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EDITORIAL COMMENT

PCSK9 in South African Variants of Familial Hypercholesterolemia*

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An elevated low-density lipoprotein cholesterol (LDL-C) is a major established risk factor for the development of atherosclerotic cardiovascular disease. The standard regimen for the treatment of elevated LDL-C relies on the statins, which inhibit endogenous cholesterol synthesis, reducing the regulatory pool of intracellular cholesterol, thus resulting in increased transcription of the LDL receptor (*LDLR*) gene. The LDLR, especially that present on the surface of hepatocytes, is mainly responsible for clearance of LDL from the plasma. The transcription factor mediating this change is sterol regulatory element-binding protein (SREBP), especially SREBP2. In addition to increased transcription of the *LDLR* gene, many other genes are transcribed at a higher rate. Among these genes is the gene encoding proprotein convertase subtilisin/kexin type 9 (PCSK9) (1).

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PCSK9 is an inhibitor of the LDLR, mostly as a result of the enhancement of the receptor degradation in the lysosome. Thus, attenuation of PCSK9 function reduces LDLR degradation. Inhibition of PCSK9 represents a strategy in addition to statin treatment to lower LDL-C levels to a healthy range and represents a treatment for those who do not tolerate high-dose statin therapy. Recent years have seen much progress in the development of approaches to the reduction of PCSK9 levels, whether using antisense technology or monoclonal antibodies (2,3).

Classical familial hypercholesterolemia (FH) is attributable to mutations in the gene for *LDLR*, and a large number of such mutants have been catalogued (4). Inheritance of the phenotype is by a dominant mechanism. The LDLR is a multidomain protein containing a signal peptide (exon 1), 7 ligand-binding domains (exons 2 to 6); an epidermal growth factor–like (EGF-like) repeat (exons 7 to 14), a domain that plays a role in the trafficking of the receptor from the endoplasmic reticulum to the Golgi as well as in the recycling of the receptor from the endosome back to the plasma membrane, an O-linked glycosylation domain (exon 15), and a cytoplasmic tail (exons 17 and 18) that is involved in receptor internalization. On the plasma membrane, the LDLR is found in coated pits, where it binds LDL and the complex is internalized, trafficking to the endosomal compartment. Acidification in the endosome promotes dissociation of LDL from the receptor, which recycles to the plasma membrane, while the LDL moves to the lysosome, where it is degraded. There is an average of about 20 such cycles before the receptor itself is degraded.

The interactions between LDL, PCSK9, and the LDLR are complex. PCSK9 is a secreted protein and associates with the extracellular domain of the LDLR. Its effect on LDLR degradation, however, is intracellular. The catalytic activity of the kinase is not required for PCSK9 to influence LDLR turnover, either steering the complex with the receptor to the lysosomes where it is degraded or inhibiting its recycling (5,6). In the study in this issue of the *Journal*, Lambert et al. (7) asked whether PCSK9 is equally effective in reducing the level of LDLR, whether of normal structure or altered by mutations sufficiently to generate the FH phenotype. They studied 3 receptor missense mutations originally described in South African Afrikaners (designated as Afrikaner mutations [4]). These mutations fall into 2 of the 5 functional classes of LDLR mutations. Two mutations, D154N and D206E (exon 4), are class 2 mutations (i.e., defective in maturation and/or transport from the endoplasmic reticulum to the Golgi), whereas the third mutation, V408M (exon 9), is defective in recycling and belongs to class 5. Homozygote cells from the D206E mutants exhibit 5% to 15% of LDL activity, whereas V408M has <2% activity (4). A relatively large number of heterozygous patients bearing each of these mutant receptors were assessed for baseline lipoprotein characteristics (i.e., before any statin treatment was initiated). The researchers also studied primary fibroblasts and lymphocytes derived from a smaller subset of each of the mutant patients. These cells were cultured in a medium to which recombinant PCSK9 was added. They noted that PCSK9 addition reduced cell surface expression of mutant and normal receptors in both cell types (measured by flow cytometry) by about the same percentage. In heterozygous FH cells, the cell surface contained a mixture of normal and mutant receptors, with the former predominating to various extents depending on the nature of the mutation. To avoid this complexity, the researchers transfected HEK cells, either expressing or not expressing PCSK9, with plasmids encoding either normal or mutant receptor. In this case, the receptors expressed were homogeneous, either wild type or mutant. For the 2 class 2 mutants, coexpression of PCSK9 resulted again in the same percentage reduction of

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cell surface receptor levels as was observed in the wild type–expressing cells. For the class 5 mutant, V408M, the cells expressed very few surface receptors and were quite unresponsive to PCSK9 presence. Being a recycling receptor mutant, LDLR-V408M does not mature intracellularly. Maturation can be detected by sizing the receptor. The intracellular immature form is 120 kDa, whereas the fully glycosylated mature form is 160 kDa. Despite the ineffectiveness of PCSK9 on the class 5 cell surface receptor mutant, it did reduce the level of immature 120-kDa protein, showing that PCSK9 can act intracellularly.

This study is valuable in correlating patients' in vivo lipoprotein metabolism before treatment with the behavior of their fibroblasts and lymphocytes in culture and the response to added PCSK9. All 3 groups of heterozygous patients with FH exhibited a significant increase in plasma PCSK9 concentration. The excursions and regulation of PCSK9 are complex. Approximately 30% of the circulating PCSK9 is bound to LDL (8) and is cleared as a complex by the LDLR. PCSK9 may also bind to the LDLR independently of LDL and may be internalized with the receptor. The half-life of injected PCSK9 varies as a function of the LDLR, being fast with normal levels of functional LDLR, slower with heterozygous mutant LDLR, and slowest when the LDLR is completely absent. Thus, in patients, there is a good correlation between LDL-C and PCSK9 in the plasma. This relationship is disrupted with statin treatment when the levels of LDL-C decline and PCSK9 increase.

PCSK9 independently of LDL binds to the LDLR at the N-terminal portion of the EGF-like repeat (EGFA exon 7), consisting of residues 293 to 332. Clearly, none of the mutants in this study fell within this interval. Two residues that significantly influence PCSK9 binding are Leu318 (9) and Asp310. (10). It would be of interest to study PCSK9 function in mutants falling within this interval, especially those affecting the 2 named residues. For such experiments, the outcome measure might be the stability of the 120-kDa intracellular precursor. The synthetic peptide of residues 293 to 332 would be expected to block the interaction of PCSK9 with the receptor and hence improve receptor stability in vivo or in a culture system similar to that employed by Lambert et al. (7). Strategic residues within this 40–amino acid stretch could be altered to dissect effects in vivo on PCSK9 binding to the cell surface receptor.

Most of the PCSK9 function is at the liver. The precise basis for this organ specificity is not clear. LDLR function is important in the adrenal gland, yet the injection of PCSK9 has no effect on the adrenal receptor. Also, knockout of PCSK9 has no effect on the adrenal receptor. The pathway for LDLR internalization is not precisely the same for hepatocytes, lymphocytes, and fibroblasts. Thus, the cytoplasmic adaptor protein autosomal recessive hypercholesterolemia (ARH) is required for LDLR internalization in hepatocytes and lymphocytes. PCSK9 can promote degradation of LDLR in fibroblasts lacking ARH (6). One of the limitations of this study, recognized by the researchers, was that they were unable to study the interaction of LDLR and its mutants with PCSK9 in liver cells.

The authors concluded that elevated PCSK9 levels were equally detrimental for heterozygous patients with FH and patients with nonfamilial hypercholesterolemia (non-FH). Their assessment of the cell surface receptor was accomplished by immunofluorescence in a flow cytometer, measuring the amount of receptor, rather than the amount of functional receptor. Actually, despite their culture experimental results, in vivo elevated levels of PCSK9 in heterozygous patients with FH may be more deleterious than in patients with non-FH. This is because a smaller fraction of the total cell surface receptor is functional in heterozygotes, and a larger number of functional cell surface receptors may be degraded in the presence of the elevated PCSK9 levels. If this is indeed the case, PCSK9 inhibition may be functionally more beneficial in heterozygous patients.

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