The Influence of *PCSK9* Polymorphisms on Serum Low-Density Lipoprotein Cholesterol and Risk of Atherosclerosis

Jean Davignon · Geneviève Dubuc · Nabil G. Seidah

Abstract Pro-protein-convertase-subtilisin-kexin-9 (PCSK9) enhances the degradation of the low-density lipoprotein receptor (LDLR) that plays a major role in cholesterol homeostasis. Recent advances have revealed a large number of genetic variants of PCSK9 that may modulate plasma cholesterol levels either positively or negatively, therefore influencing the risk of atherosclerosis. Recognition of these mutants may have clinical implication in assessing severity of disease, prognosis, or response to drug therapy. PCSK9's expression, secretion, and plasma levels maybe modulated by the proprotein convertase furin, by natural inhibitors (annexin-A2), or influenced by lipid-altering agents such as statins, fibrates, ezetimibe, and berberine. It is now a prime target for therapy, prompting the development of various approaches to reduce its LDLR degrading activity, including antibody neutralization, anti-sense oligonucleotides such as phosphorothioates, locked nucleic acids, and RNA interference, and eventually small molecule inhibitors. Which one will be clinically applicable will depend on long-term effects, cost, and ease of administration.

Introduction

The discovery that autosomal co-dominant familial hypercholesterolemia (FH), in majority attributed to mutations of the LDL-receptor (LDLR) or more rarely of apolipoprotein (apo) B (dominant), could also be associated with mutations in the proprotein convertase-subtilisin/kexin type 9 (*PCSK9*) gene was a breakthrough [1]. The importance of these findings was amplified by the demonstration that PCSK9 acted by enhancing the degradation of the LDLR [2]. The demonstration that loss-of-function (LOF) mutations could be associated with low plasma cholesterol concentrations and protect against coronary artery disease (CAD), in contrast to the opposite effects for the gain-of-function (GOF) mutations [3], further kindled the interest in PCSK9. Altogether, these observations changed our clinical perspective for the diagnosis and management of FH but also added to our knowledge of the LDLR pathway and fostered a large number of research initiatives. This review focuses on recent advances of the influence of genetic variations of PCSK9 on LDL cholesterol (LDL-C) levels and on the risk and progression of atherosclerosis.

PCSK9 Protein and Gene Structure

The unraveled structures of both the gene and the protein greatly enhanced our understanding of PCSK9's role in

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cholesterol homeostasis [1, 4, 5]. The *PCSK9* gene on the short arm of chromosome 1 (1p32.3) is 25-kb long and comprises 12 exons and 11 introns. It encodes a 692-amino acid serine protease PCSK9, formerly called NARC1 (for neural apoptosis regulated convertase-1), which is a member of the proprotein convertase family of enzymes. These convertases act as molecular scissors for the tissue-specific processing of multiple precursor proteins. The domain organization of PCSK9 is depicted in Fig. 1 [6, 7]. The N-terminal signal peptide necessary for secretion is followed by a pro-domain that inhibits the catalytic activity when cleaved. The catalytic domain, responsible for its autocatalytic intramolecular processing in the endoplasmic reticulum (ER) (the only known catalytic function of PCSK9), is followed by a cysteine-histidine rich domain (CHRD). After cleavage, the N-terminal inhibitory pro-segment remains associated with the catalytic domain of PCSK9 and forms a stable secreted heterodimeric pro-segment–PCSK9 complex. The catalytic domain contains the main binding structure of PCSK9 to the epidermal growth factor–like repeat A domain (EGF-A) of the LDLR. The major function of the heterodimeric pro-segment PCSK9 is to degrade the LDLR intracellularly and extracellularly, acting as a chaperone that binds to the LDLR to promote its lysosomal degradation [8].

In adults, PCSK9 is produced primarily by the liver and intestine, but only the liver releases it into the circulation [9]. It is degraded in plasma where it is cut at Arg_{218} by furin, another proprotein convertase, generating an inactive 474-amino acid protein [10]. The gene is polymorphic and a large number of LOF and GOF mutations have been reported in the past few years [7, 11] (also see http://www.ucl.ac.uk/ldlr/Current/index.php?select_db=PCSK9). The fact that lowered plasma LDL-C and apolipoprotein (apo) B from birth with LOF mutations in *PCSK9* were associated with reduced coronary artery disease (CAD) risk in both whites and blacks [3] markedly increased the excitement for this protein.

PCSK9 Single Nucleotide Polymorphisms/Variants/Mutations Influencing LDL-C Levels and Atherosclerosis

To date, more than 50 amino acid variants of PCSK9 have been shown to affect plasma cholesterol levels in humans (Fig. 1) [4, 11, 12]. GOF mutations result in mild to severe hypercholesterolemia. In the most severe Anglo-Saxon mutation, D374Y, total cholesterol (TC) reaches values as high as 13.1 mmol/L (506 mg/dL) [13]. The onset of CAD in patients with D374Y maybe 10 years sooner than in heterozygous FH patients with severe LDLR mutations [14]. In a recent transgenic mouse study by Herbert et al. [15•], the expression of human *PCSK9* gene (WT) or of the human D374Y mutant gene and their endogenous regulatory sequences at physiologic levels produced a phenotype
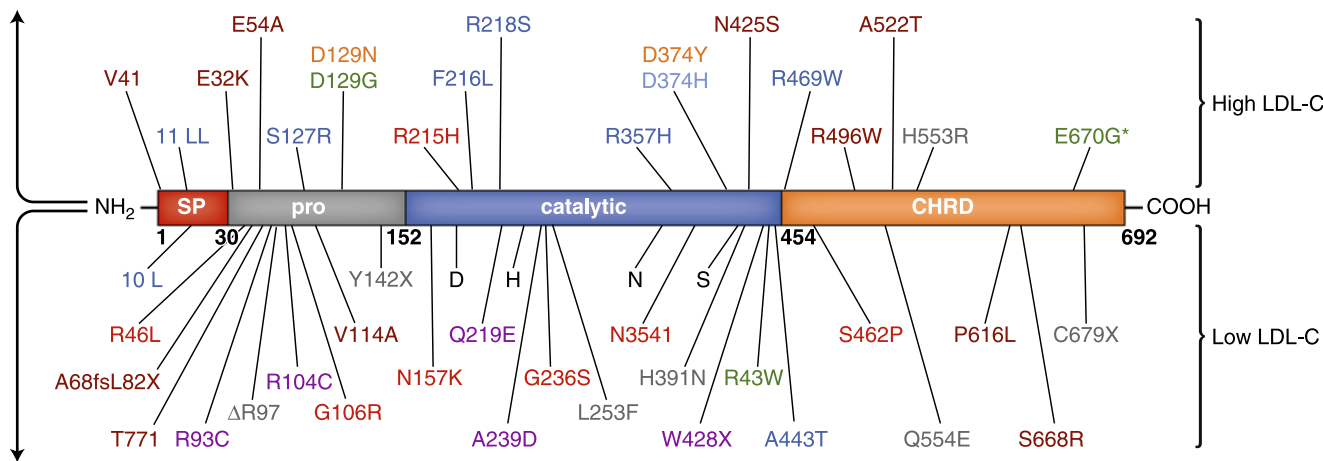
Fig. 1 The PCSK9 protein and mutations influencing plasma low-density lipoprotein cholesterol (LDL-C). The PCSK9 protein is in the center, the key amino acids of the catalytic pocket containing the nucleophiles aspartic acid (D), histidine (H), asparagine (N) and serine (S) are depicted; it takes an active conformation after cleavage of the pro-segment. The position and the amino acid substitutions of the mutations that are associated with high LDL-C levels are shown above the protein, those that are associated with a reduced plasma LDL-C are shown below. The color code indicates the origin of the mutation: France (*blue*), Great Britain (*orange*), Canada (*green*), Norway (*red*), Italy (*dark red*), Japan (*purple*), New Zealand (*dark green*), and Portugal (*light blue*). The mutations associated with low LDL-C reported by Lakoski et al. [18] in the Dallas Heart Study are shown in gray. *Asterisk* indicates that the E670G coding the single nucleotide protein reported in whites also influences LDL-C levels (increased with the rare GG genotype). (*Data from* Davignon and Dufour [6] and Leigh et al. [7]. See also the LOVD database at http://www.ucl.ac.uk/ldlr/Current/index.php?select_db=PCSK9)

that closely matched that found in heterozygous D374Y patients. Both transgenes increased serum cholesterol level and reduced hepatic LDLR protein; however, the effects were greater in D374Y mice. Extensive atherosclerotic plaques were found only in the D374Y on a high-cholesterol diet. They also secreted more triglyceride-rich lipoproteins into the circulation than WT mice, suggesting that reduced LDLR activity is not the sole cause of their hypercholesterolemia and propensity to dietary-induced atherosclerosis. This is the first study to use the endogenous *PCSK9* promoter, and not *apoE* or albumin promoters as in previous studies, to drive PCSK9 D374Y expression.

On the other hand, a retrospective study has shown a significantly reduced risk of CAD in carriers of PCSK9 LOF variants R46L (partial LOF) and Y142X or C679X (complete LOF). Together, the latter two nonsense mutations were associated with a 28% reduction of plasma LDL-C and an 88% reduction in the frequency of coronary events in blacks, whereas in whites the R46L was associated with a 15% reduction in LDL-C and a 47% reduction in the risk of CAD [3]. Although that study supported the cardioprotective role of long-term reduction of cholesterol levels, a direct protective effect of reduced PCSK9 was not excluded. Furthermore, a compound heterozygous black American woman exhibiting two inactivating mutations (Y142X and ΔR97) in PCSK9 had strikingly low plasma levels of LDL-C (0.36 mmol/L; 14 mg/dL) and no immunodetectable circulating PCSK9 [12]. Another woman homozygous for the C679X mutation in Zimbabwe had a plasma LDL-C of 0.41 mmol/L (15.8 mg/dL) [16]. All these findings support the hypothesis that levels and/or higher activity of plasma PCSK9 modulate LDL-C and TC levels, suggesting that long-term lowering of PCSK9 might be beneficial in reducing the incidence of CAD [17]. Recently, the discovery of new mutations and methods to regulate PCSK9 led to the deeper analysis of the influence of PSCK9 on various lipid and metabolic parameters and to the probing of the therapeutic benefits of silencing PCSK9 in cardiovascular diseases at large.

Lakoski et al. [18] in a study of plasma PCSK9 in the large multi-ethnic population of the Dallas Heart study (*n*=3,138) found that individuals with LOF mutations and reduced levels of LDL-C had significantly lower plasma levels of PCSK9 after adjusting for age and gender (*P*<0.0001). A 100-ng/mL increase in plasma PCSK9 level was associated with a 4.5-mg/dL (0.12 mmol/L) increase in plasma LDL-C concentration in women and a 3.2-mg/dL (0.08 mmol/L) increase in LDL-C in men. Women had approximately 15% higher plasma concentrations than men and post-menopausal women higher levels than pre-menopausal women, regardless of estrogen status. They also observed that plasma triglycerides, insulin, and glucose

correlated with plasma PCSK9. They did not find any relationship between *apoE* polymorphism and plasma concentrations of PCSK9. They observed the usual genotypic effect of increasing levels of LDL-C from E2/2 to E4/4 across a population subset of 3,096 individuals, but no difference among those genotypes for PCSK9 plasma levels. However, they did not look at the impact of PCSK9 variants on LDL-C as a function of variation in apoE. Two recent reports addressed this question.

Huang et al. [19••] investigated the associations of six PCSK9 variants with LDL-C over 20 years in 1,750 blacks and 1,828 whites from the Coronary Artery Risk Development In Young Adults (CARDIA) study. This longitudinal study of normal young adults aimed at studying development of cardiovascular disease risk factors revealed that for blacks, LDL-C levels at age 18 years were significantly lower (*P*<0.001) among those with one of the three variants L253F, C679X, and Y142X, (81.5 mg/dL; 2.1 mmol/L) or A443T (95.5 mg/dL; 2.47 mmol/L) compared with non-carriers (109.6 mg/dL; 2.83 mmol/L). White carriers of the R46L variant had lower mean LDL-C concentrations at age 18 years (84.4 mg/dL; 2.18 mmol/L) than non-carriers (100.9 mg/dL; 2.6 mmol/L; *P*<0.001), and the increase in LDL-C with age was similar to that of non-carriers. Importantly, the four cholesterol-lowering variants in black men were associated with lower carotid intima-media thickness and lower prevalence of coronary artery calcifications (CAC) measured once at year 20 in participants aged 38 to 50 years. Interestingly, the authors observed that *apoE* genotypes exerted their respective effects on LDL-C in an additive manner to that of the *PCSK9* variants. This is the first demonstration of an *apoE* by *PCSK9* LOF mutation interaction in a cohort of normal individuals and the relative protective effect of the *apoE2* allele. The tracking of LDL-C over time in carriers of LOF mutations has been shown also in the Bogalusa Heart Study in a lower age range (4–38 years) in both whites (*n*=1,086, R46L LOF) and blacks (*n*=478, Y142X and C679X LOF) [20]. These results also showed that these LOF variants are associated with significantly lower LDL-C levels starting in childhood.

Norata et al. [21••] investigated the effects of two common *PCSK9* mutations (E670G and I474V) on the intima media thickness of the common carotid artery and the possible relation to apoE polymorphism. Their database included 1,541 middle-aged individuals selected from the general population enrolled in the Progression of Lesion in the Intima of the Carotid artery (PLIC) study. They confirmed their major findings in 776 individuals from a second free-living population enrolled in the Ventimiglia in Sicilia study for whom a DNA sample was available. The E670G polymorphism of the *PCSK9* gene was associated with increased IMT progression in the general population

but the I474V was neutral. When the presence of 670G allele was stratified according to the apoE gene alleles, *apoE2–PCSK9-670EE* carriers showed a more favorable plasma lipid profile and decreased intima media thickness compared with the *apoE4–PCSK9-670G* carriers. This is the first demonstration of an apoE by PCSK9 gain-of-function mutation interaction in a general population and supports the relative protective effect of the *apoE2* allele. It is consistent with the findings of Chen et al. [22] both for the effect of the E670E variation as part of a haplotype on LDL-C and on severity of CAD in the Lipoprotein Coronary Atherosclerosis Study (LCAS) in Houston.

There is no consensus, however, on the effect of the E670G mutation on plasma cholesterol or LDL-C levels and one should keep in mind that variation in the effect of PCSK9 on a lipid parameter maybe population-specific or influenced by other genetic or environmental factors. Hsu et al. [23] failed to find a cholesterol-raising effect of the E670G in a Taiwanese population sample, in contrast to the findings Norata [21••] observed in an Italian population. Moreover, in their observation, the polymorphism was associated with lower LDL-C levels. A gender effect may also be responsible for some discrepancies. Evans and Beil [24] determined that the *PCSK9* 670G allele was associated with increased LDL-C levels in men, but not in women, of European origin. The study of Kotowski et al. [11] failed to find such an association between plasma levels of LDL-C and the E670G substitution in the Dallas Heart Study. Finally, one must consider an age effect as a source of phenotypic modulation. Polisecki et al. [25], in the elderly population of the Prospective Study of Pravastatin in the Elderly (PROSPER) study ($n=5,783$, 43% with a history of vascular disease at baseline), found significantly reduced LDL-C associated with the T allele of the G to T substitution accounting for the LOF R46L mutation (3.5% of sample; 10% reduction). There was a non-significant (19% unadjusted and 9% adjusted) decreased risk of vascular disease at baseline, with no on-trial effect. In this study, the 6% carriers of the E670G G allele did not exhibit a significant relationship with baseline LDL-C, response to pravastatin, or vascular disease risk.

Peripheral Vascular Disease

The impact of *PCSK9* variation on peripheral arterial disease (PAD; defined by an ankle-brachial index <0.9 or a history of leg claudication) was evaluated by Folsom et al. [26•] in the Atherosclerosis Risk in Communities (ARIC) study, which assessed risk factors and PCSK9 variants Y142X and C679X (relevant to blacks) and R46L (relevant to whites) in a cohort of individuals 45 to 64 years of age in the years from 1987 to 1989 ($n=13,634$). Greater LDL-C

was a risk factor for prevalent and incident PAD. Carriers of one of the race-specific PCSK9 variants (2.4% of blacks and 3.1% of whites) had lower prevalence of PAD compared with non-carriers (2.3% vs 4.6%). The corresponding age-adjusted and sex-adjusted odds ratio of PAD was 0.47 (95% CI, 0.24–0.92). In contrast with the cross-sectional findings, however, there was no association between PCSK9 variants and incident PAD, providing only mixed evidence that variation in *PCSK9* may contribute to genetic risk of PAD. A longitudinal study focused on patients with established PAD in a vascular clinic with extensive assessment of severity of disease might help to clarify this issue.

Cerebrovascular Disease

Two independent studies, one Belgian and one Finnish, were combined to obtain clinical and autopsy data [27]. They provided evidence that *PCSK9* variations associate with the risk of large-vessel atherosclerosis (LVA) stroke subtype and suggest that the risk is mediated by the severity of intracranial atherosclerosis. The Belgium Stroke Study included 237 middle-aged (45–60 years of age) patients with small-vessel occlusion (SVO) and large-vessel athero-sclerosis stroke (LVA), and 326 gender-matched and ethnicity-matched controls (>60 years of age) without a history of stroke. In multi-variate analysis, the minor allele (G) of the E670G *PCSK9* polymorphism was a significant predictor of LVA (odds ratio=3.52; 95% CI, 1.25–9.85; $P=0.017$). In the Finnish cross-sectional population-based study of consecutive autopsy series of 604 men and women (mean age of 62.5 years), the G-allele carriers tended to have more severe allele copy number–dependent ($P=0.095$) atherosclerosis in the circle of Willis and in its branches. The genetic variation is not associated with small vessel occlusion, a phenomenon that is unrelated to atherosclerosis. LDL-C values were not reported. These findings support the involvement of the E670G in determining serum lipids (higher frequency of hyperlipidemia in affected) and atherosclerosis (gene–dosage effect).

PCSK9 Plasma Levels Modulators

Because PCSK9 degrades the LDLR, contributes to cholesterol homeostasis, and may impact on atherosclerotic vascular disease, it is a potential therapeutic target [4]. This notion has prompted a major effort to identify natural and synthetic agents that affect the *PCSK9* gene and its protein expression and metabolism. In human studies, the accent was put recently on plasma levels and effects of lipid-lowering agents.

Natural Modulators

To date, two natural modulators have been shown to interact with PCSK9. As mentioned previously, furin is capable of cleaving PCSK9 at residue Arg_{218}, resulting in an inhibition of PCSK9-induced degradation of the LDLR [10]. Recently, Mayer et al. [28] demonstrated that the C-terminal Cys-His rich domain (CHRD) of PCSK9 interacts specifically with the N-terminal repeat R1 of annexin-A2 (AnxA2) and inhibits the degradation of the LDLR induced by PCSK9, thereby identifying the first natural inhibitor of PCSK9. AnxA2 is a calcium-dependent phospholipid-binding protein whose function is to help organize exocytosis of intracellular proteins to the extracellular domain and that is involved in pleiotropic functions such as cell motility, linkage of membrane-associated protein complexes to the actin cytoskeleton, endocytosis, fibrinolysis, ion channel formation, and cell matrix interactions [29]. Mayer et al. [28] reported that the LOF mutant Q554E binds at least threefold better to the R1 repeat of AnxA2, leading to a tighter binding of PCSK9 to AnxA2, resulting in loss of ability of PCSK9 to induce the degradation of the LDLR. This discovery should prompt the search for mutations of annexin-A2 in orphan forms of inherited hypercholesterolemia and should pave the way toward the development of PCSK9 inhibitory molecules [5].

Artificial Modulators and Therapeutic Agents

Dubuc et al. [30] previously demonstrated that PCSK9 mRNA is upregulated in vitro by statins via a pathway involving reduced intracellular cholesterol concentration and increased SREBP-2 levels [31, 32]. Recently, in a cross-sectional study, Dubuc et al. [33••] evaluated plasma levels of PCSK9 and their relationship with plasma lipids and lipoproteins in normal ($n=254$) and hypercholesterolemic ($n=200$) individuals treated or not treated with statins or a statin plus ezetimibe (two hypocholesterolemic agents with complementary modes of action). In hypercholesterolemic participants, PCSK9 levels were higher than in controls (99.3 ± 31.7 ng/mL; $P<0.04$) and increased in proportion to the statin dose, combined or not with ezetimibe. In treated patients ($n=139$), those with FH due to LDL receptor gene mutations had higher PCSK9 values than patients without FH (147.01 ± 42.5 ng/mL vs 127.2 ± 40.8 ng/mL; $P<0.005$). This finding is in line with the observation of Careskey et al. [34] that plasma PCSK9 increases as LDL-C goes down during 16 weeks of atorvastatin therapy. Dubuc et al. [33••] also showed that plasma PCSK9 is further increased when ezetimibe and statins are used in combination. However, studies examining the effect of another common lipid-lowering medication, fenofibrate, on PCSK9 plasma levels seem to be contradictory. Troutt et al. [35] studied the effect of fenofibrate on circulating PCSK9 protein levels in patients with an atherogenic dyslipidemia treated with fenofibrate or placebo for 12 weeks. They observed that fenofibrate (200 mg/d) significantly increased circulating PCSK9 levels by 25% compared with baseline. Interestingly, fenofibrate-induced increases in serum PCSK9 levels were highly correlated with fenofibrate-induced changes in HDL-C and triglyceride levels, as well as with fenofibrate-induced changes in LDL-C levels. Mayne et al. [36] also observed an increase in PCSK9 plasma level in 19 patients on the American Heart Association NCEP step-1 diet after a 24-week treatment with gemfibrozil or fenofibrate (17.01%; $P=0.031$). In contrast, Lambert et al. [37] measured circulating PCSK9 concentrations in 115 diabetic patients from the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study before and after a 6-week fenofibrate treatment and found an 8.5% decrease ($P=0.041$ vs pretreatment). These divergent findings are difficult to reconcile. In vitro studies with immortalized hepatocytes by Kourimate et al. [38] showing that fenofibric acid represses PCSK9 both by reducing *PCSK9* expression and increasing furin expression (a PCSK9 inhibitor) are in favor of the lowering effect. Differences in methods and the patient subset studied might contribute to better understanding.

Berberine

Berberine (BBR), is a new cholesterol-lowering agent derived from a Chinese herb (*Coptis sinensis*) that upregulates LDLR expression via mRNA stabilization, implicating novel regulatory proteins located downstream of the extracellular signal regulated kinase (ERK) pathway and able to interact with sequences in the proximal section of the LDLR mRNA 3′ untranslated region (UTR) [39]. Cameron et al. [40] reported that in HepG2 cells, berberine reduces PCSK9 expression and secretion in a dose-dependent manner. Also, it suppresses the *PCSK9* mRNA–raising effect of mevastatin while increasing its LDLR-raising effect. In hepatic cell lysates, however, berberine and mevastatin both increase LDLR protein, acting in synergy when combined. Recently, Li et al. [41•] identified the mRNA binding proteins that regulate the stability of LDLR mRNA induced by berberine through AU-rich (ie, a region with frequent A and U bases in mRNA) elements. Heterogeneous nuclear ribonucleoprotein D (hnRNP D), hnRNP I, and KH-type splicing regulatory protein (KSRP) were found to be key modulators of LDLR mRNA stability in liver cells. These interact with AU-rich elements of the LDLR 3′UTR with sequence specificity. Silencing the expression of these proteins increased LDLR mRNA and protein levels. This work provides new insight on the regulation of LDLR gene expression at the post-

transcriptional level. Importantly, Li et al. [42••], in studying mechanisms underlying the transcriptional suppression of *PCSK9* by berberine in HepG2 cells, also identified a highly conserved hepatocyte nuclear factor 1-α (HNF1α) binding site upstream from the sterol regulatory element (SRE) as a critical sequence motif for *PCSK9* transcription and its regulation by berberine. They discovered that HNF1α, from among an array of transcription factors, was the predominant transactivator of *PCSK9*, and mutation of its binding site reduced PCSK9 activity by more than 90%. They found that HNF1α works cooperatively with SRE, as HNF1α site mutations significantly attenuated the activity of nuclear SREBP-2 to transactivate the *PCSK9* promoter. A coordinate modest reduction of HNF1α and nuclear SREBP-2 by berberine led to a strong suppression of *PCSK9* transcription through these two critical regulatory sequences. These findings further improve our understanding of the role of PCSK9 in cholesterol homeostasis and of the regulatory networks that modulate its transcription. It provides a new tool for the development of selective repressors of *PCSK9* via inhibition of its transcriptional enhancers and may help to improve statin efficacy.

Interfering Antibody, RNA Interference, and Anti-Sense Oligonucleotides

PCSK9 deficiency causes a 40% to 50% decrease in total plasma cholesterol and a threefold increase in hepatic LDLR in mice [9, 43, 44]. These observations and the lifetime reduction of LDL-C in individuals with *PCSK9* LOF mutations reported above suggest that pharmacologic inhibition of PCSK9 would result in increased LDLR activity and enhanced plasma LDL-C clearance. This hypothesis is reinforced by the demonstration of a hypersensitivity to statin in *PCSK9*-deficient mice, suggesting a possible synergic interaction between a statin's cholesterol-lowering effects and an eventual PCSK9 inhibitor [44]. Recently, inhibition of *PCSK9* expression using anti-sense oligonucleotides (ASO) [45], locked nucleic acids [46], and RNA interference (RNAi) [47], or inhibition of PCSK9 activity on LDLR using neutralizing antibodies [48] was tested with success in animals models, including mice and monkeys, and a very recent early report evaluated a PCSK9 mAb in humans, with positive results on LDL-C lowering (http://www.fiercebiotech.com/press-releases/regeneron-provides-initial-data-two-antibody-product-candidates-0). These therapeutic approaches have been reviewed recently [5]. ASO administered in subcutaneous injections in mice on a fat-rich diet during 6 weeks provoked a 90% reduction in *PCSK9* mRNA, a 53% reduction in total plasma cholesterol, and a 38% reduction in LDL-C [45]. This was the first demonstration that injectable ASOs could be a useful therapeutic approach to reduce PCSK9 hepatic levels and as a possible complement to statin treatment. Moreover, RNAi in injectable lipidoid nanoparticles (LNP) dispensed in mice and rat produced a 50% to 70% reduction in PCSK9 mRNA and significant LDL-C reductions in cynomolgus monkeys without affecting HDL-C or TG levels [47]. This gene-silencing effect lasted for 3 weeks. Finally, smaller ASOs from the "locked nucleic acid" technology have shown safety and efficacy in mice and non-human primates, and a neutralizing effect that lasts for 21 days with an injection twice weekly [46]. These results are very promising and studies in humans are undergoing.

PCSK9 Polymorphisms and Response to Statin Therapy

Statins are effective at lowering LDL-C and reducing cardiovascular disease risk, but variability in response is not well understood. To address this, Thompson et al. [49] genotyped 5,745 individuals from the Treating to New Targets (TNT) trial in a candidate gene approach to identify associations with response to atorvastatin treatment. Twenty-three candidate genes previously associated with statin response were analyzed and three single nucleotide polymorphisms in *apoE* were most highly associated with LDL-C response, followed by one in *PCSK9* with a similar effect size. Single nucleotide polymorphisms in *HMGCR* were also significant, although the effect was less than with those in *apoE* and *PCSK9*. rs7412/*apoE* had the most significant association ($P=6\times10^{(-30)}$), and its high significance in the whole-genome study ($P=4\times10^{(-9)}$) confirmed the suitability of this population for detecting effects. Age and gender were found to influence LDL-C response to a similar extent as the most pronounced genetic effect. This study confirmed previous results of Berge et al. [50], suggesting that a disruption in the normal function of PCSK9 could result in increased number of LDLRs and improved response to statin therapy. The authors screened 38 unrelated hypocholesterolemic individuals as well as 25 unrelated familial hypercholesterolemia (FH) heterozygotes who responded particularly well to statin therapy for mutations in the 12 exons of the *PCSK9* gene by DNA sequencing. Six of the 38 (15.8%) hypocholesterolemic individuals were heterozygous for one of the three mutations R46L, G106R, or R237W in PCSK9. In the group of 25 FH heterozygotes who responded particularly well to statin therapy, three (8.8%) were heterozygous for mutations R46L or N157K in PCSK9.

Conclusions

The discovery of PCSK9 has stimulated a large number of research initiatives, resulting in an improved understanding

of LDLR biology and cholesterol homeostasis. PCSK9 is becoming a hub in a network of metabolic interactions that are just beginning to be unraveled. The field is expanding exponentially as the focus moves from mechanisms to clinical and therapeutic applications. This review, which highlights but a few of the recent advances in PCSK9 physiology and pathology, is a witness to the growing interest for and importance of this field.

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