PCSK9 and its Inhibitors

Past, Present, and Future Implications

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Disclosures: Consulting/Research/Speaking

- Amgen
- Regeneron/Sanofi
- Aegerion
- Merck

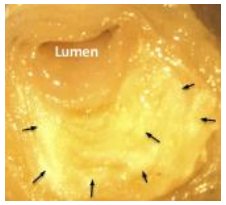
Understanding Cholesterol Metabolism

Historic Changes

History of Cholesterol Understanding

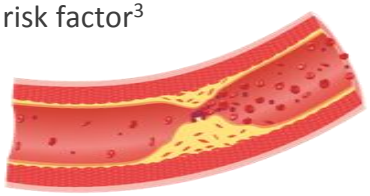
1908–1913

Cholesterol related to atherosclerosis[1]



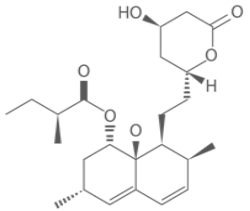
1961

Cholesterol identified as cardiovascular risk factor[3]



1987

First statin approved[5]



1948

Framingham Heart Study begins[2]

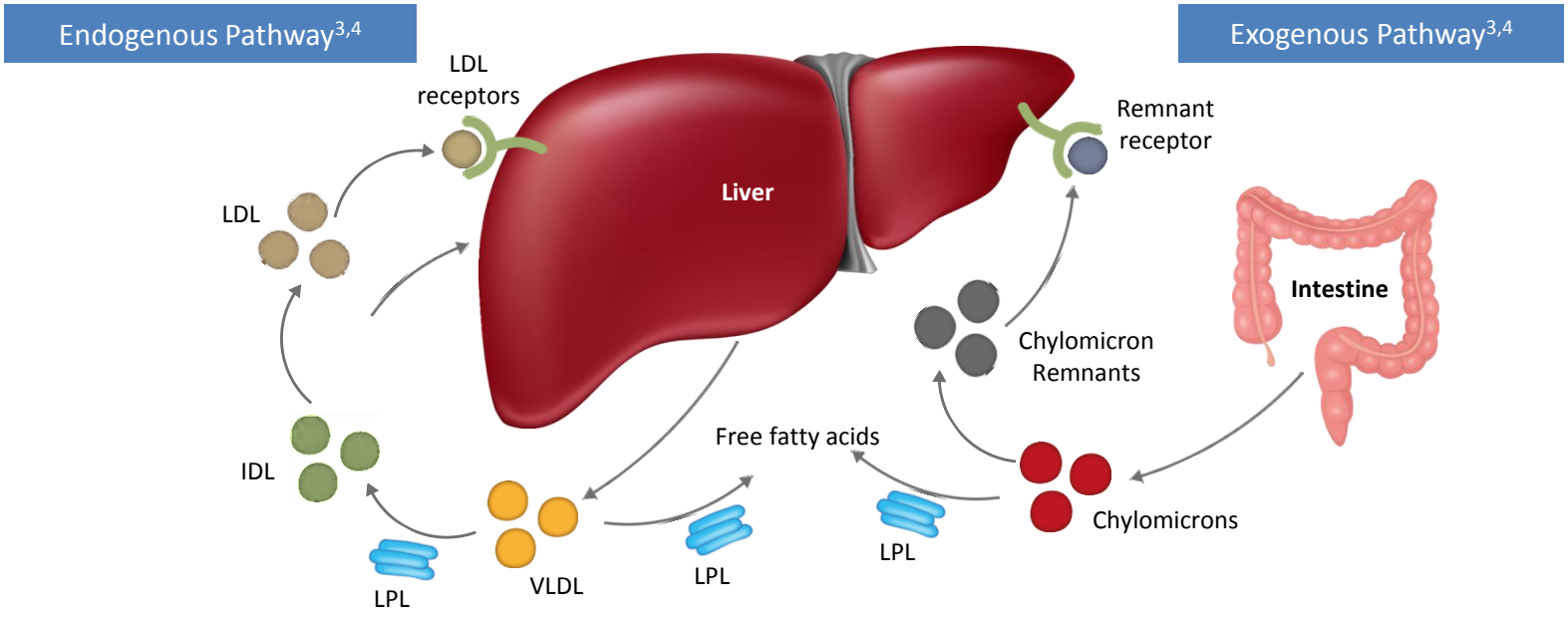
1985

Brown and Goldstein awarded nobel prize for cholesterol metabolism (LDL receptor)[4]

1. Konstantinov IE, et al. *Tex Heart Inst J.* 2006;33:417-423. 2. Framingham Heart Study. https://www.framinghamheartstudy.org/about-fhs/index.php. Accessed January 13, 2016. 3. Kannel WB, et al. *Ann Intern Med*. 1961;55:33-50. 4. Brown MS, Goldstein JL. Nobel lecture 1985. http://www.med.harvard.edu/md_phd/news/Brown%20and%20Goldstein%20Nobel%20Lecture.pdf. Accessed January 13, 2016. 5. Tobert JA. *Nat Rev Drug Discov*. 2003;2:517-526. 5. Nobelprize.org. The Nobel prize in physiology or medicine 1985. http://www.nobelprize.org/nobel_prizes/medicine/laureates/1985/. Accessed January 13, 2016.

LDL Is the End Product of Endogenous Lipoprotein Metabolism

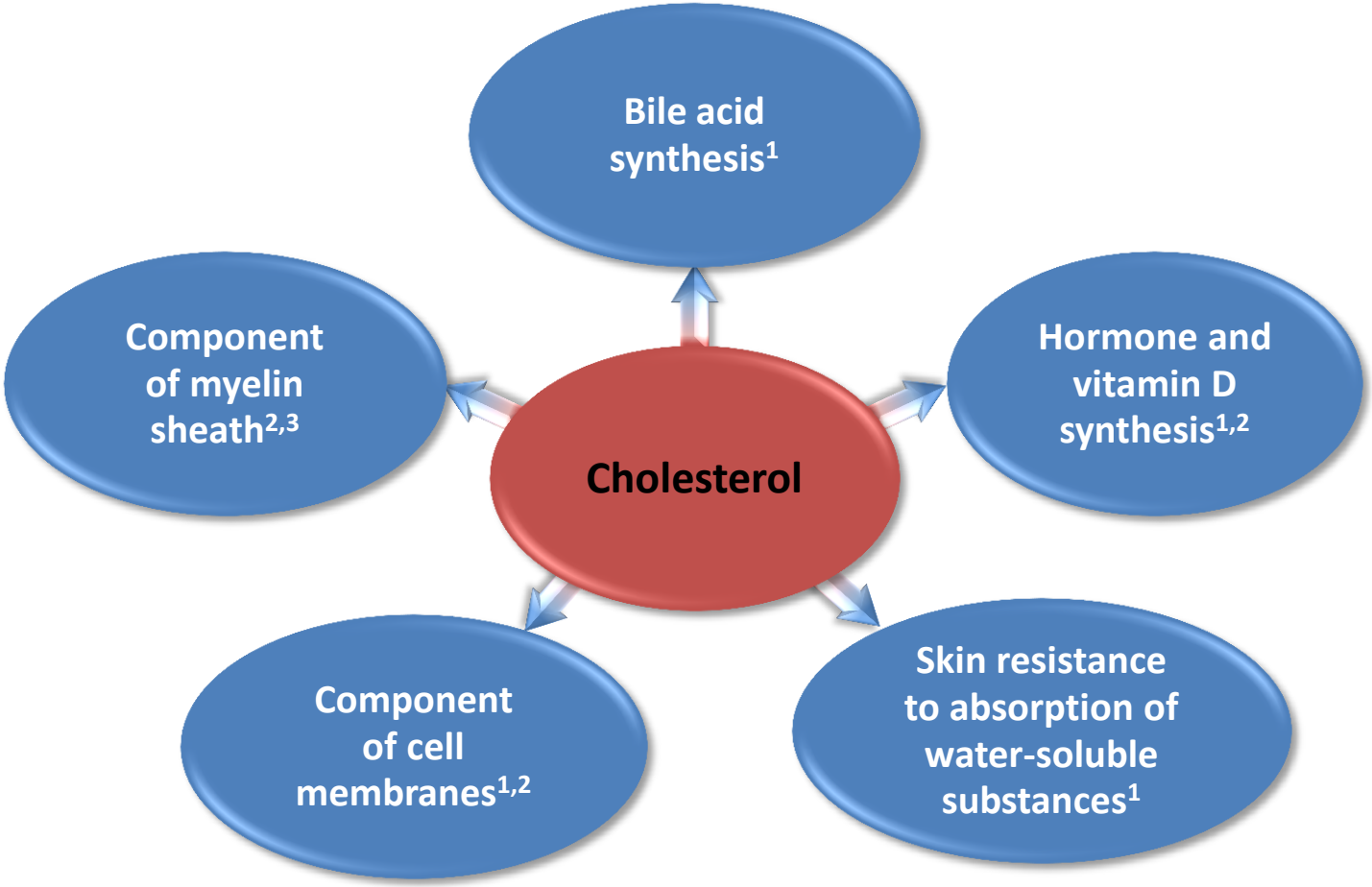
- LDL particles are the end product of endogenous lipoprotein metabolism[1]
  - LDL receptors remove LDL from the circulatory system[1]
  - LDL minimally delivers cholesterol to peripheral tissues[2]



HDL = high-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; LPL = lipoprotein lipase; VLDL = very low-density lipoprotein

1. Goldstein JL, Brown MS. *Arterioscler Thromb Vasc Biol*. 2009;29;431-438. 2. Rader DJ, et al. *J Clin Invest*. 2003;111:1795-1803.
3. Dietschy JM, Turley SD. *J Lipid Res.* 2004;45:1375-1397; 4. Mc Auley MT, et al. *BMC Syst Biol*. 2012;6:130.

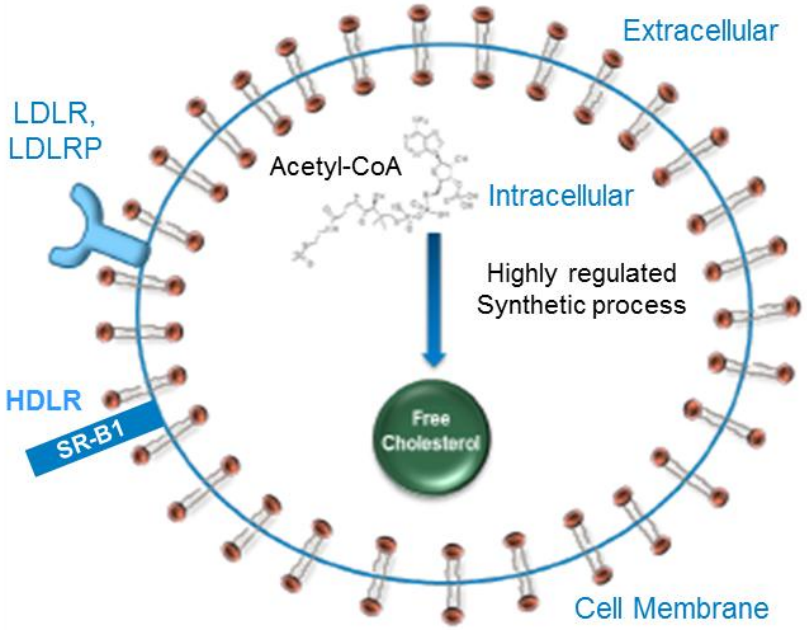
Cholesterol Plays a Role in Many Important Physiologic Functions



1. Hall JE, Guyton AC. In: *Guyton and Hall Textbook of Medical Physiology*. 12th ed. Philadelphia, PA: Saunders; 2011:819-830.
2. Goldstein JL, Brown MS. *Arterioscler Thromb Vasc Biol*. 2009;29:431-438. 3. Saher G, et al. *Nat Neurosci*. 2005;8:468-475.

Cellular Acquisition of Cholesterol Can Be From Multiple Sources

- Cholesterol for cellular physiologic functions can be from intra and/or extracellular pathways[1–5]
  - Systemic distribution of cholesterol is important, but cells are not dependent on circulating plasma LDL-C[3]



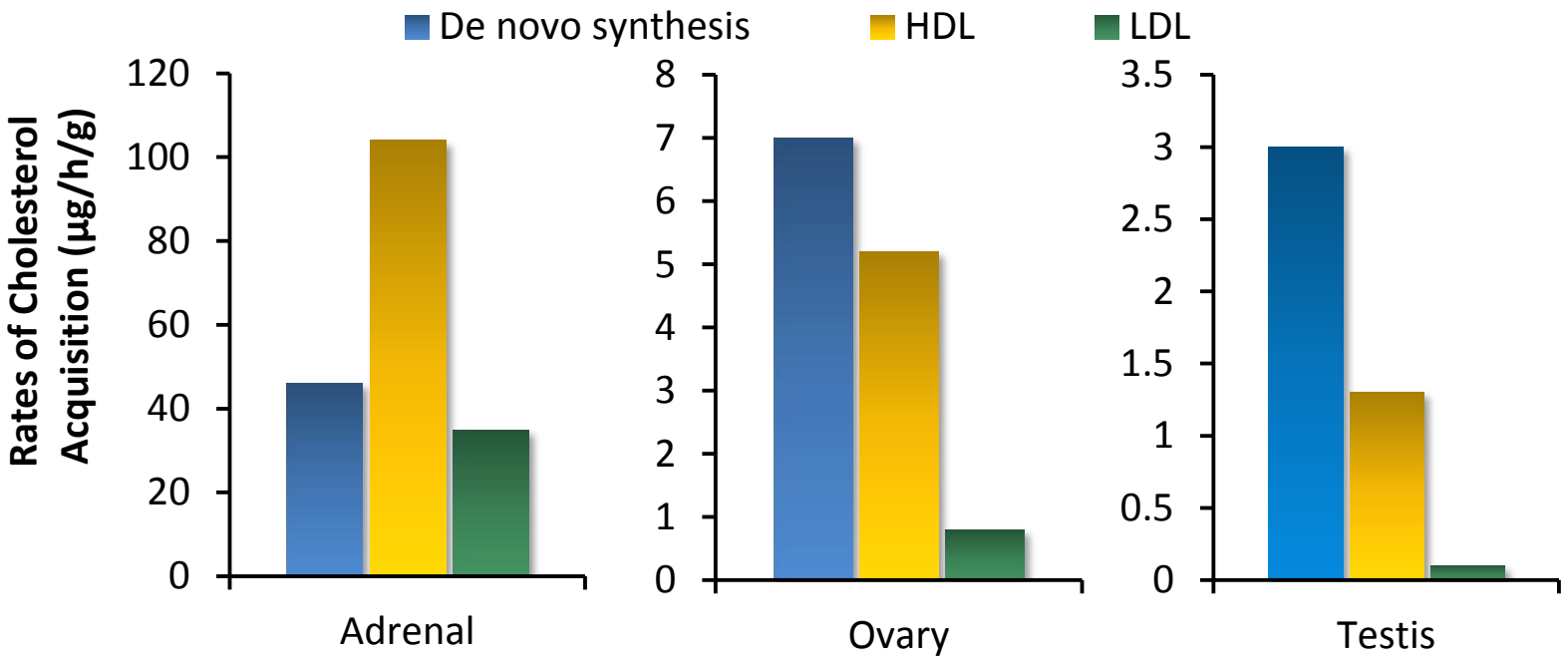
HDLR = high-density lipoprotein cholesterol receptor; LDL-C = low-density lipoprotein cholesterol; LDL-R = LDL receptor; LDLRP = LDLR protein; SR-B1 = scavenger receptor class B type 1.

1. Mc Auley MT, et al. *BMC Syst Biol.* 2012;6:130. 2. Xie C, et al. *J Lipid Res.* 2006;47:953-963. 3. Hu J, et al. *Nutr Metab (Lond).* 2010;7:47. 4. Orth M, Bellosta S. *Cholesterol.* 2012;2012:292598. 5. Dietschy JM, Turley SD. *J Lipid Res.* 2004;45:1375-1397.
Figure adapted from Dietschy 2004.

Animal Data Demonstrate Steroidogenic Tissues Predominantly Acquire Cholesterol via HDL and De Novo Synthesis[1,2]

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

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



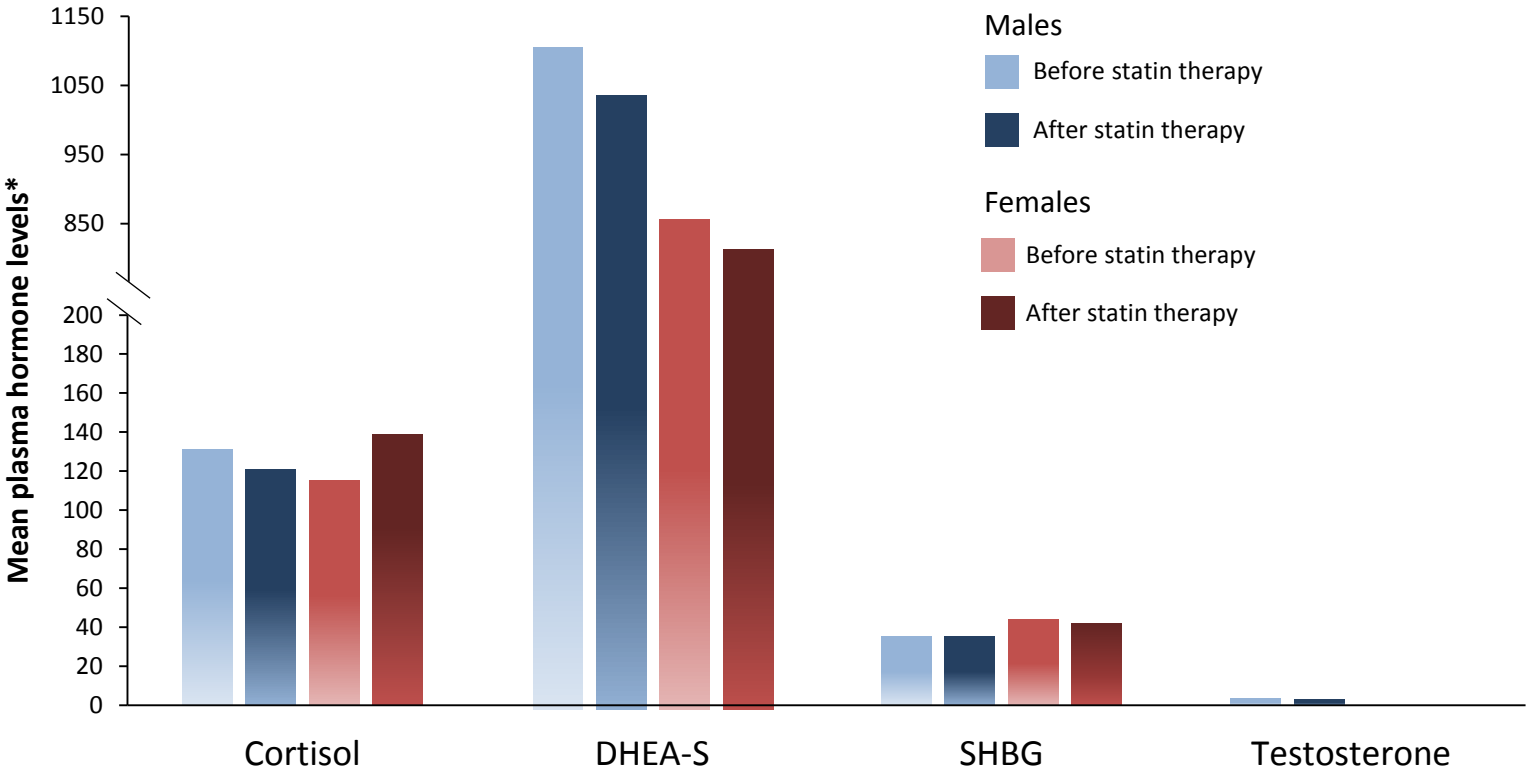
*Data were calculated from measurements made in 49-day-old control mice with LDL receptor activity.

HDL = high-density lipoprotein; LDL = low-density lipoprotein.

1. Xie C, et al. *J Lipid Res.* 2006;47:953-963. 2. Hu J, et al. *Nutr Metab (Lond).* 2010;7:47.

Despite Reducing LDL-C, Statins Do Not Alter Gonadal or Adrenal Steroid Hormones in Humans

Plasma hormone levels before and 3 months after treatment with statin[1]



- Reduction in LDL-C with statins without changing steroid hormones has been consistently shown[1–3]

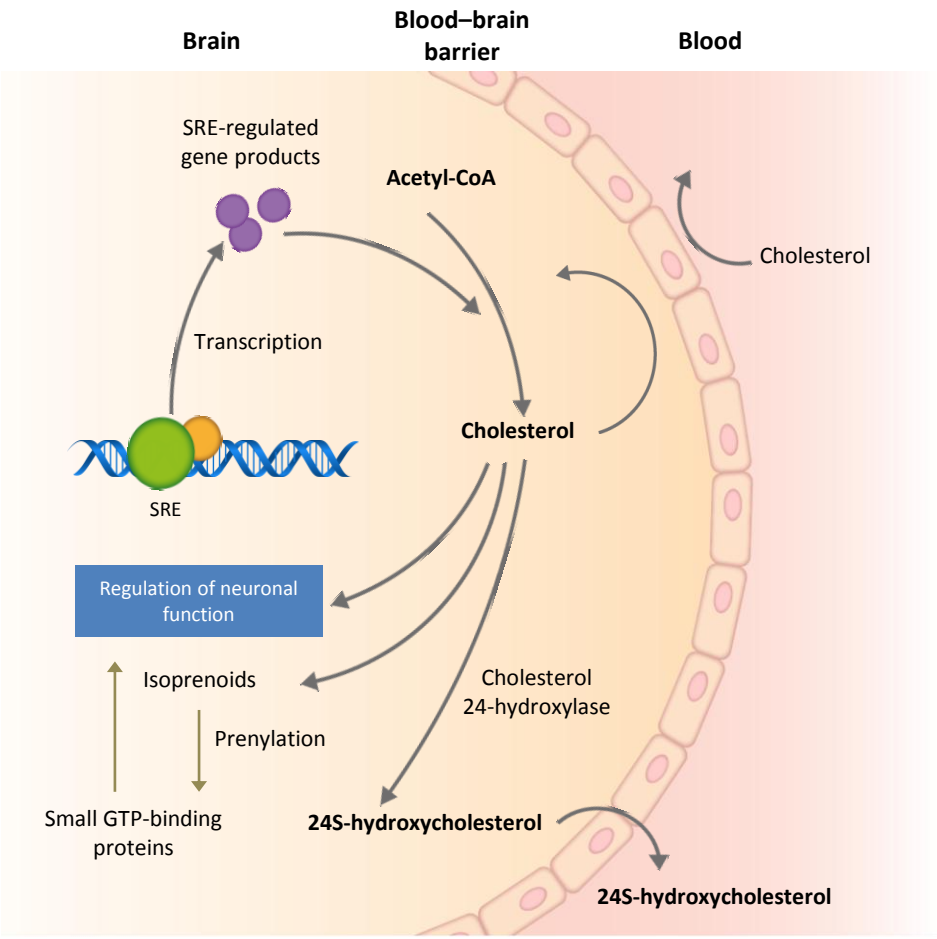
*ng/mL for cortisol, DHEA-S and testosterone and nmol/L for SHBG. Effect of statin on gonadal and adrenal hormones studied on 24 patients with type 2 diabetes, studied before and after a 3-month treatment with statin.

DHEA-S = dehydroepiandrosterone sulfate; SHBG = sex hormone binding globulin.

1. Santini SA, et al. *J Atheroscler Thromb*. 2003;10:160-164. 2. Sezer K, et al. *J Endocrinol Invest*. 2008;31:1075-1078.
3. Bohm M, et al. *Z Kardiol*. 2004;93:43–48.

The Central Nervous System Synthesizes Cholesterol De Novo

- The central nervous system synthesizes cholesterol de novo[1,2]
- The blood–brain barrier prevents the uptake of systemic lipoprotein cholesterol[1,2]
- 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.

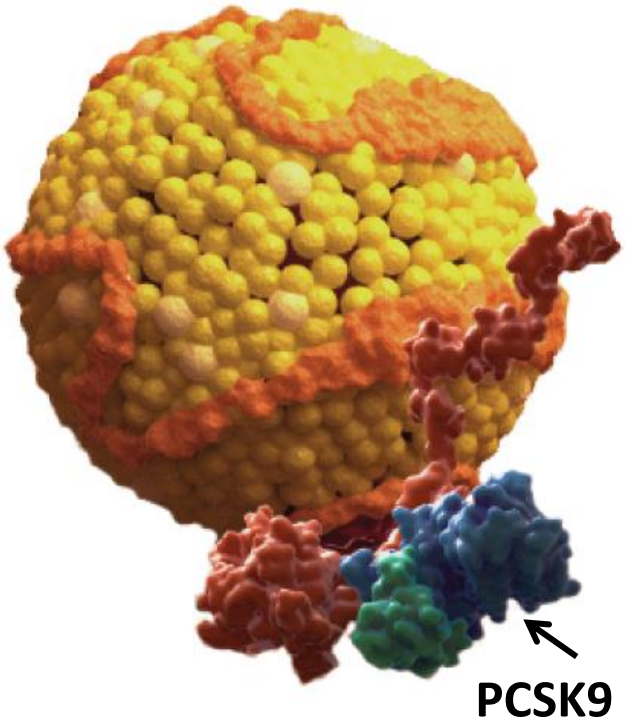
A century of cholesterol research

- Our knowledge of cholesterol's role and trafficking mechanisms has dramatically evolved over the last century
- Cholesterol plays a role in various vital physiologic functions
- Cellular cholesterol is minimally dependent on extracellular acquisition; and not at all in the brain
- LDL is an insignificant source of cholesterol for steroid hormone synthesis
- LDL carries cholesterol destined for excretion

Recent Discovery in Cholesterol Metabolism – The Role of PCSK9

A Powerful Revelation

PCSK9 Is a Regulator of LDL Metabolism

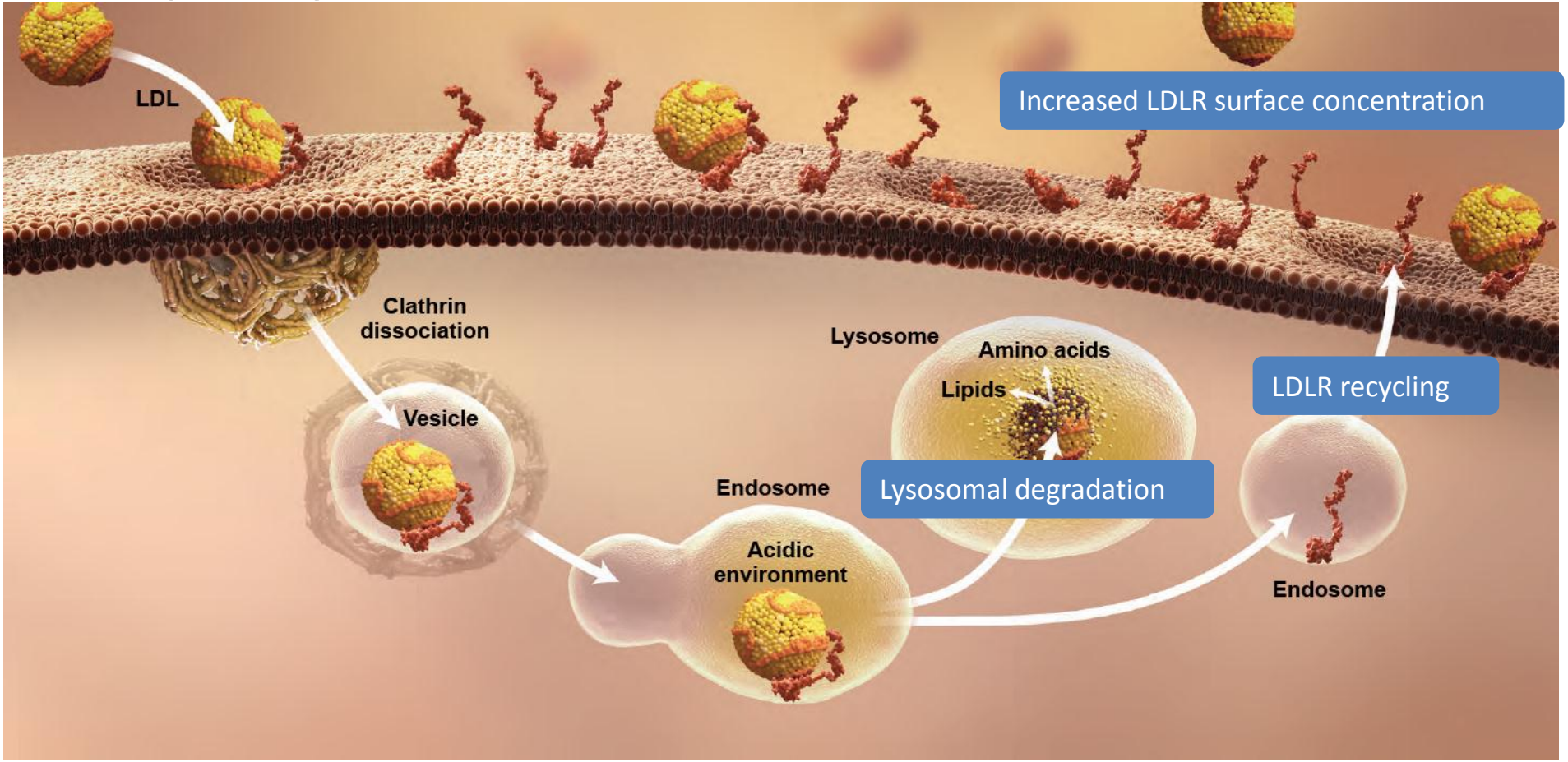


PCSK9

- **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, Witztum JL. *Proc Natl Acad Sci U S A.* 2009;106:9546-9547.

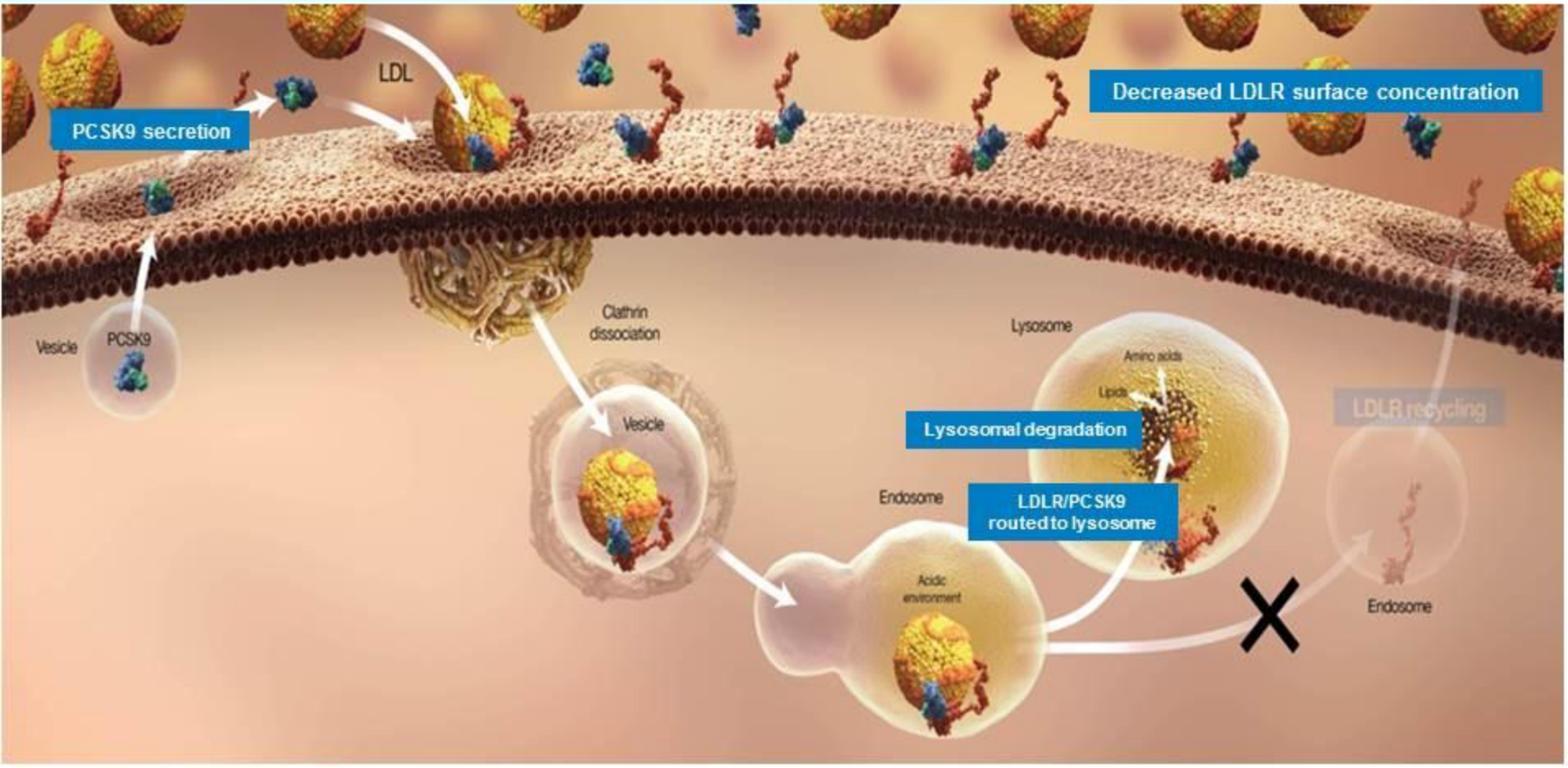
LDL Particles Are Cleared From the Plasma by Binding to LDL Receptors and Being Internalized by the Hepatocyte[1-3]



Recycled LDL receptors continue to clear plasma LDL

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

PCSK9 Regulates the Surface Expression of LDLRs by Targeting for Lysosomal Degradation



PCSK9 = proprotein convertase subtilisin/kexin type 9

1. Qian YW, et al. *J Lipid Res*. 2007;48:1488-1498. **2.** Horton JD, et al. *J Lipid Res*. 2009;50:S172-S177. **3.** Brown MS, et al. *Proc Natl Acad Sci*. 1979;76:3330-3337. **4.** Steinberg D, et al. *Proc Natl Acad Sci*. 2009;106:9546-9547. **5.** Goldstein JL, et al. *Arterioscler Thromb Vasc Biol*. 2009;29:431-438. **6.** Zhang DW, et al. *J Biol Chem*. 2007;282:18602-18612.

Genetic Variants Establish PCSK9 as a Modulator of LDL-C

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

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]

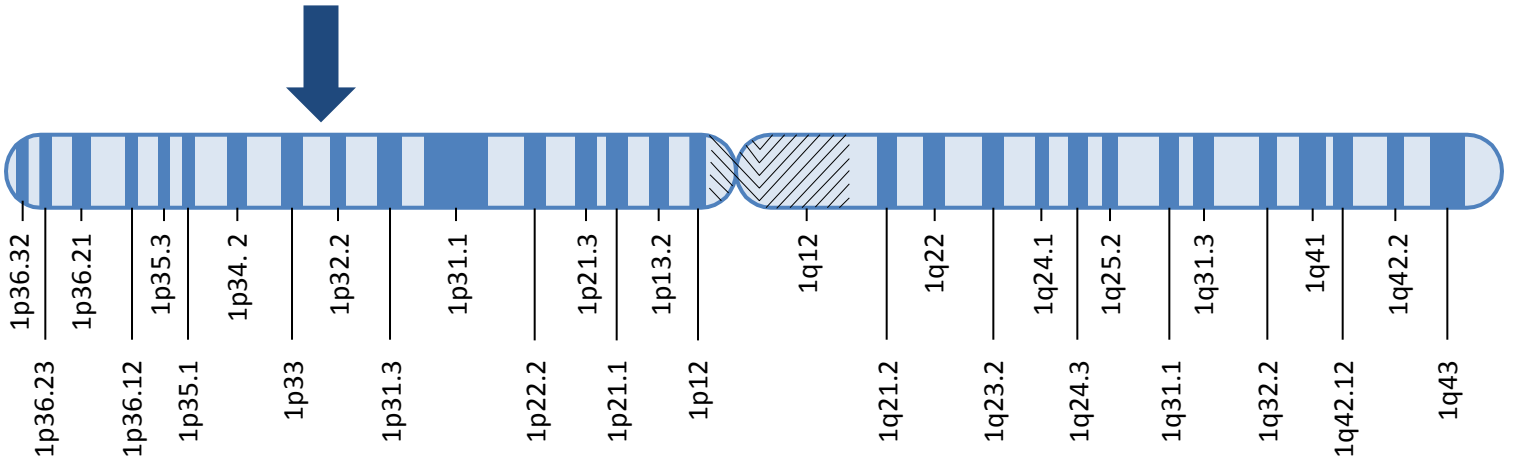
FH = familial hypercholesterolemia; GOF = gain of function; LDL-C = low-density lipoprotein cholesterol; LOF = loss of function; TC = total cholesterol.

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.

Gene for PCSK9 Associated With Hypercholesterolemia in 2003

- In 2003, Nabil Seidah was studying a protease called NARC-1 that mapped to the short arm of chromosome 1[1,2]
- Catherine Boileau's group in Paris was following families with FH[1,2]
  - Spent five years looking for gene linked to FH that mapped to same region
  - Nabil Seidah suggested that the gene encoding NARC-1 might be that gene

NARC-1 Location on Chromosome 1[3]

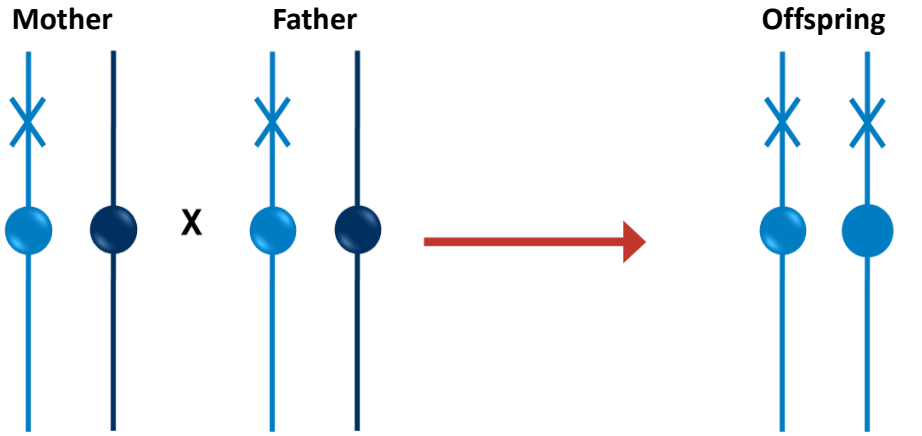


NARC = neural apoptosis-regulated convertase; PCSK9 = proprotein convertase subtilisin/kexin type 9.

1. Abifadel M, et al. *Hum Mutat*. 2009;30:520-529. 2. Hall SS. *Nature.* 2013;496:152-155. 3. Figure reprinted from Genetics Home Reference. Available at: http://ghr.nlm.nih.gov/gene/PCSK9; published October 26, 2015. Accessed January 16, 2016.

Familial Hypercholesterolemia (FH)

- Individuals with FH have inherited genetic differences that result in significantly elevated LDL-C levels[1,2]



FH Heterozygotes

- **~ 1 in 200 to 1:500 persons worldwide[1,2]**
- **1 mutated allele[1]**
- **TC: 350–500 mg/dL[3]**
- **LDL-C: 200–400 mg/dL[1,4]**

FH Homozygote

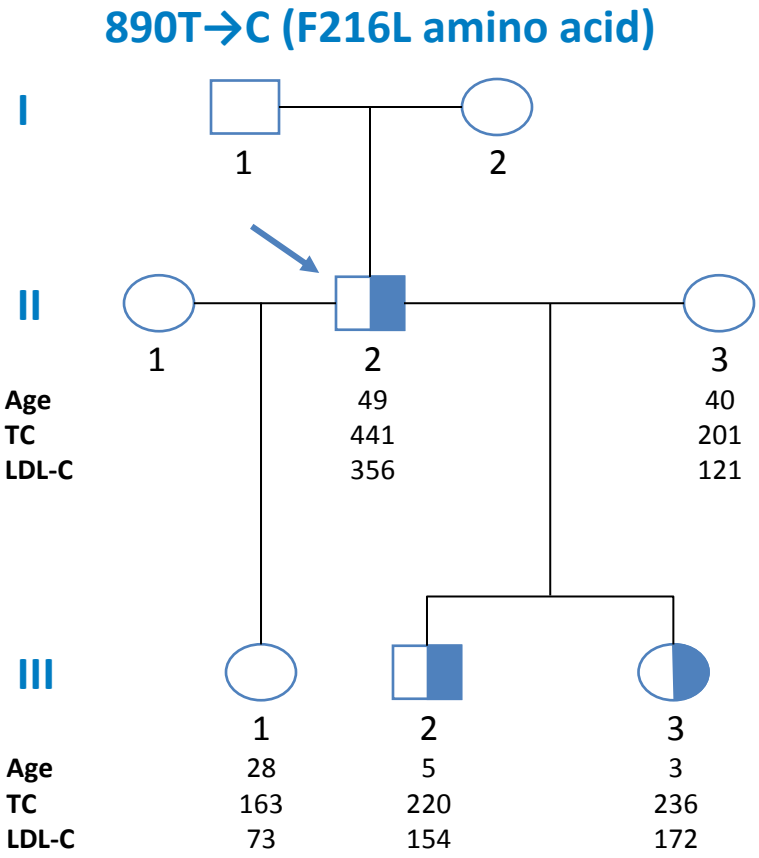
- **~ 1 in 160,000 to 1,000,000 persons worldwide[1]**
- **2 mutated alleles[1]**
- **TC: > 500 to > 1,000 mg/dL[1]**
- **LDL-C: > 300 mg/dL[4-6]**

- Mutations in *LDLR* and *APOB* genes are the most common cause of FH[7]

1. Rader DJ, Hobbs HH. In: Longo DL, et al, eds. Harrison's Principles of Internal Medicine. Vol I.18th ed. 2012:1-22.
2. Nordestgaard BG, et al. *Eur Heart J*. 2013;34:3478–3490a. 3. National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Final Report. *Circulation*. 2002;106:3143-3421. 4. Robinson JG. *J Manag Care Pharm.* 2013;19:139-149. 5. Raal FJ, et al. *Atherosclerosis.* 2012;223:262-268. 6. Sjouke B, et al. *Eur Heart J.* 2014;doi:10.1093/eurheartj/ehu058. 7. Abifadel M, et al. *Hum Mutat*. 2009;30:520-529.

PCSK9 Mutations Associated With Familial Hypercholesterolemia

- In 2003 Marianne Abifadel, a member of Dr. Boileau's team, performed positional cloning[1]
  - Using families from the French Research Network for ADH
  - Identified the third gene implicated in FH to be *PCSK9*
  - NARC-1 was more precisely characterized as the ninth member of subtilisin family of kexin-like proconvertases, or PCSK9
- Found 2 mutations in the gene *PCSK9* associated with FH*[2]

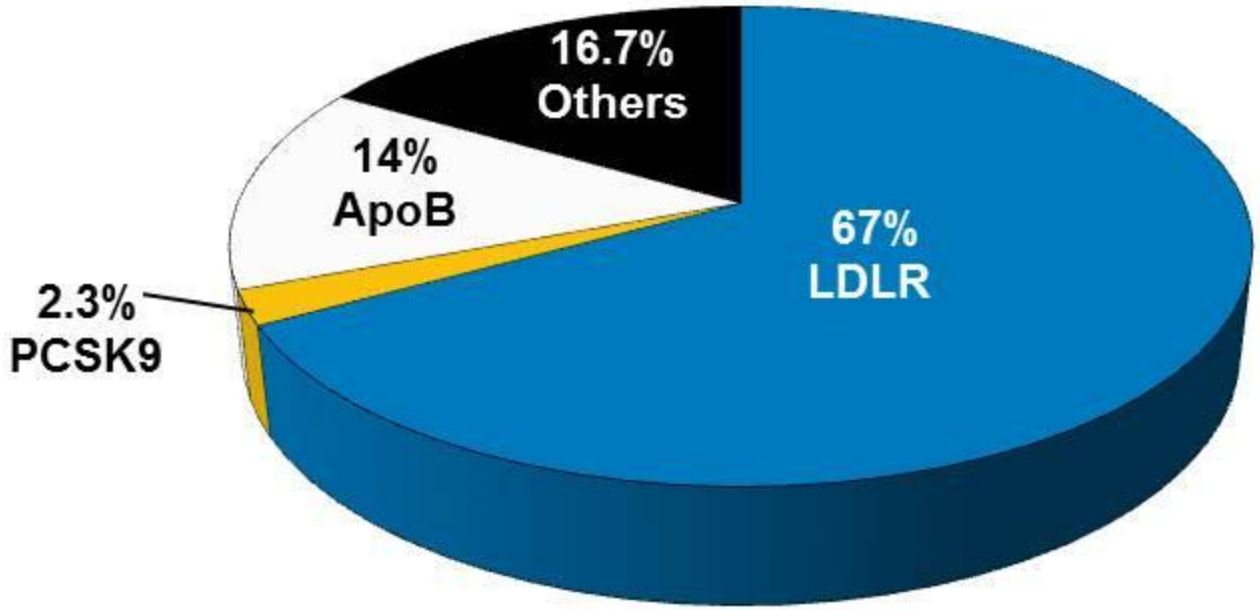


Filled bars indicate the mutated allele.
Age (in years) at lipid measurement, TC, and LDL-C (in mg/dL; untreated values for affected individuals) are given.
*ADH.
ADH = autosomal dominant hypercholesterolemia; FH = familial hypercholesterolemia; LDL-C = low-density lipoprotein cholesterol; TC = total cholesterol.

1. Abifadel M, et al. *Hum Mutat*. 2009;30:520-529. 2. Abifadel M, et al. *Nat Genet.* 2003;34:154-156.

PCSK9 Mutations Are Involved in Familial Hypercholesterolemia

- Familial hypercholesterolemia (FH) characterized by[1]:
  - Severely elevated LDL-C levels
- Mutations of three genes are primarily responsible for FH*[2]



*Autosomal Dominant Hypercholesterolemia form of FH

1. van der Graaf A, et al. *Circulation*. 2011;123:1167-1173. **2.** Seidah NG, et al. *J Mol Med*. 2007;85:685-696.

PCSK9 GOF Mutations Associated With FH*[1]

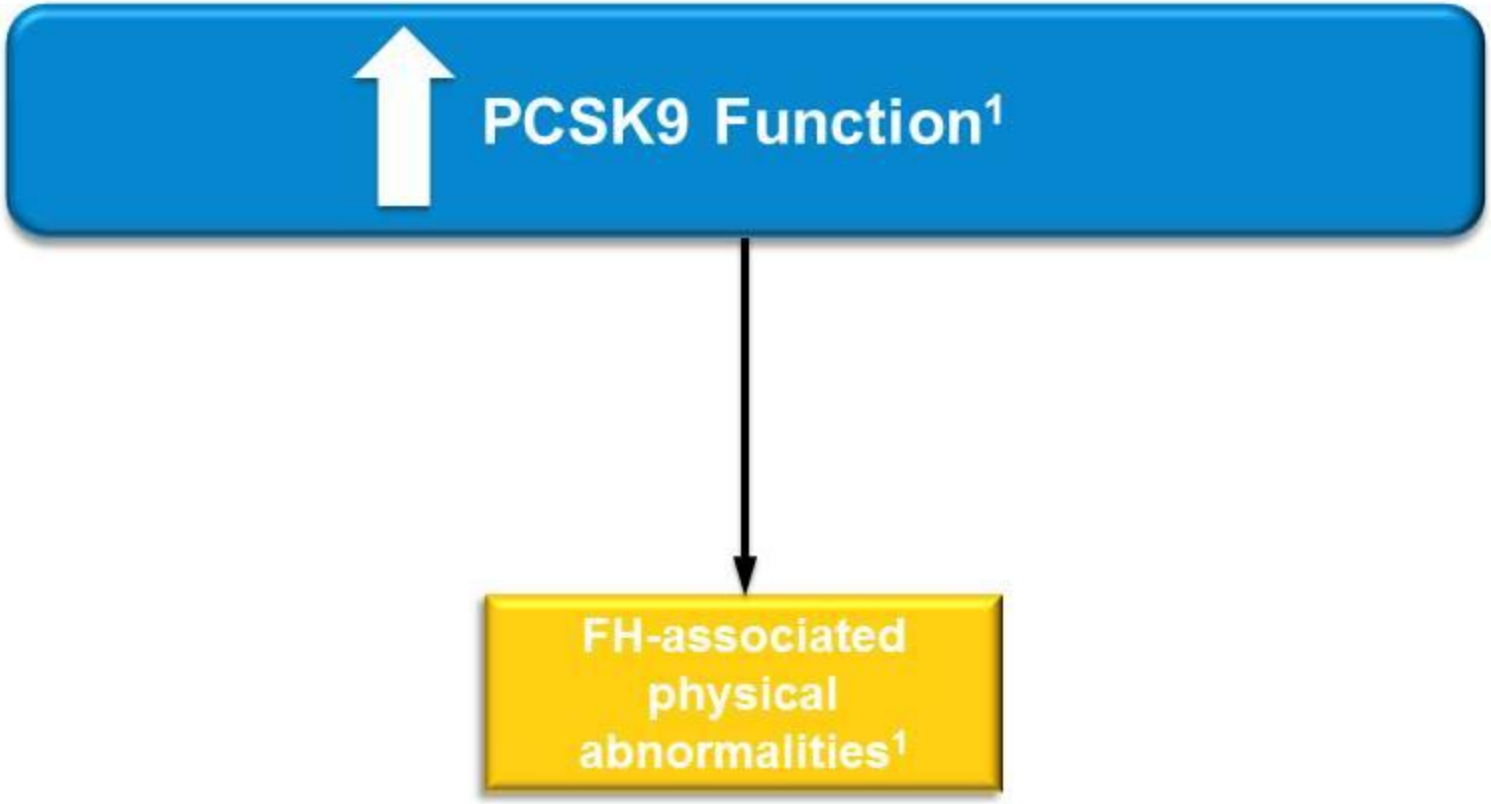
PCSK9 Genotype	Mutation Type	Biochemical Phenotype	Clinical/Biochemical Phenotype
S127R	Missense	5x higher affinity for LDLR; decreased LDLR expression and activity; may interfere with trafficking of LDLR to the cell surface[1,2]	Cholesterol levels in 90th percentile; tendon xanthomas[3]
D129G	Missense	Leads to decreased LDLR expression and activity[1]	Elevated LDL-C[1]
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 LDLR; decreased LDLR recycling and increased degradation[1,5]	Tendon xanthomas[4]

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

*Autosomal Dominant Hypercholesterolemia form of FH

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.

Clinical Outcomes Associated With Genetic Mutations for Gain of PCSK9 Function



1. Abifadel M, et al. In: Toth PP. *The Year in Lipid Disorders.* Vol. 2. Oxford, UK: Atlas Medical Publishing Ltd. 2010:3-23.

Case Reports Highlight Hypercholesterolemia Associated With PCSK9 GOF Mutations

F216L mutation[1,2]

French proband

Age: 49 years

TC: 441 mg/dL

LDL-C: 356 mg/dL

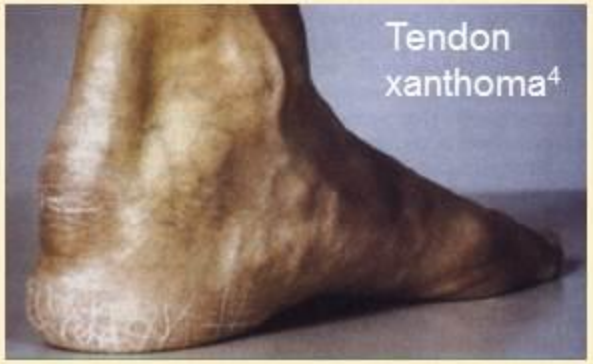
R218S mutation[3]

French proband presented with tendinous xanthoma and arcus corneae

Age: 45 years

TC: 402 mg/dL

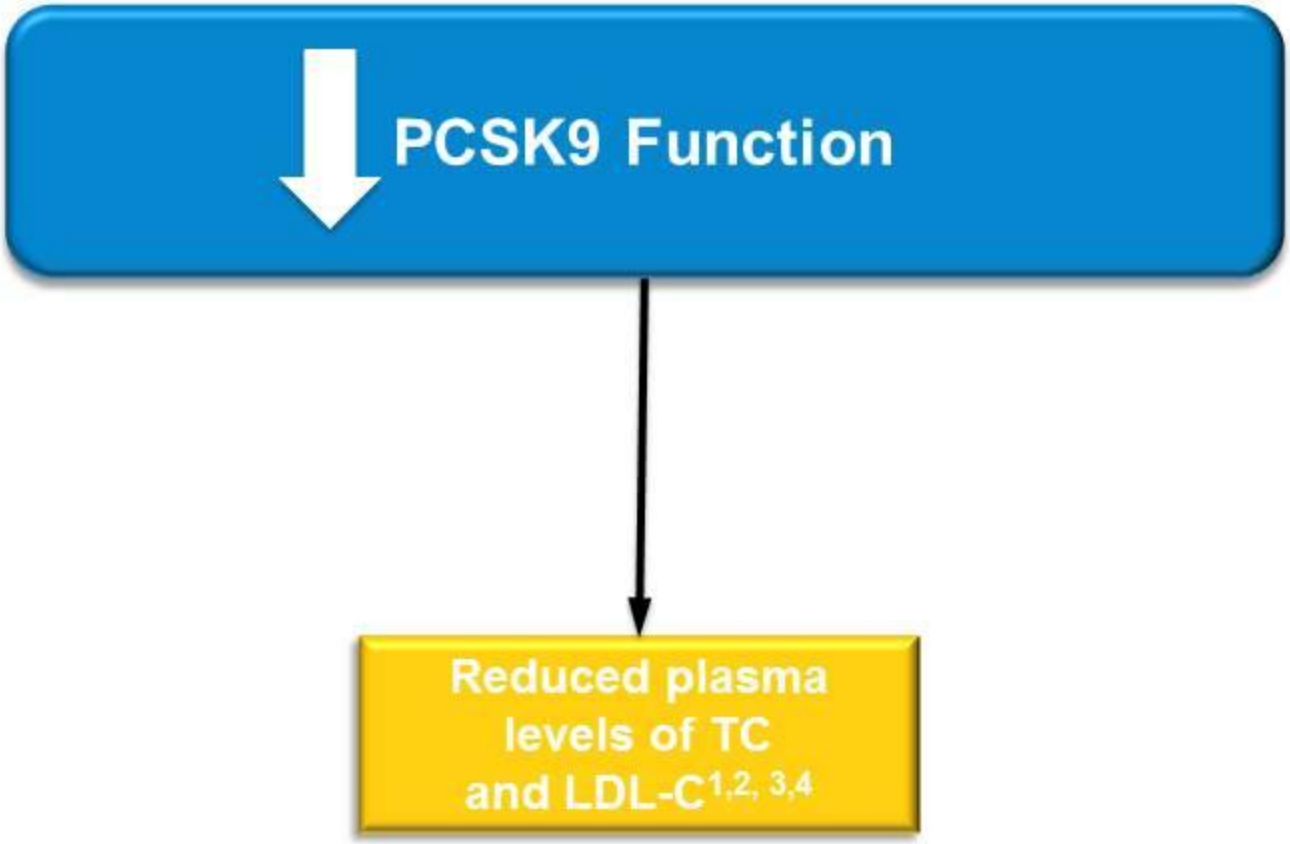
LDL-C: 293 mg/dL



TC = total cholesterol

1. Abifadel M, et al. *Nat Genet*. 2003;34:154-156. **2.** Abifadel M, et al. In: Toth PP. *The Year in Lipid Disorders*. Vol. 2. Oxford, UK: Atlas Medical Publishing Ltd. 2010:3-23. **3.** Abifadel M, et al. *Hum Mutat*. 2009;30:520-529. **4.** Durrington P. *Lancet*. 2003;362:717-731.

Clinical Outcomes Associated With Genetic Mutations for Loss of PCSK9 Function



1. Abifadel M, et al. *Hum Mutat*. 2009;30:520-529. **2.** Abifadel M, et al. *Hum Mutat*. 2009;30:supplementary information. **3.** Abifadel M, et al. In: Toth PP. *The Year in Lipid Disorders*. Vol. 2. Oxford, UK: Atlas Medical Publishing Ltd. 2010:3-23. **4.** Benn M, et al. *J Am Coll Cardiol*. 2010;55:2833:2842.

The NEW ENGLAND JOURNAL of MEDICINE

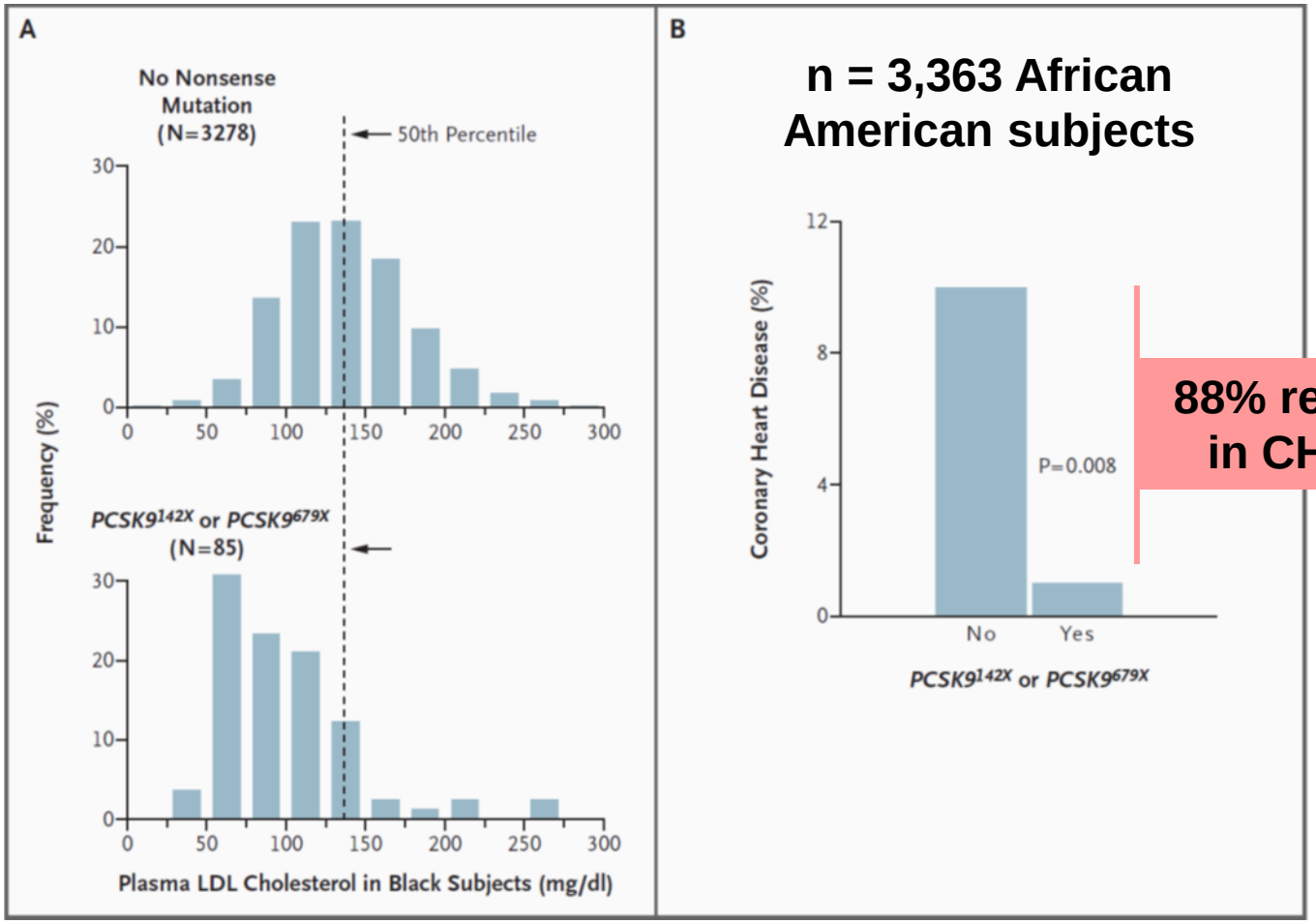
ORIGINAL ARTICLE

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

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

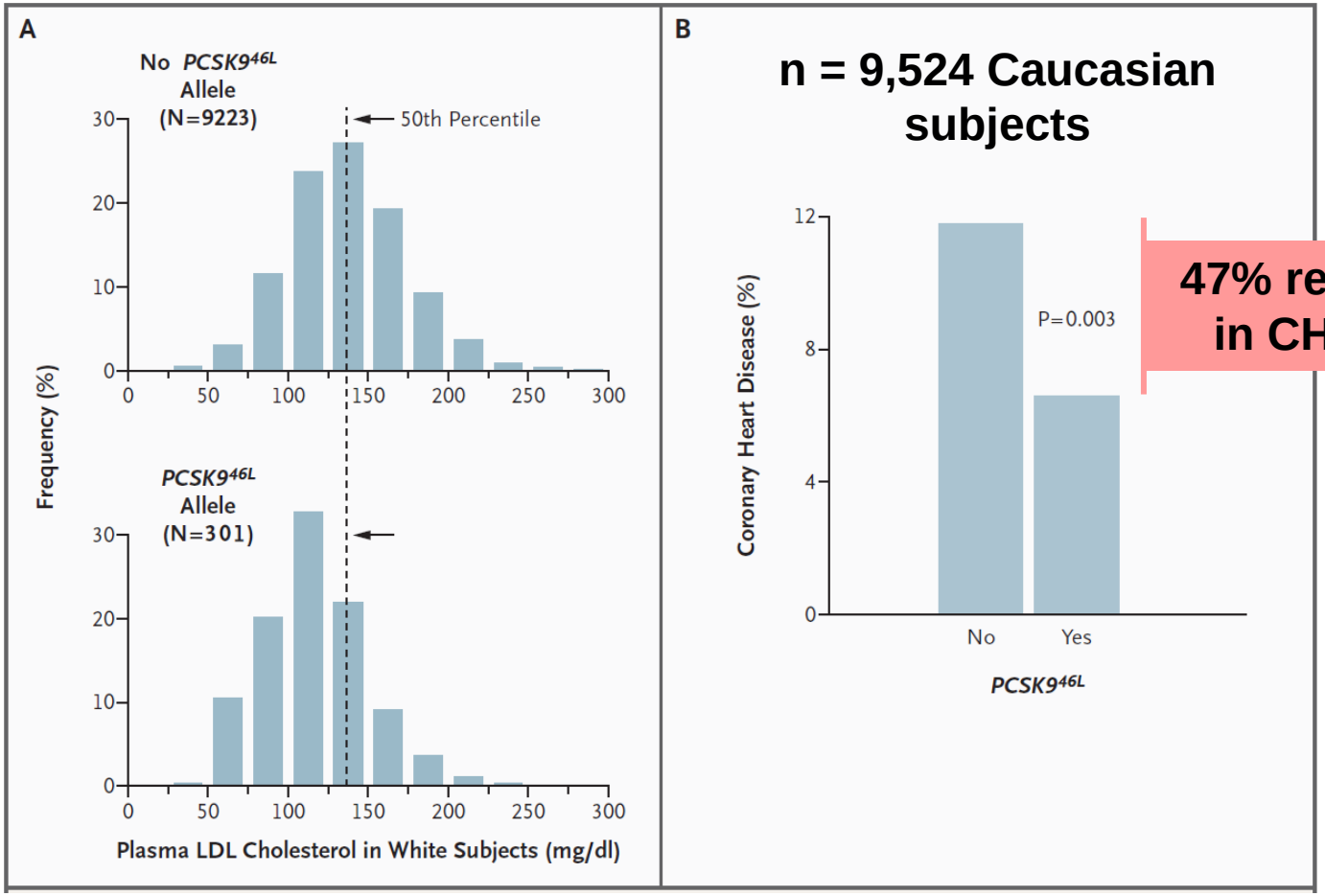
Compared the incidence of CHD (MI, fatal CHD, or coronary revascularization) over a 15-year interval in the ARIC study according to the presence or absence of sequence variants in the PCSK9 gene that are associated with reduced plasma levels of LDL cholesterol.

Plasma LDL cholesterol levels and incidence of CHD in African Americans



Cohen JC et al. *N Engl J Med*. 2006; 354: 1264-1272.

Plasma LDL cholesterol levels and incidence of CHD in Caucasians



Cohen JC et al. *N Engl J Med*. 2006; 354: 1264-1272.

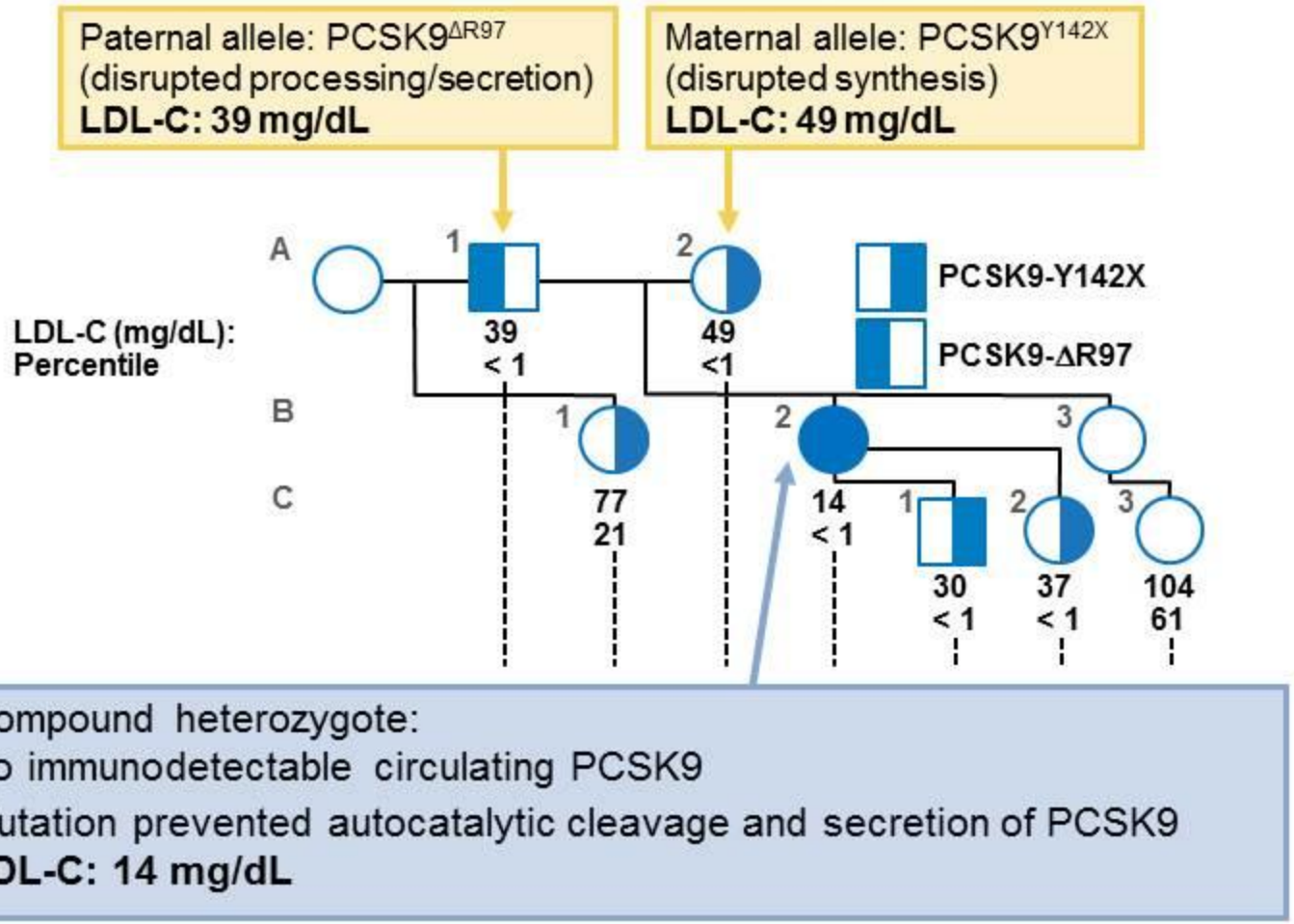
PCSK9 LOF Mutations and Variants Associated With Hypocholesterolemia

PCSK9 Genotype	Mutation Type	Biochemical Phenotype	Clinical/Biochemical Phenotype
R46L	Missense Polymorphism	No effect on processing or secretion[1]	11%–16% reduction in LDL-C[2]
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[1]
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[3,5]
A443T	Missense Polymorphism	Normally cleaved and secreted; higher susceptibility to cleavage[1]	Modest (2%) reduction in LDL-C[6]
Q554E	Missense	Poorly cleaved and secreted[1]	Reduced LDL-C[7]
C679X	Nonsense	Disrupted protein folding; impaired protein secretion[1]	40% reduction in LDL-C[1]

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.** Benn M, et al. *J Am Coll Cardiol*. 2010;55:2833-2842.
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. Abifadel M, et al. *Hum Mutat*. 2009;30:520-529. **6.** Zhao Z, et al. *Am J Hum Genet*. 2006;79:514-523.
7. Abifadel M, et al. In: Toth PP. *The Year in Lipid Disorders*. Vol. 2. Oxford, UK: Atlas Medical Publishing Ltd. 2010:3-23.

PCSK9 LOF Compound Heterozygote With No Detectable Circulating PCSK9



Adapted from Zhao Z, et al. *Am J Hum Genet*. 2006;79:514-523.

Summary

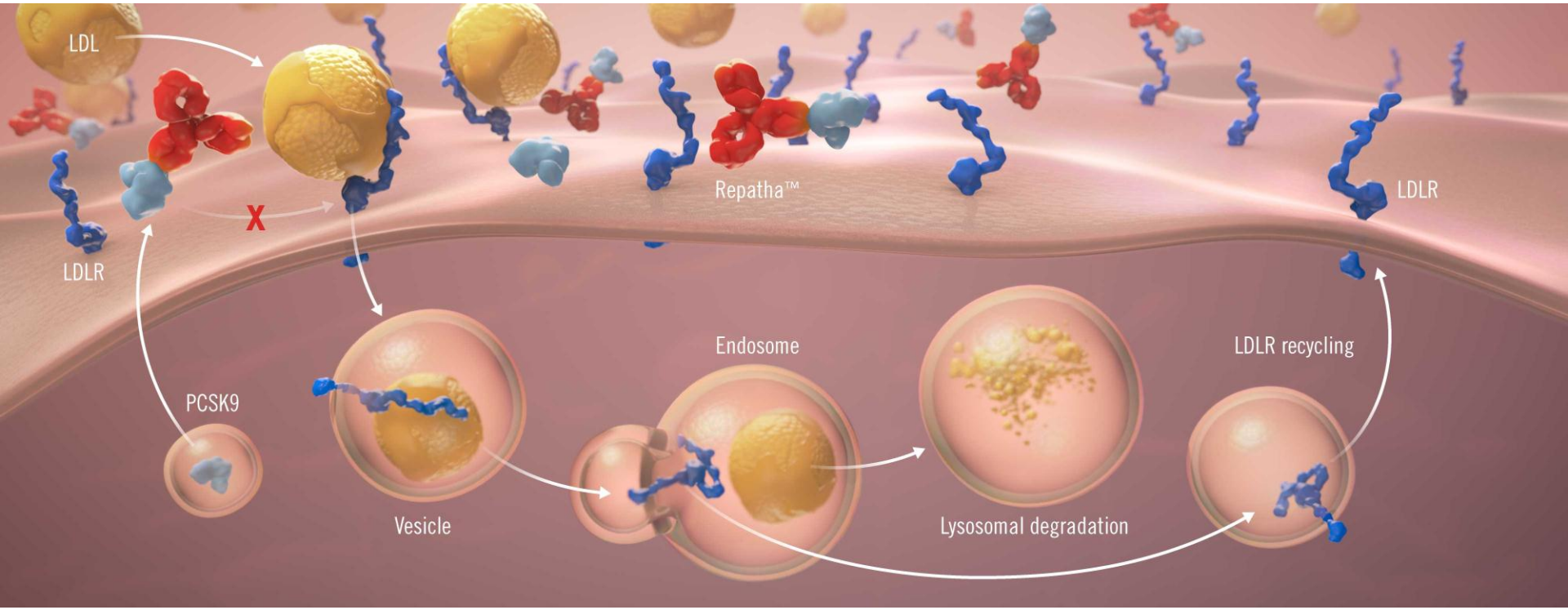
- PCSK9 regulates hepatic surface expression of LDLR and, subsequently, systemic LDL-C levels
- Mutations in PCSK9 are associated with effects on LDL-C levels which in turn translate into effects on incident ASCVD

PCSK9 Inhibitors: A revolution in lipid control

A new era of LDL-C reduction: PCSK9i

- **Evolocumab** and **Alirocumab**
  - Both are human monoclonal antibodies that bind to PCSK9 and inhibit its interaction with LDL receptors
  - When PCSK9 binds to the LDL receptor, the receptor is degraded and can no longer remove LDL cholesterol from the blood.
  - If PCSK9 is blocked, more LDL receptors will be present on the surface of the liver which will remove more LDL cholesterol from the blood.
  - Both FDA approved for clinical ASCVD & HeFH when maximally tolerated statin is insufficient

PCSK9i Is a Human mAb Directed Against PCSK9 and Inhibits PCSK9/LDLR Interaction Preventing Degradation of the LDLR Thereby Reducing Serum LDL-C Levels

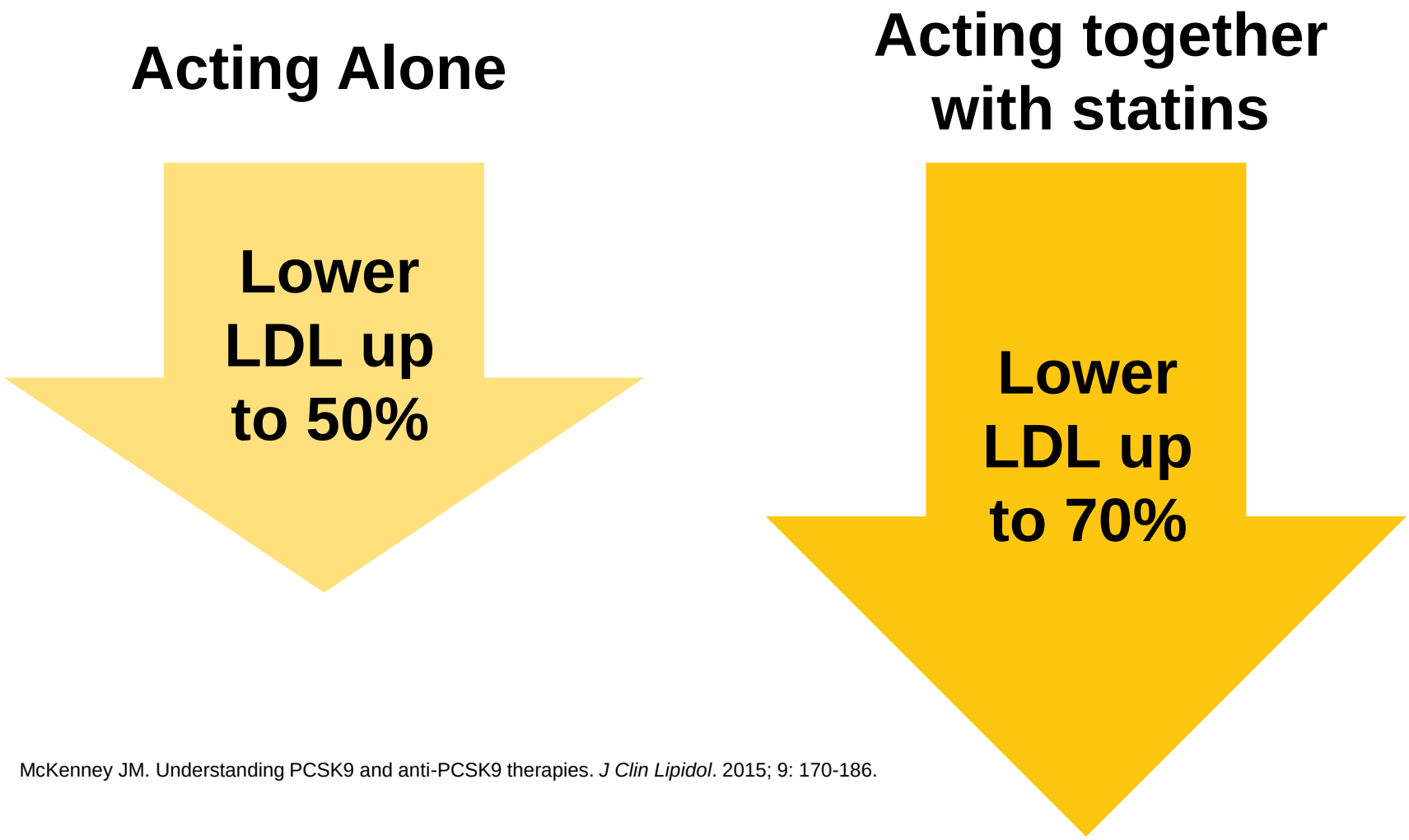


PCSK9i, a human mAb directed against PCSK9, binds to PCSK9 and inhibits circulating PCSK9 from binding to the LDLR, preventing PCSK9 mediated LDLR degradation and permitting the LDLR to recycle back to the liver cell surface. The increased number of LDLRs available to clear LDL from the blood results in lower LDL-C levels.

mAb = monoclonal antibody PCSK9 = Proprotein convertase subtilisin/kexin type 9; LDL = low density lipoprotein; LDLR = low density lipoprotein receptor

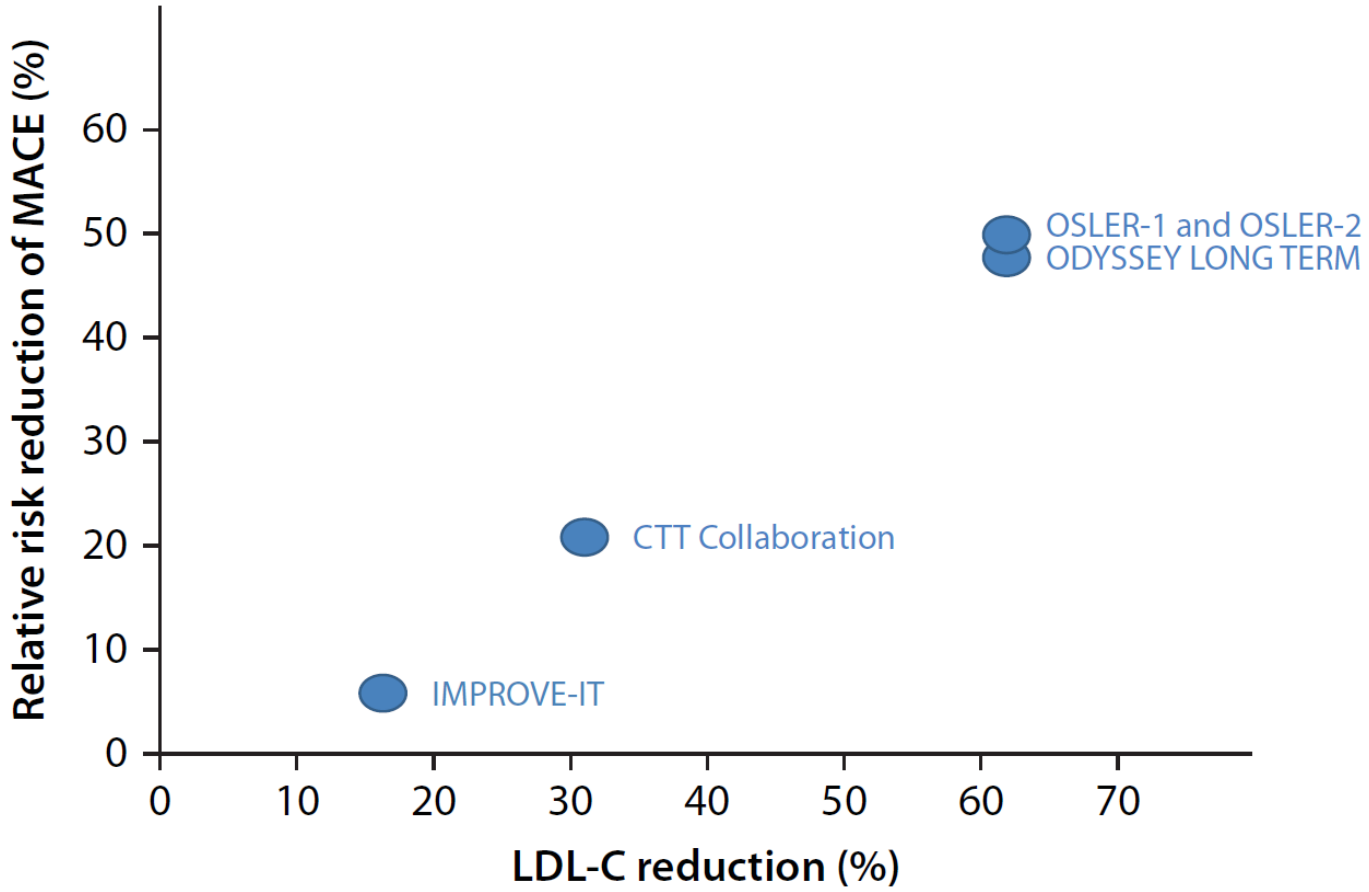
1. Repatha™ (evolocumab) Prescribing Information v2, Amgen. 2. Kwon HJ, et al. *Proc Natl Acad Sci U S A.* 2008;105:1820-1825. 3. Nassoury N, et al. *Traffic.* 2007;8:718-732. 4. McNutt MC, et al. *J Biol Chem.* 2009;284:10561-10570. 5. Chan JC, et al. *Proc Natl Acad Sci U S A.* 2009;106:9820-9825.

Both PCSK9i intensively and predictably reduce LDL-C, either alone or when added to statin therapy in short term studies



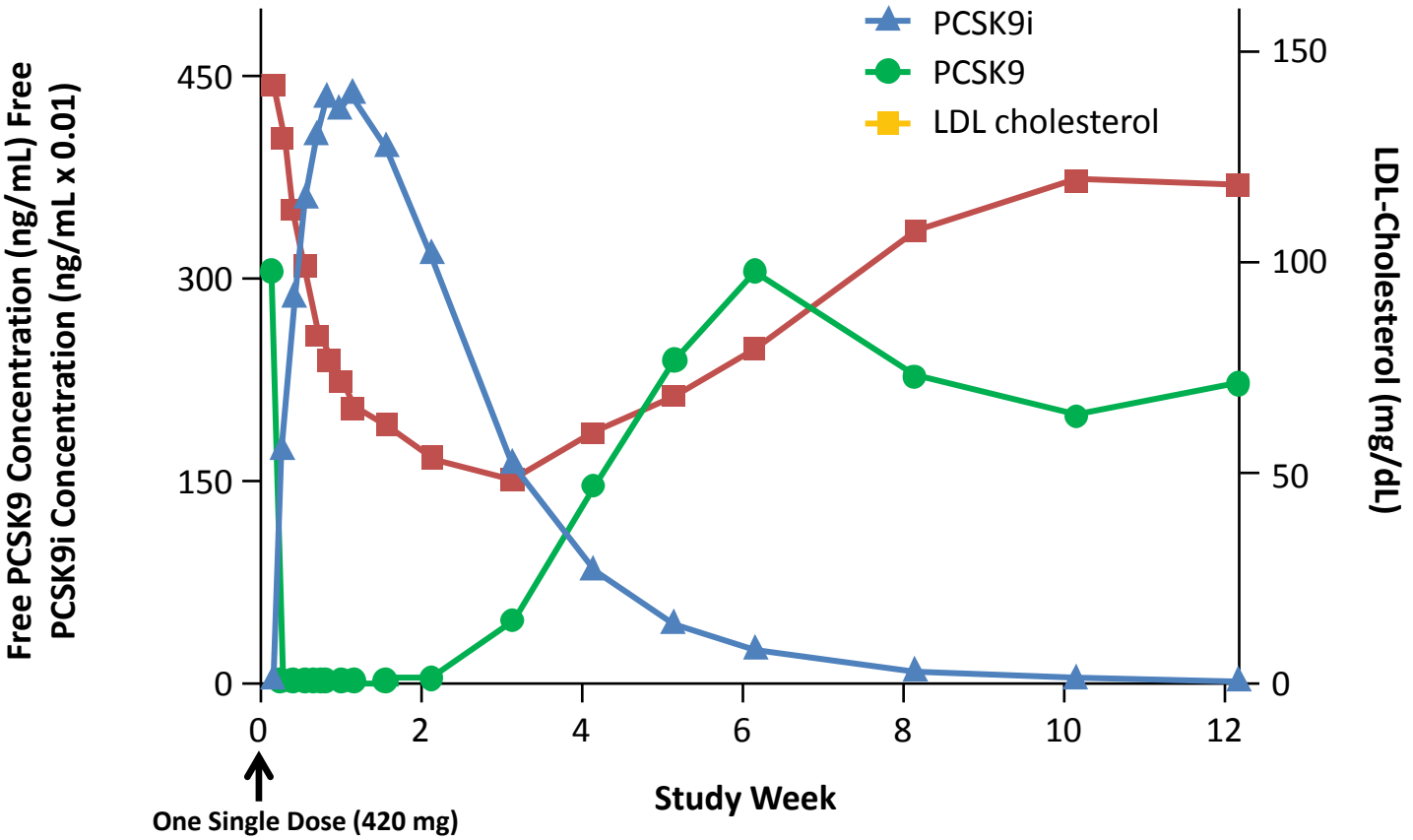
McKenney JM. Understanding PCSK9 and anti-PCSK9 therapies. *J Clin Lipidol*. 2015; 9: 170-186.

Effectiveness of LDL-lowering therapies (statins, ezetimibe and PCSK9i)



Hassan M. *Global Cardiology Science and Practice. 2015: 20:.*

Pharmacokinetics and Pharmacodynamics



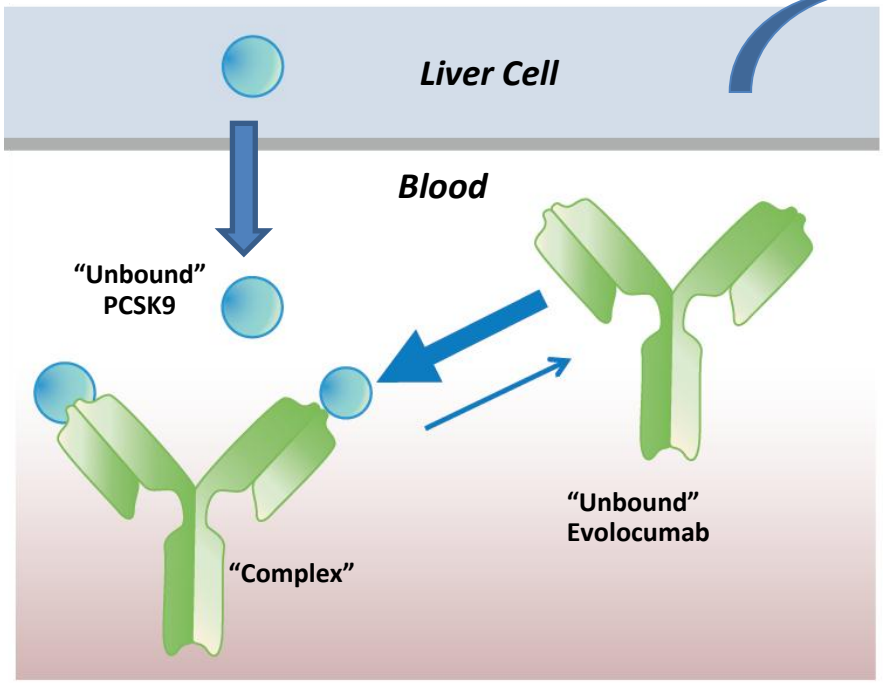
- **PCSK9i single SC administration of 140 mg or 420 mg results in maximum suppression of circulating unbound PCSK9 by 4 hours**
- **Unbound PCSK9 concentrations return towards baseline when PCSK9i concentrations decrease below the limit of quantitation**

LDL-C = low-density lipoprotein cholesterol.

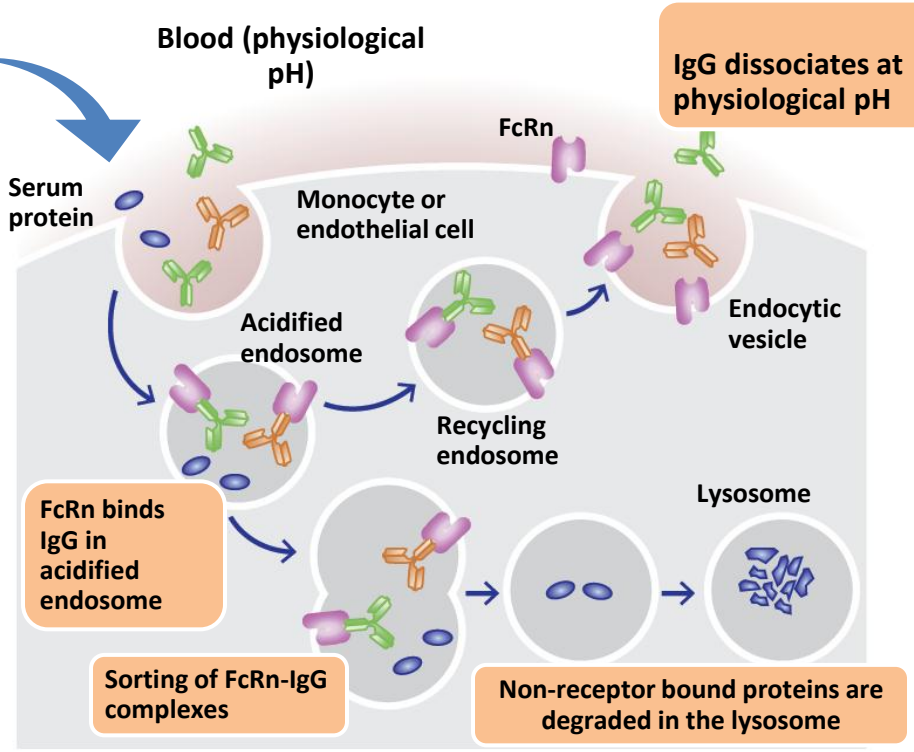
Adapted from Stein AE, et al, *Drugs of the Future.* 2013;38(7):451-459. 2. Repatha™ (evolocumab) Prescribing Information v2, Amgen.

Two mechanisms of PCSK9i *clearance*

Nonlinear Pathway ("Target-Mediated")



Linear Pathway ("Reticuloendothelial System (RES)"*)



The non-linear pathway is saturable (when all PCSK9 is bound), resulting in faster elimination at lower concentrations

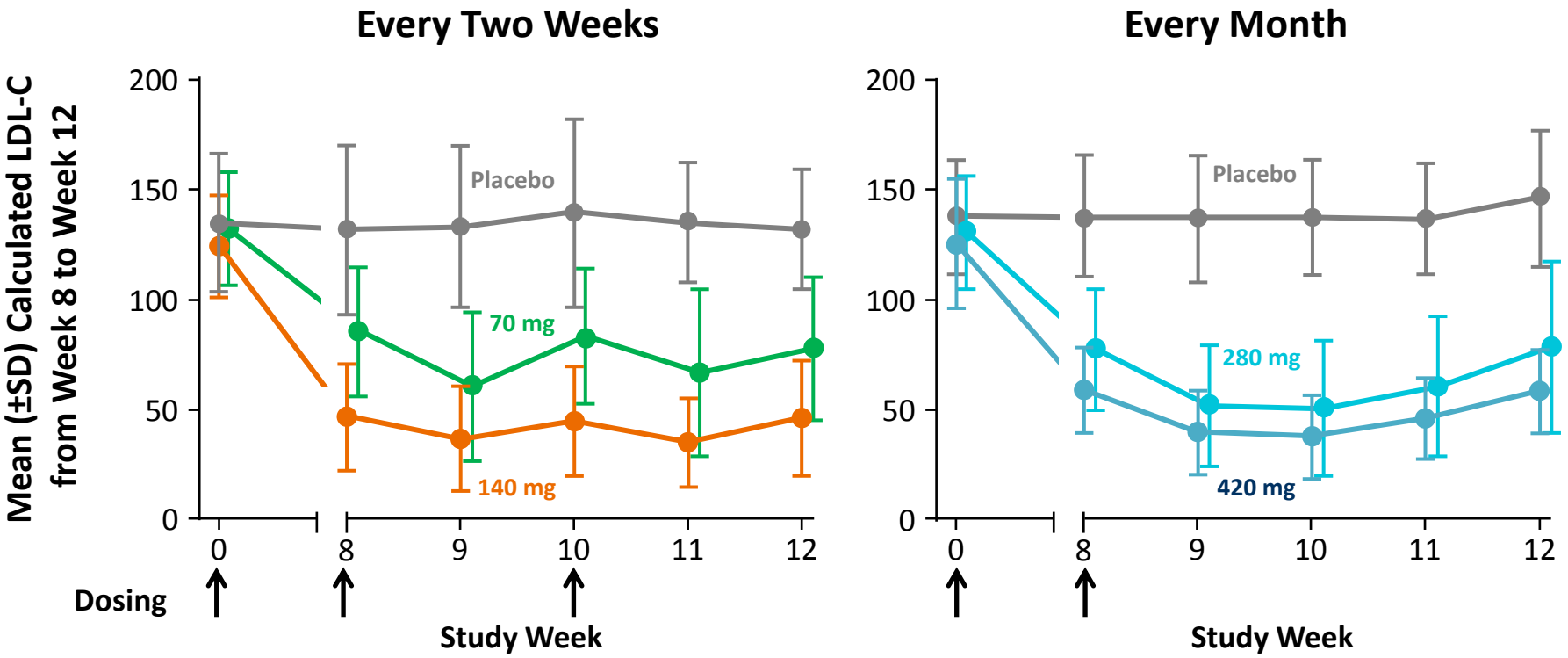
* non-saturable proteolytic pathway

FcRn = neonatal Fc receptor; IgG = immunoglobulin G; PCSK9 = proprotein convertase subtilisin/kexin type 9

1. Repatha™ (evolocumab) Prescribing Information v2, Amgen. 2. Roopenian DC, et al. *Nat Rev Immunol*. 2007;7:715-725. 3. Langslet G, et al. *Expert Rev Cardiovasc Ther*. 2015; 13(5):477-488.

PCSK9 Inhibitor

Dose Selection



Dose regimens of 140 mg every 2 weeks and 420 mg once monthly were identified as the appropriate regimens to achieve LDL-C lowering with less intrapatient and interpatient variability, when compared to lower doses

Wasserman SM, et al. Integrated analysis of Phase 2 substudy. Poster presented at: 2014 American Heart Association Scientific Sessions; November 15-19, 2014; Chicago, IL.

PCSK9i are effective; yet, there is enormous insurance pushback

- Despite being effective at lowering LDL-C, PCSK9i are expensive (cost roughly $14,000/year).
- Insurance barriers are among the top reasons for not prescribing the PCSK9 inhibitors
  - Either insurer does not cover one or both drugs, or
  - Insurer requires patients to first try other medicines (prior authorization)
  - To date, 90% of prescriptions have been denied!

Summary

- PCSK9 regulates hepatic surface expression of LDLR and, in turn, systemic LDL-C levels
- Mutations in PCSK9 (both GOF and LOF) influence LDL-C levels as well as ASCVD risk
- The PCSK9 inhibitors represent the realization of a prior revelation: PCSK9 represents an excellent therapeutic target for LDL reduction