ΙΑΤΡΙΚΗ ΧΗΜΕΙΑ

ΠΑΝΕΠΙΣΤΗΜΙΟ ΙΩΑΝΝΙΝΩΝ
ΣΧΟΛΕΣ ΘΕΤΙΚΩΝ ΕΠΙΣΤΗΜΩΝ – ΕΠΙΣΤΗΜΩΝ ΥΓΕΙΑΣ
ΤΜΗΜΑΤΑ ΧΗΜΕΙΑΣ– ΙΑΤΡΙΚΗΣ-ΒΙΟΛΟΓΙΚΩΝ ΕΦΑΡΜΟΓΩΝ ΚΑΙ ΤΕΧΝΟΛΟΓΙΩΝ
ΔΠΜΣ«ΙΑΤΡΙΚΗ ΧΗΜΕΙΑ»

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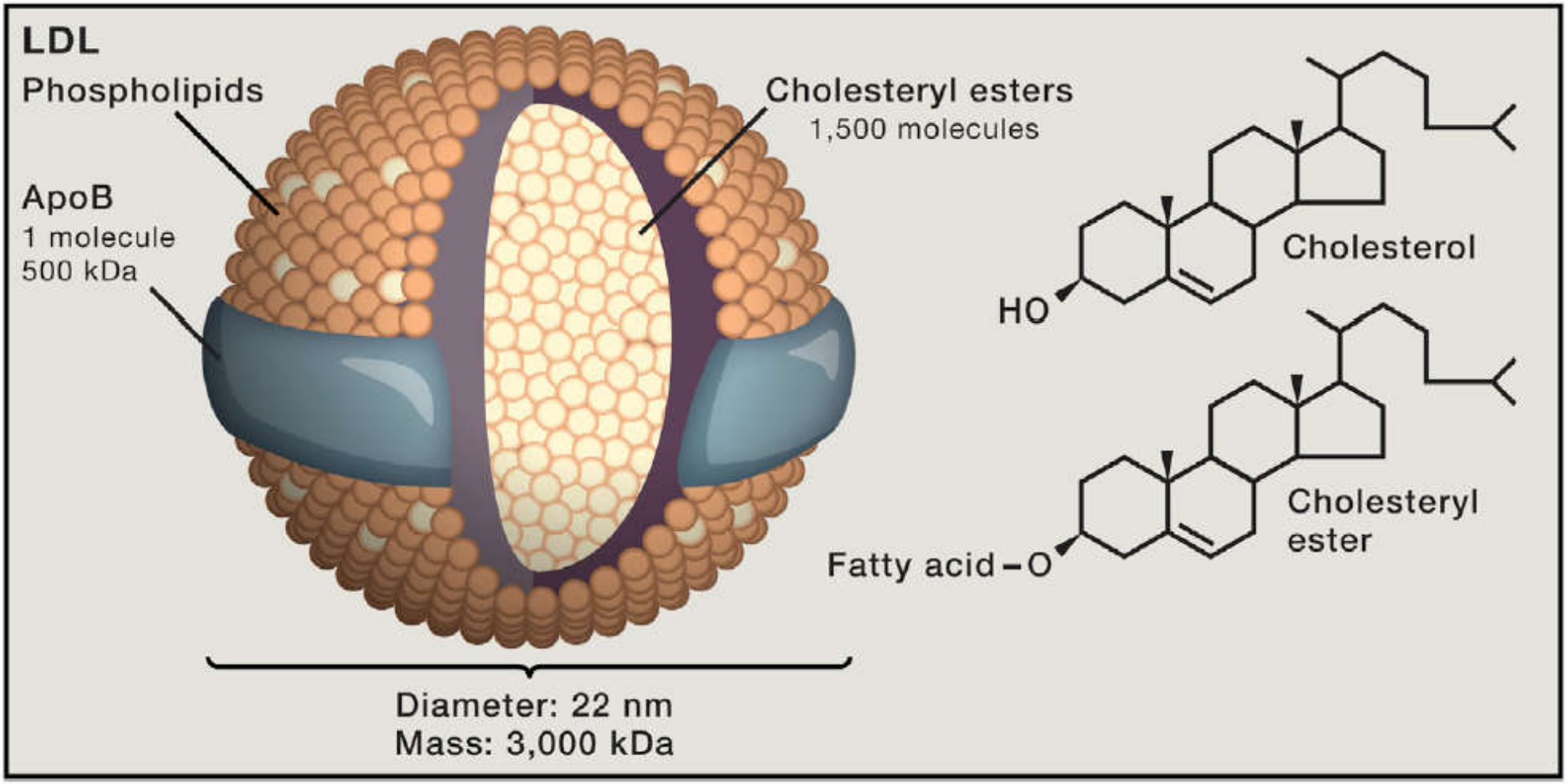
Ο ΡΟΛΟΣ ΤΗΣ PCSK9 ΣΤΟ ΜΕΤΑΒΟΛΙΣΜΟ ΤΩΝ ΛΙΠΟΠΡΩΤΕΪΝΩΝ

Ευάγγελος Λυμπερόπουλος-Μωυσής Ελισάφ

Τομέας Παθολογίας Ιατρικής Σχολής Παν/μίου Ιωαννίνων
www.bpath.gr, www.atherosclerosis.gr

LDL: A Cholesterol Carrier



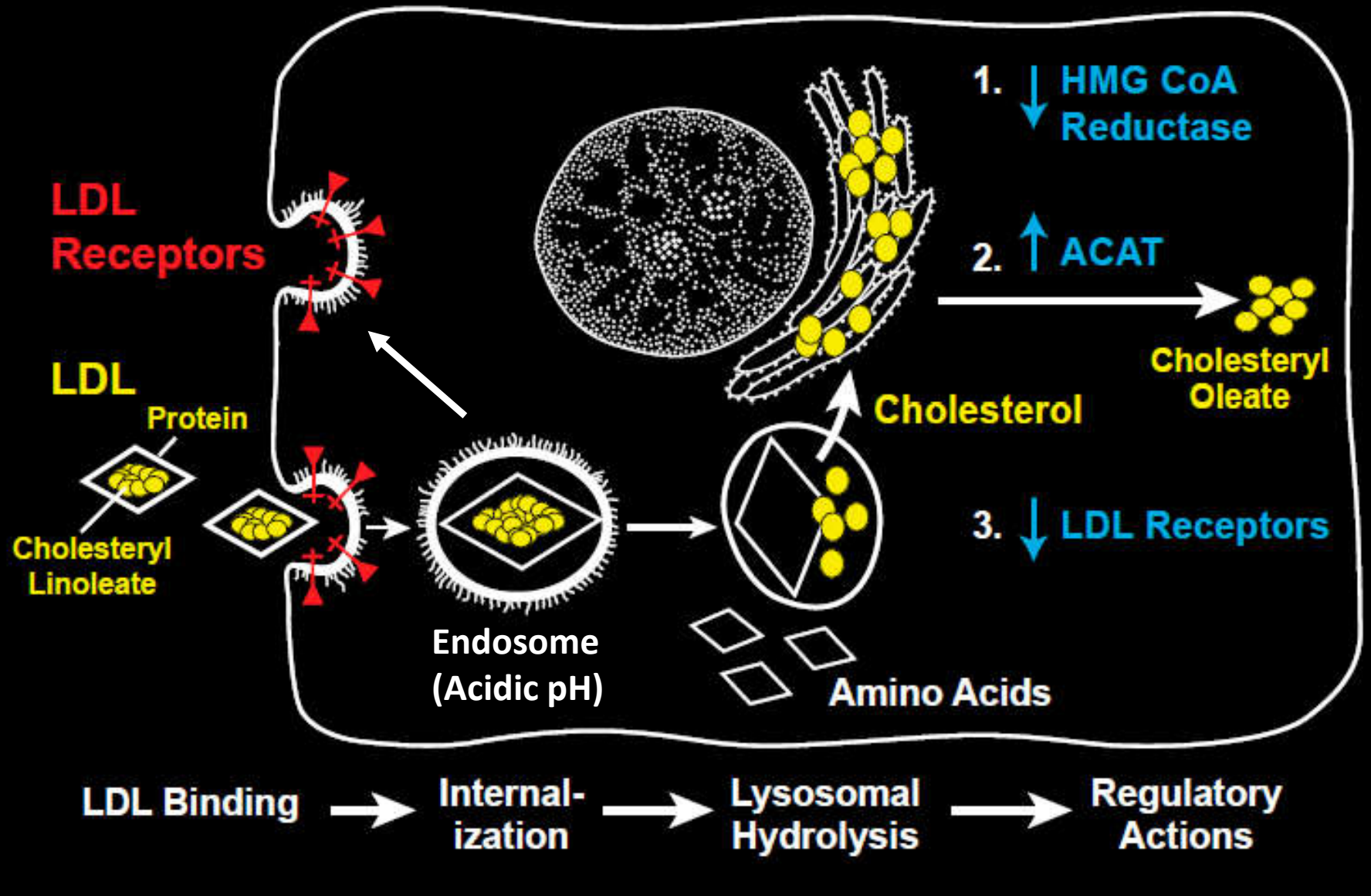
Goldstein JL, Brown MS. Cell. 2015

I- PCSK9 (Proprotein Convertase Subtilisin Kexin 9) DISCOVERY

What is PCSK9 ?

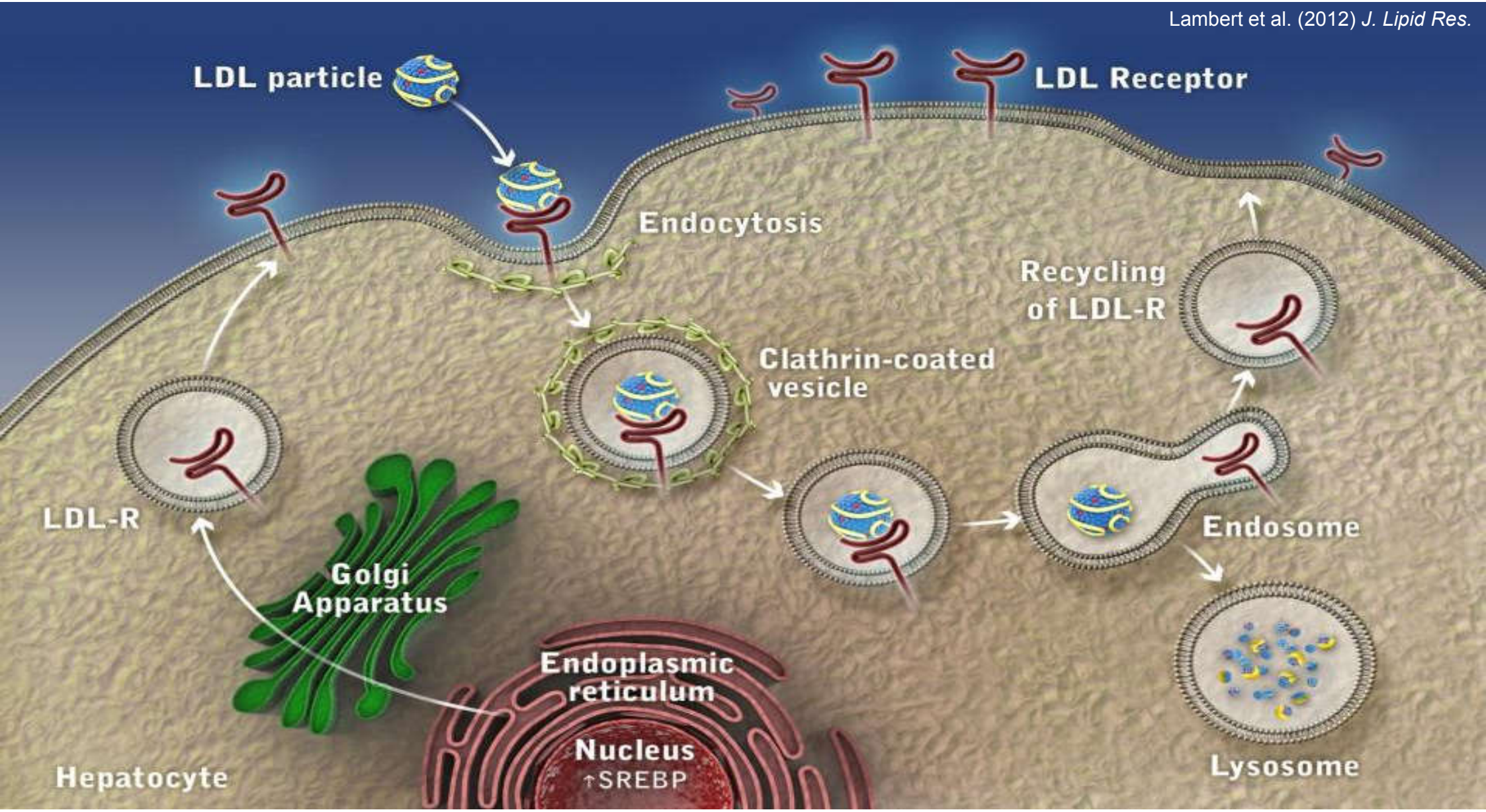
How was PCSK9 identified as a key gene in cholesterol homeostasis ?

Sequential steps in the LDL receptor pathway of mammalian cells

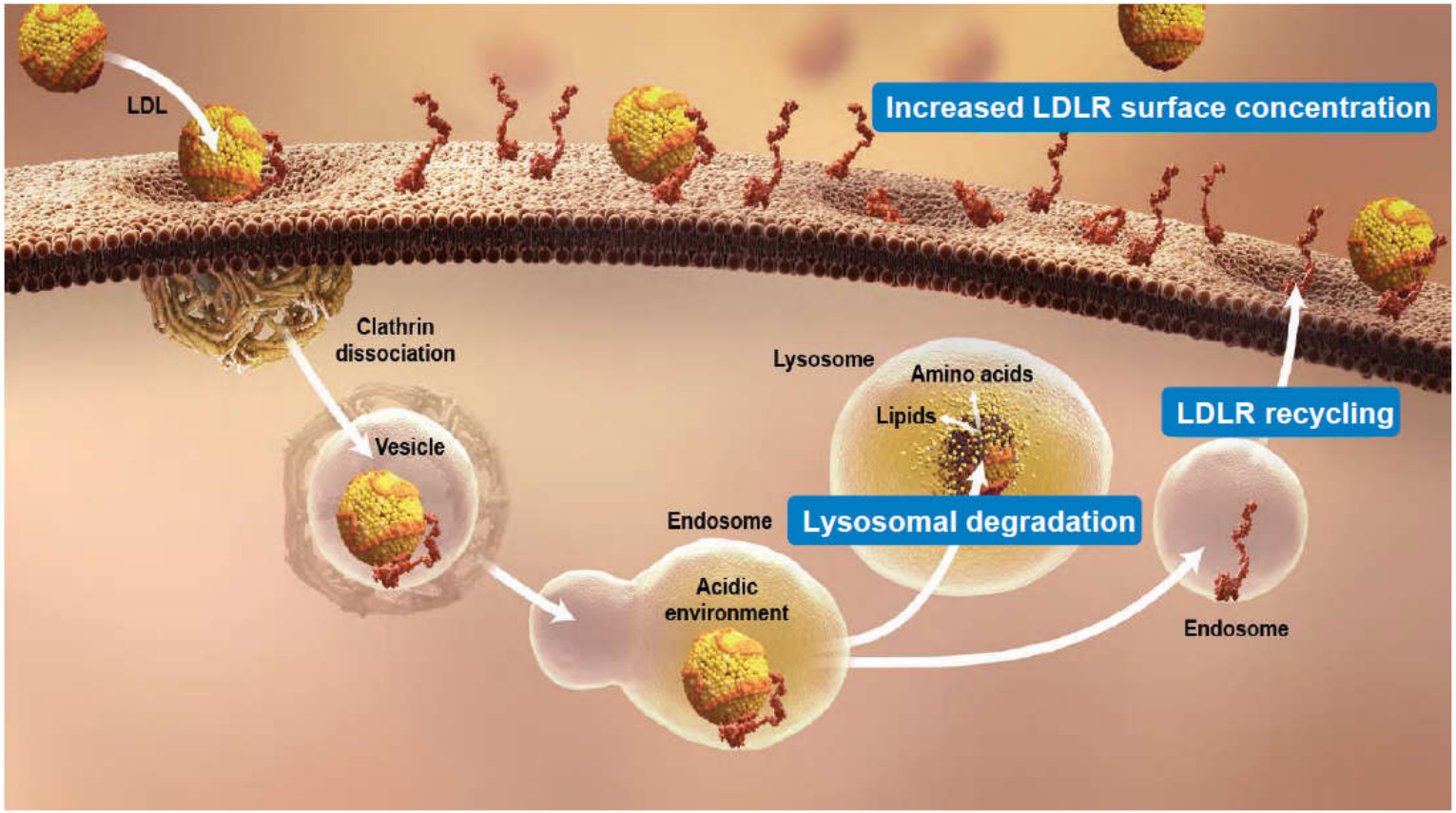


Brown MS, Goldstein JL. Proc Natl Acad Sci U S A. 1979;76: 3330-3337

LDL-Receptor Function and Life Cycle



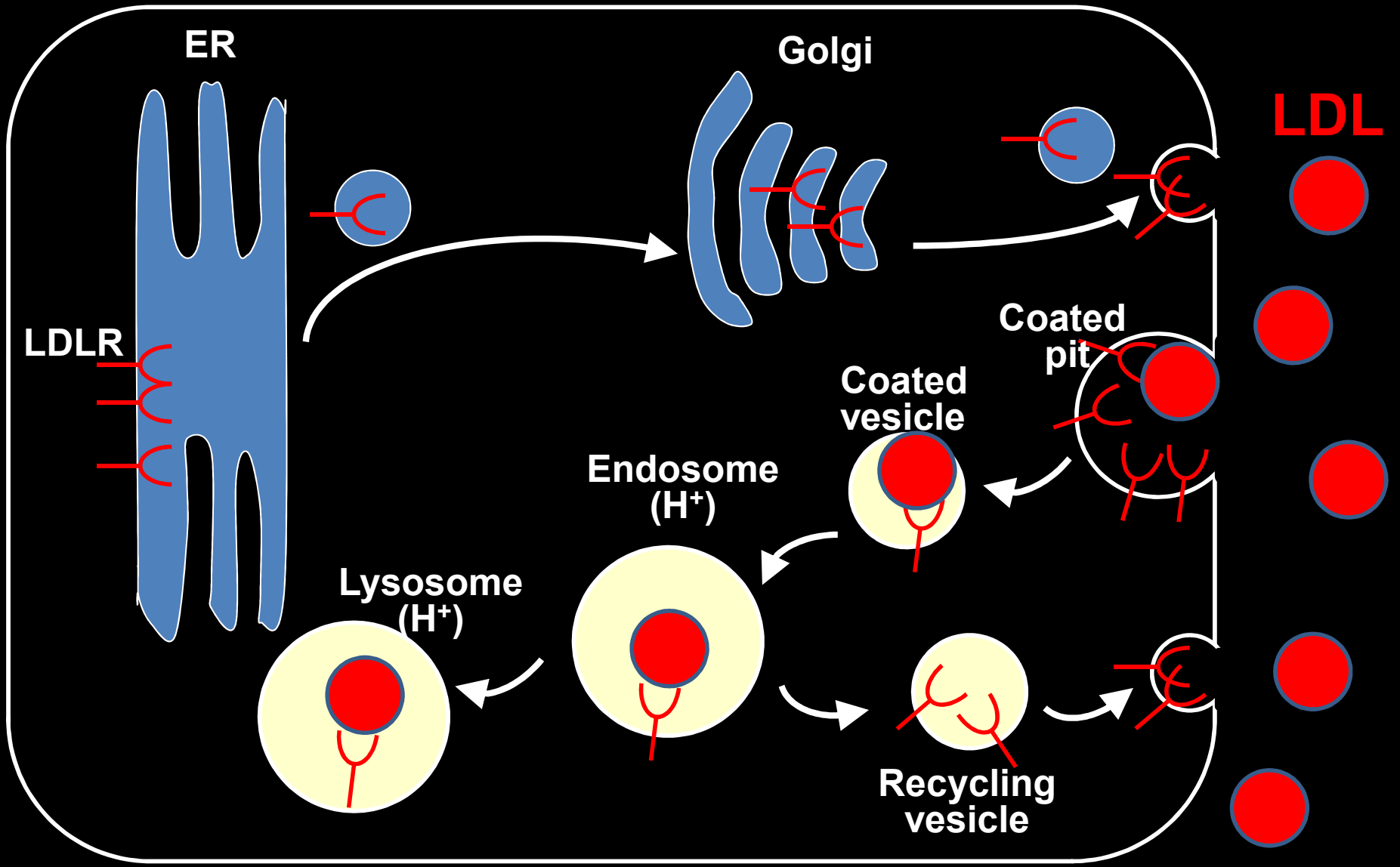
LDL Particles Are Cleared From the Plasma by Binding to LDL Receptors and Being Internalized by the Hepatocyte[1-3]



Recycled LDL receptors continue to clear plasma LDL

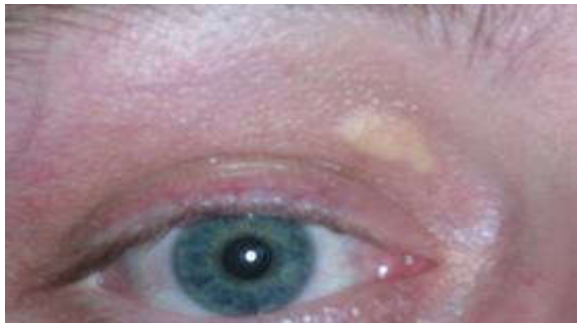
1. Brown MS, et al. *Proc Natl Acad Sci U S A*. 1979;76:3330-3337. 2. Brown MS, et al. *Science*. 1986;232:34-47. 3. Steinberg D, et al. *Proc Natl Acad Sci U S A*. 2009;106:9546-9547.

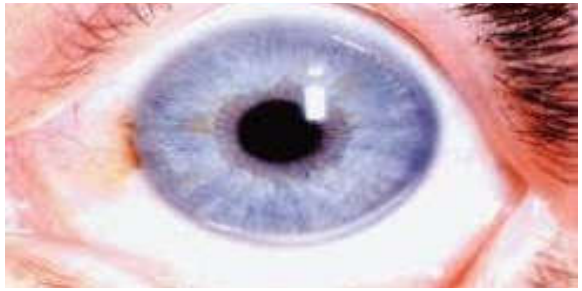
Plasma LDL degradation by the LDL Receptor pathway

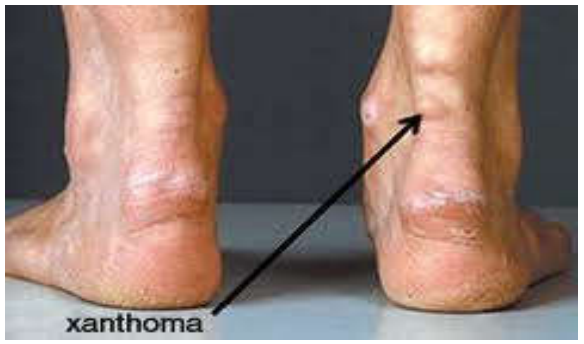


Familial Hypercholesterolaemia (FH)

- Plasma Total Cholesterol > 8mM (3g/L)
- Tendon Xanthomas
- Corneal Arcus
- Xanthelasma
- Coronary Syndromes (Family)
- LDL Receptor 90%
- ApoB100 (LBD) 5%

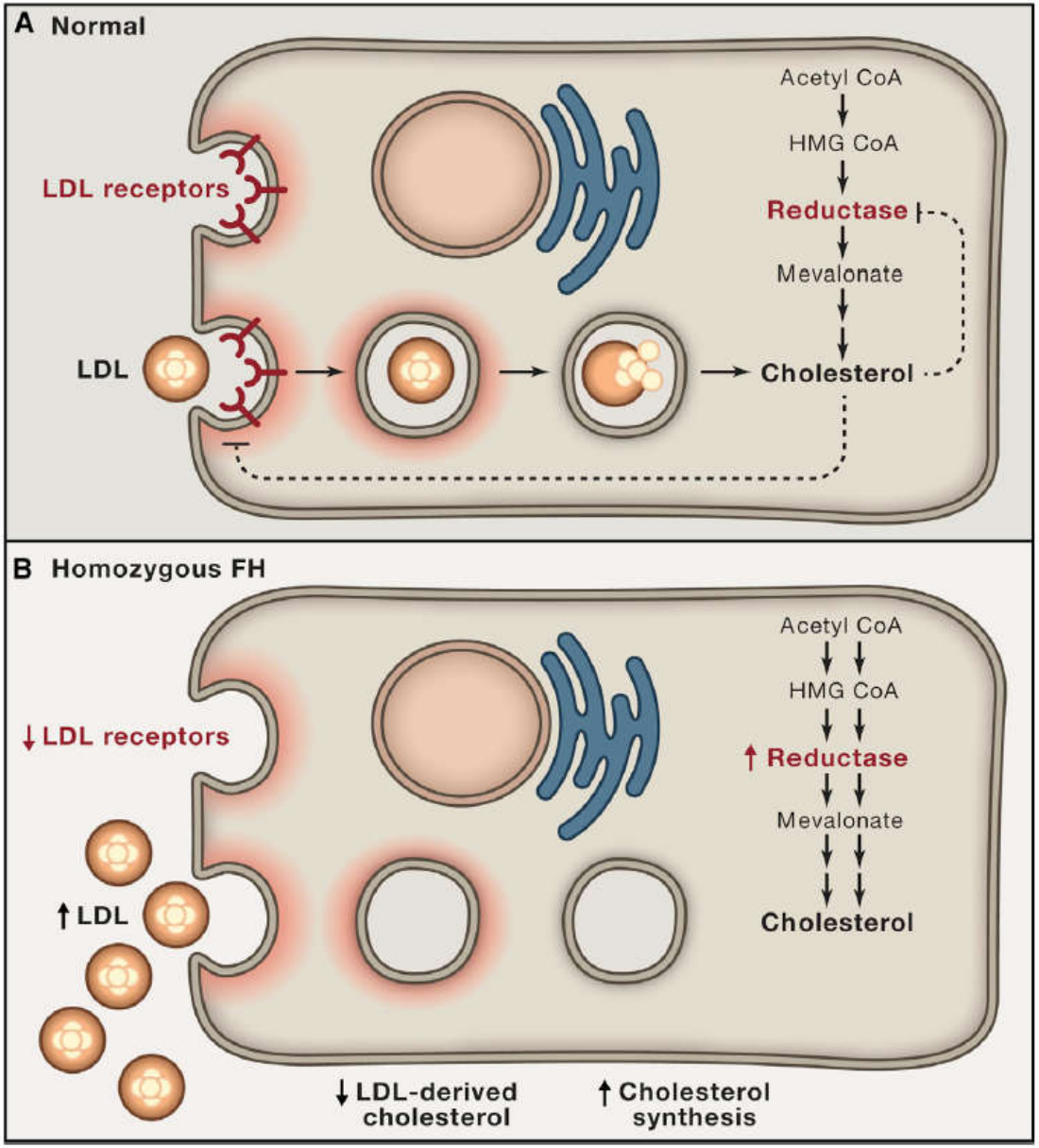






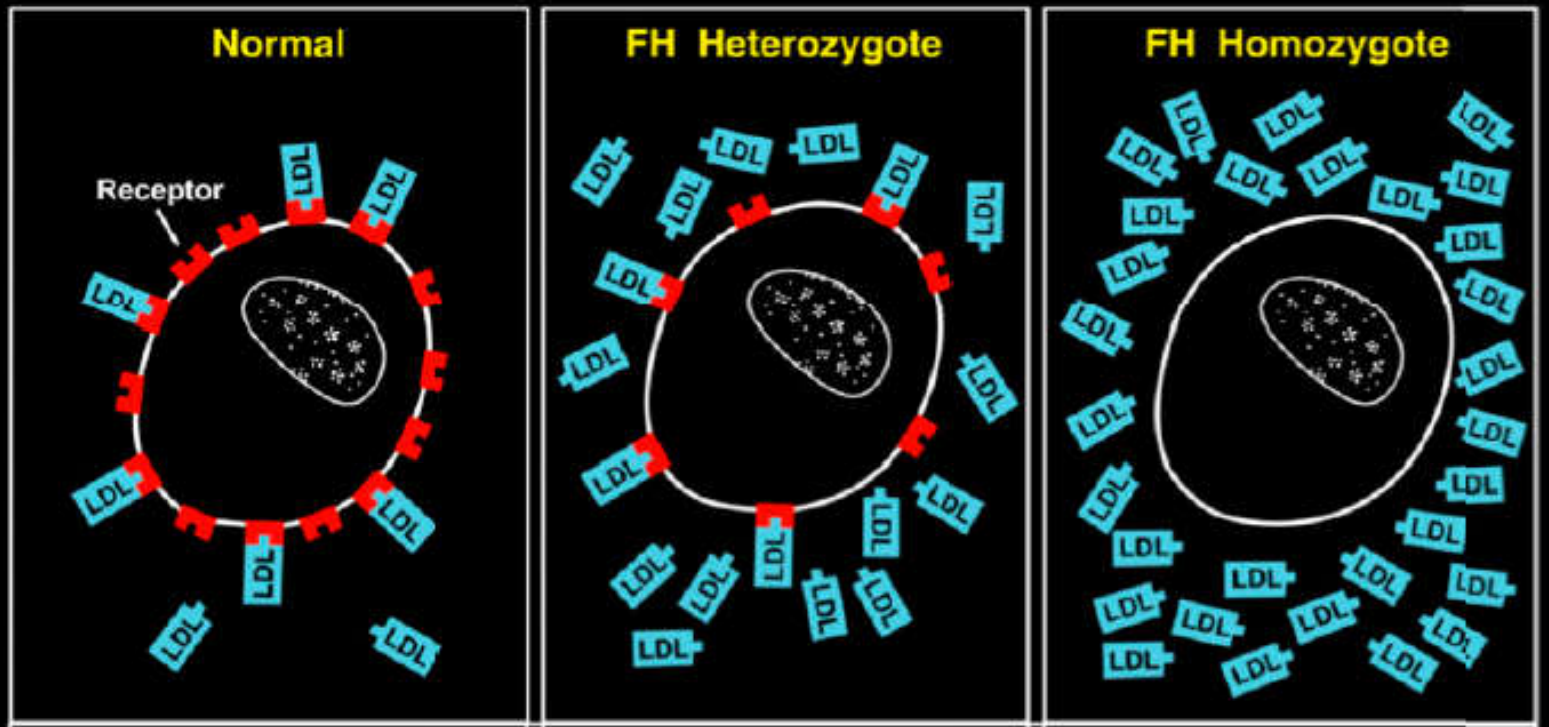
Genest J, Libby P. Lipoprotein disorders and cardiovascular disease. In: Bonow RO, Mann DL, Zipes DP, Libby P, eds. *Braunwald's Heart Disease: A Textbook of Cardiovascular Medicine* . 9th ed. Philadelphia, PA:Saunders Elsevier; 2011:chap 47.
Semenkovich, CF. Disorders of lipid metabolism. In: Goldman L, Schafer AI, eds. *Cecil Medicine* . 24th ed. Philadelphia, PA: Saunders Elsevier; 2011:chap 213

Feedback Regulation of Cholesterol Synthesis and LDL Receptors



Goldstein JL, Brown MS. Cell. 2015

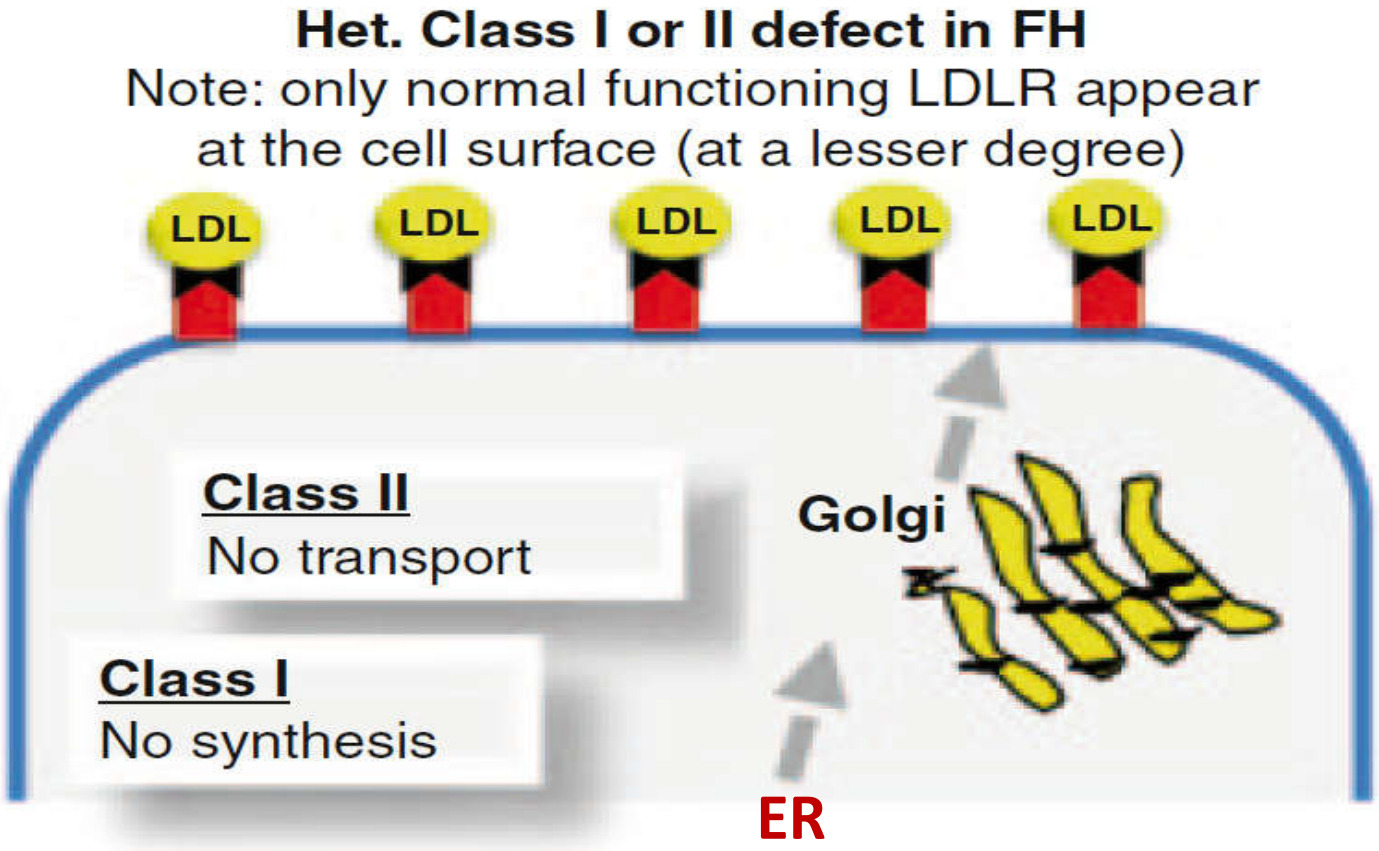
LDL Receptors on Liver Cells



Plasma LDL Level	↑ 2-3 Fold	↑ 8-10 Fold
Population Frequency	1 in 500	1 in 10^6
Age for Heart Attacks	35-65 years	5-15 years

Prashant Nair. PNAS 2013

Defects of LDL receptor function

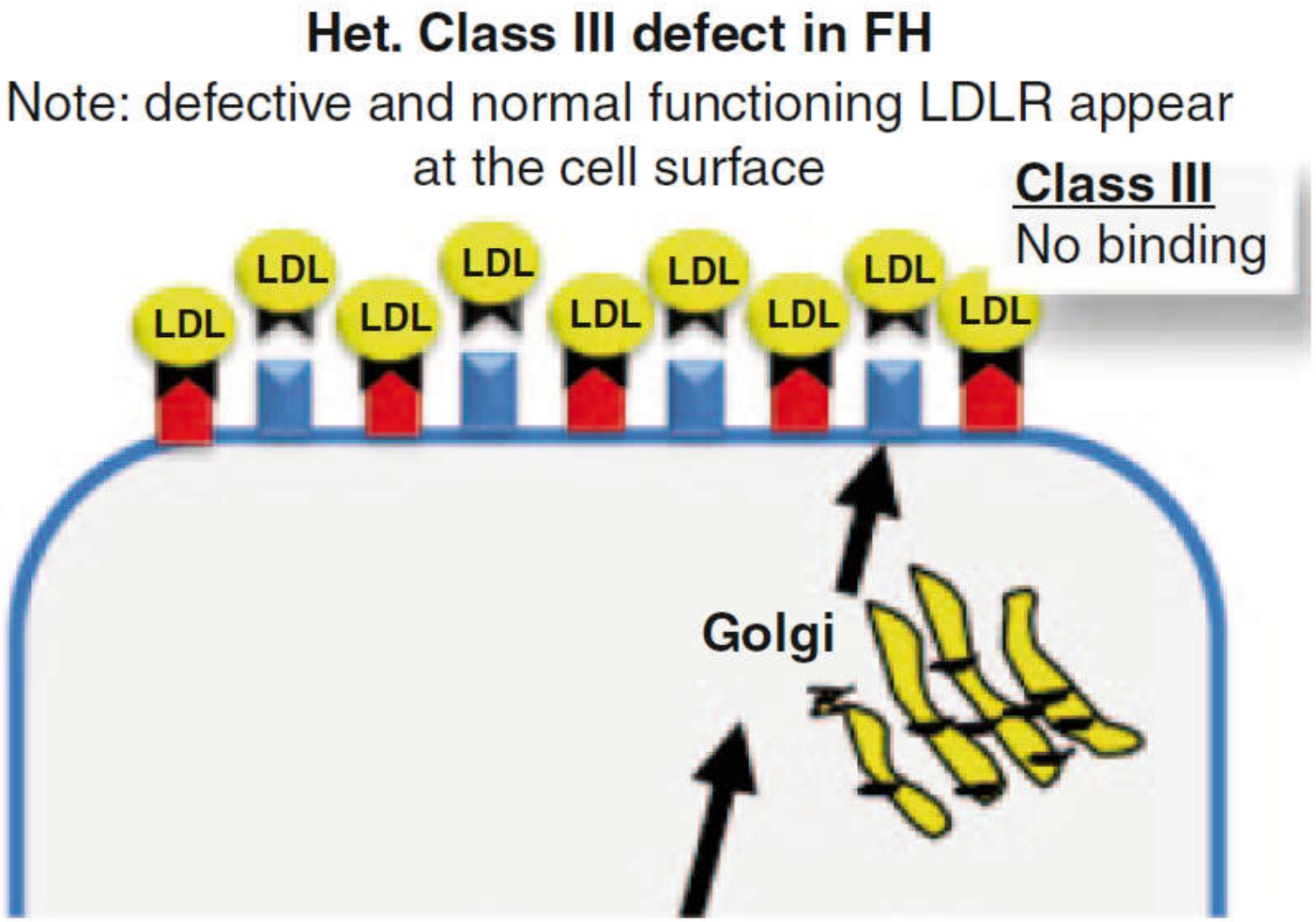


Class I mutations: Include null alleles with no detectable LDLR protein

Class II mutations: Produce transport-defective LDLR proteins that are either completely (class IIa) or partially blocked (class IIb or leaky LDLRs) in their transport from the ER to the Golgi apparatus due to impaired glycosylation

Schaefer JR, et al. Clin Res Cardiol Suppl. 2012;7:2–6

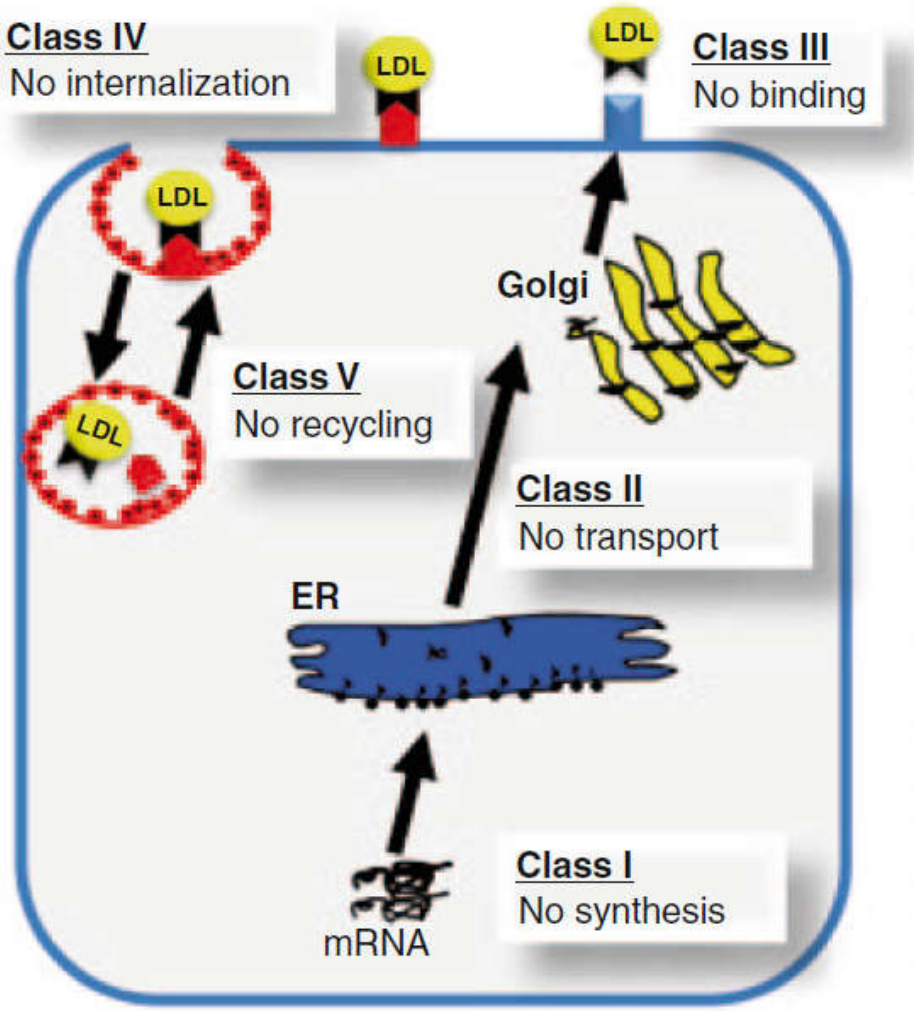
Defects of LDL receptor function



Class III mutations: Encode LDLR receptors with normal intracellular transport but defective LDL binding

Schaefer JR, et al. Clin Res Cardiol Suppl. 2012;7:2–6

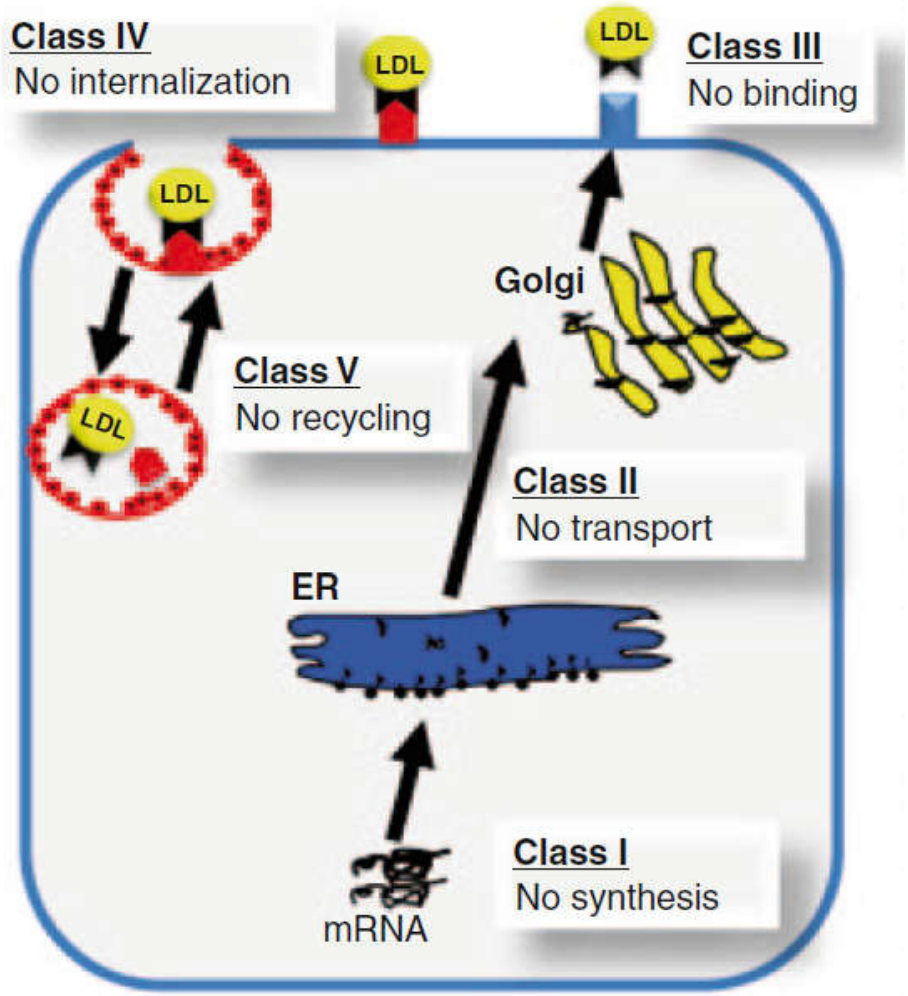
Defects of LDL receptor function



Class IV mutations: Produce LDLR with normal transport and cell surface LDL binding but defective clustering in clathrin-coated pits for internalization

Schaefer JR, et al. Clin Res Cardiol Suppl. 2012;7:2–6

Defects of LDL receptor function



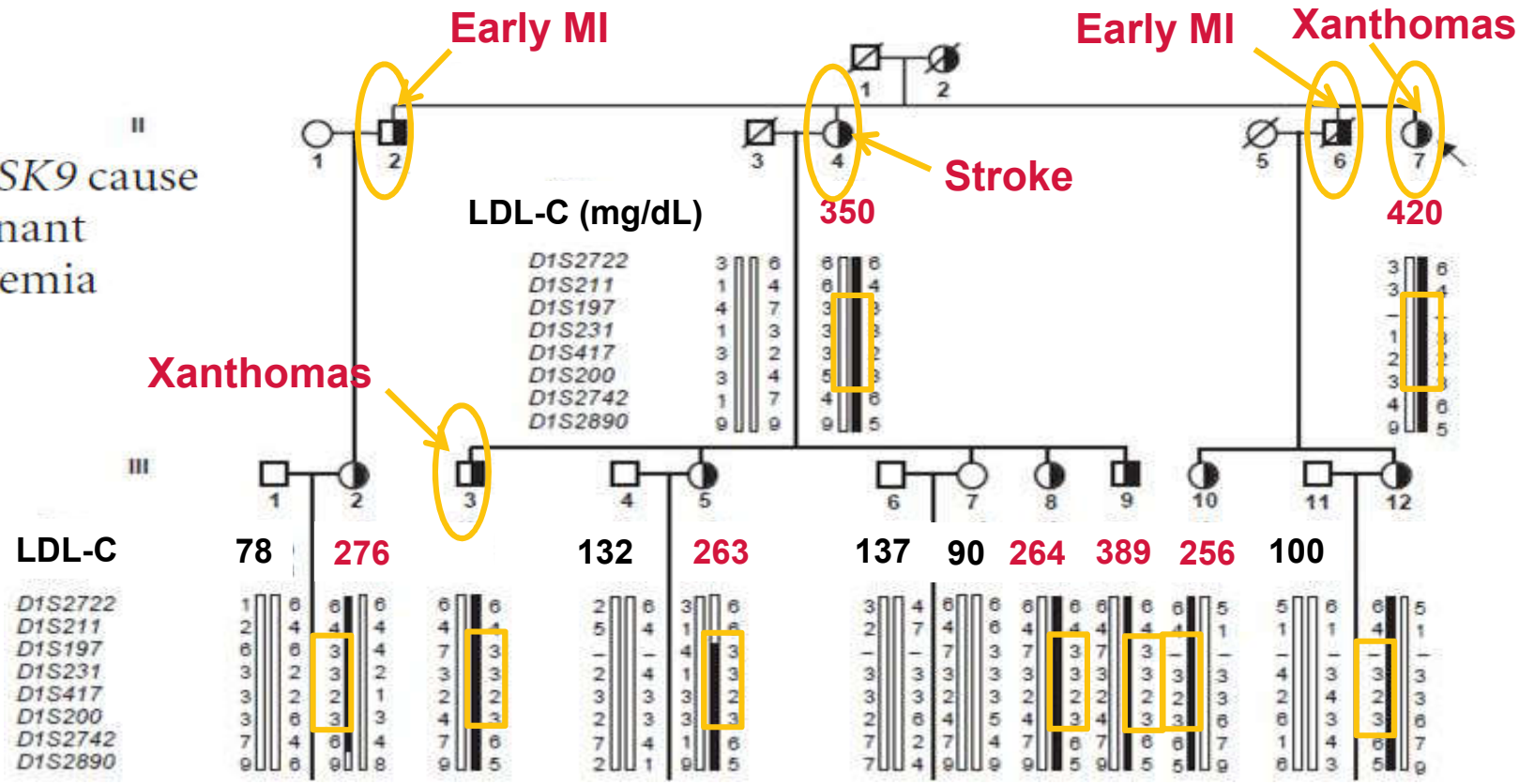
Class V mutations: Produce recycling defective receptors that internalize normally, but are unable to release bound ligand within the acidic environment of the endosome, and thus do not recycle to the cell surface

Schaefer JR, et al. Clin Res Cardiol Suppl. 2012;7:2–6

Discovery of Proprotein Convertase Subtilisin Kexin Type 9 (*PCSK9*)

nature

Mutations in *PCSK9* cause autosomal dominant hypercholesterolemia



PCSK9 is the third locus for autosomal dominant hypercholesterolemia (ADH): Gain-of-Function mutations in PCSK9

Cardiovascular

1. *Abifadel M, et al. Nature Genet. 34: 154-156, 2003.*

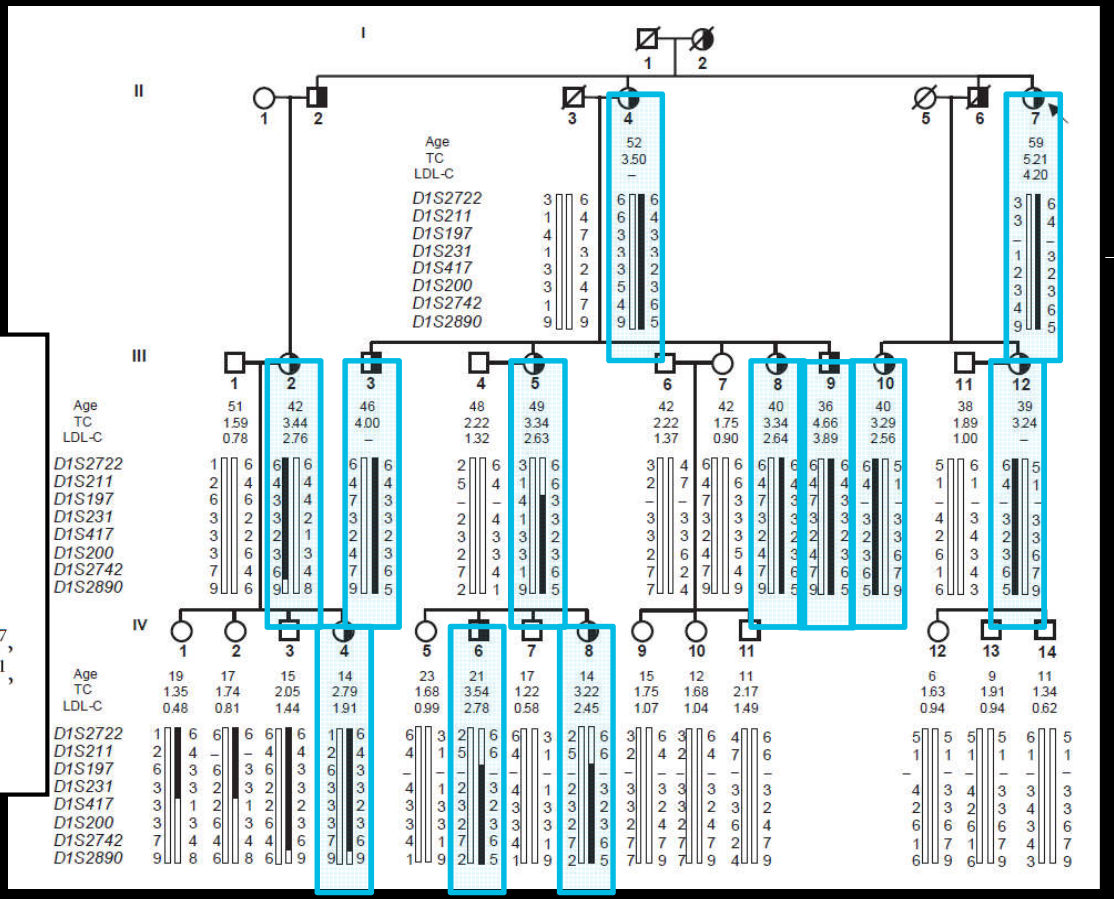
A PCSK9 GAIN-of-Function mutation causes Autosomal Dominant Hypercholesterolaemia

Affected family members with:

- Total cholesterol in 90th percentile
- Tendon xanthomas
- CHD
- Early MI
- Stroke

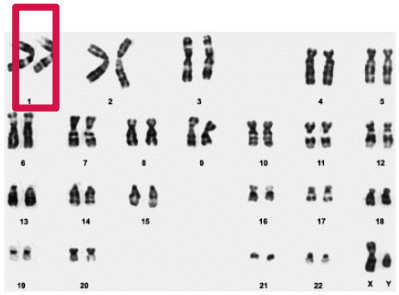
Mutations in *PCSK9* cause autosomal dominant hypercholesterolemia

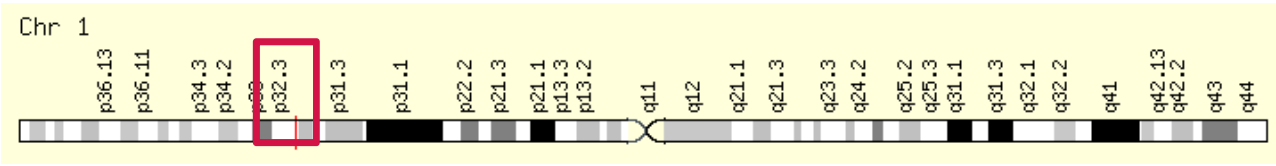
Marianne Abifadel[1,2], Mathilde Varret[1], Jean-Pierre Rabès[1,3], Delphine Allard[1], Khadija Ouguerram[4], Martine Devillers[1], Corinne Cruaud[5], Suzanne Benjannet[6], Louise Wickham[6], Danièle Erlich[1], Aurélie Derré[1], Ludovic Villéger[1], Michel Farnier[7], Isabel Beucler[8], Eric Bruckert[9], 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]



Abifadel M, et al. Nat Genet 2003;34:154–6.

What is PCSK9?

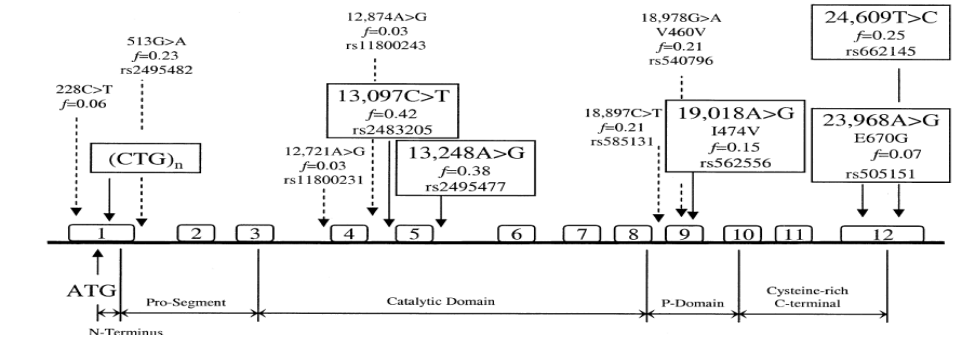


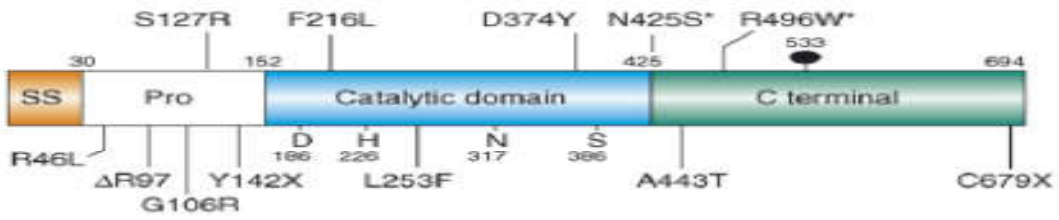


Chromosome:
- 1p32.3

Gene:
- 12 exons
- cDNA 3617 bp

Protein:
- 692 amino acids



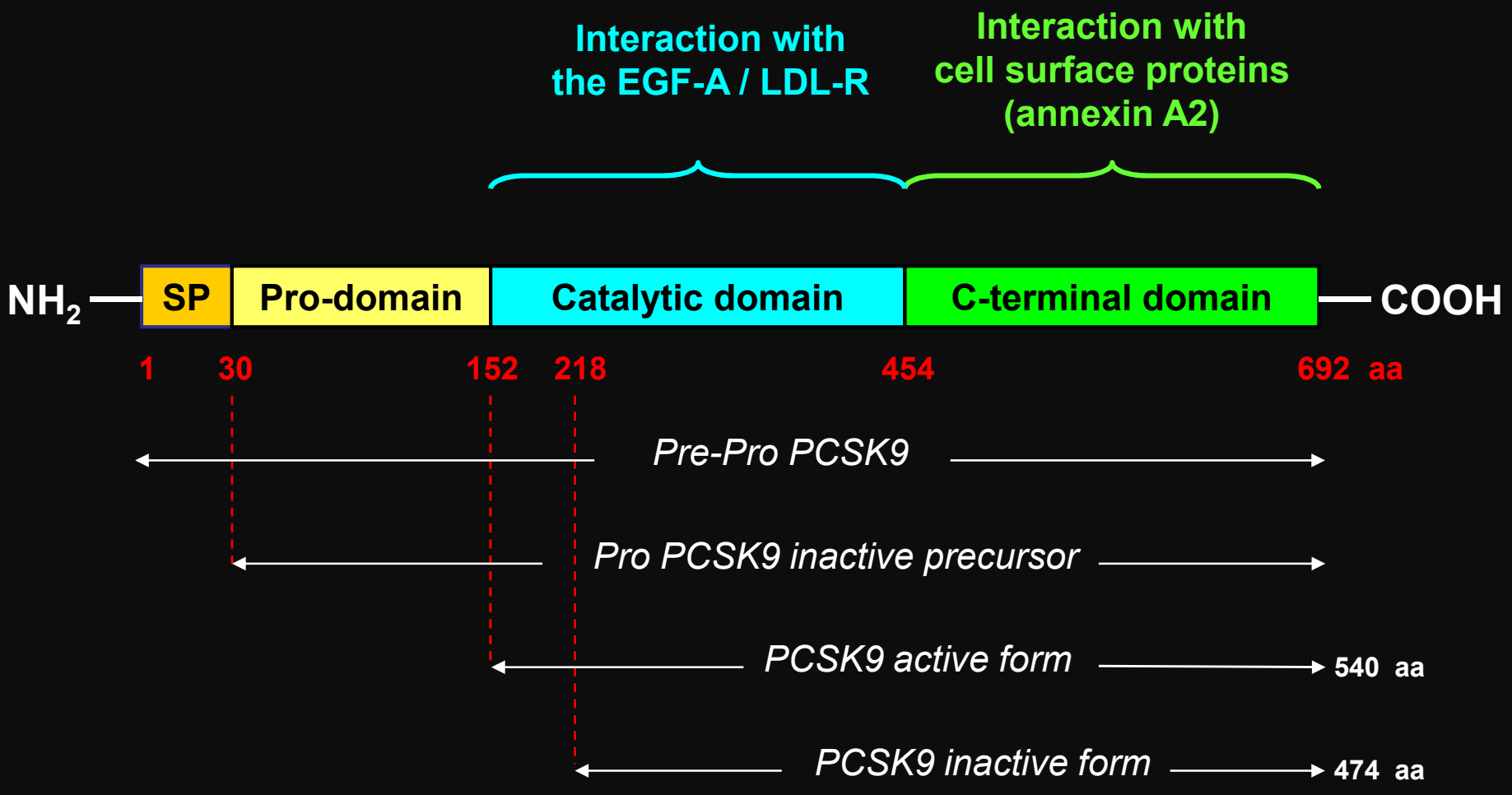


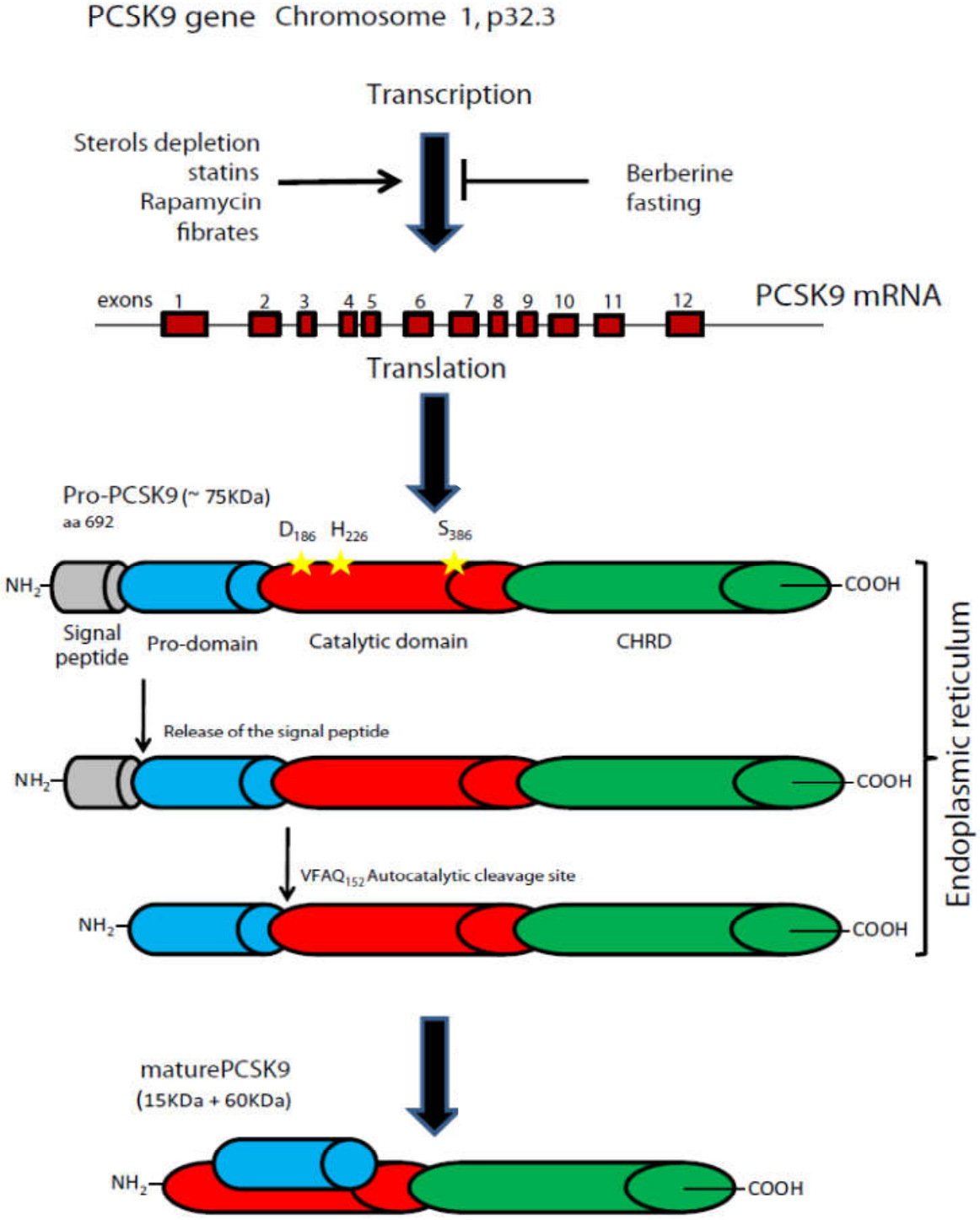
- NARC-1: Neural apoptosis regulated convertase 1; neurogenesis
- Expressed in liver, kidney, intestine
- Undergoes autocatalytic cleavage in the ER to active conformation
- Appears to play the role of an intracellular protein chaperone

http://www.genecards.org/cgi-bin/carddisp.pl?gene=PCSK9/. [Accessed 19 July 2011]
Abifadel M, et al. Nature Genet 2003;34:154–6.
Abifadel M, et al. Hum Mutat 2009;30:520–9.
Horton JD, et al. Trends Biochem Sci 2007;32:71–7.
Chen SN, et al JACC 2005;45:1611–9.

II- PCSK9 BIOLOGY

Maturation of PCSK9

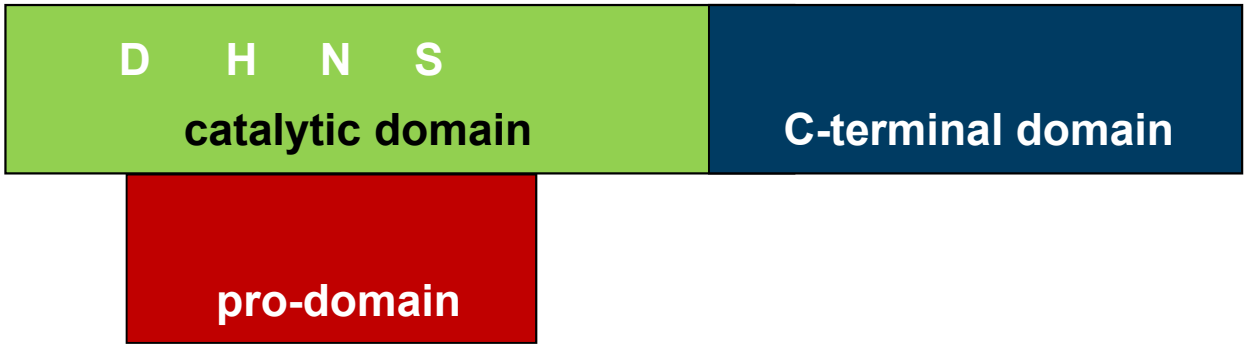


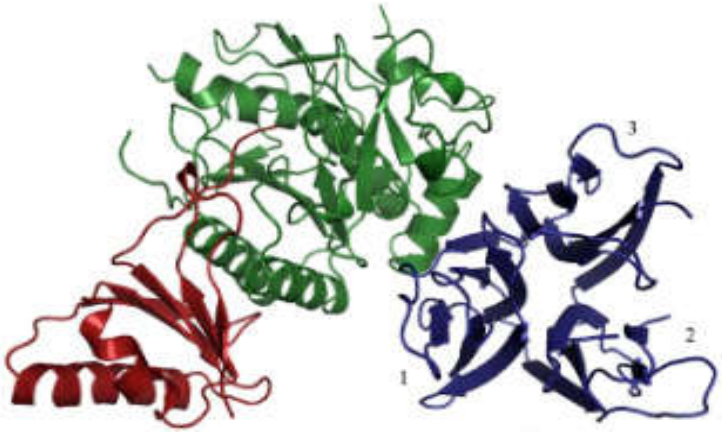

PCSK9 gene Chromosome 1, p32.3
Transcription
Sterols depletion
statins
Rapamycin
fibrates
Berberine
fasting
exons 1 2 3 4 5 6 7 8 9 10 11 12
PCSK9 mRNA
Translation
Pro-PCSK9 (~ 75KDa)
aa 692
D186 H226 S386
NH2
COOH
Signal peptide
Pro-domain
Catalytic domain
CHRD
Release of the signal peptide
VFAQ152 Autocatalytic cleavage site
Endoplasmic reticulum
maturePCSK9
(15KDa + 60KDa)

PCSK9: The Enzyme (Proprotein Convertase)

Endoplasmic Reticulum

Golgi

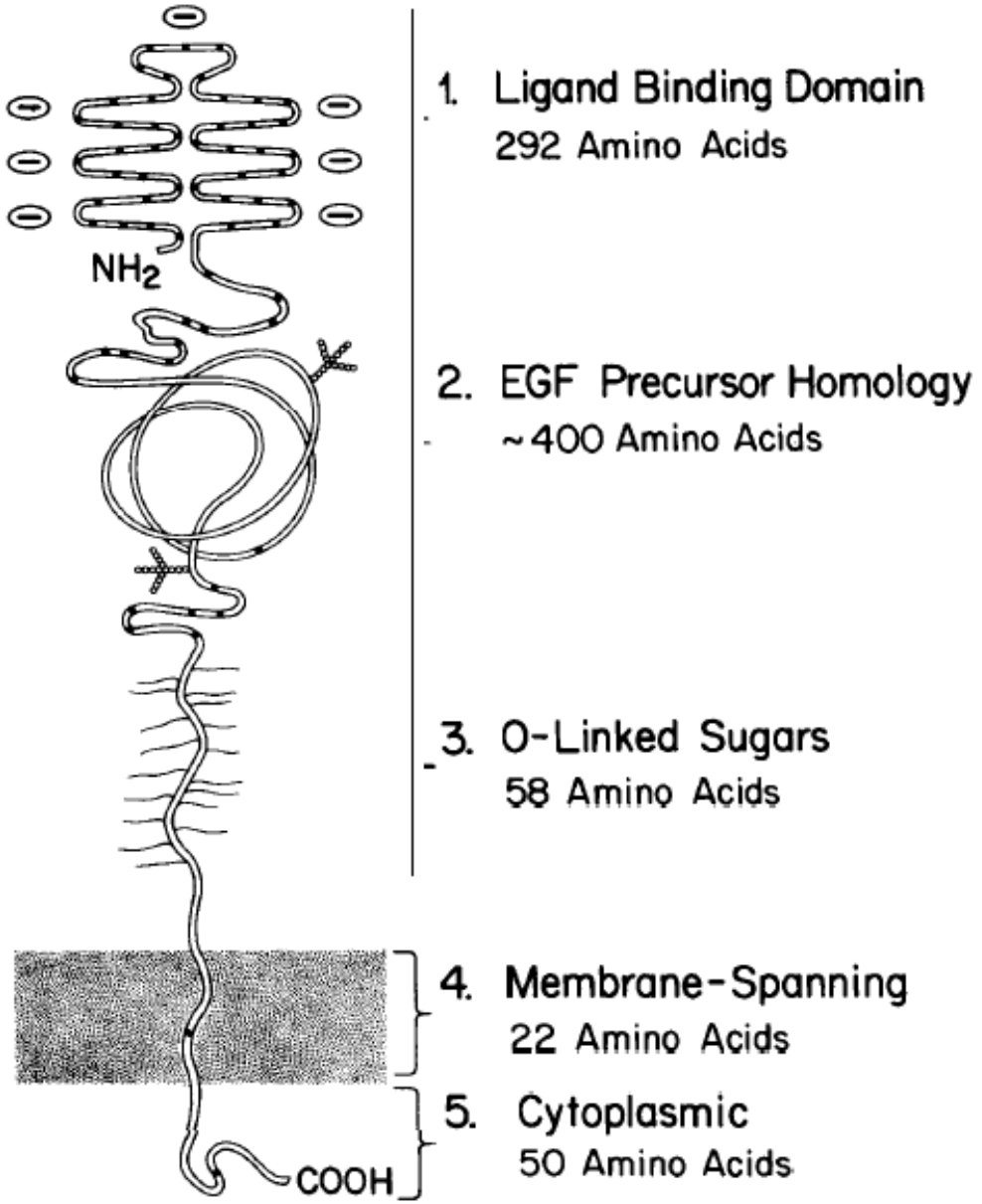




Secretion

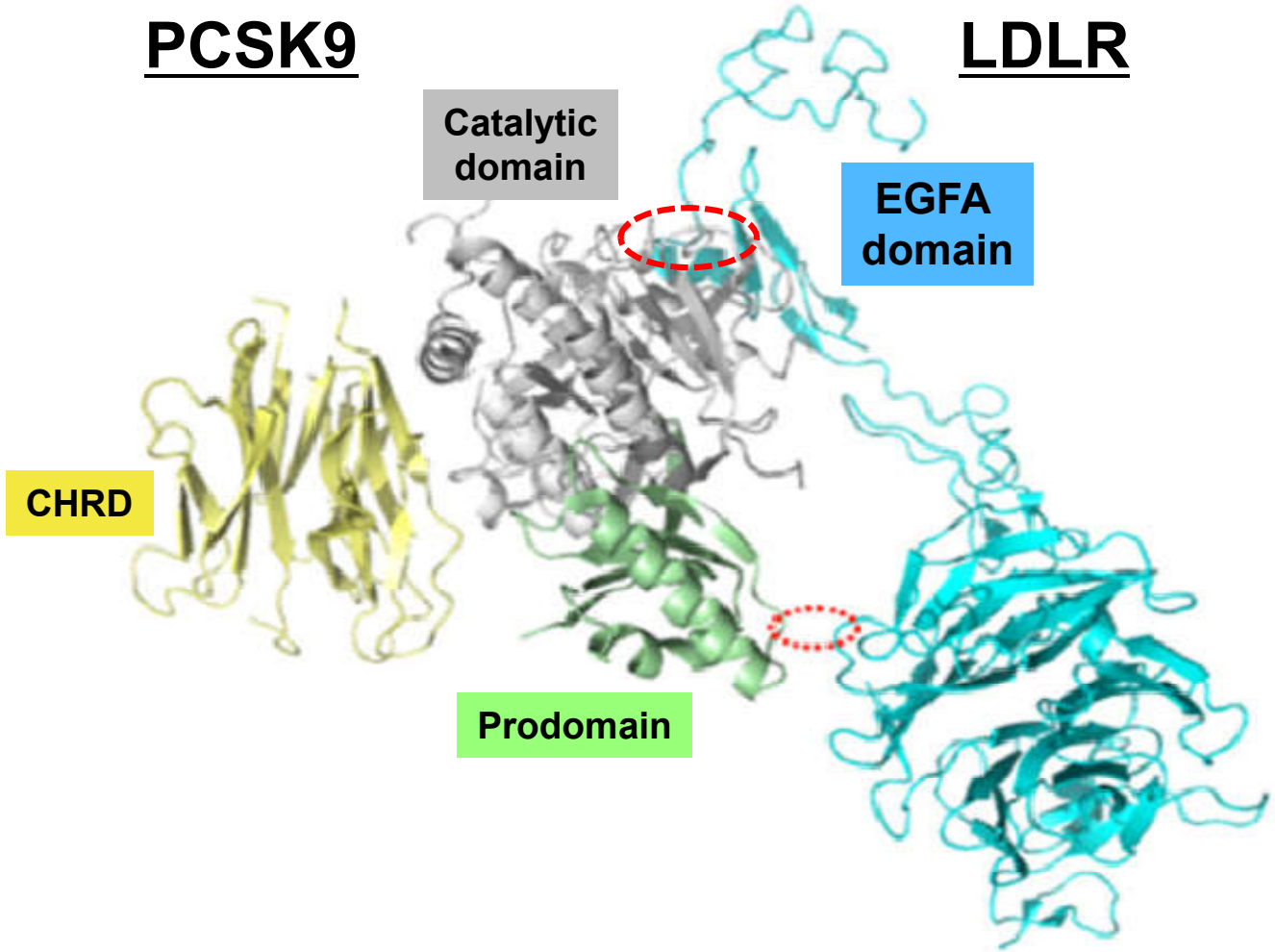
Lambert et al. (2009) *Atherosclerosis*

The LDL receptor: a single protein with five domains



MICHAEL S. BROWN AND JOSEPH L. GOLDSTEIN Nobel lecture, 9 December, 1985

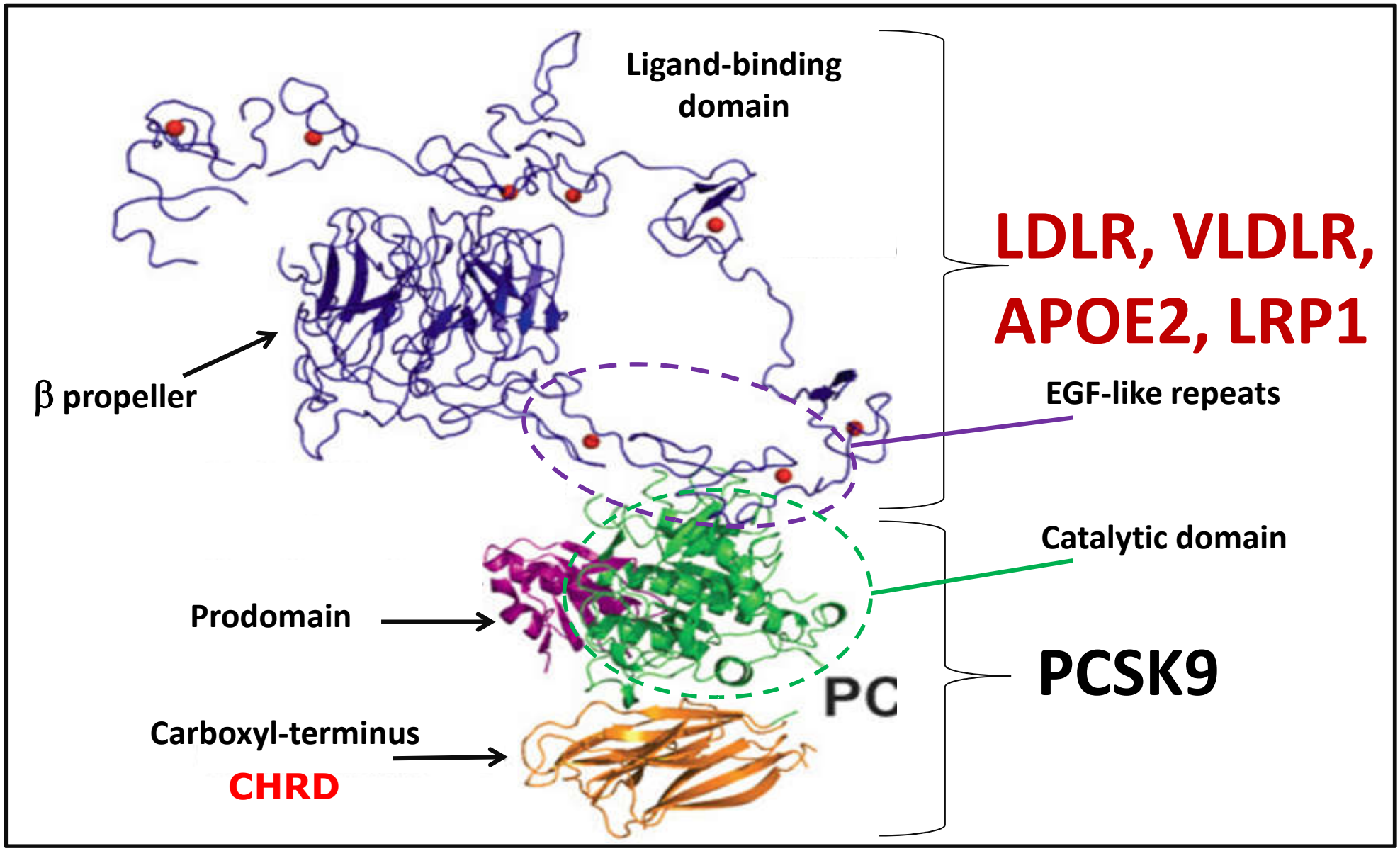
PCSK9: The Chaperone (Binds to the LDLR)



Endocytosis

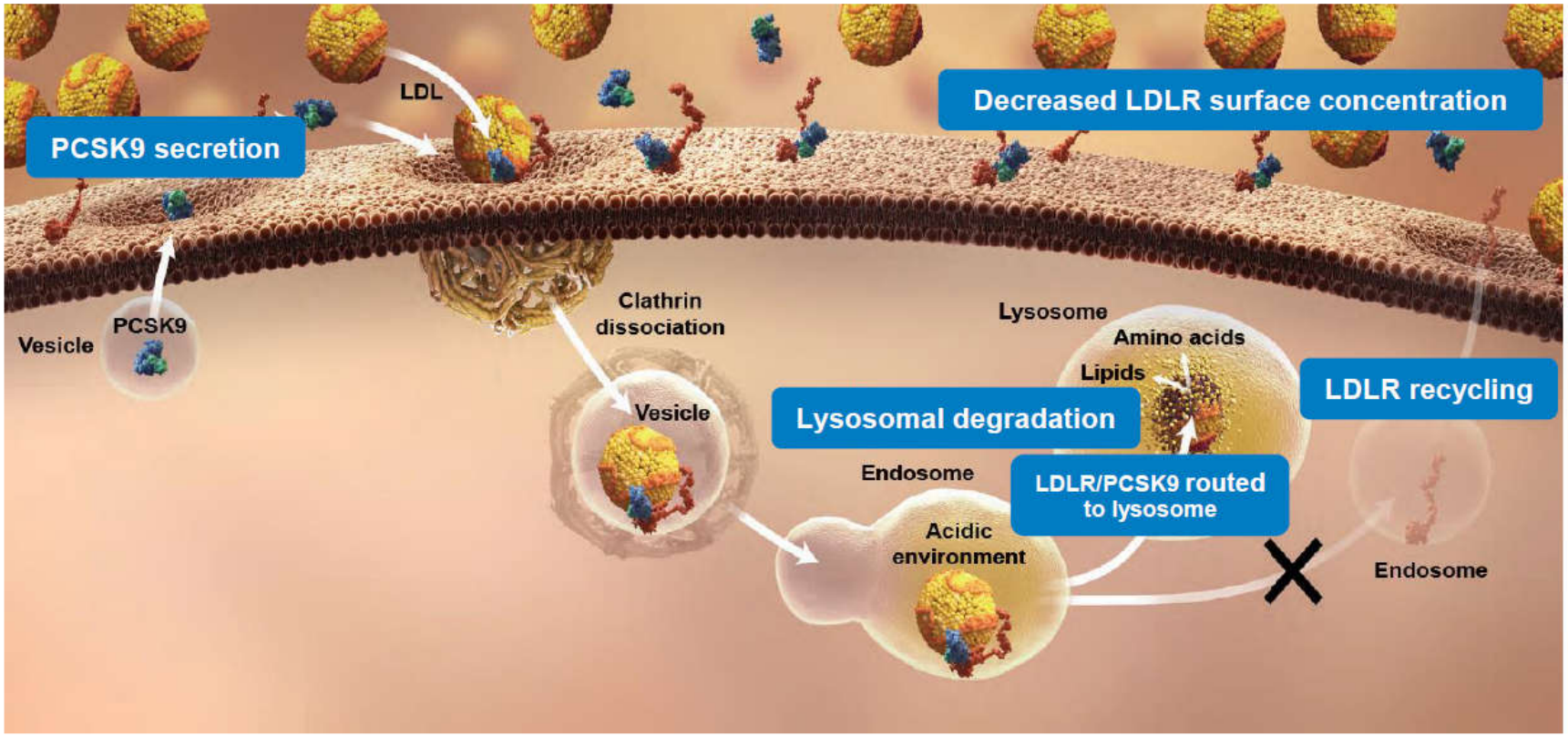
Seidah et al. (2014) *Circ Res*

PCSK9 and the LDL Receptor



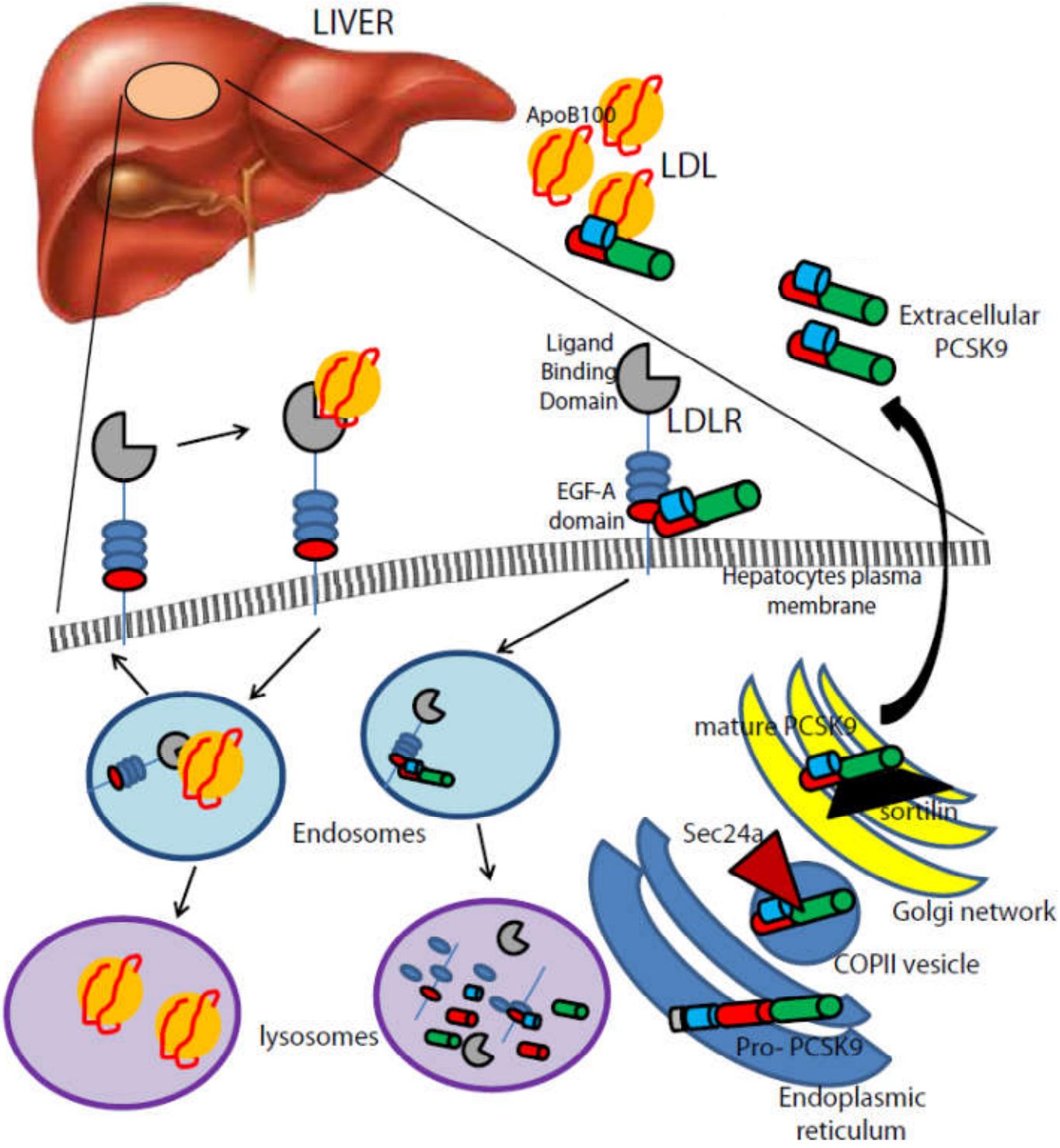
Horton JD, et al. *J Lipid Res*. 2009;50:S172-S177.

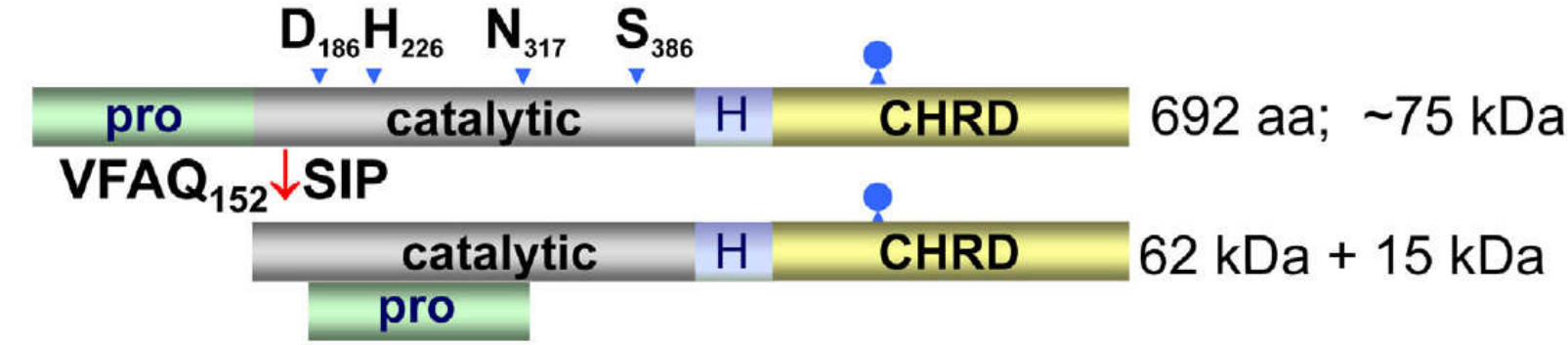
PCSK9 Binds to the LDL Receptor and Targets the LDL Receptor for Degradation[1-3]

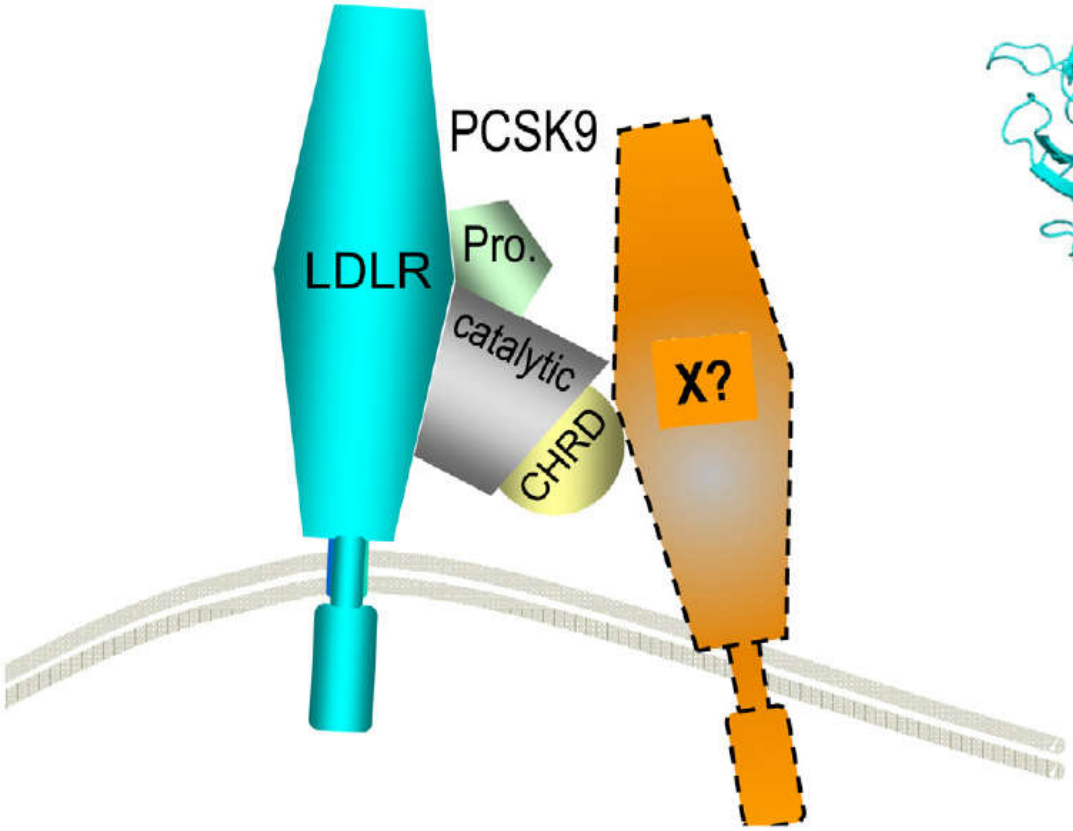


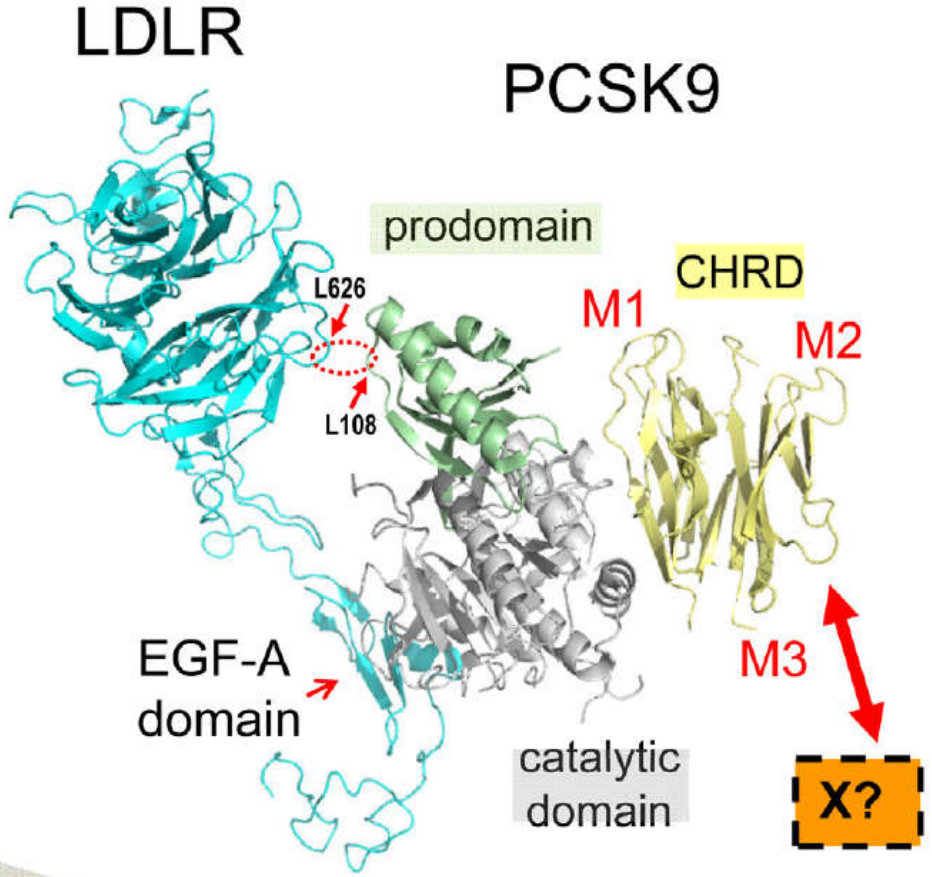
Fewer LDL receptors on hepatocyte surface result in increased plasma LDL-C

1. Abifadel M, et al. *Hum Mutat*. 2009;30:520-529. 2. Seidah NG, et al. *Circ Res*. 2014;114:1022-1036. 3. Steinberg D, et al. *Proc Natl Acad Sci U S A*. 2009;106:9546-9547.


LIVER
ApoB100
LDL
Extracellular
PCSK9
Ligand
Binding
Domain
LDLR
EGF-A
domain
Hepatocytes plasma
membrane
mature PCSK9
sortilin
Sec24a
Golgi network
COPII vesicle
Endosomes
lysosomes
Pro- PCSK9
Endoplasmic
reticulum

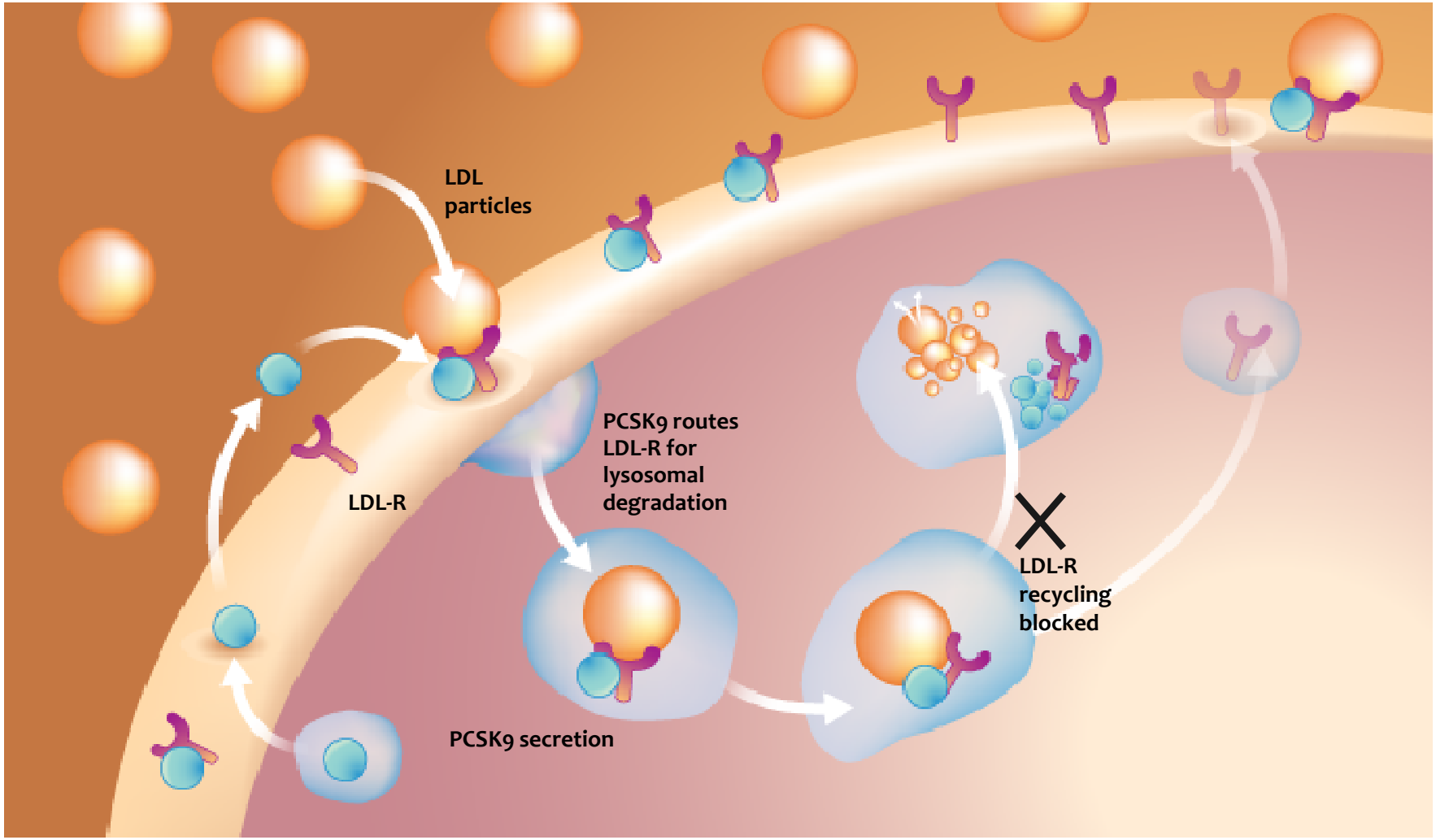




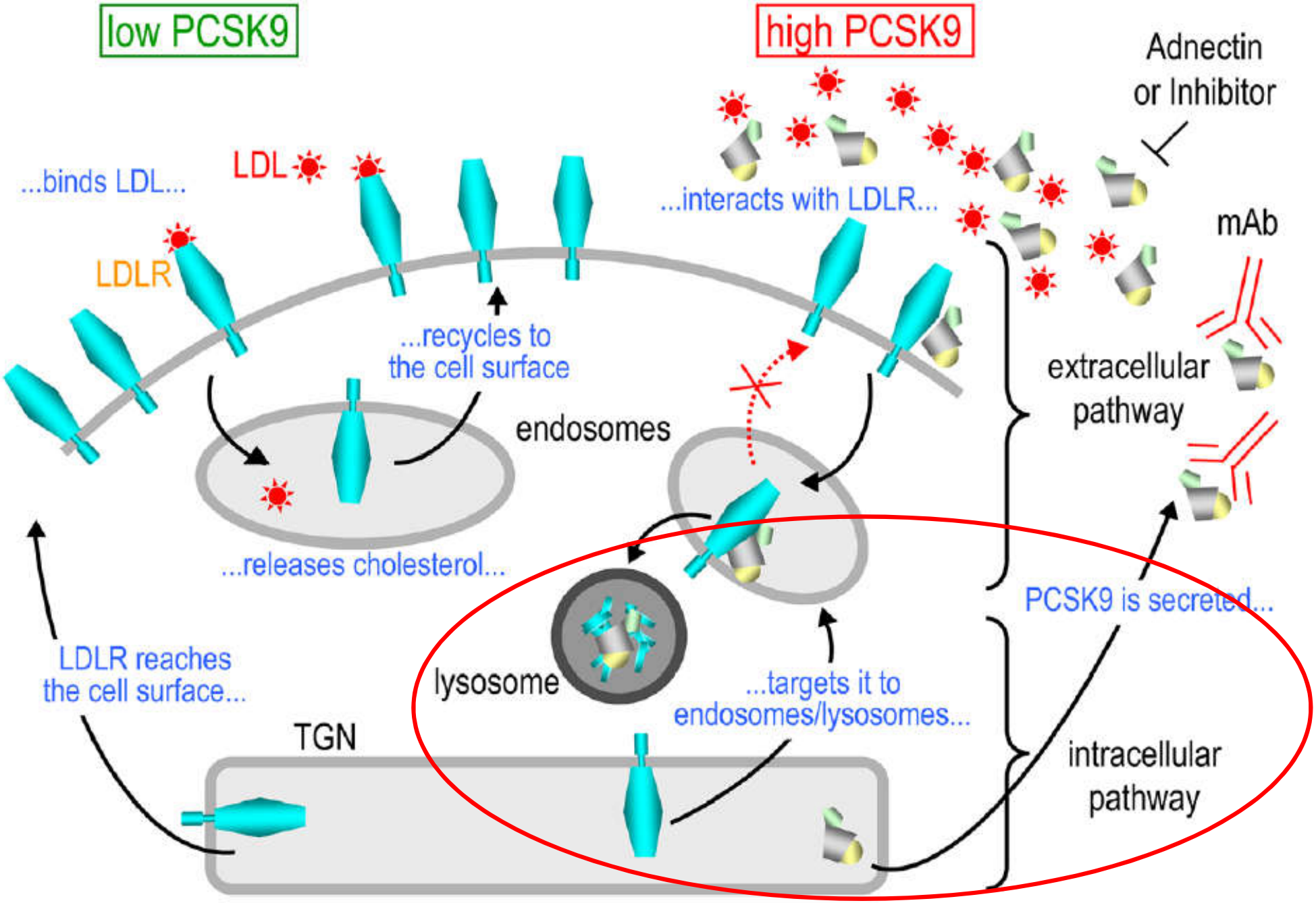


Circ Res. 2014;114:1022-1036

Role of PCSK9 in regulation of the surface expression of LDL receptors



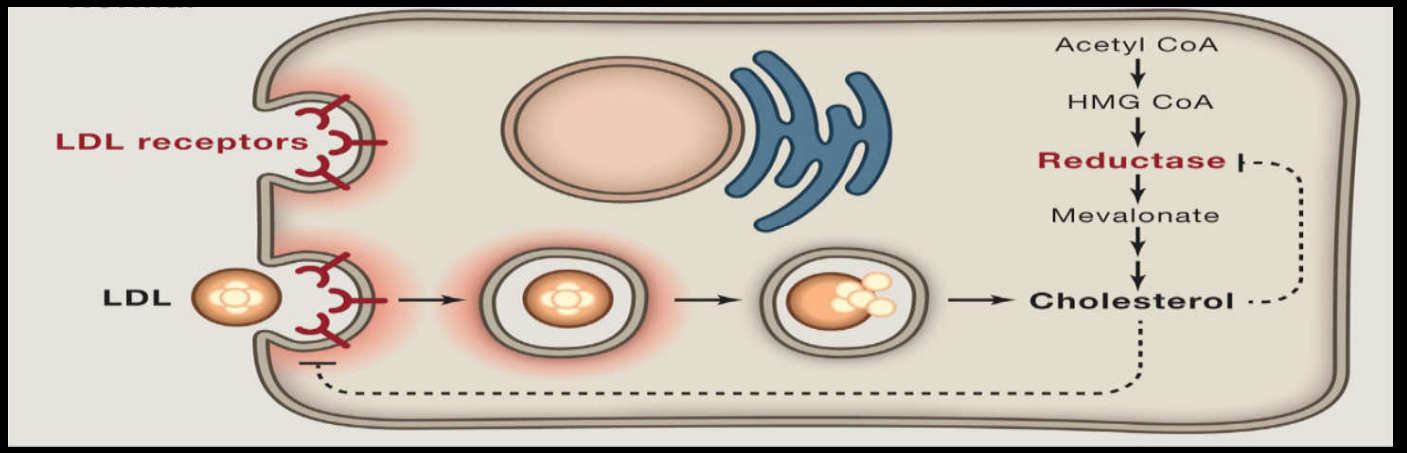
Chan JC, et al. Proc Natl Acad Sci USA 2009;106:9820–5..



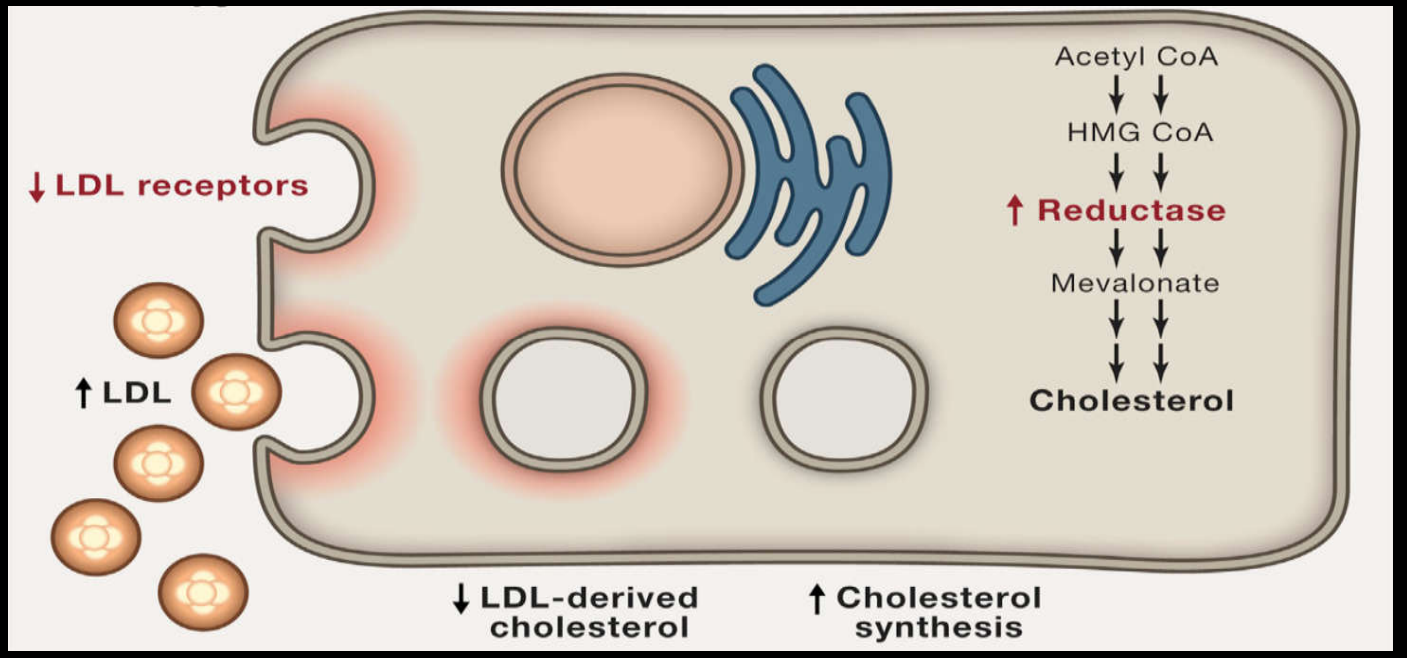
Circ Res. 2014;114:1022-1036

Feedback regulation of the LDL receptor and cholesterol synthesis

Normal



HoFH

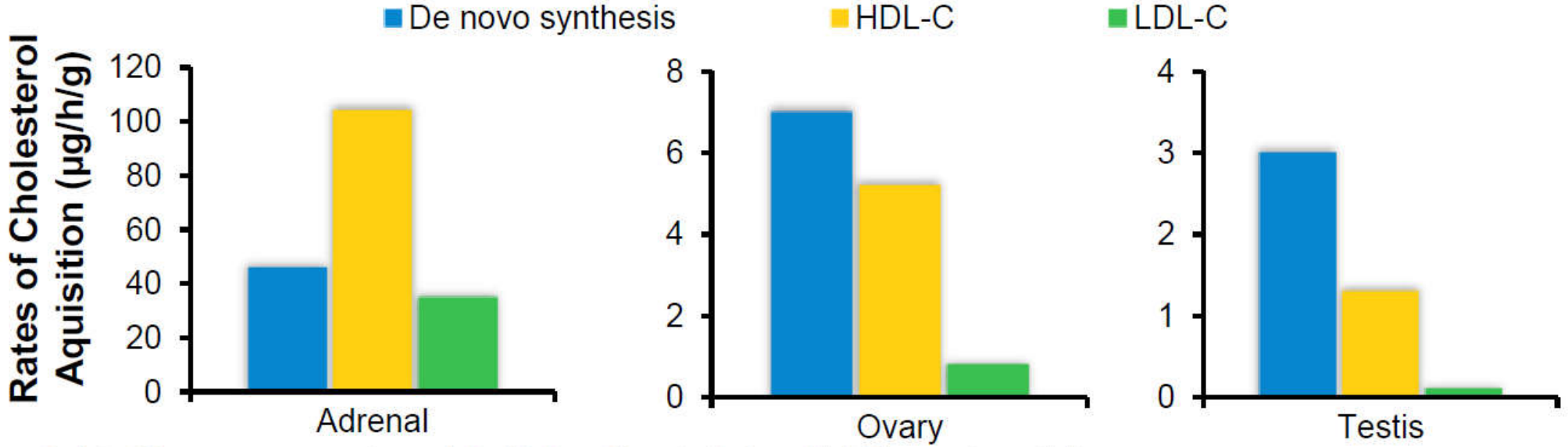


Goldstein JL, Brown MS. Cell 2015;161:161–72.

Animal Data Demonstrate Steroidogenic Tissues Predominantly Acquire Cholesterol Via HDL-C and De Novo Synthesis[1]

- Adrenal, ovarian, and testicular tissue can acquire cholesterol via LDL-C, HDL-C, and de novo synthesis
  - Predominant pathway is HDL-C and de novo synthesis[1,2]

Rates of cholesterol acquisition from *de novo* synthesis, HDL-C and LDL-C*[1]



*Data were calculated from measurements made in 49-day-old control mice with LDL receptor activity.
HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.
1. Xie C, et al. *J Lipid Res.* 2006;47:953-963. 2. Hu J, et al. *Nutr Metab (Lond).* 2010;7:47.

Control of hepatic LDL-Receptor activity

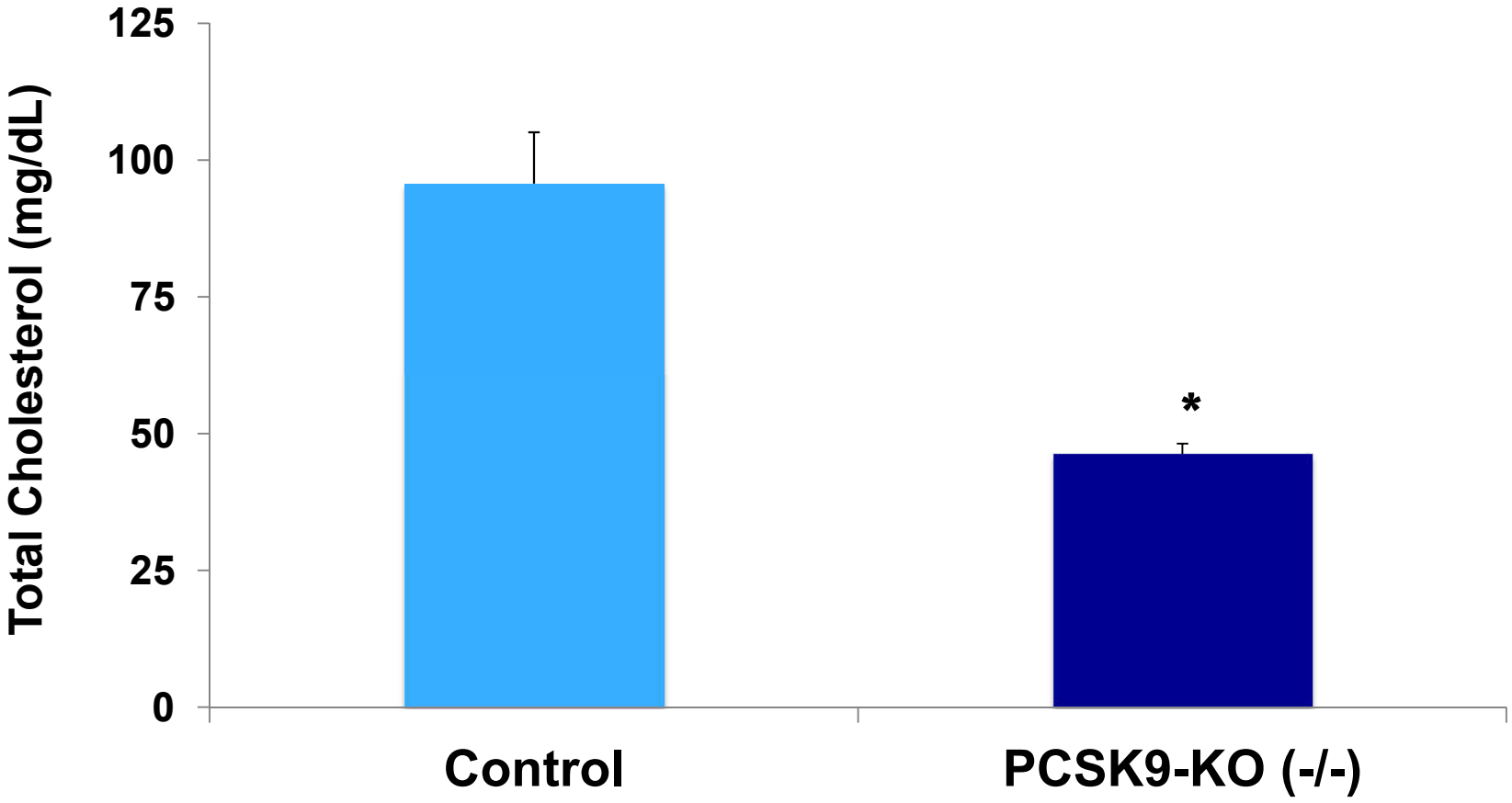
- **Intracellular levels of cholesterol**

(reflecting uptake of cholesterol contained in LDL, VLDL and chylomicron remnants, and HDL), endogenous cholesterol synthesis, cholesterol conversion to bile acids, and excretion of bile acids and biliary cholesterol)

via the SREBP pathway

- **the IDOL pathway**

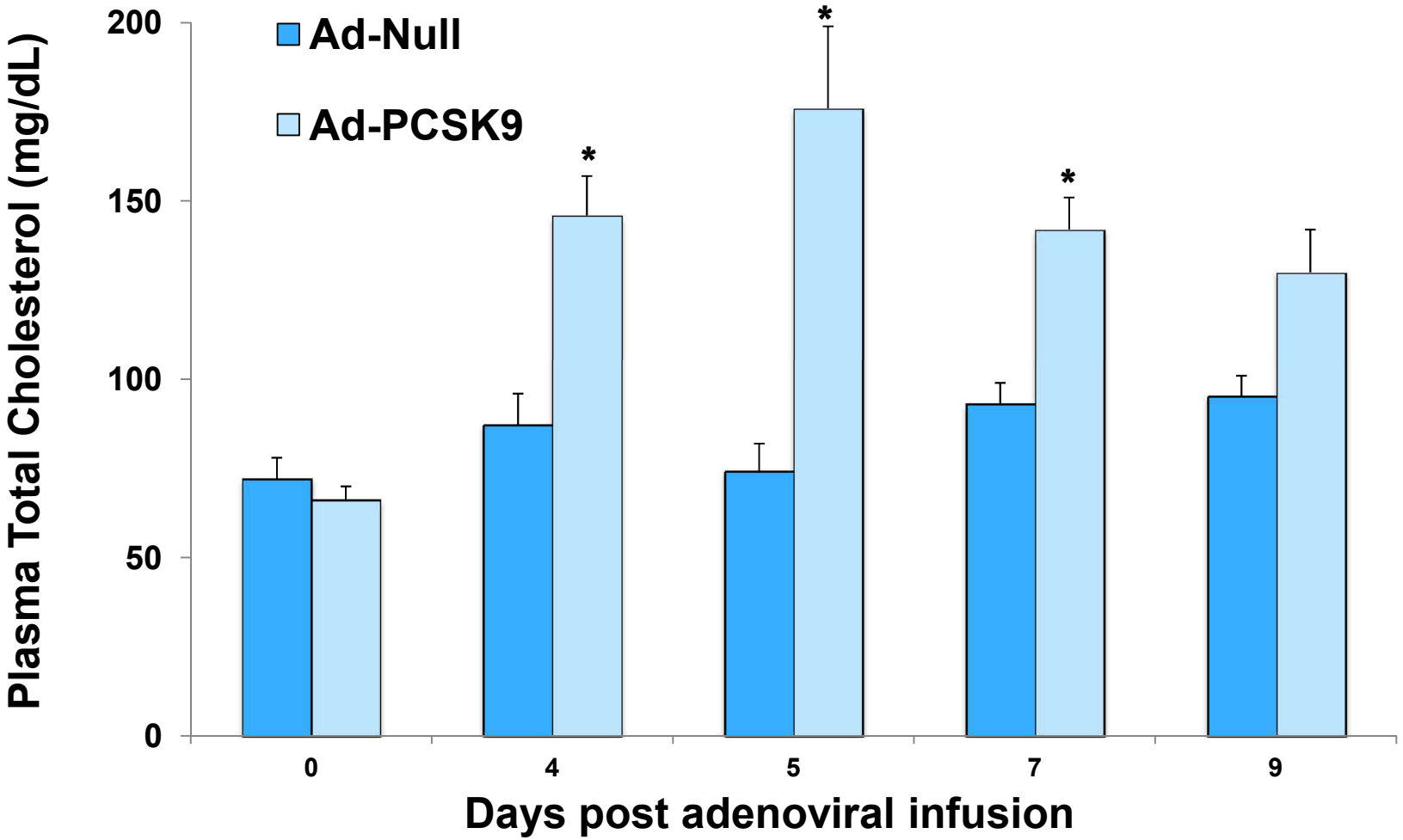
PCSK9 Knockout Mice Have Decreased Plasma Cholesterol



* p<0,05 vs. Control

Rashid et al. (2005) *Proc Natl Acad Sci USA*

PCSK9 Expression Increases Circulating LDL Cholesterol Levels in Mice



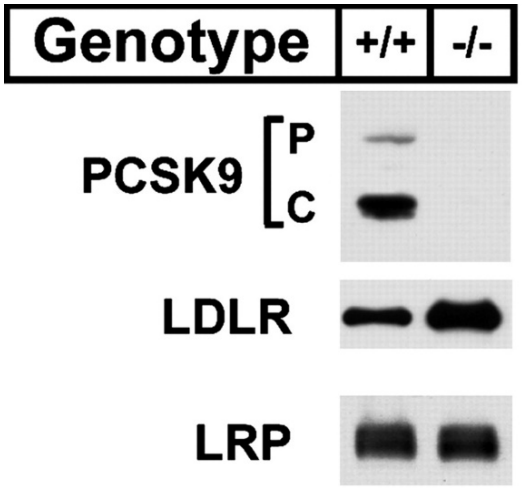
* p<0,05 vs. Ad-null

Maxwell and Breslow (2004) *Proc Natl Acad Sci USA*
Benjannet et al. (2004) *J Biol Chem*
Park et al. (2004) *J Biol Chem*
Lalanne et al. (2005) *J Lipid Res*

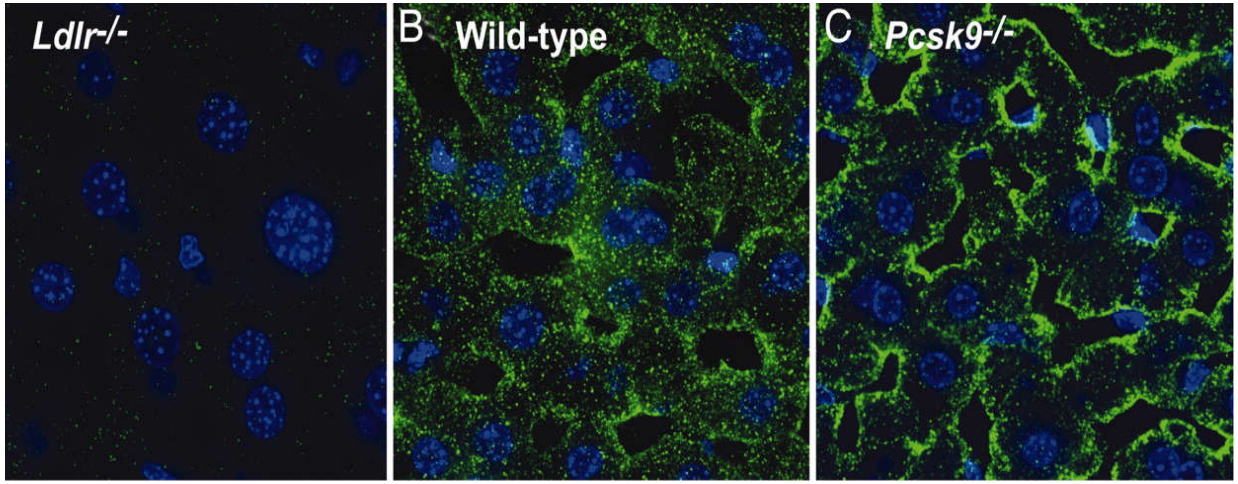
Absence of PCSK9 Leads to Marked Increase in LDL-R

PCSK9 knockout mice : ↑ hepatic LDL-R levels

Immunoblot



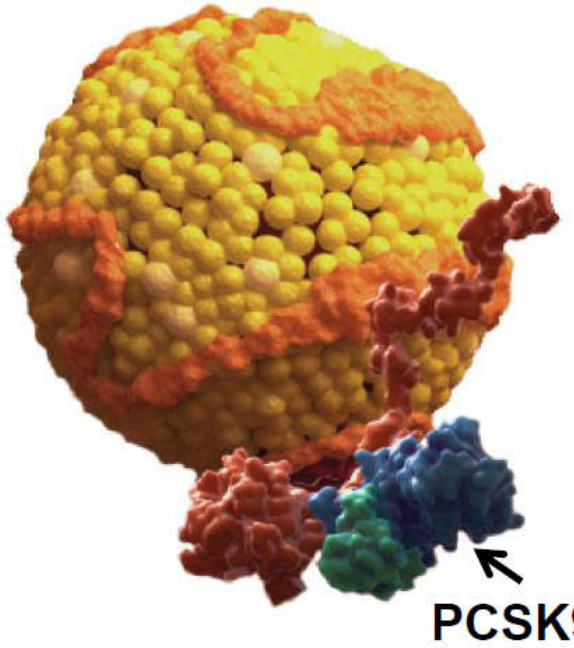
Immunofluorescence against LDL-R



PCSK9 decreases number of LDL-R : ↑ LDL-C

Rashid S et al. *PNAS* 2005;102:5374–5379

PCSK9 Is a Regulator of LDL Metabolism

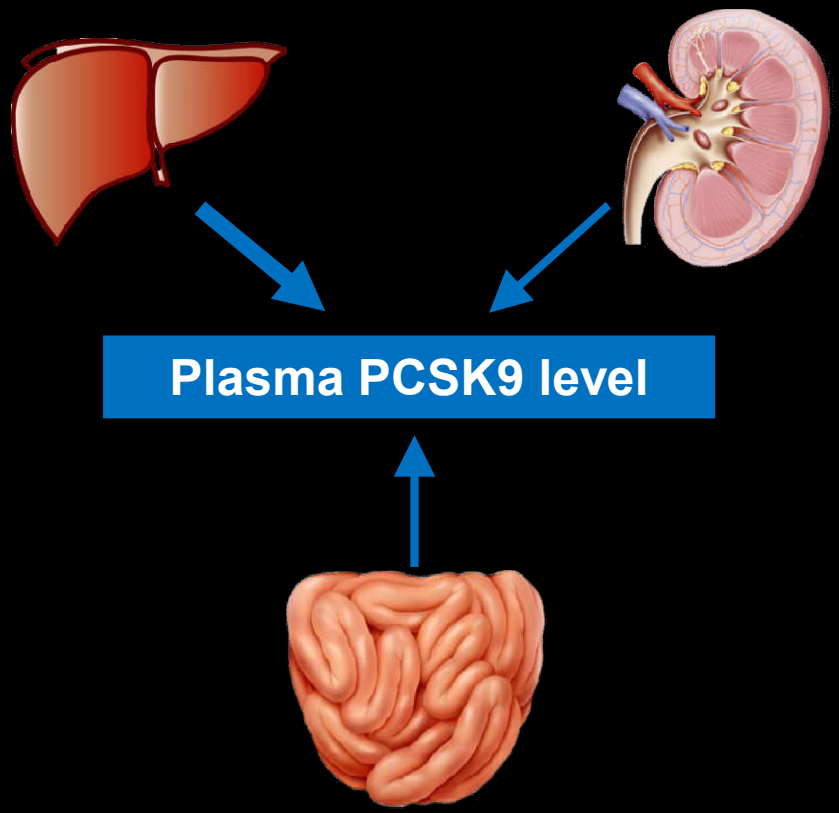


PCSK9

- Proprotein convertase subtilisin/kexin type 9[1]
- Secreted by liver into plasma[1]
- Binds LDL receptor on surface of hepatocyte[1,2]
- Targets LDL receptor for degradation[1,2]

1. Seidah NG, et al. *Circ Res.* 2014;114:1022-1036. 2. Steinberg D, et al. *Proc Natl Acad Sci U S A.* 2009;106:9546-9547.

Regulation of PCSK9 is dynamic



Upregulates PCSK9

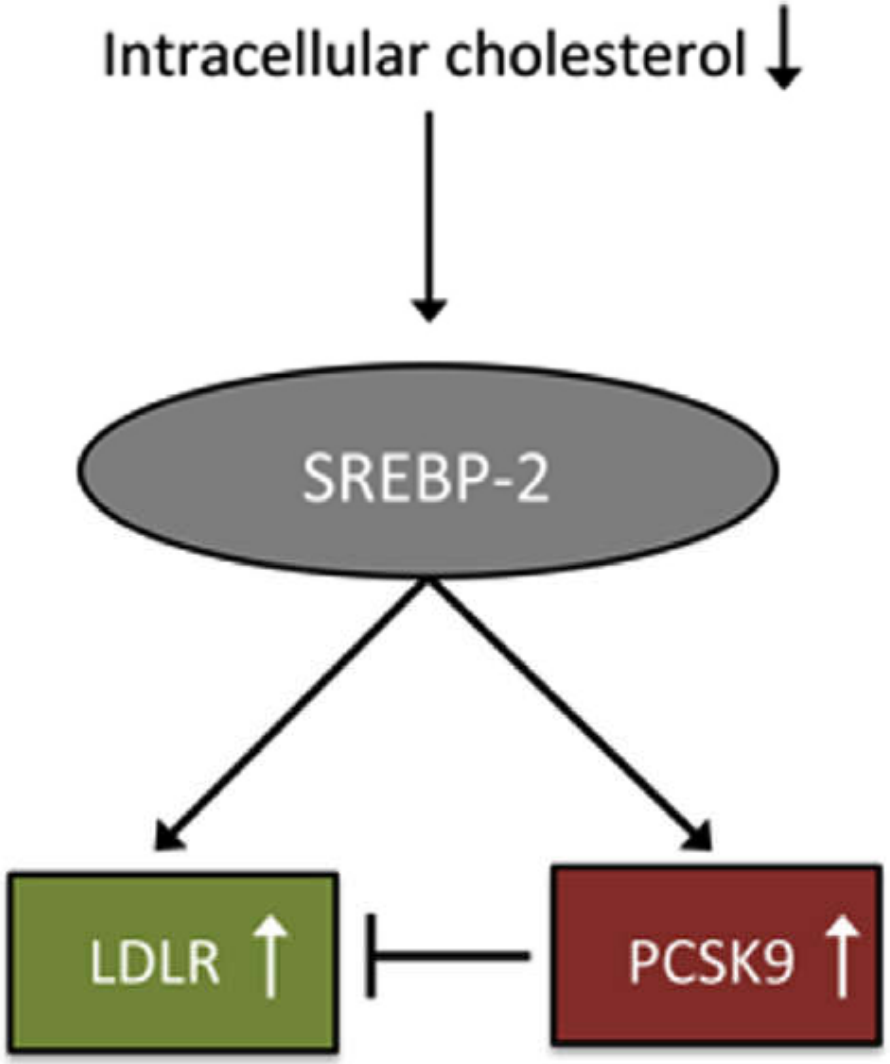
- Cholesterol depletion[2,3]
- SREBP2 [1,3,4]
- Statins[3,4]

Downregulates PCSK9

- Dietary and cellular cholesterol[4]
- Bile acids[3,4]

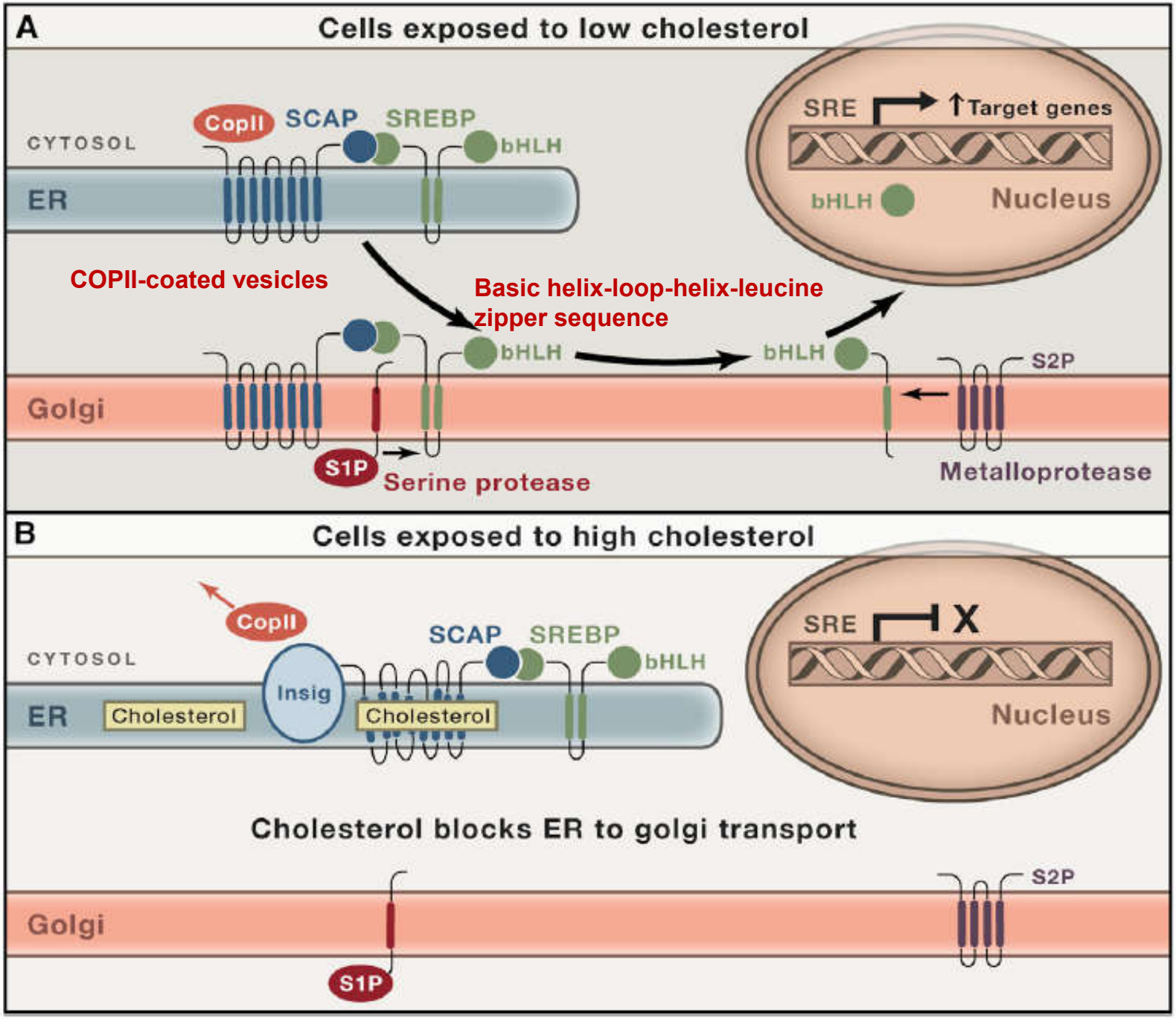
1. Horton JD, et al. J Lipid Res. 2009;50:S172–7.
2. Lopez D. Biochem Biophys Acta 2008;1781:184–91.
3. Abifadel M, et al. Hum Mutat. 2009;30: supplementary information.
4. Abifadel M, et al. In: Toth PP. The Year in Lipid Disorders. Vol. 2. Oxford, UK: Atlas Medical Publishing Ltd. 2010:3–23.
5. Miao et al, ATVB. 2015;35:1589-1596.

SREBP-2-Mediated Coexpression of LDLR and PCSK9



Urban D, et al. J Am Coll Cardiol. 2013;62:1401–8

The SREBP Pathway for Cholesterol Homeostasis in Animal Cells



LDLR
HMG CoA Reductase

Goldstein JL, Brown MS. Cell. 2015

PCSK9 ΚΑΙ ΛΙΠΟΠΡΩΤΕΪΝΕΣ ΠΟΥ ΠΕΡΙΕΧΟΥΝ Apo B

- **Το 40% της PCSK9 συνδέεται με τα LDL σωματίδια**

1/500-1000 σωματίδια που έχουν Apo B μεταφέρουν 1 μόριο PCSK9

- **Η PCSK9 υπάρχει στο πλάσμα σε 2 μορφές**

- **Αθικτη πρωτεΐνη (62KDa)**
- **Furin-cleared τύπος (55KDa) [λιγότερο ενεργός]**

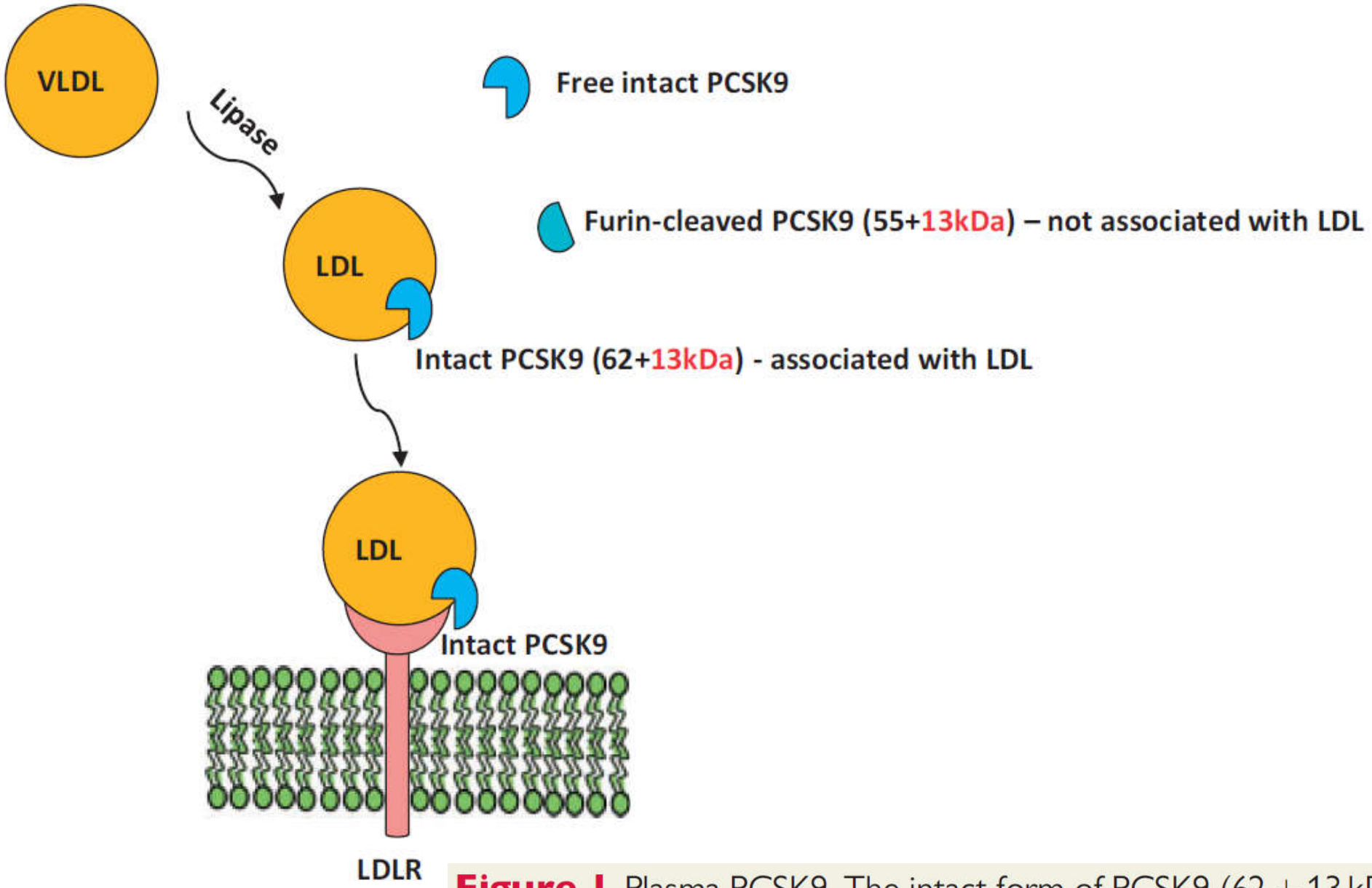


Figure 1 Plasma PCSK9. The intact form of PCSK9 (62 + 13 kDa) in plasma is found predominantly on LDL but not on VLDL. The furin-cleaved form of PCSK9 (55 + 13 kDa) in plasma is found predominantly as apoB-free.

Cardiovascular Research Advance Access published August 22, 2016

PCSK9 Association With Lipoprotein(a)

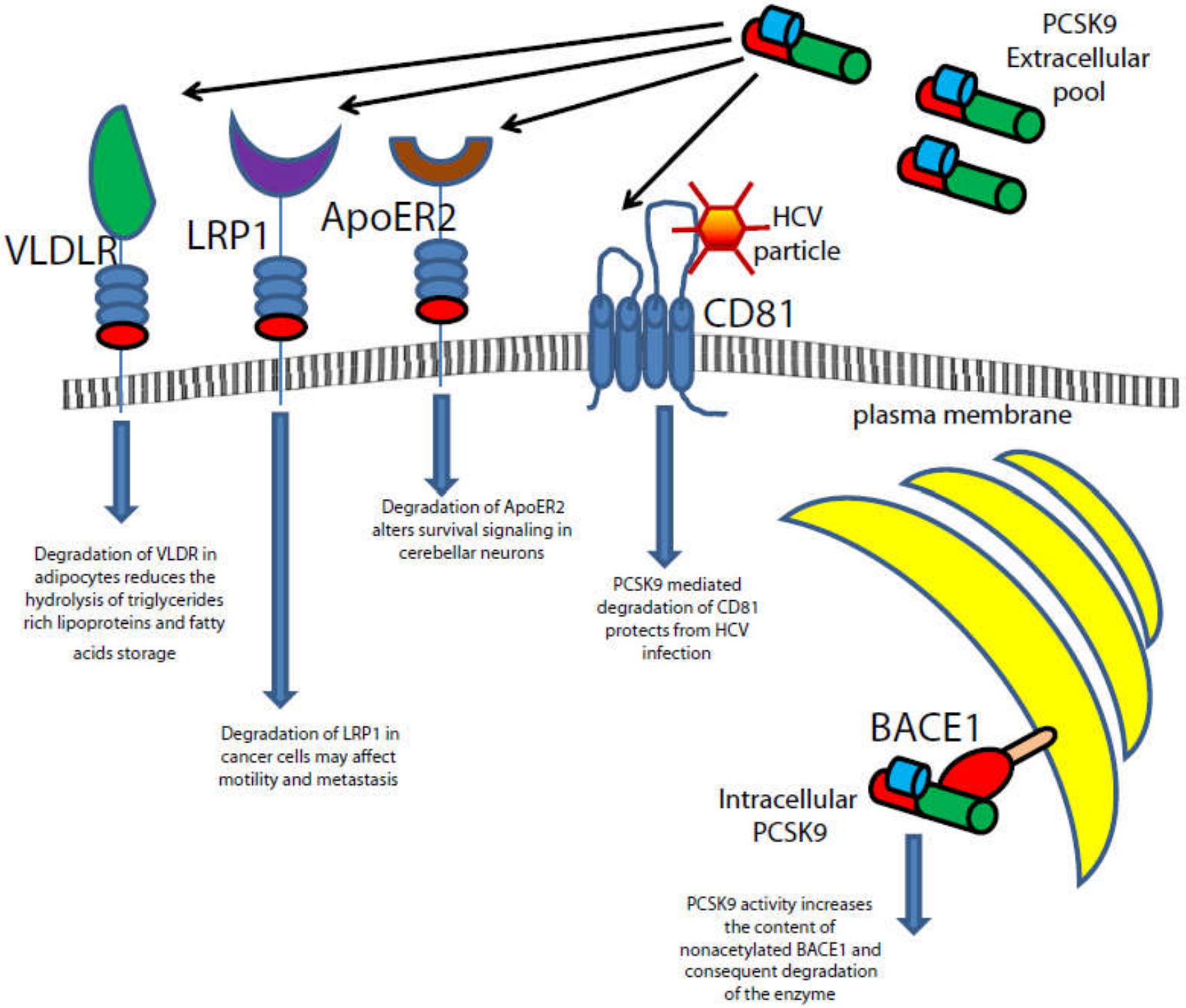
Hagai Tavori, Devon Christian, Jessica Minnier, Deanna Plubell, Michael D. Shapiro, Calvin Yeang, Ilaria Giunzioni, Mikael Croyal, P. Barton Duell, Gilles Lambert, Sotirios Tsimikas, Sergio Fazio

Rationale: **Lipoprotein(a) [Lp(a)] is a highly atherogenic low-density lipoprotein–like particle characterized by the presence of apoprotein(a) [apo(a)] bound to apolipoprotein B. Proprotein convertase subtilisin/kexin type 9 (PCSK9) selectively binds low-density lipoprotein; we hypothesized that it can also be associated with Lp(a) in plasma.**

Objective: **Characterize the association of PCSK9 and Lp(a) in 39 subjects with high Lp(a) levels (range 39–320 mg/dL) and in transgenic mice expressing either human apo(a) only or human Lp(a) (via coexpression of human apo(a) and human apolipoprotein B).**

Methods and Results: **We show that PCSK9 is physically associated with Lp(a) in vivo using 3 different approaches: (1) analysis of Lp(a) fractions isolated by ultracentrifugation; (2) immunoprecipitation of plasma using antibodies to PCSK9 and immunodetection of apo(a); (3) ELISA quantification of Lp(a)-associated PCSK9. Plasma PCSK9 levels correlated with Lp(a) levels, but not with the number of kringle IV-2 repeats. PCSK9 did not bind to apo(a) only, and the association of PCSK9 with Lp(a) was not affected by the loss of the apo(a) region responsible for binding oxidized phospholipids. Preferential association of PCSK9 with Lp(a) versus low-density lipoprotein (1.7-fold increase) was seen in subjects with high Lp(a) and normal low-density lipoprotein. Finally, Lp(a)-associated PCSK9 levels directly correlated with plasma Lp(a) levels but not with total plasma PCSK9 levels.**

Conclusions: **Our results show, for the first time, that plasma PCSK9 is found in association with Lp(a) particles in humans with high Lp(a) levels and in mice carrying human Lp(a). Lp(a)-bound PCSK9 may be pursued as a biomarker for cardiovascular risk.** (*Circ Res*. 2016;119:29-35. DOI: 10.1161/CIRCRESAHA.116.308811.)


PCSK9
Extracellular
pool
VLDLR
LRP1
ApoER2
HCV
particle
CD81
plasma membrane
Degradation of VLDR in adipocytes reduces the hydrolysis of triglycerides rich lipoproteins and fatty acids storage
Degradation of LRP1 in cancer cells may affect motility and metastasis
Degradation of ApoER2 alters survival signaling in cerebellar neurons
PCSK9 mediated degradation of CD81 protects from HCV infection
BACE1
Intracellular
PCSK9
PCSK9 activity increases the content of nonacetylated BACE1 and consequent degradation of the enzyme

III- PCSK9 MUTATIONS

PCSK9

How do genetic mutations/variants in PCSK9 relate to LDL-C levels and CV risk?

Can PCSK9 be targeted to reduce LDL-C and CVD?

Genetic Variants Establish PCSK9 as a Modulator of LDL-C

- FH-associated physical abnormalities[1]
- Increased plasma levels of TC and LDL-C[1,2]

Increasing PCSK9 (GOF)

- Fewer LDL receptors[1,2]
- Higher LDL-C[1,2]

Plasma LDL-C

Decreasing PCSK9 (LOF)

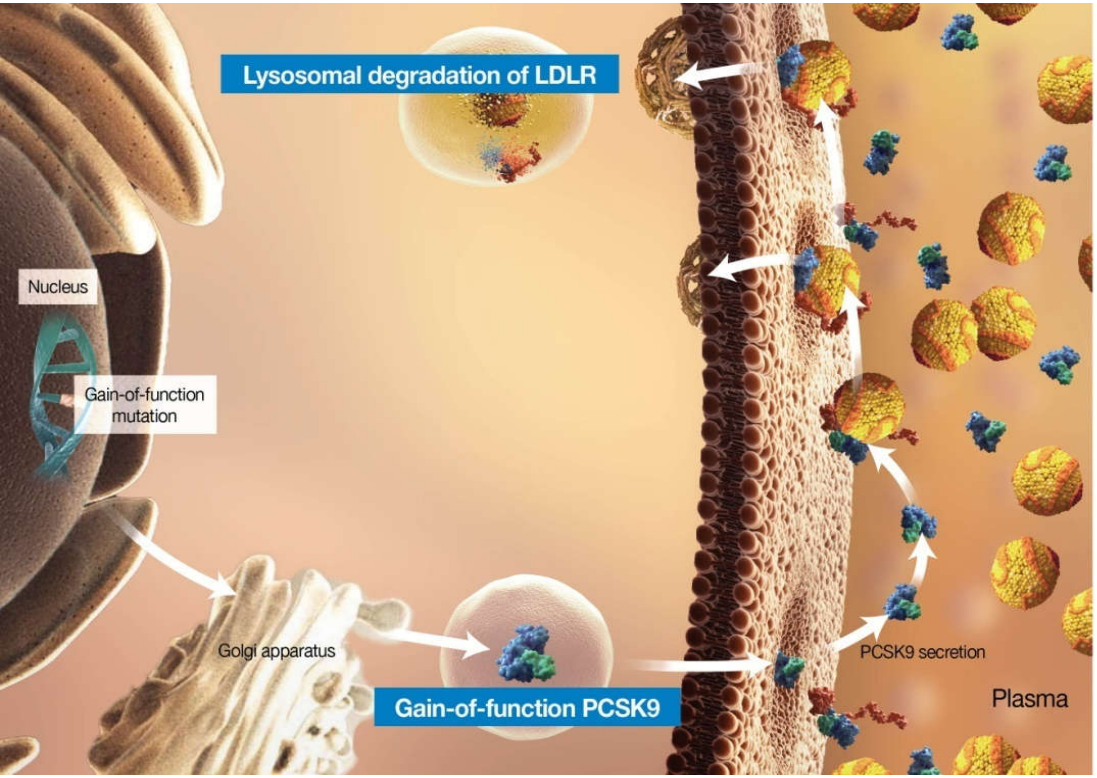
- More LDL receptors[1,2]
- Lower LDL-C[1,2]

- Reduced plasma levels of TC and LDL-C[1,3]

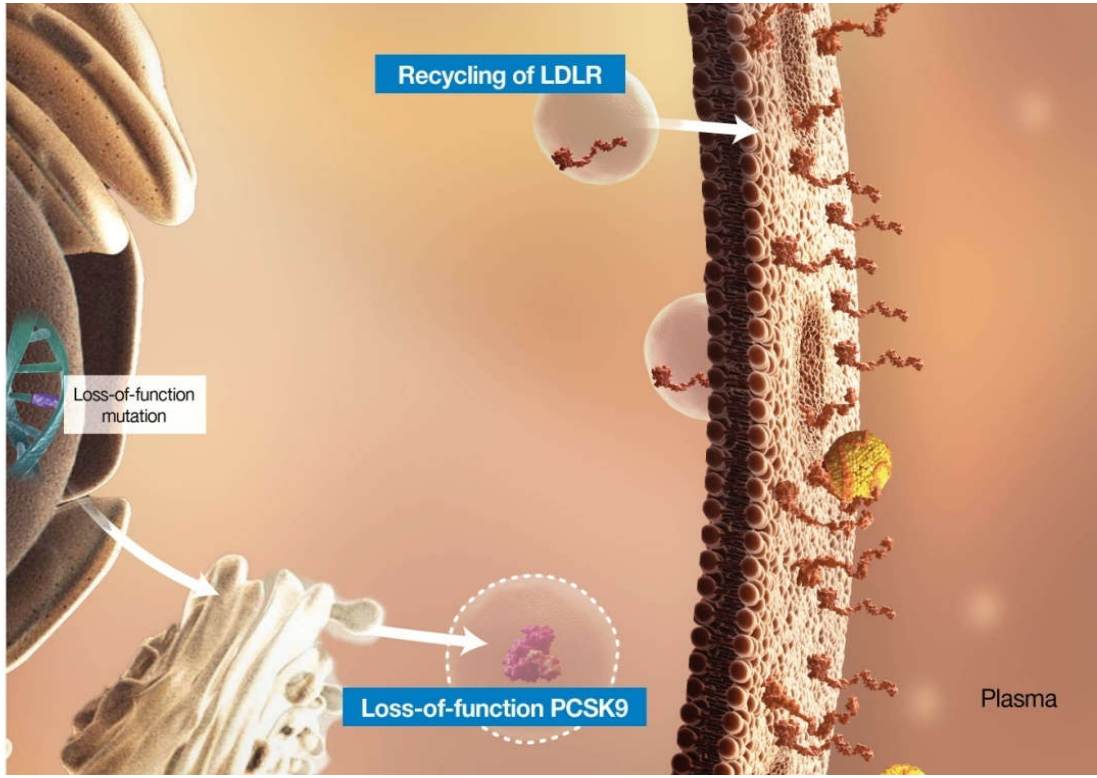
FH = familial hypercholesterolemia; GOF = gain of function; LDL-C = low-density lipoprotein cholesterol; LOF = loss of function; TC = total cholesterol.

1. Abifadel M, et al. *Hum Mutat*. 2009;30:520-529. 2. Seidah NG, et al. *Circ Res*. 2014;114:1022-1036. 3. Benn M, et al. *J Am Coll Cardiol*. 2010;55:2833:2842.

Genetic Variants of PCSK9 Demonstrate Its Importance in Regulating LDL Levels



PCSK9 Gain of Function (GOF) = <u>Less</u> LDL-Rs[1,3,5]

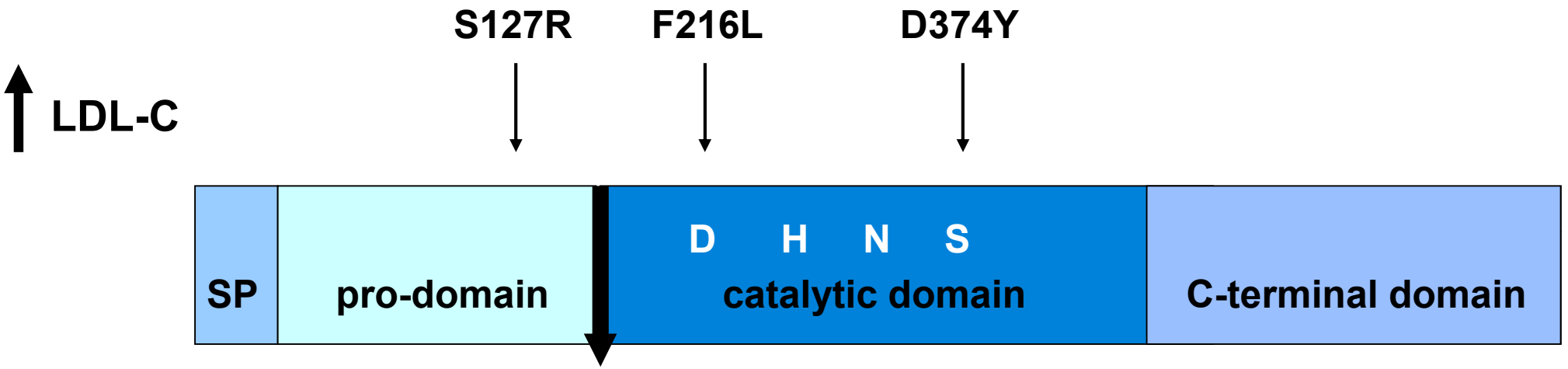


PCSK9 Loss of Function (LOF) = <u>More</u> LDL-Rs[2,4,5]

Mutations in the human PCSK9 gene that lead to a loss of PCSK9 function are found in 1% to 3% of the population[6,7]

1. Horton JD, et al. *J Lipid Res*. 2009;50:S172-S177. **2.** Lakoski SG, et al. *J Clin Endocrinol Metab.* 2009;94: 2537-2543. **3.** Abifadel M, et al. *Hum Mutat.* 2009;30:520-529. **4.** Cohen J, et al. *Nat Genet.* 2005;37:161-165. **5.** Steinberg D, et al. *PNAS.* 2009;106:9546-9547. **6.** Cohen JC, et al. *N Engl J Med*. 2006;354:1264-1272. **7.** Benn M, et al. *J Am Coll Cardiol*. 2010;55:2833-2842.

PCSK9: A Recently Identified Gene Associated with FH (Gain of Function Mutations)



Lambert et al. (2009) *Atherosclerosis*

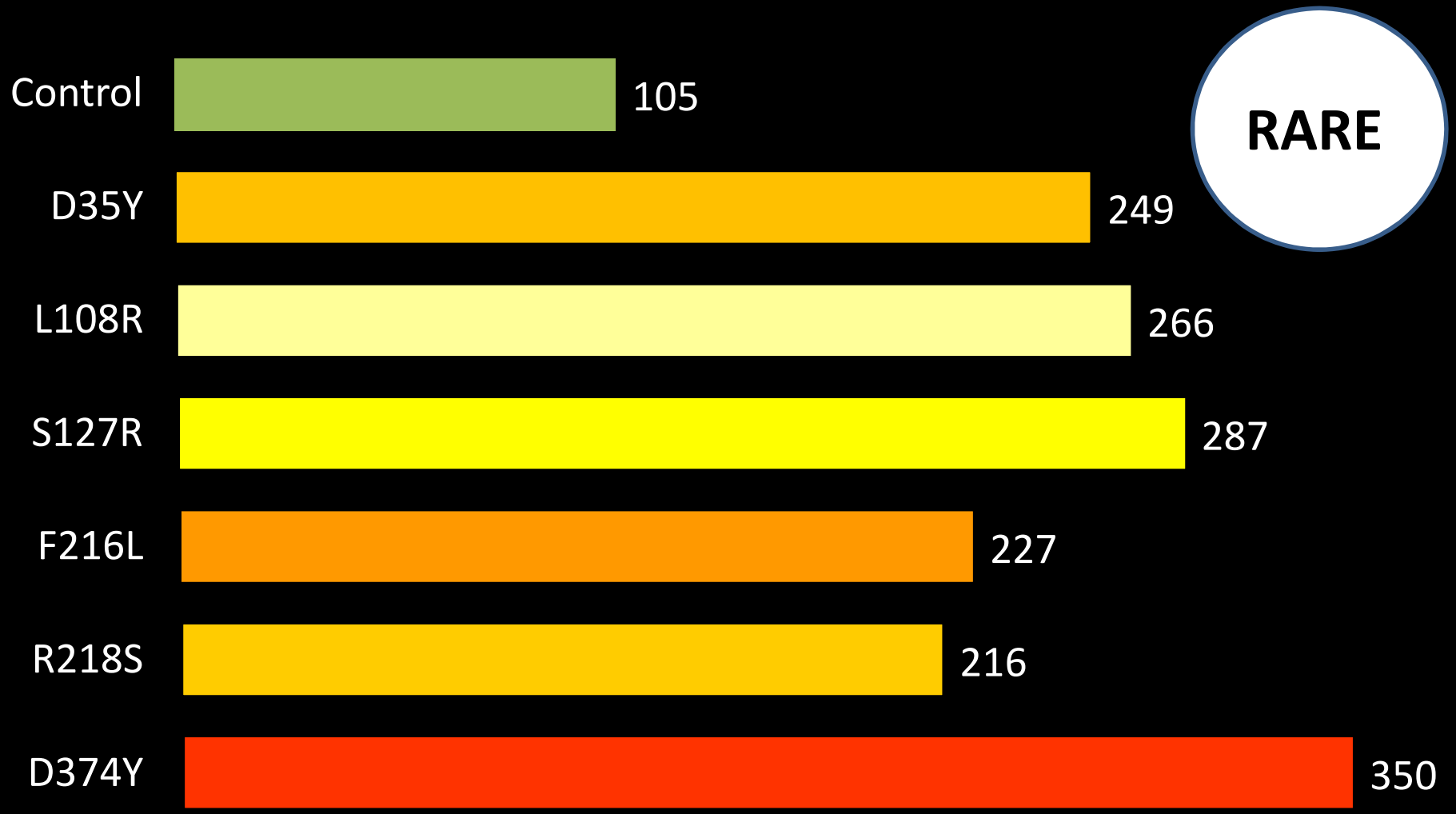
PCSK9 GOF Mutations Associated With ADH[1]

PCSK9 Genotype	Mutation Type	Biochemical Phenotype	Clinical Phenotype
S127R	Missense	5x higher affinity for LDL-R; decreased LDL-R expression and activity; may interfere with trafficking of LDL-R to the cell surface[1,2]	Cholesterol levels in 90th percentile; tendon xanthomas, CHD, early MI, and stroke[3]
D129G	Missense	Leads to decreased LDL-R expression and activity[1]	Elevated LDL-C, identified in family with strong history of CV disease[2]
F216L	Missense	Loss of PCSK9 activation; increased LDL-R degradation; may prolong PCSK9 half-life, causing higher circulating PCSK9[2,3]	Premature CHD; Early MI[3]
R218S	Missense	Normal processing and secretion but loss of PCSK9 enzymatic activity[1]	Tendon xanthomas, arcus corneae[4]
D374Y	Missense	10–25x higher affinity for LDL-R; decreased LDL-R recycling and increased degradation[1,5]	Tendon xanthomas; premature atherosclerosis[4]

Please refer to Lopez et al. (2008) and Abifadel et al. (2009) for comprehensive lists of PCSK9 mutations and variants.

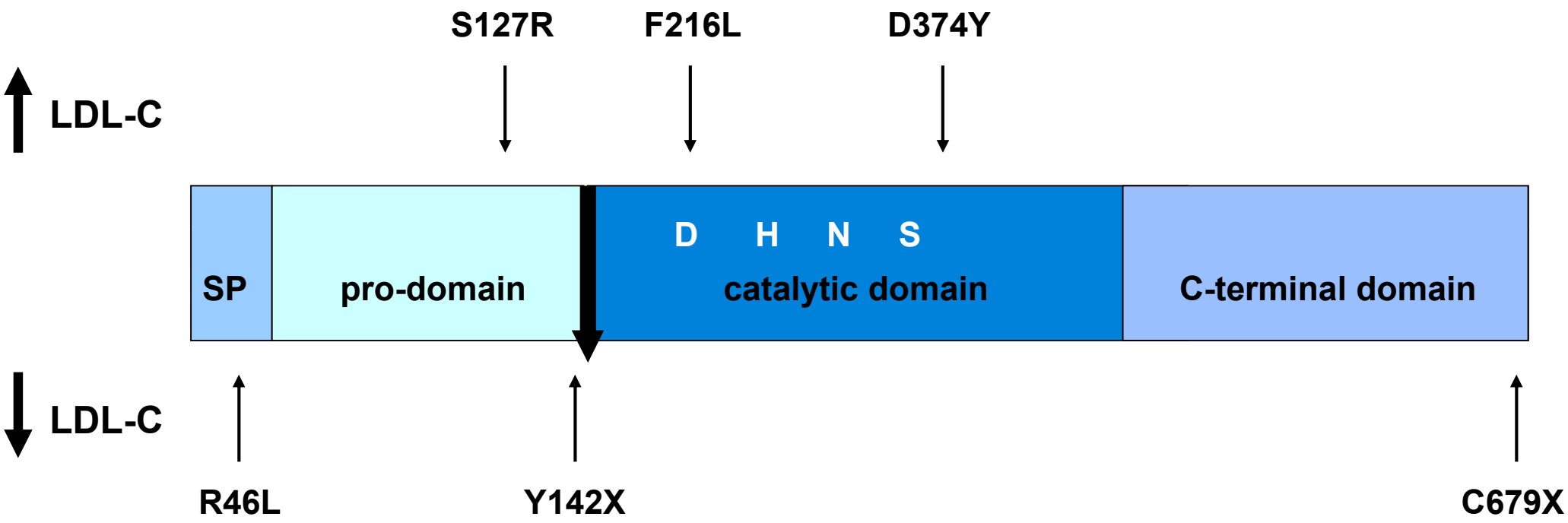
1. Lopez D. *Biochem Biophys Acta*. 2008;1781:184-191. **2.** Horton JD, et al. *J Lipid Res*. 2009;50: S172-S177. **3.** Abifadel M, et al. *Nat Genet.* 2003;34:154-156. **4.** Abifadel M, et al. *Hum Mutat*. 2009;30:520-529. **5.** Cunningham D, et al. *Nat Struct Mol Biol*. 2007;14:413-419.

Elevated LDL-C levels in patients with GAIN-of-Function PCSK9 mutations



Poirier S, Mayer G. Drug Des Devel Ther 2013;7:1135–48.

PCSK9: A Gene Associated with Familial Hypobetalipoproteinaemia (Loss of Function Mutations)



Lambert et al. (2009) *Atherosclerosis*

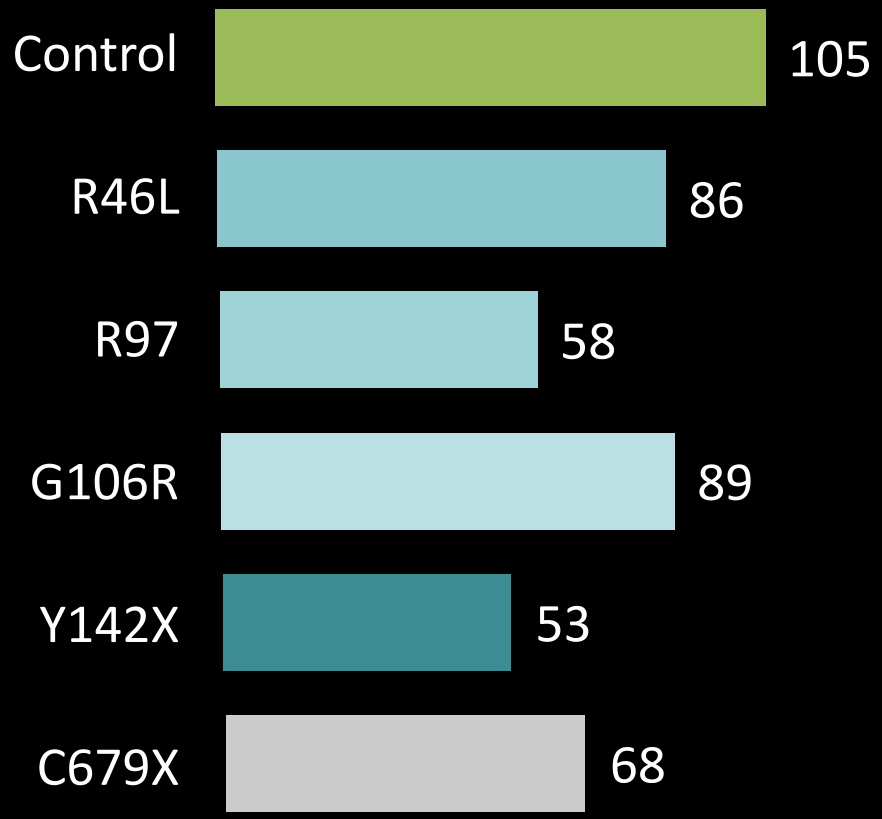
PCSK9 LOF Mutations and Variants Associated With Hypocholesterolemia

PCSK9 Genotype	Mutation Type	Biochemical Phenotype	Clinical Phenotype
R46L	Missense Polymorphism	No effect on processing or secretion[1]	11%–16% reduction in LDL-C[5]; 30% reduction in IHD[5]; reduced risk of early onset MI[6]; 47% reduction of CHD[1]
G106R	Missense	Defective protein that is not secreted[1]	Reduced LDL-C[1]
Y142X	Nonsense	Disrupted protein synthesis resulting in no detectable protein[3]	40% reduction in LDL-C; 88% reduction in CHD[1,2]
Q152H	Missense	Defective autocatalytic cleavage and secretion[4]	48% decrease in LDL-C; 79% decrease in plasma PCSK9[4]
L253F	Missense	Poorly cleaved and secreted[1]	30% reduction in LDL-C[2,3]; Reduced risk of CHD[3]
A443T	Missense Polymorphism	Normally cleaved and secreted; higher susceptibility to cleavage[1]	Modest (2%) reduction in LDL-C[7]
Q554E	Missense	Poorly cleaved and secreted[1]	Reduced LDL-C[8]
C679X	Nonsense	Disrupted protein folding; impaired protein secretion[1,2]	40% reduction in LDL-C; 88% reduction in CHD[1,2]

Please refer to Lopez et al. (2008) and Abifadel et al. (2009) for comprehensive lists of PCSK9 mutations and variants.

1. Lopez D. *Biochem Biophys Acta*. 2008;1781:184-191. **2.** Abifadel M, et al. *Hum Mutat*. 2009;30:520-529. **3.** Cunningham D, et al. *Nat Struct Mol Biol*. 2007;14:413-419. **4.** Mayne J, et al. *Clin Chem*. 2011;57:1415-1423. **5.** Benn M, et al. *J Am Coll Cardiol*. 2010;55:2833-2842. **6.** Kathiresan S. *N Engl J Med*. 2008;358:2299-3200. **7.** Zhao Z, et al. *Am J Hum Genet*. 2006;79:S14-S23. **8.** Abifadel M, et al. In: Toth PP. *The Year in Lipid Disorders.* Vol. 2. Oxford, UK: Atlas Medical Publishing Ltd. 2010:3-23.

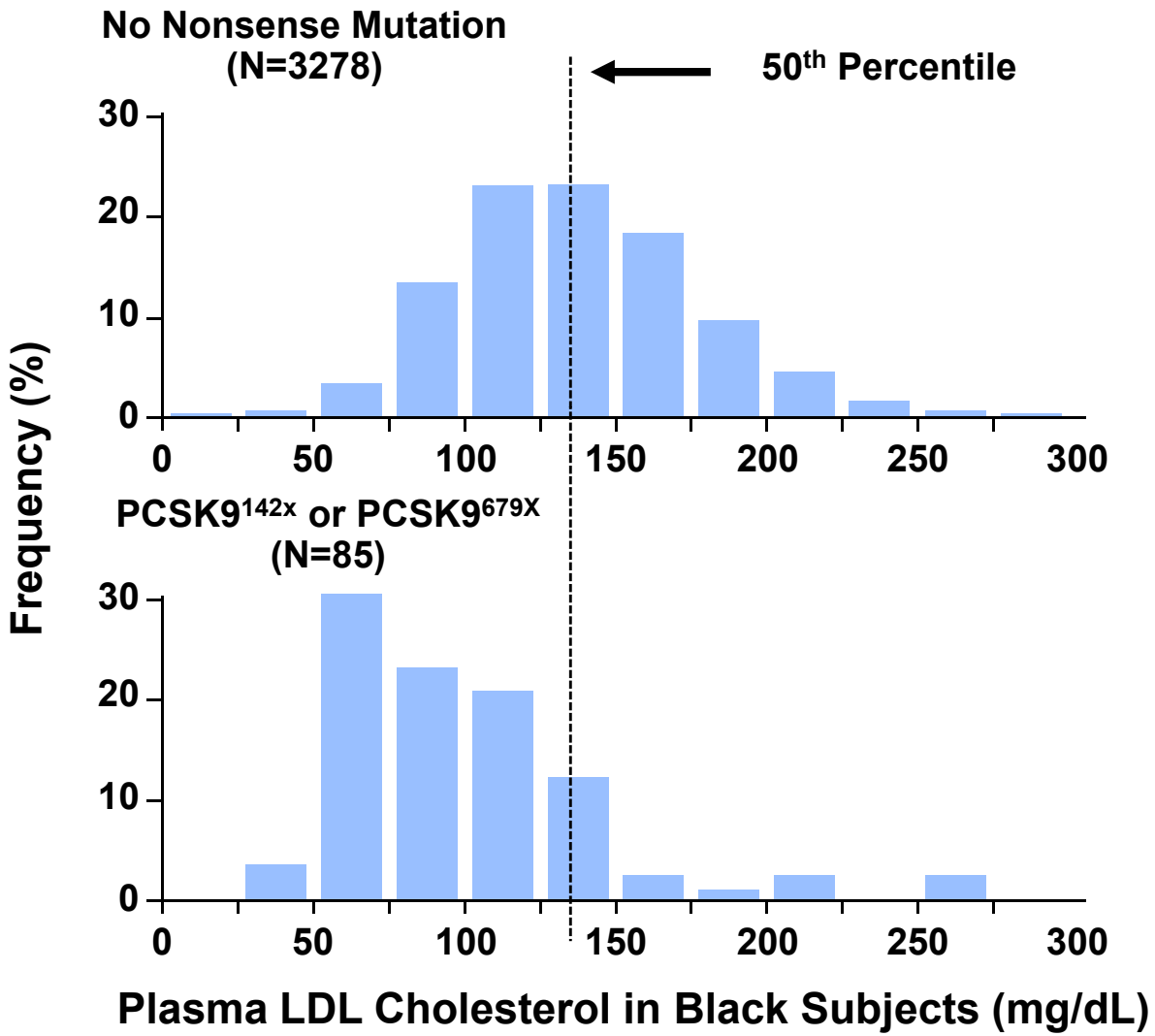
Low LDL-C levels in patients with LOSS-of-Function PCSK9 mutations

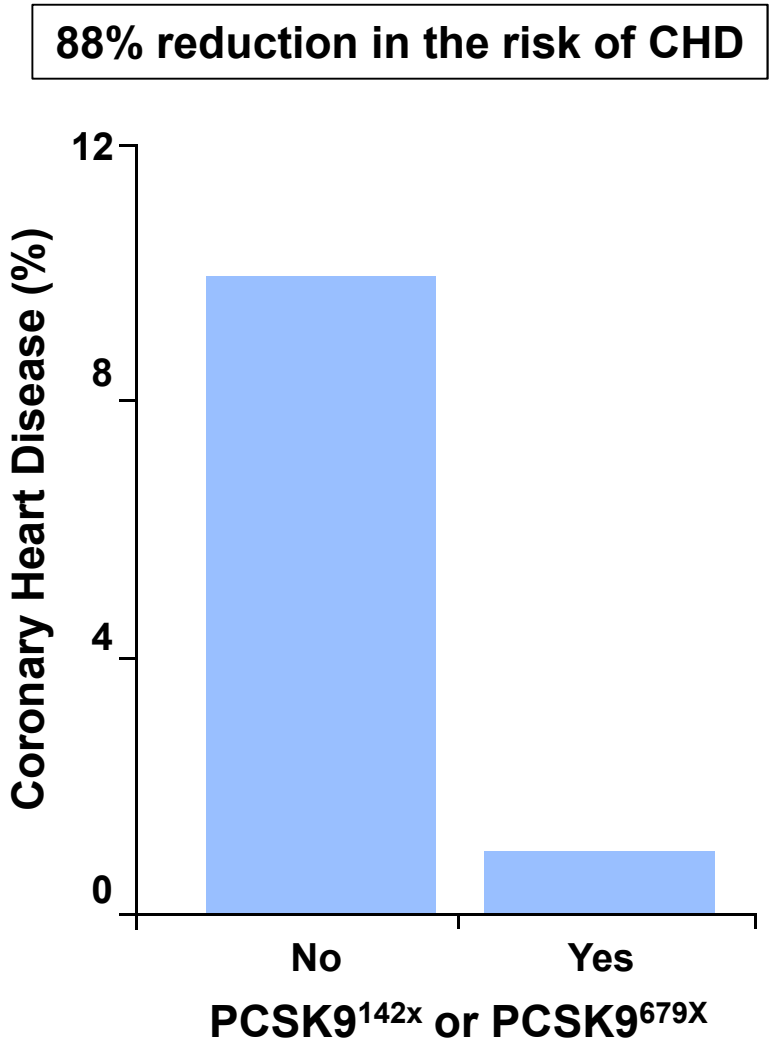


FREQUENT

Poirier S, Mayer G. Drug Des Devel Ther 2013;7:1135–48.

Cardiovascular Benefits of PCSK9 Loss of Function Mutations

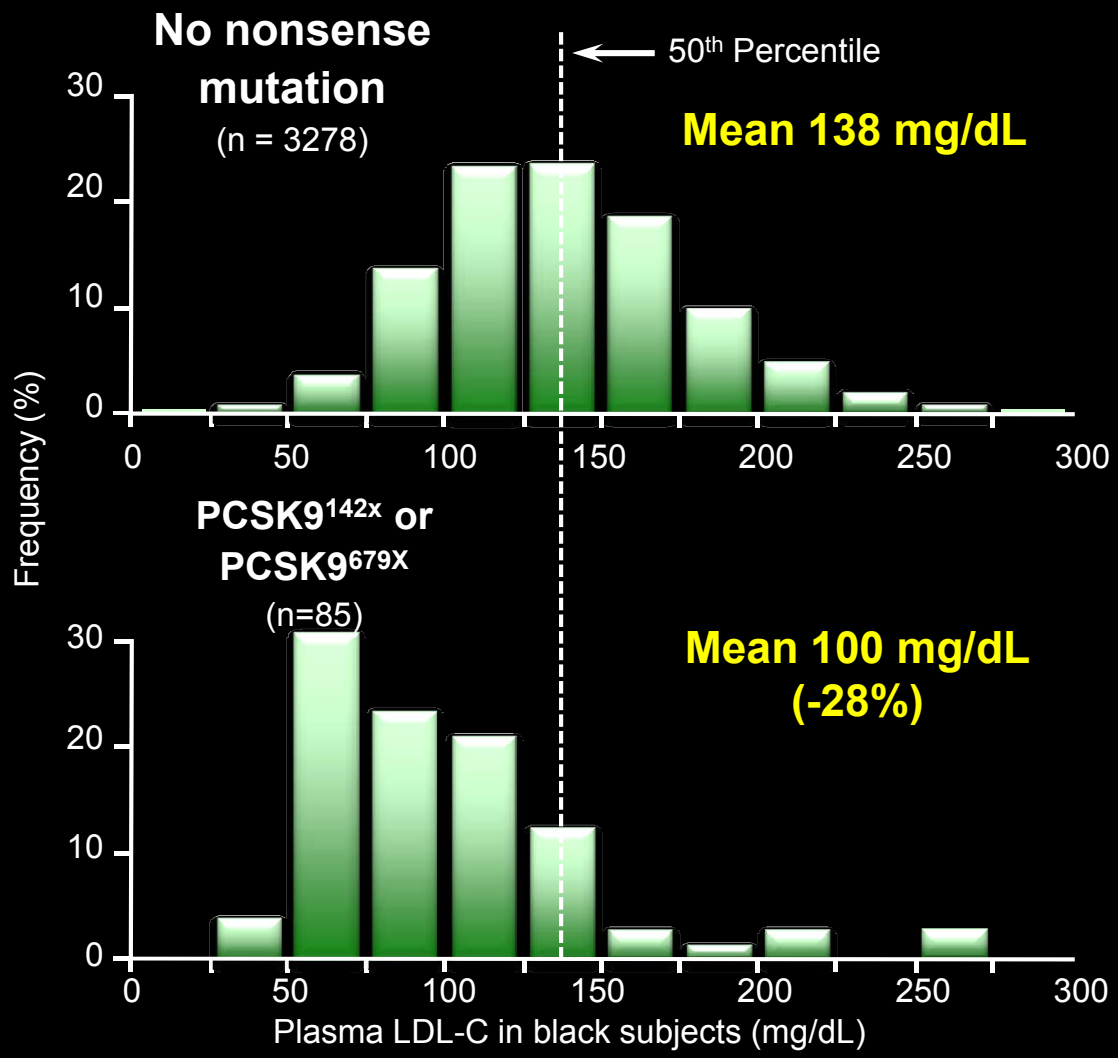


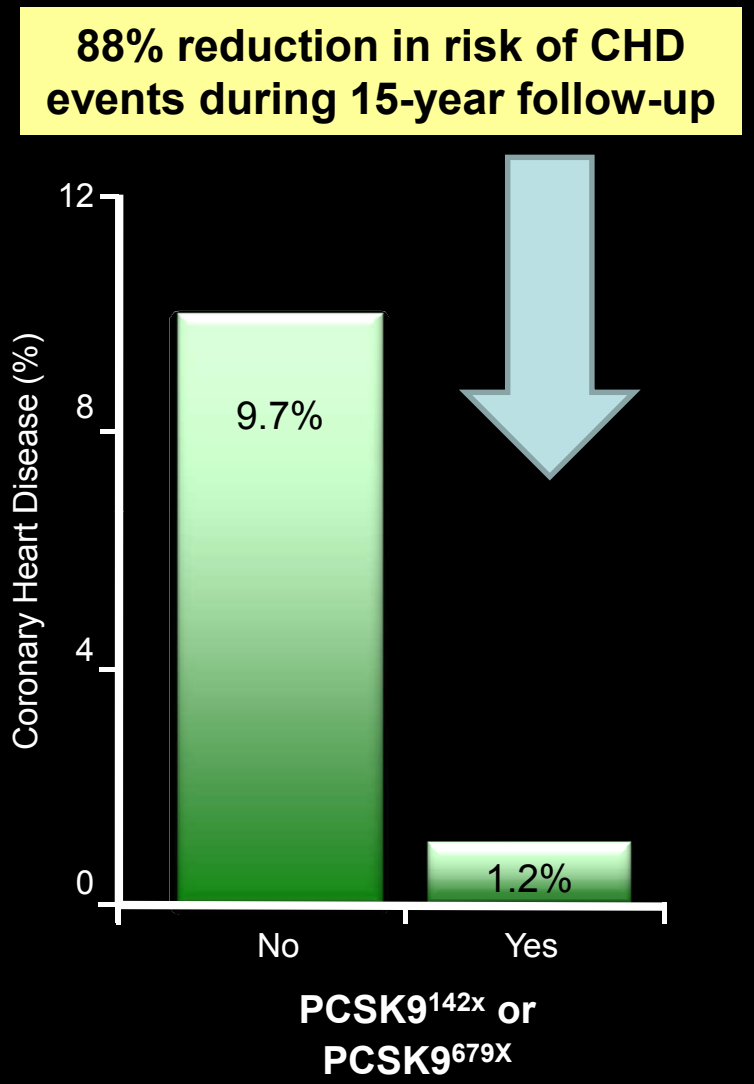


Cohen et al. (2005) *Nat Genet*
Cohen et al (2006) *N Engl J Med*

ARIC=Atherosclerosis Risk in the Community

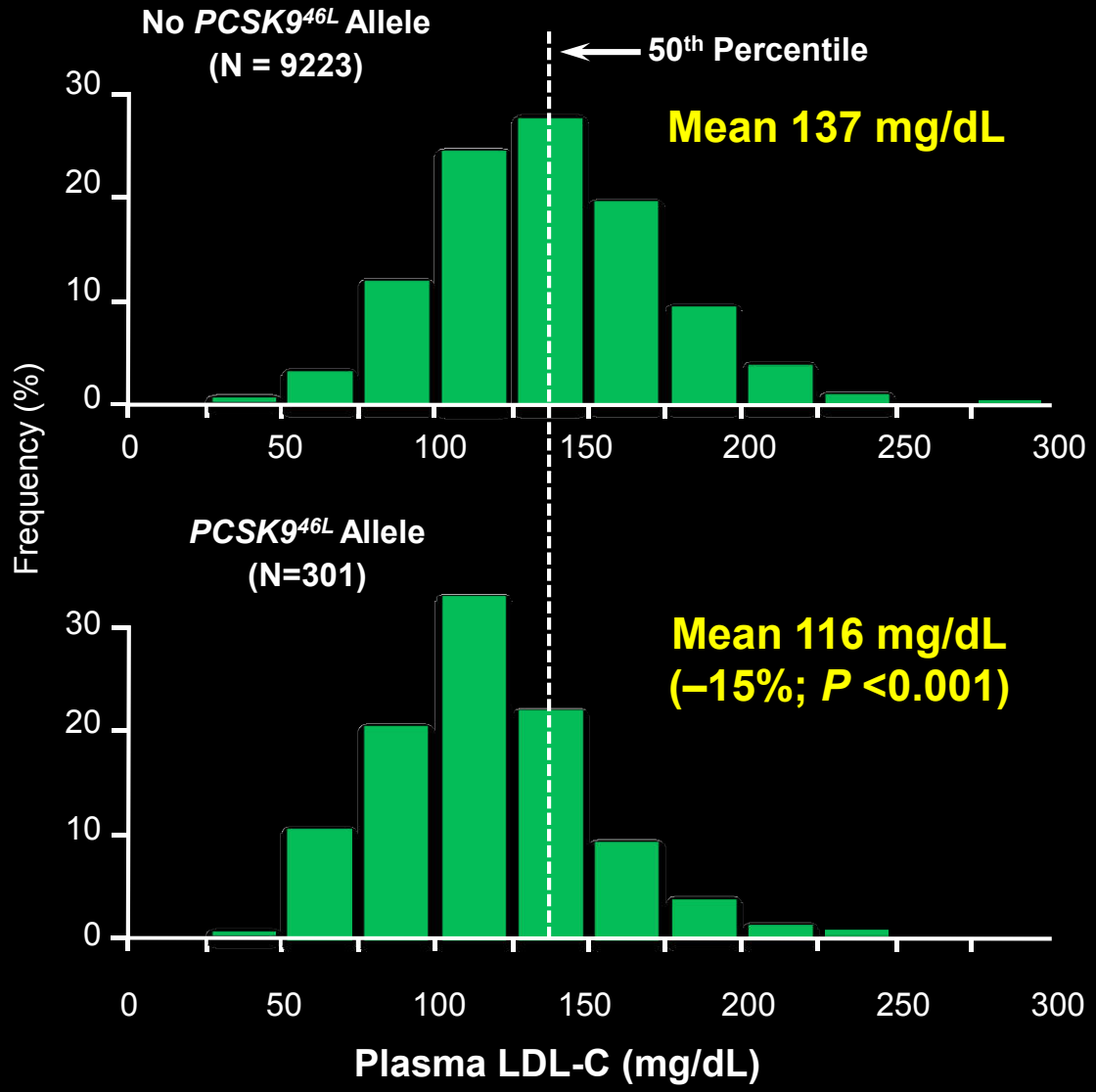
Loss-of-Function PCSK9 mutations are associated with low LDL-C and low prevalence of CHD events

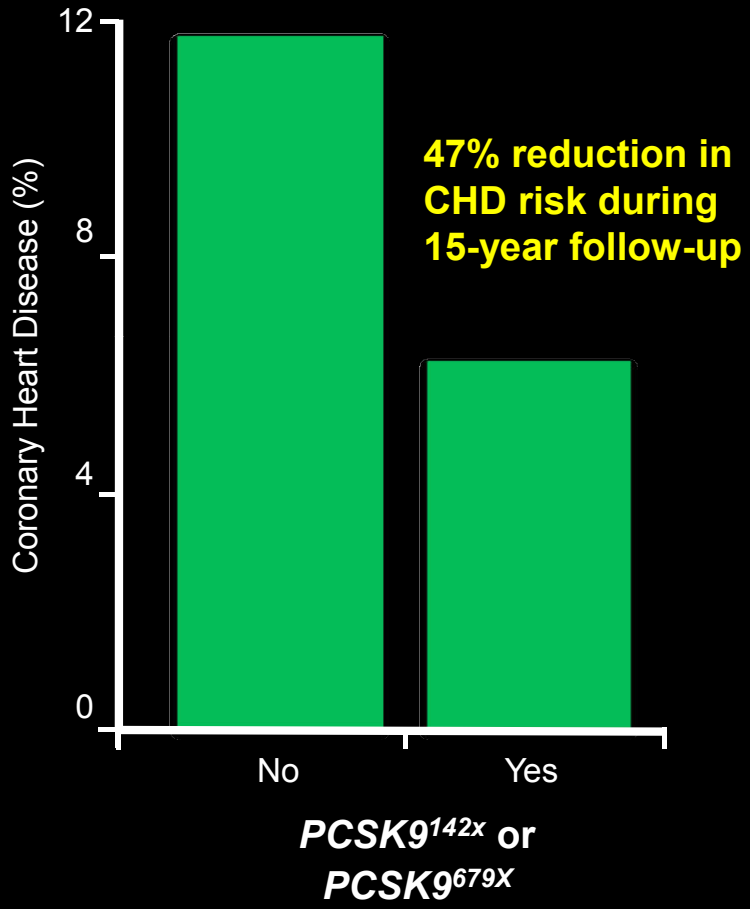




Adapted from Cohen JC et al. N Engl J Med 2006;354:1264–72.

Loss-of-Function PCSK9 Mutations in Whites Are Associated with Low LDL-C and Low Prevalence of CHD Events





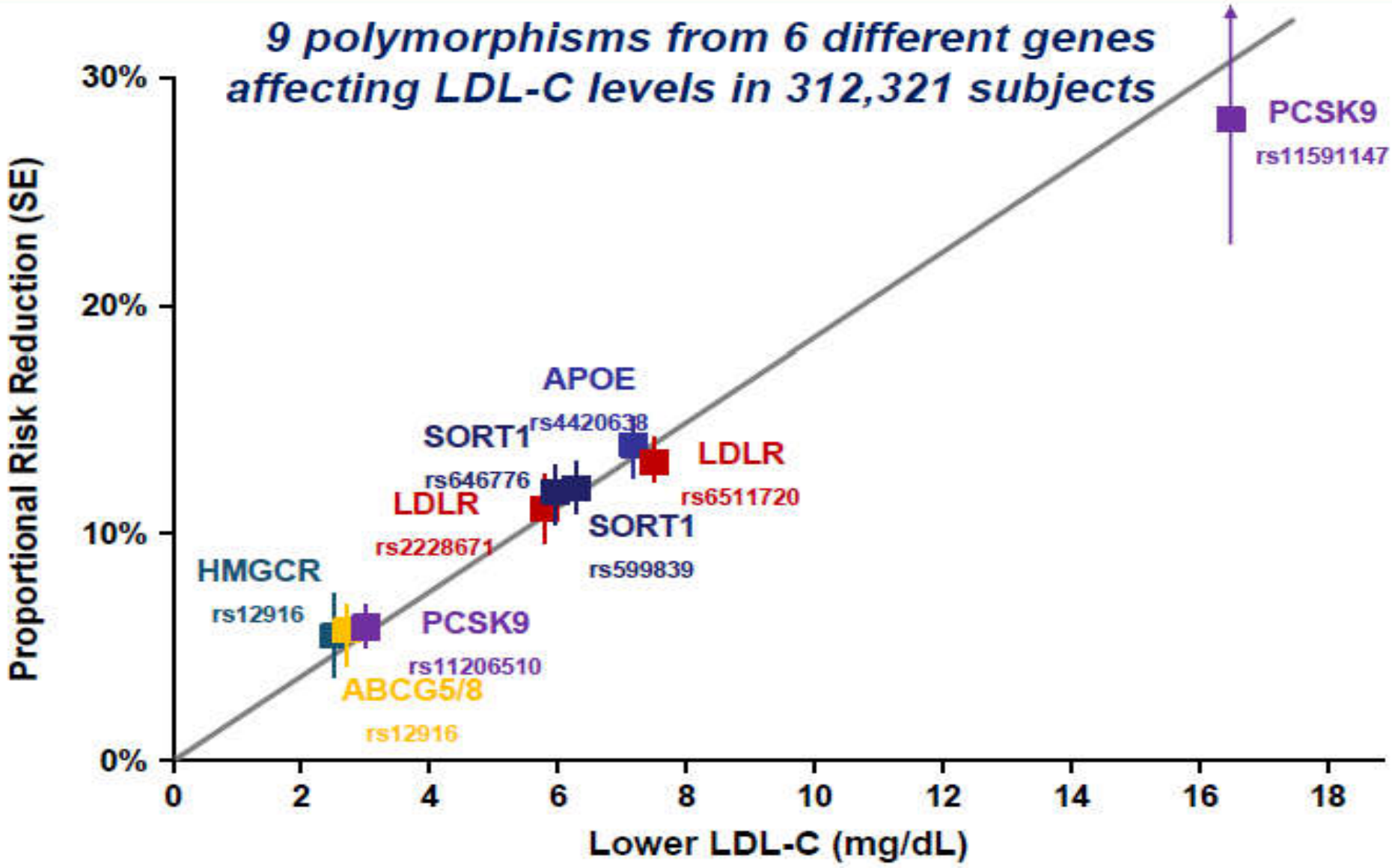
Impact of PCSK9 Loss of Function Mutation on Risk of MI

Lifelong Impact of 16% Lower LDL translates into 60% Lower Risk

Site	Study	OR for MI	95% CI	p-value
Finland	FINRISK	**0.30**	**(0.11, 0.84)**	**0.02**
Sweden	Malmo Diet and Cancer Study CV cohorts	**0.32**	**(0.07, 1.61)**	**0.17**
Spain	Registre Gironi del Cor (REGICOR)	**0.35**	**(0.15, 0.82)**	**0.02**
Seattle	Heart Attack Risk in Puget Sound	**0.45**	**(0.21, 0.98)**	**0.049**
Boston	MGH Premature CAD Study	**0.59**	**(0.21, 1.69)**	**0.46**
Combined analysis		**0.40**	**(0.26, 0.61)**	**0.0002**

N Engl J Med. 2008;358:2299-2300

Lower Risk of Cardiovascular Events via Multiple Genetic Variants Affecting LDL-C



Ference BA et al. JACC 2012;60:2631–9

Two People Identified With Inactivating Mutations in Both *PCSK9* Alleles

PCSK9 Genotype	$PCSK9^{Y142X/\Delta R97}$	$PCSK9^{C679X/C679X}$
Total Cholesterol	96 mg/dL (2.5 mmol/L)	85mg/dL (2.2 mmol/L)
LDL	**14 mg/dL** (0.4 mmol/L)	**15 mg/dL** (0.4 mmol/L)
TG	119 mg/dL (1.3 mmol/L)	71 mg/dL (0.8 mmol/L)
HDL	65 mg/dL (1.7 mmol/L)	54 mg/dL (1.4 mmol/L)
Plasma [PCSK9]	Undetectable	N/A
Clinical	• Apparent good health • Normal fertility (mother) • No developmental abn. • College graduate • Aerobics instructor	• Apparent good health • Normal fertility (mother)

Humans with very low LDL-C levels from PCSK9 deficiency appear generally healthy, with normal fertility and development

- *PCSK9* knockout mice also have normal fertility and development[3]

1. *Zhao Z, Tuakli-Wosornu Y,Lagace TA, et al.,Am J Hum Genet. 2006, 79: 514-523.*
2. *Hooper AJ , Marais AJ, Tanyanyiwa DM, Burnett JR. Atherosclerosis. 2007:193:445-448*
3. *Rashid S. et al. PNAS, 2005;102:5374-79*

3 Individuals With Double Loss-of-Function PCSK9 Mutations

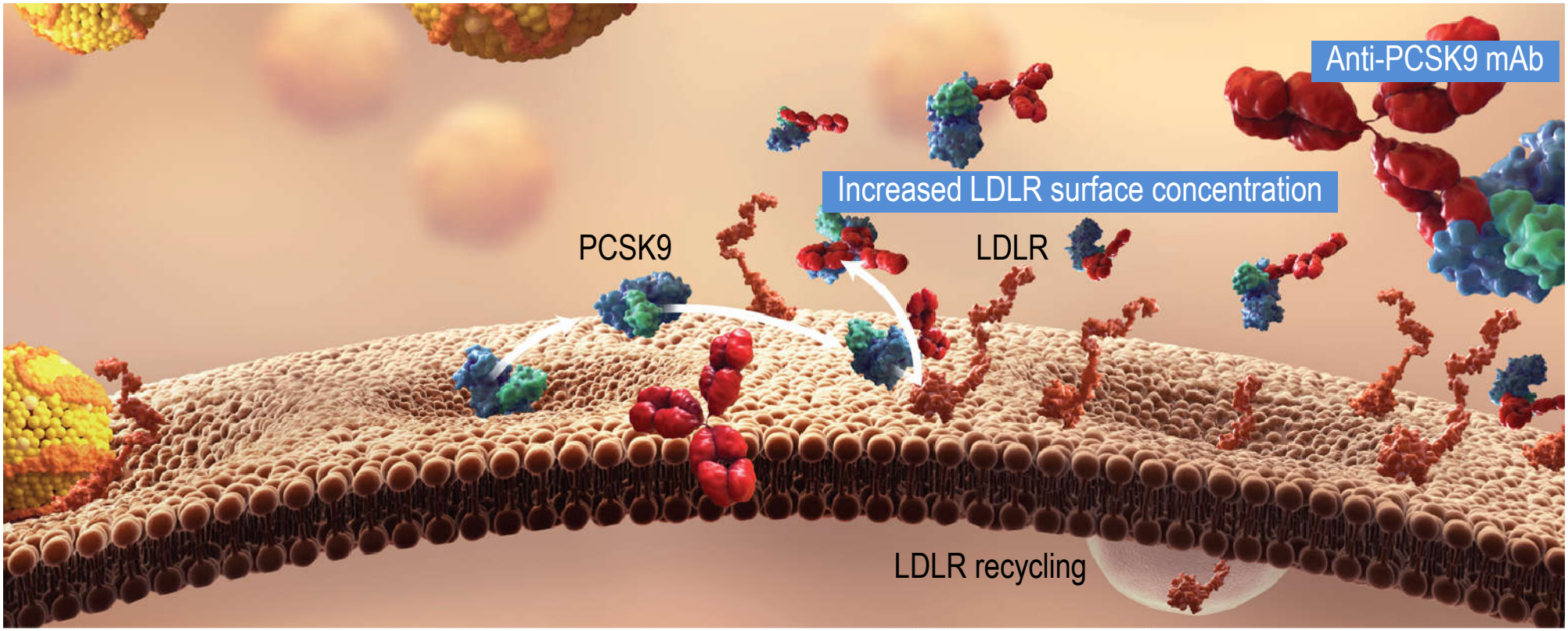
- **Two cases of homozygous loss-of-function mutations in PCSK9 :**
  - 32-year-old woman with compound mutations, no measurable PCSK9, LDL-C of 14 mg/dL, was healthy and normotensive, and had normal liver and renal function[1]
  - 21-year-old Zimbabwean black woman with homozygous C679X mutation of PCSK9 and LDL-C of 15 mg/dL [2]
- One case with heterozygous mutations for 2 PCSK9 missense mutations, R104C and V114A :[3]
  - 49-year-old French male hospitalized for new-onset diabetes, with no detectable PCSK9 levels, LDL-C of 16 mg/dL, apo B of 25mg/dL, was healthy and had normal liver function
- The finding that individuals who completely lack PCSK9 and have very low LDL-C can be healthy suggests that inhibition of PCSK9 may be a safe pharmacologic approach to dyslipidemia management[4]

LDL-C=low-density lipoprotein cholesterol; PCSK9=proprotein convertase subtilisin/kinexin type 9.

1. Zhao Z et al. *Am J Hum Genet*. 2006;79:514-523; 2. Hooper AJ et al. *Atherosclerosis.* 2007;193:445-448;
3. Cariou B et al. *Arterioscler Thromb Vasc Biol*. 2009;29(10):2191-2197; 4. Lambert G et al. *J Lipid Res.* 2012. 53: 2515-2524.

- Individuals who completely lack PCSK9 and have very low LDL-C levels are healthy
  - Therefore, inhibition of PCSK9 may be a safe pharmacologic approach to dyslipidaemia management

Impact of an anti-PCSK9 monoclonal antibody on LDL-R expression



IV- PCSK9 AND LIPOPROTEIN SYNTHESIS

PCSK9 ΚΑΙ ΗΠΑΤΙΚΗ ΣΥΝΘΕΣΗ ΛΙΠΟΠΡΩΤΕΪΝΩΝ (1)

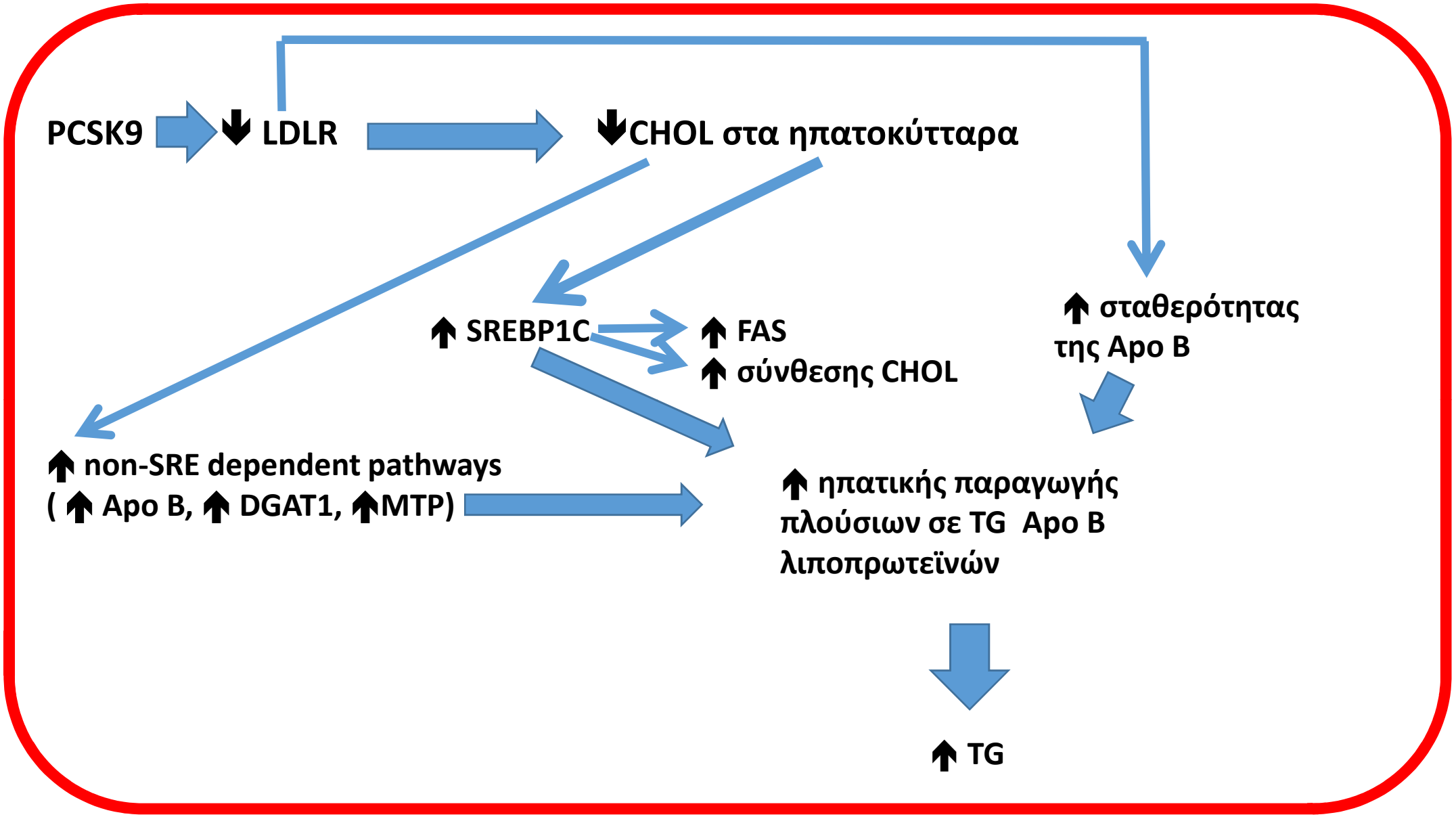
Οι LDL υποδοχείς διαδραματίζουν σημαντικό ρόλο στην έκκριση και αποδόμηση των πλούσιων σε TRG λιποπρωτεϊνών που παράγονται από τα ηπατοκύτταρα

J Biol Chem 2008;283: 11374

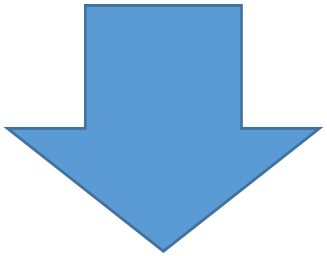
PCSK9 ⇨ ↓LDLR ⇨ ↑ TRG

Αναστολείς της PCSK9 ⇨ ↑LDLR ⇨ ↓TRG

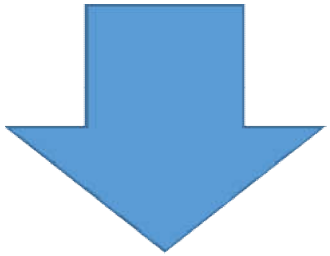
PCSK9 ΚΑΙ ΗΠΑΤΙΚΗ ΣΥΝΘΕΣΗ ΛΙΠΟΠΡΩΤΕΪΝΩΝ



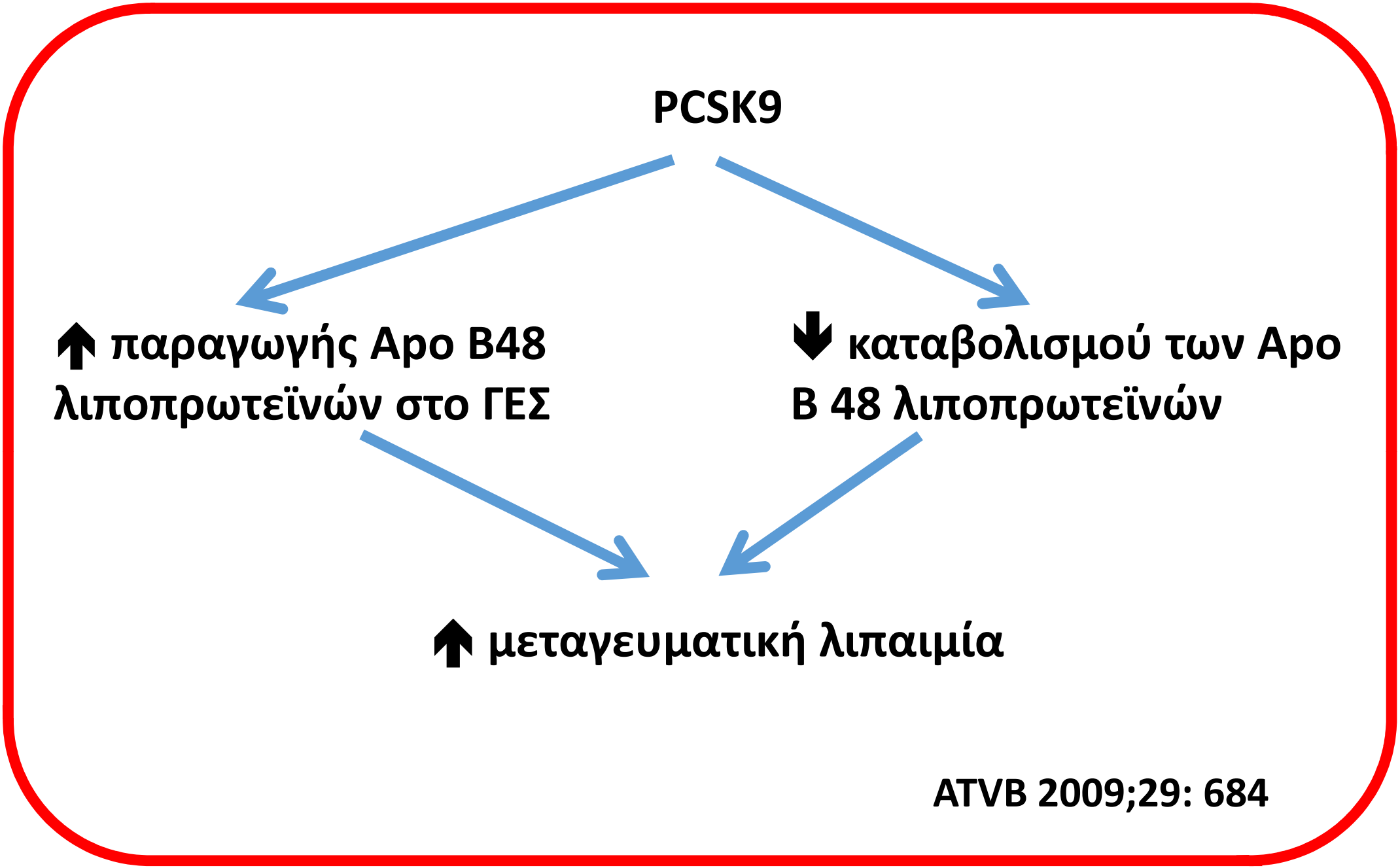
PCSK9 (εκφράζεται στα εντεροκύτταρα)



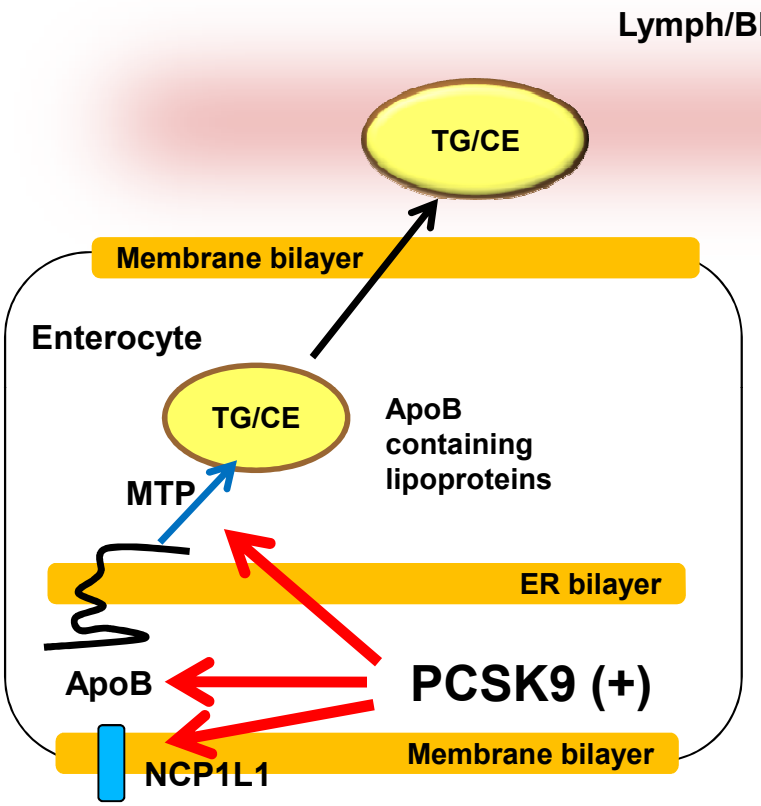
↑ παραγωγή στον εντερικό σωλήνα πλούσιων σε TG ApoB 48 λιποπρωτεϊνών



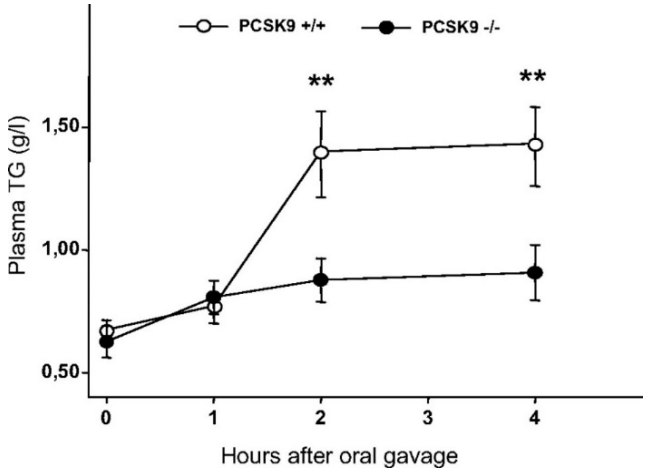
↑ μεταγευματική λιπαιμία
(με παρόμοιους μηχανισμούς με τους αντίστοιχους στα ηπατοκύτταρα)


PCSK9
↑ παραγωγής Apo B48 λιποπρωτεϊνών στο ΓΕΣ
↓ καταβολισμού των Apo Β 48 λιποπρωτεϊνών
↑ μεταγευματική λιπαιμία
ATVB 2009;29: 684

PCSK9 and TGs

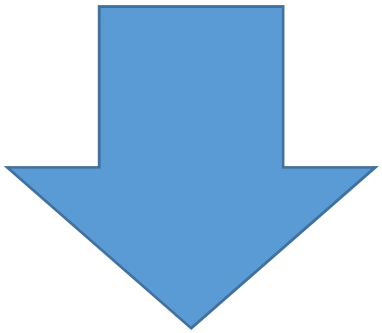


Le May C et al. Arterioscler Thromb Vasc

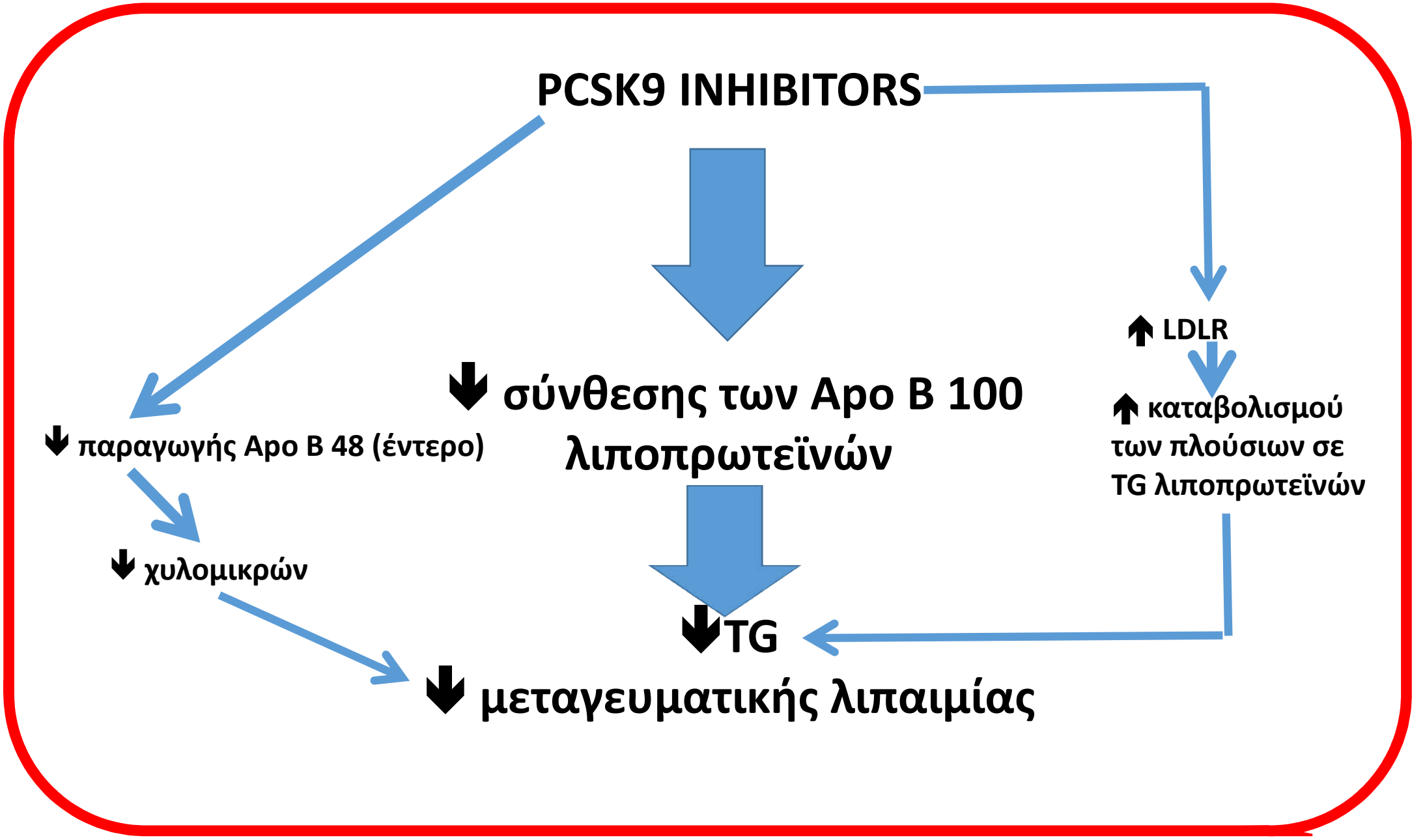


Η PCSK9 αυξάνει τη μεταγευματική υπερλιπιδαιμία

ΑΝΑΣΤΟΛΕΙΣ ΤΗΣ PCSK9



⬇ μεταγευματικής λιπαιμίας ;


PCSK9 INHIBITORS
↓ παραγωγής Apo B 48 (έντερο)
↓ χυλομικρών
↓ σύνθεσης των Apo B 100 λιποπρωτεϊνών
↑ LDLR
↑ καταβολισμού των πλούσιων σε TG λιποπρωτεϊνών
↓TG
↓ μεταγευματικής λιπαιμίας

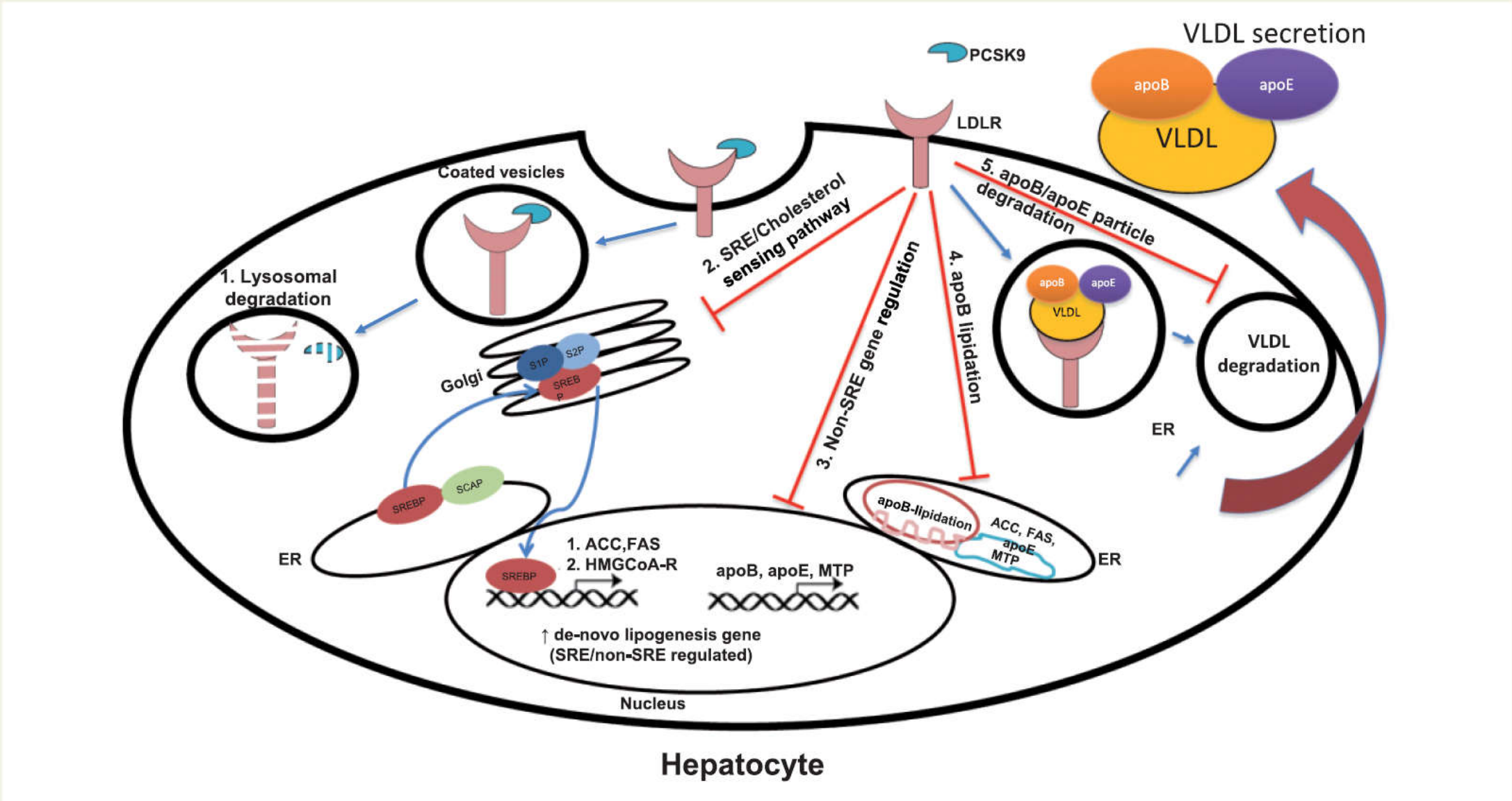


Figure 2 PCSK9 and apoB-containing lipoproteins metabolism. PCSK9 regulates surface LDLR levels via targeting of both proteins to lysosomal degradation (1). The main consequence of the decreased PCSK9 interaction with the LDLR is the increase in the intracellular cholesterol pool which promotes: (2) a reduction of the activity of the SRE-dependent pathway and of intracellular cholesterol synthesis, (3) a reduction in the expression of non-SRE genes involved in lipogenesis, (4) a reduction of apoB lipidation, and (5) a reduction of apoB/apoE particle uptake and degradation.

Cardiovascular Research Advance Access published August 22, 2016

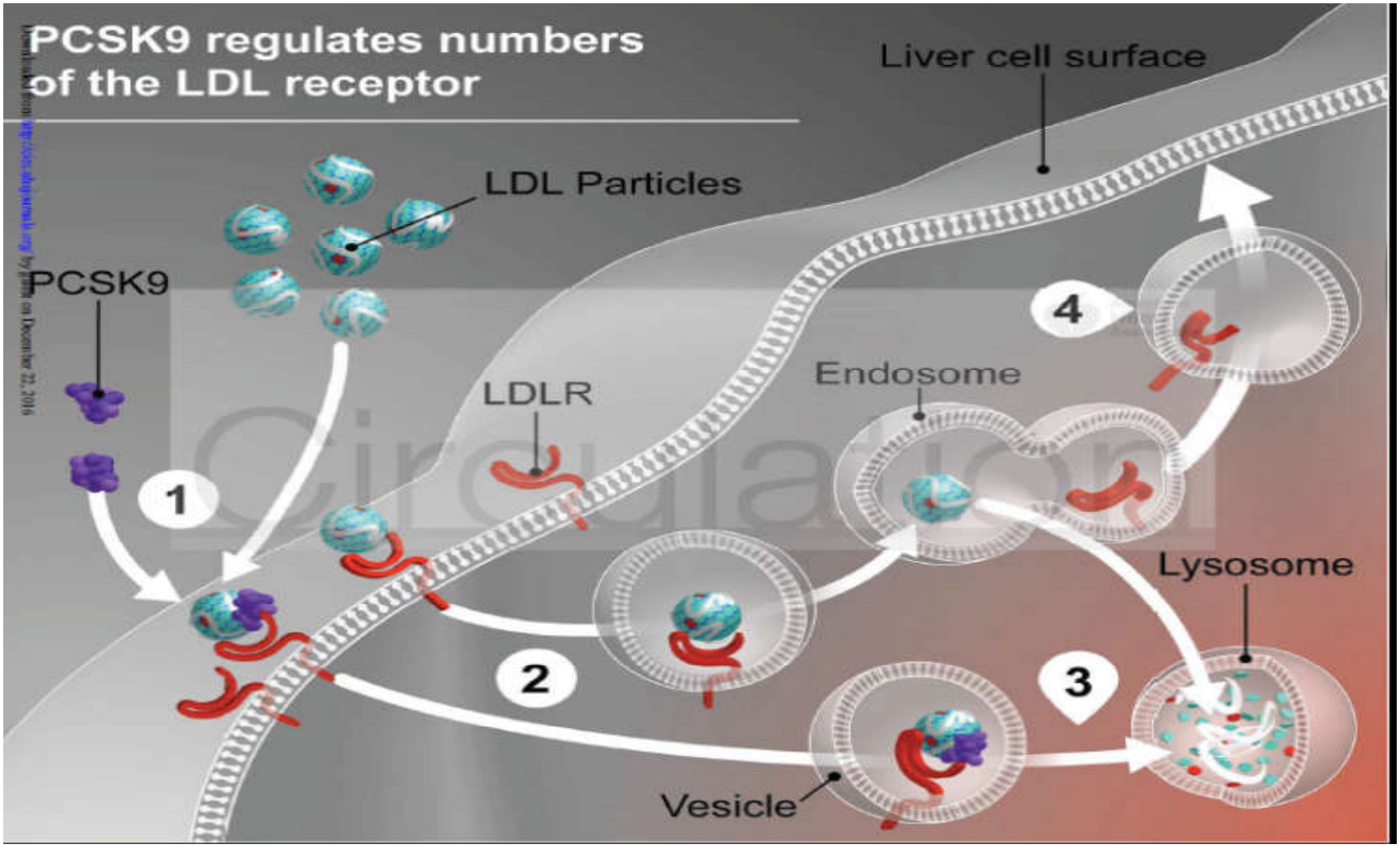
Circulation

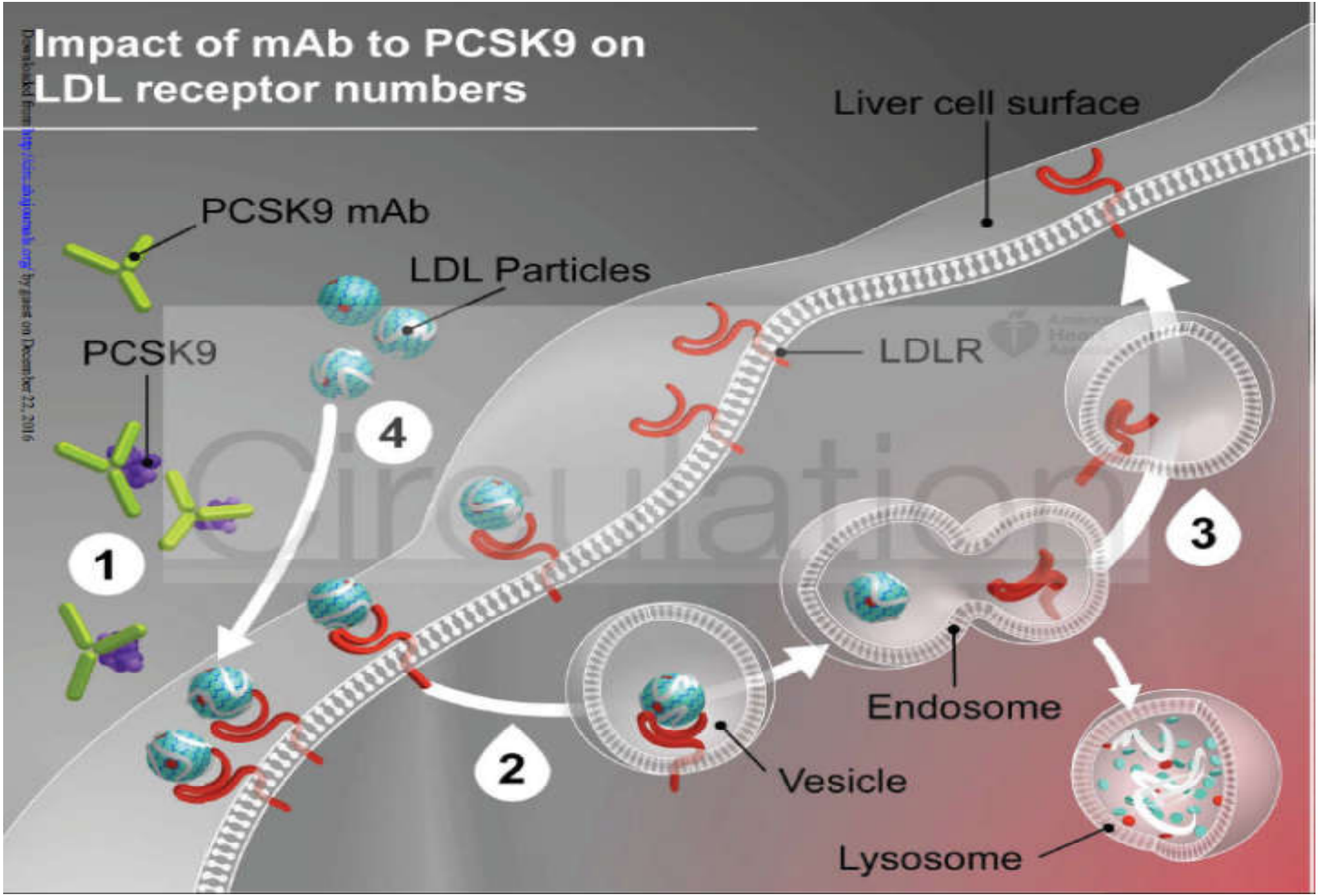
Effects of PCSK9 Inhibition with Alirocumab on Lipoprotein Metabolism in Healthy Humans

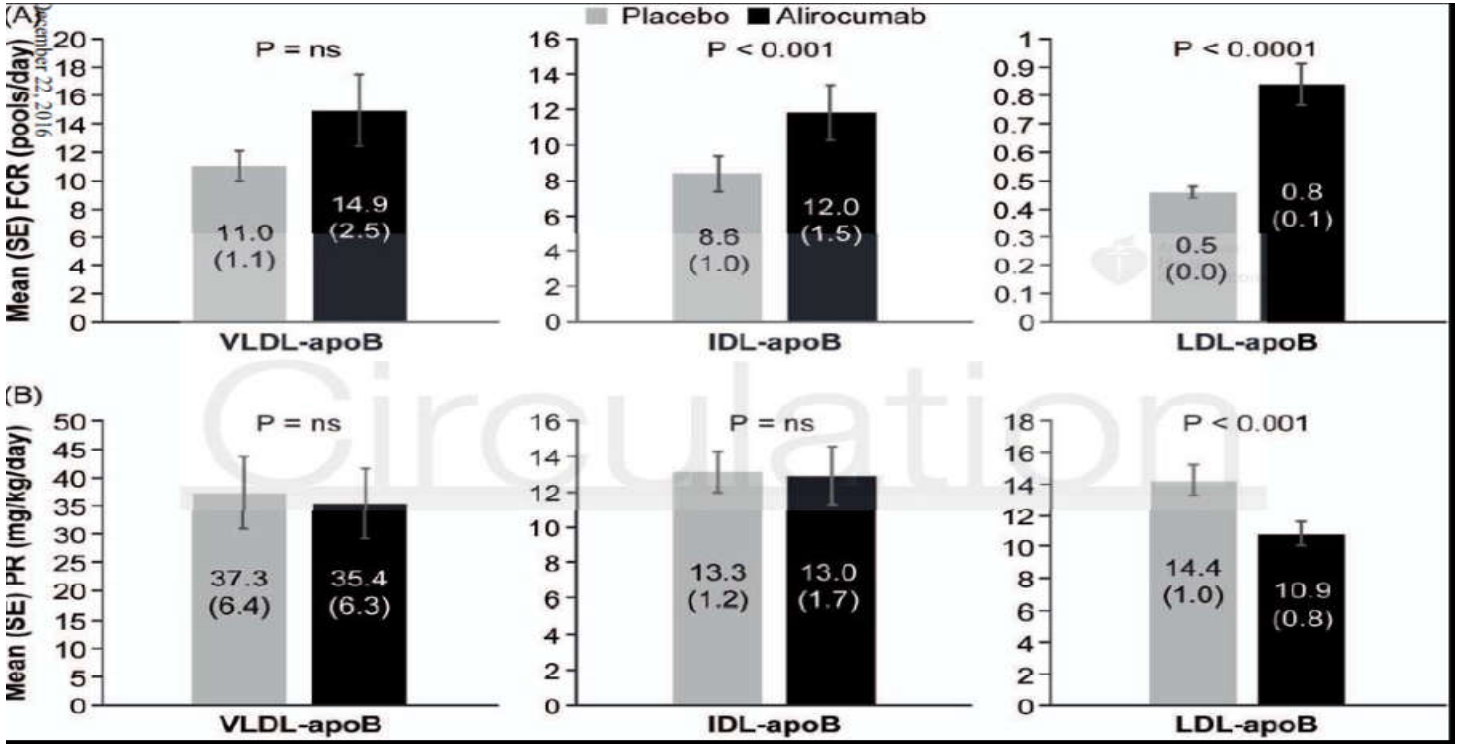
Gissette Reyes-Soffer, Marianna Pavlyha, Colleen Ngai, Tiffany Thomas, Stephen Holleran, Rajasekhar Ramakrishnan, Wahida Karmally, Renu Nandakumar, Nelson Fontanez, Joseph C. Obunike, Santica M. Marcovina, Alice H. Lichtenstein, Nirupa R. Matthan, James Matta, Magali Maroccia, Frederic Becue, Franck Poitiers, Brian Swanson, Lisa Cowan, William J. Sasiela, Howard K. Surks and Henry N. Ginsberg

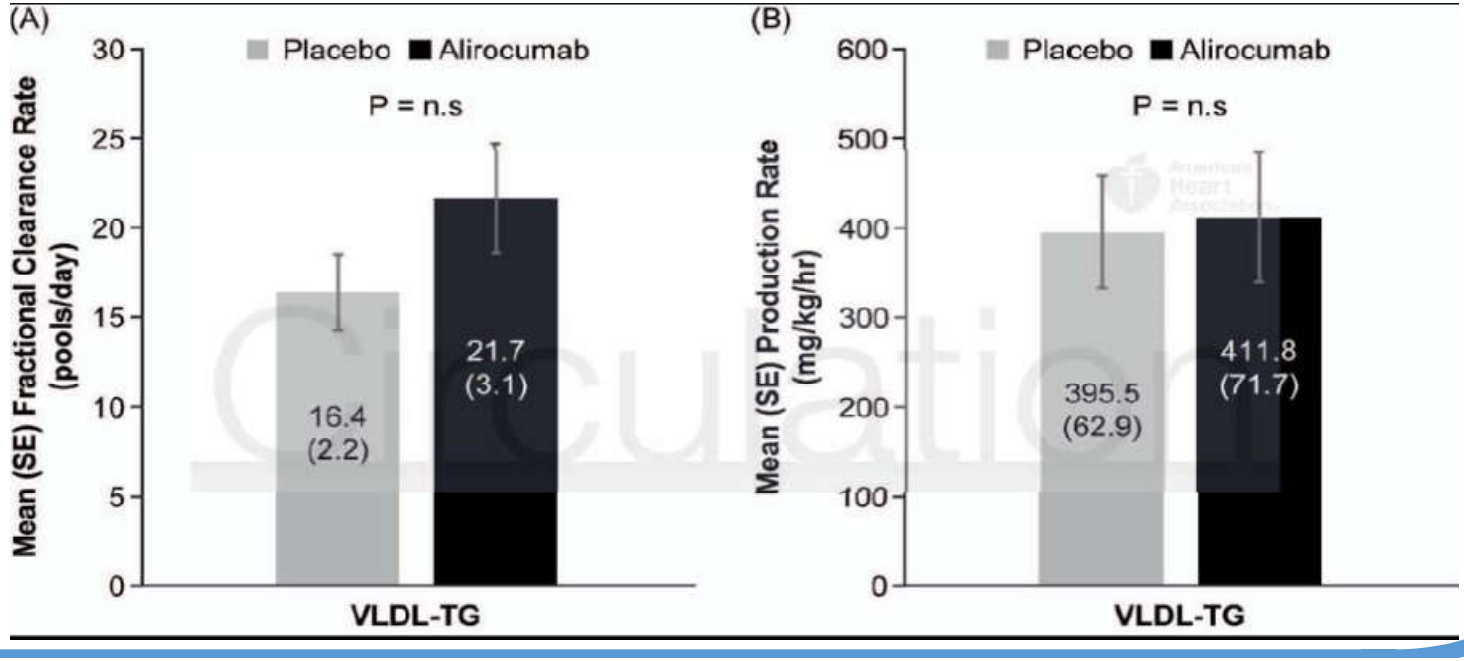
Circulation. published online December 16, 2016;
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

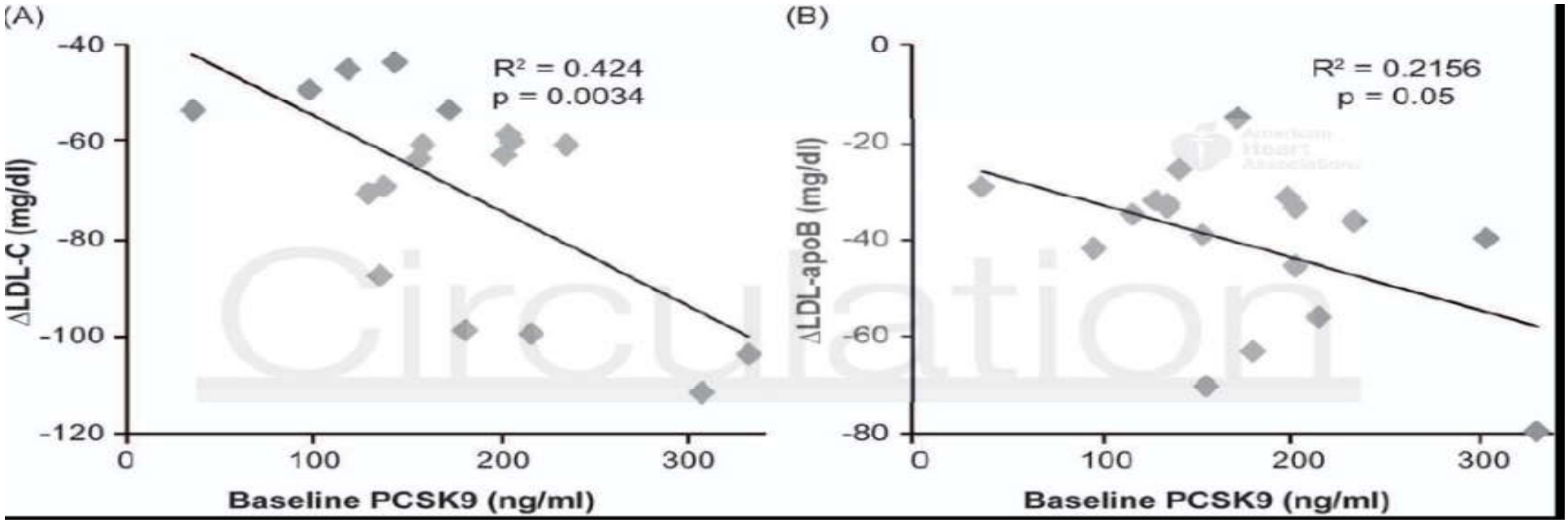
Print ISSN: 0009-7322. Online ISSN: 1524-4539

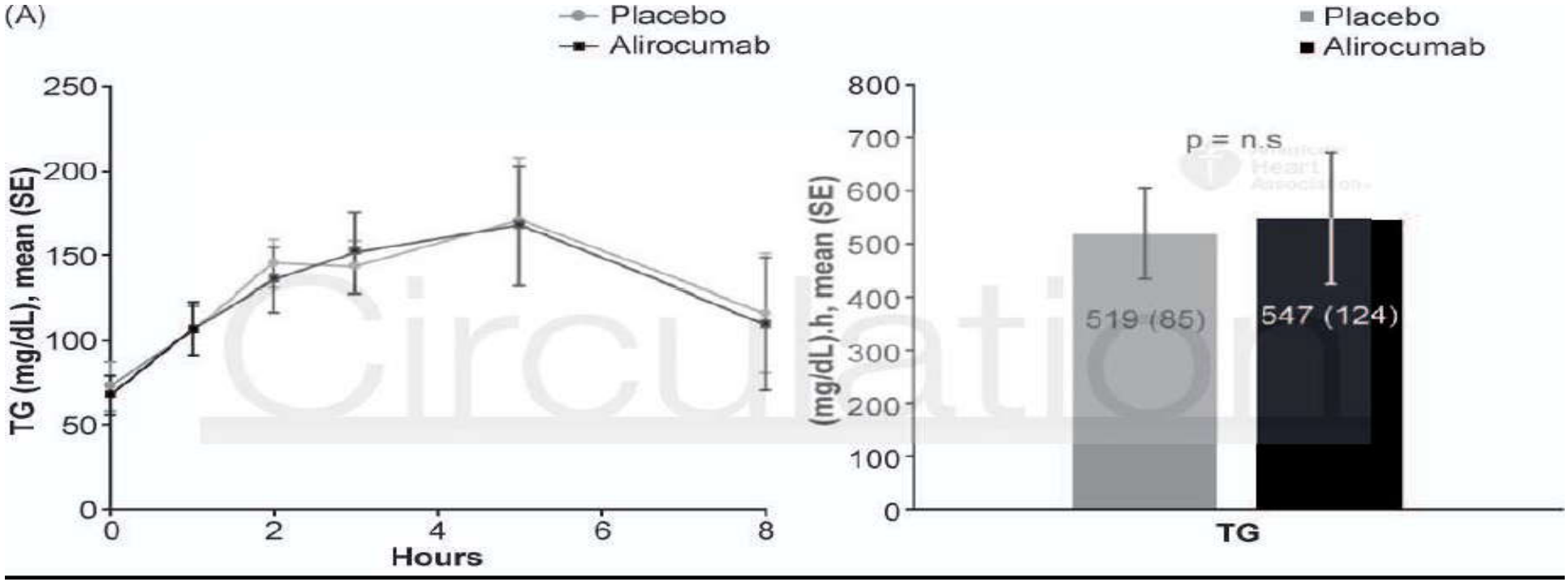

PCSK9 regulates numbers of the LDL receptor
Liver cell surface
LDL Particles
PCSK9
LDLR
Endosome
Lysosome
Vesicle
1
2
3
4

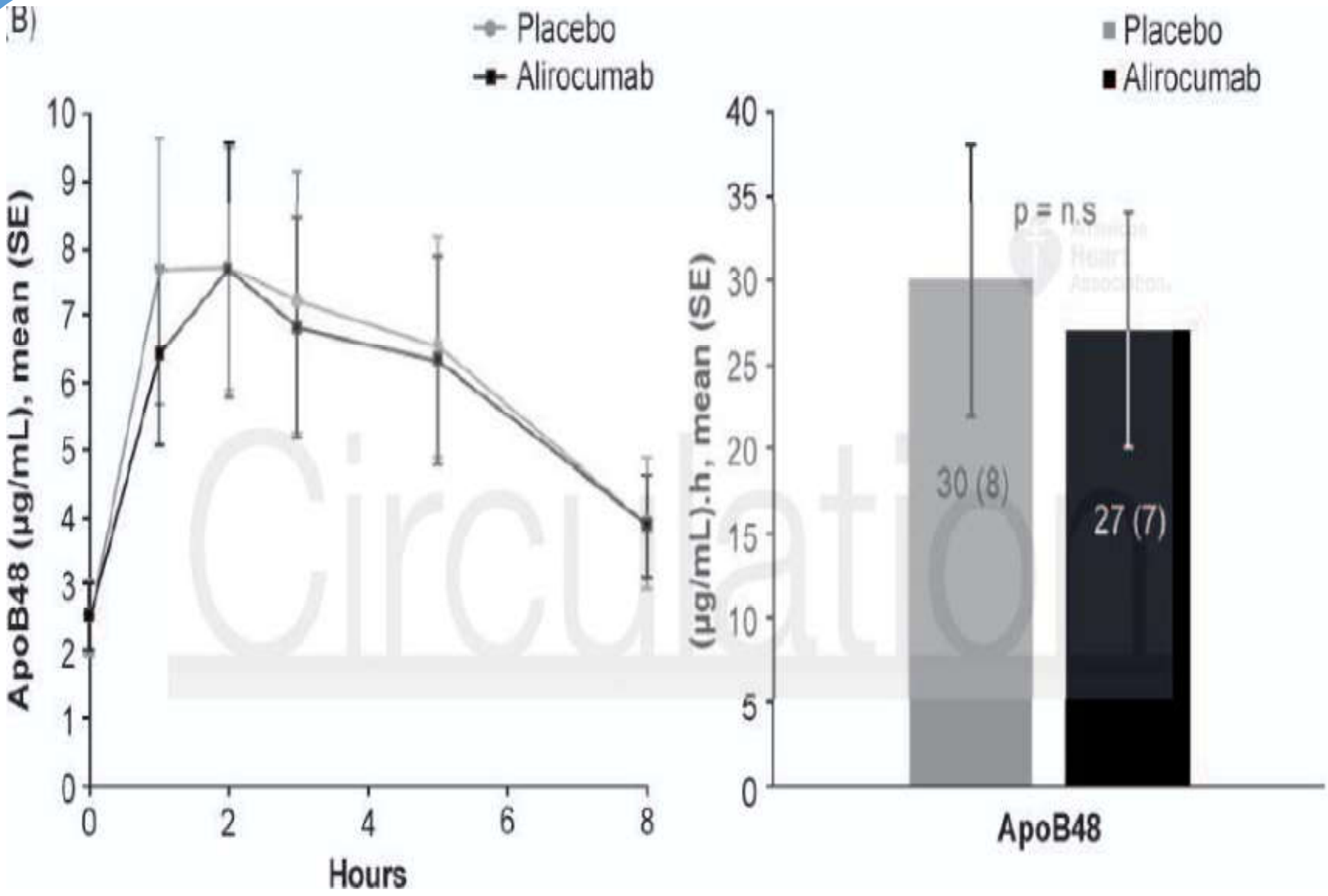

Impact of mAb to PCSK9 on LDL receptor numbers
Liver cell surface
PCSK9 mAb
LDL Particles
PCSK9
LDLR
1
2
3
4
Endosome
Vesicle
Lysosome


(A)
Placebo
Alirocumab
Mean (SE) FCR (pools/day)
P = ns
11.0 (1.1)
14.9 (2.5)
VLDL-apoB
P < 0.001
8.6 (1.0)
12.0 (1.5)
IDL-apoB
P < 0.0001
0.5 (0.0)
0.8 (0.1)
LDL-apoB
(B)
Mean (SE) PR (mg/kg/day)
P = ns
37.3 (6.4)
35.4 (6.3)
VLDL-apoB
P = ns
13.3 (1.2)
13.0 (1.7)
IDL-apoB
P < 0.001
14.4 (1.0)
10.9 (0.8)
LDL-apoB


(A)
Placebo
Alirocumab
Mean (SE) Fractional Clearance Rate (pools/day)
P = n.s
16.4 (2.2)
21.7 (3.1)
VLDL-TG
(B)
Placebo
Alirocumab
Mean (SE) Production Rate (mg/kg/hr)
P = n.s
395.5 (62.9)
411.8 (71.7)
VLDL-TG


(A)
-40
-60
-80
-100
-120
ΔLDL-C (mg/dl)
R² = 0.424
p = 0.0034
0
100
200
300
Baseline PCSK9 (ng/ml)
(B)
0
-20
-40
-60
-80
ΔLDL-apoB (mg/dl)
R² = 0.2156
p = 0.05
0
100
200
300
Baseline PCSK9 (ng/ml)


(A)
Placebo
Alirocumab
250
200
150
100
50
0
TG (mg/dL), mean (SE)
0
2
4
6
8
Hours
Placebo
Alirocumab
800
700
600
500
400
300
200
100
0
(mg/dL).h, mean (SE)
p = n.s
519 (85)
547 (124)
TG


(B)
Placebo
Alirocumab
ApoB48 (µg/mL), mean (SE)
Hours
0
2
4
6
8
0
1
2
3
4
5
6
7
8
9
10
Placebo
Alirocumab
(µg/mL).h, mean (SE)
p = n.s
30 (8)
27 (7)
ApoB48
0
5
10
15
20
25
30
35
40

Circulation

American Heart Association®

Effects of PCSK9 Inhibition with Alirocumab on Lipoprotein Metabolism in Healthy Humans

Gissette Reyes-Soffer, Marianna Pavlyha, Colleen Ngai, Tiffany Thomas, Stephen Holleran, Rajasekhar Ramakrishnan, Wahida Karmally, Renu Nandakumar, Nelson Fontanez, Joseph C. Obunike, Santica M. Marcovina, Alice H. Lichtenstein, Nirupa R. Matthan, James Matta, Magali Maroccia, Frederic Becue, Franck Poitiers, Brian Swanson, Lisa Cowan, William J. Sasiela, Howard K. Surks and Henry N. Ginsberg

Conclusions—Alirocumab decreased LDL-C and LDL-apoB by increasing IDL- and LDL-apoB FCRs, and decreasing LDL-apoB PR. These results are consistent with increases in LDLRs available to clear IDL and LDL from blood during PCSK9 inhibition. The possible increase in apo(a) FCR during alirocumab treatment suggests that increased LDLRs may also play a role in the reduction of plasma Lp(a).

Circulation. published online December 16, 2016;

Circulation

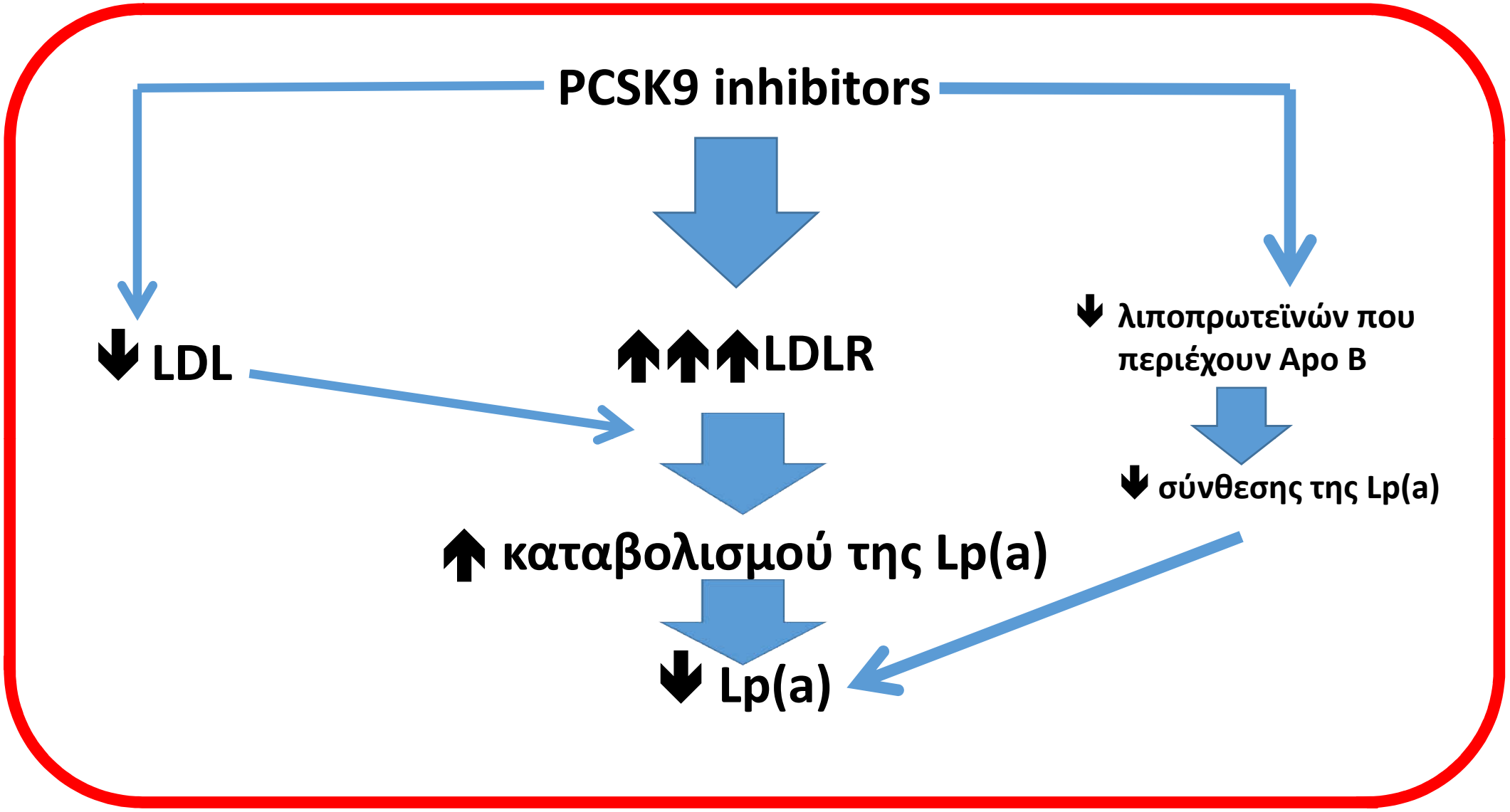
American Heart Association®

Factorial Effects of Evolocumab and Atorvastatin on Lipoprotein Metabolism

Gerald F. Watts, Dick C. Chan, Ricardo E. Dent, Ransi Somaratne, Scott M. Wasserman, Rob Scott, Sally Burrows and Peter Hugh R. Barrett

Conclusions—In healthy, normolipidemic subjects, evolocumab decreased the concentration of atherogenic lipoproteins, particularly LDL, by accelerating their catabolism. Reductions in IDL and LDL production also contributed to the decrease in LDL particle concentration with evolocumab by a mechanism distinct from that of atorvastatin. These kinetic findings provide a metabolic basis for understanding the potential benefits of PCSK9 mAbs incremental to statins in on-going clinical endpoint trials.

Circulation. published online December 9, 2016


PCSK9 inhibitors
↓ LDL
↑↑↑LDLR
↓ λιποπρωτεϊνών που περιέχουν Apo B
↓ σύνθεσης της Lp(a)
↑ καταβολισμού της Lp(a)
↓ Lp(a)

V- PCSK9 AND FAT ACCUMULATION

PCSK9 ΚΑΙ ΣΥΣΣΩΡΕΥΣΗ ΛΙΠΟΥΣ

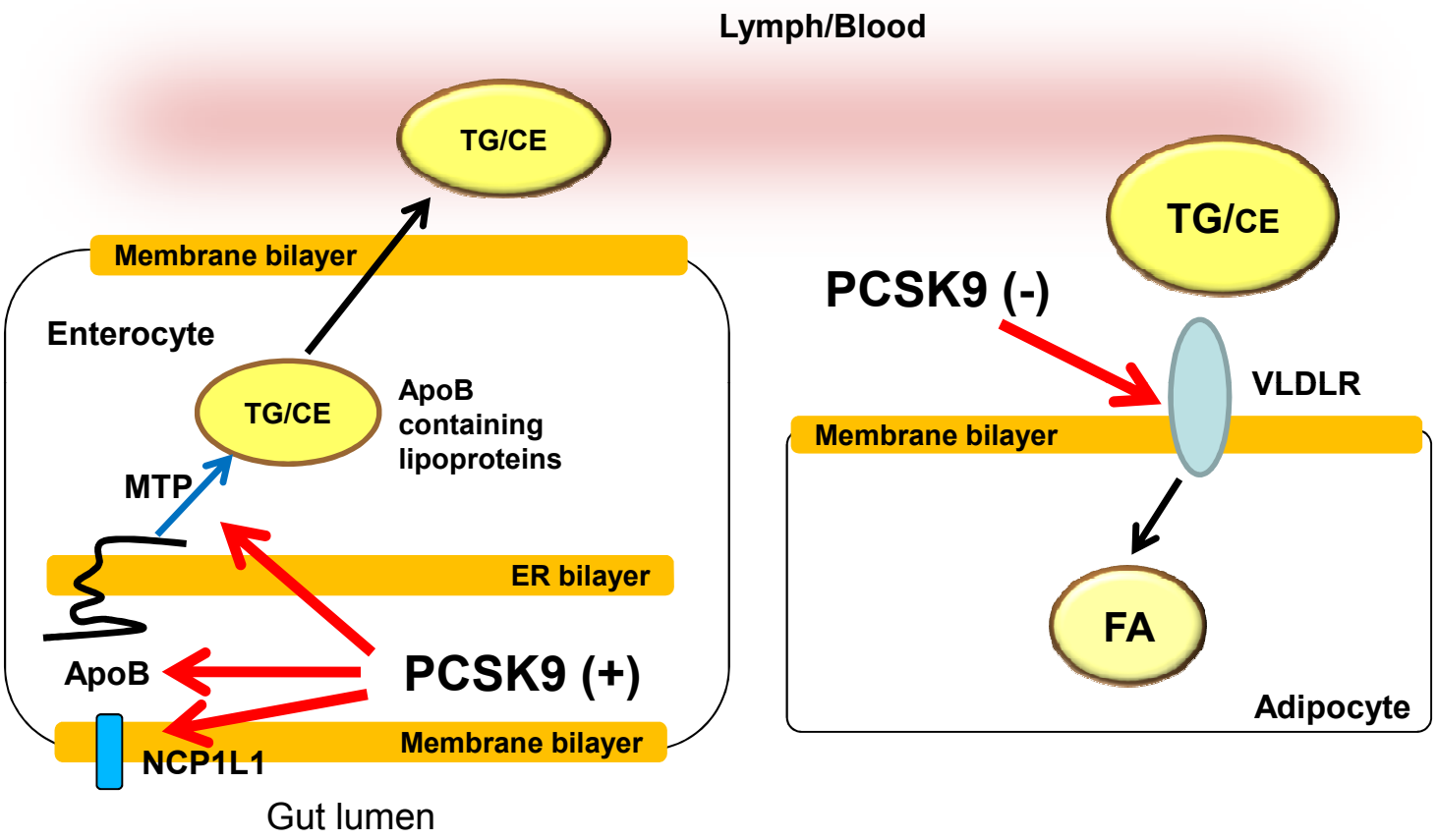
PCSK9

↓ VLDL R expression → **↓ συσσώρευσης TG στα ηπατοκύτταρα + στα κύτταρα του λιπώδους ιστού**

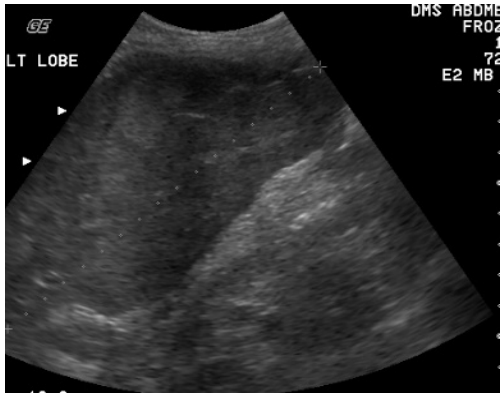
↓ ApoER2 →

PCSK9 inhibitors → ↑ συσσώρευσης λίπους;

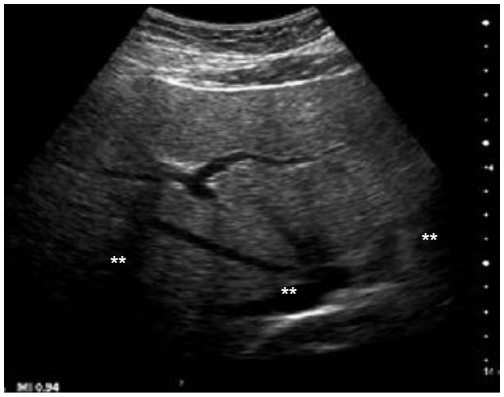
PCSK9 and TGs



PCSK9 and hepatic steatosis

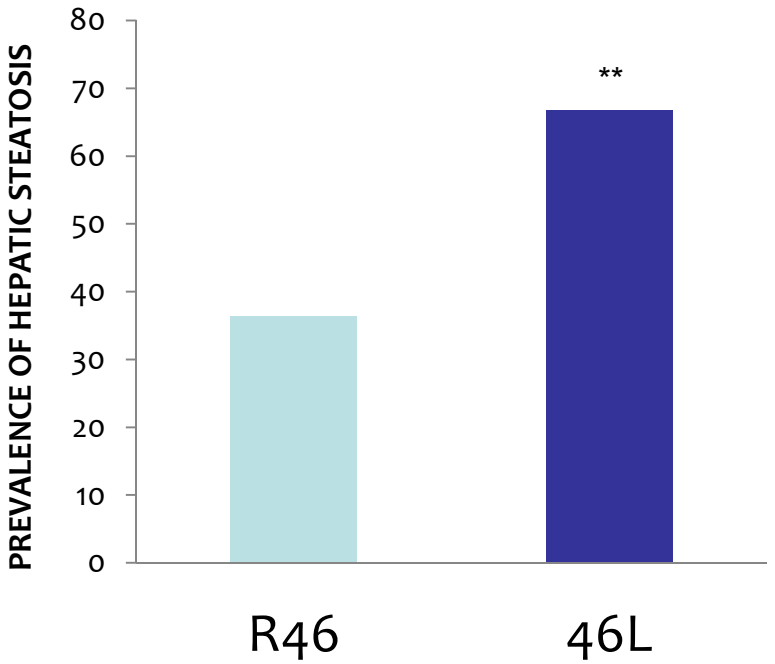


46R



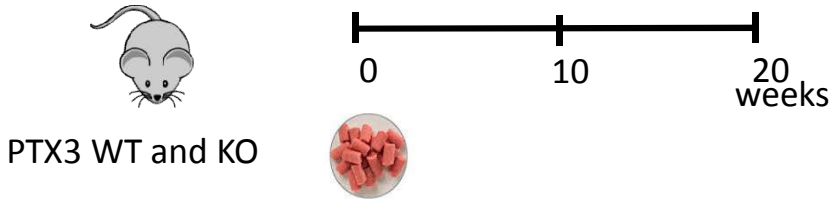
LIVER

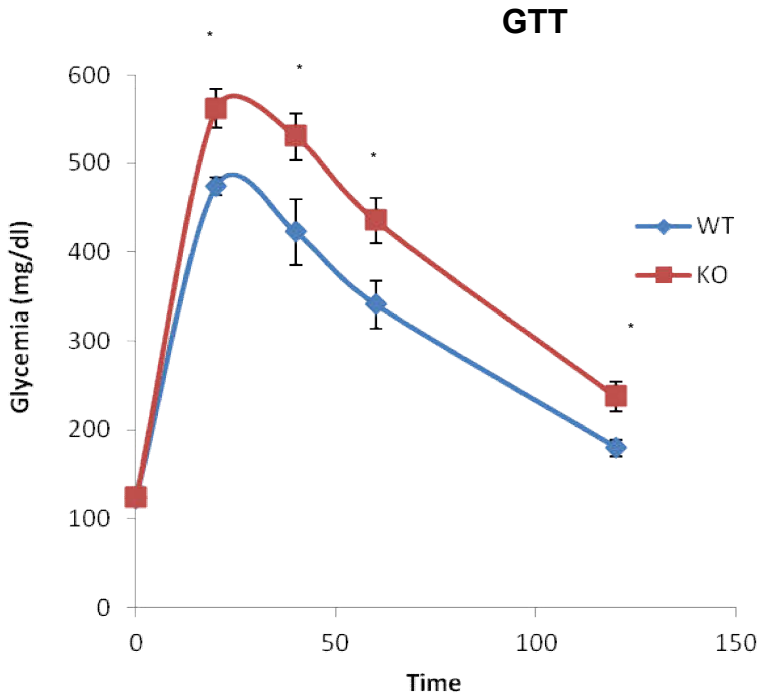
R46L → LOF mutation

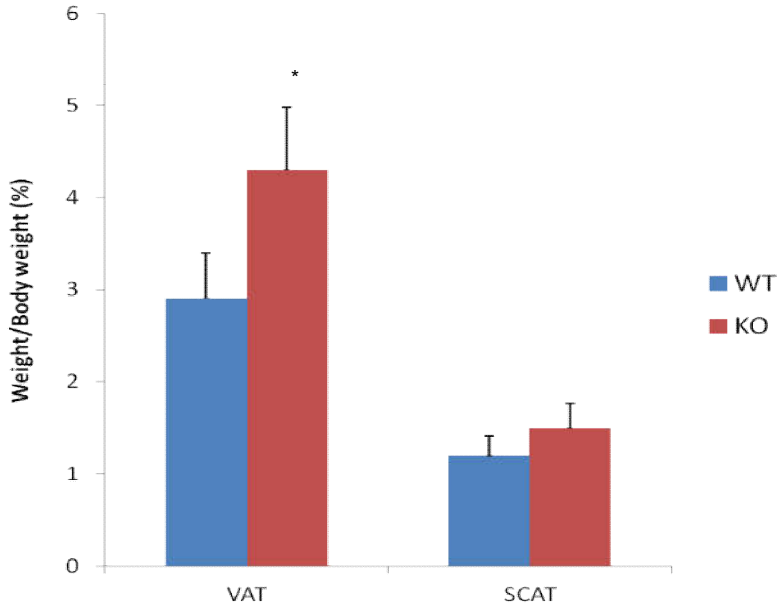


**Logistic Regression Model, adjusted for all variables

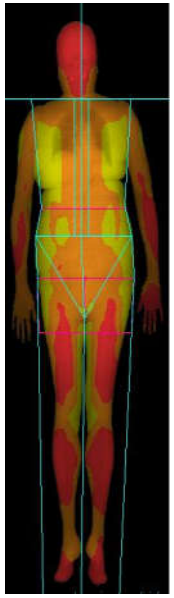
PCSK9 and metabolic dysfunction





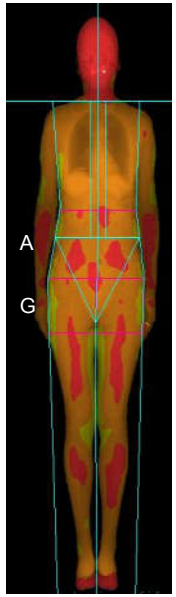


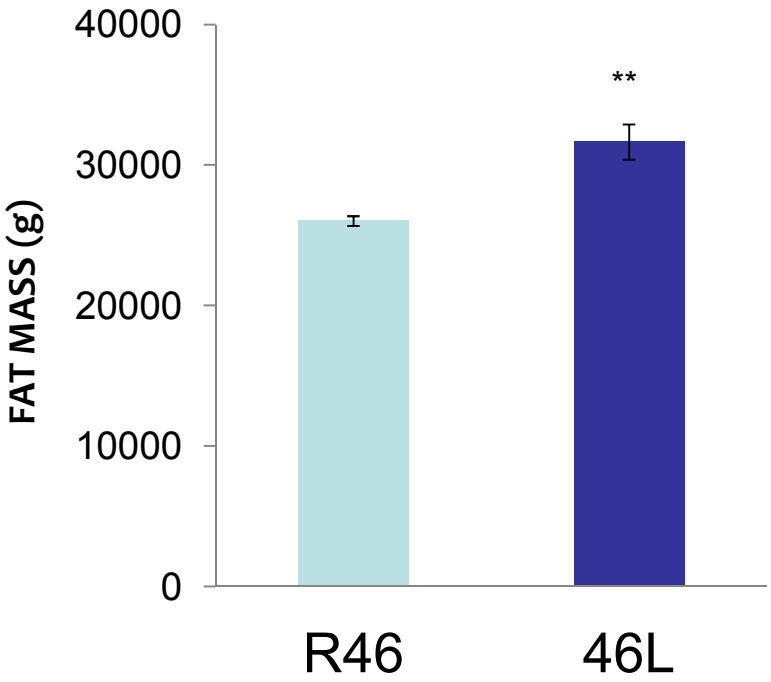
PCSK9, and body fat mass



Female 65 years
BMI: 24.3 Kg/m²
Android fat: 61.1%
Gynoid fat: 47.4 %
A/G_r : 1.29

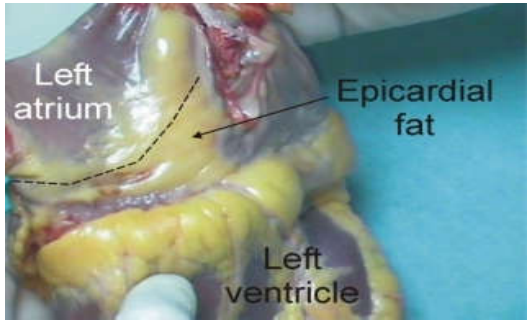
Female 66 years
BMI: 23.7 Kg/m²
Android fat: 43.2%
Gynoid fat: 44.6 %
A/G_r : 0.97

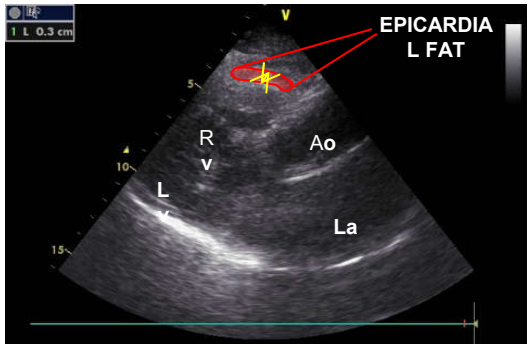


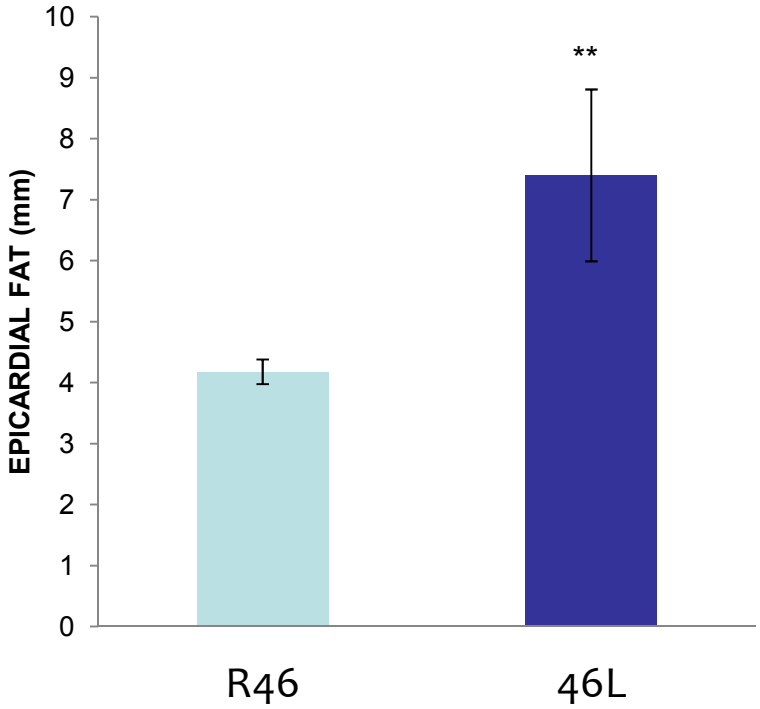


*Univariate analysis- T-test.
**Logistic Regression Model. PCSK9 R46L genotype as dependent variable. Including as covariates: age, gender, BMI, waist, systolic blood pressure, lipid profile, glucose levels, therapies.

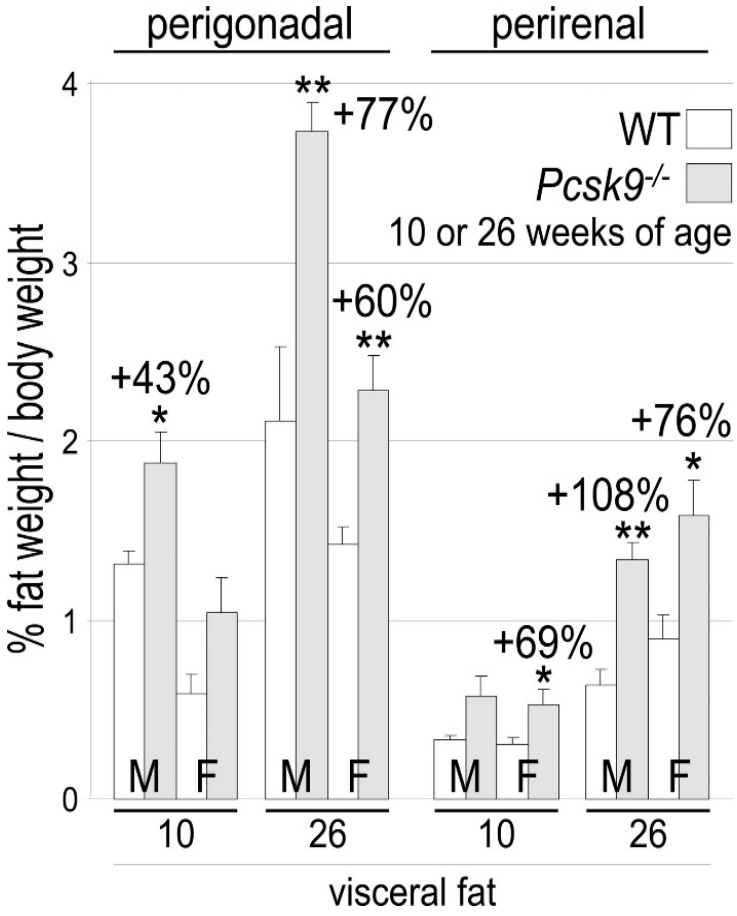
PCSK9 and epicardial fat

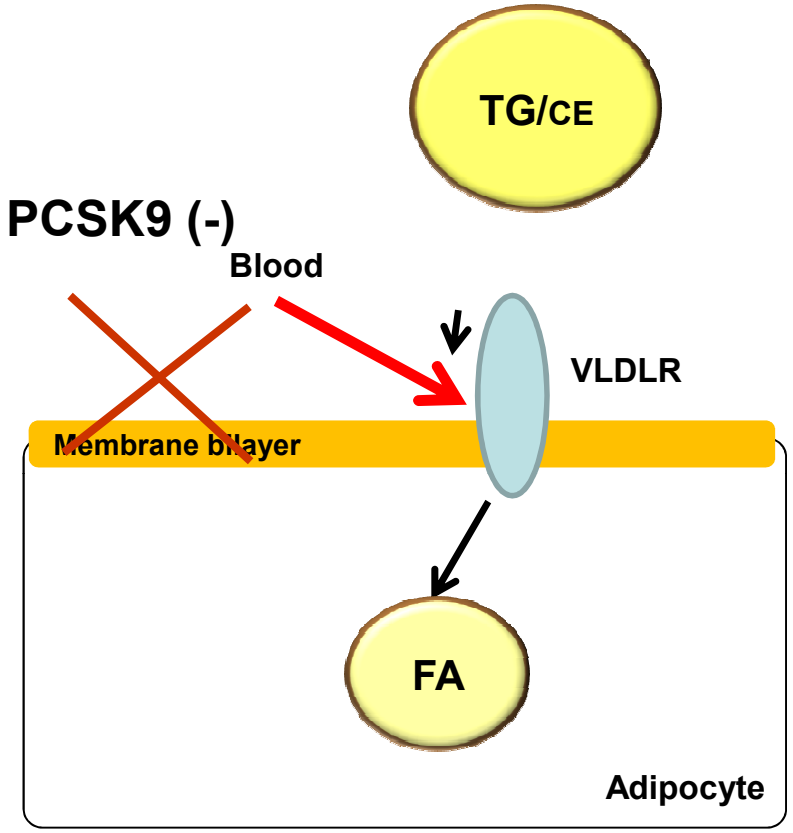






PCSK9 and TG accumulation





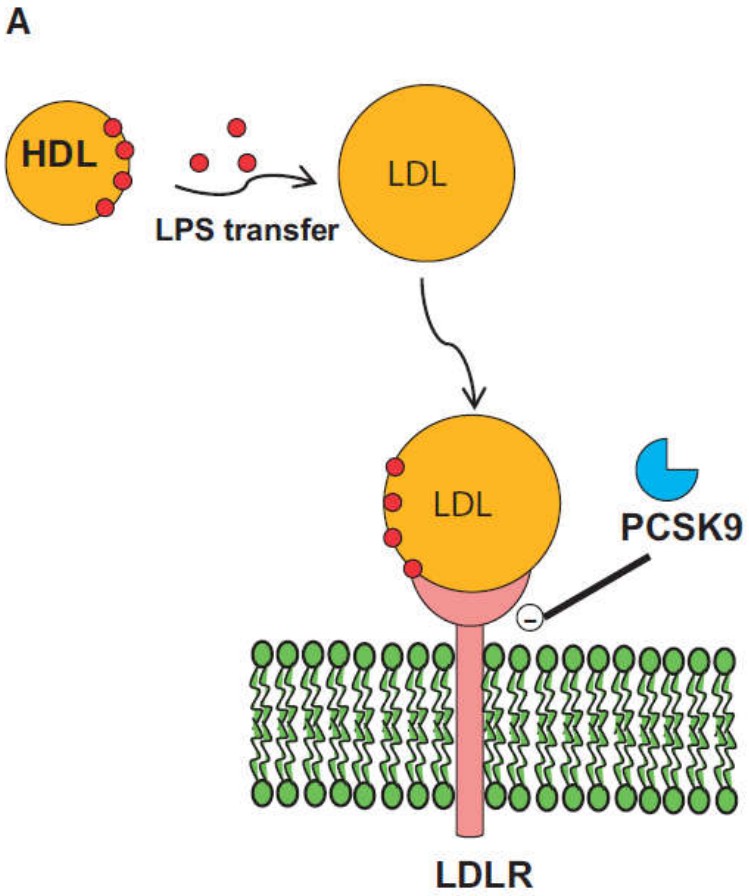
Roubtsova A et al. ATVB 2011;31:785-791

Η PCSK9 περιορίζει τη συσσώρευση λίπους (διαμέσου των VLDLR)

PCSK9, TG and obesity

- PCSK9 promotes intestinal production of apoB containing lipoproteins
- PCSK9 deficency is associated with reduced postprandial trygliceridemia
- PCSK9 and ApoCIII levels are associated with increased post prandial response and reduced apoB48 catabolism
- R46L LOF mutation is associated with reduced plasma cholesterol levels but increased lipids deposition in liver, body mass fat and epicardial adipose tissue.

VI- PCSK9 AND INFECTION



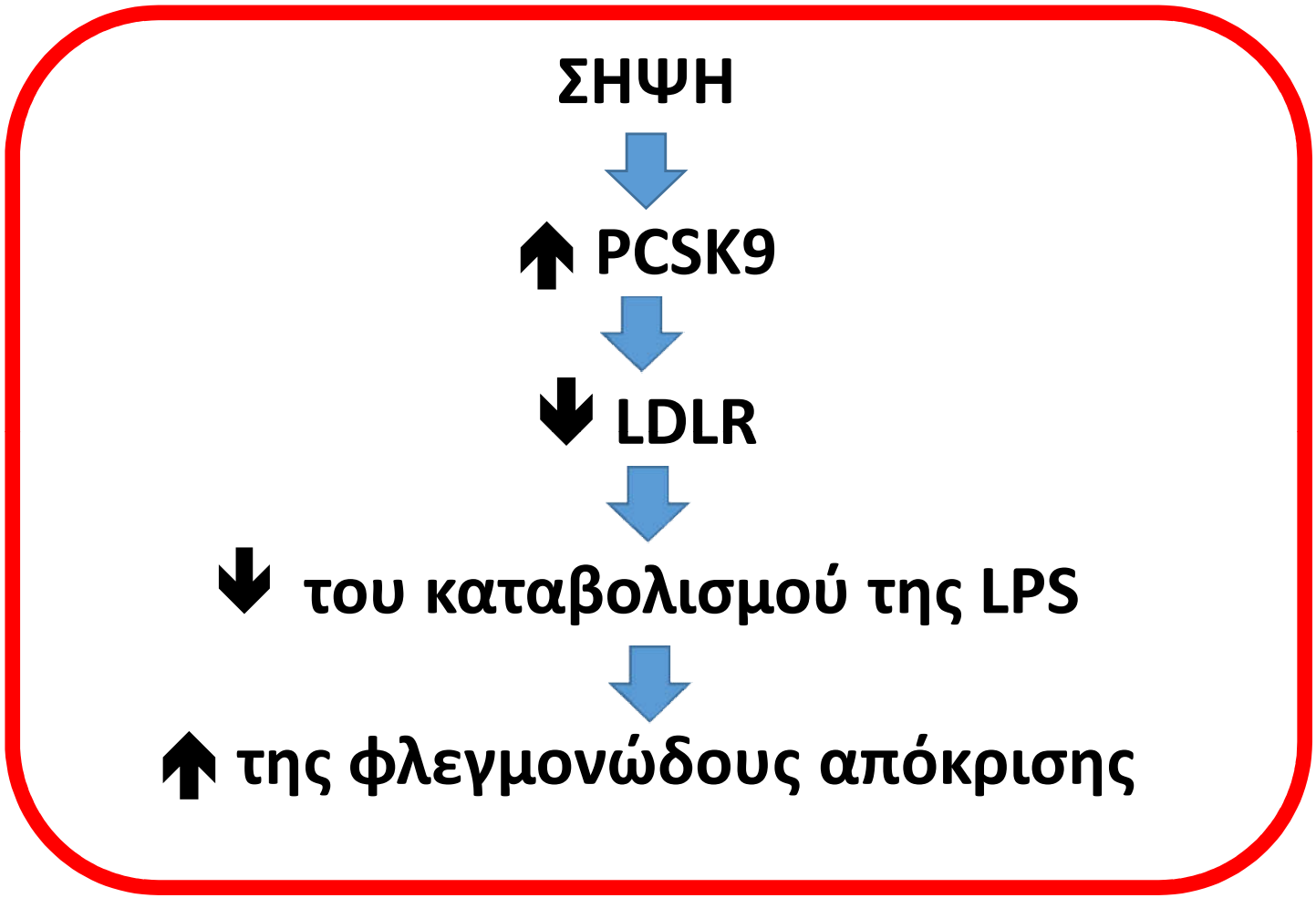
Cardiovascular Research Advance Access published August 22, 2016

Figure 5 PCSK9 and infection. (*A*) PCSK9 reduces LDL uptake thus reducing LPS clearance and increasing inflammatory response during sepsis. (*B*) PCSK9 reduces LDLR thus resulting in reduced LDL-associated HCV uptake and decreased viral infection.

Ο ΡΟΛΟΣ ΤΗΣ PCSK9 ΣΤΗ ΦΛΕΓΜΟΝΗ

- Τα επίπεδα της PCSK9 συσχετίζονται με τον αριθμό των λευκών αιμοσφαιρίων σε ασθενείς με σταθερή στεφανιαία νόσο
- Η έλλειψη της PCSK9 προστατεύει από το σηπτικό shock που οφείλεται στη χορήγηση LPS

PCSK9 ΚΑΙ ΣΗΨΗ



Τα επίπεδα της PCSK9 στο πλάσμα συσχετίζονται με την εμφάνιση πολυοργανικής ανεπάρκειας σε σηπτικούς ασθενείς

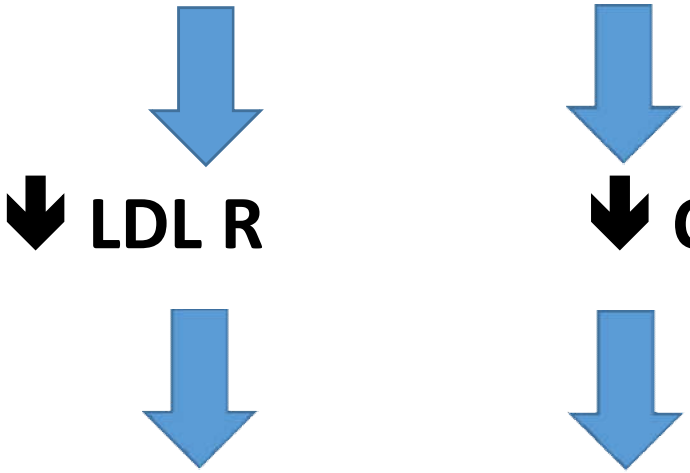
J Innate Immunol 2016;8: 211

PCSK9 loss of function mutations → **↑ επιβίωσης**

PCSK9 gain of function mutations → **↓ επιβίωσης**

Sci Transl Med 2014;6: 258

PCSK9 ΚΑΙ ΛΟΙΜΩΞΗ ΑΠΟ ΤΟΝ ΙΟ ΤΗΣ ΗΠΑΤΙΤΙΔΑΣ C

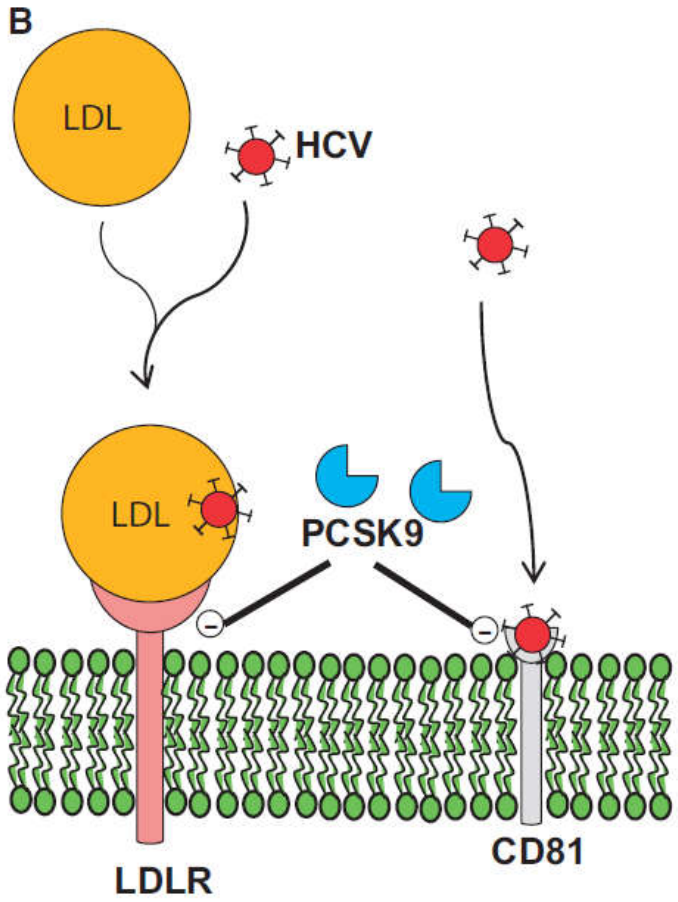


↓ LDL R

↓ CD 81

↓ της εισόδου του HCV στα ηπατοκύτταρα

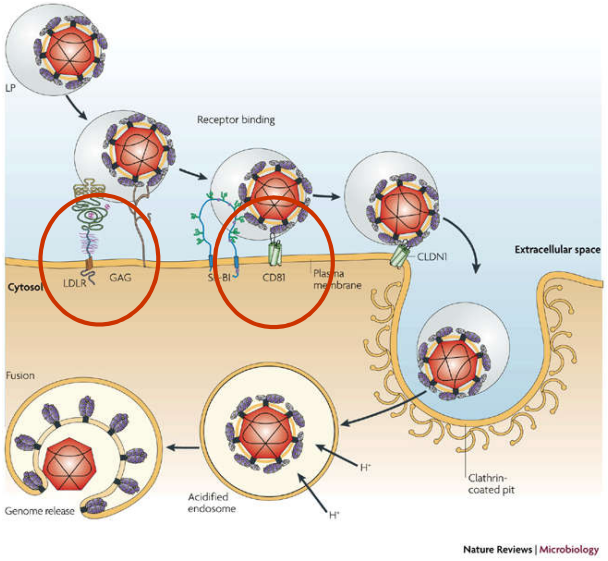
J BIOL CHEM 2015;290: 23385



Cardiovascular Research Advance Access published August 22, 2016

Figure 5 PCSK9 and infection. (*A*) PCSK9 reduces LDL uptake thus reducing LPS clearance and increasing inflammatory response during sepsis. (*B*) PCSK9 reduces LDLR thus resulting in reduced LDL-associated HCV uptake and decreased viral infection.

PCSK9 open questions: immune system



PCSK9 Impedes Hepatitis C Virus Infection *In Vitro* and Modulates Liver CD81 Expression

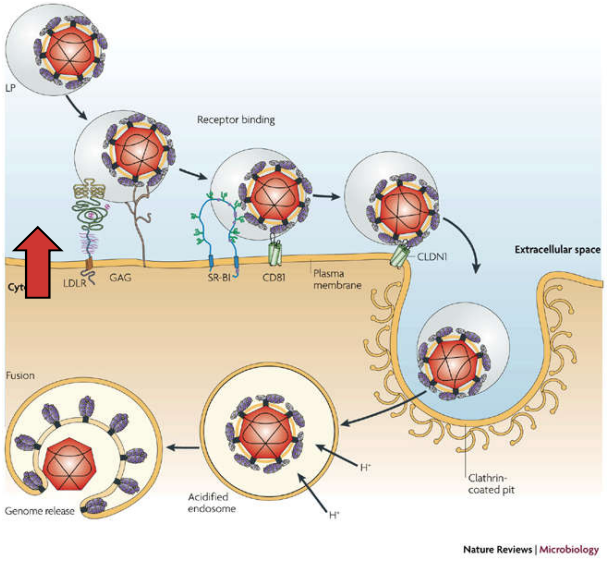
(HEPATOLOGY 2009;50:17-24.)

Plasma Membrane Tetraspanin CD81 Complexes with Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) and Low Density Lipoprotein Receptor (LDLR), and Its Levels Are Reduced by PCSK9*

Received for publication, February 3, 2015, and in revised form, July 2, 2015 Published, JBC Papers in Press, July 20, 2015, DOI 10.1074/jbc.M115.642991

Quoc-Tuan Le[‡§], Matthieu Blanchet[‡], Nabil G. Seidah[¶], and Patrick Labonté[‡1]

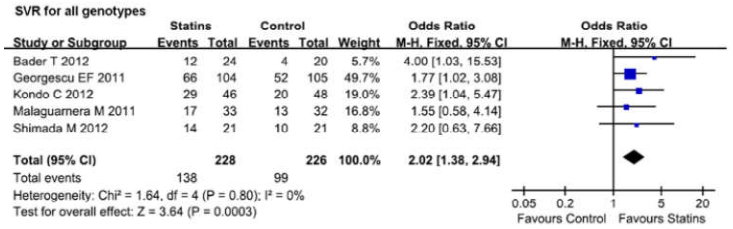
PCSK9 open questions: immune system



LDL-R → Hepatitis C infection

Atorvastatin Does Not Exhibit Antiviral Activity Against HCV at Conventional Doses: A Pilot Clinical Trial

(HEPATOLOGY 2007;45:895-898.)



VII- PCSK9 AND VASCULAR TISSUES

PCSK9 ΚΑΙ ΑΓΓΕΙΑ

Η PCSK9 εκφράζεται στα κύτταρα του αγγειακού τοιχώματος (ενδοθηλιακά κύτταρα, κύτταρα των λείων μυϊκών ινών, μακροφάγα)

PCSK9 ΚΑΙ ΦΛΕΓΜΟΝΗ

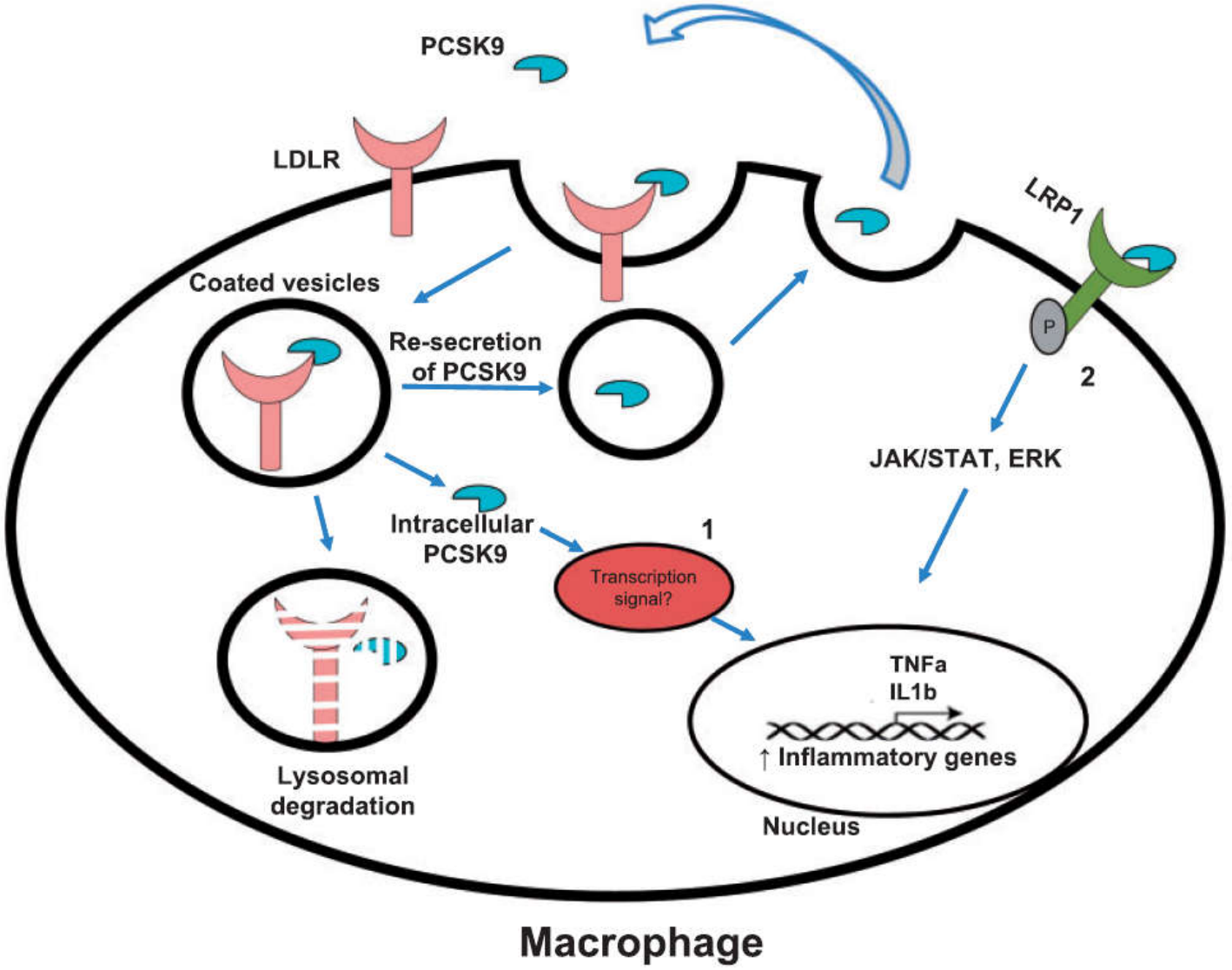


Figure 4 Pathways involved in PCSK9-mediated inflammation. Intracellular PCSK9, including the internalized one which escapes lysosomal degradation could eventually undergoes re-secretion but exerts cytoplasmic effects that might regulate the expression of genes controlling inflammation (1). PCSK9 could also target LRP-1 which is involved in the activation of JAK/STAT and ERK pathways (2).

Cardiovascular Research Advance Access published August 22, 2016

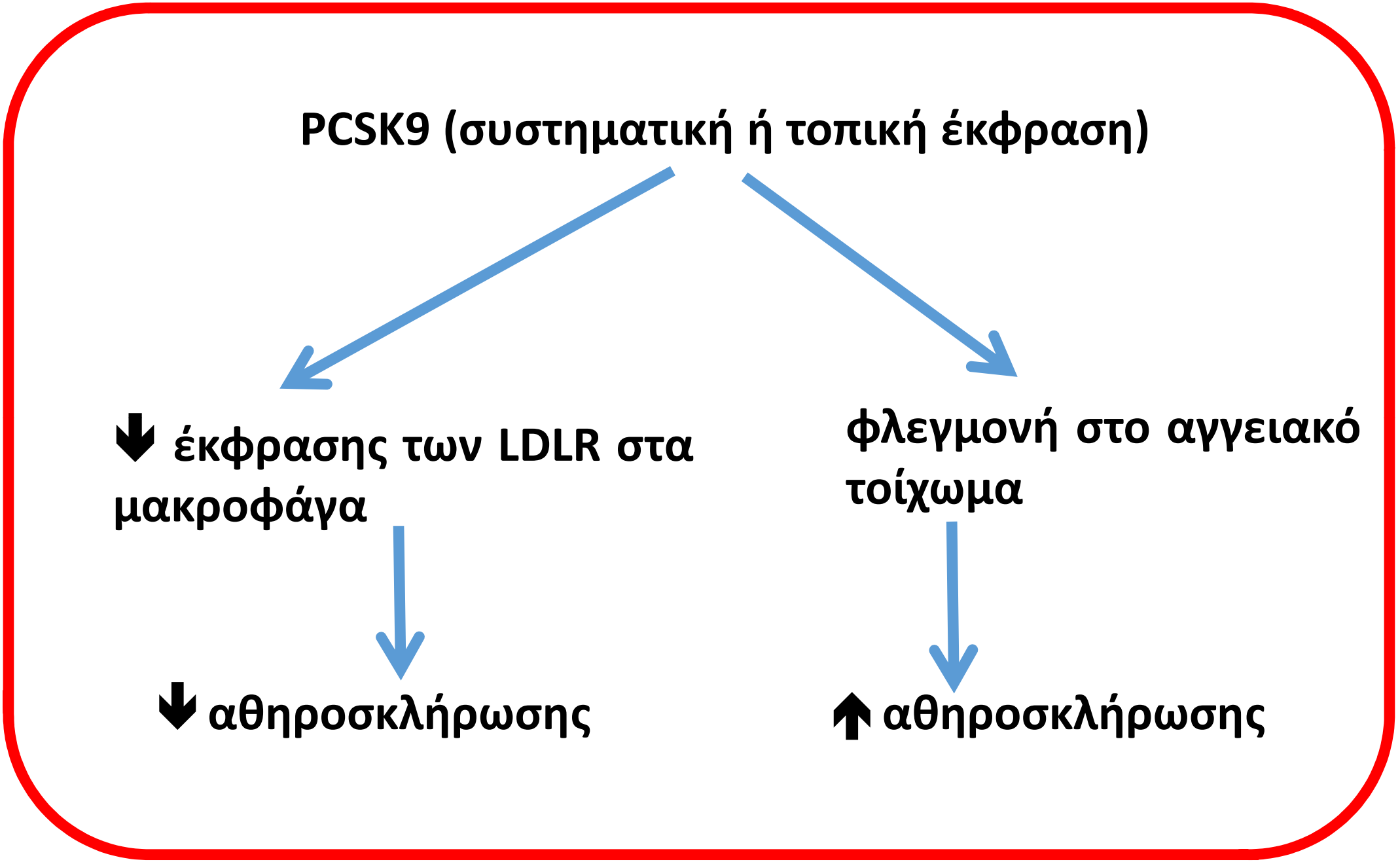
PCSK9 ΚΑΙ ΦΛΕΓΜΟΝΗ ΤΟΥ ΑΓΓΕΙΑΚΟΥ ΤΟΙΧΩΜΑΤΟΣ

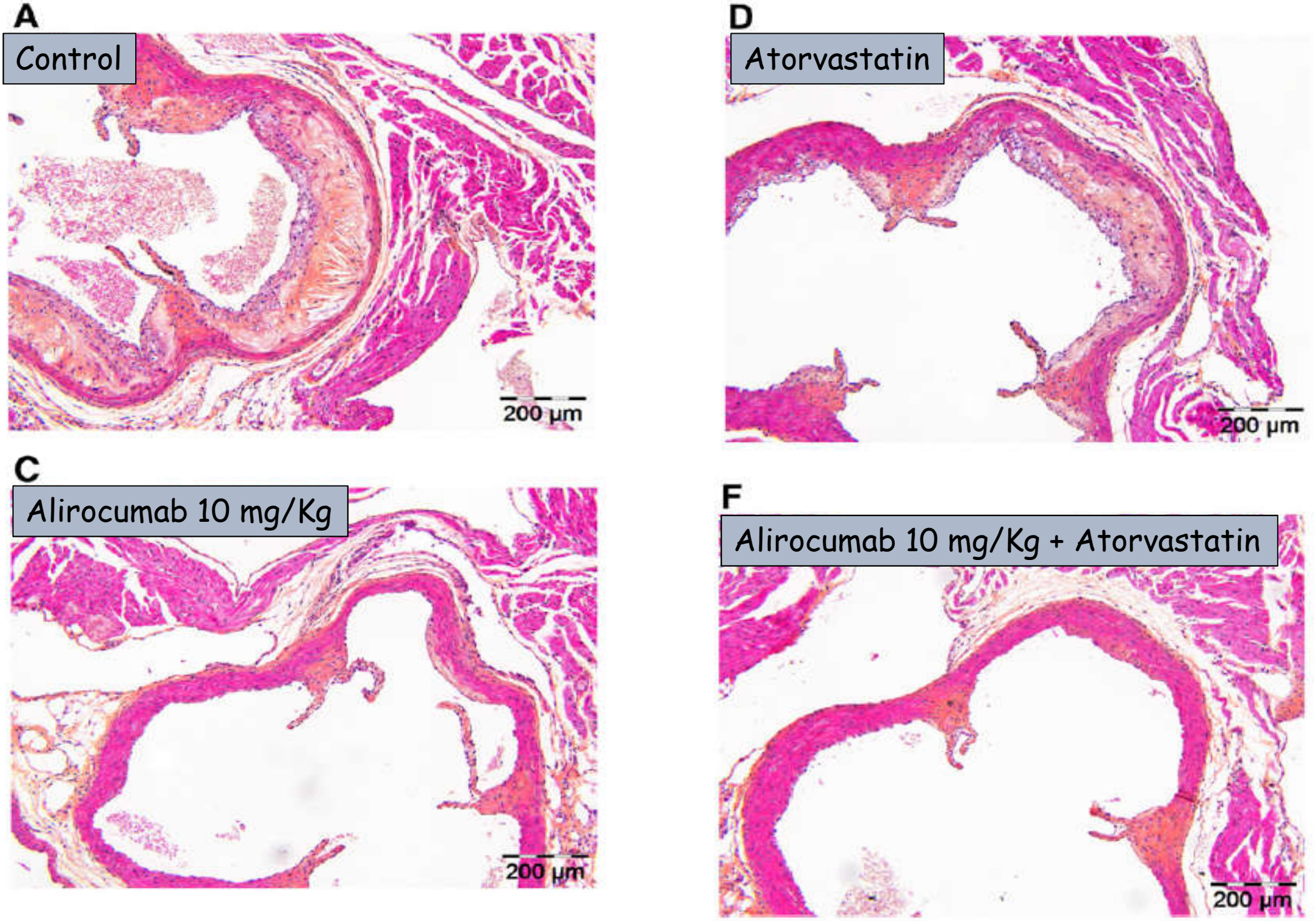
PCSK9 (στο αγγειακό τοίχωμα)

Επάγει τη διήθηση από ενεργοποιημένα μακροφάγα (χωρίς μεταβολή των λιπιδίων)

Φλεγμονή του αγγειακού τοιχώματος

J Pathol 2016;238: 52


PCSK9 (συστηματική ή τοπική έκφραση)
↓ έκφρασης των LDLR στα μακροφάγα
↓ αθηροσκλήρωσης
φλεγμονή στο αγγειακό τοίχωμα
↑ αθηροσκλήρωσης



J. Lipid Res. 2014. 55: 2103–2112

ALIROCUMAB

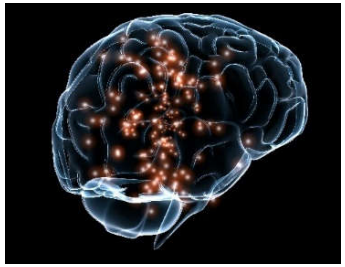
⬇ αθηρωματικών βλαβών και βελτίωση της μορφολογίας της αθηρωματικής πλάκας (⬇ μακροφάγων και του νεκρωτικού πυρήνα, ⬆ του κολλαγόνου και των λεμφοκυττάρων)

J LIPID RES 2014;55: 2013

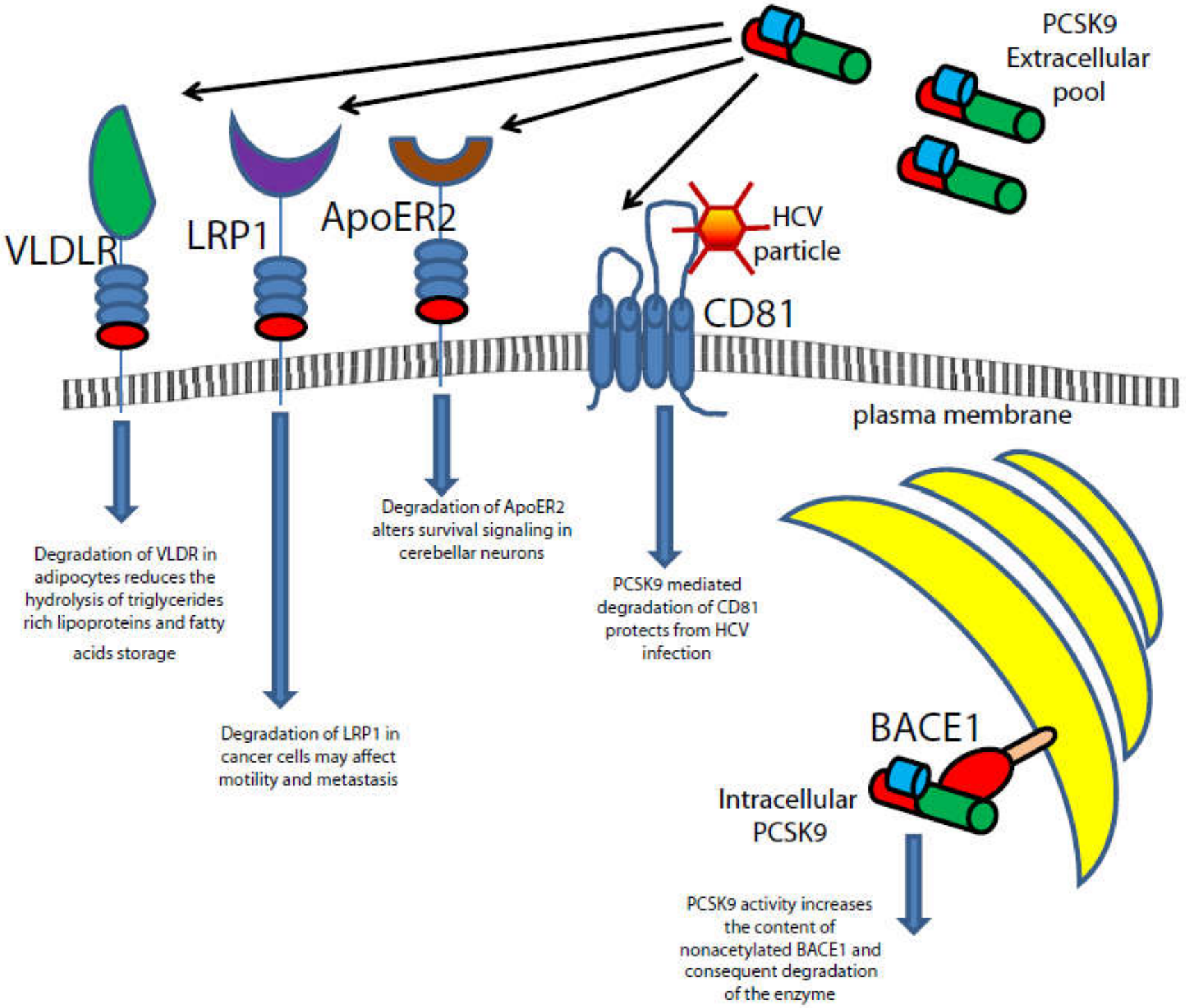
VIII- PCSK9 AND THE BRAIN

PCSK9 open questions: brain

- PCSK9 initially described as Neural Apoptosis-Regulated Convertase-1 or NARC-1
  (upregulated in primary cerebellar neurons that undergo apoptosis induced by serum deprivation; has a proapoptotic effect in cerebellar granule neurons that is mediated by the degradation of the apoER2)
- PCSK9 is directly involved in the degradation of BACE1 [β-site amyloid precursor protein (APP)-cleaving enzyme 1] and the generation of amyloid β-peptide (Aβ). This observation was not confirmed in PCSK9 KO mice.



- In the PROSPER study no association of SNP in PCSK9 (which influence LDL-C levels) with cognitive performance.


PCSK9
Extracellular
pool
VLDLR
LRP1
ApoER2
HCV
particle
CD81
plasma membrane
Degradation of VLDR in adipocytes reduces the hydrolysis of triglycerides rich lipoproteins and fatty acids storage
Degradation of LRP1 in cancer cells may affect motility and metastasis
Degradation of ApoER2 alters survival signaling in cerebellar neurons
PCSK9 mediated degradation of CD81 protects from HCV infection
BACE1
Intracellular
PCSK9
PCSK9 activity increases the content of nonacetylated BACE1 and consequent degradation of the enzyme

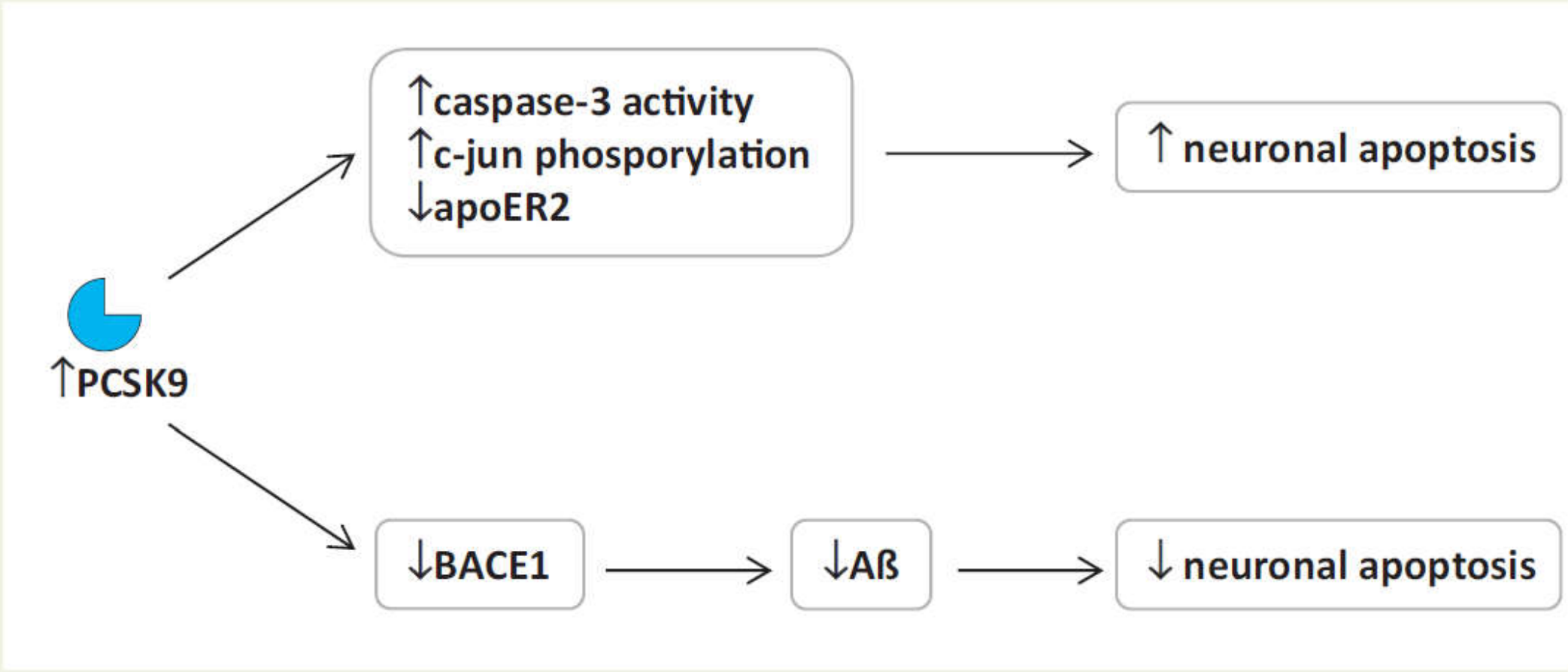
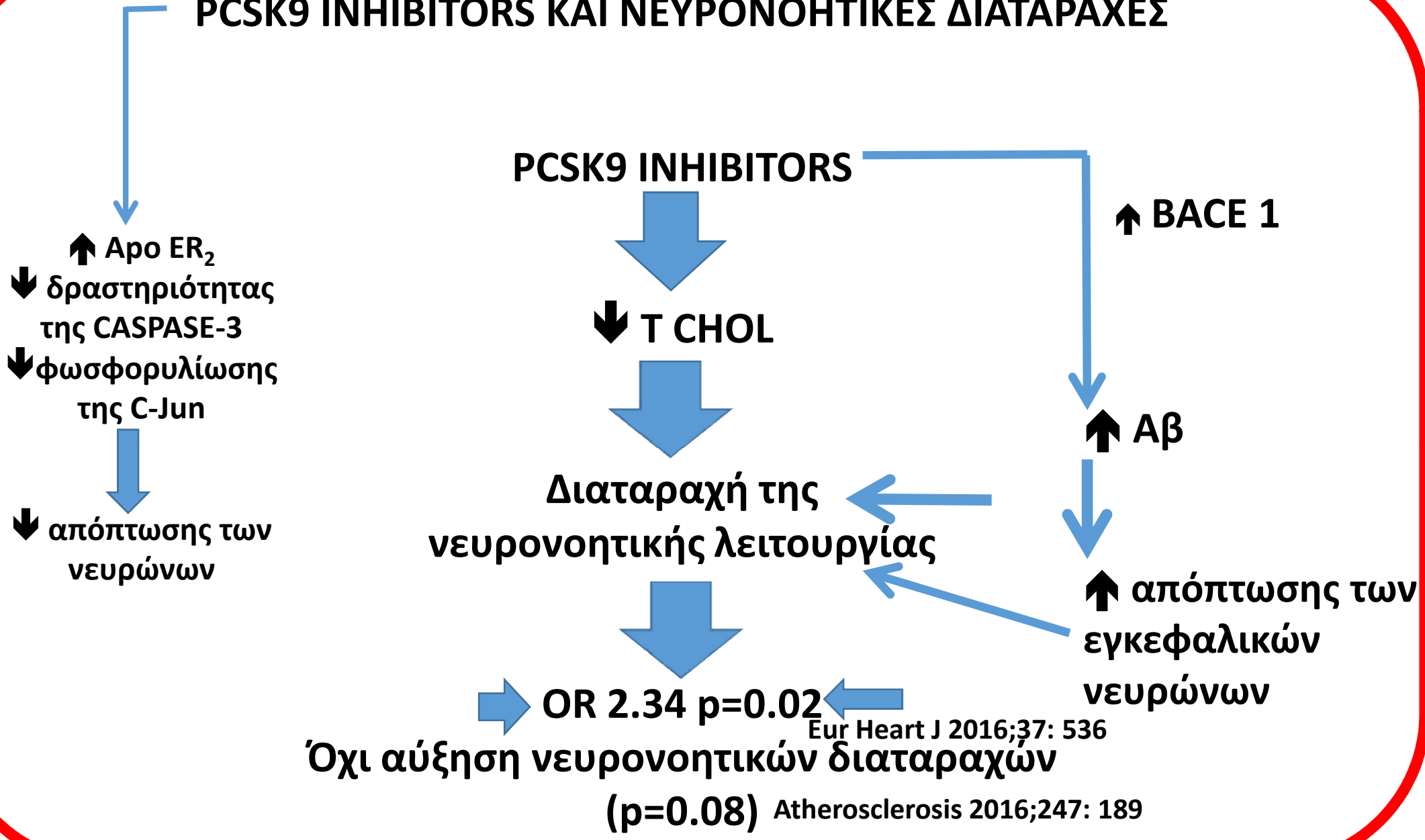


Figure 6 PCSK9 and the brain. Role of PCSK9 in neuronal apoptosis and AD progression.

Cardiovascular Research Advance Access published August 22, 2016

PCSK9 INHIBITORS ΚΑΙ ΝΕΥΡΟΝΟΗΤΙΚΕΣ ΔΙΑΤΑΡΑΧΕΣ



Eur Heart J 2016;37: 536

Atherosclerosis 2016;247: 189

Ανάλυση 28 μελετών δεν επιβεβαίωσε την εμφάνιση νευρονοητικών διαταραχών

0.7% placebo vs 0.7% alirocumab

0.3% ομάδα ελέγχου vs 0.1% evolocumab

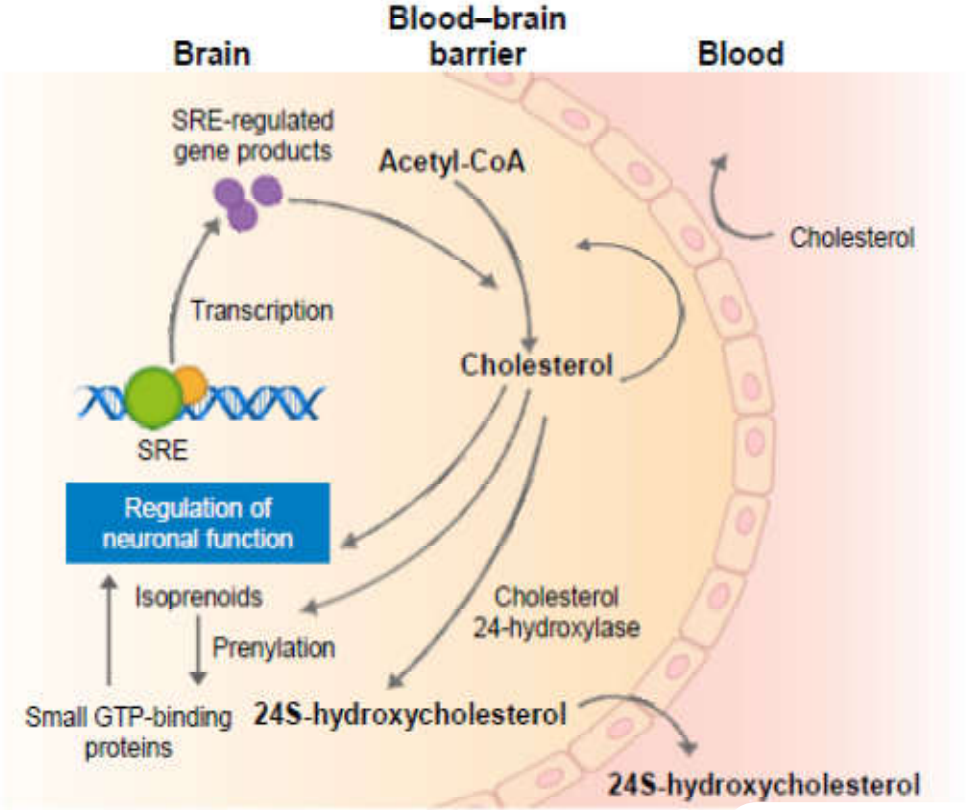
FDA BRIEFING DOCUMENT

PCSK9 INHIBITORS ΚΑΙ ΝΕΥΡΟΝΟΗΤΙΚΕΣ ΔΙΑΤΑΡΑΧΕΣ

- Όχι συσχέτιση μείωσης της LDL CHOL με την εμφάνιση νευρονοητικών διαταρχών
- Οι LDL δεν περνάν τον αιματοεγκεφαλικό φραγμό τοπική de novo σύνθεση χοληστερόλης
- Τα μονοκλωνικά αντισώματα δεν περνούν τον αιματοεγκεφαλικό φραγμό

The Central Nervous System Predominantly Synthesizes Cholesterol De Novo

- Cholesterol is a major component of the central nervous system[1,2]
  - The central nervous system predominantly synthesizes cholesterol de novo[1,2]
  - The blood–brain barrier prevents the uptake of systemic lipoprotein cholesterol[2]
  - This segregation ensures that cholesterol metabolism within the brain is isolated from changes in the circulating lipid levels[2]



1. Björkhem I, Meaney S. *Arterioscler Thromb Vasc Biol.* 2004;24:806-815. 2. Katsuno M, et al. *Nat Med.* 2009;15:253-254.
Figure adapted from Katsuno M et al. 2009.

IX- PCSK9 AND THE KIDNEYS

PCSK9 ΚΑΙ ΝΕΦΡΟΙ

PCSK9

↓

↓ επιθηλιακών διαύλων Na^+ (↑ αποδόμησης) [ENaC]

↓

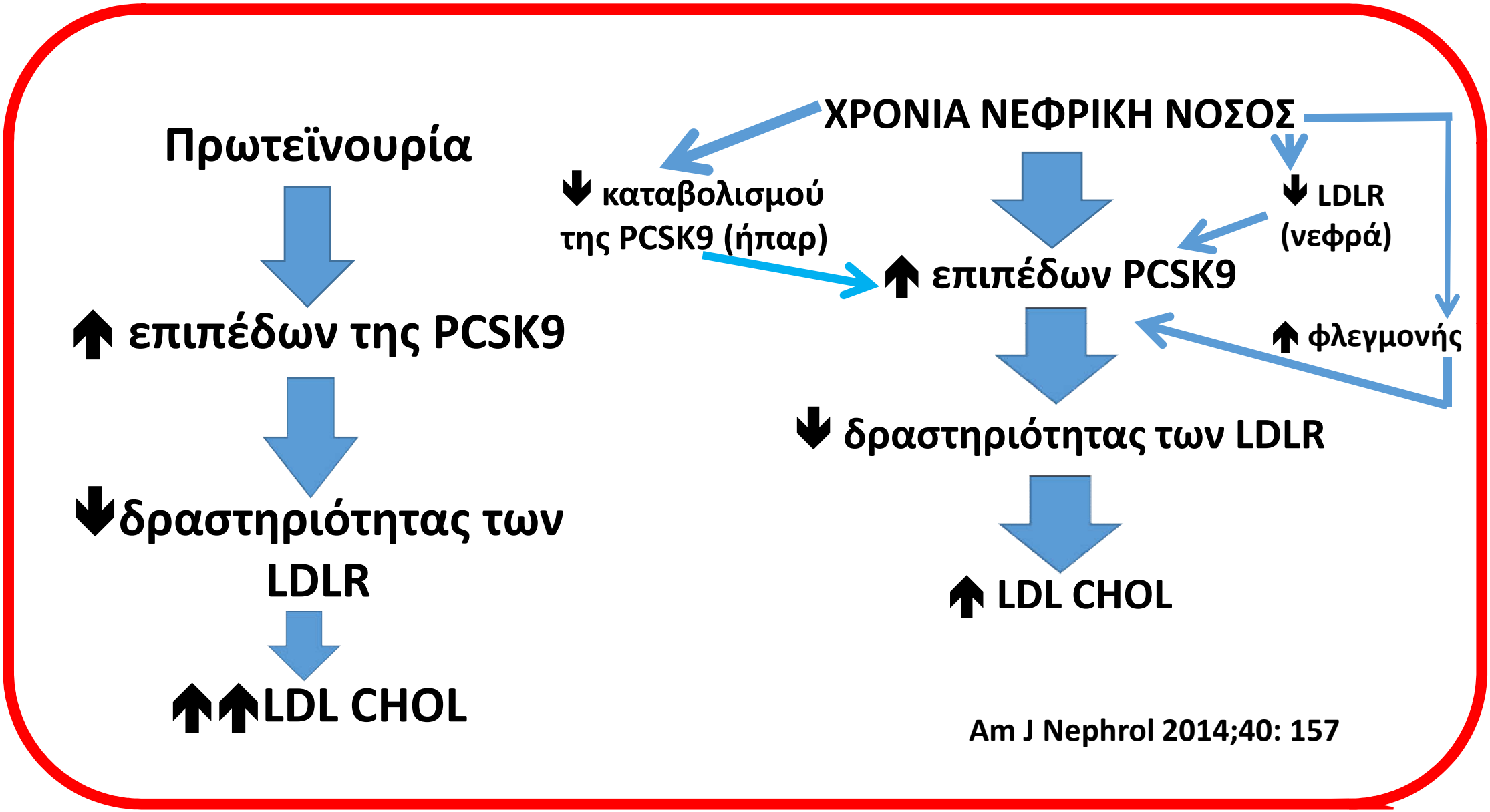
Νατριούρηση

Αναστολείς της PCSK9

↓

↑ επαναρρόφησης Na^+;; (δεν επιβεβαιώνεται από τις κλινικές μελέτες)

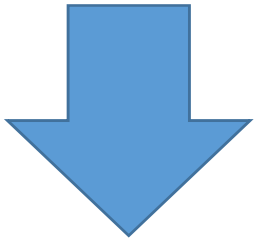
ΑΥΞΗΜΕΝΑ ΕΠΙΠΕΔΑ PCSK9 ΣΕ ΑΣΘΕΝΕΙΣ ΜΕ ΝΕΦΡΙΚΗ ΝΟΣΟ/ΠΡΩΤΕΪΝΟΥΡΙΑ



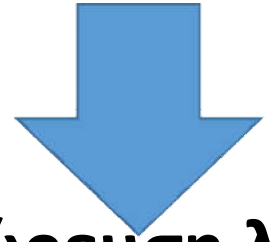
Am J Nephrol 2014;40: 157

X- PCSK9 AND DIABETES

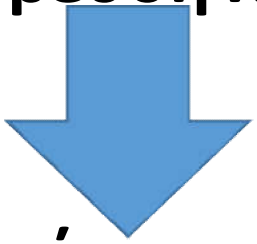
ΥΠΟΛΙΠΙΔΑΙΜΙΚΑ ΦΑΡΜΑΚΑ
(στατίνες /ezetimibe/αναστολείς της PCSK9)



↑ LDL υποδοχέων στα παγκρεατικά κύτταρα



Συσσώρευση λιπιδίων



Δυσλειτουργία των β-κυττάρων

Σακχαρώδης διαβήτης

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Variation in *PCSK9* and *HMGCR* and Risk of Cardiovascular Disease and Diabetes

Brian A. Ference, M.D., Jennifer G. Robinson, M.D., M.P.H., Robert D. Brook, M.D., Alberico L. Catapano, Ph.D., M. John Chapman, Ph.D., David R. Neff, D.O., Szilard Voros, M.D., Robert P. Giugliano, M.D., George Davey Smith, M.D., D.Sc., Sergio Fazio, M.D., Ph.D., and Marc S. Sabatine, M.D., M.P.H.

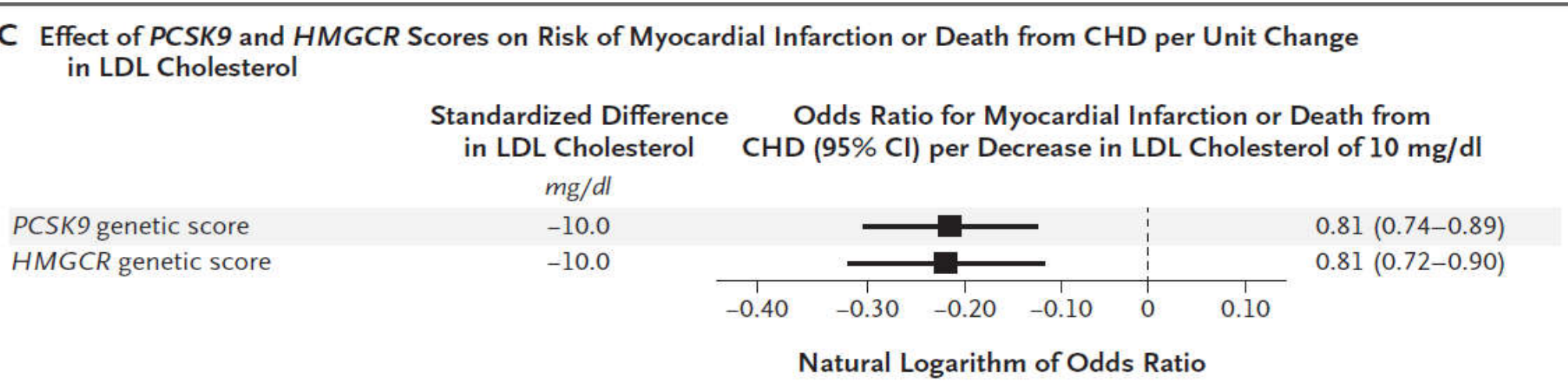


Figure 1. Effect of *PCSK9* and *HMGCR* Genetic Scores on the Risk of Myocardial Infarction or Death from Coronary Heart Disease.

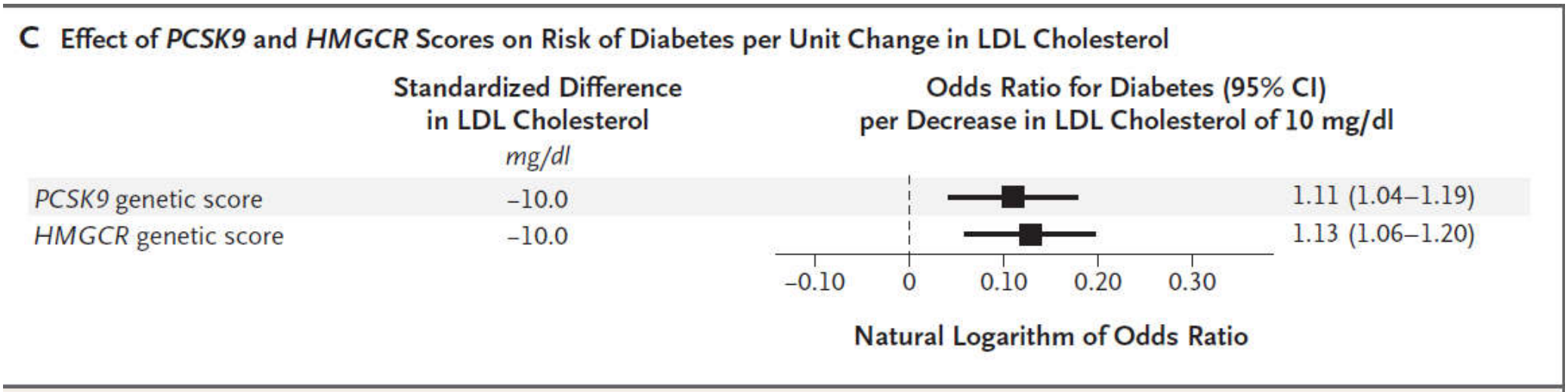


Figure 3. Dose–Response Relationship between *PCSK9* and *HMGCR* Scores and Risk of Diabetes.

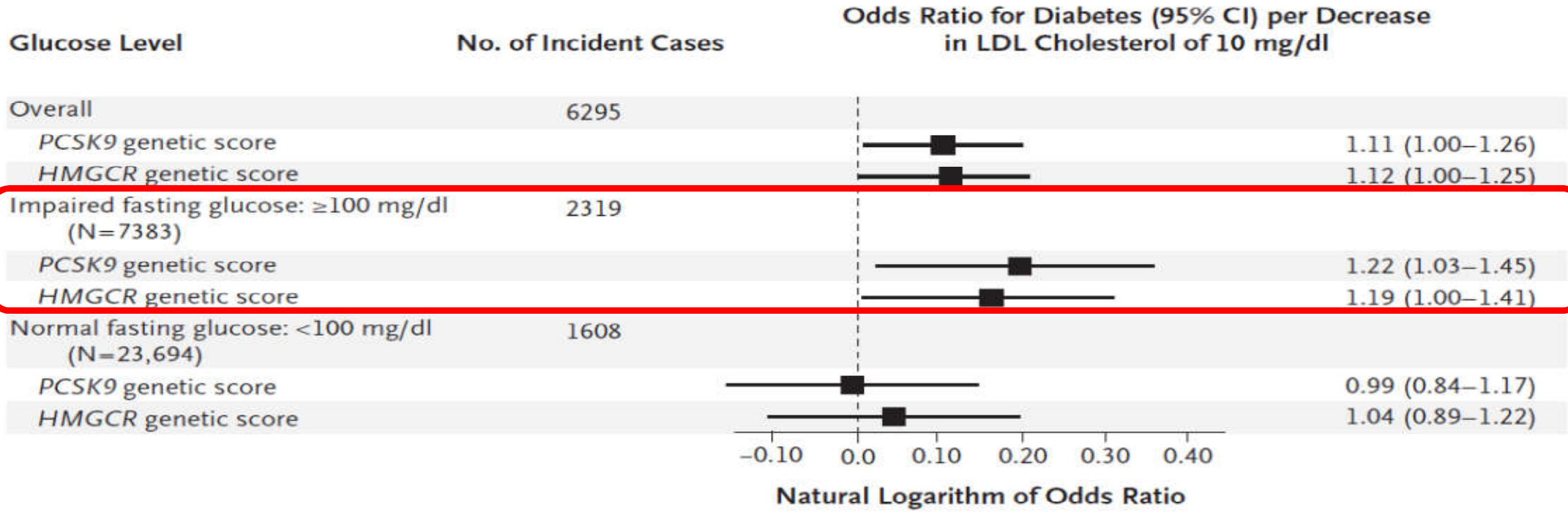


Figure 4. Effect of *PCSK9* and *HMGCR* Scores on the Risk of Incident Diabetes.

A total of 6295 incident cases of diabetes occurred during follow-up in the prospective cohort studies. After the exclusion of participants with prevalent diabetes, baseline fasting plasma glucose levels were available for 31,077 participants. The main analysis included all the participants after the exclusion of 4340 participants with prevalent diabetes; the subgroup analysis that was stratified according to fasting plasma glucose level included the 31,077 participants without prevalent diabetes for whom baseline fasting plasma glucose levels were available. Boxes represent point estimates of effect. Lines represent 95% CIs.

N Engl J Med 2016;375: 2144-53

PCSK9 ΚΑΙ ΣΑΚΧΑΡΩΔΗΣ ΔΙΑΒΗΤΗΣ: A MENDELIAN RANDOMISATION STUDY

n=550.000 άτομα / 51.623 διαβητικοί ασθενείς

4 PCSK9 variants → 1 mmol/L (38.4 mg/dL) μείωση της LDL CHOL:

- **↑ γλυκόζης κατά 1.62 mg/dL**
- **↑ σωματικού βάρους κατά 1.03 kg**
- **↑ waist to hip ratio (0.0001)**
- **↑ επίπτωσης διαβήτη (OR 1.29)**

Lancet Diabetes Endocrinol, 2016

PCSK9 genetic variants and risk of type 2 diabetes: a mendelian randomisation study

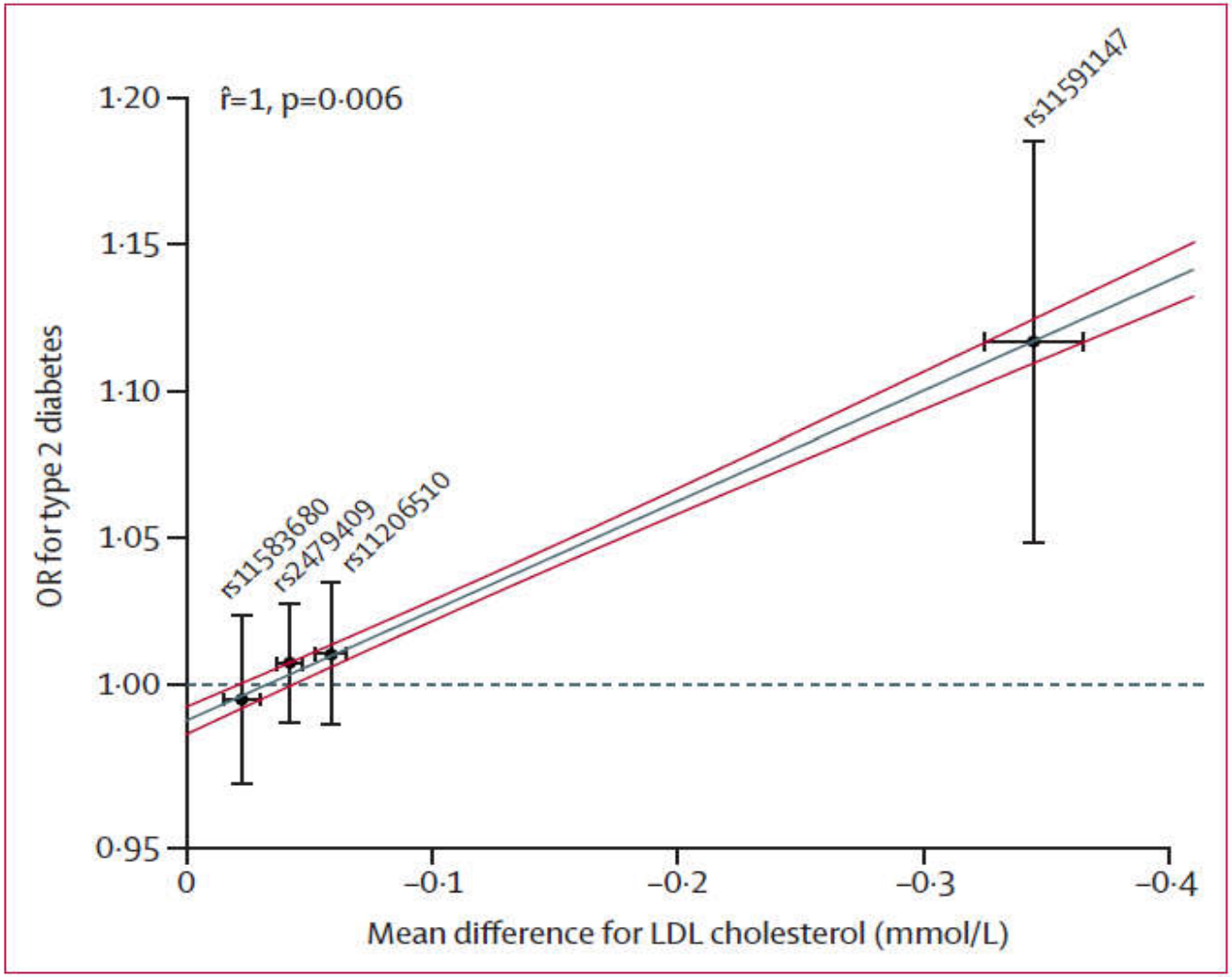


Figure 4: Correlation between *PCSK9* associations with LDL cholesterol concentration and type 2 diabetes

ALIROCUMAB AND NEW ONSET DIABETES MELLITUS

Pooled analysis of 10 ODYSSEY phase 3 trials (n=4974)
n= 3448 individuals without diabetes

	PLACEBO-CONTROLLED GROUP	EZETIMIBE-CONTROLLED GROUP
Incident diabetes mellitus or diabetic complications TEAE	0.64	0.55

Prediabetic individuals (39.6%):
The HR associated for transition from prediabetes to new-onset diabetes for alirocumab was 0.90 vs placebo and 1.10 vs ezetimibe

Eur Heart J 2016

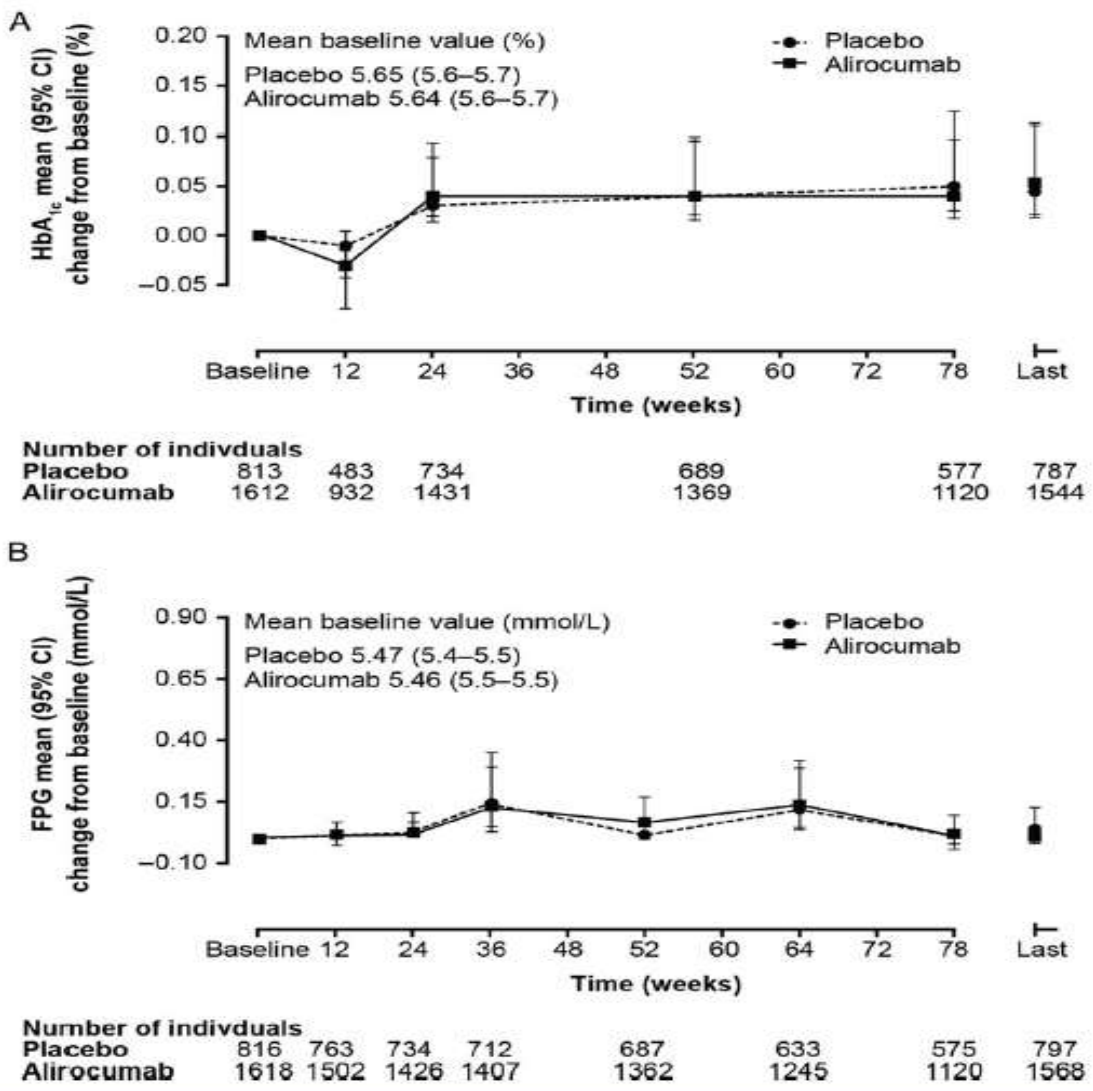
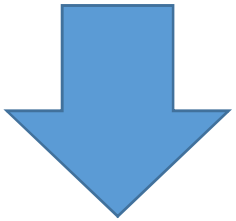


Figure 2 HbA_{1C} (%) and fasting plasma glucose (mmol/L) trajectories (change from baseline) in those without diabetes at baseline: alirocumab vs. placebo. Placebo-controlled studies: Phase 3 (LONG TERM, FH I, FH II, HIGH FH, COMBO I). At Week 12, HbA_{1C} was measured only in the LONG TERM. The last on-treatment value is defined as the last value collected up to 21 days after the last double-blind IMP injection. Patients who had that parameter assessed at baseline and/or at follow-up are included. Individuals without diabetes at baseline defined as those not assigned CMQ code 'diabetes' recorded in medical history. CMQ, Custom MedDRA Query; FPG, fasting plasma glucose; HbA_{1C}, glycated haemoglobin A_{1C}; IMP, Investigational Medicinal Product; CI, confidence interval.

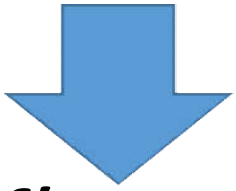
PCSK9 ΚΑΙ ΠΑΓΚΡΕΑΣ

⬇ PCSK9 (π.χ. σε PCSK9 null mice)



δ cells of pancreas

⬇ έκκρισης ινσουλίνης (δυσπλασία, απόπτωση και φλεγμονή των παγκρεατικών κυττάρων)



δυσανεξία στη γλυκόζη

FEBS Lett 2010;584: 701

PCSK9 inhibitors

↑ LDL-R (πάγκρεας)

↑ CHOL στα παγκρεατικά κύτταρα

↓ λειτουργίας των β-κυττάρων

FEBS Lett 2010;584: 701

PCSK9 loss of function mutations και διαβήτης: conflicting data

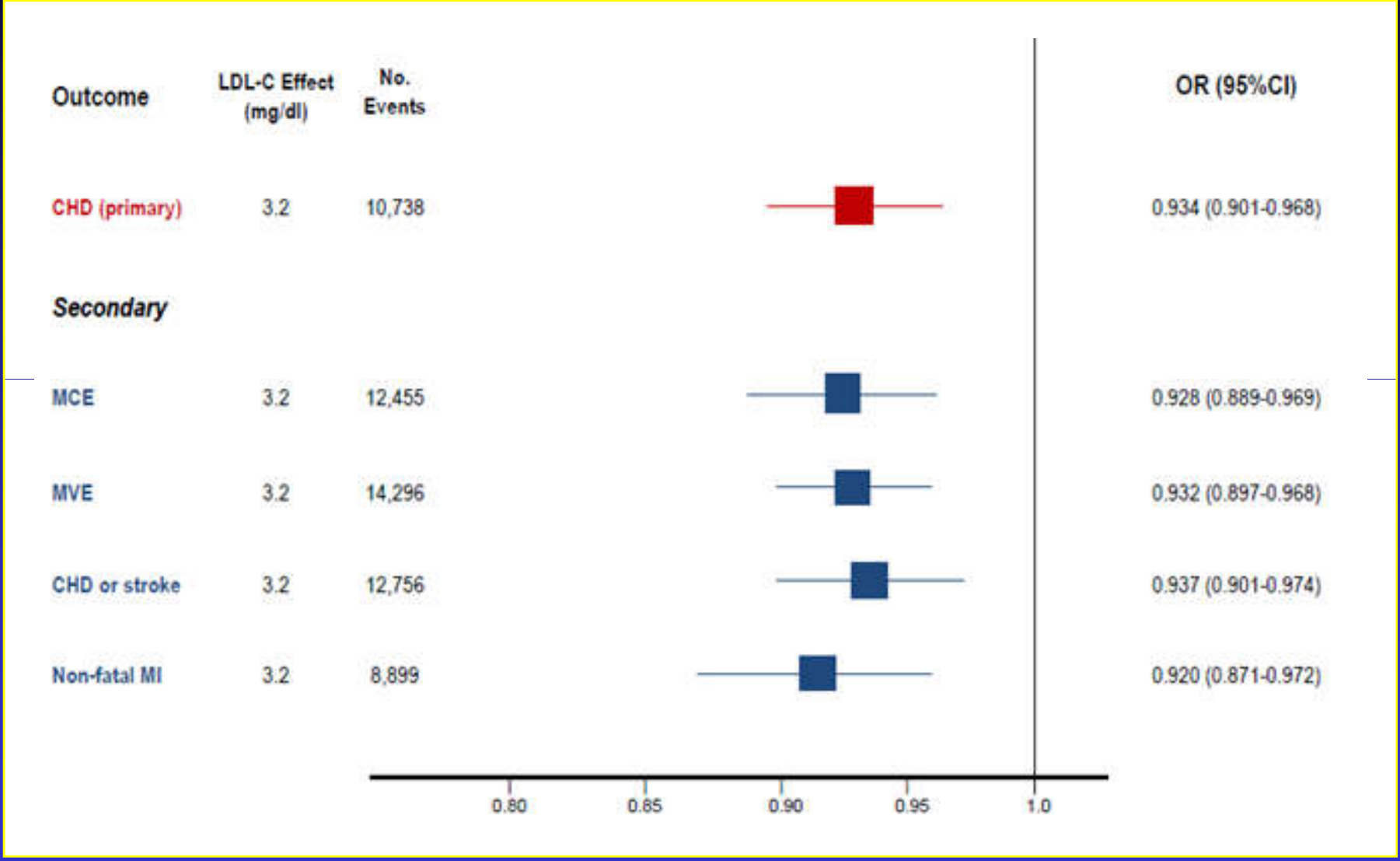
Diabetologia 2015;58: 2051
J Clin Lipidol 2015;9: 786

PCSK9 Polymorphisms, HMGCR Polymorphisms, Protection from Cardiovascular Disease, and Risk of Diabetes

Brian A. Ference, Jennifer G. Robinson, Robert D. Brook, Alberico L. Catapano, M. John Chapman, David R. Neff, Szilard Voros, Robert P. Giugliano, George Davey Smith, Sergio Fazio, Marc S. Sabatine

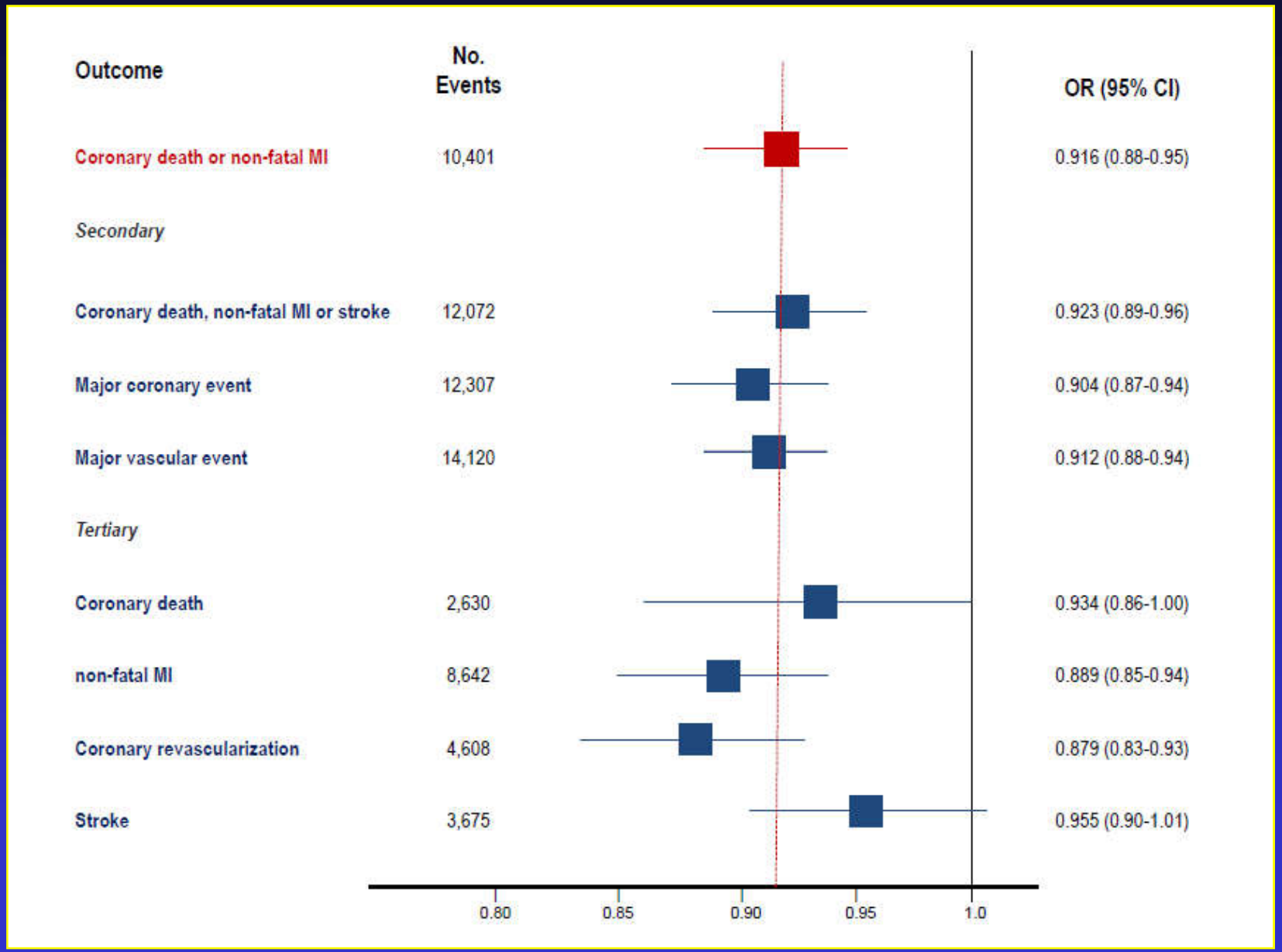
Ference B.A. et al. Submitted manuscript

Effect of HMGCR genetic score on the risk of cardiovascular events



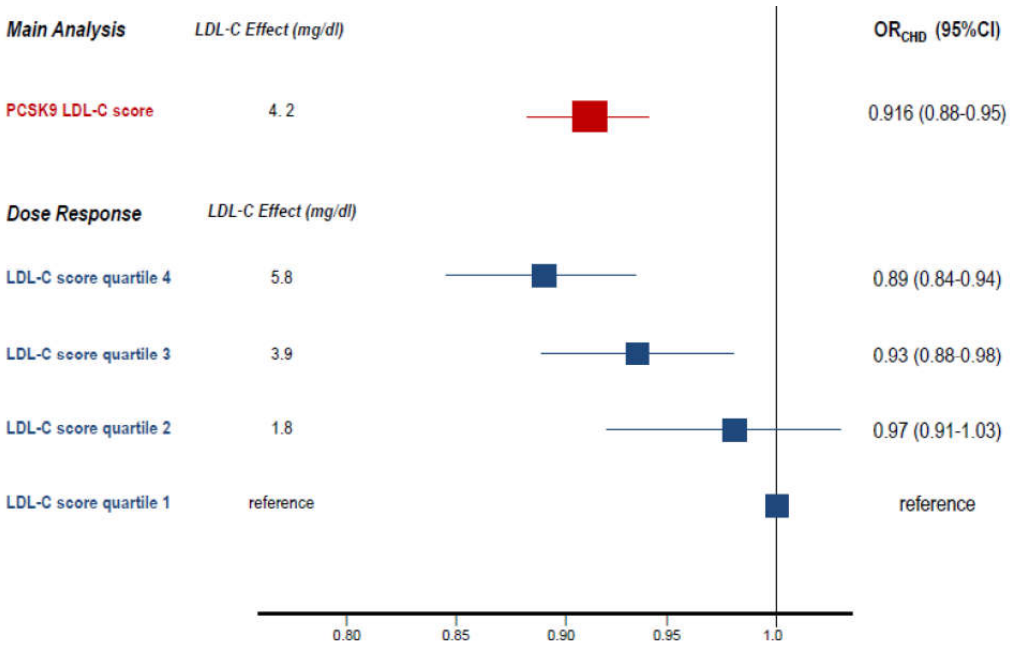
Ference B.A. et al. Submitted manuscript – Supplementary App.

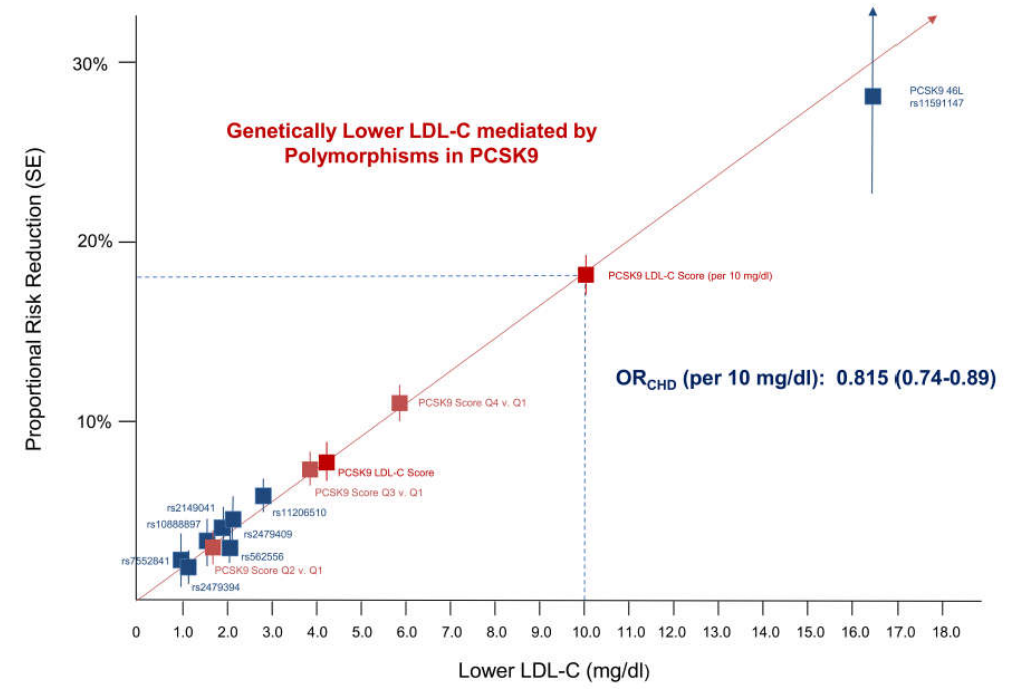
Effect of PCSK9 genetic score on the risk of cardiovascular events



Ference B.A. et al. Submitted manuscript

Dose-response effect of PCSK9 quartile scores on LDL-C and the risk of coronary death or MI



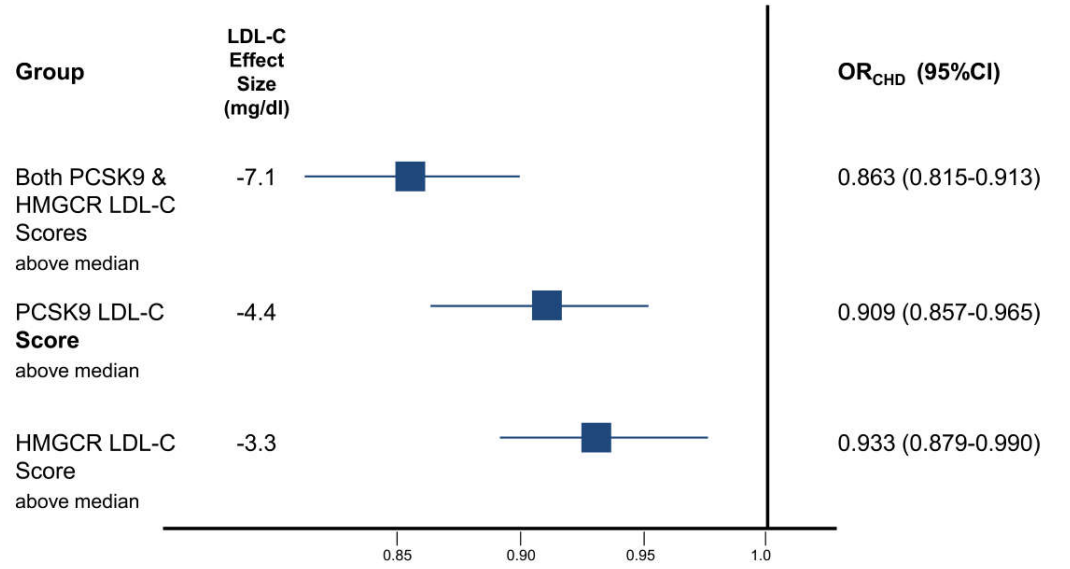


Ference B.A. et al. Submitted manuscript

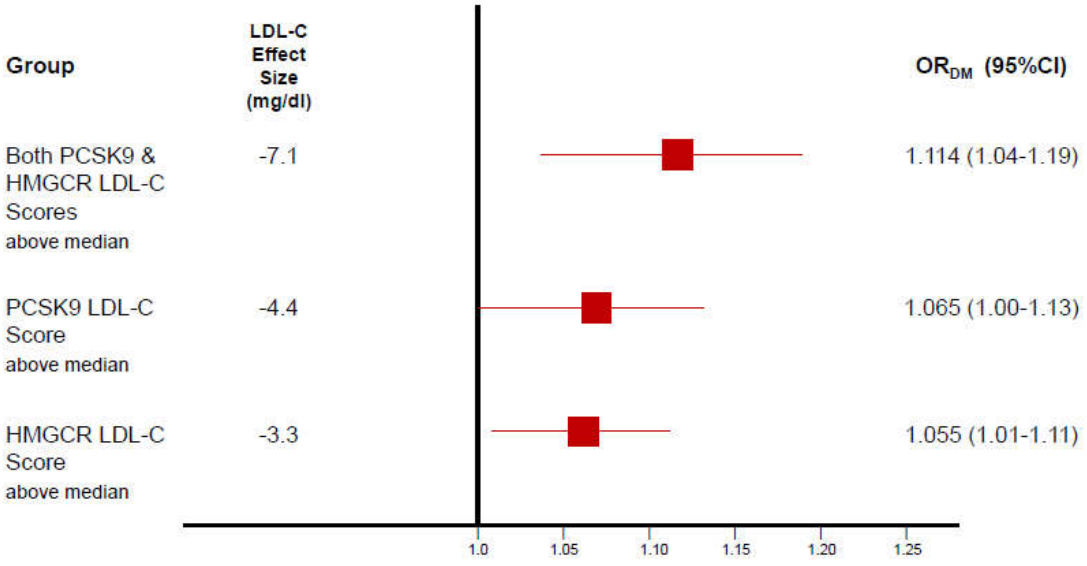
2x2 Factorial Analysis:

Separate and combined effect of PCSK9 and HMGCR genetic scores on the risk of cardiovascular events and diabetes

A. Coronary Death or Myocardial Infarction



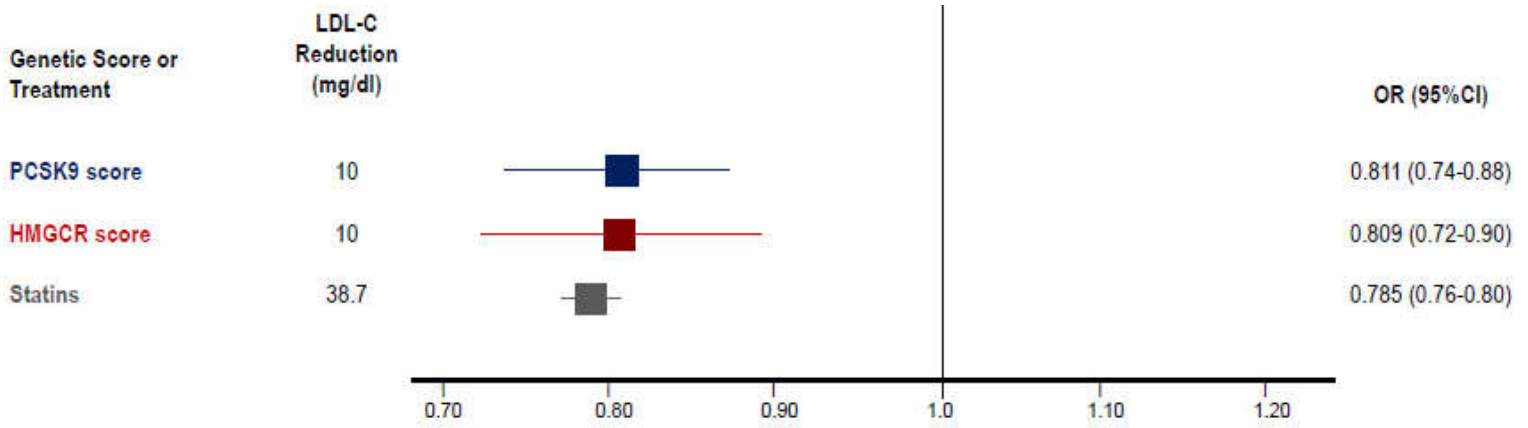
B. Diabetes



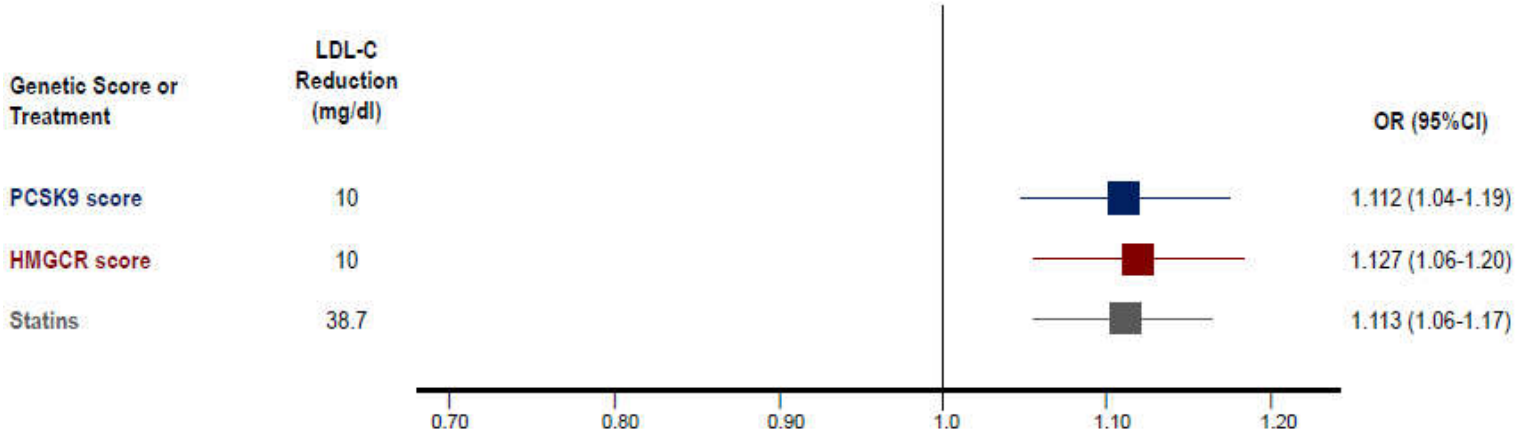
Ference B.A. et al. Submitted manuscript

Comparison of the effect of genetic and pharmacologic reduction in LDL-C on risk of cardiovascular events and diabetes

A. Coronary Death or Myocardial Infarction



B. Diabetes



Ference B.A. et al. Submitted manuscript

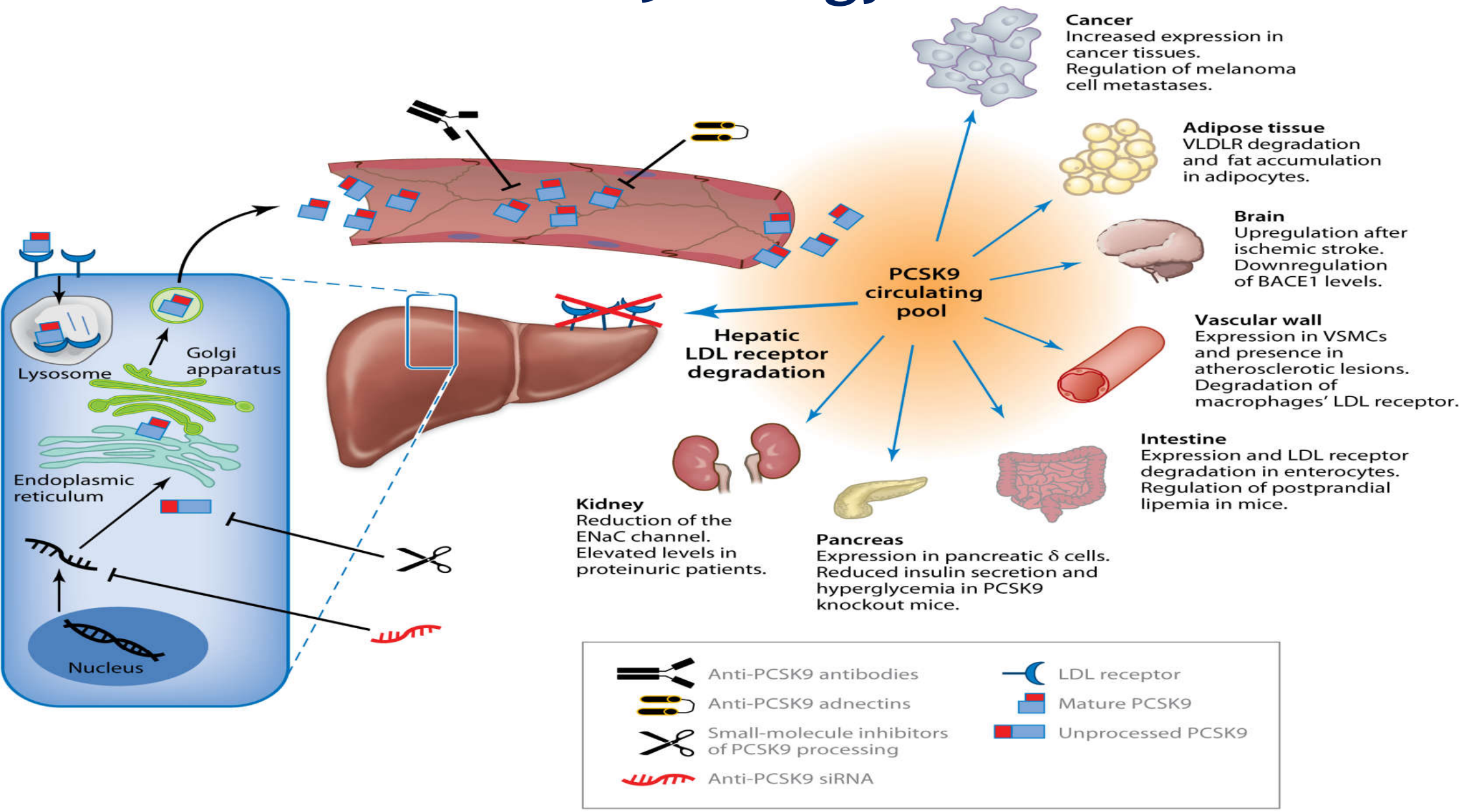
PCSK9 ΚΑΙ ΣΑΚΧΑΡΩΔΗΣ ΔΙΑΒΗΤΗΣ

	OR για διαβήτη
↓ LDL CHOL κατά 40 mg/dL (HMGCR gene/στατίνες)	1.39
	JAMA, 2016
↓ LDL CHOL κατά 40 mg/dL (PCSK9 gene/ αναστολείς της PCSK9	1.29
	Lancet Diab Endocrinol, 2016

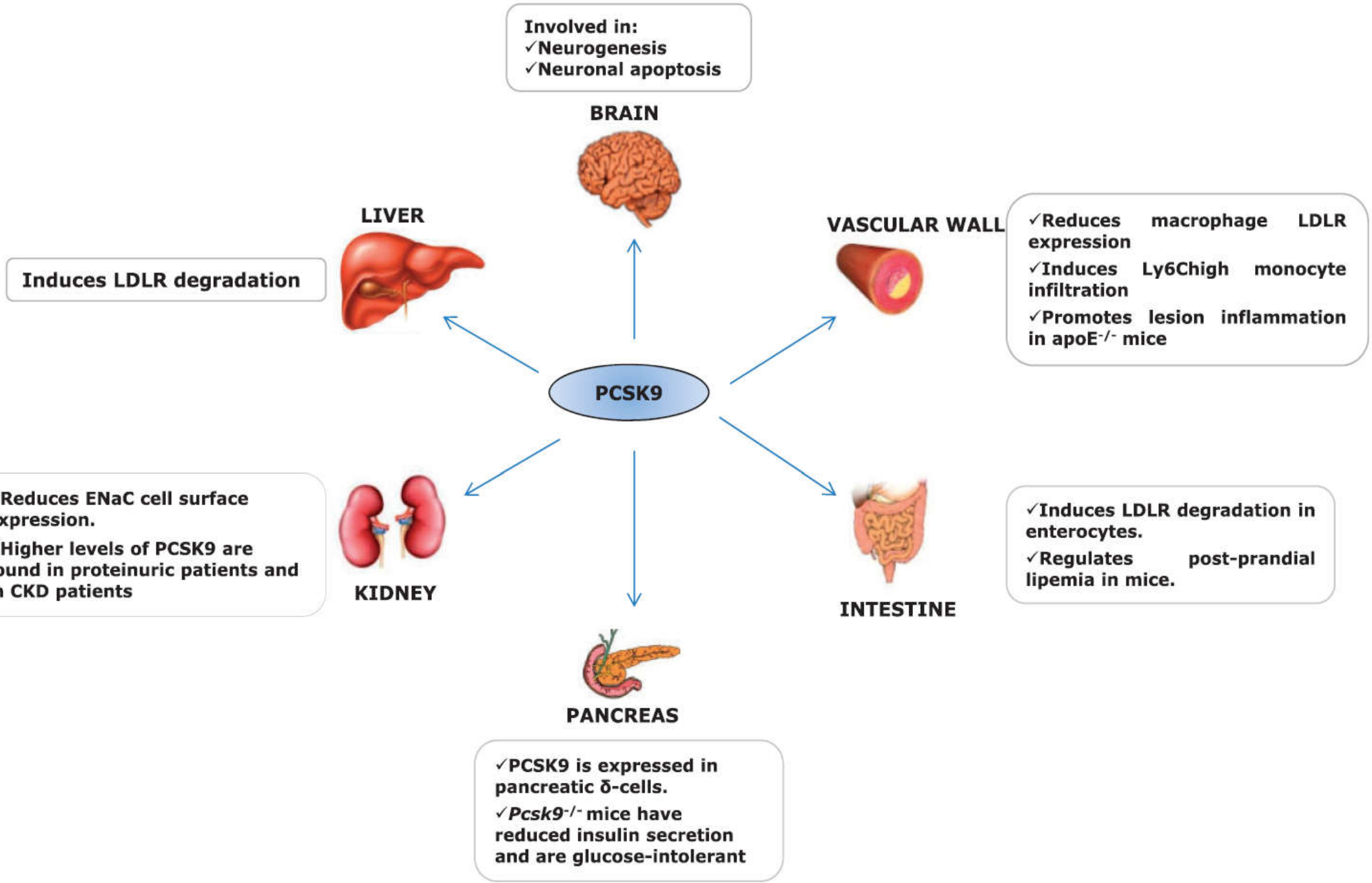
1 single SNP in PCSK9: OR για διαβήτη τύπου 2 = 1.19 για κάθε μείωση της LDL CHOL κατά 40 mg/dL

ΣΥΜΠΕΡΑΣΜΑΤΑ

PCSK9 biology



Norata GD, et al. 2014.
Annu. Rev. Pharmacol. Toxicol. 54:273–93



Cardiovascular Research Advance Access published August 22, 2016

Figure 3 Expression and function of PCSK9. PCSK9 is mainly expressed in the liver, where it is involved in the binding and degradation of LDLR. PCSK9 is expressed also in other tissues and organs, where it plays additional functions.

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