Louis and Artur Lucian Award Lecture-IV

Montreal Cholesterol Summit

May 30, 2019

IRCM

MONTREAL , CANADA
Laboratory of Biochemical Neuroendocrinology

"PCSK9: from Discovery to Therapeutic Applications"

NABIL G. SEIDAH

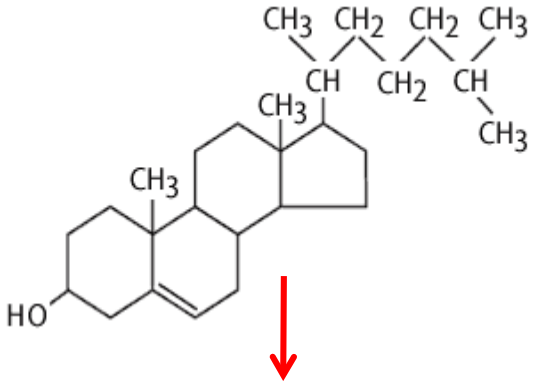
I have no disclosures or conflicts of interest to report

Learning Objectives:

At the end of this presentation, participants will be able to:

- understand if the major function of PCSK9 requires protease activity
- understand how PCSK9 lead to LDLR degradation
- understand the current strategy for PCSK9 inhibition and what the CVD outcomes are

100 g cholesterol / 70 kg body weight



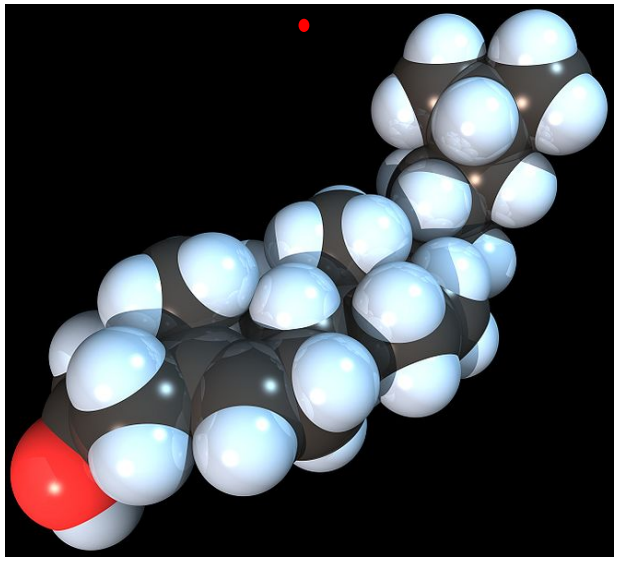
Cellular membranes:
control substances that enter or leave cells

Hormones:
testosterone & estradiol

Vitamines:
vitamin D

Biliary acids:
cholic acid

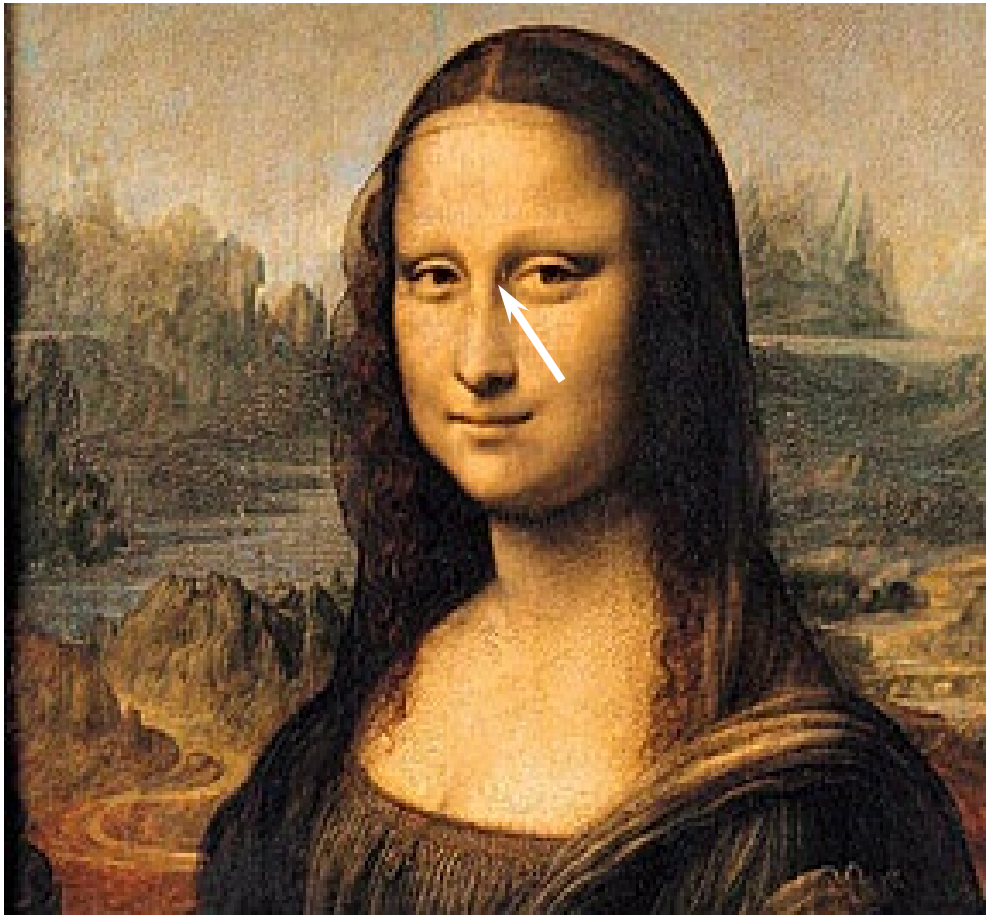
- discovered in 1812
- present in all animal tissues
- absent in plants & vegetables



Cholesterol : 75% synthesized by the human body
25% obtained from ingested food

Xanthoma/Xanthelasma are associated with hyperlipidemia

Franz Hals (1583-1666)
Noble Haarlem woman



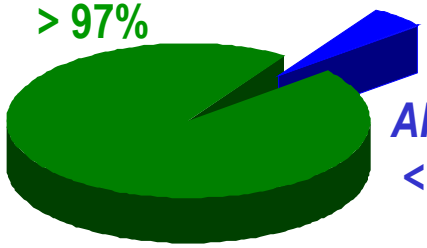
Leonardo da Vinci (1452-1519)
Mona Lisa (died at 37 years)

Familial Hypercholesterolemia

AKIRA ENDO
Compactin, 1982

LDLR
> 97%

APOB
< 3%



POMERANTZ et al.
1951

C. MULLER
1939

INNERARITY
1987

FREDERICKSON
et al.
1967

EPSTEIN
et al.
1959

BROWN & GOLDSTEIN
1974…

Statins save lives
1992

LDLR

APOB

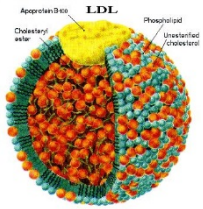
PCSK9

TUTTLE
1950

PIPER
& ORRILD
1956

KHACHADURIAN
ADH
1964

Lovostatin
(Commercialization
Merck)
1986

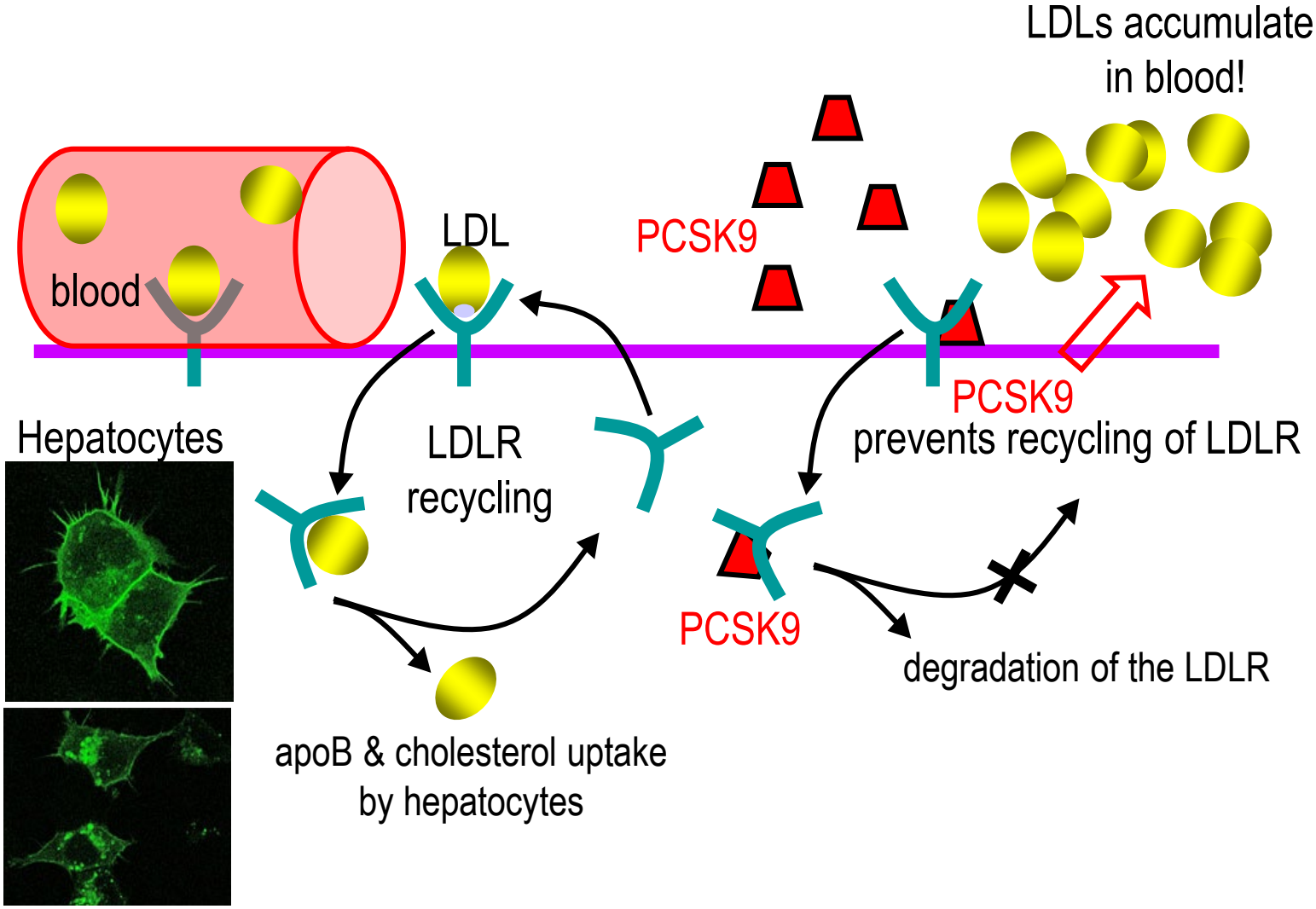


ABIFADEL et al.
2003

FH is essentially due to mutations in the *LDLR* gene

2003

LDL-cholesterol clearance



January

PCSK9 (NARC-1) is the 9th and last member of the PC family

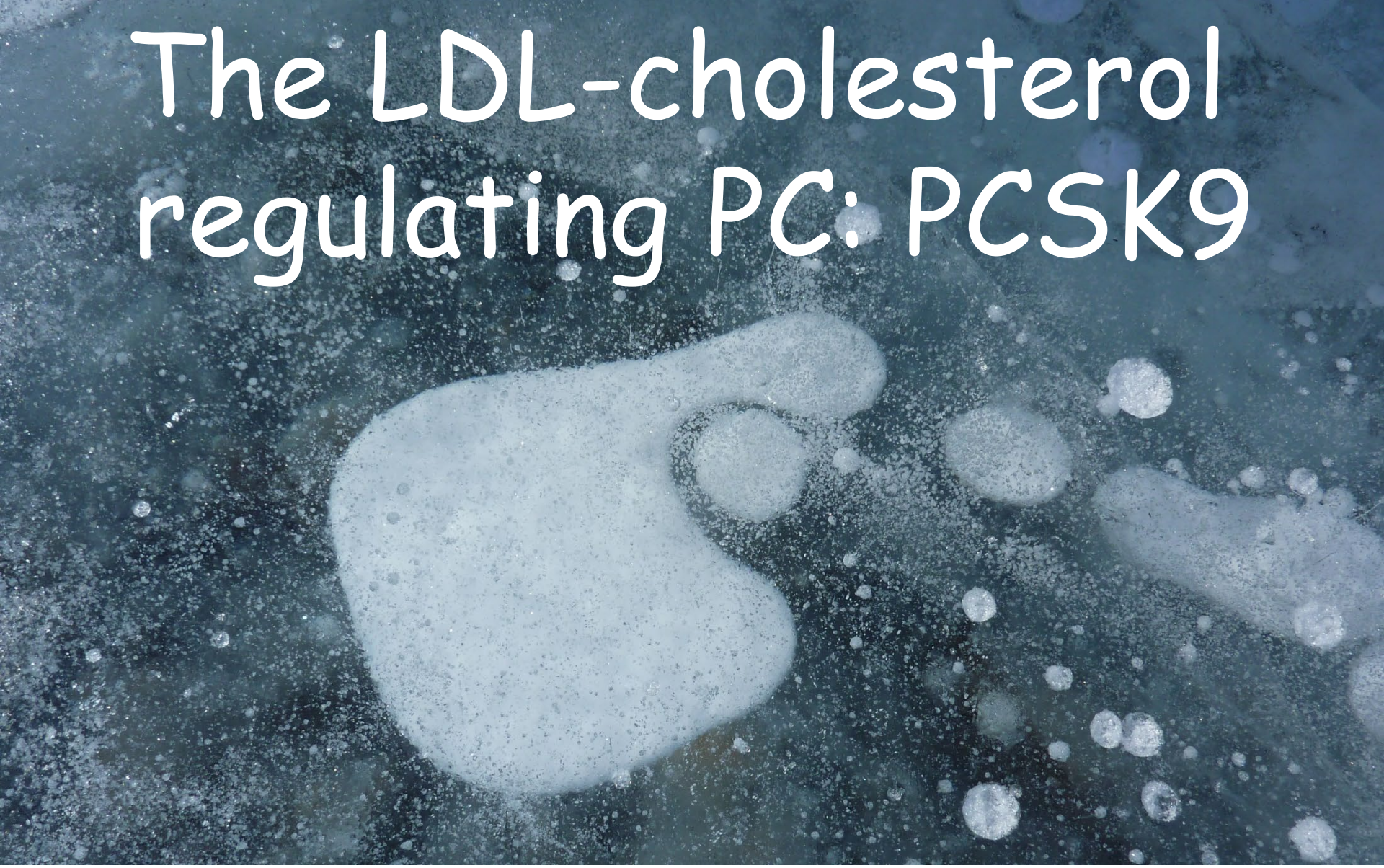
Seidah NG et al. *PNAS* (2003) **100**, 928

PCSK9 is the third locus linked to ADH

May

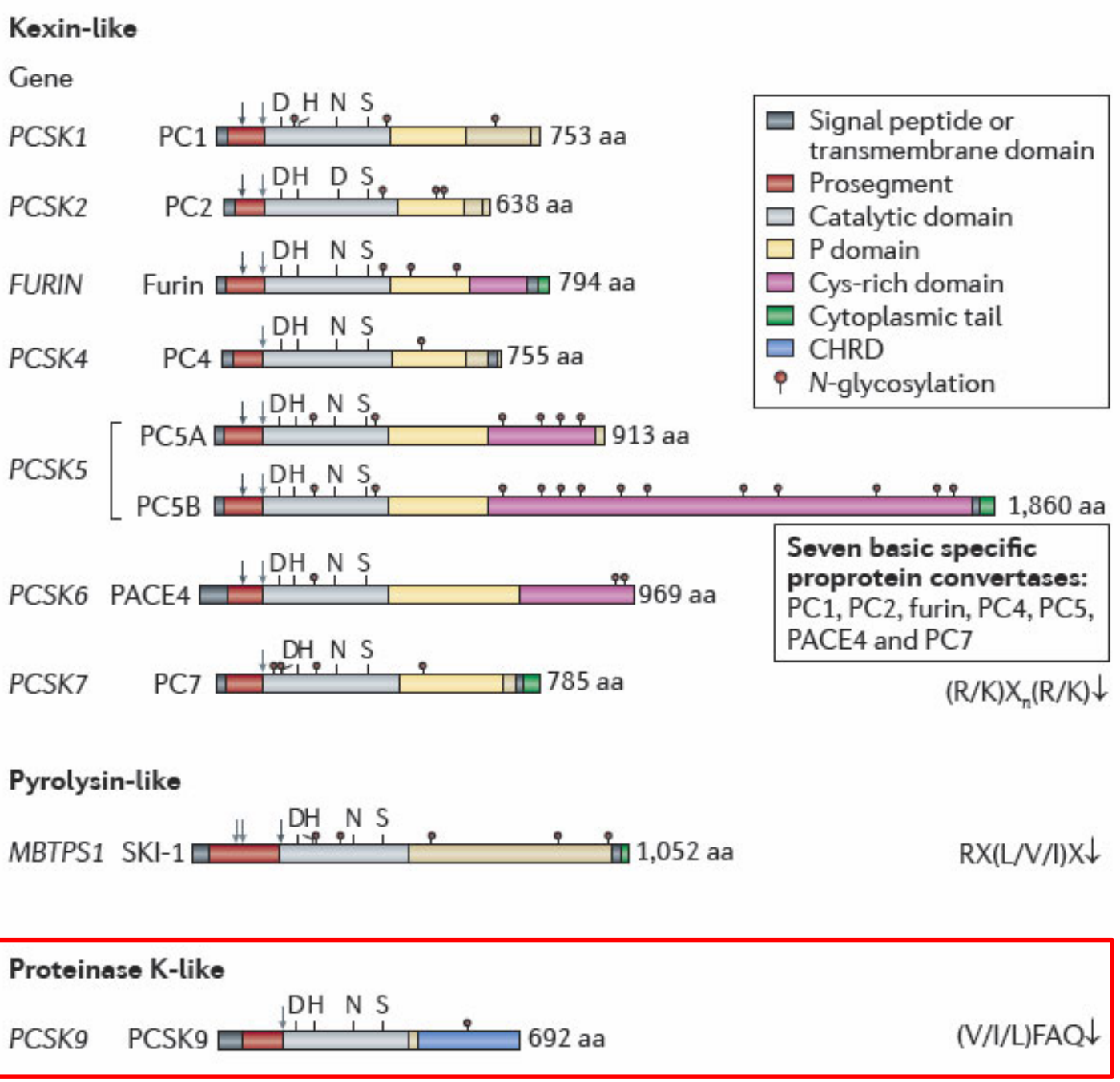
Abifadel M et al. *Nature Gen* (2003) **34**, 154

The LDL-cholesterol regulating PC: PCSK9



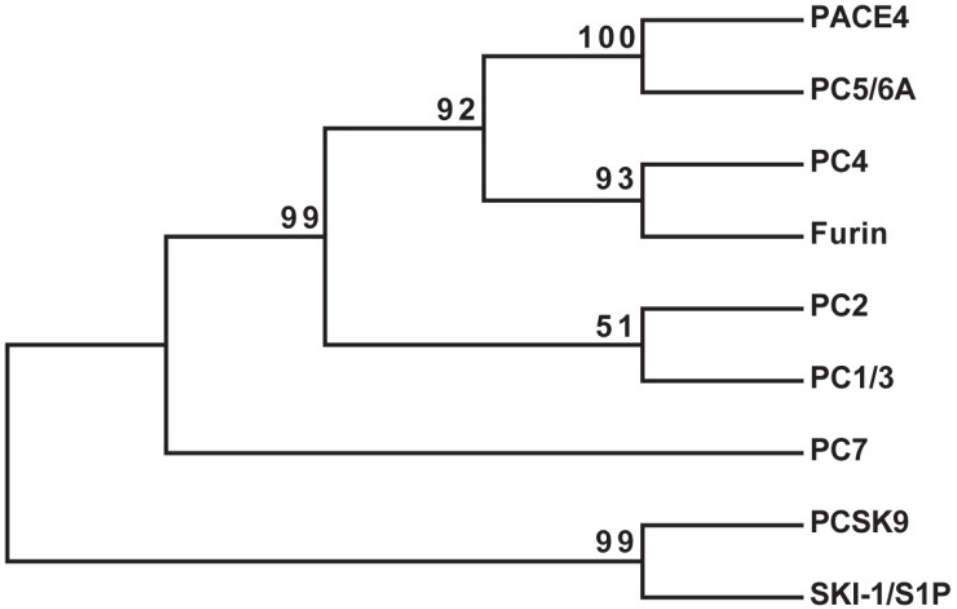
IRCM

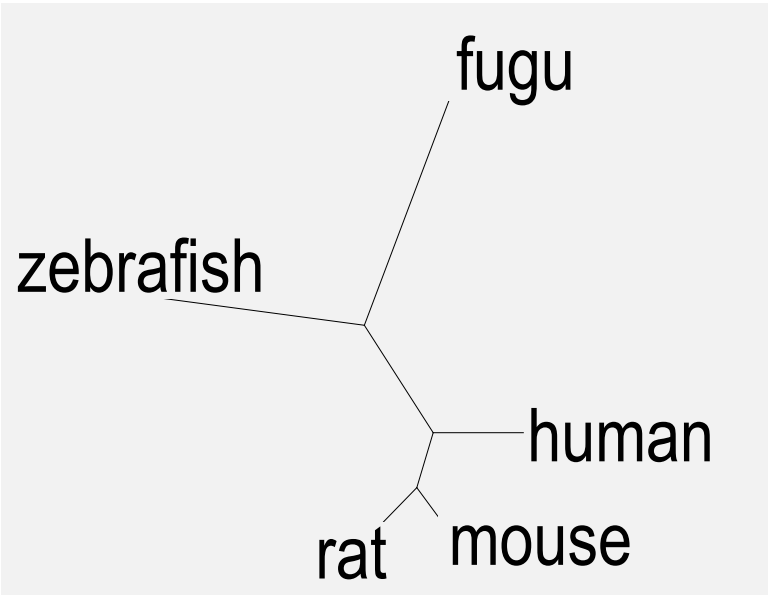
Proprotein Convertases of the Subtilisin/Kexin type (PCSK)



Seidah NG & Prat A. Nat Rev Drug Discov (2012) **11**, 367

Phylogenetic analysis of the nine PCs including PCSK9



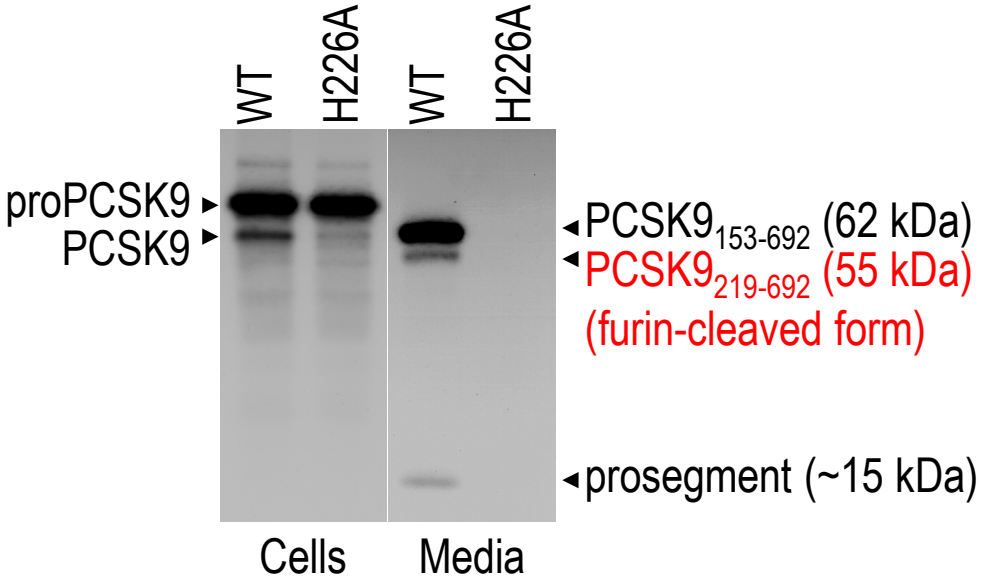


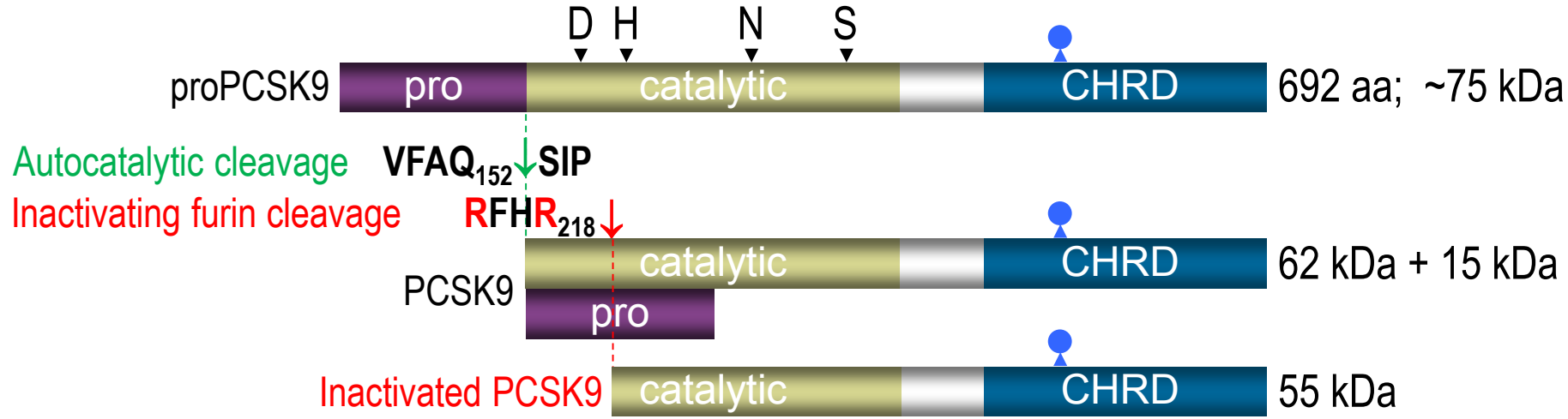
PCSK9

Seidah NG. et al. *Pharmacological Reviews* (2017) **69**, 33

Autocatalytic processing of proPCSK9 and secetion of PCSK9-prosegment complex

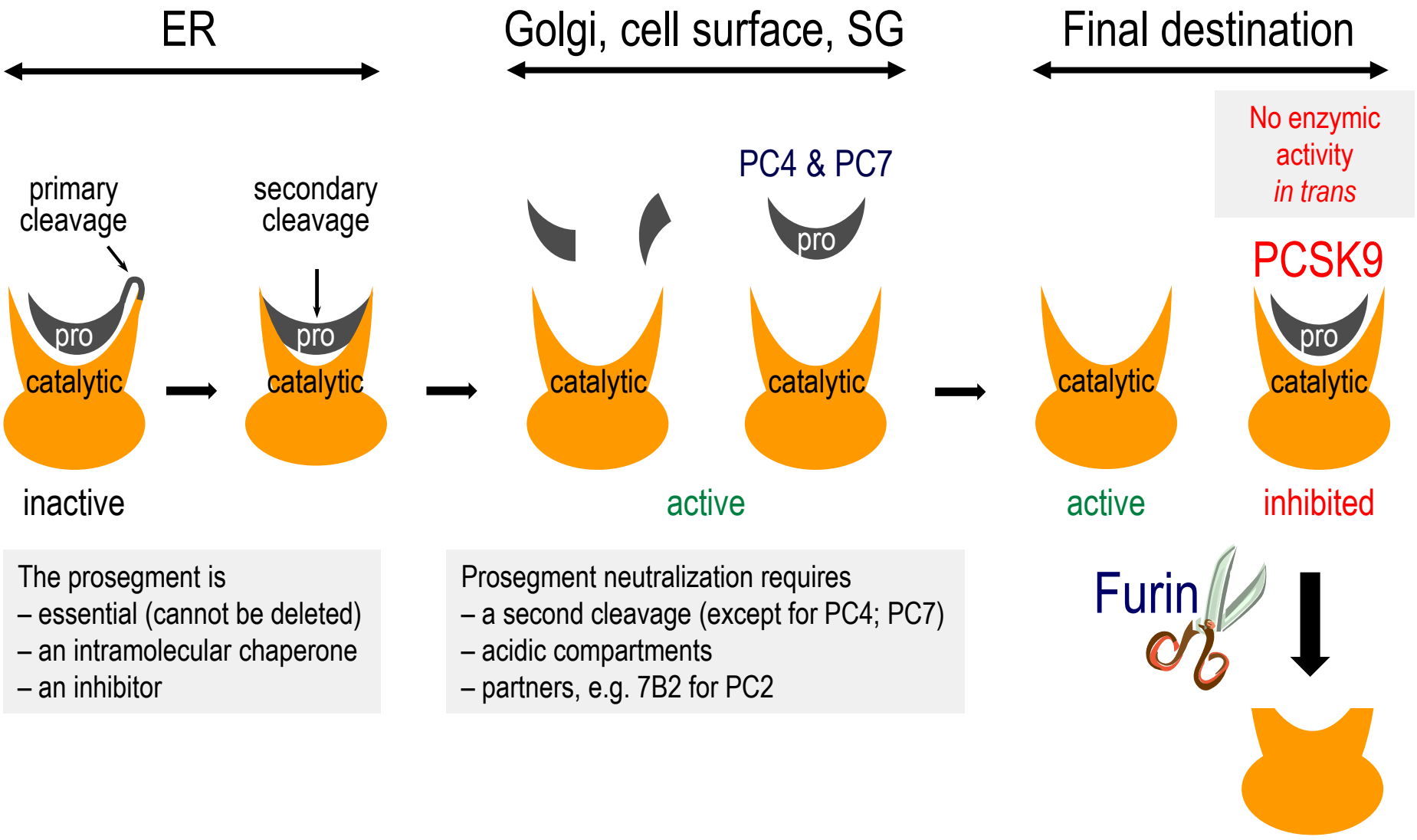
HEK293 cells
4h pulse
^{35}S-Met/Cys
C-terminal V5 tag



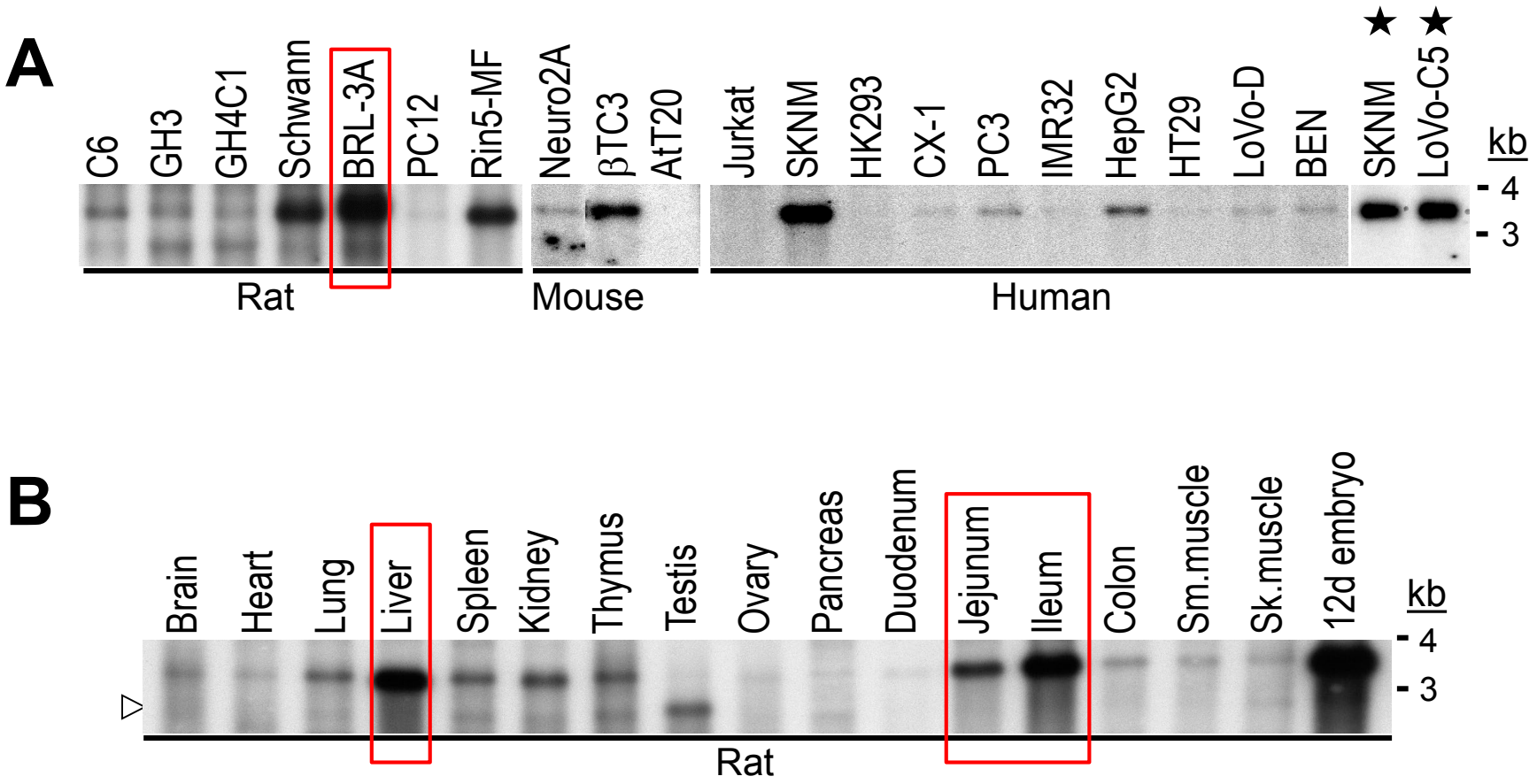


Seidah NG et al. *PNAS* (2003) **100**, 928

PCSK9 is a class of its own



Cellular and tissue expression of PCSK9

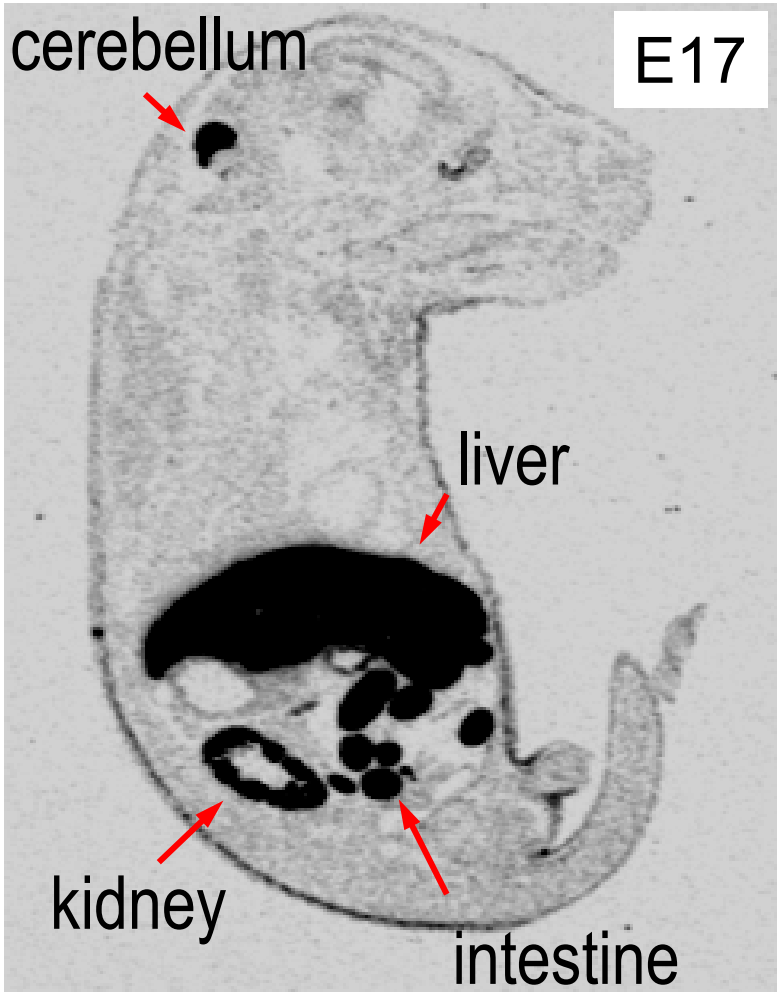


Seidah NG et al. *PNAS* (2003) **100**, 928

PCSK9 biological functions ?

Clues

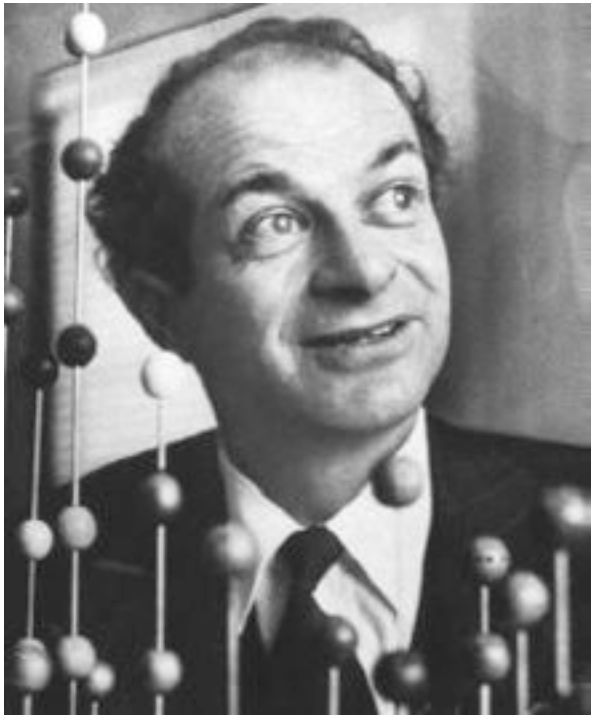
1. Rich in liver & intestine
2. Chrom. 1p32



Seidah NG et al. *PNAS* (2003) **100**, 928

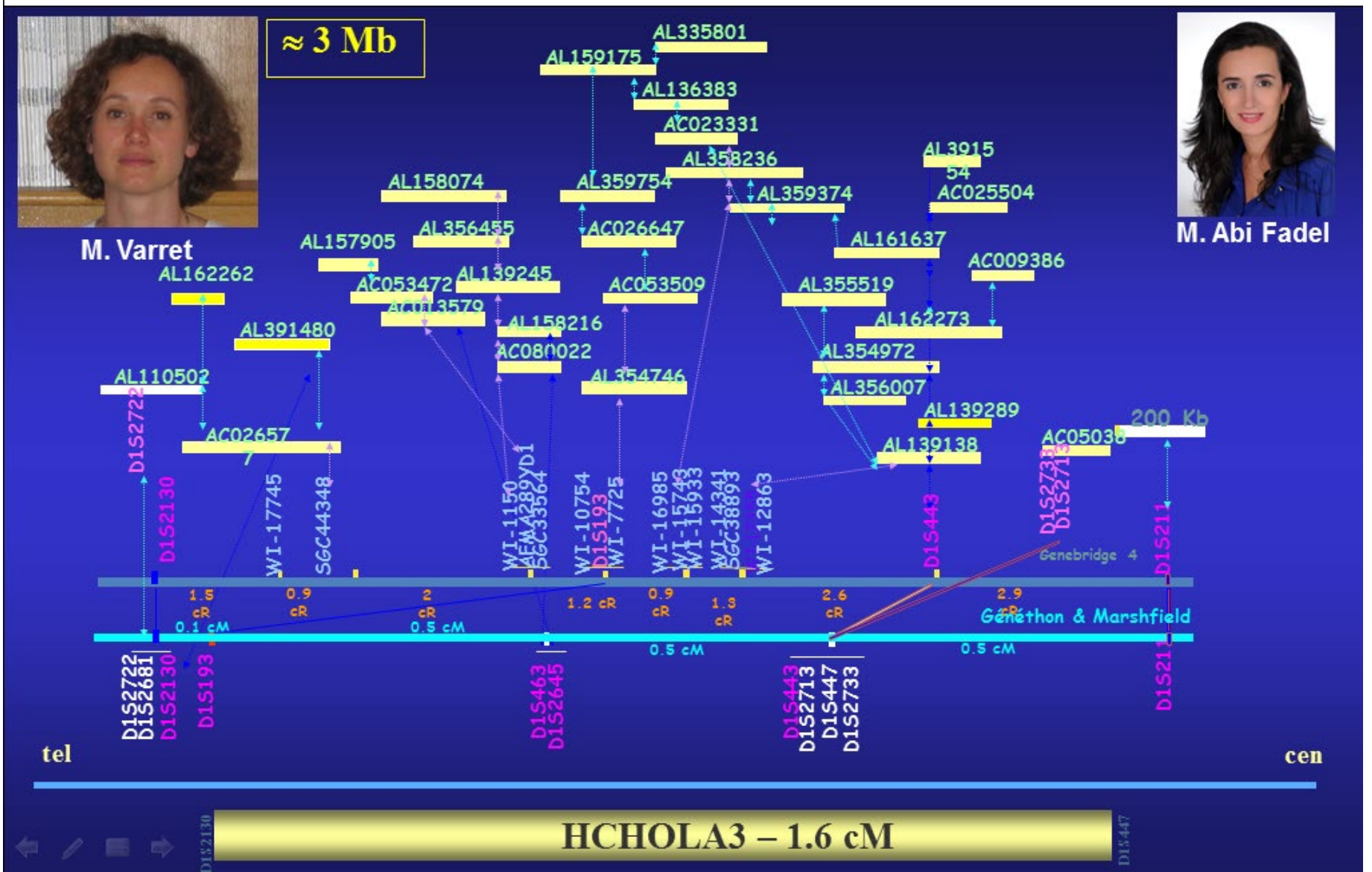
“The best way to have a really good idea is to have lots of ideas”

Linus Pauling

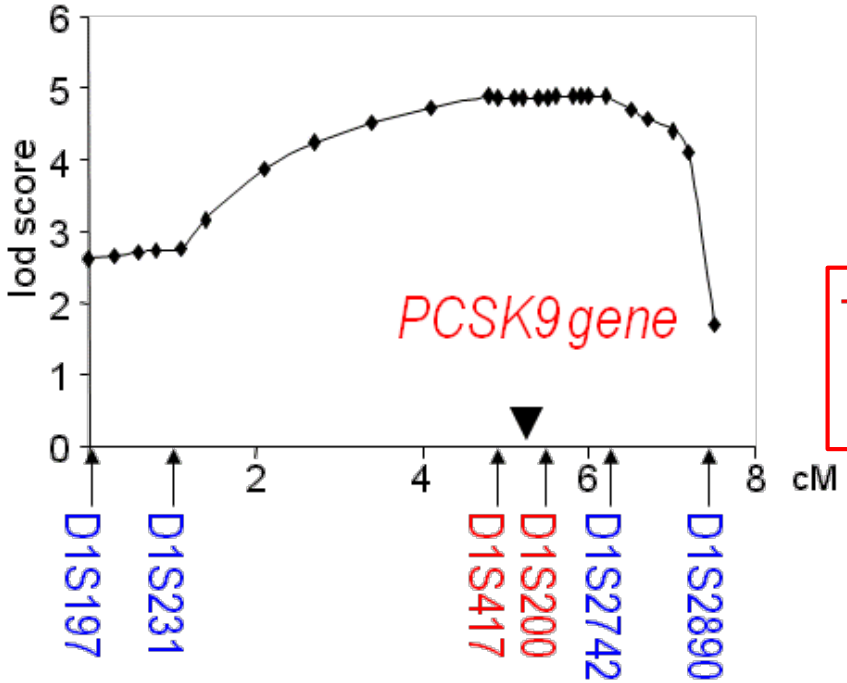


Familial Hypercholesterolemia

Physical map at 1p32 in 2002



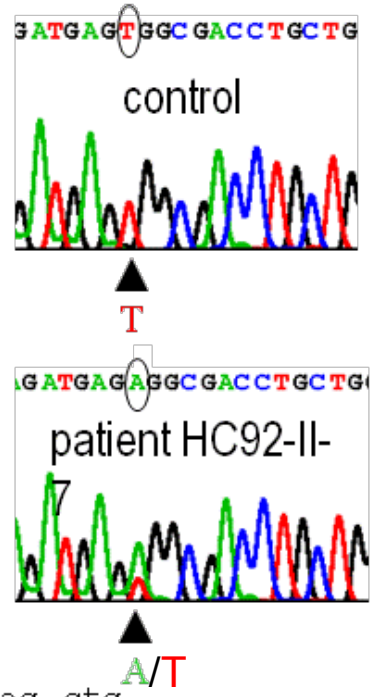
In families HC92 and HC2, PCSK9 exhibits a S127R mutation in exon 2



Homozygote
(T/T)

T⟶ A at position 625:
Ser127Arg

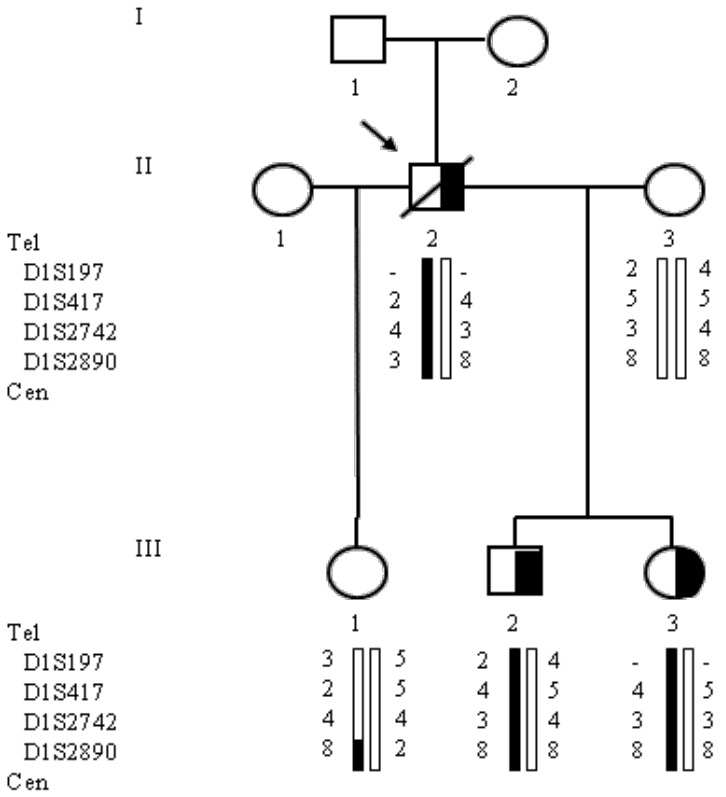
Heterozygote
(A/T)



Human wt sequence	cct	ggc	ttc	ctg	gtg	aag	atg	agT	ggc	gac	ctg	ctg	gag	ctg
	P	G	F	L	V	K	M	S	G	D	L	L	E	L
Mus musculus	P	G	F	L	V	K	M	S	S	D	L	L	G	L
Rattus norvegicus	P	G	F	L	V	K	M	S	S	D	L	L	G	L
	P	G	F	L	V	K	M	R	G	D	L	L	E	L
Patient T625A-S127R	cct	ggc	ttc	ctg	gtg	aag	atg	agA	ggc	gac	ctg	ctg	gag	ctg

Abifadel M et al. *Nature Gen* (2003) **34**, 154

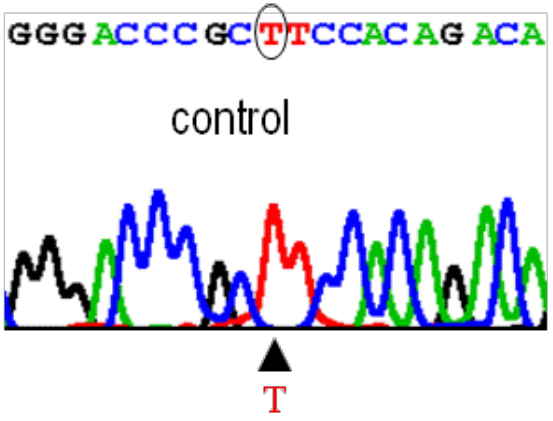
In family HC60, PCSK9 exhibits a F216L mutation in exon 4

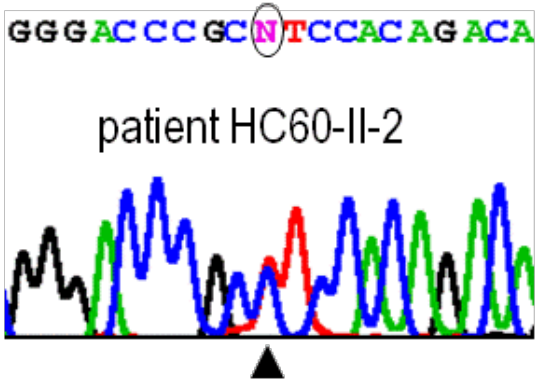


Homozygote
(T/T)

T⟶ C at position 890:
Phe216Leu

Heterozygote
(C/T)





Human wt sequence	ccc	gag	gag	gac	ggg	acc	cgc	Ttc	cac	aga	cag	gcc	agc	aag	tgt
	P	E	E	D	G	T	R	F	H	R	Q	A	S	K	C
Mus musculus	P	E	E	D	G	T	R	F	H	R	Q	A	S	K	C
Rattus norvegicus	P	E	E	D	G	T	R	F	H	R	Q	A	S	K	C
	P	E	E	D	G	T	R	L	H	R	Q	A	S	K	C
Patient T890C-F216L	ccc	gag	gag	gac	ggg	acc	cgc	Ctc	cac	aga	cag	gcc	agc	aag	tgt

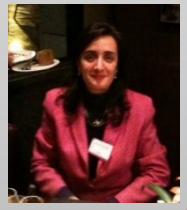
Human PCSK9 mutations

Cause Autosomal Dominant Hypercholesterolemia (ADH)
or Hypocholesterolemia

M. Abifadel

C. Boileau

gain of function — **hypercholesterolemia** — Abifadel *et al.* Nat. Genet. 2003



E32K

L108R
S127R

N157K

R215H
F216L
R218S

R237W

D374Y

R469W; R496W

D35Y

1 kb

1 2 3 4 5 6 7 8 9 10 11 12

0 — 22 kb

D199

H239

N330 S399

R104C/V114A
(16 mg/dL; 0.4 mM)

Q152H

R46L

G106R

ΔR97 Y142X
14 mg/dL
0.36 mM

L253F

G236S

N354I

A443T
Kotowski et al.

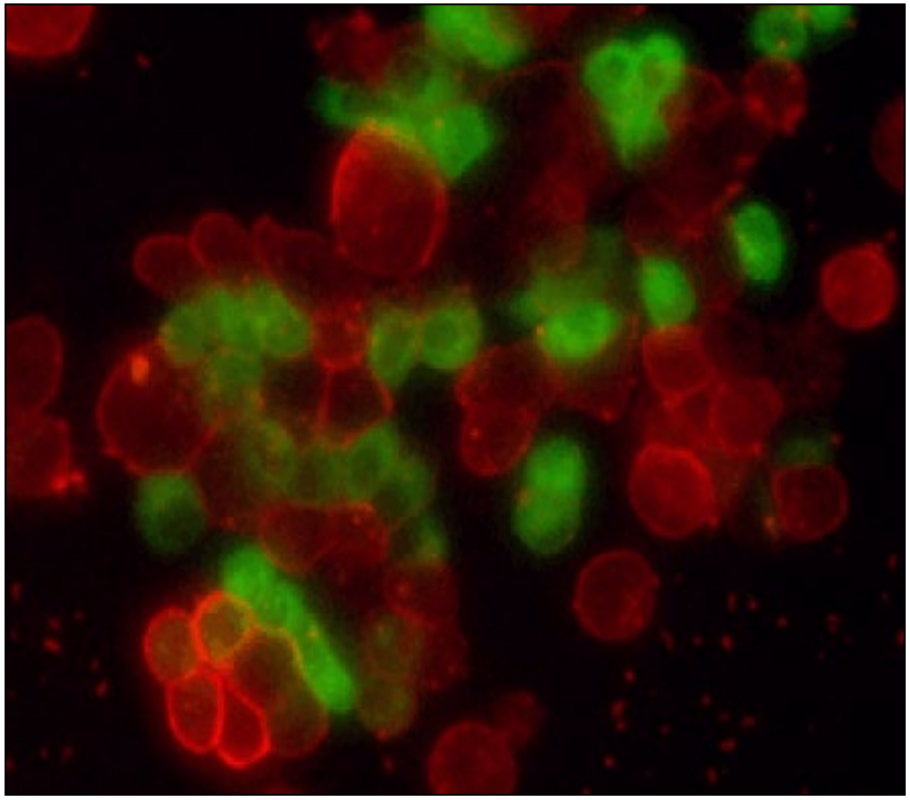
R434W

C679X
(XX Hom: 0.4 mM)
Zimbabwe

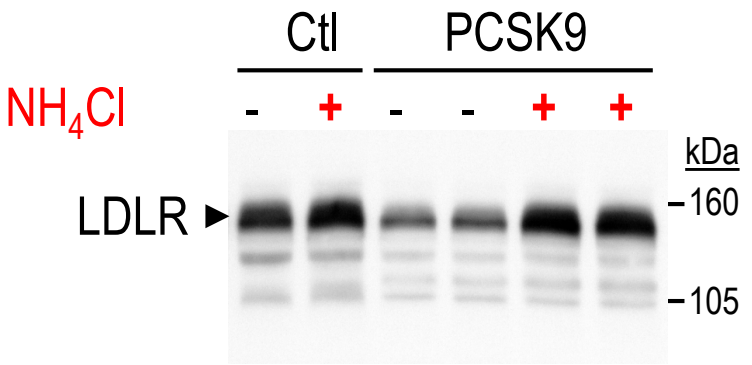
loss of function — **hypocholesterolemia** — Cohen *et al.* Nature Gen. 2005

In a non-enzymatic fashion, PCSK9 leads to LDLR degradation in lysosomes

DiI-LDL labeling of the cell surface
PCSK9 and EGFP coexpression



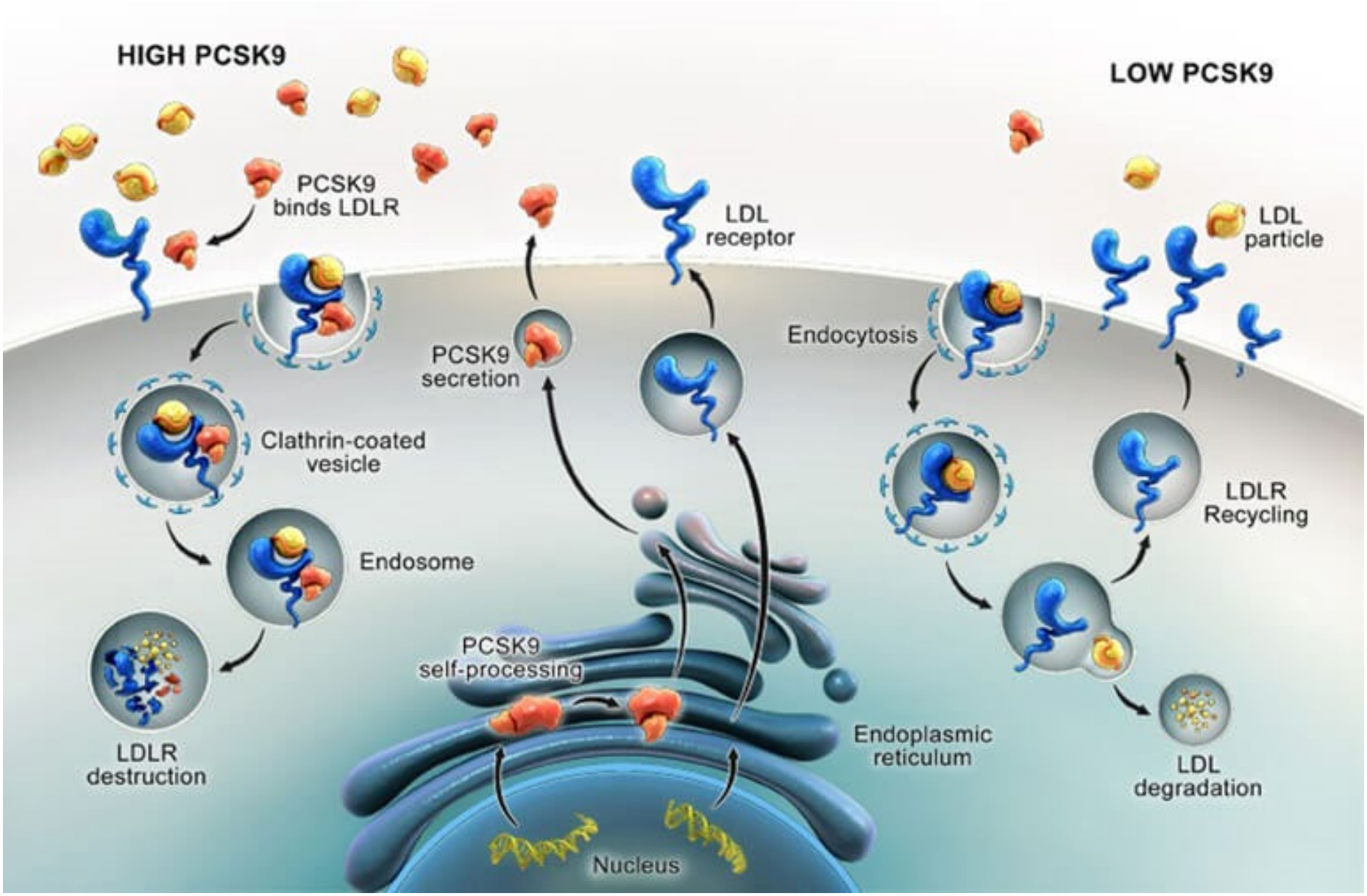
PCSK9 target proteins:
LDLR, VLDLR, LRP1, LRP8 (apoER2) & CD36



Maxwell KN & Breslow JN. *PNAS* (2004) **101**, 7100

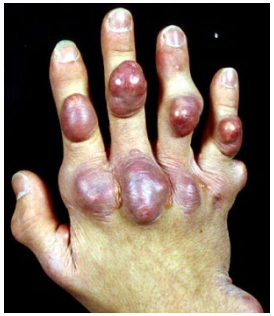
Benjannet S et al. *JBC* (2004) **79**, 48865

PCSK9 binds the LDLR and triggers its degradation in endosomes/lysosomes



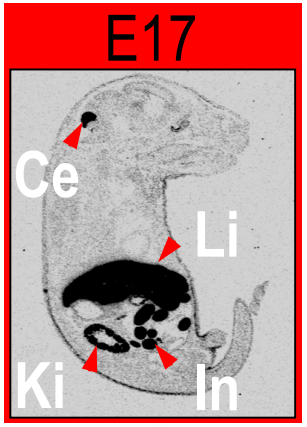
Seidah NG et al. Pharmacol Rev (2017) **69**, 33

PCSK9 is the third gene implicated in hypercholesterolemia



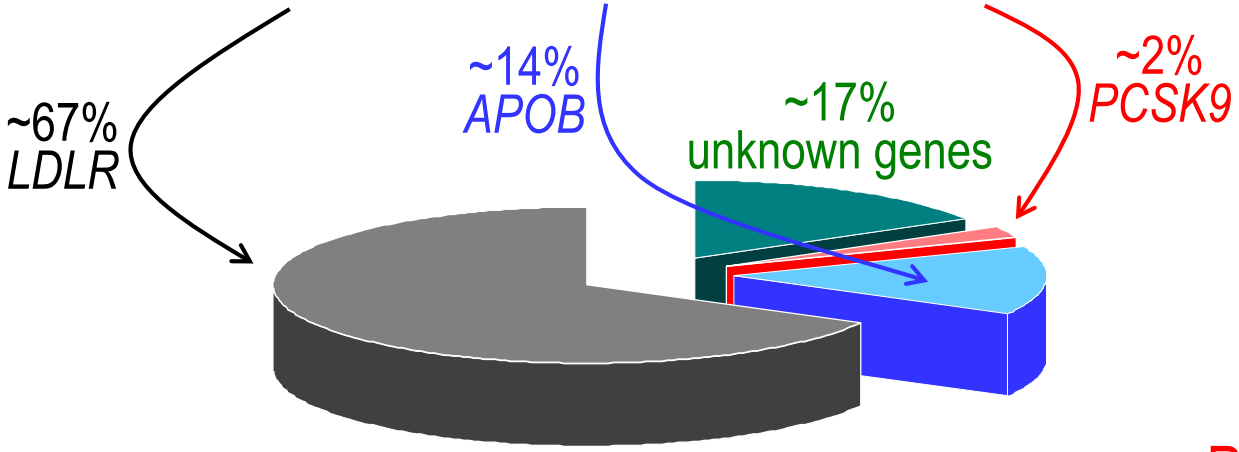
- 1/500
- high LDL levels
- xanthomas
- early cardiovascular desease

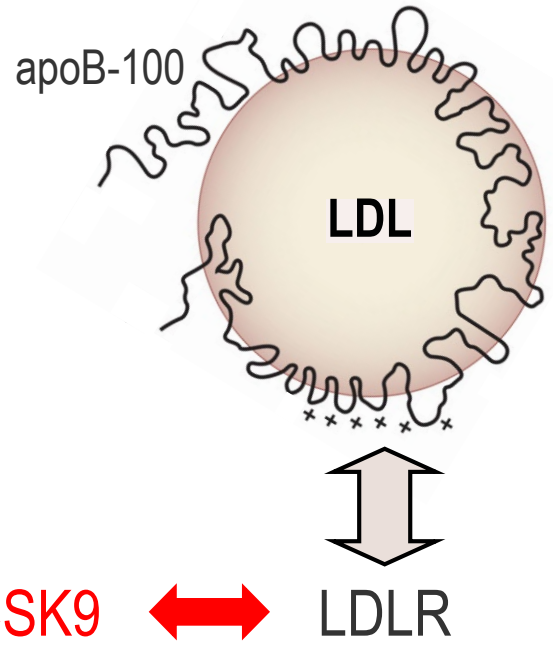
PCSK9



Seidah et al. *PNAS* 2003

	FH1	FH2	FH3…
chromosome	19p13	2p23-24	**1p32**
gene	*LDLR*	*APOB*	***PCSK9***

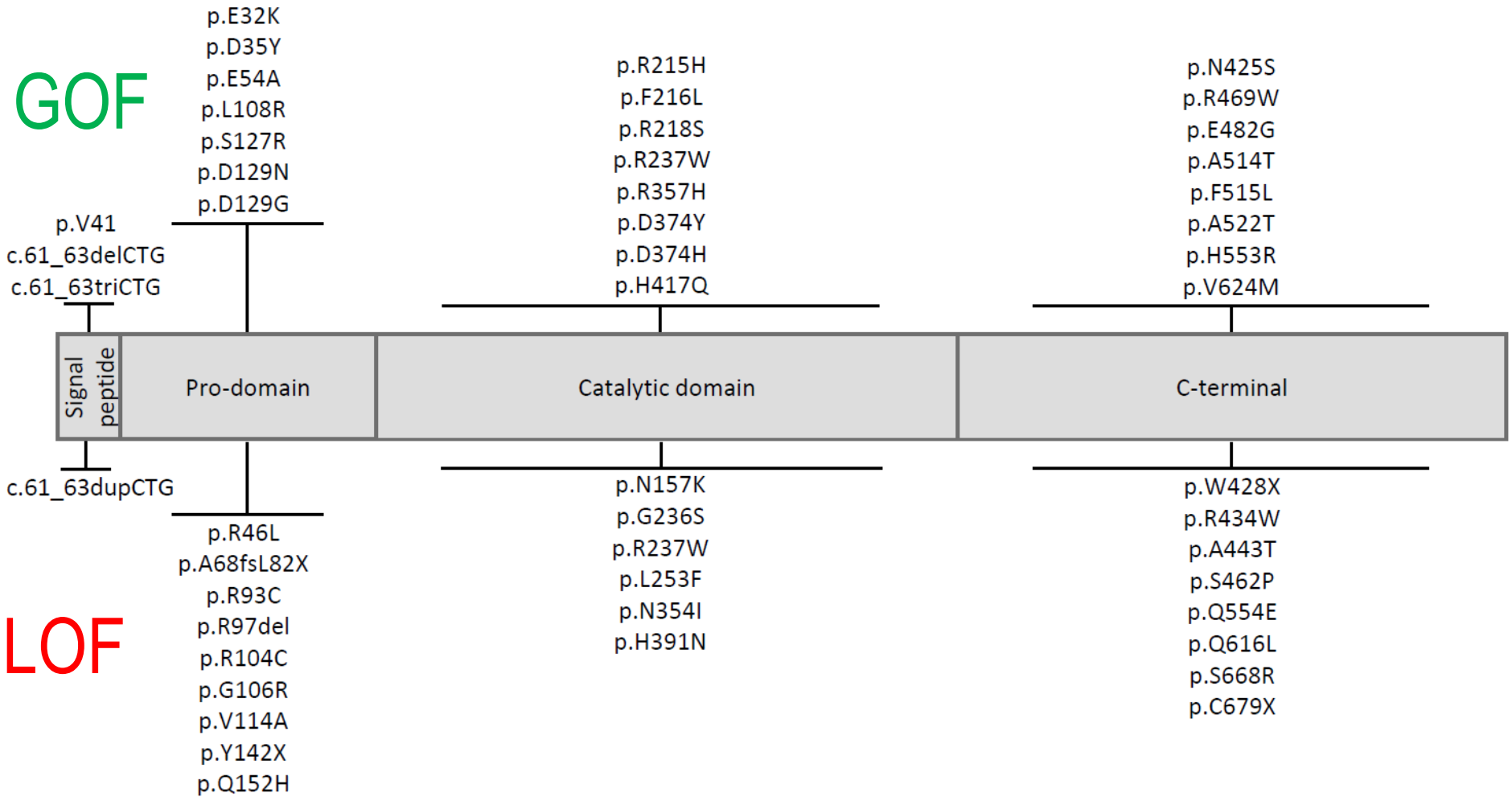




Abifadel et al. *Nature Genetics* (2003)
(Catherine Boileau's lab; Necker; Paris)

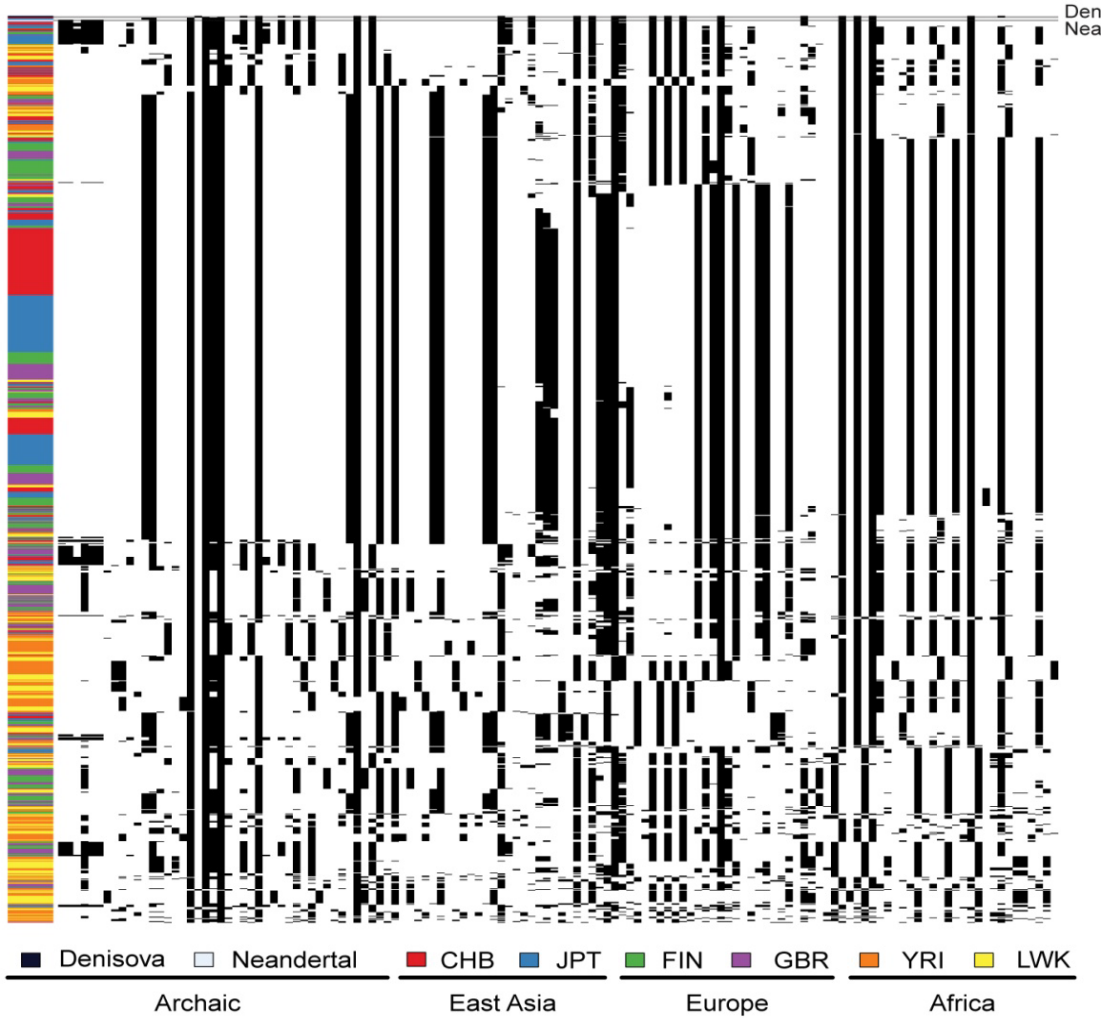
Maxwell & Breslow *PNAS* (2004)

Natural PCSK9 mutations

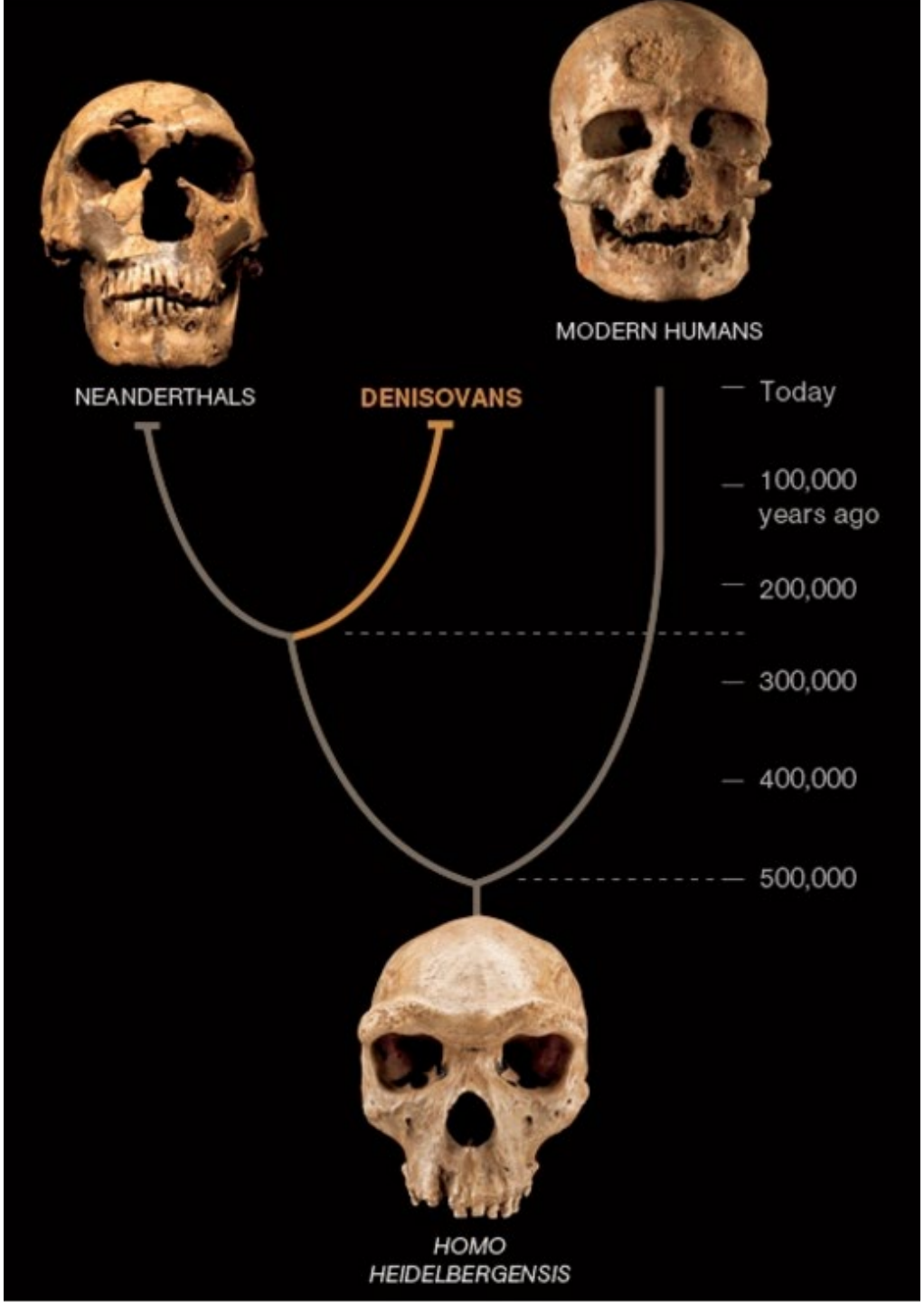


Dron JS & Hegele RA. Curr Opin Lipidol (2017) 28, 161

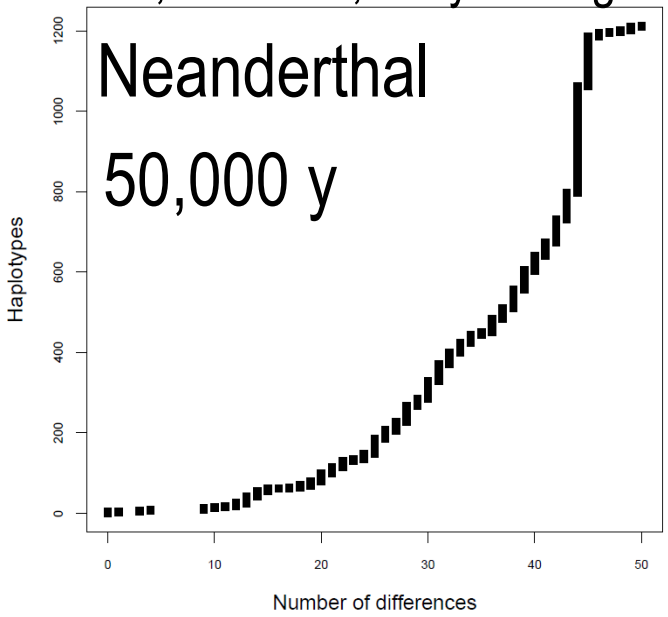
Haplotype structure of PCSK9 in modern and extinct human species



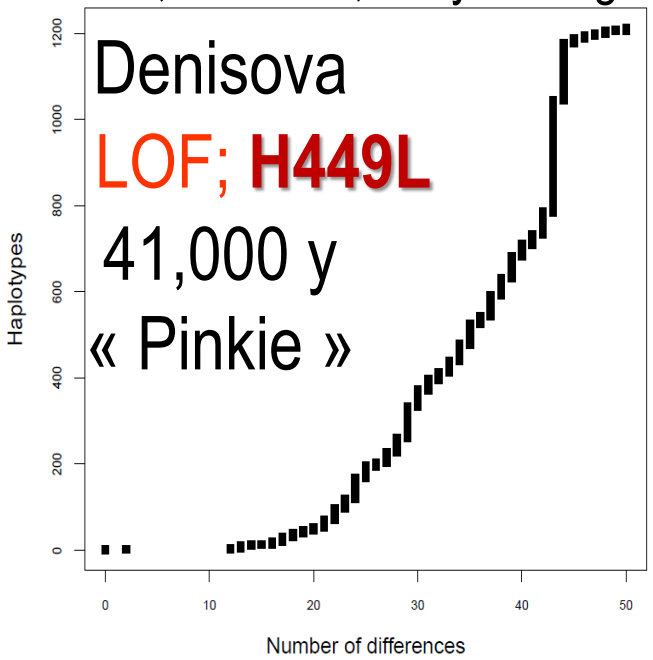
Seidah NG. et al. *Pharmacological Reviews* (2017) **69**, 33



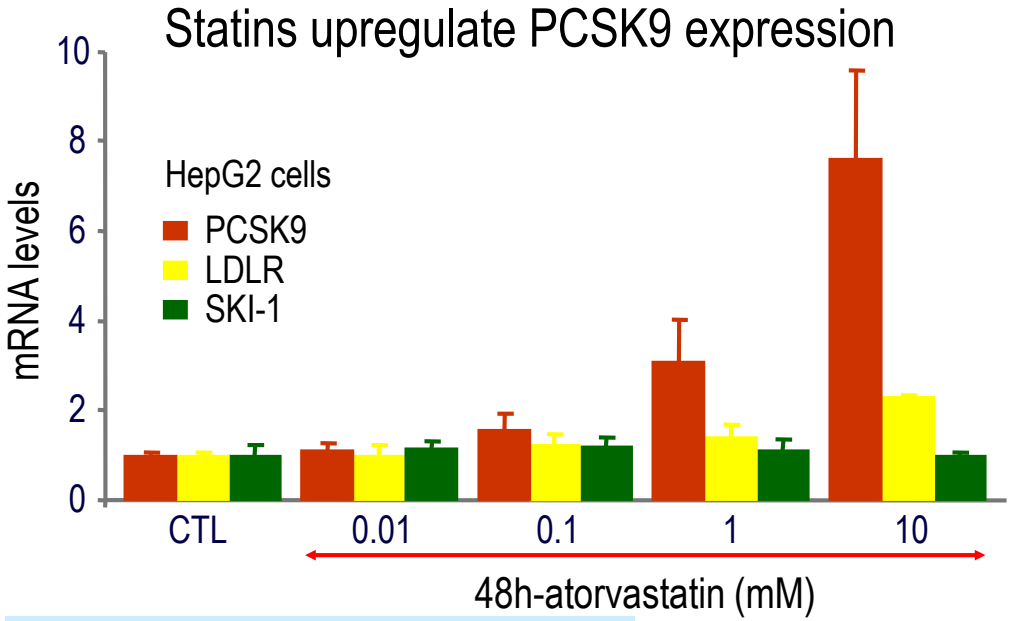
550,000 - 765,000 years ago



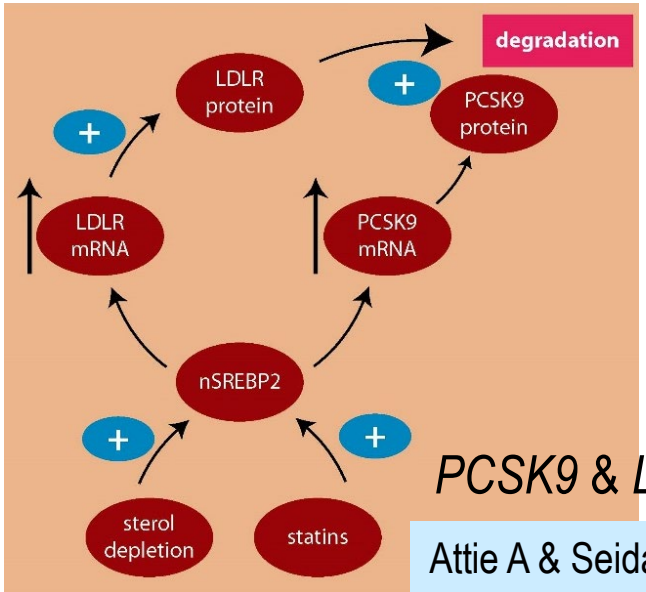
380,000 - 473,000 years ago

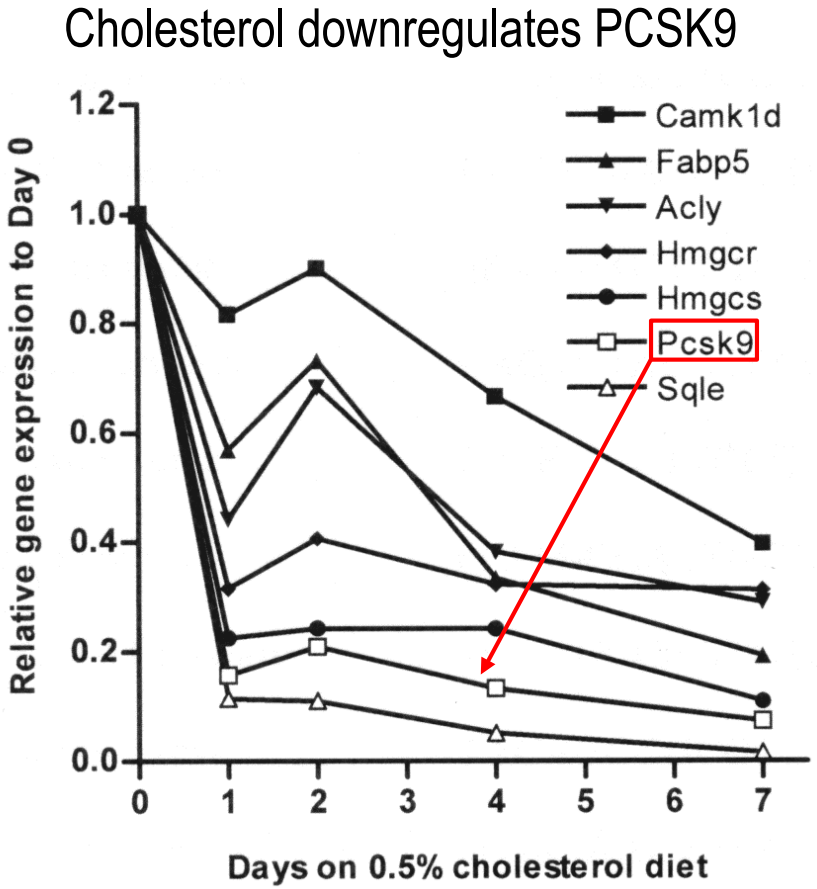


The PCSK9/LDLR paradox



Dubuc G. et al. *ATVB* (2004) **24**,1454



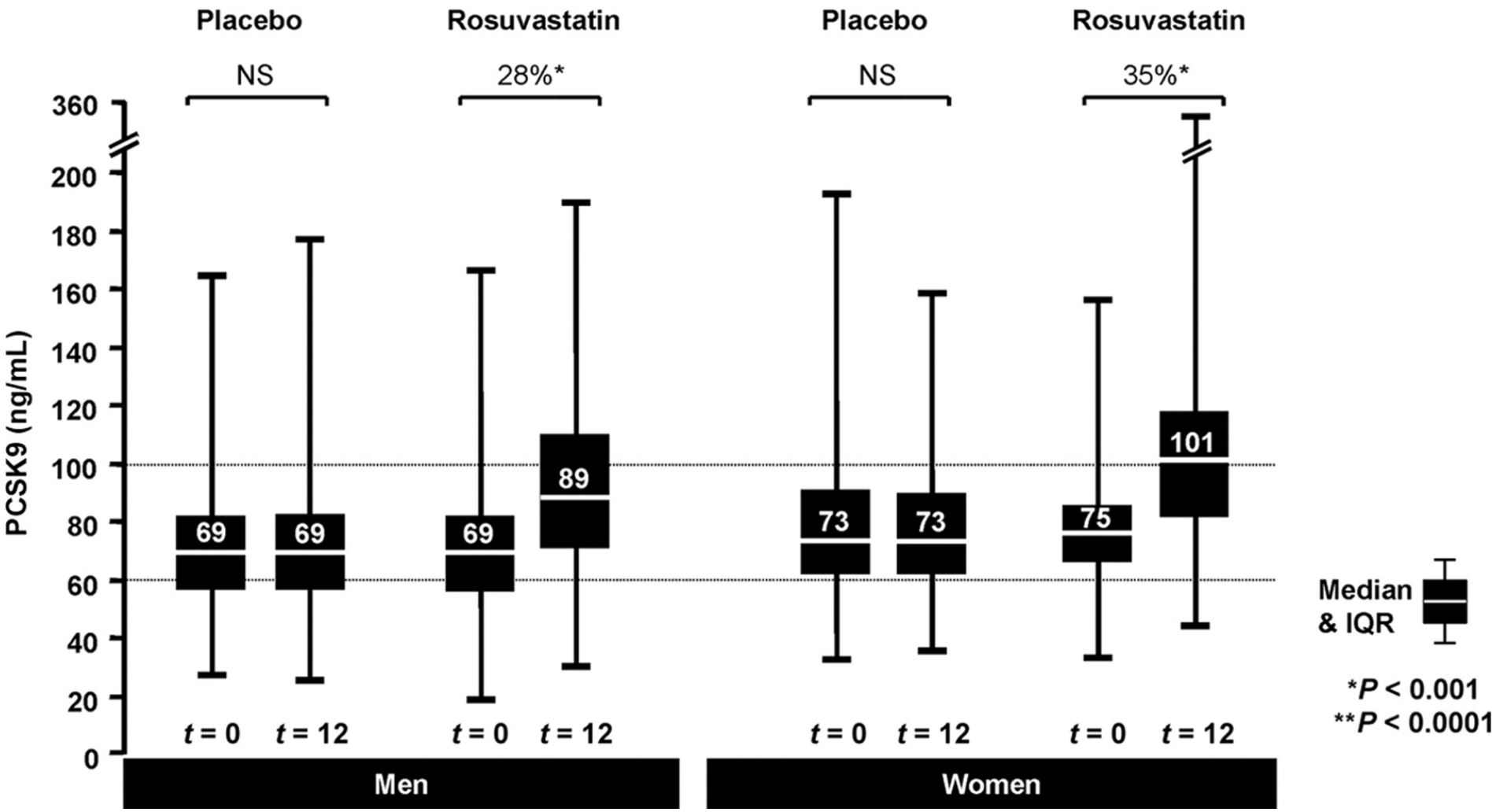


Maxwell KN et al., *JLR* (2003) 44, 2109

PCSK9 & *LDLR* are co-regulated by cholesterol

Attie A & Seidah NG. *Cell Metab* (2005) **1**, 290

Rosuvastatin increased plasma concentration of PCSK9 in proportion to the magnitude of LDLc reduction

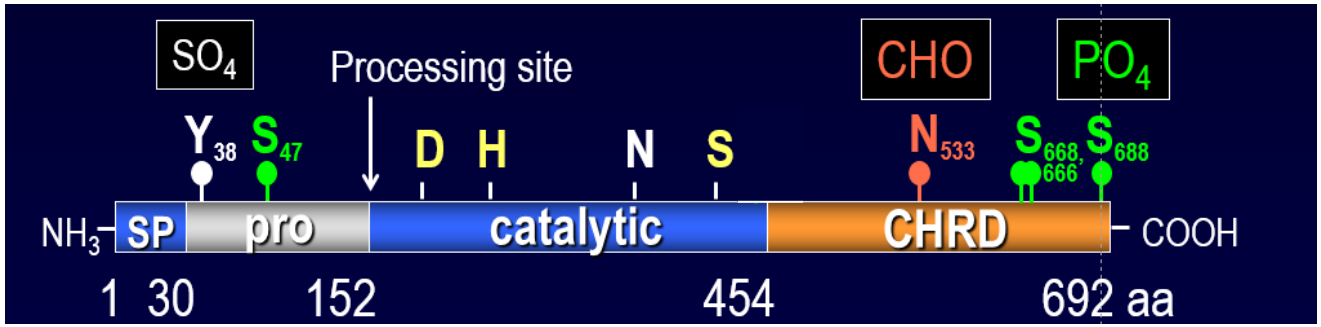


JUPITER

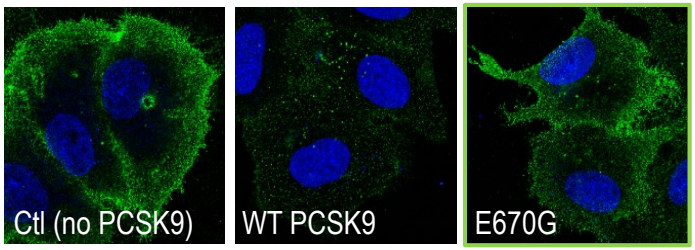
Awan Z. et al. *Clin Chem* (2012) **58**, 183

Ser-phosphorylation of PCSK9 enhances its function on LDLR

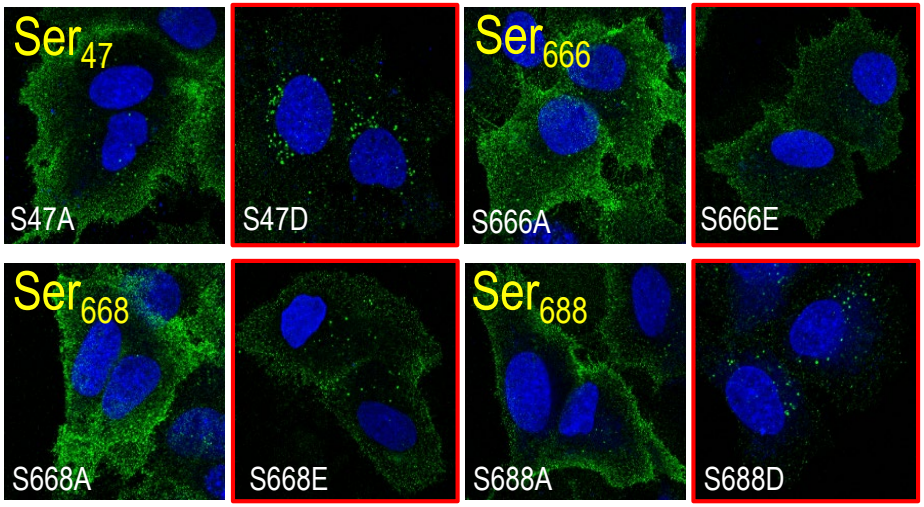
PCSK9 post-traductional modifications

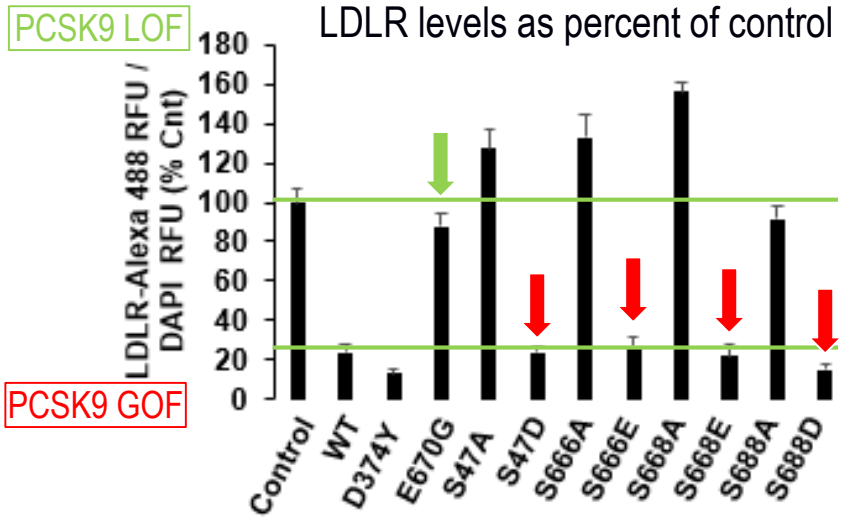


LDLR labeling

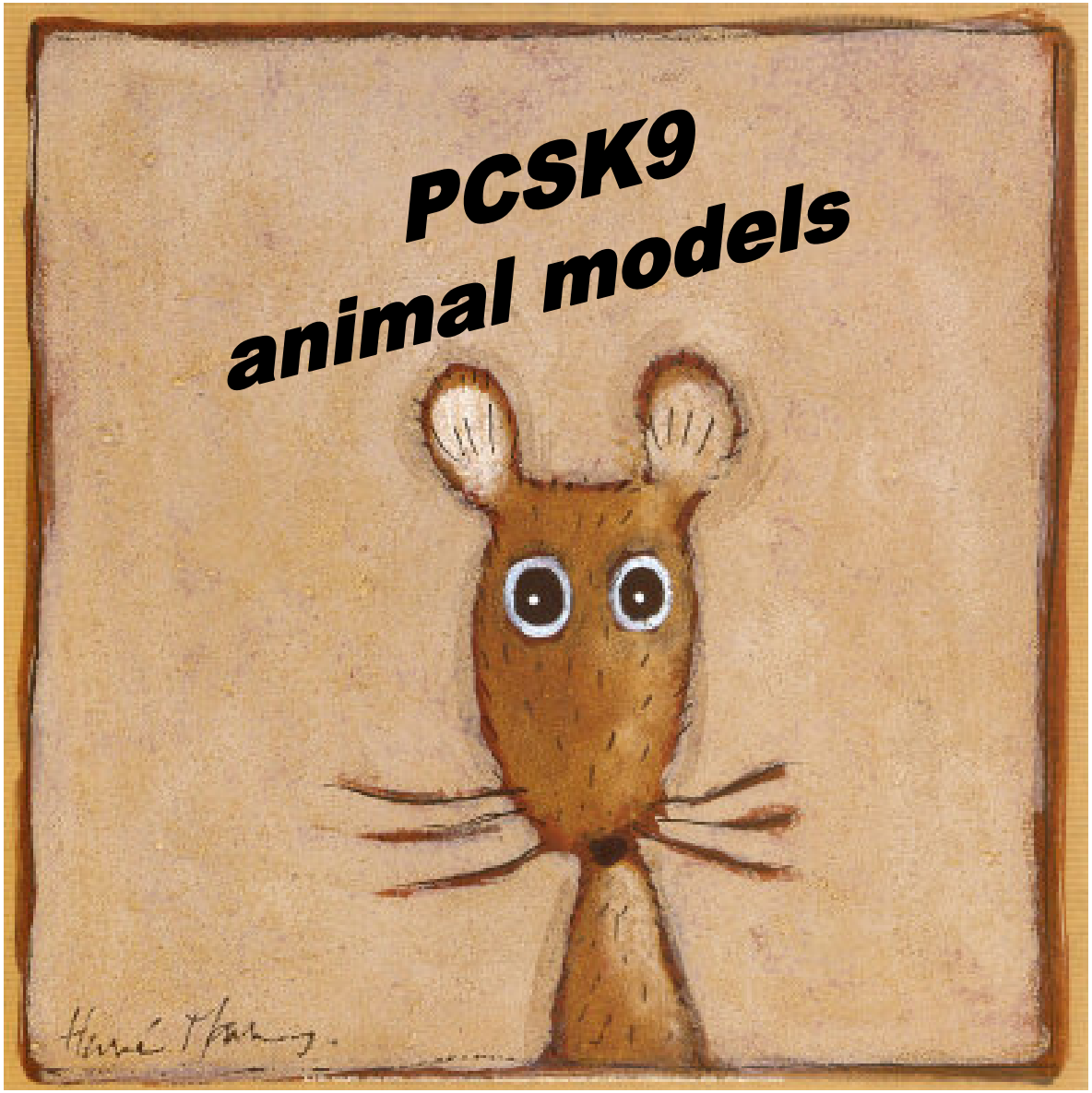


Loss-of-function R46L & E670G reduce $pSer_{46}$ & $pSer_{668}$

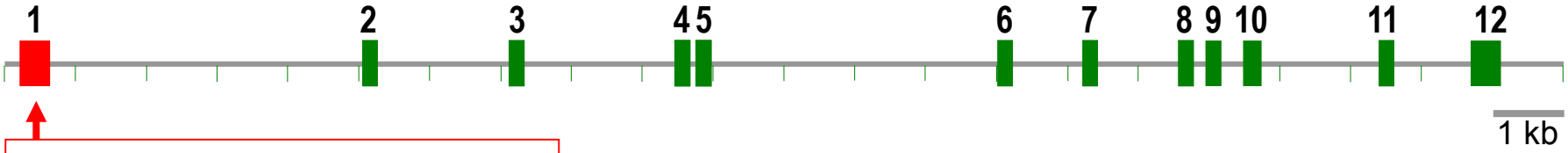




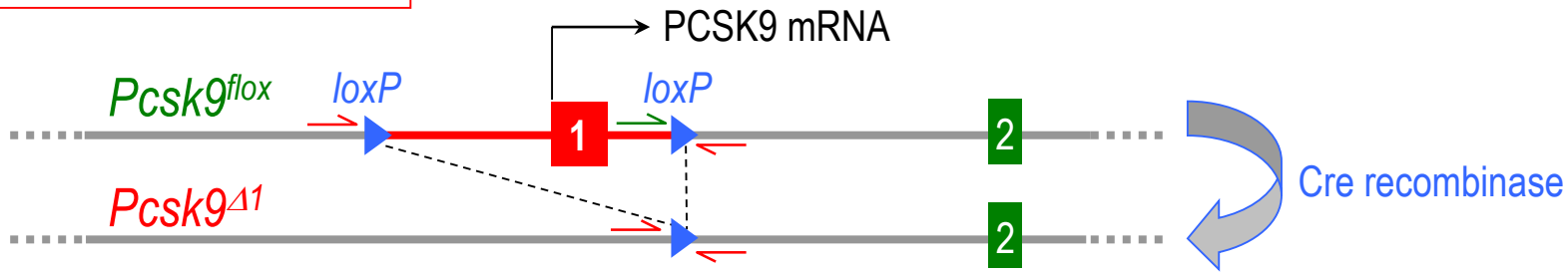
Ben Djoudi Ouadda A. et al. *ATVB* (2019) (*in revision*)


PCSK9
animal models

PCSK9 conditional inactivation (Cre/lox)



Exon 1 flanked with *loxP* sites:



Exon 1 deleted in a Cre-dependent manner → no mRNA, no protein

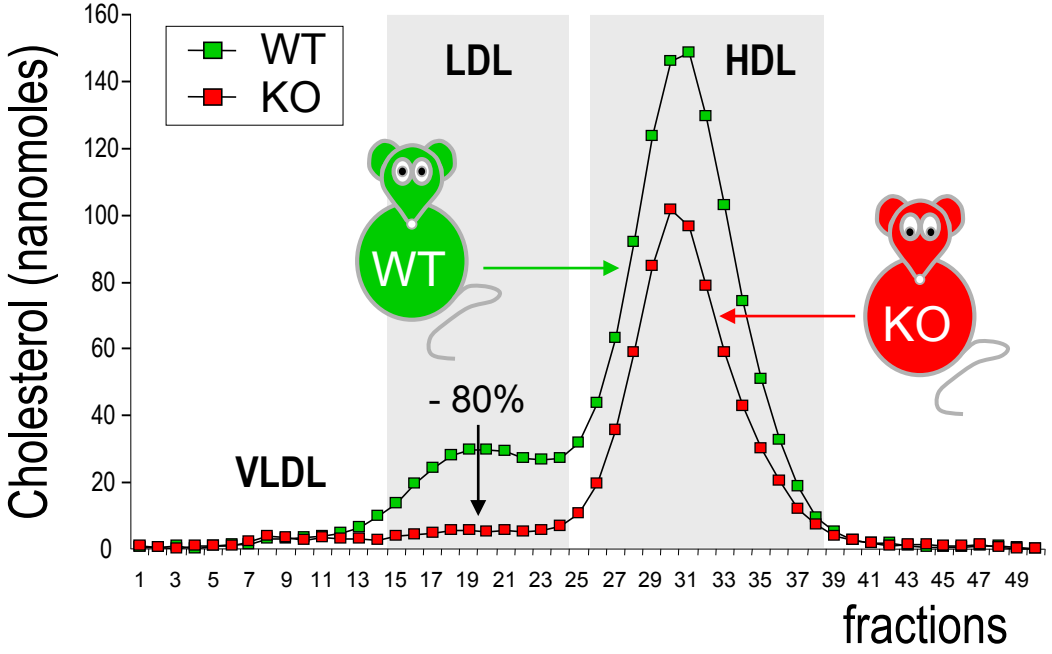
PCSK9 KO mice :
breeding with CMV-cre mice
expressing Cre ubiquitously

no PCSK9

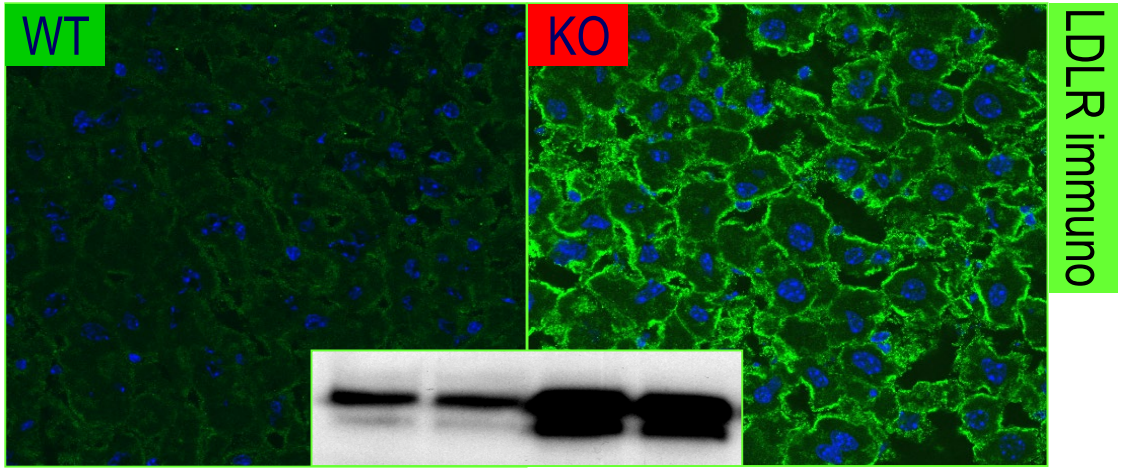
Hepatocyte-specific KO mice :
breeding with Tg(Albumin-cre) mice
expressing Cre only in hepatocytes

no liver PCSK9

PCSK9 inactivation leads to severe hypocholesterolemia



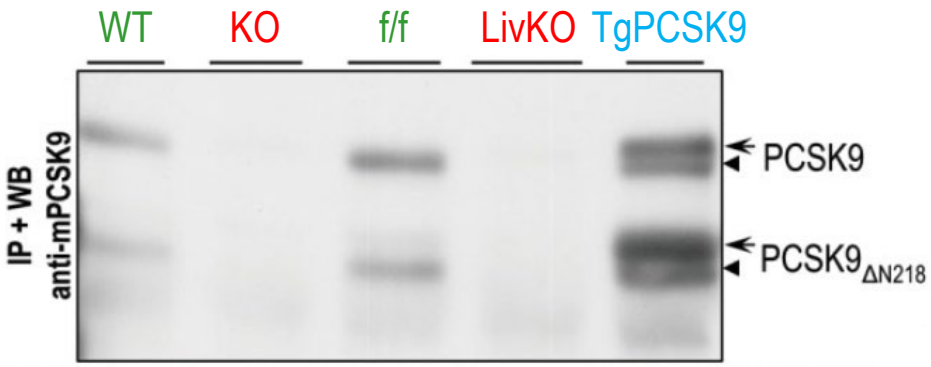
- ~40% drop in TC
- ~80% drop in LDL-C



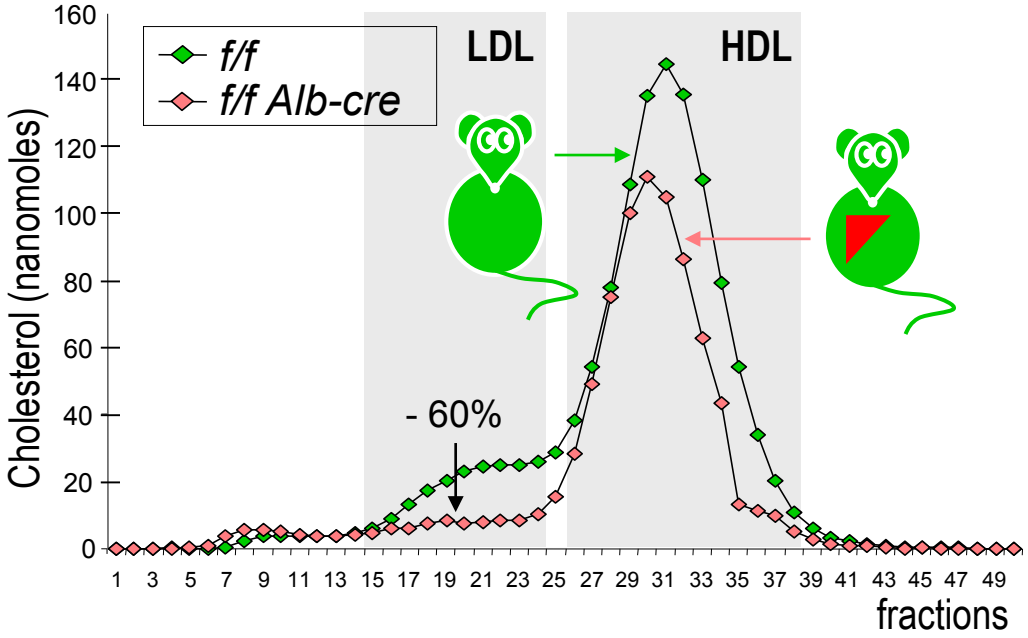
- LDLR accumulation in the liver
- LDLR accumulation at the hepatocyte cell surface

Zaid A et al. *Hepatology* (2008) **48**, 646

Hepatocyte-specific *Pcsk9* inactivation (hepKO)



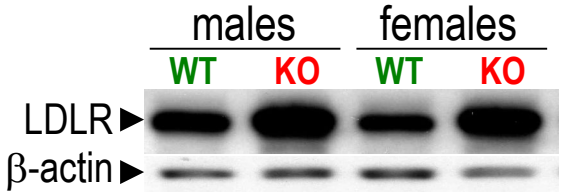
Liver-specific PCSK9 KO :
no circulating PCSK9 (also by ELISA)
→ exclusively secreted by hepatocytes



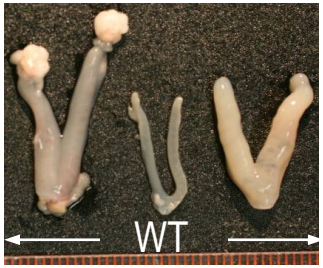
Zaid A et al. *Hepatology* (2008) **48**, 646

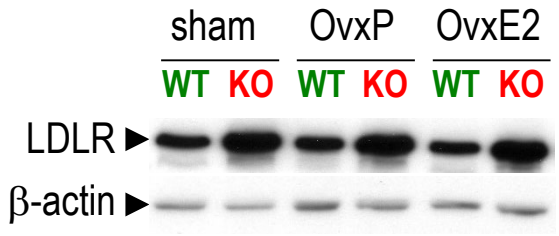
Roubstova A & Prat A et al. *ATVB* (2011) **31**, 785

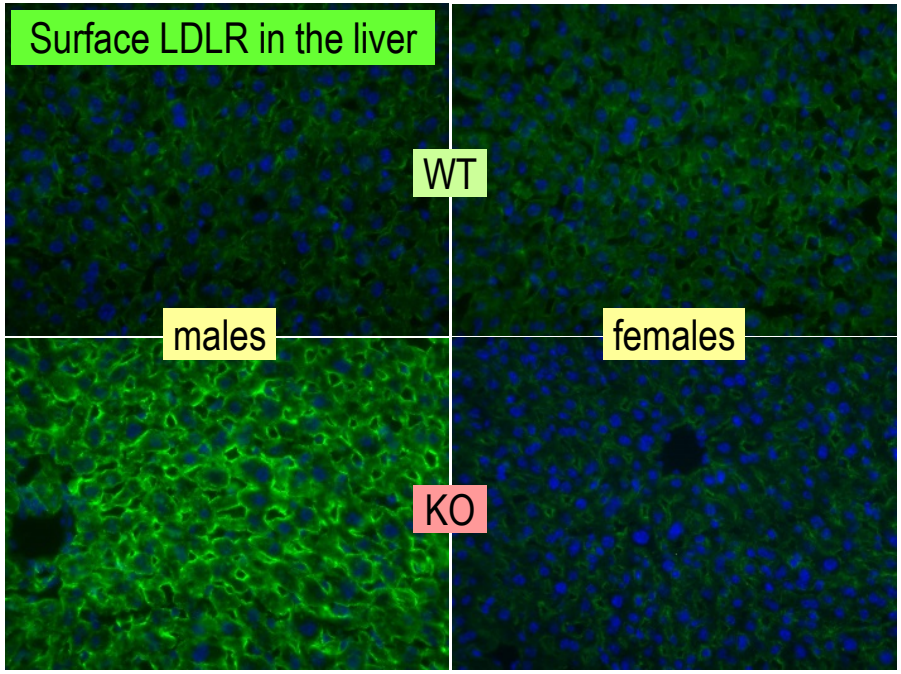
The absence of PCSK9 unmasks an estrogen-dependent subcellular distribution of the LDLR in mouse liver

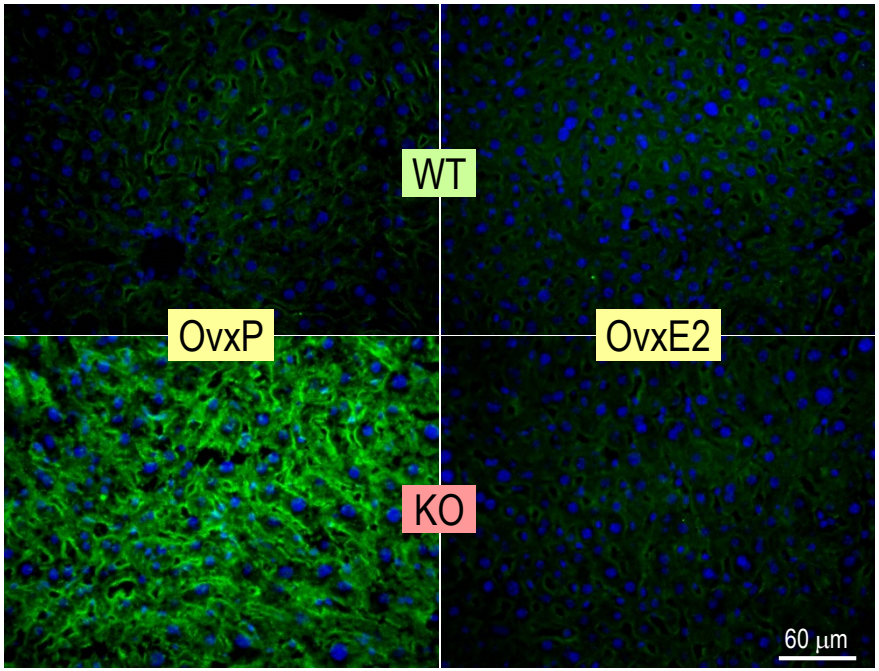


(same LDLR mRNA levels)







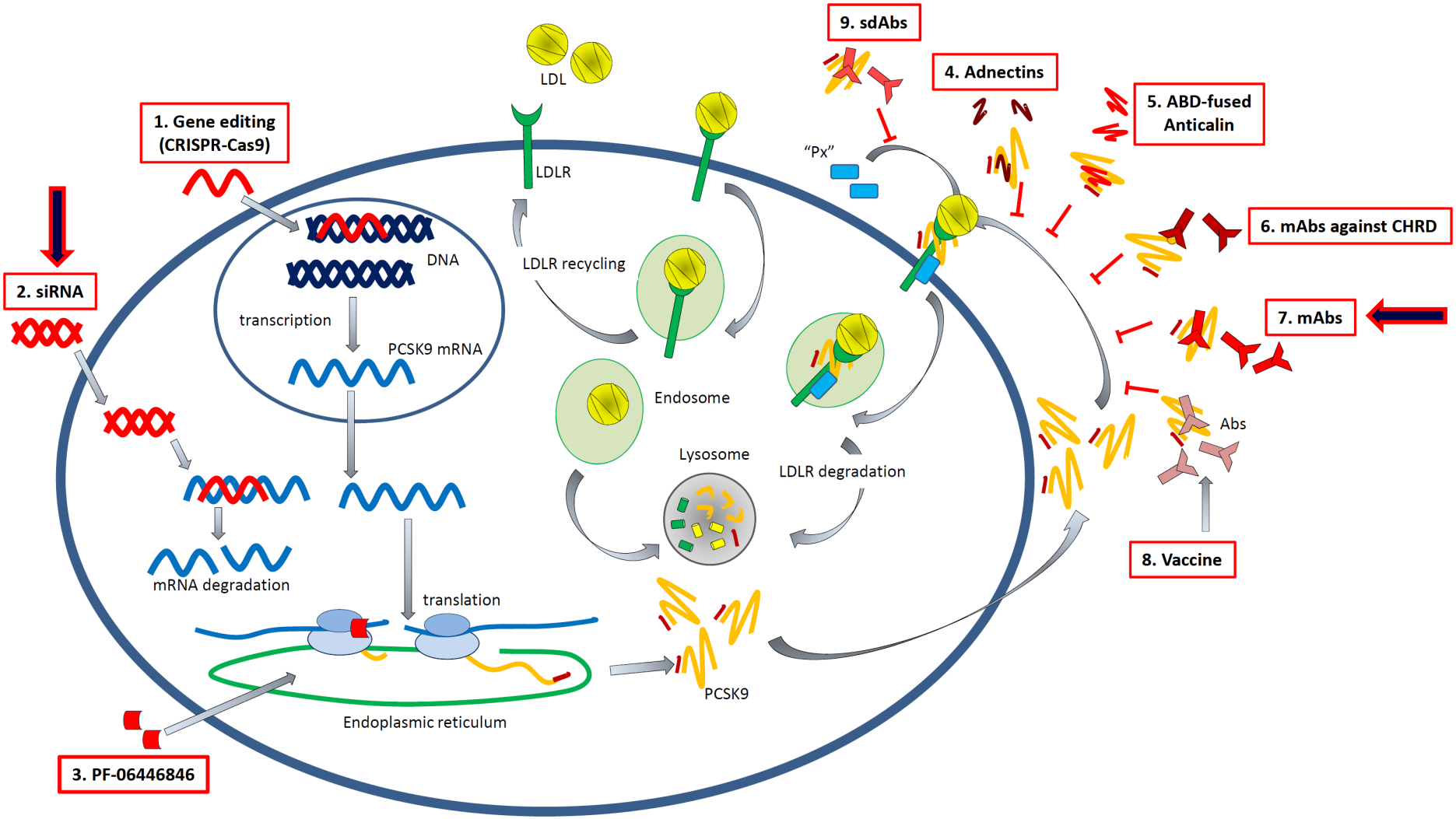


Roubstova A & Prat A et al. JLR (2015) **56**, 2133

Clinical applications of PCSK9 inhibition

IRCM

Strategies to target PCSK9



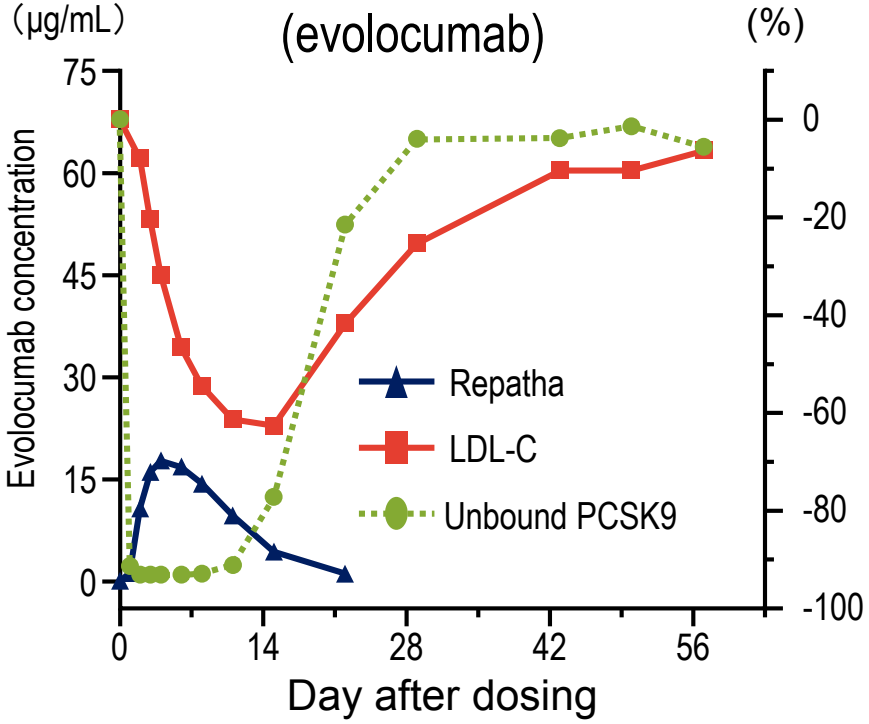
Seidah NG et al. *Cardiovasc Res* (2019) **115**, 510

Humanized PCSK9 monoclonal antibodies:

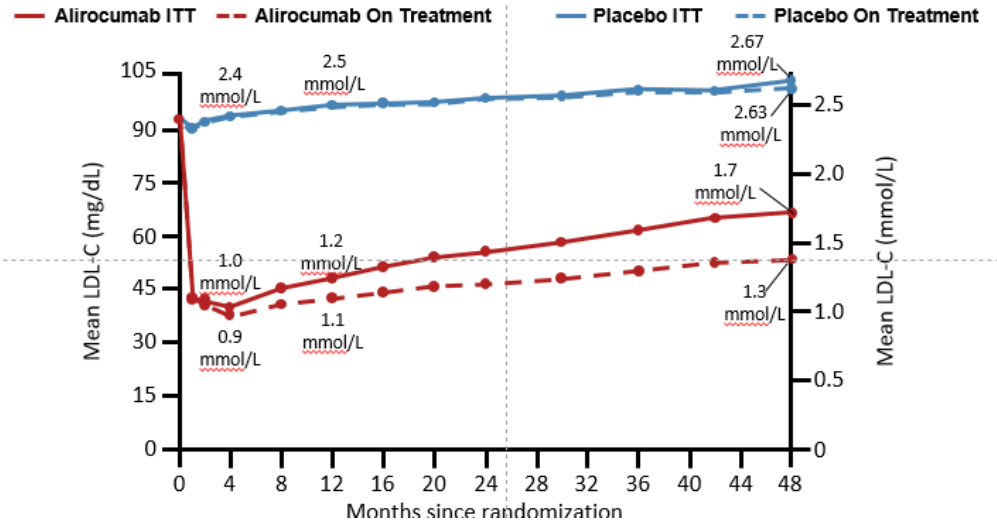
Repatha (140 mg SC) versus Praluent (75 mg SC)

mAb of the human IgG2 subclass can form covalent dimers *in* vivo (ideal), whereas IgG1 are monomers

Repatha 140 mg (evolocumab)



Praluent 75-150 mg (alirocumab)

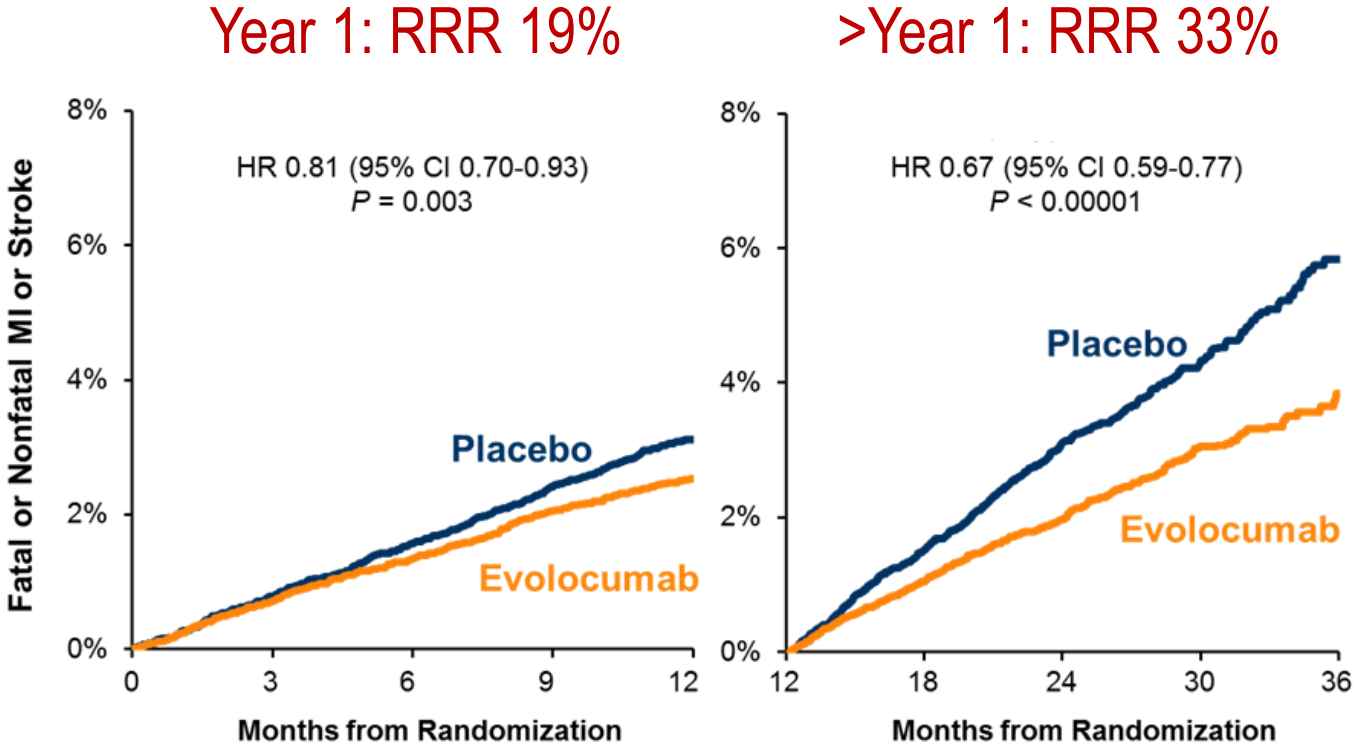


IgG2-λ

IgG1

Benefit of continuing aggressive lipid-lowering therapy to prevent recurrent cardiovascular events

FOURIER: Landmark analysis of fatal or nonfatal MI or stroke

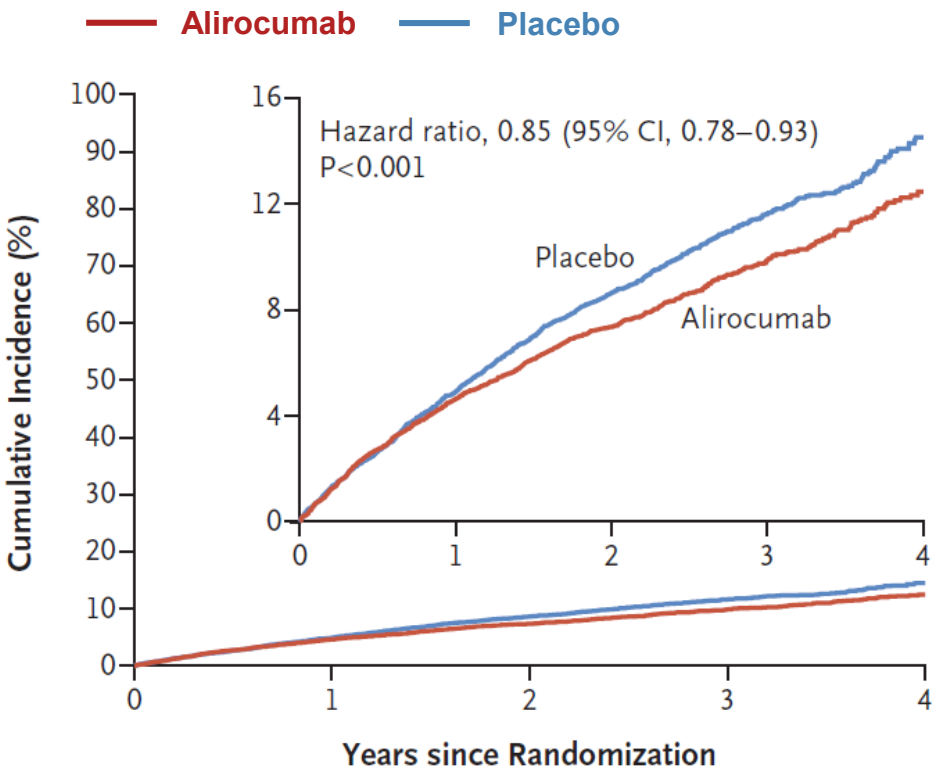


Longer duration of treatment and follow-up suggests larger risk reduction

Adapted from Sabatine MS *et al*. American College of Cardiology 2017 Scientific Sessions. March 17, 2017, Washington, DC.

Main Secondary Endpoints: Any Cardiovascular Event and Death, Nonfatal MI, or Nonfatal Ischemic Stroke

Composite death from:
- CAD,
- non fatal MI,
- non fatal ischemic stroke,
- or unstable angina

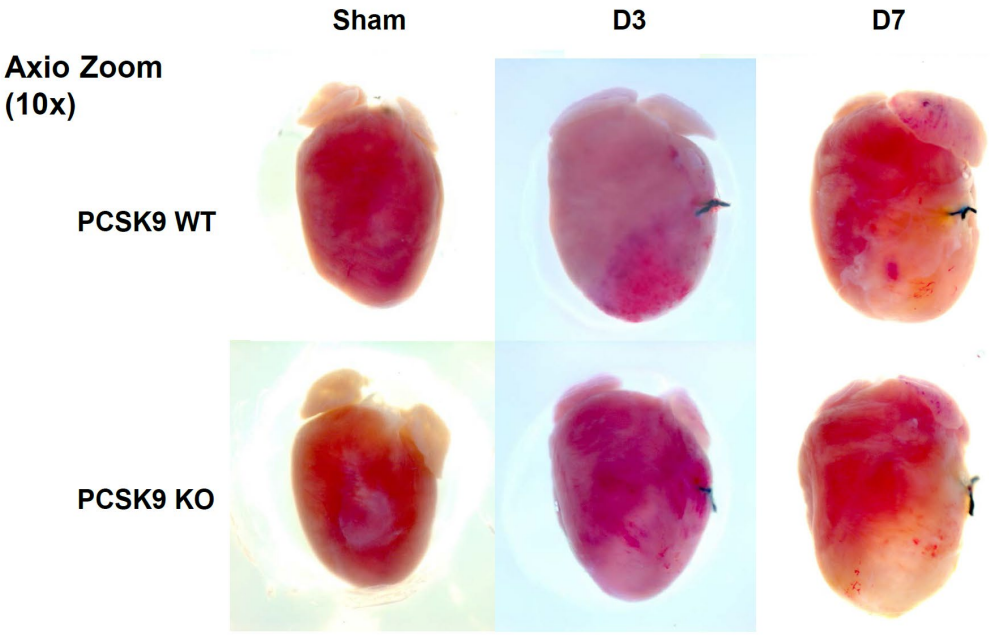


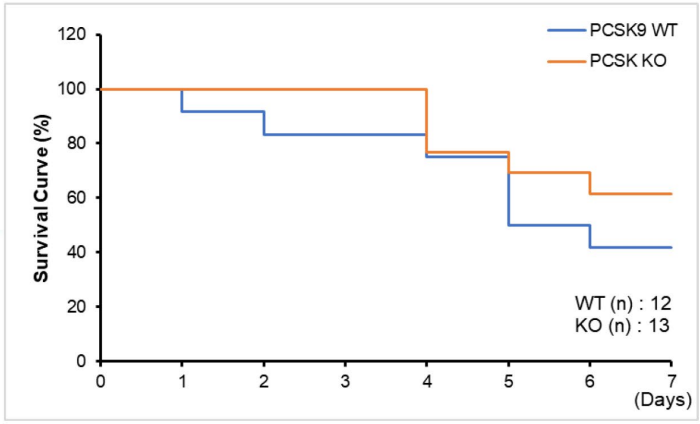
No. at Risk					
Placebo	9462	8805	8201	3471	629
Alirocumab	9462	8846	8345	3574	653

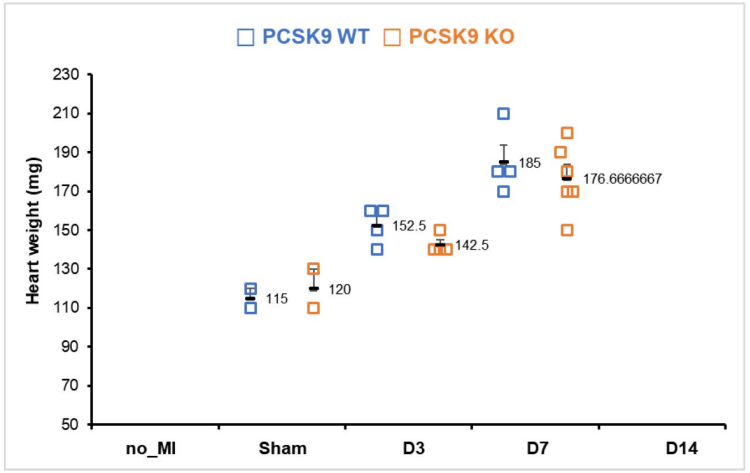
Schwartz GG et al. N Engl J Med 2018 (epub ahead of print).
Alirocumab and Cardiovascular Outcomes After Acute Coronary Syndrome

ODYSSEY OUTCOMES

After acute myocardial infarction (MI), survival is higher in PCSK9 KO mice than PCSK9 WT ones





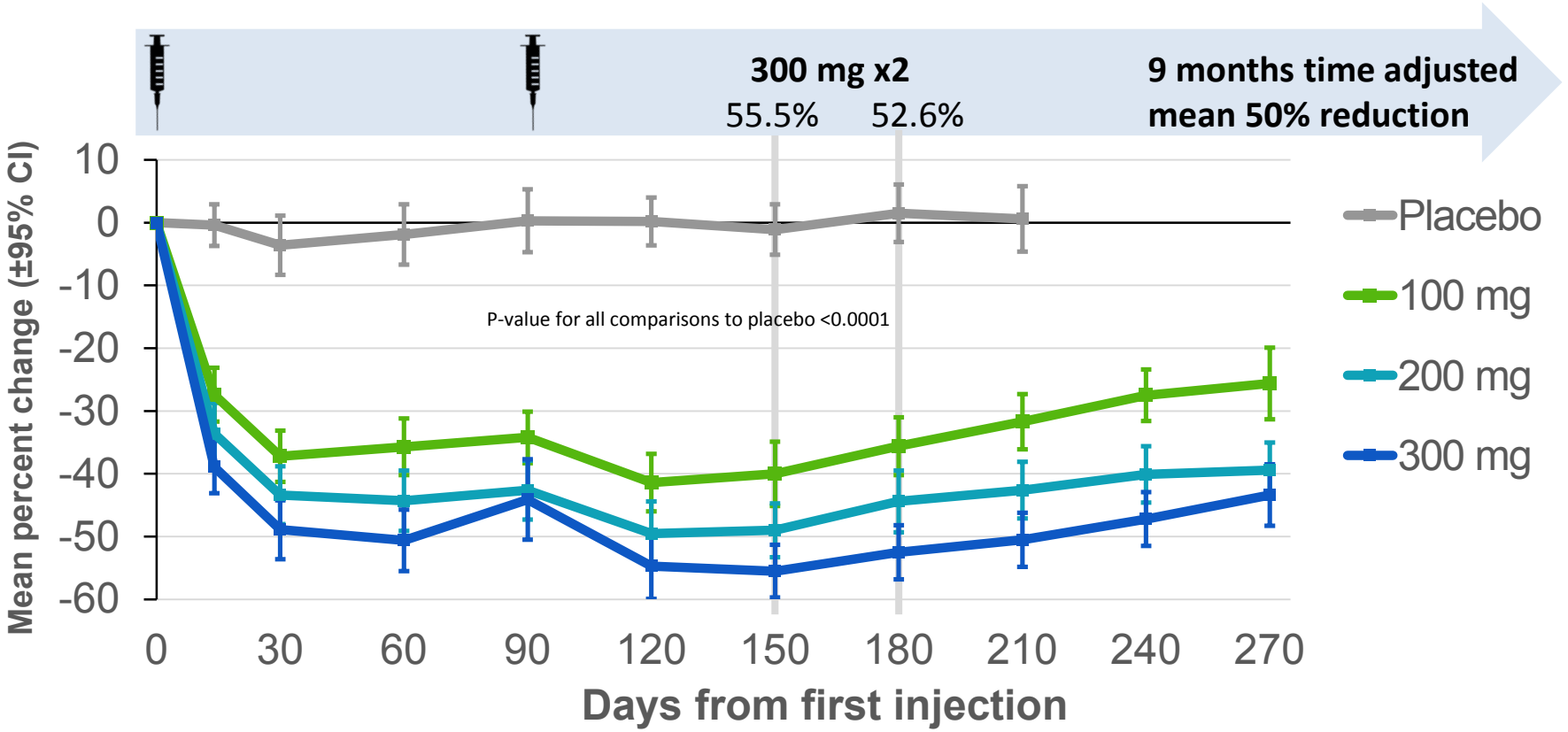


MI is induced by
ligation of the left coronary artery

https://www.jove.com/video/3093/modified-technique-for-coronary-artery-ligation-in-mice

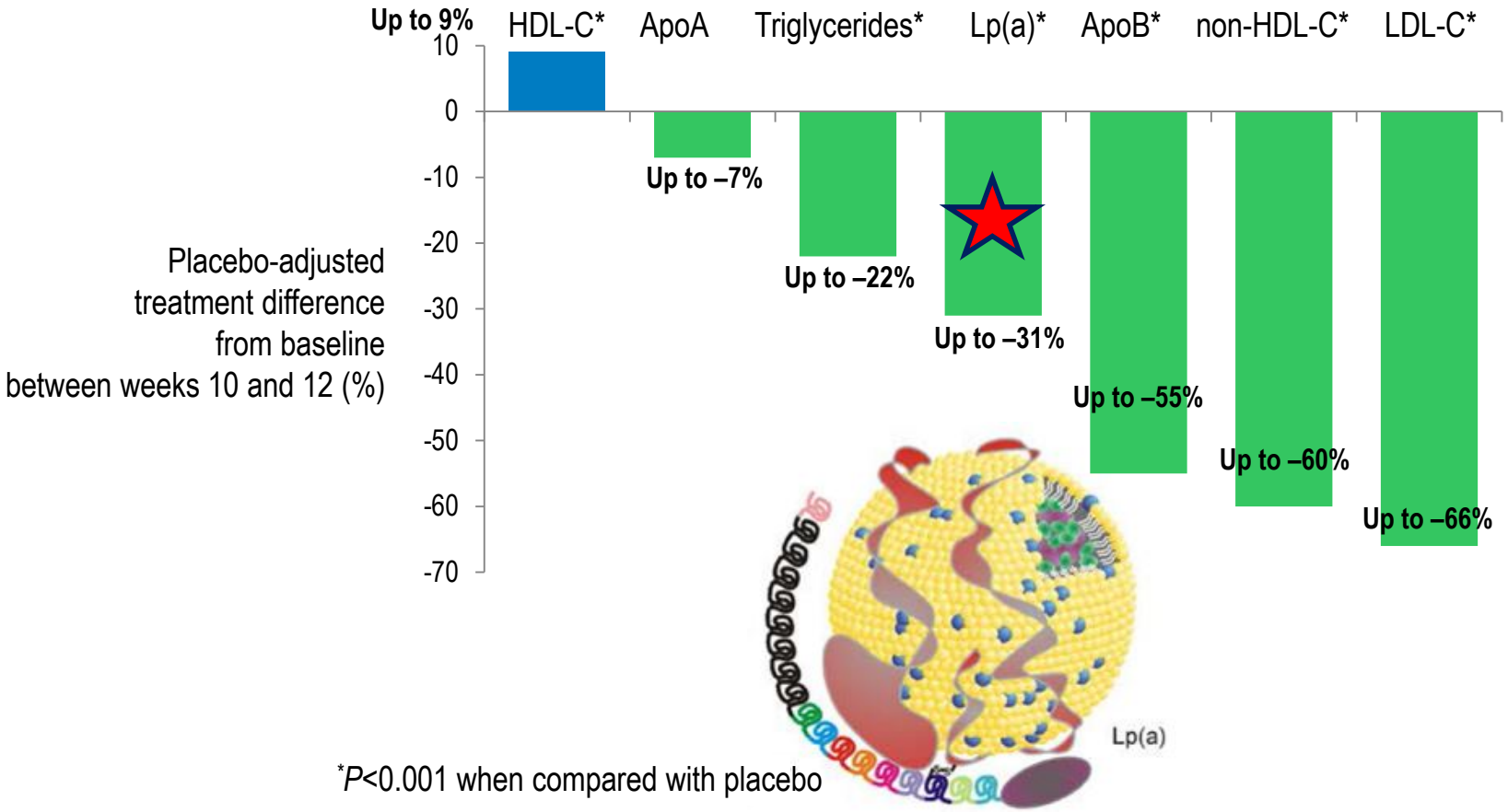
Goo Taeg Oh et al. (*In preparation*)

Inclisiran (siRNA) efficacy (two dose-starting regimen) : robust, sustained LDL-C reductions



Ray KK et al. *N Engl J Med* (2017) **376**, 1430

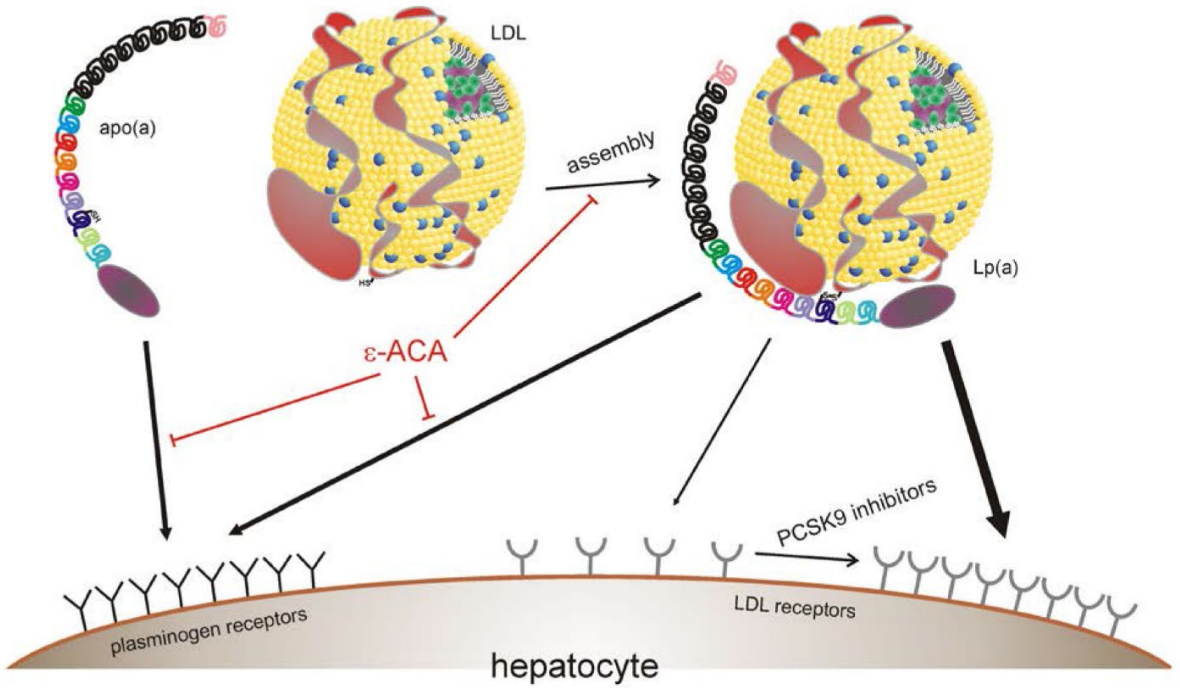
In addition to lowering LDL-C, evolocumab reduced other atherogenic lipids and modestly increased HDL-C in patients with HeFH



*P<0.001 when compared with placebo

Raal FJ et al. Lancet (2015) **385**, 331

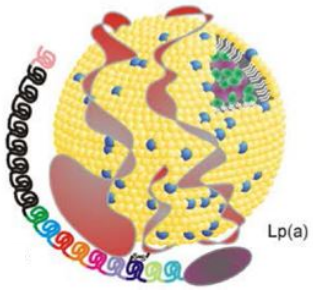
Under supra-physiological levels of the LDLR, as with PCSK9 mAbs, the LDLR is the receptor of Lp(a)



Romagnuolo R. et al. JBC (2015) **290**, 11649

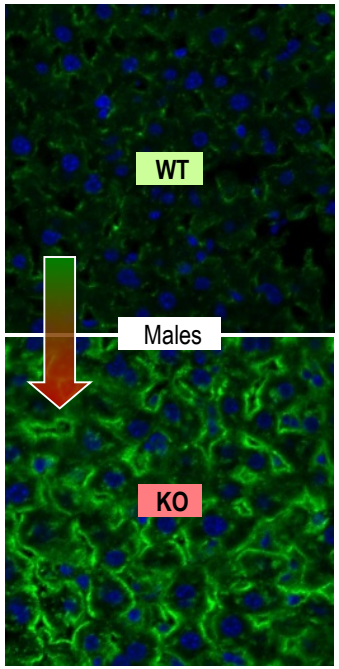
Romagnuolo R. et al. PLoS One (2017) e0180869, 2017

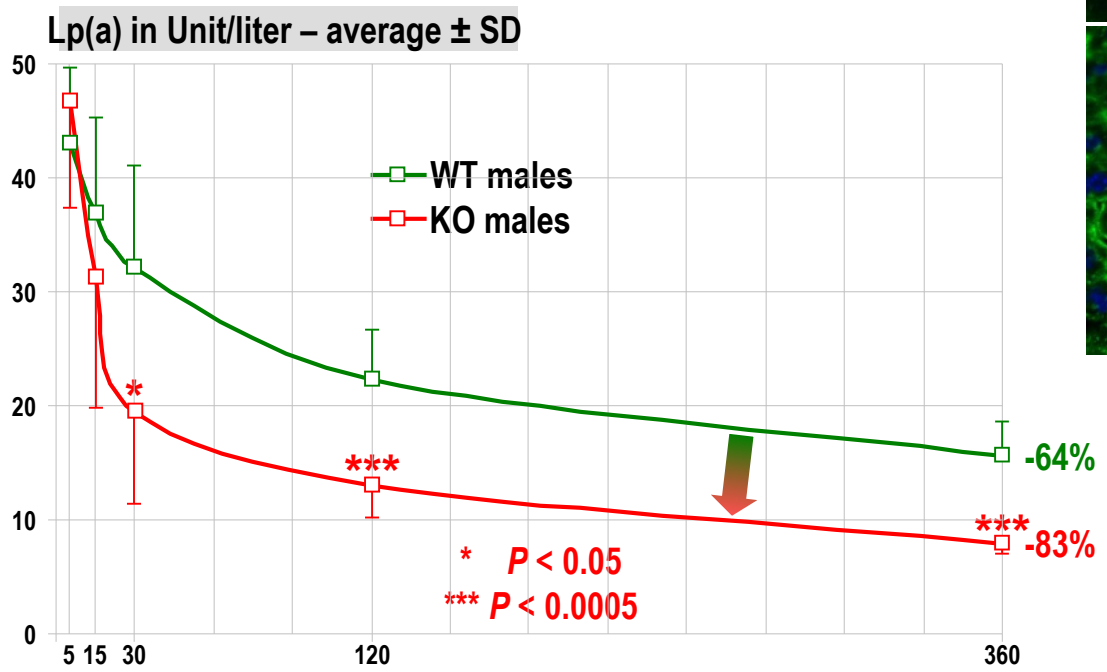
Lp(a) half life is shorter in PCSK9 KO mice compared to WT mice



KO versus WT male mice (n=9) – 25 μg/mouse

- 3 to 4 months of age
- 3hrs fasting
- Injection 100 uL at 0.25 mg/mL (25 μg/mouse)
- Bleeds at t = 5, 15, 30, 120 and 360 min (re-feeding after the t = 2 h bleeding)
- Lp(a) ELISA (Mercodia)

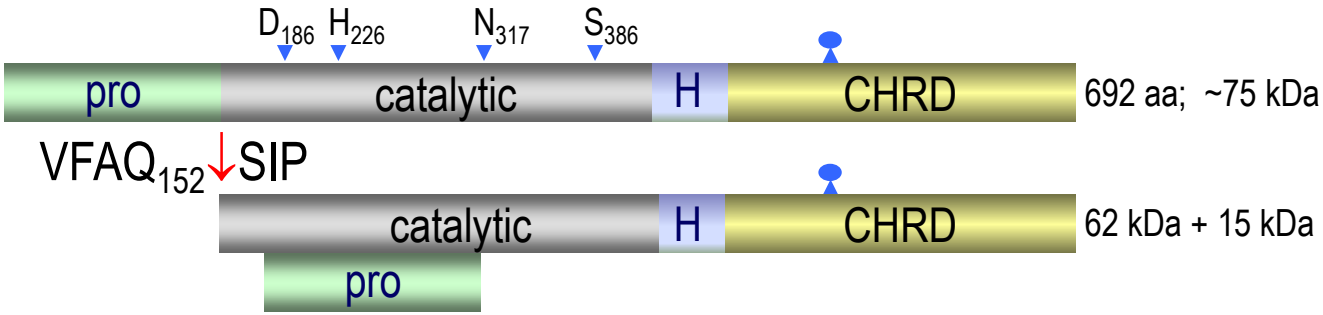




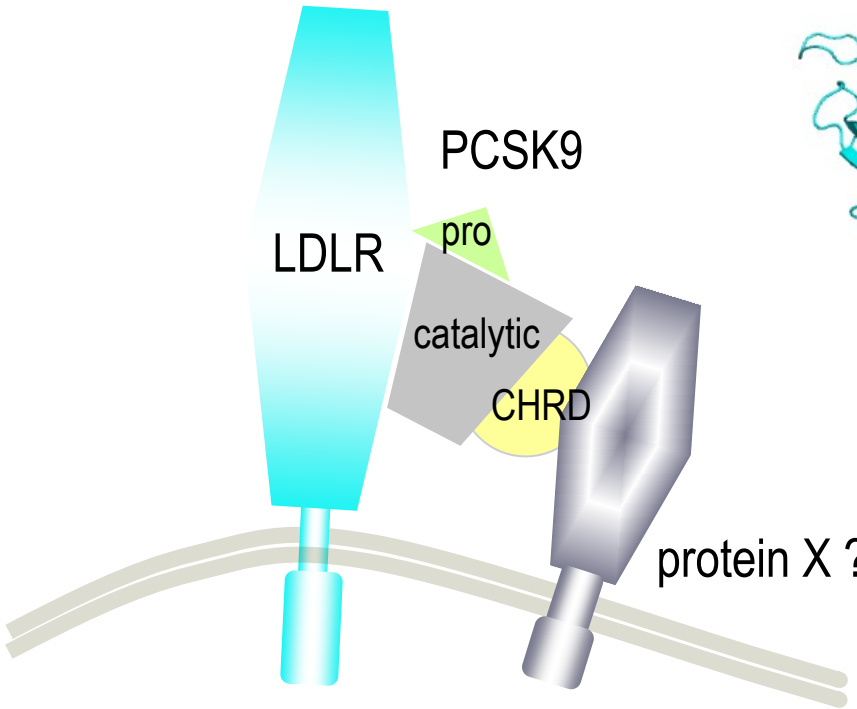
Initial velocity of clearance is 2.5-fold faster in KO males
(slope between the points 5 and 15 min: 1.56 versus 0.64 U/min)

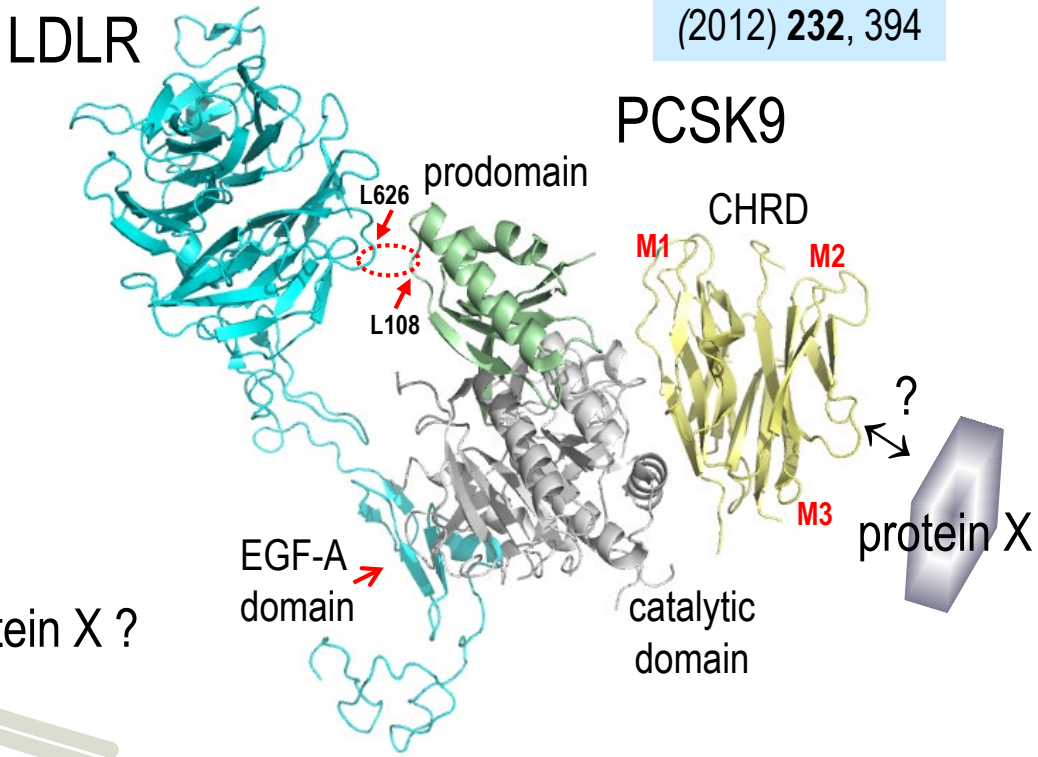
Roubstova A. et al. (in preparation)

PCSK9 binds the LDLR and triggers its degradation



Abifadel M et al. *Atherosclerosis* (2012) **232**, 394

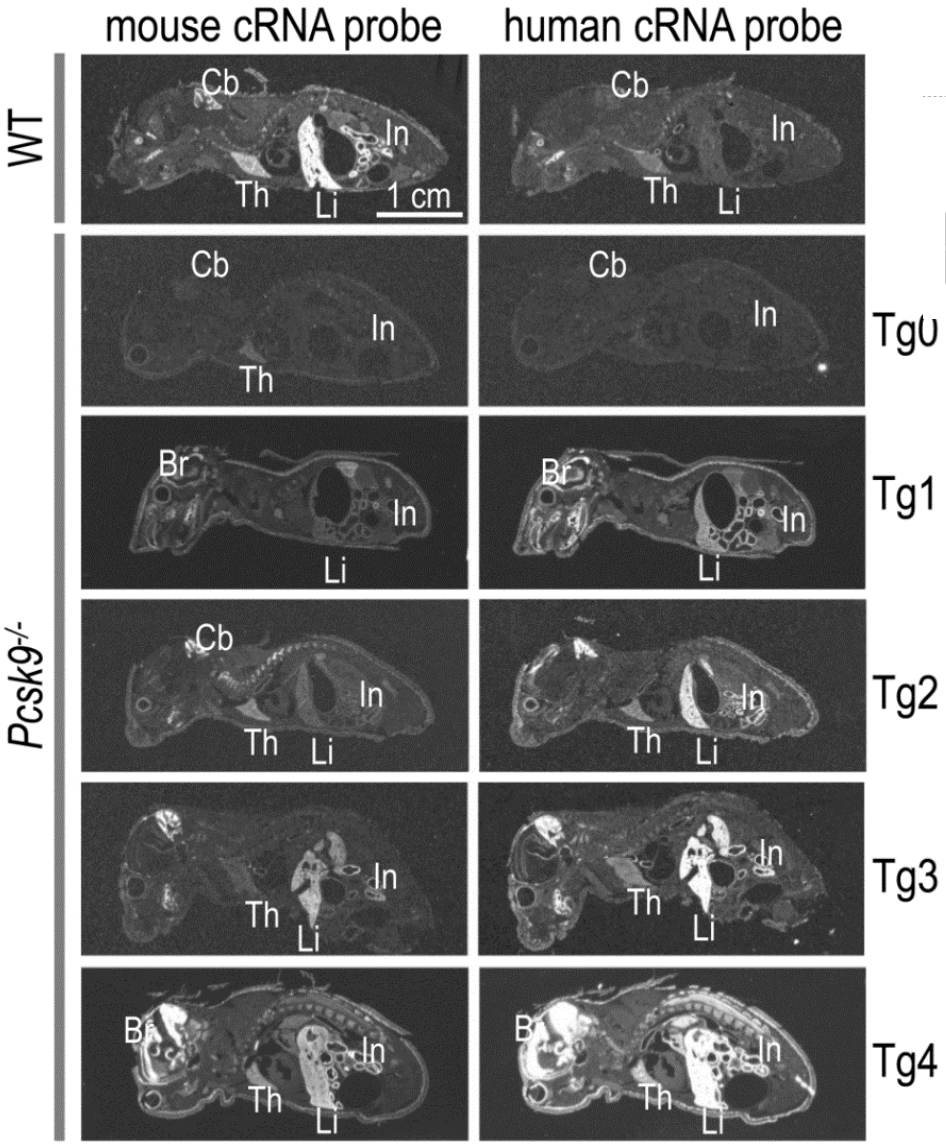


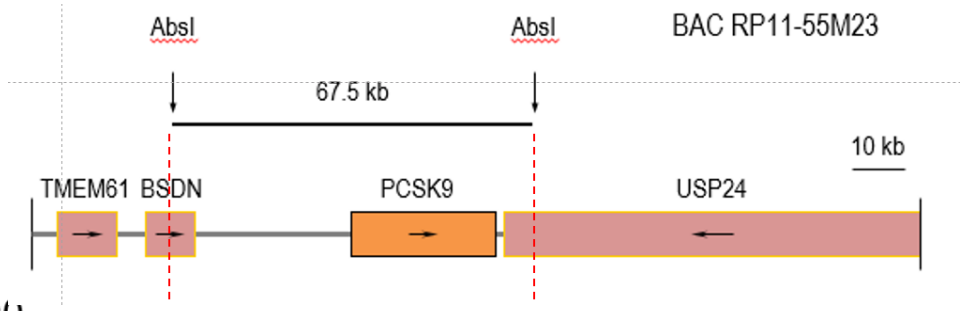


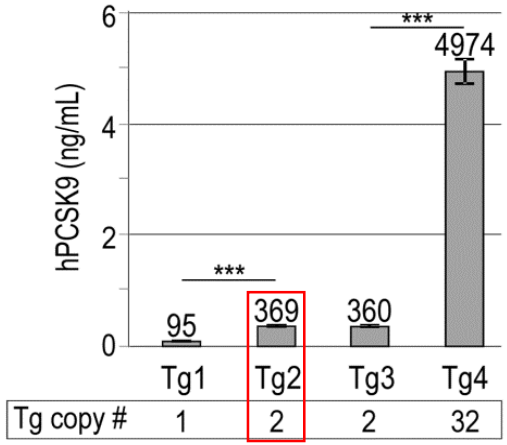
Zhang DW et al. *JBC* (2007) **82**, 18602

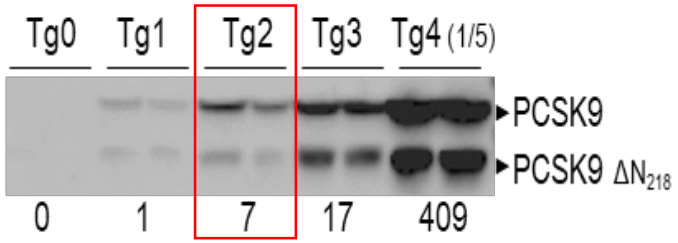
Lo Surdo P et al. *EMBO Rep* (2011) **12**, 1300

Mice solely expressing human PCSK9





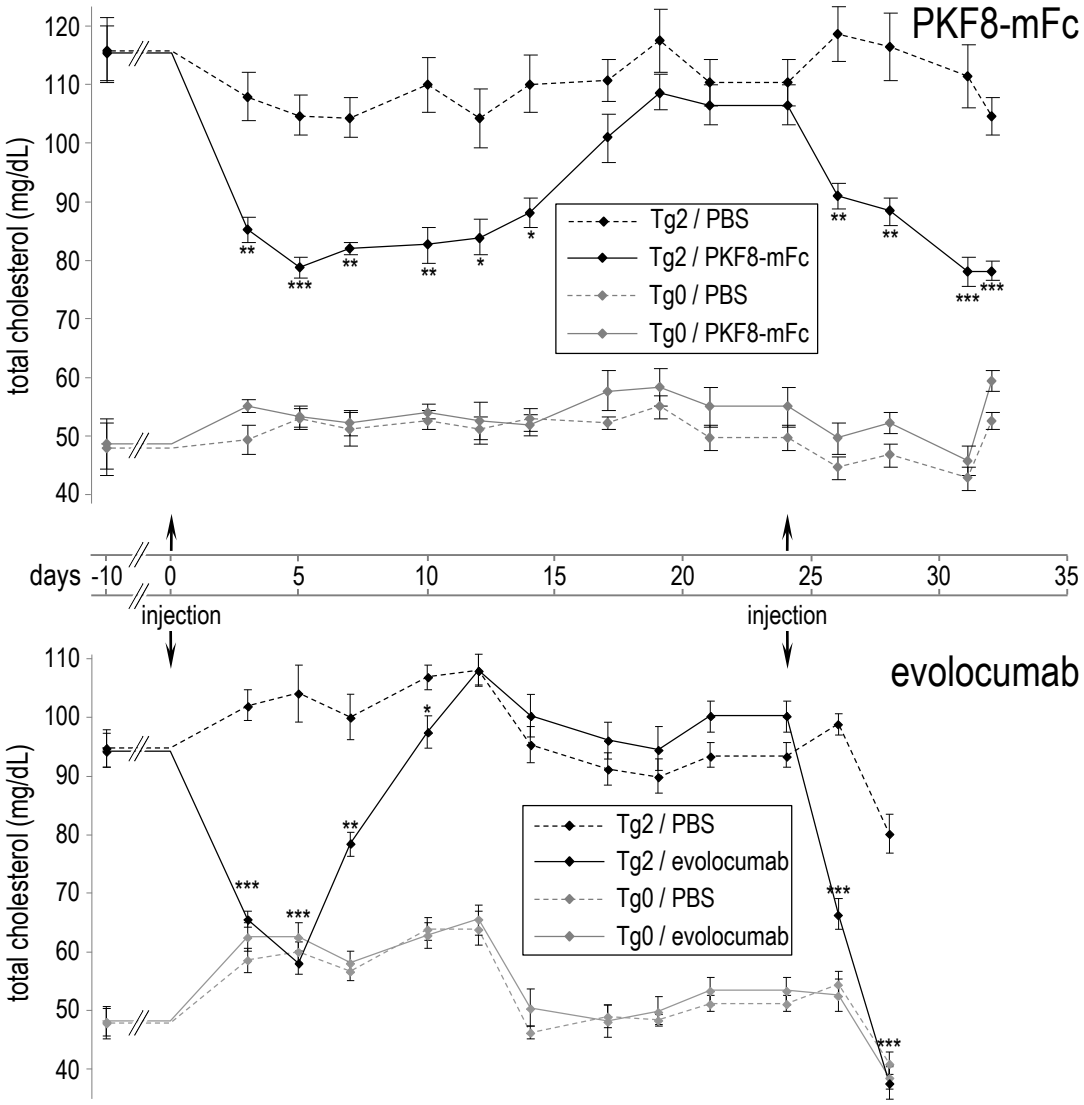




Weider E. et al. *JBC* (2016) **291**, 16659

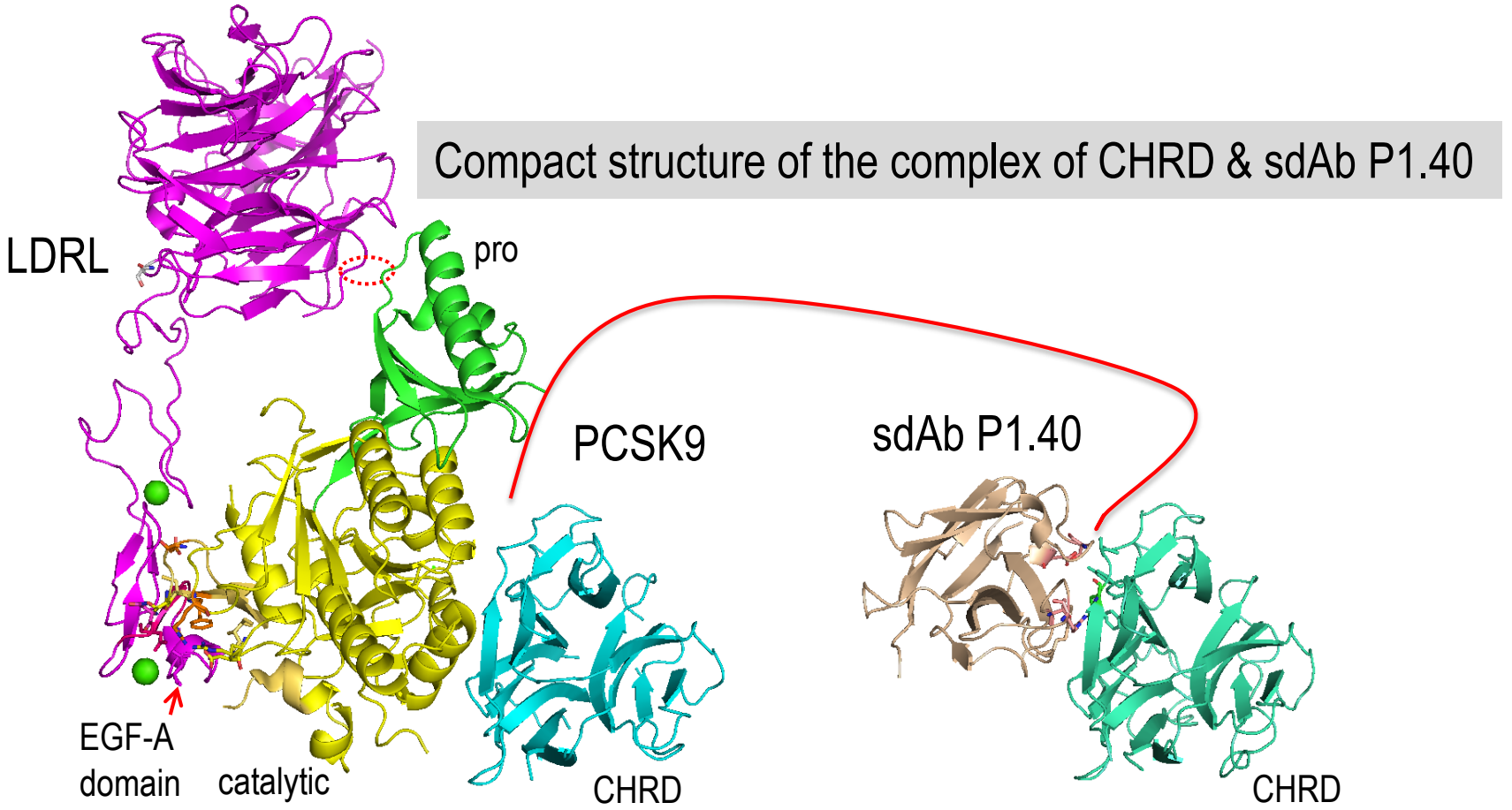
Essalmani R. et al. *Biol Chem* (2018) **399**, 13631

sdAbs against human the CHRD domain of PCSK9 inhibits its activity



Essalmani R. et al. *Biol Chem* (2018) **399**, 13631

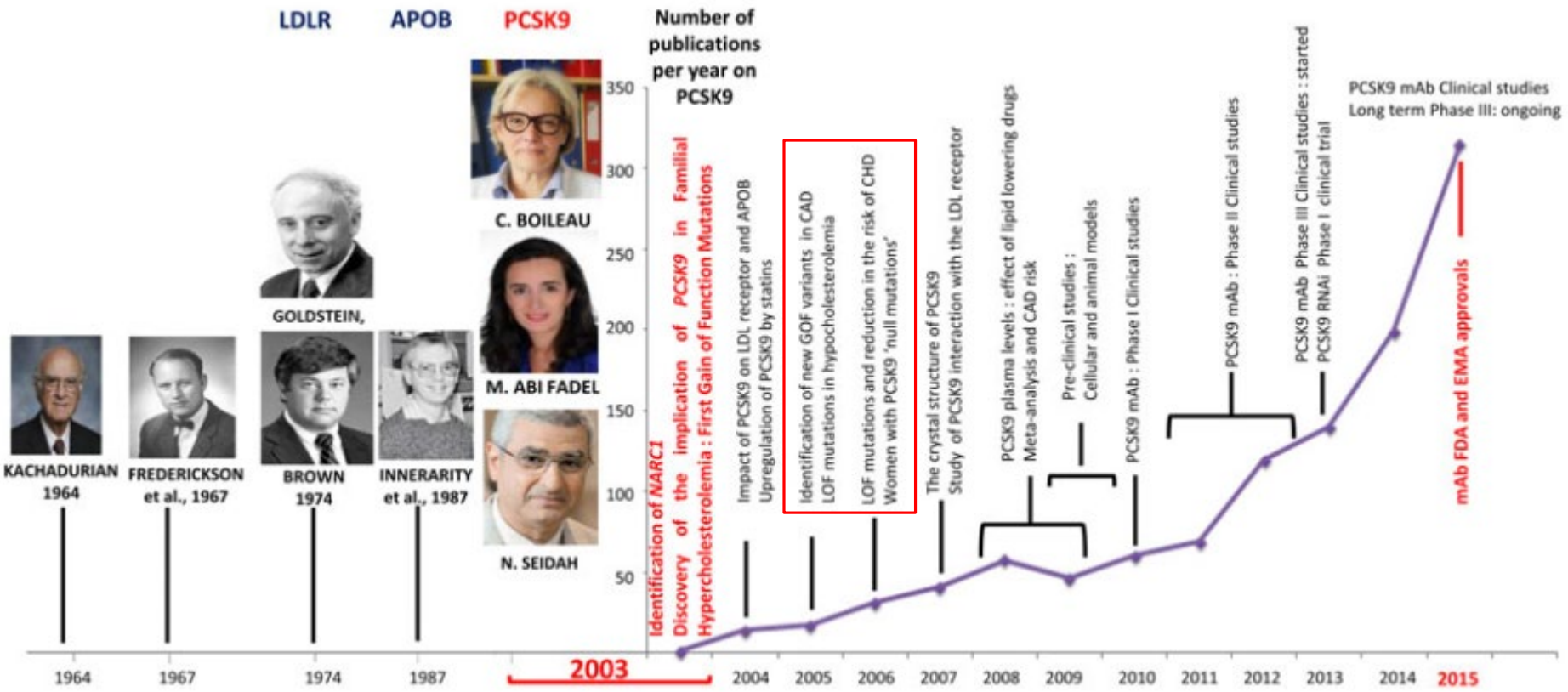
Could the sdAb P1.40 compete with « protein X » ?



Carole Fruchart-Gaillard & Vincent Dive (collab., France)

Lo Surdo P et al. *EMBO Rep* (2011) **12**, 1300

Autosomal dominant hypercholesterolaemia and the PCSK9 adventure



Eur Heart J (2016) **37**,1341-1352


DIET
LDLC
TG
LDLR
LRP8
CD36
VLDLR
BBR
ANXA2
HDL
OM
FoxO3
TICE
LOF
Lp(a)
STATIN
HDL
HCV
CHD
ATHER
SEPSIS
ENaC
VIRUS
OXIDATION
FIBROSIS
$?

Martin Waldseemüller incomplete world map 1508

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- Jean Davignon (IRCM)
- Vincent Dive (Paris)

Learning objectives

Objective 1

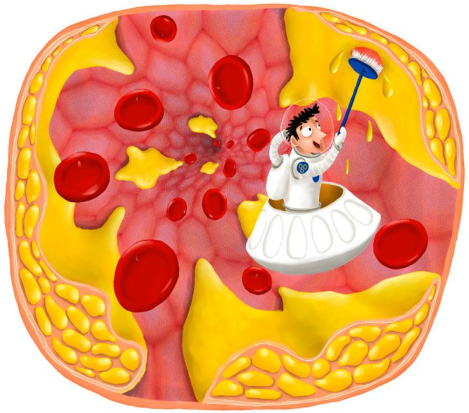
- Is PCSK9 acting as a protease and why ?

Objective 2

- How does PCSK9 lead to LDLR degradation ?

Objective 3

- What is the current strategy for PCSK9 inhibition and what are the CVD outcomes ?



merci / thank you!