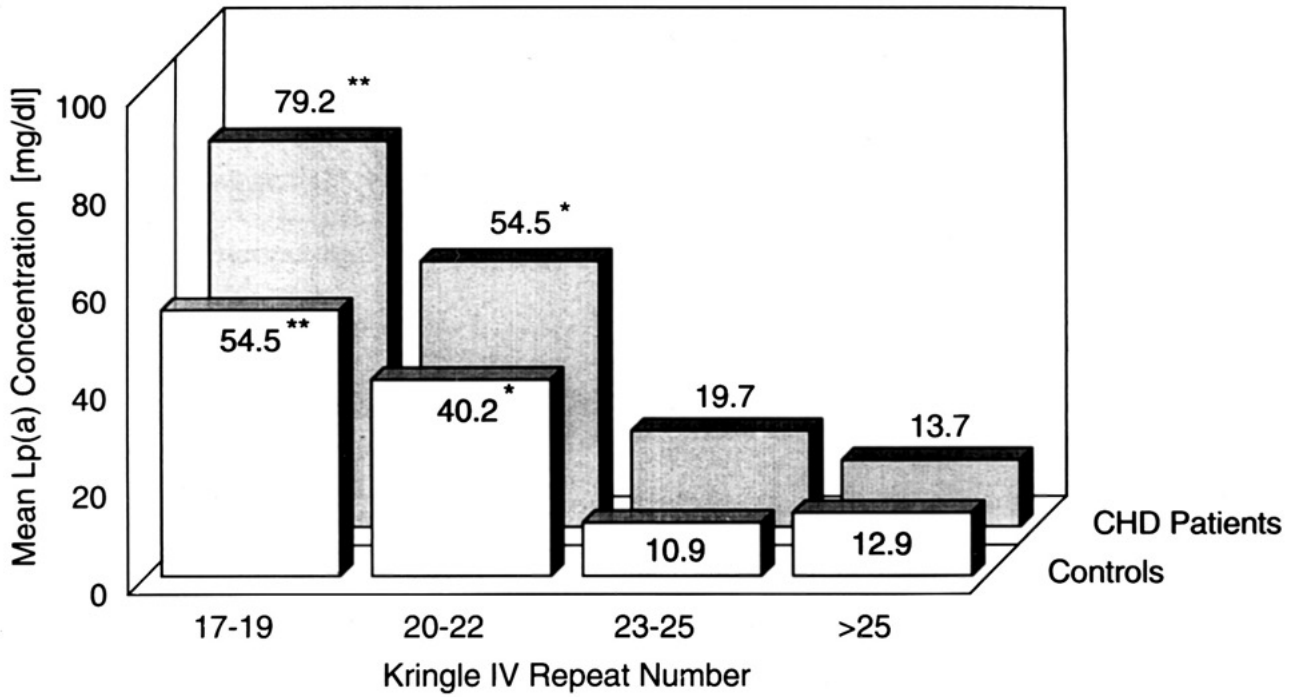
Possède 10 isoformes de KIV:

1 copie de KIV_1, KIV_{3-10}
1 à 40 copies de KIV_2
1 copie de KV
1 domaine protéase inactif

Tsimikas S. JACC. 2017. 69 (6) : 692 – 711.

Existence d'un lien inverse entre la taille de Lp(a) et sa concentration plasmatique



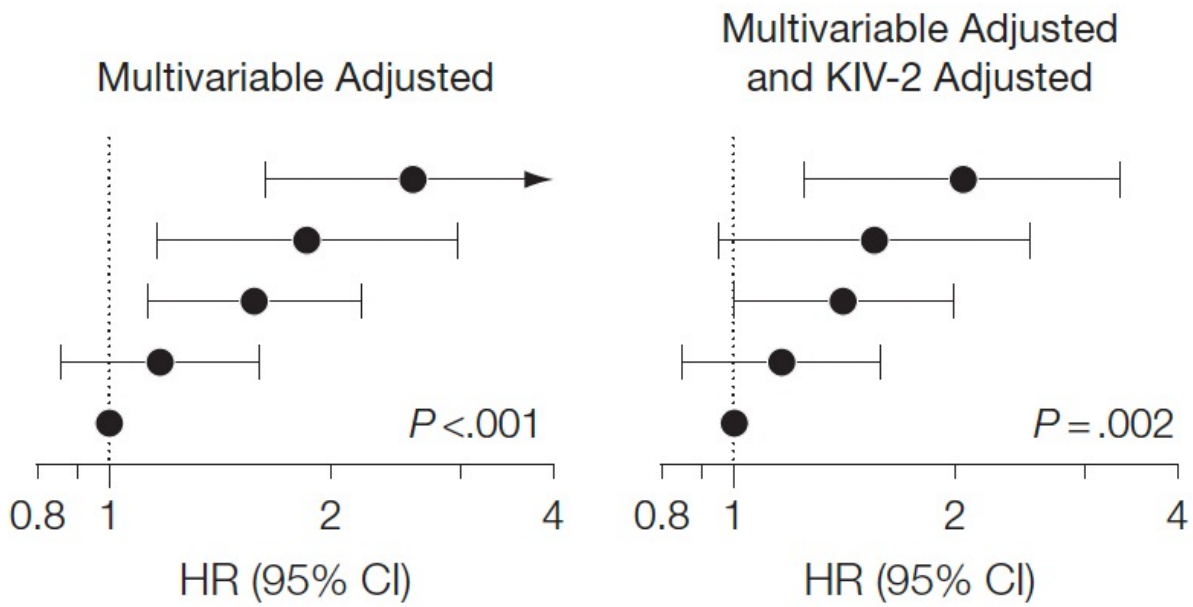


Arterioscler Thromb Vasc Biol. 1996 Jun;16(6):713-9.

Des concentrations élevées en Lp(a) sont associées à un risque accru de maladies cardiovasculaires

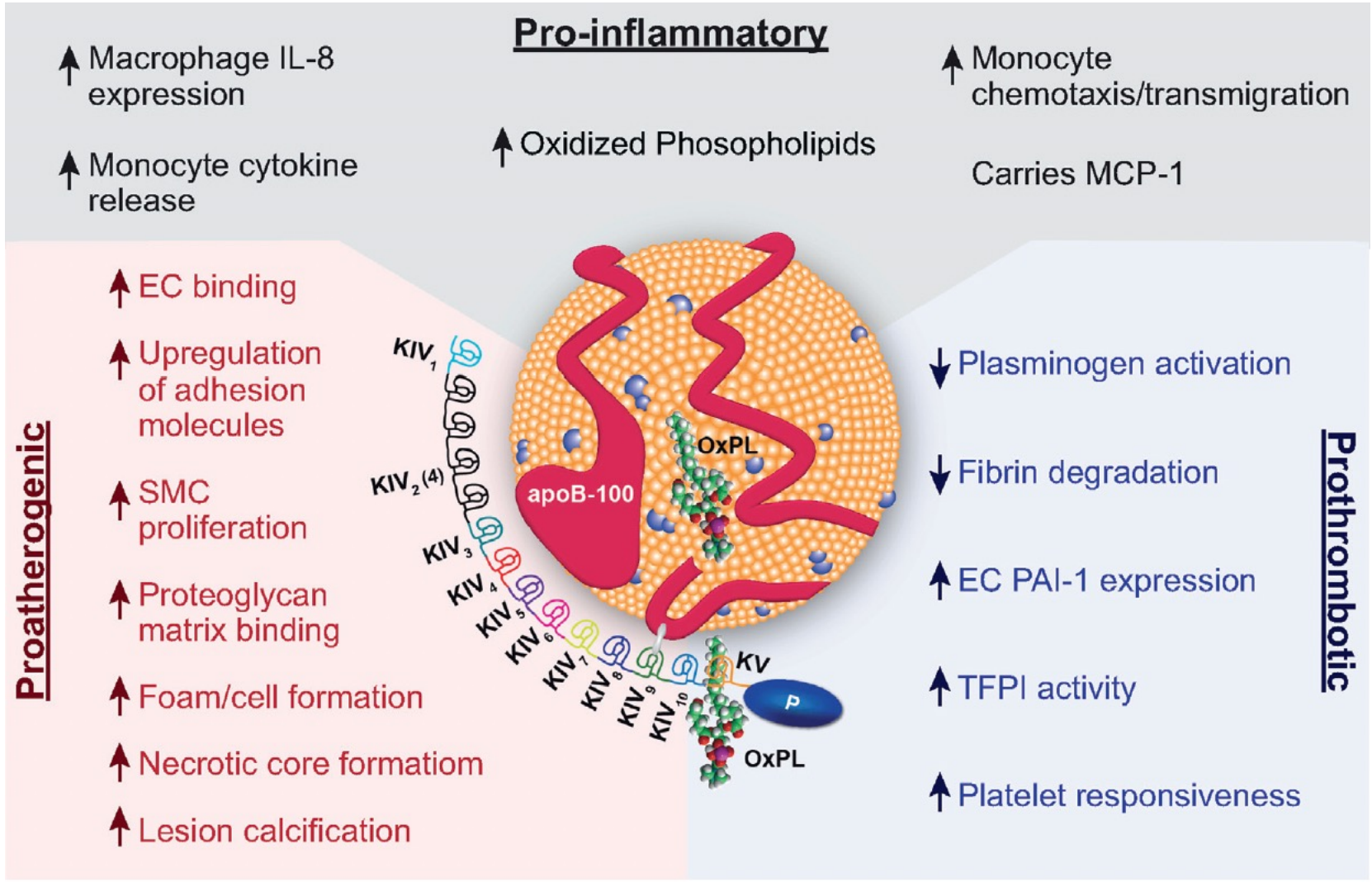
Figure 1. Risk of Myocardial Infarction by Extreme Levels of Lipoprotein(a) in the General Population

Lipoprotein(a)		Participants, No.	Events, No.
Percentile	mg/dL		
>95th	>117	376	46
90th-95th	77-117	450	46
67th-89th	30-76	1731	155
22nd-66th	5-29	3385	241
<22nd [Reference]	<5	1582	104



Kamstrup PR et al. JAMA. 2009;301(22):2331-2339

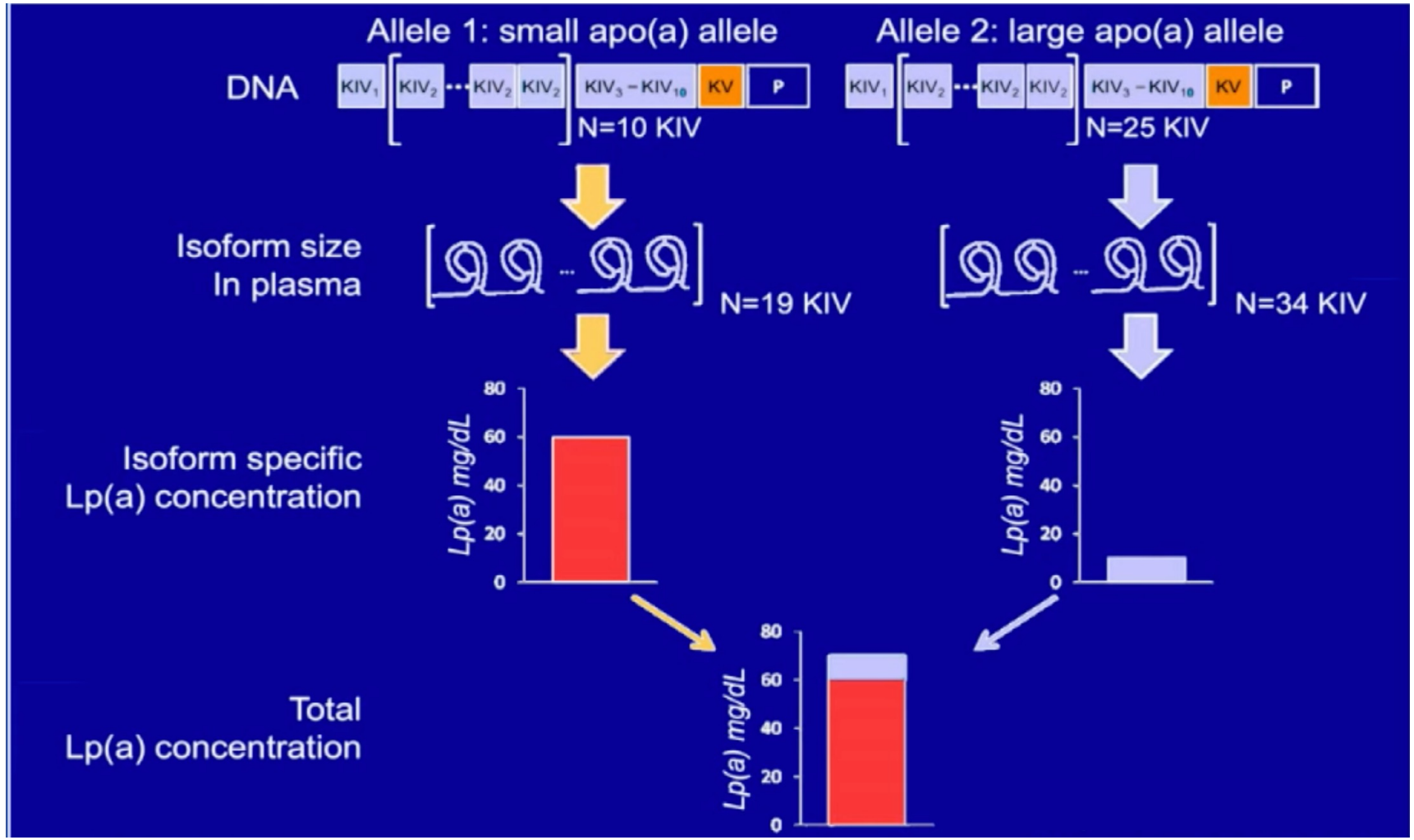
La Lp(a) exerce des actions pro-athérogènes, pro-inflammatoires et pro-thrombotiques



Tsimikas S. JACC. 2017. 69 (6) : 692 – 711.

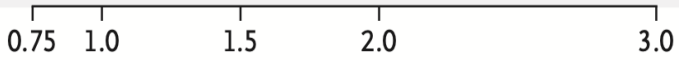
Les concentrations en Lp(a) sont majoritairement déterminées par la génétique

Les interventions alimentaires et/ou sur l'hygiène de vie n'ont pas/peu d'impact



Les variants génétiques de Lp(a) sont associés à des concentrations élevées de Lp(a) et à un risque élevé de maladies coronaires

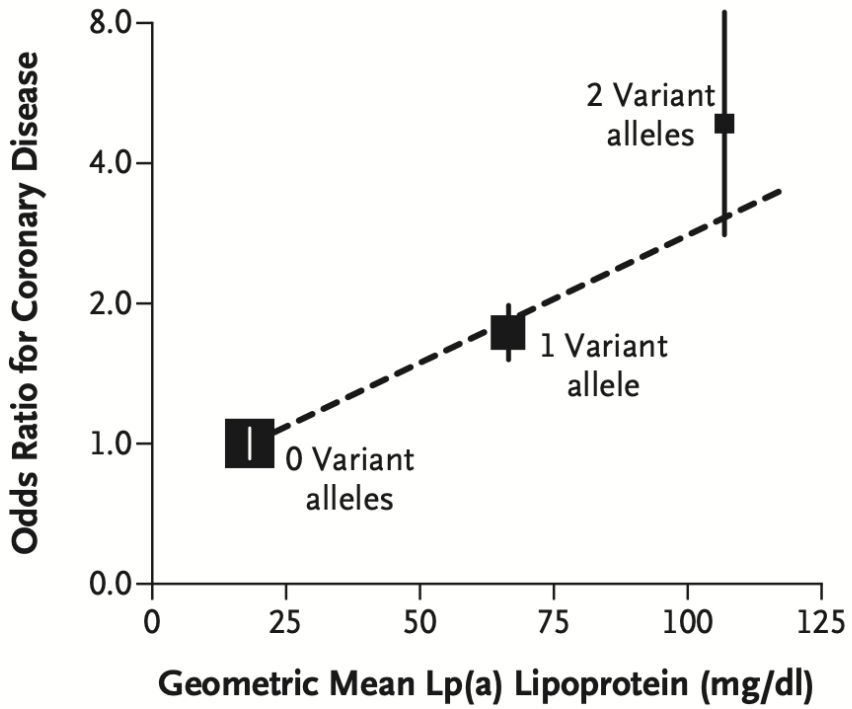
SNP	Risk Allele (Frequency)	Regression Coefficient (SE)	Odds Ratio for Coronary Disease (95% CI)
rs3798220*†	C (0.02)	1.27 (0.08)	1.92 (1.48–2.49)
rs10455872*†	G (0.07)	1.18 (0.04)	1.70 (1.49–1.95)
rs4708871	T (0.93)	0.53 (0.11)	1.16 (0.93–1.45)
rs11751605*†	C (0.16)	0.50 (0.04)	1.21 (1.10–1.34)
rs6919346*†	C (0.83)	0.43 (0.05)	1.11 (1.00–1.24)
rs13202636*	G (0.78)	0.33 (0.04)	1.09 (0.99–1.20)
rs9355813*	T (0.64)	0.32 (0.04)	1.08 (1.00–1.18)
rs10945682*	G (0.64)	0.32 (0.04)	1.08 (1.00–1.18)
rs3127596*†	G (0.30)	0.30 (0.04)	1.13 (1.04–1.23)
rs3798221*	G (0.81)	0.28 (0.05)	1.08 (0.97–1.19)
rs10755578*†	G (0.48)	0.27 (0.04)	1.11 (1.03–1.20)
rs6923877*	A (0.67)	0.26 (0.04)	1.08 (1.00–1.18)
rs7765781*	G (0.67)	0.26 (0.04)	1.08 (1.00–1.18)
rs7765803*	G (0.67)	0.26 (0.04)	1.08 (1.00–1.17)
rs1321195*	G (0.86)	0.26 (0.05)	1.01 (0.90–1.14)
rs1652507	T (0.84)	0.00 (0.05)	0.97 (0.88–1.08)
rs783149	C (0.84)	0.00 (0.05)	0.97 (0.88–1.08)



Associations of Single-Nucleotide Polymorphisms (SNPs) in *LPA* with the Lp(a) Lipoprotein Level and the Risk of Coronary Disease in the PROCARDIS Cohort.

Clarke R. et al. N Engl J Med 2009;361:2518-28.

Les variants génétiques de Lp(a) sont associés à des concentrations élevées de Lp(a) et à un risque élevé de maladies coronaires



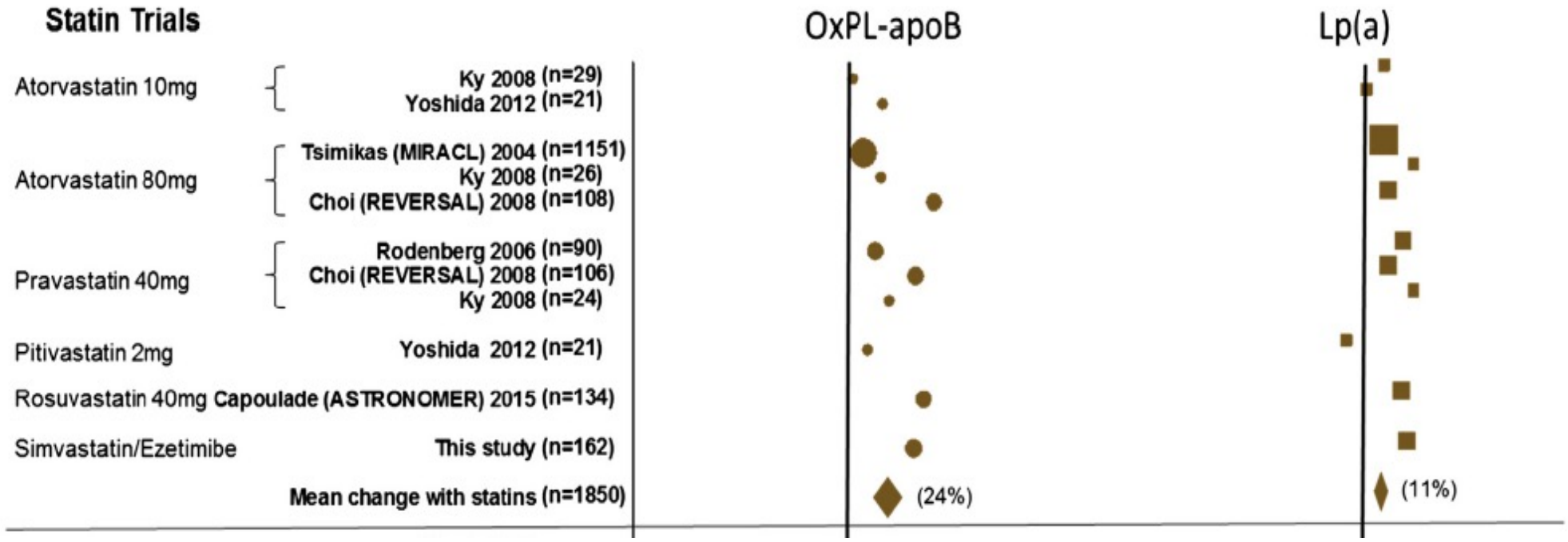
Association of the *LPA* Genotype Score with the Lp(a) Lipoprotein Level and the Risk of Coronary Disease in the PROCARDIS Cohort.

The odds ratios (squares, with the size inversely proportional to the sampling variation) are for the association of the *LPA* genotype score (no variant alleles, one variant allele, or two variant alleles) with the risk of coronary disease, as measured with the use of "floating absolute risks" which summarize the sampling variation for the three genotype scores without the selection of an arbitrary baseline genotype score. The vertical lines indicate 95% confidence intervals.

Clarke R. et al. N Engl J Med 2009;361:2518-28.

Lp(a) et lipid lowering therapies

Statins have no effect on Lp(a) levels (or even a slighter increase)



Yeang C et al. J Clin Lipidology 2016

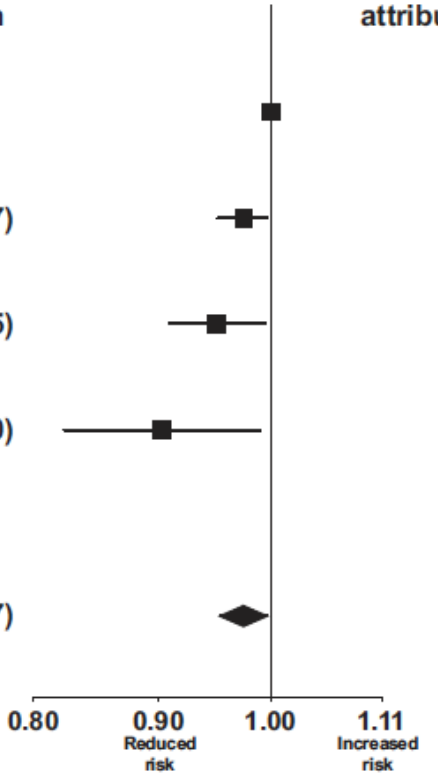
Lp(a) et lipid lowering therapies

ESC European Society of Cardiology
European Heart Journal (2020) **41**, 4245–4255
doi:10.1093/eurheartj/ehaa649

Lipoprotein(a) lowering by alirocumab reduces the total burden of cardiovascular events independent of low-density lipoprotein cholesterol lowering: ODYSSEY OUTCOMES trial

PCSK9 inhibitors reduce Lp(a) levels

Baseline Lp(a) quartile	Month 4 median Lp(a) reduction	HR (95% CI) for Lp(a) median reduction	Joint risk reduction attributable to Lp(a)
Quartile 1 (<6.7 mg/dL)	0 mg/dL	1.00	0%
Quartile 2 (6.7 to <21.2 mg/dL)	5.1 mg/dL	0.975 (0.952, 0.997)	13%
Quartile 3 (21.2 to <59.6 mg/dL)	9.9 mg/dL	0.951 (0.909, 0.995)	23%
Quartile 4 (≥59.6 mg/dL)	20.1 mg/dL	0.903 (0.824, 0.990)	39%
Overall	5.0 mg/dL	0.975 (0.953, 0.997)	13%



Lipid lowering therapies targeting Lp(a) in developpement

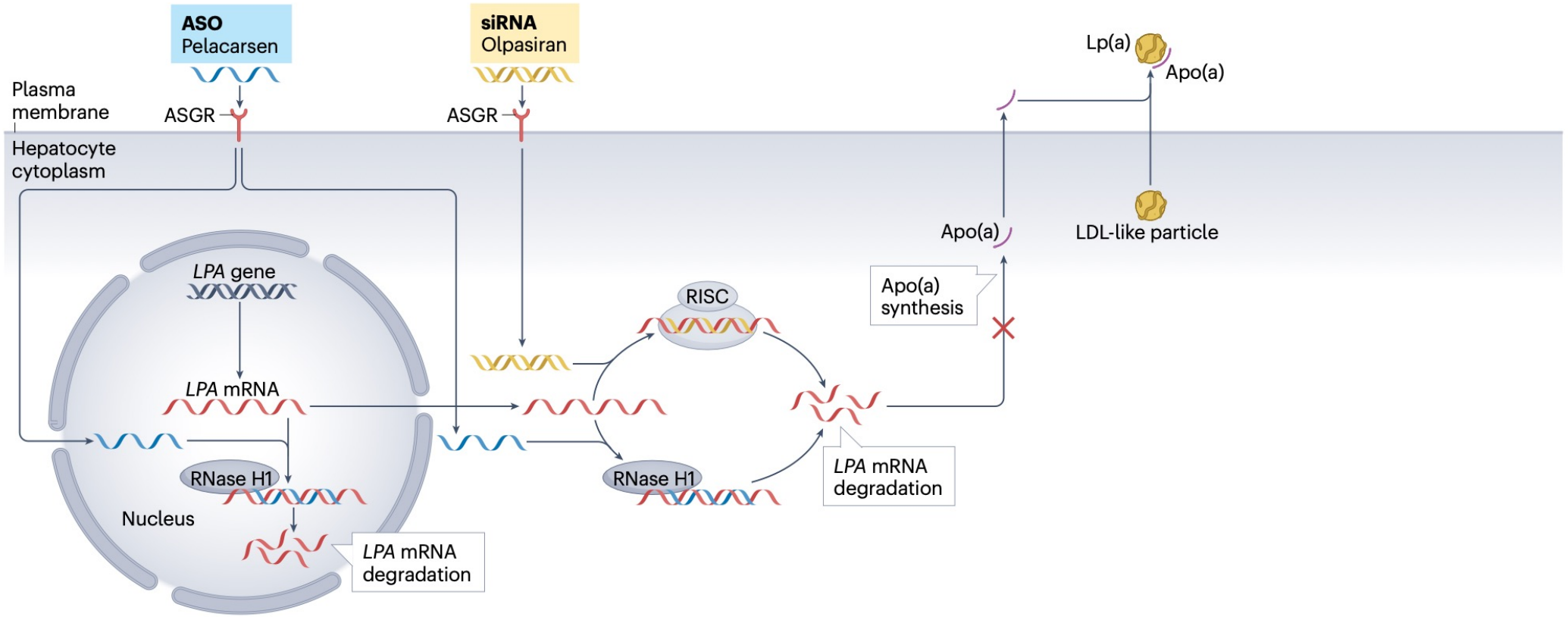
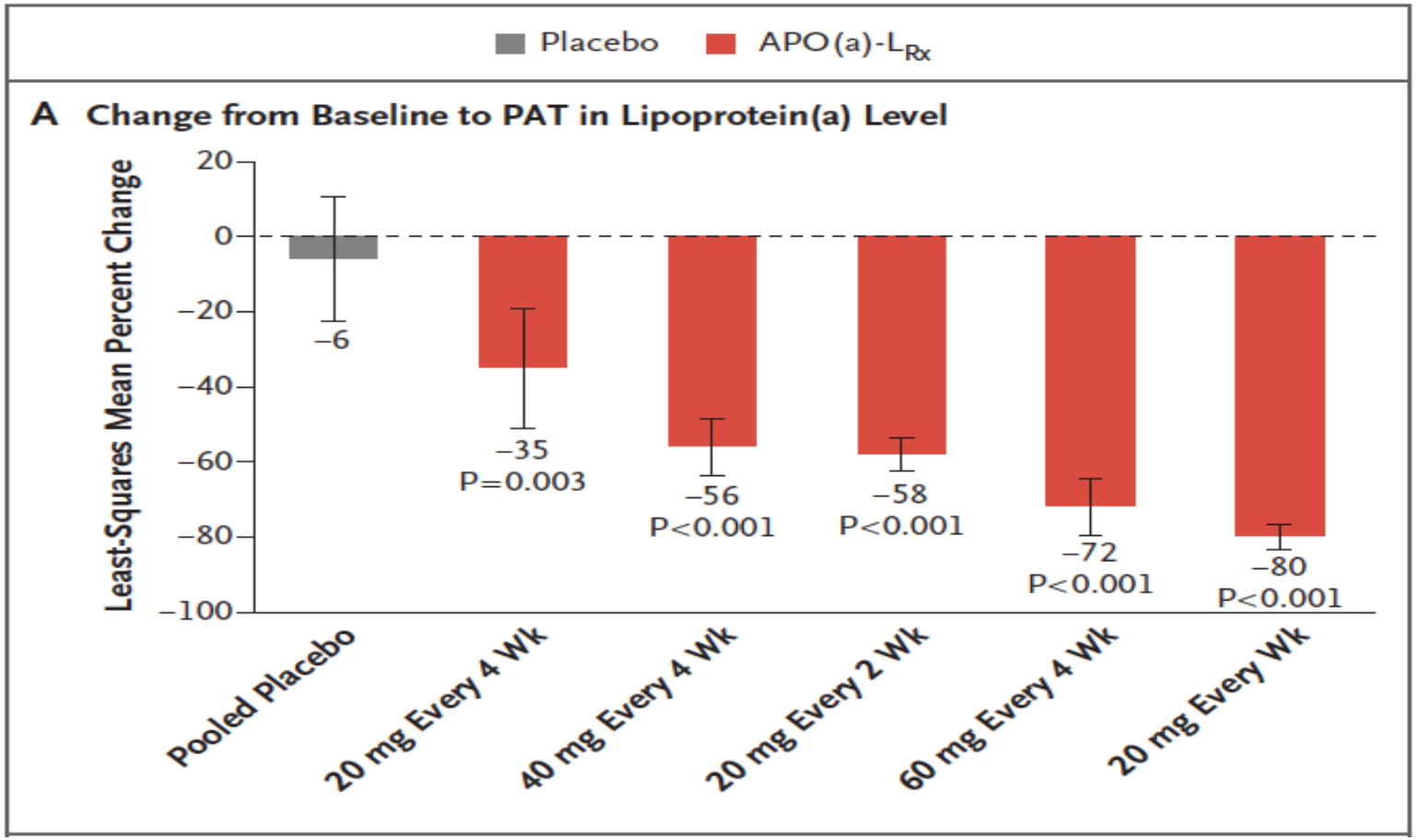


Fig. 4 | Emerging lipid-lowering therapies targeting Lp(a). Therapeutic strategies to lower plasma lipoprotein(a) (Lp(a)) levels that are currently being tested in clinical trials include an antisense oligonucleotide (ASO) and a small interfering RNA (siRNA). The ASO pelacarsen binds to *LPA* mRNA in the nucleus and the cytoplasm, causing RNase H1-mediated *LPA* mRNA degradation. The siRNA olpasiran binds to *LPA* mRNA in the cytoplasm to induce *LPA* mRNA degradation through the RNA-induced silencing complex (RISC). Degradation of *LPA* mRNA suppresses the translation and synthesis of apolipoprotein(a) (apo(a)), which in turn prevents the formation of Lp(a). Pelacarsen and olpasiran are conjugated to triantennary *N*-acetylgalactosamine, which confers hepatocyte-specific uptake through asialoglycoprotein receptors (ASGR).

Lipid lowering therapies targeting Lp(a) in developpement

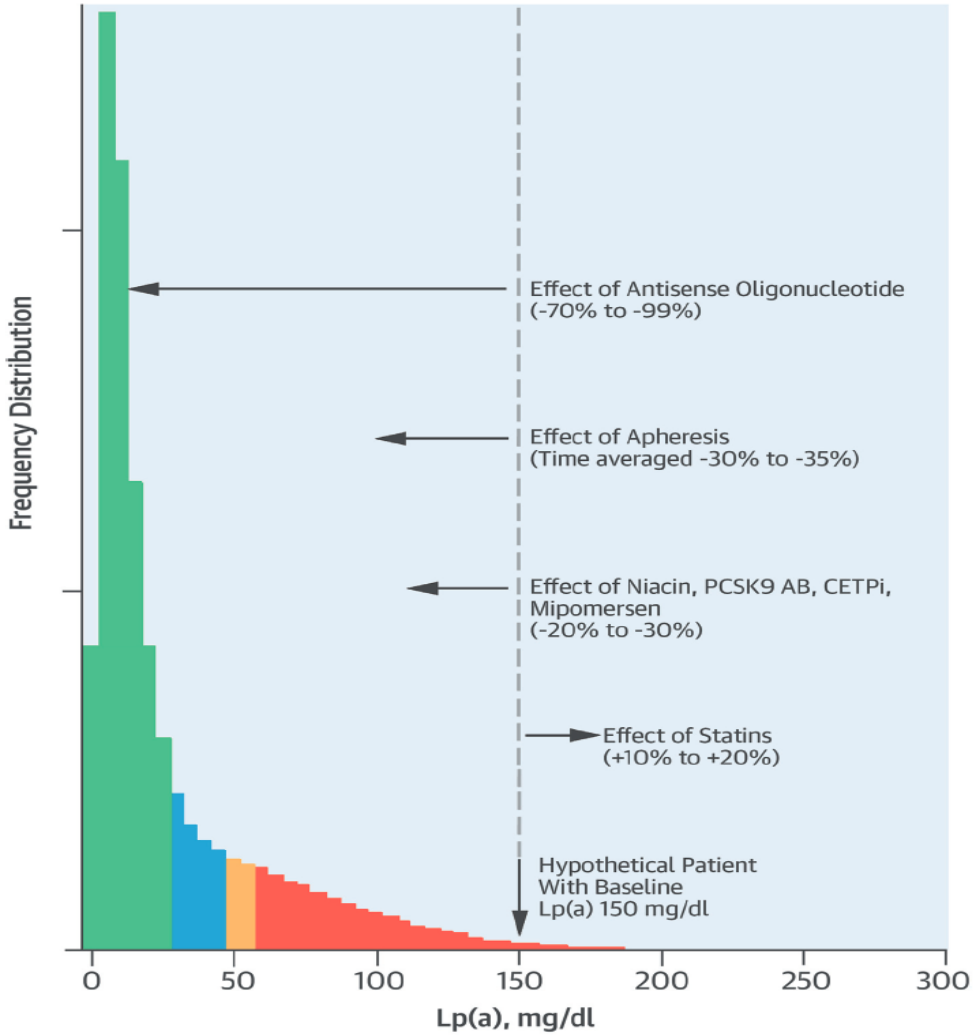
Patients in secondary CV prevention with Lp(a) ≥ 60 mg.dl (150 nmol/l))



ASO anti-Apo(a)

Tsimikas S. et al. N Eng J Med 2020; 382: 244-55

Lipid lowering therapies targeting Lp(a) in developpement



(<30 mg/dl) U.S. normal levels, represents negligible risk for CVD and CAVS

(<50 mg/dl) EAS recommendation

(<60 mg/dl) Cutoff for apheresis eligibility in Germany and U.K.

Tsimikas S. et al. J AM Coll Cardiol. 2017;69(6):692-711

Lipid lowering therapies targeting Lp(a) in developpement

WAITING FOR the Cardiovascular outcome Trials results …

ASO: **Lp(a)HORIZON** -NCT04023552

siRNA (olparsiran, AMG 890)

Merci pour votre attention

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