Expression of LDLRs (Low-Density Lipoprotein Receptors), Dyslipidemia Severity, and Response to PCSK9 (Proprotein Convertase Subtilisin Kexin Type 9) Inhibition in Homozygous Familial Hypercholesterolemia

Connecting the Dots

Raul D. Santos

Homozygous familial hypercholesterolemia (HoFH) is characterized by extremely elevated low-density lipoprotein cholesterol (LDL-C) levels (usually 4–5-fold normal) and appearance of xanthomas, aggressive atherosclerotic cardiovascular as well as aortic and supra-aortic valve diseases, before the age of 20 years.[1] The severity of the HoFH phenotype and its ominous consequences correlate with LDL-C levels. The latter are influenced primarily by the type of familial hypercholesterolemia (FH)-causing molecular defect. The gravest phenotypes result from homozygous or compound heterozygous mutations in the *LDLR* gene (low-density lipoprotein receptor) encoding null or negative alleles that are associated with <2% of LDLR activity. Less severe forms are encountered in homozygotes or compound heterozygotes for *LDLR*-defective alleles encoding LDLRs with 2% to 25% activity, or variants in other genes, such as *APOB* (apolipoprotein B), *PCSK9* (proprotein convertase subtilisin kexin type 9), or rarely *LDLRAP1* (LDL receptor adaptor protein-1).[2]

See accompanying article on page 592

HoFH patients have been treated mainly with statins, ezetimibe, and, when available, lipoprotein apheresis.[2] Unfortunately, despite best efforts with these traditional therapies, individuals with HoFH remain severely undertreated and seldom achieve target or even acceptable LDL-C levels. The residual LDLR expression and consequent function certainly play a role in LDL-C reduction induced by statins.[3] For instance, Stein et al[3] encountered significantly greater LDL-C–lowering effects of rosuvastatin in HoFH patients who had receptor-defective alleles in comparison to those with receptor-negative ones, namely, 23% and 14% lowering, respectively. On the contrary, despite effectiveness of ezetimibe in reducing LDL-C in HoFH, it is uncertain whether this is affected by *LDLR* expression.[4–7] In theory, *LDLR* expression and function should not influence LDL-C lowering by mipomersen (an apoB synthesis inhibitor)[8] or lomitapide (a microsomal triglyceride transfer protein inhibitor)[9] that reduce production of LDL precursors (Figure), and this seems to be the case from small numbers of patients in clinical trials treated with these newer agents.

Monoclonal antibodies against PCSK9, like alirocumab and evolocumab, reduce PCSK9-mediated LDLR degradation and consequently lower LDL-C concentrations on average by 50% to 60% both in the general population and in patients with heterozygous FH (HeFH).[10,11] In the long-term open-label extension of TAUSSIG (Trial Assessing Long-Term Use of PCSK9 Inhibition in Subjects With Genetic LDL Disorders), evolocumab reduced LDL-C in HoFH patients on average by a lesser extent, ≈20%, but with wide variability as indicated by an SD of response of 24%. This means that some HoFH patients treated with evolocumab had >50% LDL-C reduction, whereas others did not respond to treatment at all.[12] Although subject numbers are limited, it has been suggested that HoFH patients who have 2 receptor-negative or null alleles have minimal to no response to PCSK9 inhibition, whereas those with at least 1 receptor-defective allele with residual LDL receptor function respond somewhat to these agents.[13] In addition, in TAUSSIG, in which >100 patients were treated, HoFH individuals who did not have 2 receptor-negative alleles also had variable responses to evolocumab treatment.[12] It was intriguing that heterogeneity in response also occurred among individuals who carried the identical molecular variants. Indeed, this variability in LDL-C response to PCSK9 inhibitors seems to occur not only in HoFH but also in those with HeFH[14] and even in individuals with moderate dyslipidemia, after accounting for nongenetic factors such as extremely uncommon antidrug antibodies that are known to attenuate treatment response.[15] Therefore, the ensuing question is because PCSK9 inhibitors reduce LDLR degradation, how do baseline LDLR expression and function modulate the LDL-C lowering effects of these drugs?

In this issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, Thedrez et al[16] report an elegant experiment evaluating the relationship between LDLR expression and lipid-lowering effects of PCSK9 inhibition. The authors compared ex vivo LDLR expression on the cell surface of lymphocytes (an accepted surrogate of hepatocyte LDLR expression)[17] in 22

From the Lipid Clinic Heart Institute (InCor), University of Sao Paulo Medical School Hospital, Brazil; and Preventive Medicine Centre and Cardiology Program, Hospital Israelita Albert Einstein, Sao Paulo, Brazil.

Correspondence to Raul D. Santos, MD, PhD, Unidade Clinica de Lípides InCor-Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo, Ave Doutor Enéas Carvalho de Aguiar 44, Bloco 2 Segundo Andar, Sala 4, CEP- 05403-900, São Paulo, Brazil. E-mail rdsf@uol.com.br

(*Arterioscler Thromb Vasc Biol*. 2018;38:481-483. DOI: 10.1161/ATVBAHA.117.310675.)

***Arterioscler Thromb Vasc Biol* is available at http://atvb.ahajournals.org**
DOI: 10.1161/ATVBAHA.117.310675

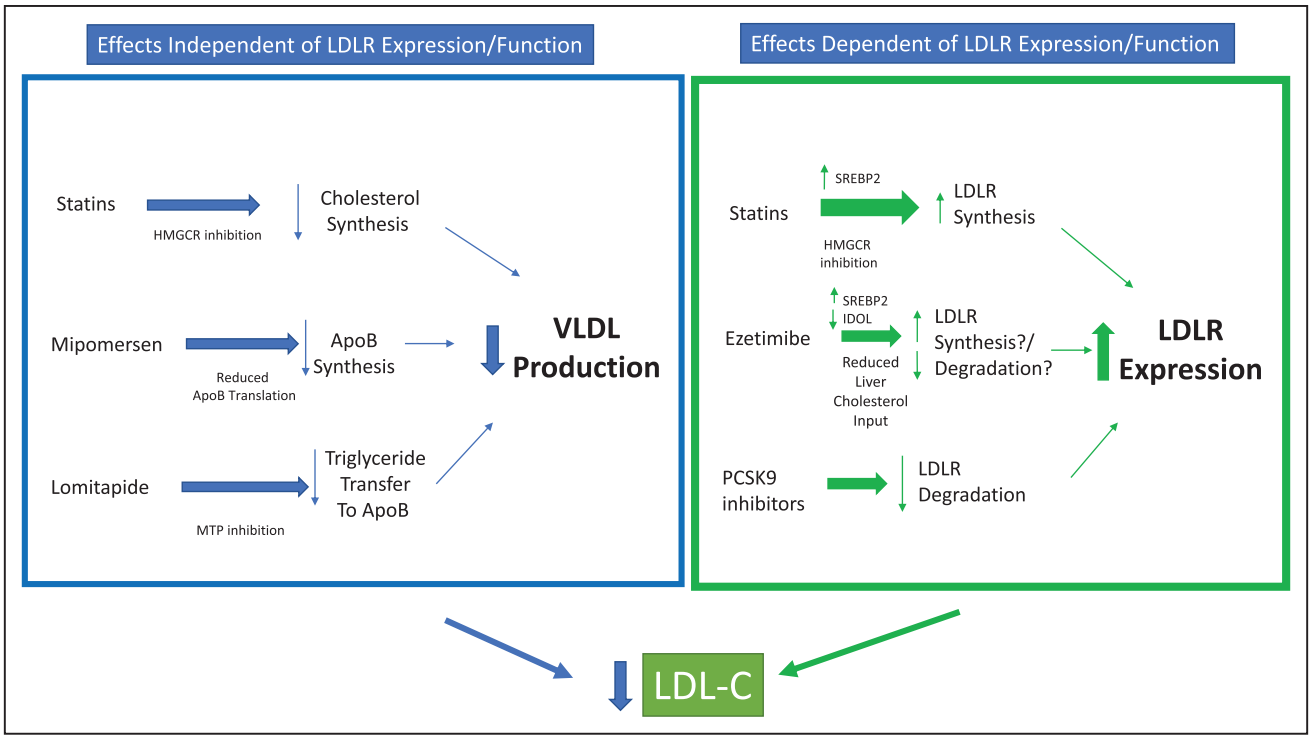

Figure. Mechanisms involved with low-density lipoprotein cholesterol (LDL-C) lowering by medications approved for homozygous familial hypercholesterolemia and their possible associations with LDLR (LDL receptor) expression/function. apoB indicates apolipoprotein B; HMGCR, 3-hydroxy-3-methylglutaryl-coenzyme reductase; IDOL, inducible degrader of LDL receptor; MTP, microsomal triglyceride transfer protein; PCSK9, proprotein convertase subtilisin kexin type 9; SREBP2, steroid regulatory element binding protein-2; and VLDL, very-low-density lipoprotein.

HoFH patients with 1 normolipidemic individual and 5 HeFH patients. Experiments followed a rigorous sequence previously used by the authors[18] that simulate lipid-lowering treatments usually prescribed to FH patients, including stimulation of LDLR expression by 3-hydroxy-3-methylglutaryl-coenzyme reductase inhibition with mevastatin, incubation with recombinant PCSK9 to facilitate LDLR degradation, and incubation with a PCSK9 neutralizing antibody. Ten patients were simple homozygotes for the same *LDLR* mutations, whereas 5 were identical compound heterozygotes and 1 was homozygous for a pathogenic *APOB* variant that reduced ligand affinity but did not affect receptor activity. There was a range of different *LDLR* mutations in the HeFH patients, whereas no mutations were found in controls. As expected, at baseline, lymphocytes from HoFH patients, except for those from the patient with the homozygous *APOB* mutation, had reduced cell surface LDLR expression compared with lymphocytes from both non-FH and HeFH. Lymphocytes from HoFH patients who carried at least 1 receptor-negative mutation showed the lowest LDLR expression, with greater expression seen in lymphocytes from patients with 2 receptor-defective alleles. Statin treatment increased, whereas PCSK9 incubation reduced, LDLR expression. The PCSK9-neutralizing antibody restored LDLR expression. For all experiments, effects on LDLR expression were attenuated in lymphocytes taken from individuals with homozygous receptor-negative mutations.

In addition, the authors were able to relate the ex vivo LDLR expression with the observed clinical impact of evolocumab treatment in the TAUSSIG. Lymphocytes that had higher LDLR expression came from patients who had the lowest plasma LDL-C and apoB concentrations, both before and after evolocumab treatment. LDLR expression was inversely correlated with baseline and on-treatment concentrations of LDL-C and apoB. Also, in a subset of individuals with the same molecular defect, there was a direct correlation between maximal LDLR expression and changes in plasma LDL-C concentrations after treatment. Interestingly, LDLR expression varied both before and after incubation with the PCSK9 inhibitor in individuals who carried the identical molecular defects, suggesting a modulatory role for nongenomic or post-translational factors.

Importantly, no consistent correlation was found between LDLR expression and Lp(a) [lipoprotein(a)] concentrations. The role of the LDLR and PCSK9 inhibition in Lp(a) clearance remains unclear and is still a matter of controversy, especially in FH individuals; further studies are necessary to clarify this issue.[19]

The findings of Thedrez et al[16] can be clinically translated to suggest that HoFH patients who have the most severe form of receptor defect (ie, receptor-negative mutations) had less LDLR expression and activity, and thus responded less or not at all to PCSK9 inhibition. The results indicate that baseline LDLR expression and function are important determinants of LDL-C lowering in HoFH.

The study is limited by the small number of subjects, a common feature in studies of HoFH patients, and by the fact that LDLR expression but not function or activity were evaluated. At any rate, the authors should be commended for

these mechanistic experiments that help us understand better how PCSK9 inhibitors work in particular clinical settings, such as in HoFH. However, there is still an unmet need for acquiring more complete understanding of all factors that underlie the variability of response to lipid-lowering treatments seen in patients with HoFH. In the age of precision medicine and high costs for interventions, it may prove to be worthwhile to determine the precise molecular diagnosis to efficiently select the most appropriate lipid-lowering therapies in patients with HoFH.

Disclosures

Dr Santos has received honoraria related to consulting, lectures, and research activities from Amgen, Astra Zeneca, Akcea, Biolab, Kowa, Merck, Novo-Nordisk, Pfizer, and Sanofi/Regeneron.

References

1. Defesche JC, Gidding SS, Harada-Shiba M, Hegele RA, Santos RD, Wierzbicki AS. Familial hypercholesterolaemia. *Nat Rev Dis Primers*. 2017;3:17093. doi: 10.1038/nrdp.2017.93.
2. Santos RD, Gidding SS, Hegele RA, et al; International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. Defining severe familial hypercholesterolaemia and the implications for clinical management: a consensus statement from the International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. *Lancet Diabetes Endocrinol*. 2016;4:850–861. doi: 10.1016/S2213-8587(16)30041-9.
3. Stein EA, Dann EJ, Wiegman A, Skovby F, Gaudet D, Sokal E, Charng MJ, Mohamed M, Luirink I, Raichlen JS, Sundén M, Carlsson SC, Raal FJ, Kastelein JJP. Efficacy of rosuvastatin in children with homozygous familial hypercholesterolemia and association with underlying genetic mutations. *J Am Coll Cardiol*. 2017;70:1162–1170. doi: 10.1016/j.jacc.2017.06.058.
4. Gagné C, Gaudet D, Bruckert E; Ezetimibe Study Group. Efficacy and safety of ezetimibe coadministered with atorvastatin or simvastatin in patients with homozygous familial hypercholesterolemia. *Circulation*. 2002;105:2469–2475. doi: 10.1161/01.CIR.0000018744.58460.62.
5. Telford DE, Sutherland BG, Edwards JY, Andrews JD, Barrett PH, Huff MW. The molecular mechanisms underlying the reduction of LDL apoB-100 by ezetimibe plus simvastatin. *J Lipid Res*. 2007;48:699–708. doi: 10.1194/jlr.M600439-JLR200.
6. Gouni-Berthold I, Berthold HK, Gylling H, Hallikainen M, Giannakidou E, Stier S, Ko Y, Patel D, Soutar AK, Seedorf U, Mantzoros CS, Plat J, Krone W. Effects of ezetimibe and/or simvastatin on LDL receptor protein expression and on LDL receptor and HMG-CoA reductase gene expression: a randomized trial in healthy men. *Atherosclerosis*. 2008;198:198–207. doi: 10.1016/j.atherosclerosis.2007.09.034.
7. Engelking LJ, McFarlane MR, Li CK, Liang G. Blockade of cholesterol absorption by ezetimibe reveals a complex homeostatic network in enterocytes. *J Lipid Res*. 2012;53:1359–1368. doi: 10.1194/jlr.M027599.
8. Raal FJ, Santos RD, Blom DJ, Marais AD, Charng MJ, Cromwell WC, Lachmann RH, Gaudet D, Tan JL, Chasan-Taber S, Tribble DL, Flaim JD, Crooke ST. Mipomersen, an apolipoprotein B synthesis inhibitor, for lowering of LDL cholesterol concentrations in patients with homozygous familial hypercholesterolaemia: a randomised, double-blind, placebo-controlled trial. *Lancet*. 2010;375:998–1006. doi: 10.1016/S0140-6736(10)60284-X.
9. Cuchel M, Meagher EA, du Toit Theron H, et al; Phase 3 HoFH Lomitapide Study Investigators. Efficacy and safety of a microsomal triglyceride transfer protein inhibitor in patients with homozygous familial hypercholesterolaemia: a single-arm, open-label, phase 3 study. *Lancet*. 2013;381:40–46. doi: 10.1016/S0140-6736(12)61731-0.
10. Ito MK, Santos RD. PCSK9 inhibition with monoclonal antibodies: modern management of hypercholesterolemia. *J Clin Pharmacol*. 2017;57:7–32. doi: 10.1002/jcph.766.
11. Santos RD, Watts GF. Familial hypercholesterolaemia: PCSK9 inhibitors are coming. *Lancet*. 2015;385:307–310. doi: 10.1016/S0140-6736(14)61702-5.
12. Raal FJ, Hovingh GK, Blom D, Santos RD, Harada-Shiba M, Bruckert E, Couture P, Soran H, Watts GF, Kurtz C, Honarpour N, Tang L, Kasichayanula S, Wasserman SM, Stein EA. Long-term treatment with evolocumab added to conventional drug therapy, with or without apheresis, in patients with homozygous familial hypercholesterolaemia: an interim subset analysis of the open-label TAUSSIG study. *Lancet Diabetes Endocrinol*. 2017;5:280–290. doi: 10.1016/S2213-8587(17)30044-X.
13. Stein EA, Honarpour N, Wasserman SM, Xu F, Scott R, Raal FJ. Effect of the proprotein convertase subtilisin/kexin 9 monoclonal antibody, AMG 145, in homozygous familial hypercholesterolemia. *Circulation*. 2013;128:2113–2120. doi: 10.1161/CIRCULATIONAHA.113.004678.
14. Raal FJ, Stein EA, Dufour R, et al; RUTHERFORD-2 Investigators. PCSK9 inhibition with evolocumab (AMG 145) in heterozygous familial hypercholesterolaemia (RUTHERFORD-2): a randomised, double-blind, placebo-controlled trial. *Lancet*. 2015;385:331–340. doi: 10.1016/S0140-6736(14)61399-4.
15. Ridker PM, Tardif JC, Amarenco P, et al; SPIRE Investigators. Lipid-reduction variability and antidrug-antibody formation with bococizumab. *N Engl J Med*. 2017;376:1517–1526. doi: 10.1056/NEJMoa1614062.
16. Thedrez A, Blom DJ, Ramin-Mangata S, Blanchard V, Croyal M, Chemello K, Nativel B, Pichelin M, Cariou B, Bourane S, Tang L, Farnier M, Raal FJ, Lambert G. Homozygous familial hypercholesterolemia patients with identical mutations variably express the LDLR (low-density lipoprotein receptor): implications for the efficacy of evolocumab. *Arterioscler Thromb Vasc Biol*. 2018;38:592–598. doi: 10.1161/ATVBAHA.117.310217.
17. Lambert G, Petrides F, Chatelais M, Blom DJ, Choque B, Tabet F, Wong G, Rye KA, Hooper AJ, Burnett JR, Barter PJ, Marais AD. Elevated plasma PCSK9 level is equally detrimental for patients with nonfamilial hypercholesterolemia and heterozygous familial hypercholesterolemia, irrespective of low-density lipoprotein receptor defects. *J Am Coll Cardiol*. 2014;63:2365–2373. doi: 10.1016/j.jacc.2014.02.538.
18. Thedrez A, Sjouke B, Passard M, et al. Proprotein convertase subtilisin kexin type 9 inhibition for autosomal recessive hypercholesterolemia-brief report. *Arterioscler Thromb Vasc Biol*. 2016;36:1647–1650. doi: 10.1161/ATVBAHA.116.307493.
19. Santos RD. Familial hypercholesterolaemia: beware of lipoprotein(a). *Lancet Diabetes Endocrinol*. 2016;4:553–555. doi: 10.1016/S2213-8587(16)30082-1.

Arteriosclerosis, Thrombosis, and Vascular Biology

JOURNAL OF THE AMERICAN HEART ASSOCIATION

Expression of LDLRs (Low-Density Lipoprotein Receptors), Dyslipidemia Severity, and Response to PCSK9 (Proprotein Convertase Subtilisin Kexin Type 9) Inhibition in Homozygous Familial Hypercholesterolemia: Connecting the Dots

Raul D. Santos

Arterioscler Thromb Vasc Biol. 2018;38:481-483
doi: 10.1161/ATVBAHA.117.310675
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

Print ISSN: 1079-5642. Online ISSN: 1524-4636