

Review

PCSK9 inhibition for the treatment of hypercholesterolemia: Promises and emerging challenges



Giuseppe Danilo Norata<sup>a,b</sup>, Gianpaolo Tibolla<sup>a,c</sup>, Alberico Luigi Catapano<sup>a,c,\*</sup>

<sup>a</sup> Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy

<sup>b</sup> Center for the Study of Atherosclerosis, Società Italiana Studio Aterosclerosi, Bassini Hospital, Cinisello Balsamo, Italy

<sup>c</sup> I.R.C.C.S. Multimedica, Milan, Italy

ARTICLE INFO

Article history:

Received 30 May 2014

Accepted 31 May 2014

Available online 9 June 2014

Keywords:

PCSK9

Hypercholesterolemia

LDL cholesterol

LDL receptor

Monoclonal antibodies

ABSTRACT

Hypercholesterolemia, is a prominent risk factor for cardiovascular disease (CVD). Understanding of the biochemical mechanisms that regulate the expression of the low density lipoproteins receptor (LDLR) and the hepatic clearance of LDL cholesterol (LDL-C) paved the way to the statin therapy as the gold standard for CVD prevention. The discovery of proteins that regulate – at a post-translational level – the activity of the LDLR has been a major breakthrough in developing new cholesterol-lowering drugs. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a key modulator of the LDLR degradation in the liver. Genetic studies confirmed that in humans PCSK9 mutations associate with hypercholesterolemia and hypocholesterolemia (gain-of-function or loss-of-function variants respectively). Moreover, PCSK9 is up-regulated by statin treatment and limits the efficacy of these agents. These findings led to the development of PCSK9 inhibitors. Anti-PCSK9 monoclonal antibodies showed encouraging results and are currently being evaluated in phase III clinical trials. The aim of this short review is to describe the new frontier of PCSK9 inhibition in the treatment of hypercholesterolemia. Emphasis here is given to critical emerging issues linked to PCSK9 physiology and pharmacology, which will require future investigation to definitely address the potential of anti-PCSK9 drugs in clinical practice.

© 2014 Elsevier Inc. All rights reserved.

Contents

1. Introduction	103
2. Discovery and validation of PCSK9 as a new target for treating hypercholesterolemia	104
2.1. PCSK9 mediated degradation of the LDLR and LDLC hepatic clearance	104
3. PCSK9 physiology	104
3.1. Modulation of PCSK9 secretion and plasma levels	104
3.2. PCSK9 and triglyceride-rich lipoprotein metabolism	106
3.3. PCSK9 target proteins and PCSK9 extra-hepatic activity	107
4. Pharmacological strategies for inhibiting PCSK9: where do we stand?	108
5. Conclusions	109
Disclosure statement	109
References	109

1. Introduction

Hypercholesterolemia, characterized by elevated levels of circulating low-density lipoprotein cholesterol (LDL-C), is a key risk factor in

determining cardiovascular disease (CVD) [1]. The standard of care for the treatment of hypercholesterolemia is based on the administration of statins, that have been proven to be effective in reducing LDL-C and cardiovascular risk. However a considerable number of patients, particularly at high and very-high risk, require LDL-C lowering larger than currently achievable with statins alone or combination with other lipid-lowering drugs [2,3]. In addition, some patients are intolerant or respond very little to statins. Studies of the mechanisms that regulate low density lipoprotein receptor (LDLR) activity at the post-translational

DOI of original article: <http://dx.doi.org/10.1016/j.vph.2014.03.004>.

\* Corresponding author at: Department of Pharmacological and Biomolecular Sciences, University of Milan, Via Balzaretti 9, 20133 Milan, Italy. Tel.: +39 02 50318302; fax: +39 02 50318386.

E-mail address: [alberico.catapano@unimi.it](mailto:alberico.catapano@unimi.it) (A.L. Catapano).

level, allowed the identification of proteins that may be pharmacologically targeted to improve plasma lipid profile in patients with dyslipidemia. Of particular relevance has been the discovery of PCSK9 as the third locus associated with autosomal dominant hypercholesterolemia (ADH) [4]. Genetic studies demonstrated that gain-of-function PCSK9 variants lead to hypercholesterolemia, whereas loss-of-function mutations associate with low LDL-C and reduced CVD risk. Biological research concomitantly elucidated mechanisms involved in the PCSK9-induced LDLR degradation, showing that PCSK9 is secreted and binds the LDLR at the cell surface of hepatocytes, thus inhibiting the recycling of the LDLR to the plasma membrane and dampening LDL clearance [5]. Interestingly PCSK9 is up-regulated by statins, thus limiting the efficacy of treatment with these agents [6]. The observation that genetic deletions of PCSK9 in mice do not interfere with the development and life of the adult organism [7] and the identification of two healthy women with complete loss-of-function mutations in the PCSK9 gene [8,9], leading to the absence of the circulating protein, proved the viability of this approach. PCSK9 inhibitors are currently under clinical development using different strategies [10]. The encouraging results obtained with anti-PCSK9 monoclonal antibodies (mAbs), that reduce LDL-C levels by 70% and are now being evaluated in phase III clinical trials, have generated great excitement for the possibility to open, after a long time, a new avenue to reduce cholesterol levels [10]. Nonetheless, the global physiological function of PCSK9 is still under scrutiny, and recent findings suggest that this protein may have still unrecognized roles other than the regulation of LDL-C homeostasis. In this issue of *Vascular Pharmacology* Werner et al. [11] show that circulating levels of PCSK9 predict cardiovascular events in patients with coronary artery disease (CAD), and that the PCSK9 predictive power depends on its positive correlation with serum triglycerides. This finding suggests that, in CAD patients, PCSK9 may be used as a biomarker for CVD risk assessment, and that this protein may also play a role in triglyceride metabolism, possibly regulating atherogenic apolipoprotein B (apoB)-containing lipoprotein secretion and clearance. The degradation of alternative targets, such as members of the LDLR-related protein family, and the relative contribution of systemic versus locally produced PCSK9 in determining protein activity in the liver and extra-hepatic tissues are further key points that need to be further explored for a better understanding of the role of PCSK9 in humans and to gain further insight on the effects of PCSK9 inhibition.

## 2. Discovery and validation of PCSK9 as a new target for treating hypercholesterolemia

PCSK9 was initially discovered as a protein up-regulated after apoptosis induction in primary cerebellar neurons, and for this reason called neural apoptosis regulated convertase 1 (NARC-1) [12]. Nevertheless the interest about PCSK9 originated from the description of PCSK9 mutations associated with autosomal dominant hypercholesterolemia in two French families where mutations in the usual candidate genes encoding the LDL receptor (LDLR) and apoB were previously excluded [4]. Further studies showed that, in humans, genetic variations in the PCSK9 gene consistently contributed to the regulation of LDL-C plasma levels, with – specifically – rare gain-of-function variants leading to hypercholesterolemia, whereas loss-of-function variants induced hypocholesterolemia (a detailed and updated description of PCSK9 genetic variants can be found at [http://www.ucl.ac.uk/ldlr/LOVDv.1.1.0/index.php?select\\_db=PCSK9](http://www.ucl.ac.uk/ldlr/LOVDv.1.1.0/index.php?select_db=PCSK9)). These observations prompted intensive research on the molecular mechanisms involved in PCSK9 activity that eventually described the ability of PCSK9 to regulate the levels of circulating LDL-C by enhancing the degradation of the hepatic LDLR.

### 2.1. PCSK9 mediated degradation of the LDLR and LDLC hepatic clearance

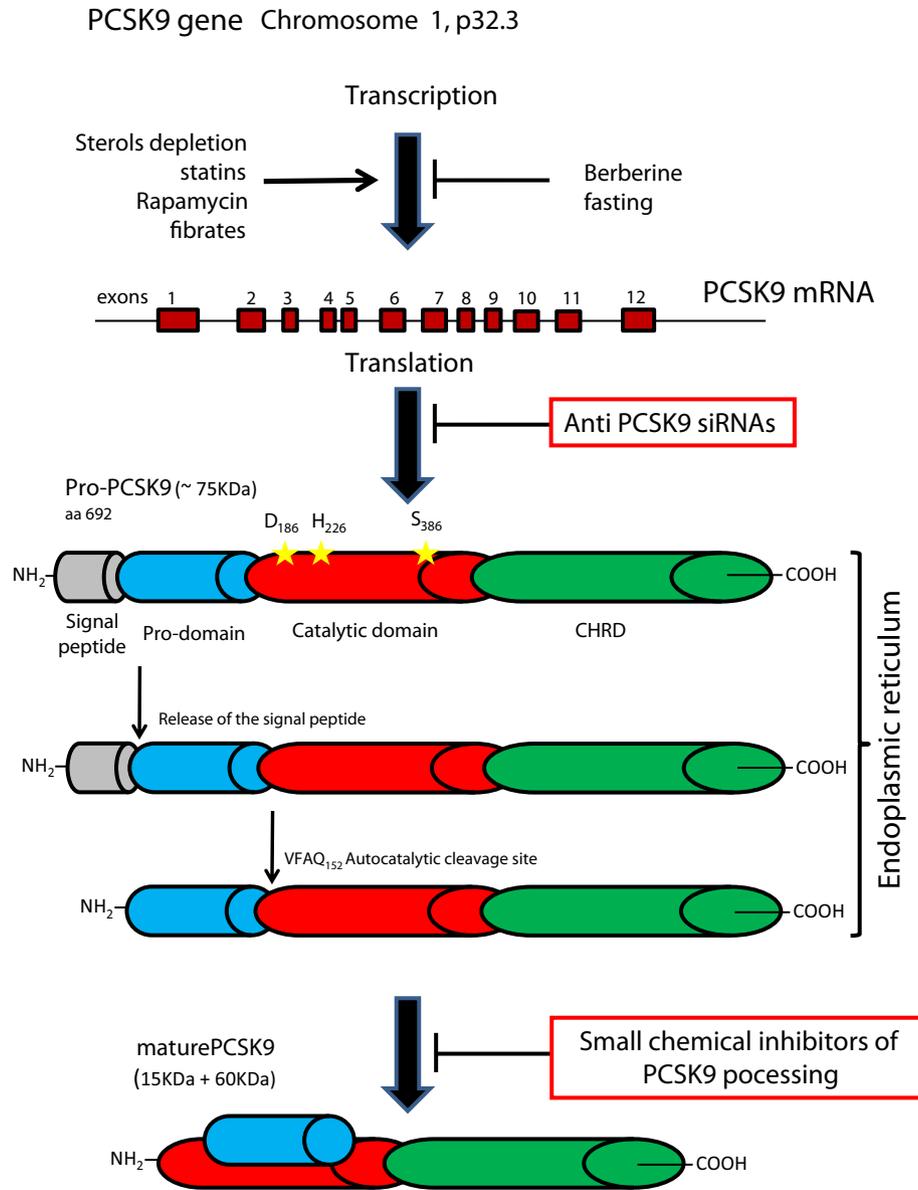
PCSK9 is a 692 amino-acid glycoprotein belonging to the family of proprotein convertases (PC) [13], predominantly expressed not only in the liver but also in the small intestine, the kidney and the central

nervous system. PCSK9 is synthesized as a soluble zymogen (proPCSK9) (Fig. 1) structured with a signal peptide (amino acids 1–30), a pro-domain (amino acids 31–152), a catalytic domain, an exposed hinge region, and a Cys- and His-rich C-terminal domain (CHRD). The protein is characterized by structural homology with resistin [14]. ProPCSK9 catalyzes its self-cleavage at position 152 (Asp<sub>151</sub>Gln<sub>152</sub>) in the endoplasmic reticulum. The autocatalytic cleavage is required for PCSK9 folding and secretion, but – in contrast with other PCs – the pro-domain remains associated, through hydrogen bonds, with the catalytic site of the protein, leading to the formation of a PCSK9/pro-domain complex that prevents the access of protein substrates to the catalytic site and inhibits PCSK9 protease activity [15]. Upon secretion, extracellular PCSK9 acts as a chaperone protein and binds the first EGF-like repeat (EGF-A) of the LDLR present at the plasma membrane of the hepatocytes (Fig. 2) [16,17]. This activity is responsible for the PCSK9-mediated degradation of the LDLR, as demonstrated by *in vivo* parabiosis experiments showing that circulating PCSK9 derived from PCSK9 transgenic mice through the shared circulation reduces the levels of hepatic LDLR once transferred to recipient wild-type mice [18]. The PCSK9–LDLR complex is internalized through clathrin-mediated endocytosis, and then moves to the endosomes, where lowering of the pH strengthens the interaction between the two proteins [19–21]. The PCSK9–LDLR protein association inhibits the physiological recycling of the receptor to the cell surface while promoting degradation in the lysosomes [22]. Whether the concomitant binding of the LDL particle to the receptor affects PCSK9 efficacy in the degradation of the LDLR is still unclear, and is a key point that warrants further investigation. The CHRD of PCSK9 is critical for this process, as shown by the complete loss of LDLR degrading activity of truncated PCSK9 protein lacking this region [22]. The exact role of the CHRD is unknown, and both interactions in the endosomes with the negatively charged ligand-binding domain of the receptor [23] and the presence of plasma membrane PCSK9 binding proteins, such as APLP-2 [24] have been proposed. In this regard, the investigation of PCSK9 partner proteins that modulate PCSK9 secretion or its LDLR degrading activity is a key area for the development of innovative anti-PCSK9 strategies. Recent findings identified two important regulators of PCSK9 sorting and maturation. To exit from the endoplasmic reticulum, the cleaved PCSK9 needs to interact with an unknown membrane-bound protein that mediates the binding of sec24a, a cytosolic protein associated with Coat Protein-II-coated vesicles [25]. Sortilin has also been established as a critical regulator of the late PCSK9 secretory pathway. Indeed, sortilin co-localizes with PCSK9 in the trans-Golgi network, and a sortilin genetic deletion leads to a change in PCSK9 sub-cellular distribution and secretion [26]. PCSK9 can also bind to circulating LDL, and this association reduces PCSK9 ability to degrade the LDLR [27]. Of note it was recently shown that in hepatocytes PCSK9 binds apoB, the major protein constituent of LDL, and reduces its intra-cellular degradation [28], thus suggesting that apoB may represent the key factor involved in PCSK9 interaction with LDL. Finally, compelling evidence showed that PCSK9 degrades the LDLR in hepatocytes, enterocytes and macrophages [29], but is inactive in the kidney and adrenals; in neurons PCSK9 activity is triggered during development and after ischemic stroke [30]. These results may be explained by the different expression of regulatory proteins such as annexin A2, that is secreted in extra-hepatic tissues where it binds the C-terminal domain of PCSK9 and inhibits LDLR degradation [31].

## 3. PCSK9 physiology

### 3.1. Modulation of PCSK9 secretion and plasma levels

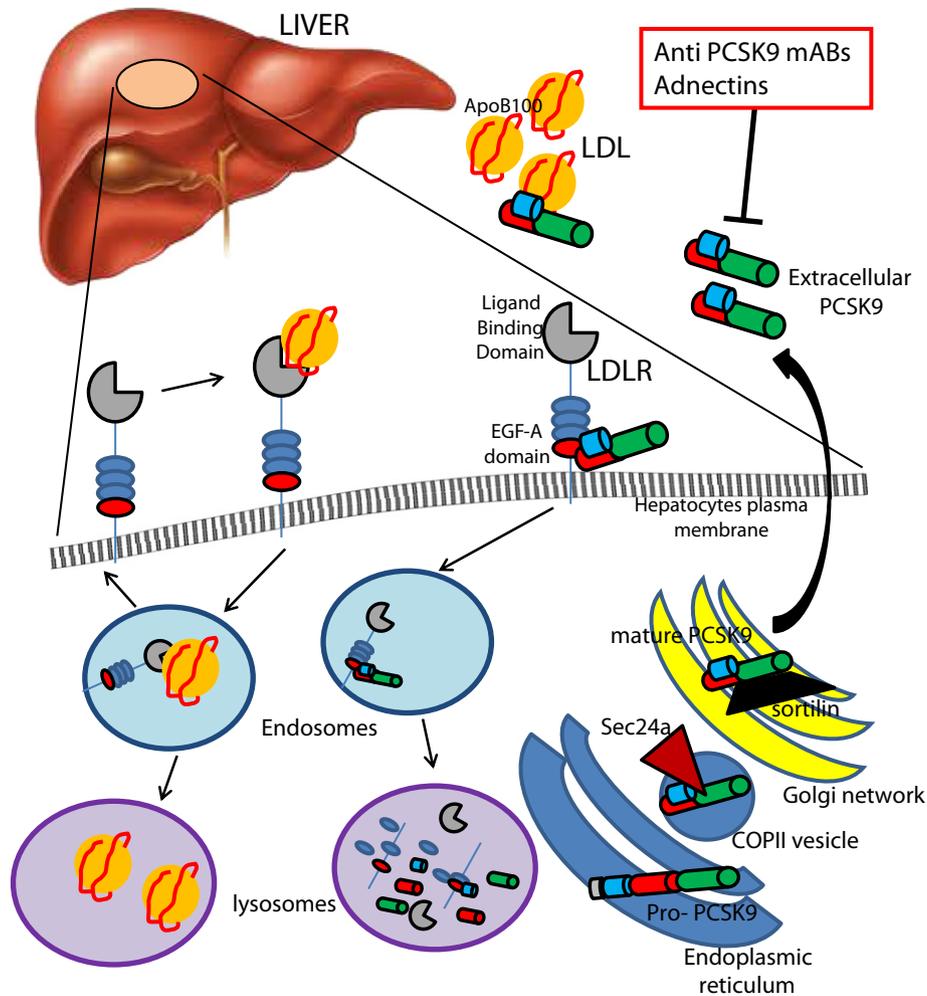
Werner et al. in this issue of *Vascular Pharmacology* [11] report for the first time that PCSK9 predicts cardiovascular events in statin-treated patients with well controlled LDL levels and documented stable coronary artery disease. This finding supports the relevance and the clinical interest for the determination of PCSK9 plasma levels in various



**Fig. 1.** Synthesis and maturation of PCSK9. The genetic locus and the primary PCSK9 mRNA transcript are schematically represented. Translation of the mature mRNA results in a proPCSK9 protein that undergoes autocatalytic cleavage in the endoplasmic reticulum at the amino-acid position 152 (Asp<sub>151</sub>,Gln<sub>152</sub>), creating a pro-domain of ~15 kDa and a mature protein of ~60 kDa, containing the catalytic triad (Asp<sub>186</sub>, His<sub>226</sub>, Ser<sub>386</sub>, represented as stars) and a C-terminal Cys-His rich domain (CHR D). The pro-domain remains covalently bound to the mature protein and covers the catalytic site, inhibiting the protease activity of the mature secreted protein. The main inducers (arrow-line) and inhibitors (dashed-line) of PCSK9 gene expression are listed. The therapeutic approaches that inhibit intracellular PCSK9 are highlighted in red boxes.

populations or after pharmacological treatment, in addition to the monitoring of anti-PCSK9 therapies. PCSK9 hepatic expression is the key determinant of PCSK9 circulating levels. As a consequence, circulating PCSK9 is mainly regulated by cholesterol level changes in the liver, and parallels cholesterol biosynthesis with a marked diurnal rhythm [32]. The analysis of the PCSK9 gene promoter region showed the presence of a Sp1 site, an HNF1 $\alpha$  site and two sterol responsive elements (SRE-1). The latter are responsible for the sterol-dependent regulation of PCSK9 transcription, indeed the key factor that regulates PCSK9 gene expression is intracellular cholesterol availability, that modulates the nuclear translocation of the sterol responsive element binding protein 2 (SREBP-2) transcription factor [33]. PCSK9 mature protein also undergoes post-translational modifications that may modulate its function. For instance furin and PC5/6A, two members of the PC family, cleave mature PCSK9 in the extracellular space, releasing a truncated protein of about 60 kDa. The activity of furin-cleaved PCSK9 is debated.

In humans the PCSK9 F216L and Arg218Ser mutations shield the secreted protein from furin protease activity and result in hypercholesterolemia, suggesting that furin physiologically reduces LDLR degrading activity by PCSK9 [34]. In contrast it was recently shown that furin-cleaved PCSK9, purified from the plasma of normo-lipidemic donors, still down-regulates hepatic LDLR levels, thus increasing total cholesterol levels after injection in mice [35]. This information is important, because PCSK9 quantification is based on immunoassays that do not discriminate between the different forms of the circulating protein. As a consequence, results show large variations and do not provide a direct and full information on the activity of PCSK9 [36–39]. This may contribute to explaining the weak positive association between circulating PCSK9 and LDLC plasma levels found in the populations enrolled in the JUPITER trial and the Dallas Heart Study, where only 7% and 2.25% of the variance in LDLC are explained by PCSK9 plasma levels, respectively [36,40]. PCSK9 plasma levels increase following cholesterol-



**Fig. 2.** PCSK9 mediated degradation of the LDLR. The secreted PCSK9 binds the EGFA domain of the LDLR present on the plasma membrane of hepatocytes. The complex undergoes clathrin-mediated endocytosis and is then escorted to lysosomes for degradation, in contrast to the physiological recycling of the LDLR to the plasma membrane, after binding the circulating LDL particle. The therapeutic approaches that inhibit circulating PCSK9 are highlighted in red boxes.

lowering pharmacological treatments. The most relevant example is represented by statins [40–42], which by blocking intracellular cholesterol synthesis induces SREBP-2 mediated pathways, thus concomitantly increasing the expression of LDLR and PCSK9. This effect reduces the pharmacological effect of statins and abolishes the positive correlation between circulating PCSK9 and LDLC [40,42]. Thus, PCSK9 is an indirect marker of statins efficacy, as recently demonstrated by analysis in a cohort of patients enrolled in the JUPITER trial, where the greatest reduction of LDLC was observed in patients presenting the strongest PCSK9 induction [40]. The effect of fibrates on plasma PCSK9 is debated, with either reduced or increased PCSK9 levels reported [43–45]. Both a direct PPAR $\alpha$ -mediated or an indirect action, linked to lipoprotein metabolism and hepatocyte intracellular cholesterol level regulation, have been proposed to explain these findings. The nutritional and hormonal status represent additional factors involved in the regulation of PCSK9 plasma levels that are reduced by fasting, glucagon and growth hormone [46,47]. Sex hormones have been implicated in the regulation of PCSK9 levels: indeed, premenopausal women have higher PCSK9 compared with men, and in boys PCSK9 decreases during puberty. However the responsible mechanisms for these effects have not yet been clarified [36–38].

### 3.2. PCSK9 and triglyceride-rich lipoprotein metabolism

An intriguing aspect of the observation of Werner et al. published in this issue of the journal [11] is also the effect of plasma triglycerides on

the circulating PCSK9 predictive power. To date, the involvement of PCSK9 in triglyceride metabolism, and particularly in the regulation of post-prandial lipidemia and apoB-containing lipoprotein output from the liver, is controversial. PCSK9 is expressed in the intestine, and in the enterocytes promotes the degradation of the LDLR expressed at the baso-lateral surfaces. The exposure of polarized Caco 2–15 cells to exogenous PCSK9 reduces the levels of LDLR, while increasing cholesterol uptake from the luminal surface and apoB48 synthesis and output [48]. PCSK9 knockout mice show reduced intestinal apoB secretion, increased chylomicrons clearance and decreased plasma triglycerides following olive oil gavage [49]. In two subjects bearing loss-of-function mutations leading to up to 50% reduced or absent levels of the circulating protein [50], post-prandial triglyceride levels were similar compared with control subjects [51]. The latter study suggests that circulating PCSK9 is not involved in the regulation of post-prandial lipidemia in humans, but does not exclude a yet to be discovered activity of the locally produced protein. Nevertheless the limited number of subjects investigated makes it difficult to draw a firm conclusion on this, and further studies in larger populations are warranted. The carriers of the S127R and D374Y mutations in the PCSK9 gene [8,9] are affected by severe forms of hypercholesterolemia, characterized by an increased production of VLDL. In agreement, the over-expression of these mutated forms of PCSK9 in hepatocytes and in mice results in increased apoB secretion and VLDL plasma levels [52–54]. The overexpression of the wild-type PCSK9 in mice can also upregulate the hepatic VLDL output by a mechanism dependent on

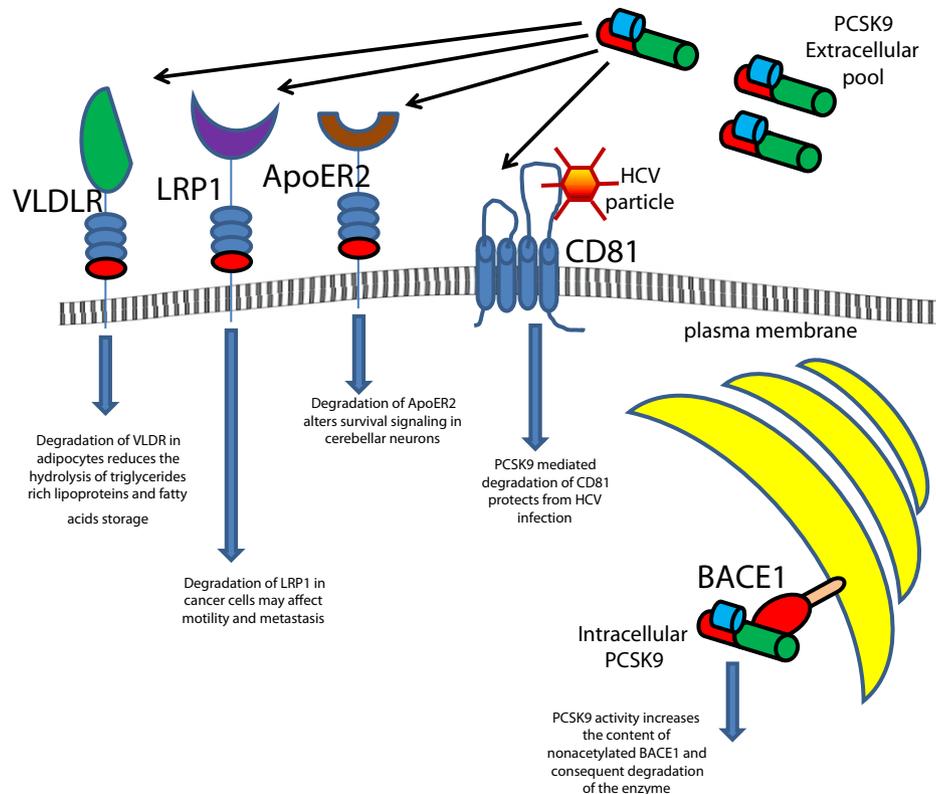
LDLR degradation, PPAR $\alpha$  activity and the nutritional status, with marked hypertriglyceridemia in the fasting but not in the fed state [55]. In contrast, the genetic deletion of PCSK9 in mice and the knock-down mediated by short interfering RNA in cultured hepatocytes fail to affect apoB containing lipoproteins secretion [56]. Furthermore, PCSK9 plasma levels are not correlated with VLDL secretion or clearance in obese patients [57]. This scenario is further complicated by the recent discovery that in hepatocytes PCSK9 binds and reduces the intracellular degradation of apoB100, this activity being independent on the LDLR levels and positively modulating the apoB output [58]. The contrasting data presented may be reconciled by the analysis of the experimental methodologies used in the different studies. Indeed the levels of PCSK9 transgene over-expression achieved by adenoviral infections are heterogeneous, being more than 50-fold higher in the study of Sun et al. [28] and only 3-fold higher in the study by Lambert et al. [55]. Finally, studies performed in familial combined hyperlipidemia (FCHL) patients showed that plasma PCSK9 is positively associated with both triglycerides and apoB levels, the two hallmarks of this genetic dyslipidemia [59,60]. These results suggest that FCHL patients, actually a consistent portion of hypercholesterolemic subjects characterized by high CVD risk, might benefit from PCSK9 inhibition-based therapies.

### 3.3. PCSK9 target proteins and PCSK9 extra-hepatic activity

Most of the efforts of basic and clinical research on PCSK9 during this decade have been devoted to the characterization of the PCSK9-LDLR pathway, given the enormous potential of its pharmacological blockade. Nonetheless, considerations on the physiological functions of the protein, even unrelated to the control of LDL uptake in the liver, are now emerging, paralleling the encouraging results obtained in clinical trials with anti-PCSK9 mAb. Experimental evidence is accumulating showing that extra-hepatic tissues, such as the kidney, the small intestine, the endocrine pancreas, the central nervous system and vascular tissues, express PCSK9 [12,29,49,61]. Of note, the PCSK9 activity is not restricted to

the LDLR, and alternative targets have been identified, such as the LDLR-related receptors VLDLR (very low density lipoprotein receptor), ApoER2 (LRP8) and LRP1, the enzyme BACE1 ( $\beta$ -site amyloid precursor protein (APP)-cleaving enzyme 1), and the hepatic receptor CD81 [62–65]. The degradation of these proteins may require specific cellular pathways and may have biological roles not directly linked with cholesterol homeostasis (Fig. 3). It is interesting to note that the over-expression of PCSK9 modulates not only genes involved in cholesterol metabolism, but also in proliferative, apoptotic and inflammatory pathways [66,67]. The PCSK9-mediated degradation of the VLDLR has been recently shown to affect adipocyte size and adipose tissue distribution in mice. Indeed, PCSK9 knockout mice show, at 6 months of age, 80% increased visceral adipose tissue compared with wild-type mice. Adipocytes do not express PCSK9, and this effect is mediated by circulating endogenous PCSK9, which lowers the levels of VLDLR in the adipose tissue [68], thus dampening the binding and hydrolysis of triglyceride-rich lipoproteins and the disposal of fatty acids to be stored in the adipose tissue. In humans the correlation between plasma PCSK9 and adiposity indices, such as the body mass index (BMI), is modest [36], and specific studies aimed at investigating the role of PCSK9 in visceral adipose tissue metabolism are needed. Interestingly, PCSK9 expression is activated in vitro by resistin, a protein that in humans is mostly derived from macrophages and plays a key role in the development of insulin resistance and the associated dyslipidemia. However the plasma levels of resistin show an inverse correlation with circulating PCSK9 in lean, but not obese, subjects, thus making the relevance of resistin in the determination of PCSK9 plasma levels questionable, even if a role in the control of the expression and the activity of the locally produced protein cannot be excluded [69,70].

PCSK9 interacts with ApoER2 and enhances its intracellular degradation [62]. In vitro studies in cerebellar neurons confirmed this finding and showed that treatment with exogenous PCSK9 reduces the plasma levels of ApoER2 and alters ApoER2-mediated signaling, thus inducing apoptosis [71]. PCSK9 gene is physiologically silent in the central



**Fig. 3.** PCSK9 target proteins. PCSK9 synthesized in the liver or locally produced contributes to the degradation of different receptors that are present not only in the liver but also in extra-hepatic tissues, as described in the text. The major PCSK9 physiological functions mediated by the activity on the different receptors are briefly summarized.

nervous system, but can be induced following ischemic stroke in mice; however this inducible pool of PCSK9 does not affect the lesion volume even if it triggers the degradation of the LDLR in the cerebral tissues [30]. Interestingly, the only genetic trait that has been associated with the incidence of sporadic Alzheimer's disease (AD) is the presence of the ApoE  $\epsilon$ 4 genotype [72,73]. An interaction between PCSK9 SNPs and ApoE genotype was also reported [74]. Furthermore, PCSK9 is detectable in the human cerebrospinal fluid [75] and negatively regulates the activity of BACE1, the enzyme responsible for the generation of the amyloid  $\beta$ -peptide (A $\beta$ ) present in the Alzheimer's disease (AD) plaques [64]. These findings suggest the possible involvement of PCSK9 in neurodegenerative processes implicated in the development of AD, even if the global PCSK9 knockout in mice does not interfere with BACE1 activity and A $\beta$ <sub>1–40</sub> peptide formation [76], and even if the analysis of the rs11591147 SNP in the PCSK9 gene, associated with a loss of function of the protein and lower LDLC in the carriers, showed no association with cognitive performance in patients enrolled in the PROSPER (PROspective Study of Pravastatin in the Elderly at Risk) study [77]. Among the different LDLR-related receptors, PCSK9 has been recently shown to recognize LRP1 in murine B16F1 melanoma cells and Chinese hamster ovary (CHO) cells and to mediate its degradation [63]. LRP1 is a large endocytic and signaling receptor, characterized by a wide expression and highly heterogeneous function [78]. LRP1 can indeed modulate cell motility, the inflammatory response, the clearance of coagulation and other circulating proteins, and has been involved in many diseases such as AD, cancer metastasis, aortic aneurisms, diabetes and atherosclerosis [79–81]. Of note, we recently showed that proliferating vascular smooth muscle cells (VSMC) express PCSK9, that is functionally active and degrades the LDLR in macrophages, thus reducing in vitro foam cell formation [29]. VSMC and macrophages express high levels of LRP1, that in these cells is crucial for the regulation of proliferation, migration and the inflammatory response [79]. These findings suggest a possible role of PCSK9 in the regulation of vascular integrity, and particularly on atherosclerotic plaque development. In apolipoprotein E (ApoE) knockout mice the PCSK9 deficiency improves the plasma lipid profile and reduces the atherosclerotic burden in the aorta, whereas in compound PCSK9 and LDLR knockout mice atherosclerosis development is not affected, suggesting that the protective effect of PCSK9 deficiency is mostly derived from the increased hepatic levels of LDLR [82]. In contrast, in human patients affected by familial hypercholesterolemia [83] and hypertension [84] circulating PCSK9 is inversely correlated with carotid intima media thickness (IMT), a surrogate marker of pre-clinical atherosclerosis, and the association is independent of the plasma lipid profile. Large clinical trials aimed at assessing atherosclerosis development in patients treated with anti-PCSK9 drugs, such as the GLAGOV (Global Assessment of Plaque regression With a PCSK9 antibody as Measured by intravascular Ultrasound, [www.clinicaltrials.gov](http://www.clinicaltrials.gov), NCT01813422) will help assessing the impact of PCSK9 on plaque phenotype. The regulation of liver receptors such as CD81, LDLR and LRP1 opens questions on the effect of anti-PCSK9 therapies on the infectivity of viruses such as hepatitis C virus [65] and the vesicular stomatitis virus [85]. Particularly, HCV viral particles require the presence of LDLR and LRP1 on hepatocytes, and for this reason anti-PCSK9 therapies may potentially increase the vulnerability to HCV infection, an aspect that should be carefully considered during long-term therapies.

#### 4. Pharmacological strategies for inhibiting PCSK9: where do we stand?

Given that PCSK9 acts as a chaperone directing the LDL-R and thus promoting LDL-R degradation [5], the possibility of inhibiting PCSK9 represents a logical step to enhance the lipid-lowering effect of conventional agents [5] (Fig. 1). To this end, at least six different human mAbs and three gene-silencing approaches are under development. Among the mAb developed against PCSK9, clinical trial results are available for the three of them, alirocumab (SAR236553/REGN727), evolocumab

(AMG145) and bococizumab (RN316/PF-04950615), while for RG7652, 1B20 and LGT209 no data were published yet. A large body of evidence is available so far for alirocumab and evolocumab. Results from phase I and phase II studies on alirocumab and evolocumab have been discussed elsewhere [2], while the results of phase II trials with bococizumab were recently presented (<http://www.abstractsonline.com/pp8/#!/3392/presentation/29479>). In all cases, phase II studies have shown an LDL-C cholesterol reduction following PCSK9 therapy up to 60–70% [2]. Here we will review in details the data from the first phase III clinical trials, which were recently reported.

The Mendel-2 study [86] enrolled 614 hypercholesterolemic subjects not on statin treatment. The results show that three months of treatment with evolocumab decrease LDL-C levels about 55–57% and, compared with ezetimibe treatment, of an additional 38–40%. The results from the LAPLACE-2 study (oral communication at ACC Sessions 2014), which aimed at evaluating the efficacy of 12 weeks of subcutaneous evolocumab (vs placebo) administered every 2 weeks or every month in combination with a statin in patients with hypercholesterolemia and mixed dyslipidemia, showed an LDL-C reduction from 55% to 76% compared with placebo, and from 38% to 47% compared with ezetimibe. On the same line, 52 weeks of treatment with evolocumab of more than 900 hyperlipidemic patients at elevated cardiovascular risk (DESCARTES Study) [87] showed that evolocumab, added to diet alone, to low-dose atorvastatin, or to high-dose atorvastatin with or without ezetimibe, significantly reduced LDL cholesterol levels in patients with a range of cardiovascular risks, by 48–62%. Of note, evolocumab treatment also significantly reduced levels of apoB, non-high-density lipoprotein cholesterol, lipoprotein(a), and triglycerides. The most common adverse events were nasopharyngitis, upper respiratory tract infection, influenza, and back pain.

The GAUSS-2 study aimed at evaluating the efficacy and safety of subcutaneous evolocumab compared with oral ezetimibe in hypercholesterolemic subjects which were unable to tolerate effective statin doses [88]. Evolocumab reduced LDL-C from baseline by 53–56%, corresponding to treatment differences versus ezetimibe of 37–39%. Muscle adverse events occurred in 12% of evolocumab- and 23% of ezetimibe-treated patients. Treatment-emergent adverse events and laboratory abnormalities were comparable across treatment groups. Preliminary data from the RUTHERFORD-2 study, where evolocumab was administered in patients with familial hypercholesterolemia, showed an average reduction of LDL-C by about 60% (oral communication at ACC Sessions 2014). Also the first data from a phase III study with alirocumab were recently reported, and the ODISSEY-MONO study (oral communication at ACC Sessions 2014) showed that the antibody administered in patients not on statin therapy shows efficiency and safety profiles similar to what was reported for evolocumab. Alirocumab resulted to be effective also when administered with fibrates, which are also known to increase PCSK9 levels. The association between alirocumab and fibrates slightly affected the efficacy of the antibody in decreasing LDL-C levels (Oral communication at ACC Sessions 2014). Overall data from phase I to phase III trials indicate that the mAbs result in an average reduction of LDL-C levels in the range of 50–60% either as a monotherapy or in combination with statins.

An important consideration relates to the ability of anti-PCSK9 mAbs to decrease the Lp(a) plasma levels. A recent analysis, pooling data from 1359 patients in 4 phase II trials, showed a dose-dependent reduction of circulating Lp(a) in patients treated with evolocumab versus placebo (–29.5% and –24.5% with 140 mg and 420 mg respectively) [89]. Future studies are needed to characterize the mechanism underlying this effect. However current therapies effective in reducing Lp(a) are limited to anti-apoB antisense oligonucleotide (mipomersen) or lomitapide [90]. PCSK9 inhibitors might therefore represent an optimal therapeutic option to improve the lipid profile of subjects at high CVD risk characterized by elevated Lp(a) plasma levels. Data on hard cardiovascular endpoints are clearly warranted. However, based on the epidemiological observations, which suggest that the benefit achieved is directly

related to LDL-C reduction, a decrease by 40 to 50% or larger in the relative risk of cardiovascular events is expected. In addition to monoclonal antibodies, other strategies are under development, and include anti-PCSK9 adnectins, that bind and block circulating PCSK9, small molecules that inhibit the PCSK9 processing, and antisense oligonucleotide or short interfering RNA which favor the degradation of the messenger RNA coding for PCSK9 [91] (Fig. 4). Phase I data with the siRNA ALN-PCS were recently released [92], and showed that the inhibition of PCSK9 synthesis by RNA interference (RNAi) provides a potentially safe mechanism to reduce LDL cholesterol concentration in healthy individuals with raised cholesterol. These results set the stage for further assessment of ALN-PCS in patients with hypercholesterolemia, including those treated with statins.

## 5. Conclusions

The rapid development and the interest in lipids and lipoproteins biology lead to the identification of PCSK9 as a critical target for the development of novel pharmacological approaches for the treatment of hypercholesterolemia. Some open questions however remain: i) the long term safety of a therapy with PCSK9 monoclonal antibodies in relation to immune system activation, despite data so far available from 52 weeks of treatment look promising; ii) as PCSK9 inhibitors look so effective in reducing LDL-C to very low levels will this situation be free of any negative consequence? Data from patients with genetic mutations leading to a complete loss of function of PCSK9, which show a normal life, are encouraging; iii) PCSK9 expression has been detected in extra-hepatic tissues, such as the central nervous system, and a better understanding of the physiological role of the protein and its possible involvement in neurological diseases is needed; iv) In contrast to statins, PCSK9 inhibition so far did not result in muscular side effects. This peculiarity, if confirmed in long term trials, may help in elucidating the mechanisms underlying muscle toxicity linked to statins. It is therefore reasonable to expect that PCSK9 inhibitors will be first available for patients with genetic hypercholesterolemia or intolerant to statins.

## Disclosure statement

A.L.C is a consultant for AstraZeneca, Bristol-Myers Squibb, Genentech, Kowa, Merck, Novartis, Pfizer, Roche, Sanofi-Synthelabo,

and Takeda. G.D.N and G.T do not have any financial conflict of interest to declare.

## References

- [1] Nordestgaard BG, Chapman MJ, Humphries SE, Ginsberg HN, Masana L, Descamps OS, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J* 2013;34:3478–940.
- [2] Norata GD, Ballantyne CM, Catapano AL. New therapeutic principles in dyslipidaemia: focus on LDL and Lp(a) lowering drugs. *Eur Heart J* 2013;34:1783–9.
- [3] Reiner Z, Catapano AL, De Backer G, Graham I, Taskinen MR, Wiklund O, et al. ESC/EAS guidelines for the management of dyslipidaemias: the Task Force for the management of dyslipidaemias of the European Society of Cardiology (ESC) and the European Atherosclerosis Society (EAS). *Eur Heart J* 2011;32:1769–818.
- [4] Abifadel M, Varret M, Rabes JP, Allard D, Ouguerram K, Devillers M, et al. Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat Genet* 2003;34:154–6.
- [5] Tibolla G, Norata GD, Artali R, Meneghetti F, Catapano AL. Proprotein convertase subtilisin/kexin type 9 (PCSK9): from structure–function relation to therapeutic inhibition. *Nutr Metab Cardiovasc Dis* 2011;21:835–43.
- [6] Dubuc G, Chamberland A, Wassef H, Davignon J, Seidah NG, Bernier L, et al. Statins upregulate PCSK9, the gene encoding the proprotein convertase neural apoptosis-regulated convertase-1 implicated in familial hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 2004;24:1454–9.
- [7] Rashid S, Curtis DE, Garuti R, Anderson NN, Bashmakov Y, Ho YK, et al. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking PCSK9. *Proc Natl Acad Sci U S A* 2005;102:5374–9.
- [8] Zhao Z, Tuakli-Wosornu Y, Lagace TA, Kinch L, Grishin NV, Horton JD, et al. Molecular characterization of loss-of-function mutations in PCSK9 and identification of a compound heterozygote. *Am J Hum Genet* 2006;79:514–23.
- [9] Hooper AJ, Marais AD, Tanyanyiwa DM, Burnett JR. The C679X mutation in PCSK9 is present and lowers blood cholesterol in a Southern African population. *Atherosclerosis* 2007;193:445–8.
- [10] Norata GD, Tibolla G, Catapano AL. Targeting PCSK9 for hypercholesterolemia. *Annu Rev Pharmacol Toxicol* 2014;54:273–93.
- [11] Werner C, Hoffmann MM, Winkler K, Bohm M, Laufs U. Risk prediction with proprotein convertase subtilisin/kexin type 9 (PCSK9) in patients with stable coronary disease on statin treatment. *Vascul Pharmacol* 2014;62(2):94–102 (in this issue).
- [12] Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, et al. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): liver regeneration and neuronal differentiation. *Proc Natl Acad Sci U S A* 2003;100:928–33.
- [13] Benjannet S, Rhainds D, Essalmani R, Mayne J, Wickham L, Jin W, et al. NARC-1/PCSK9 and its natural mutants: zymogen cleavage and effects on the low density lipoprotein (LDL) receptor and LDL cholesterol. *J Biol Chem* 2004;279:48865–75.
- [14] Hampton EN, Knuth MW, Li J, Harris JL, Lesley SA, Spraggon G. The self-inhibited structure of full-length PCSK9 at 1.9 Å reveals structural homology with resistin within the C-terminal domain. *Proc Natl Acad Sci U S A* 2007;104:14604–9.

compound	company	Pre-clinical	Phase I	Phase II	Phase III
Evolocumab-mAb-AMG145	Amgen	→	→	→	→
Alirocumab - mAb-REG727/SAR236553	SA / Regeneron	→	→	→	→
Bococizumab- mAb-RN316(PF-04950615)	Pfizer-Rinat	→	→	→	
mAb - LGT209	Novartis	→	→	→	
mAb - RG7652	Roche / Genentech	→	→	→	
Adnectin Ad BMS962476	BMS / Adnexus	→	→		
siRNA ALN-PCS	Alnylam	→	→		
Small chemical inhibitors	Shifa Biomedical Corp.	→			

Fig. 4. Anti PCSK9 therapeutic approaches. Pharmacological agents developed for inhibiting PCSK9 and their corresponding pre-clinical or clinical development state.

- [15] Cunningham D, Danley DE, Geoghegan KF, Griffor MC, Hawkins JL, Subashi TA, et al. Structural and biophysical studies of PCSK9 and its mutants linked to familial hypercholesterolemia. *Nat Struct Mol Biol* 2007;14:413–9.
- [16] Qian YW, Schmidt RJ, Zhang Y, Chu S, Lin A, Wang H, et al. Secreted PCSK9 downregulates low density lipoprotein receptor through receptor-mediated endocytosis. *J Lipid Res* 2007;48:1488–98.
- [17] Nassoury N, Blasiote DA, Tebon-Oler A, Benjannet S, Hamelin J, Poupon V, et al. The cellular trafficking of the secretory proprotein convertase PCSK9 and its dependence on the LDLR. *Traffic* 2007;8:718–32.
- [18] Lagace TA, Curtis DE, Garuti R, McNutt MC, Park SW, Prather HB, et al. Secreted PCSK9 decreases the number of LDL receptors in hepatocytes and in livers of parabiotic mice. *J Clin Invest* 2006;116:2995–3005.
- [19] Zhang DW, Lagace TA, Garuti R, Zhao Z, McDonald M, Horton JD, et al. Binding of proprotein convertase subtilisin/kexin type 9 to epidermal growth factor-like repeat a of low density lipoprotein receptor decreases receptor recycling and increases degradation. *J Biol Chem* 2007;282:18602–12.
- [20] Bottomley MJ, Cirillo A, Orsatti L, Ruggeri L, Fisher TS, Santoro JC, et al. Structural and biochemical characterization of the wild type PCSK9-EGF(AB) complex and natural familial hypercholesterolemia mutants. *J Biol Chem* 2009;284:1313–23.
- [21] Zhang DW, Garuti R, Tang WJ, Cohen JC, Hobbs HH. Structural requirements for PCSK9-mediated degradation of the low-density lipoprotein receptor. *Proc Natl Acad Sci U S A* 2008;105:13045–50.
- [22] Yamamoto T, Lu C, Ryan RO. A two step binding model of PCSK9 interaction with the low density lipoprotein receptor. *J Biol Chem* 2011 Feb 18;286(7):5464–70.
- [23] Holla OL, Cameron J, Tveten K, Strom TB, Berge KE, Laerdahl JK, et al. Role of the C-terminal domain of PCSK9 in degradation of the LDL receptors. *J Lipid Res* 2011;52:1787–94.
- [24] DeVay RM, Shelton DL, Liang H. Characterization of proprotein convertase subtilisin/kexin type 9 (PCSK9) trafficking reveals a novel lysosomal targeting mechanism via amyloid precursor-like protein 2 (AβL2). *J Biol Chem* 2013;288:10805–18.
- [25] Chen XW, Wang H, Bajaj K, Zhang P, Meng ZX, Ma D, et al. SEC24A deficiency lowers plasma cholesterol through reduced PCSK9 secretion. *eLife* 2013;2:e00444.
- [26] Gustafsen C, Kjolby M, Nyegaard M, Mattheisen M, Lundhede J, Buttenschon H, et al. The hypercholesterolemia-risk gene SORT1 facilitates PCSK9 secretion. *Cell Metab* 2014;19:310–8.
- [27] Kosenko T, Golder M, Leblond G, Weng W, Lagace TA. Low-density lipoprotein binds to proprotein convertase subtilisin/kexin type-9 (PCSK9) in human plasma and inhibits PCSK9-mediated LDL receptor degradation. *J Biol Chem* 2013 Mar 22;288(12):8279–88.
- [28] Sun H, Samarghandi A, Zhang N, Yao Z, Xiong M, Teng BB. Proprotein convertase subtilisin/kexin type 9 interacts with apolipoprotein B and prevents its intracellular degradation, irrespective of the low-density lipoprotein receptor. *Arterioscler Thromb Vasc Biol* 2013;33:1585–95.
- [29] Ferri N, Tibolla G, Pirillo A, Cipollone F, Mezzetti A, Pacia S, et al. Proprotein convertase subtilisin kexin type 9 (PCSK9) secreted by cultured smooth muscle cells reduces macrophages LDLR levels. *Atherosclerosis* 2012;220:381–6.
- [30] Rousselet E, Marcinkiewicz J, Kriz J, Zhou A, Hatten ME, Prat A, et al. PCSK9 reduces the protein levels of the LDL receptor in mouse brain during development and after ischemic stroke. *J Lipid Res* 2011;52:1383–91.
- [31] Mayer G, Poirier S, Seidah NG. Annexin A2 is a C-terminal PCSK9-binding protein that regulates endogenous low density lipoprotein receptor levels. *J Biol Chem* 2008;283:31791–801.
- [32] Persson L, Cao G, Stahle L, Sjoberg BG, Troutt JS, Konrad RJ, et al. Circulating proprotein convertase subtilisin kexin type 9 has a diurnal rhythm synchronous with cholesterol synthesis and is reduced by fasting in humans. *Arterioscler Thromb Vasc Biol* 2010;30:2666–72.
- [33] Jeong HJ, Lee HS, Kim KS, Kim YK, Yoon D, Park SW. Sterol-dependent regulation of proprotein convertase subtilisin/kexin type 9 expression by sterol-regulatory element binding protein-2. *J Lipid Res* 2008;49:399–409.
- [34] Essalmani R, Susan-Resiga D, Chamberland A, Abifadel M, Cremers JW, Boileau C, et al. In vivo evidence that furin from hepatocytes inactivates PCSK9. *J Biol Chem* 2011 Feb 11;286(6):4257–63.
- [35] Lipari MT, Li W, Moran P, Kong-Beltran M, Sai T, Lai J, et al. Furin-cleaved proprotein convertase subtilisin/kexin type 9 (PCSK9) is active and modulates low density lipoprotein receptor and serum cholesterol levels. *J Biol Chem* 2012;287:43482–91.
- [36] Lakoski SG, Lagace TA, Cohen JC, Horton JD, Hobbs HH. Genetic and metabolic determinants of plasma PCSK9 levels. *J Clin Endocrinol Metab* 2009;94:2537–43.
- [37] Baass A, Dubuc G, Tremblay M, Delvin EE, O'Loughlin J, Levy E, et al. Plasma PCSK9 is associated with age, sex, and multiple metabolic markers in a population-based sample of children and adolescents. *Clin Chem* 2009;55:1637–45.
- [38] Cui Q, Ju X, Yang T, Zhang M, Tang W, Chen Q, et al. Serum PCSK9 is associated with multiple metabolic factors in a large Han Chinese population. *Atherosclerosis* 2010;213:632–6.
- [39] Dubuc G, Tremblay M, Pare G, Jacques H, Hamelin J, Benjannet S, et al. A new method for measurement of total plasma PCSK9: clinical applications. *J Lipid Res* 2010;51:140–9.
- [40] Awan Z, Seidah NG, MacFadyen JG, Benjannet S, Chasman DI, Ridker PM, et al. Rosuvastatin, proprotein convertase subtilisin/kexin type 9 concentrations, and LDL cholesterol response: the JUPITER trial. *Clin Chem* 2012;58:183–9.
- [41] Careskey HE, Davis RA, Albom WE, Troutt JS, Cao G, Konrad RJ. Atorvastatin increases human serum levels of proprotein convertase subtilisin/kexin type 9. *J Lipid Res* 2008;49:394–8.
- [42] Welder G, Zineh I, Pacanowski MA, Troutt JS, Cao G, Konrad RJ. High-dose atorvastatin causes a rapid sustained increase in human serum PCSK9 and disrupts its correlation with LDL cholesterol. *J Lipid Res* 2010;51:2714–21.
- [43] Lambert G, Ancellin N, Charlton F, Comas D, Pilot J, Keech A, et al. Plasma PCSK9 concentrations correlate with LDL and total cholesterol in diabetic patients and are decreased by fenofibrate treatment. *Clin Chem* 2008;54:1038–45.
- [44] Troutt JS, Albom WE, Cao G, Konrad RJ. Fenofibrate treatment increases human serum proprotein convertase subtilisin kexin type 9 levels. *J Lipid Res* 2010;51:345–51.
- [45] Costet P, Hoffmann MM, Cariou B, Guyomarc'h Delasalle B, Konrad T, Winkler K. Plasma PCSK9 is increased by fenofibrate and atorvastatin in a non-additive fashion in diabetic patients. *Atherosclerosis* 2010;212:246–51.
- [46] Browning JD, Horton JD. Fasting reduces plasma proprotein convertase, subtilisin/kexin type 9 and cholesterol biosynthesis in humans. *J Lipid Res* 2010;51:3359–63.
- [47] Persson L, Cao G, Stahle L, Sjoberg B, Troutt JS, Konrad RJ, et al. Circulating proprotein convertase subtilisin kexin type 9 has a diurnal rhythm synchronous with cholesterol synthesis and is reduced by fasting in humans. *Arterioscler Thromb Vasc Biol* 2010 Dec;30(12):2666–72.
- [48] Levy E, Ouadda AB, Spahis S, Sane AT, Garofalo C, Grenier E, et al. PCSK9 plays a significant role in cholesterol homeostasis and lipid transport in intestinal epithelial cells. *Atherosclerosis* 2013 Apr;227(2):297–306.
- [49] Le May C, Kourimate S, Langhi C, Chetiveaux M, Jarry A, Comera C, et al. Proprotein convertase subtilisin kexin type 9 null mice are protected from postprandial triglyceridemia. *Arterioscler Thromb Vasc Biol* 2009;29:684–90.
- [50] Cariou B, Ouguerram K, Zair Y, Guerois R, Langhi C, Kourimate S, et al. PCSK9 dominant negative mutant results in increased LDL catabolic rate and familial hyperbetaipoproteinemia. *Arterioscler Thromb Vasc Biol* 2009;29:2191–7.
- [51] Cariou B, Langhi C, Le Bras M, Bortolotti M, Le KA Theytaz F, et al. Plasma PCSK9 concentrations during an oral fat load and after short term high-fat, high-fat high-protein and high-fructose diets. *Nutr Metab (Lond)* 2013;10:4.
- [52] Sun XM, Eden ER, Tosi I, Neuwirth CK, Wile D, Naoumova RP, et al. Evidence for effect of mutant PCSK9 on apolipoprotein b secretion as the cause of unusually severe dominant hypercholesterolemia. *Hum Mol Genet* 2005;14:1161–9.
- [53] Herbert B, Patel D, Waddington SN, Eden ER, McAleenan A, Sun XM, et al. Increased secretion of lipoproteins in transgenic mice expressing human D374Y PCSK9 under physiological genetic control. *Arterioscler Thromb Vasc Biol* 2010;30:1333–9.
- [54] Ouguerram K, Chetiveaux M, Zair Y, Costet P, Abifadel M, Varret M, et al. Apolipoprotein B100 metabolism in autosomal-dominant hypercholesterolemia related to mutations in PCSK9. *Arterioscler Thromb Vasc Biol* 2004;24:1448–53.
- [55] Lambert G, Jarnoux AL, Pineau T, Pape O, Chetiveaux M, Laboisse C, et al. Fasting induces hyperlipidemia in mice overexpressing proprotein convertase subtilisin kexin type 9: lack of modulation of very-low-density lipoprotein hepatic output by the low-density lipoprotein receptor. *Endocrinology* 2006;147:4985–95.
- [56] Lalanne F, Lambert G, Amar MJ, Chetiveaux M, Zair Y, Jarnoux AL, et al. Wild-type PCSK9 inhibits LDL clearance but does not affect apoB-containing lipoprotein production in mouse and cultured cells. *J Lipid Res* 2005;46:1312–9.
- [57] Sullivan S, Fabbri E, Horton JD, Korenblat K, Patterson BW, Klein S. Lack of a relationship between plasma PCSK9 concentrations and hepatic lipoprotein kinetics in obese people. *Transl Res* 2011;158:302–6.
- [58] Sun H, Samarghandi A, Zhang N, Yao Z, Xiong M, Teng BB. Proprotein convertase subtilisin/kexin type 9 interacts with apolipoprotein B and prevents its intracellular degradation, irrespective of the low-density lipoprotein receptor. *Arterioscler Thromb Vasc Biol* 2012;32:1585–95.
- [59] Brouwers MC, van Greevenbroek MM, Troutt JS, Bonner Freeman A, Lu A, Schaper NC, et al. Plasma proprotein convertase subtilisin kexin type 9 is a heritable trait of familial combined hyperlipidaemia. *Clin Sci* 2011;121:397–403.
- [60] Brouwers MC, Konrad RJ, van Himbergen TM, Isaacs A, Otokozawa S, Troutt JS, et al. Plasma proprotein convertase subtilisin kexin type 9 levels are related to markers of cholesterol synthesis in familial combined hyperlipidemia. *Nutr Metab Cardiovasc Dis* 2013;23:1115–21.
- [61] Langhi C, Le May C, Gmyr V, Vandewalle B, Kerr-Conte J, Krempf M, et al. PCSK9 is expressed in pancreatic delta-cells and does not alter insulin secretion. *Biochem Biophys Res Commun* 2009;390:1288–93.
- [62] Poirier S, Mayer G, Benjannet S, Bergeron E, Marcinkiewicz J, Nassoury N, et al. The proprotein convertase PCSK9 induces the degradation of low density lipoprotein receptor (LDLR) and its closest family members VLDLR and ApoER2. *J Biol Chem* 2008;283:2363–72.
- [63] Canuel M, Sun X, Asselin MC, Paramithiotis E, Prat A, Seidah NG. Proprotein convertase subtilisin/kexin type 9 (PCSK9) can mediate degradation of the low density lipoprotein receptor-related protein 1 (LRP-1). *PLoS One* 2013;8:e64145.
- [64] Jonas MC, Costantini C, Puglielli L. PCSK9 is required for the disposal of non-acetylated intermediates of the nascent membrane protein BACE1. *EMBO Rep* 2008;9:916–22.
- [65] Labonte P, Begley S, Guevin C, Asselin MC, Nassoury N, Mayer G, et al. PCSK9 impedes hepatitis C virus infection in vitro and modulates liver CD81 expression. *Hepatology* 2009;50:17–24.
- [66] Lan H, Pang L, Smith MM, Levitan D, Ding W, Liu L, et al. Proprotein convertase subtilisin/kexin type 9 (PCSK9) affects gene expression pathways beyond cholesterol metabolism in liver cells. *J Cell Physiol* 2010;224:273–81.
- [67] Ranheim T, Mattingsdal M, Lindvall JM, Holla OL, Berge KE, Kulseth MA, et al. Genome-wide expression analysis of cells expressing gain of function mutant D374Y-PCSK9. *J Cell Physiol* 2008;217:459–67.
- [68] Roubtsova A, Munkonda MN, Awan Z, Marcinkiewicz J, Chamberland A, Lazure C, et al. Circulating proprotein convertase subtilisin/kexin 9 (PCSK9) regulates VLDLR protein and triglyceride accumulation in visceral adipose tissue. *Arterioscler Thromb Vasc Biol* 2011;31:785–91.
- [69] Kwakernaak AJ, Lambert G, Dullaart RP. Relationship of proprotein convertase subtilisin-kexin type 9 levels with resistin in lean and obese subjects. *Clin Biochem* 2012;45:1522–4.

- [70] Melone M, Wilsie L, Palyha O, Strack A, Rashid S. Discovery of a new role of human resistin in hepatocyte low-density lipoprotein receptor suppression mediated in part by proprotein convertase subtilisin/kexin type 9. *J Am Coll Cardiol* 2012;59:1697–705.
- [71] Kysenius K, Muggalla P, Matlik K, Arumae U, Huttunen HJ. PCSK9 regulates neuronal apoptosis by adjusting ApoE2 levels and signaling. *Cell Mol Life Sci* 2012;69:1903–16.
- [72] Casserly IP, Topol EJ. Convergence of atherosclerosis and Alzheimer's disease: cholesterol, inflammation, and misfolded proteins. *Discov Med* 2004;4:149–56.
- [73] Mahley RW, Weisgraber KH, Huang Y. Apolipoprotein E4: a causative factor and therapeutic target in neuropathology, including Alzheimer's disease. *Proc Natl Acad Sci U S A* 2006;103:5644–51.
- [74] Norata GD, Garlaschelli K, Grigore L, Raselli S, Tramontana S, Meneghetti F, et al. Effects of PCSK9 variants on common carotid artery intima media thickness and relation to ApoE alleles. *Atherosclerosis* 2010;208:177–82.
- [75] Chen YQ, Troutt JS, Konrad RJ. PCSK9 is present in human cerebrospinal fluid and is maintained at remarkably constant concentrations throughout the course of the day. *Lipids* 2014 May;49(5):445–55.
- [76] Liu M, Wu G, Baysarowich J, Kavana M, Addona GH, Bierilo KK, et al. PCSK9 is not involved in the degradation of LDL receptors and BACE1 in the adult mouse brain. *J Lipid Res* 2010;51:2611–8.
- [77] Postmus I, Trompet S, de Craen AJ, Buckley BM, Ford I, Stott DJ, et al. PCSK9 SNP rs11591147 is associated with low cholesterol levels but not with cognitive performance or noncardiovascular clinical events in an elderly population. *J Lipid Res* 2012;54:561–6.
- [78] Lillis AP, Van Duyn LB, Murphy-Ullrich JE, Strickland DK. LDL receptor-related protein 1: unique tissue-specific functions revealed by selective gene knockout studies. *Physiol Rev* 2008;88:887–918.
- [79] Boucher P, Gotthardt M, Li WP, Anderson RG, Herz J. LRP: role in vascular wall integrity and protection from atherosclerosis. *Science* 2003;300:329–32.
- [80] May P. The low-density lipoprotein receptor-related protein 1 in inflammation. *Curr Opin Lipidol* 2013;24:134–7.
- [81] Hofmann SM, Zhou L, Perez-Tilve D, Greer T, Grant E, Wancata L, et al. Adipocyte LDL receptor-related protein-1 expression modulates postprandial lipid transport and glucose homeostasis in mice. *J Clin Invest* 2007;117:3271–82.
- [82] Denis M, Marcinkiewicz J, Zaid A, Gauthier D, Poirier S, Lazure C, et al. Gene inactivation of proprotein convertase subtilisin/kexin type 9 reduces atherosclerosis in mice. *Circulation* 2012;125:894–901.
- [83] Huijgen R, Fouchier SW, Denoun M, Hutten BA, Vissers MN, Lambert G, et al. Plasma levels of PCSK9 and phenotypic variability in familial hypercholesterolemia. *J Lipid Res* 2012;53:979–83.
- [84] Lee CJ, Lee YH, Park SW, Kim KJ, Park S, Youn JC, et al. Association of serum proprotein convertase subtilisin/kexin type 9 with carotid intima media thickness in hypertensive subjects. *Metabolism* 2013 Jun;62(6):845–50.
- [85] Finkelshtein D, Werman A, Novick D, Barak S, Rubinstein M. LDL receptor and its family members serve as the cellular receptors for vesicular stomatitis virus. *Proc Natl Acad Sci U S A* 2013;110:7306–11.
- [86] Koren MJ, Lundqvist P, Bolognese M, Neutel JM, Monsalvo ML, Yang J, et al. Anti-PCSK9 monotherapy for hypercholesterolemia — the Mendel-2 randomized, controlled phase 3 clinical trial of evolocumab. *J Am Coll Cardiol* 2014 Jun 17;63(23):2531–40.
- [87] Blom DJ, Hala T, Bolognese M, Lillestol MJ, Toth PD, Burgess L, et al. A 52-week placebo-controlled trial of evolocumab in hyperlipidemia. *N Engl J Med* 2014 May 8;370(19):1809–19.
- [88] Stroes E, Colquhoun D, Sullivan D, Civeira F, Rosenson RS, Watts GF, et al. Anti-PCSK9 antibody effectively lowers cholesterol in patients with statin intolerance: the GAUSS-2 randomized, placebo-controlled phase 3 clinical trial of evolocumab. *J Am Coll Cardiol* 2014 Jun 17;63(23):2541–8.
- [89] Raal FJ, Giugliano RP, Sabatine MS, Koren MJ, Langslet G, Bays H, et al. Reduction in lipoprotein(a) with PCSK9 monoclonal antibody evolocumab (AMG 145): a pooled analysis of more than 1300 patients in 4 phase II trials. *J Am Coll Cardiol* 2014;63:1278–88.
- [90] Rader DJ, Kastelein JJ. Lomitapide and mipomersen: two first-in-class drugs for reducing low-density lipoprotein cholesterol in patients with homozygous familial hypercholesterolemia. *Circulation* 2014;129:1022–32.
- [91] Norata GD, Tibolla G, Catapano AL. Gene silencing approaches for the management of dyslipidaemia. *Trends Pharmacol Sci* 2013;34:198–205.
- [92] Fitzgerald K, Frank-Kamenetsky M, Shulga-Morskaya S, Liebow A, Bettencourt BR, Sutherland JE, et al. Effect of an RNA interference drug on the synthesis of proprotein convertase subtilisin/kexin type 9 (PCSK9) and the concentration of serum LDL cholesterol in healthy volunteers: a randomised, single-blind, placebo-controlled, phase 1 trial. *Lancet* 2013 Jan 4;383(9911):60–8.