

Review

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# PCSK9 and lipid metabolism and atherosclerosis: animal models

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## Abstract

PCSK9 is a secreted protein that binds to the LDL receptor on the cell surface, is endocytosed along with the receptor, and interferes with its ability to recycle to the cell surface. Instead the receptor is directed to the lysosome where it is degraded, leading to decreased levels of the receptor on the cell surface and decreased internalization of LDL. In this review, we will discuss how mice deficient in the *Pcsk9* gene or hepatic overexpression due to transgenesis or viral vector mediated expression contributed to our understanding of the *in vivo* role of PCSK9. In addition, we will discuss how the discovery of PCSK9 and its gain of function mutants has greatly facilitated the manipulation of animals modelling atherosclerosis to induce hypercholesterolemia and atherosclerosis.

**Keywords:** PCSK9, LDL receptor, hyperlipidemia, atherosclerosis, mice, pig, animal models of atherosclerosis, knockout, transgenic, viral mediated

## INTRODUCTION

Much of the concern with the approach to atherosclerotic cardiovascular disease hinges on the role of the LDL receptor (LDLR), stemming from the seminal work of Drs. Goldstein and Brown<sup>[1]</sup>. Two children with homozygous familial hypercholesterolemia, the most common form of autosomal dominant hypercholesterolemia, stimulated their interest in understanding the genetic basis for their hypercholesterolemia. Such subjects are at risk of early death from atherosclerotic cardiovascular disease due to mutations that affect the level or function of the LDLR. Other forms of autosomal dominant



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hypercholesterolemia are related to increased ApoB100 production and secretion and gain of function mutants of proprotein convertase subtilisin/kexin type 9 (PCSK9). For the most part, the function of PCSK9 with respect to hypercholesterolemia depends on its interaction with the LDLR. This will be the thrust of this review.

## PCSK9 AND LIPID METABOLISM AND ATHEROSCLEROSIS

### Pathways regulating cell surface levels of the LDLR

In order to understand the function of PCSK9, it is necessary to summarize the cellular trajectory of the LDLR. The receptor is present on cell membranes in clathrin coated pits where it interacts with extracellular LDL. The receptor-LDL complex is endocytosed and upon acidification of the endosome, the LDL is released from the receptor and is degraded in the lysosome liberating its cholesterol. The LDL derived cholesterol is used for membrane assembly, used for lipoprotein assembly, or esterified by acyl cholesteryl acyl transferase for storage. The LDLR then recycles to the cell surface for repeated cycles of LDL endocytosis. On average, each receptor recycles about 20 times. Cholesterol in the endoplasmic reticulum (ER) plays an important regulatory role by interacting with a series of membrane bound ER proteins that transport the precursors of the sterol responsive element binding protein transcription factors (SREBP1 and SREBP2) to the Golgi when cellular cholesterol levels are low or depleted. When cellular cholesterol is high, the binding proteins and the SREBP precursors are retained in the ER. The processing of the SREBP precursors in the Golgi involves 2 proteases that release the active transcription factor for transport to the nucleus. One of these proteases, site 1 protease, is a member of the subtilisin family (number 8), to which PCSK9 belongs. Among the genes induced under low cholesterol conditions are those encoding HMG-CoA reductase (the rate limiting enzyme for cholesterol biosynthesis) and the LDLR (involved in the acquisition of cholesterol packaged in the LDL particle). The rationale for the widespread use of the statins in the treatment of hypercholesterolemia relates to their inhibition of HMG-CoA reductase and hence cholesterol biosynthesis.

This very brief description indicates that cellular cholesterol homeostasis is maintained by regulated transcriptional feedback control of both cellular cholesterol biosynthesis and the acquisition of extracellular cholesterol packaged in LDL. The latter process, especially in the liver, is the most important pathway regulating plasma LDL levels. However, LDLR is expressed in most of the cells and tissues of the body. Indeed, most of the early experiments performed by Brown and Goldstein were with cultured human fibroblasts or the adrenal gland from which the receptor was first purified.

Though the transcriptional regulation of the *LDLR* gene is critical for the control of plasma cholesterol levels, there are also post-transcriptional mechanisms that modulate the level of the receptor on the cell surface. One of these involves the ubiquitination of the cytoplasmic tail of the receptor by the E3 ligase inducible degrader of the LDL receptor (IDOL) leading to proteasomal degradation of the receptor<sup>[2]</sup>. IDOL expression is positively regulated by the oxysterol sensing liver X receptor transcription factor. This will not be further discussed in this review. The second mechanism involves increased lysosomal degradation of the receptor mediated by PCSK9. The importance of PCSK9 in LDL cholesterol metabolism was discovered when mutations in the *PCSK9* gene were identified in 2 French families with autosomal dominant hypercholesterolemia that were not linked to mutations in the *LDLR* and *APOB* genes<sup>[3]</sup>. There was an AT substitution at nucleotide 625 resulting in a serine to arginine change at amino acid residue 127 (p.S127R) in 1 family and a TC substitution at nucleotide 890 resulting in the amino acid alteration of p.F216L in the second family. Additional mutants have also been identified including the p.D374Y mutation<sup>[4,5]</sup>. The identification of PCSK9 mutations associated with hypercholesterolemia represented an important new chapter in the regulation of plasma LDL levels.

It should be noted that the *PCSK9* gene was originally determined to be identical to the neuronal apoptosis factor *NARC-1* gene. While *PCSK9* (*NARC-1*) is highly expressed in the liver where it plays an important role in regulating plasma cholesterol levels, the gene is ubiquitously expressed. The protein is known to regulate neuronal survival in the central nervous system, may regulate renal physiology in the kidney, and properties of cells in the vascular wall among other functions that are not related to regulating plasma LDL levels [6,7].

### The structure and properties of PCSK9

Human PCSK9 is a 72kDa secreted protein, containing 692 amino acids. It has 3 domains in addition to the 30 amino acid signal peptide: the prodomain (residues 31-152), the catalytic domain (residue 153-425), and a C terminal cysteine and histidine rich domain (residues 426-692)[8]. The original mutations described by Abifadel *et al.*[3] are in the prodomain and catalytic domain, respectively. Pro-PCSK9 facilitates the transport of the LDLR from the ER to the Golgi where the latter is matured by glycosylation. Pro-PCSK9 is autocatalytically cleaved in the Golgi at residue Q152, though the prodomain (13kDa) remains non-covalently attached to the rest of the protein. This blocks its catalytic activity. In addition, autocatalytic cleavage is important for the secretion of PCSK9. A naturally occurring p.Q152H mutation prevents the autocatalytic cleavage and protein secretion. This mutation is associated with a 48% reduction of plasma LDL cholesterol (LDL-C) levels in humans[9]. In the Golgi, PCSK9 associates with sortilin which facilitates its secretion from the cell[10].

PCSK9 heterodimer (62 kDa plus 13 kDa prodomain) circulates in the plasma in free form or bound to LDL. In the plasma, there is 1 molecule of PCSK9 for each 500-1000 LDL particles. It also binds lipoprotein(a)[11], an independent risk factor for cardiovascular disease. However, it has little affinity for VLDL, chylomicrons, or remnants of chylomicrons.

### PCSK9 disrupts LDLR recycling

PCSK9 regulates the number of LDLR molecules on the cell surface. The catalytic domain of PCSK9 binds to the extracellular epidermal growth factor-like repeat A domain of the LDLR on the cell surface and is endocytosed along with the receptor. In the endosome, the acidic pH leads to an interaction between the C-terminal domain of PCSK9 and the ligand binding domain of the LDLR leading to increased affinity between the molecules and preventing the LDLR from assuming the conformation needed for it to recycle to the plasma membrane. As a result, instead of recycling to the plasma membrane, the LDLR is directed to the lysosome where it is degraded, leading to decreased levels of cell surface LDLR and decreased internalization of LDL. PCSK9 mediated degradation of the LDLR is independent of its catalytic activity since similar reduction of hepatic LDLR was observed by the infusion of both wild type human PCSK9 (hPCSK9) and a catalytically inactive hPCSK9 mutant (p.S386A) into *Pcsk9* knockout (*Pcsk9*<sup>-/-</sup>) mice[12]. Antibodies to the C-terminal domain of PCSK9 prevents the internalization of the PCSK9-LDLR complex[13].

The majority of LDLR degradation occurs via the extracellular interaction of PCSK9 with the receptor. However, some degradation occurs in the secretory pathway in hepatocytes. It has been shown that a large percentage of ApoB, the major apoprotein on LDL and its precursor VLDL, is degraded within hepatocytes prior to secretion[14]. This occurs before ApoB is fully lipidated in the ER and is dependent on the LDLR[15]. PCSK9 expression has an impact on plasma triglycerides as well. The mechanism is not entirely clear, but it may be related to the decrease in cell surface LDLR leading to reduced degradation of ApoB in the presecretory pathway and to reduced uptake of nascent VLDL particles secreted by hepatocytes[16,17].

PCSK9 regulates LDL-C levels in humans. Polymorphisms in the *PCSK9* gene generate either gain of function or loss of function resulting in hypercholesterolemia or hypocholesterolemia respectively<sup>[18,19]</sup>. Inhibition of the action of plasma PCSK9 by treatment with monoclonal antibodies that block its ability to bind to the LDLR reduces LDL-C levels<sup>[20,21]</sup>. Lipoprotein(a) levels are also reduced by PCSK9 monoclonal antibody treatment<sup>[22]</sup>. The effect on lipoprotein(a) may involve both LDLR-dependent and independent pathways. A newer approach uses antisense oligonucleotides to reduce hepatic PCSK9 synthesis by inhibiting translation results in sustained reductions in LDL-C<sup>[20,23]</sup>.

### Regulation of LDLR and PCSK9 genes

The regulation of the *LDLR* and *PCSK9* genes can be complex depending on the setting. They are both regulated by cellular cholesterol homeostasis as they contain sterol regulatory elements in their promoters to which SREBP2 binds<sup>[24]</sup>. However, when stimulated by adiponectin receptor agonists, they are both increased in wild type mice, though the mechanisms differ<sup>[25]</sup>. In agonist treated human hepatoma HepG2 cells, *PCSK9* transcription is activated through a PPAR dependent pathway while *LDLR* transcription involves SREBP2.

### Impact of PCSK9 on lipid metabolism in mouse models

Mice have been useful to understand how PCSK9 influences lipid metabolism and atherosclerosis. Mice with global deficiency of the *Pcsk9* gene (*Pcsk9*<sup>-/-</sup>) have a 2-3-fold increase in hepatic LDLR protein and very low plasma levels of LDL-C<sup>[26,27]</sup>. In contrast, transgenic expression of human *PCSK9* in the liver using the ApoE promoter with liver specific enhancer results in an increment in plasma LDL-C levels close to levels in global *Ldlr*<sup>-/-</sup> mice<sup>[28]</sup>. Additionally, LDLR protein in the liver was essentially eliminated<sup>[28]</sup>. Similar increase in LDL-C was observed in transgenic mice expressing human *PCSK9* under the control of its endogenous promoter<sup>[29]</sup>. Recombinant adenoviral vectors have also been used to increase hepatic expression of murine<sup>[30]</sup> and human<sup>[31,32]</sup> *PCSK9*. Following injection of the recombinant virus into wild type mice, PCSK9 protein levels were increased in the liver in a dose-dependent manner and hepatic LDLR levels were reduced. The mice became hypercholesterolemic with a lipoprotein profile similar to that seen in chow fed *Ldlr*<sup>-/-</sup> mice. These effects on LDL-C levels are dependent on the presence of the LDLR since neither decreased nor increased expression of *Pcsk9* in *Ldlr*<sup>-/-</sup> mice had an effect on LDL-C levels<sup>[30,33,34]</sup>.

PCSK9 is widely expressed in many tissues<sup>[27,35]</sup>. The tissue with the highest level of expression in mice is the liver. It is also highly expressed in the intestine with lower levels of expression observed in the kidney, spleen and aorta. Essentially all plasma PCSK9 is derived from the liver. In mice with liver specific deletion of *Pcsk9*, plasma levels of PCSK9 are undetectable. Despite the absence of plasma PCSK9, plasma LDL-C levels were only reduced by 60%, suggesting a role of extrahepatic PCSK9 in regulating LDL-C levels<sup>[16,27]</sup>. PCSK9 is widely expressed in the intestine, and the small intestine is responsible for the assembly of triglyceride-rich lipoproteins containing dietary lipids. Extracellular recombinant PCSK9 is taken up by polarized human epithelial CaCo-2 cells resulting in stimulation of the synthesis and secretion of ApoB100 and ApoB48 due to increased ApoB mRNA and increased ApoB protein stability<sup>[36,37]</sup>. Increased ApoB stability is likely due to PCSK9 mediated increase in microsomal triglyceride transfer protein (MTP) and activity which facilitate the lipidation of ApoB leading to decreased intracellular ApoB degradation. Lipogenic gene expression was also increased by PCSK9. *In vivo* studies with wild type mice, *Pcsk9*<sup>-/-</sup> mice and mice transgenic for *hPCSK9* reinforced the observations related to MTP expression in the small intestine made with the CaCo-2 cells. The stimulation of MTP occurred independent of LDLR. PCSK9 also enhanced Niemann-Pick C1-like (NPC1L1) levels and cholesterol uptake in CaCo-2 cells suggesting that PCSK9 might increase the absorption of cholesterol from the intestinal lumen into enterocytes for assembly into lipoproteins. LDLR degradation under the influence of PCSK9 is seen in lymphocytes which like the hepatocytes requires the autosomal recessive hypercholesterolemia adaptor protein that binds to the

receptor's cytoplasmic tail<sup>[38,39]</sup>.

The ligands on lipoproteins recognized by the LDLR are ApoB100 and ApoE. As a subfraction of HDL contains ApoE, the level of HDL-C may be influenced by PCSK9. Indeed, the concentration of HDL, particularly the ApoE-containing HDL subfraction, is reduced in mice deficient in *Pcsk9*<sup>[26,40]</sup>. PCSK9 binds other lipoprotein receptors, such as the VLDL receptor, ApoE receptor 2, and LRP-1, but how this impacts processes mediated by these receptors is less well understood.

### Impact of PCSK9 on atherosclerosis in mouse models

In almost all animal models, the presence of hypercholesterolemia due to increased levels of ApoB-containing lipoproteins is necessary to drive atherogenesis. The major lipoprotein in wild type mice is HDL. One of the most frequently employed atherosclerosis models is the *Ldlr*<sup>-/-</sup> mouse. On chow diet the mice accumulate of LDL and on a high fat, high cholesterol Western type diet (WTD), VLDL and LDL particles accumulate. As discussed above, PCSK9 overexpression leads to increased levels of LDL-C. Studies with transgenic mice expressing wild type mouse *Pcsk9* or human *PCSK9* in the liver have shown that increased synthesis of PCSK9 alone is sufficient to increase atherosclerosis in mice when fed a WTD<sup>[33,34]</sup>. However, the lesions were early fatty streak lesions. The transgene is also able to increase atherosclerosis in chow fed *ApoE*<sup>-/-</sup> mice but not in WTD fed *Ldlr*<sup>-/-</sup> mice. In mice expressing a single copy of the wild type or mutant human *PCSK9* genes under control of its own promoter led to more physiological levels of plasma PCSK9 and demonstrated the enhanced ability of the gain of function mutant PCSK9-D374Y to develop atherosclerosis on a high cholesterol diet compared to wild type PCSK9<sup>[29]</sup>.

The use of the *Ldlr*<sup>-/-</sup> mouse as a platform to assess the impact of a variety of other genes in mouse atherogenesis requires costly and time-consuming genetic crosses. This is even more difficult for the exploration of atherosclerosis in other species. A valuable and less onerous approach not requiring genetic crosses involves the injection into appropriate hosts of recombinant hepatic transducing virus expressing PCSK9, especially gain of function mutants. The most frequently employed gain of function human *PCSK9* mutant is that at residue 374 in the catalytic domain where aspartic acid is substituted for tyrosine. The endogenous corresponding mouse residue is at position 377. This mutation significantly reduces the autocatalytic cleavage of pro-PCSK9<sup>[41]</sup>. The applicability of this approach is exemplified by Bjørklund *et al.*<sup>[42]</sup> who used either human or mouse PCSK9-D374/377Y gain of function mutants in an adeno-associated virus (AAV) substrain 8 vector. Wild type C57BL/6 mice were injected with various doses of the recombinant virus and fed either the WTD or the Paigen diet which includes sodium cholate. There was a dose dependent increase in plasma lipids, mostly LDL-C, though at the highest dose of virus there was an increase in the VLDL/LDL ratio. Lipids were not as high as is seen in the *Ldlr*<sup>-/-</sup> mice fed the same diets, perhaps because the extrahepatic receptor plays a role in the regulation of plasma lipids. The aortic vessels were sampled at 12 weeks of diet feeding. A dose dependent increase in atherosclerotic lesions was observed in the upper vasculature which includes the aortic arch and brachiocephalic artery. The lesions were larger with the feeding of the Paigen diet. Although there was no dose response, the expression of PCSK9 also promoted the formation of advanced lesions with necrotic cores in the aortic root. Hamsters were also injected with the hPCSK9-D374Y AAV virus; they were not as intensively studied but they exhibited hyperlipidemia and early atherosclerotic lesions. The utility of this approach to induce atherosclerosis in a more complex phenotype was illustrated in virus injected diabetic *Akita* mice, which develop larger lesions than non-diabetic recipients of the virus. It is well known that various mouse strains exhibit differences in susceptibility to atherosclerosis. C57BL/6 mice are the most sensitive, while 129/Sv and FVB/N strains are more resistant. All 3 strains exhibited comparable hypercholesterolemia on receipt of the human PCSK9-D374Y expressing virus, but this did not alter their relative atherosensitivity<sup>[43]</sup>.

Two studies have compared the AAV8 mediated expression of PCSK9 in male and female mice. The injection of  $3 \times 10^{10}$  genome copies of AAV8-PCSK9 into wild type C57BL/6 mice showed a different tropism of the virus with much lower expression in the liver of female mice than male mice<sup>[44]</sup>. This was associated with greater hyperlipidemia in male mice. The gender differences could be equalized by increasing the viral dose administered to female mice by 3-fold. Another study<sup>[45]</sup> used an AAV dose of  $5 \times 10^{11}$  genome copies, which is higher than the highest level employed by Vozenilek *et al.*<sup>[44]</sup>, to express human PCSK9-D374Y in wild type mice. In AAV- hPCSK9 injected mice, males exhibited higher levels of plasma PCSK9 than females but the resultant decrease in hepatic LDLR was higher in females. The authors noted that there was considerable variability in the plasma levels of PCSK9 in both groups. Plasma lipids were measured every 4 weeks after initiation of WTD. Plasma cholesterol increased more slowly with time in male mice expressing hPCSK9 than females and by 20 weeks was only 50% of that observed in male *Ldlr*<sup>-/-</sup> mice. Plasma cholesterol levels in female mice expressing hPCSK9, on the other hand, were more similar to female *Ldlr*<sup>-/-</sup> mice. The distribution of PCSK9 in the plasma was assessed. In males more than half of the protein was in the HDL fraction while in females almost all of the protein was in the lipid free region. With respect to atherosclerosis assessed by the *en face* method at the end of the 20-week diet period, the lesions were ~3 fold smaller in the male AAV-hPCSK9 treated mice than *Ldlr*<sup>-/-</sup> mice. In female mice the lesions were similar in size in the *Ldlr*<sup>-/-</sup> and PCSK9 treated mice. In this study the authors also examined the ability of AAV8 mediated delivery of CRISPR/Cas9 to liver cells to disrupt the *Ldlr* gene by creating insertion or deletion mutants (INDELS). The efficiency INDEL formation was comparable in male and female mice. However, this too resulted in sex differences in the resulting hyperlipidemia and atherosclerosis. The authors state that with this complexity in virus-based modulation of the LDLR expression it is necessary that “comparisons across sex and strains should be avoided”<sup>[45]</sup>.

AAV-mediated expression of PCSK9 can also be used in atherosclerosis regression models. Wild type C57BL/6 mice were treated with AAV8-mPCSK9-D377Y and fed a WTD for 16 weeks followed by a switch to low fat chow diet with or without an inhibitor of MTP for 6 weeks. Similar to other regression models, the diet switch led to significant decrease in hyperlipidemia which was further enhanced by the MTP inhibitor. While no decrease in lesion size was observed, there was a significant loss in the macrophage content of the aortic root lesions during the regression period accompanied by a drop in blood monocytes<sup>[46]</sup>. Reduced macrophage content is often observed in regressing lesions<sup>[47]</sup>. Hypercholesterolemia promotes monocytoysis that contributes to atherogenesis<sup>[48]</sup>.

Although not directly related to PCSK9 function, an alternate approach to the reduction of LDLR levels has employed antisense oligonucleotides to the *Ldlr* mRNA with a resultant increase in plasma LDL-C levels and the development of lesions in the aortic root, aortic arch and brachiocephalic artery in mice fed a WTD<sup>[49]</sup>. The macrophage content of these lesions can be substantially reduced by the removal of the antisense oligonucleotides using a sense version of the oligonucleotide.

Endothelial cells, smooth muscle cells, and macrophages in the atherosclerotic lesion also express PCSK9, but at much lower levels<sup>[50]</sup>. Of the three cell types, smooth muscle cells appear to be a major source of PCSK9 in atherosclerotic lesions and *in vitro* studies have shown that smooth muscle cell-derived PCSK9 can promote LDLR degradation in macrophages in a paracrine fashion<sup>[51]</sup>.

Local effects of PCSK9 are evident from the study of bone marrow chimeras derived by the transfer of bone marrow from hPCSK9tg/*Apoe*<sup>-/-</sup> mice into *Apoe*<sup>-/-</sup> mice<sup>[52]</sup>. The peritoneal macrophages in the hPCSK9tg/*Apoe*<sup>-/-</sup> mice express both human and murine PCSK9 and have reduced levels of cell surface LDLR and LRP1. Although human PCSK9 is detectable in the plasma of the *Apoe*<sup>-/-</sup> recipients of the bone marrow, no

change in plasma lipids or lesion size was noted. However, hPCSK9 was detected in lesions of the *ApoE*<sup>-/-</sup> recipients as was an increase in inflammatory Ly6C<sup>hi</sup> monocytes. A similar study was performed with *Ldlr*<sup>-/-</sup> mice but human PCSK9 was not detected in the lesions nor was there an increase in Ly6C<sup>hi</sup> cells suggesting that PCSK9 mediated inflammation is LDLR dependent. Upon *in vitro* stimulation of peritoneal macrophages with LPS, increased levels of proinflammatory *Il1b* and *Tnfa* expression was observed in the transgenic macrophages compared to control macrophages. The inflammatory effects of the transgene were also dependent on the presence of the LDLR<sup>[52]</sup>.

The local influence of PCSK9 has recently been reviewed by Ding *et al.*<sup>[53]</sup>. From this it is clear that PCSK9 is pro-atherogenic at 2 physiologic levels - by controlling the level of LDL-C as discussed above and by exerting pro-inflammatory influence at the vessel wall level. Disturbed flow, sites at which lesions often develop, activates the production of PCSK9 especially by smooth muscle cells in the presence of dysfunctional endothelial cells. PCSK9 and scavenger receptor LOX-1 expression in macrophages are involved in a positive feedback loop which facilitates the uptake of oxidized LDL, the formation of foam cells and the progression of atherogenesis<sup>[54]</sup>. Upregulation of PCSK9-LOX-1 expression is associated with the activation of reactive oxygen species production that requires the activity of NADPH oxidase.

### **Atherosclerosis in larger animal models**

The study of murine atherosclerosis in genetically modified mice has provided a wealth of information on the many processes involved in atherogenesis. But this model has limitations. First, mice are small and do not readily furnish the mass of tissue required for detailed biochemical characterization. Second, they do not often develop advanced and complicated lesions that are characteristic of clinically significant atherosclerosis in humans. Third, the distribution of lesions is not exactly similar to those found in humans. For example, obstructive coronary artery lesions are not found in most models of murine atherosclerosis. Finally, the use of *in vivo* imaging of progressing lesions is not easy in mice. Other larger animal species have been employed in experimental atherosclerosis studies<sup>[55,56]</sup>. Among the models most studied are rabbits (including the Watanabe heredity hypercholesterolemic rabbit with mutant LDLR), nonhuman primates, and pigs.

Pigs are attractive animal models that overcome many of the limitations listed above for mice. A favored subspecies is the miniature Yucatan pig, which is close to the size of human subjects. The advantage of the pig as an experimental model is that pigs have the following similarities with humans: hemodynamics, heart size, cardiac vasculature anatomy, lipid and lipoprotein profile (though they lack apoprotein A-II), and ease of imaging<sup>[57]</sup>. As with mice, there are porcine subspecies that exhibit differential sensitivity to hyperlipidemia and atherosclerosis. The Yucatan pigs and Ossabaw miniature pigs are relatively sensitive strains among the studied subspecies, while the Landrace pigs are relatively resistant. This relative atherosensitivity is not altered by the overexpression of PCSK9. Transgenic Yucatan pigs expressing the human gain of function mutant PCSK9-D374Y in the liver were hyperlipidemic with increased plasma LDL levels on both low fat and high fat, high cholesterol (HFHC) diet and develop atherosclerosis on the HFHC diet<sup>[58]</sup>. Similar results were obtained by liver expression of the chimpanzee PCSK9-D374Y gain of function mutant in Ossabaw miniature pigs but not Landrace pigs<sup>[59]</sup>. Atherosclerosis is found in ascending and thoracic aorta, coronary arteries (i.e., left anterior descending and right coronary artery), abdominal aorta, and iliofemoral arteries. This is accentuated by the feeding of the atherogenic diet. Femoral artery disease may also be found especially with feeding the atherogenic diet<sup>[60]</sup>.

## CONCLUSION

The understanding of the role of PCSK9 in the regulation of the LDLR, lipid homeostasis, and inflammation has led to important therapeutic possibilities. As pointed out above, the loss of function of PCSK9 leads to an increase in the function of the LDLR and hypocholesterolemia, indicating that the protein does function under steady state. This can be induced by PCSK9 monoclonal antibody or antisense oligonucleotides treatment. Indeed, germline loss of function leads to lifetime hypocholesterolemia with very low coronary artery disease risk<sup>[1]</sup>.

## DECLARATIONS

### Authors' contributions

Made substantial contributions to conception, research and writing of the review: Getz GS, Reardon CA

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### Conflicts of interest

Both authors declared that there are no conflicts of interest.

### Ethical approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

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