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Targeting proprotein convertase subtilisin/kexin type 9 in mice and monkeys

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Low-density lipoprotein (LDL) receptors are critical contributors to lipoprotein homeostasis. Proprotein convertase subtilisin/kexin type 9 (PCSK9) regulates the abundance of LDL receptors by altering their recycling pathway during endocytosis. Since the discovery of PCSK9 in 2003 [1], many investigators have manipulated PCSK9 in animal models to garner insights into PCSK9 biology and determine its therapeutic potentials for hypercholesterolemia and atherosclerosis [2–9]. This commentary summarizes two recent publications that manipulated either LDL receptors or PCSK9 using adeno-associated virus (AAV) vector-based approaches in mice [10[■]] and monkeys [11[■]], respectively.

A human gain-of-function mutation D374Y of PCSK9 through AAV8-driven expression in C57BL/6 mice has been demonstrated to degrade LDL receptor, increase plasma cholesterol concentrations, and augment atherosclerosis [4,5]. Jarrett *et al.* [10[■]] compared this well-established AAV8 approach targeting D374Y mutation of the human PCSK9 to AAV8-clustered regularly interspaced short palindromic repeats vector targeting the mouse LDL receptor gene. The data in these AAV8-infected mice were compared to LDL receptor^{-/-} mice that lack this functional protein in all tissues [12]. All mice were fed a Western diet for 20 weeks. Plasma cholesterol concentrations were determined sequentially during the 20-week interval and atherosclerosis was assessed after termination. Both approaches led to profound increases of plasma cholesterol concentrations and development of atherosclerosis in C57BL/6 mice. The authors studied both male and female mice as sexual dimorphism has been frequently noted in cardiovascular diseases [13,14]. It is interesting that targeting LDL receptors using the AAV8-clustered regularly interspaced short palindromic repeats vector was more effective in male mice, whereas AAV8-encoded PCSK9D374Y increased plasma

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Conflicts of interest

There are no conflicts of interest.

cholesterol concentrations and atherosclerotic lesions more robustly in female mice, when compared the two approaches within each sex.

Augmenting PCSK9 function has been used to study mechanisms of hypercholesterolemia and atherosclerosis as described above [4–9,10[■]]. In contrast, inhibition of PCSK9 has been studied to explore potential therapeutic effects. A recent study determined whether meganuclease targeting PCSK9 would effectively reduce serum cholesterol concentrations in a monkey model (Rhesus macaques) [11[■]]. Initially, the authors developed a first-generation meganuclease recognizing a DNA sequence in exon 7 of the human and macaque PCSK9 gene. This was called M1PCSK9. An AAV8 vector containing the liver-specific thyroxine-binding globulin promoter and M1PCSK9 was administered in six monkeys through a single intravenous infusion. Although serum cholesterol concentrations were reduced, M1PCSK9 also had some off-target genome editing. Subsequently, the authors engineered a second-generation meganuclease, M2PCSK9. It exhibited significant effects in reducing serum PCSK9 and cholesterol concentrations with less off-target editing. Administration of this AAV resulted in transient elevation of serum transaminase concentrations in macaques, which were not accompanied by symptoms or other apparent adverse effects. This study in primates provides hope that meganuclease targeting PCSK9 in liver may ultimately lead to development of a new effective therapeutic strategy in clinical patients with hypercholesterolemia. As hypercholesterolemia is a major risk factor for atherosclerosis, it would also be important to determine whether this approach prevents or regresses atherosclerosis in primates.

There is accumulating evidence that PCSK9 not only degrades LDL receptors but also has effects beyond regulating LDL homeostasis [15[■]]. The rapid development of monoclonal antibodies against PCSK9 and its successful application to clinical patients with hypercholesterolemia will provoke research work to explore molecular mechanisms and more extensive effects of PCSK9 on cardiovascular diseases.

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

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FURTHER RECOMMENDED READING

- Cansby E, Magnusson E, Nunez-Duran E, *et al.* STK25 regulates cardiovascular disease progression in a mouse model of hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 2018; 38:1723–1737.

STK25, serine/threonine protein kinase 25, plays critical roles in inflammation and liver steatosis. This article has provided compelling evidence that STK25 contributes to atherosclerosis in hypercholesterolemic mice, as demonstrated by reduced atherosclerosis in mice with STK25 deficiency and augmented atherosclerosis in STK25 transgenic mice.

- Klarin D, Damrauer SM, Cho K, *et al.* Genetics of blood lipids among ~300,000 multiethnic participants of the Million Veteran Program. *Nat Genet* 2018; 50:1514–1523.

This study performed comprehensive genotypic analysis and phenome-wide association evaluation of the large population in The Million Veteran Program. The findings in this study predict potentially new pharmaceutical targets for abdominal aortic aneurysms, type 2 diabetes, and triglycerides and coronary disease.

- Wilson PW, Polonsky TS, Miedema MD, *et al.* Systematic review for the 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS /APhA/ASPC/NLA/PCNA Guideline on the Management of Blood Cholesterol: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol* 2018.

This systematic review has compared effects of statins alone versus adding other lipid-modifying therapies to statins in reducing atherosclerotic cardiovascular disease events using data from randomized clinical trials with a sample size of more than 1000 patients being followed up for more than 1 year.

- Zhong S, Li L, Zhang YL, *et al.* Acetaldehyde dehydrogenase 2 interactions with LDLR and AMPK regulate foam cell formation. *J Clin Invest* 2018.

Aldehyde dehydrogenase 2 (ALDH2) is a mitochondrial enzyme. This article reports that deficiency of ALDH2 attenuates atherosclerosis in LDL receptor^{-/-} mice, but augments atherosclerosis in apolipoprotein E^{-/-} mice. Further exploration discovers that ALDH2 interacts with LDL receptor and AMPK as a mechanism for its contribution to atherosclerosis.