

# Circulation Research

JOURNAL OF THE AMERICAN HEART ASSOCIATION



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*Circ Res.* 2014;114:1672-1674

doi: 10.1161/CIRCRESAHA.114.304067

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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## Adeno-Associated Viruses as a Method to Induce Atherosclerosis in Mice and Hamsters

Noam Zelcer, Marit Westerterp

Atherogenesis involves multiple cell types and organs and can therefore only be recapitulated in animal models. The most widely used mouse models to study atherosclerosis include mice deficient in apolipoprotein E (*ApoE*<sup>-/-</sup> mice) or the low-density lipoprotein receptor (*Ldlr*<sup>-/-</sup> mice).<sup>1</sup> *ApoE*<sup>-/-</sup> mice develop advanced plaques on a chow diet, which is accelerated by Western-type diet (WTD) feeding, whereas *Ldlr*<sup>-/-</sup> mice require WTD feeding to develop advanced atherosclerosis and show only foam cell-rich plaques on chow.<sup>1</sup> Wild-type mice do not develop atherosclerotic plaques unless fed with the Paigen diet, but this diet is preferably avoided in these studies because it contains cholate that induces steatohepatitis.<sup>1,2</sup>

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To study the contribution of genes and pathways to atherogenesis, crossbreeding with the above-mentioned models is required. In this issue of *Circulation Research*, Bjørklund et al<sup>3</sup> propose an alternative to this procedure and elegantly show that a single injection of recombinant adeno-associated virus 8 (rAAV8) expressing either a mouse or a human gain-of-function mutant for proprotein convertase subtilisin/kexin type 9 (PCSK9) in combination with WTD feeding induces atherosclerosis in mice and hamsters. PCSK9 is a secreted protein that is predominantly produced in the liver and binds an extracellular domain of the LDLR to target it to the lysosome for degradation.<sup>4</sup> Bjørklund et al<sup>3</sup> used rAAV8 expressing mouse D377Y-PCSK9 (rAAV8-D377Y-mPCSK9) or human D374Y-PCSK9 (rAAV8-D374Y-hPCSK9) to induce atherosclerosis in C57BL/6NTac mice.<sup>3</sup> For both rAAV8s, stable PCSK9 plasma levels were reached 7 to 14 days after injection, leading to >95% reduced hepatic LDLR abundance. As a result, on the WTD and Paigen diet, plasma cholesterol levels were increased in a rAAV8 dose-dependent manner, reaching ≈50% to 80% of cholesterol levels in *Ldlr*<sup>-/-</sup> mice on the same diet. On the WTD, the increase in cholesterol was mainly confined to the low-density lipoprotein (LDL) fraction although very LDL (VLDL) cholesterol also increased at higher rAAV8

doses. At 12 weeks after rAAV8-D377Y-mPCSK9 injection combined with WTD feeding, C57BL/6NTac mice showed advanced atherosclerotic lesions in the aortic root, which also contained necrotic cores.<sup>3</sup> Atherosclerosis in the aortic arch and branch areas was increased by rAAV8-D377Y-mPCSK9 or rAAV8-D374Y-hPCSK9 in a dose-dependent manner on the WTD, although even at the highest rAAV8 dose, the lesion area was only ≈50% compared with that in WTD-fed *Ldlr*<sup>-/-</sup> mice.<sup>3</sup> Moreover, rAAV8-D374Y-hPCSK9 increased atherosclerosis in WTD-fed diabetic Akita mice and induced small foam cell-rich lesions in WTD-fed Golden Syrian hamsters, demonstrating the applicability of the AAV-PCSK9 approach for different mouse models and species.<sup>3</sup>

Although this approach clearly forms an advance for studying early atherosclerosis, the suitability of AAV-PCSK9 for studying advanced atherogenesis may be questioned. In the aortic root, advanced lesions were observed in WTD-fed mice, but lesions in the arch and branch areas were relatively small.<sup>3</sup> These regions are particularly sensitive to shear stress and to the activity of the enzyme endothelial nitric oxide synthase that plays a key role in maintaining endothelial function in the onset of atherogenesis and has a larger effect on atherosclerosis in the aortic arch than in the root.<sup>5</sup> It thus remains to be addressed whether rAAV8-D377Y-mPCSK9 or rAAV8-D374Y-hPCSK9 also induce advanced atherosclerosis in the aortic arch and branch areas after a longer period of PCSK9 expression. Interestingly, studies in transgenic mice expressing hepatic D374Y-hPCSK9 showed that sustained PCSK9 plasma levels in combination with 15 weeks of high-cholesterol diet increased plasma VLDL/LDL cholesterol and atherosclerosis throughout the whole aorta.<sup>6</sup> It is well known that rAAV8 gives long-term expression,<sup>7</sup> suggesting that in the case of rAAV8-D374Y-hPCSK9 also long-term stable plasma levels could be accomplished.

PCSK9 has been reported to preferentially degrade the LDLR in the liver.<sup>8</sup> Nevertheless, a reduction of up to ≈60% of LDLR in adipose tissue, kidney, and lung has been reported in mice injected with a PCSK9-encoding adenovirus.<sup>9</sup> In the rAAV8-D377Y-mPCSK9 model, hepatic LDLR was reduced by >95%, whereas plasma cholesterol levels were ≈50% to 80% of those in *Ldlr*<sup>-/-</sup> mice.<sup>3</sup> Extrahepatic LDLR in the rAAV8-D377Y-mPCSK9 mice probably contributed to LDL lowering in these mice compared with *Ldlr*<sup>-/-</sup> mice. Although modest, a contribution of haematopoietic cells to plasma LDL levels has been reported,<sup>10</sup> raising the possibility that other cell types that are insensitive to PCSK9-mediated LDLR degradation contribute to plasma LDL levels. Thus, the rAAV8-D377Y-mPCSK9 model, which effectively creates a liver-specific *Ldlr* knockout mouse, offers the possibility to investigate the function of nonhepatic LDLR and its contribution to the regulation of plasma LDL levels and atherogenesis.

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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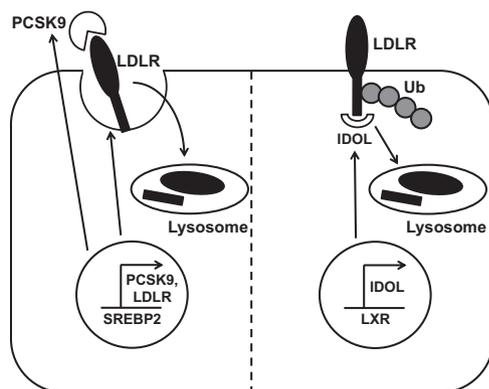
(*Circ Res.* 2014;114:1672-1674.)

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*Circulation Research* is available at <http://circres.ahajournals.org>  
DOI: 10.1161/CIRCRESAHA.114.304067

In addition to PCSK9, another pathway regulating LDLR abundance in the liver has been discovered, involving the inducible degrader of the LDLR (IDOL).<sup>11</sup> IDOL is an E3-ubiquitin ligase that specifically promotes ubiquitination and subsequent lysosomal degradation of the LDLR.<sup>11</sup> IDOL and PCSK9 share an overlapping substrate specificity that includes the LDLR, VLDLR, and APOE receptor 2,<sup>12,13</sup> yet the transcriptional pathways that govern their expression are divergent. PCSK9 is transcriptionally regulated by the sterol regulatory element-binding protein 2 pathway and is induced together with the LDLR when cellular cholesterol levels decline.<sup>14</sup> In contrast, IDOL is transcriptionally controlled by the transcription factors liver X receptors, and its expression is increased when cellular sterols increase (Figure).<sup>11</sup> Several lines of evidence indicate that while mediating a similar outcome—lysosomal degradation of the LDLR—the IDOL and PCSK9 pathways are independent of each other; PCSK9 promotes degradation of LDLR in *Idol*<sup>-/-</sup> cells,<sup>15</sup> LDLR internalizes via distinct endocytic routes in response to IDOL or PCSK9,<sup>16</sup> and an LDLR receptor lacking the intracellular tail, which is required for IDOL-dependent degradation, remains sensitive to PCSK9.<sup>17</sup> Short-term adenoviral-mediated expression of mouse IDOL in mouse liver results in a dramatic elevation in circulating levels of LDL cholesterol, which was entirely dependent on *Ldlr* expression.<sup>11</sup> Therefore, it will be interesting to test whether long-term hepatic expression of IDOL leads to sustained elevated LDL levels and atherogenesis.

The expression of a human transgene to induce atherosclerosis has been reported before in mice transgenic for *apolipoprotein B (APOB)* or *APOE\*3-Leiden*.<sup>18,19</sup> *APOB* transgenic mice show a human-like lipoprotein profile with marked increases in LDL cholesterol, leading to advanced atherogenesis with larger effects in females than in males<sup>18</sup>; female, but not male *APOE\*3-Leiden* mice show attenuated clearance of



**Figure. Regulation of low-density lipoprotein receptor (LDLR) abundance by proprotein convertase subtilisin/kexin type 9 (PCSK9) and inducible degrader of the LDLR (IDOL) in hepatocytes. Left:** When intracellular cholesterol levels decline, transcriptional activity of sterol regulatory element-binding protein 2 (SREBP2) is increased. This induces PCSK9 and LDLR expression. PCSK9 binds LDLR extracellularly and directs it to the lysosome for degradation. **Right:** When intracellular cholesterol levels increase, transcriptional activity of liver X receptor (LXR) is turned on. This induces IDOL expression. IDOL binds the intracellular tail of the LDLR and promotes ubiquitination (Ub) of the receptor, directing the receptor for lysosomal degradation.

VLDL/LDL particles and develop advanced atherosclerosis on a WTD.<sup>19</sup> Furthermore, their plasma VLDL/LDL cholesterol level mirrors the dietary cholesterol content, they are responsive to statins, and crossbreeding with mice transgenic for cholesteryl ester transfer protein has extended their application as a model for testing antiatherogenic drugs.<sup>19,20</sup> The study by Björklund et al<sup>3</sup> shows that injection with rAAV8-D374Y-hPCSK9 may be a highly promising model for atherosclerosis studies. Expression of the same mutant was also shown to increase atherogenesis in Yucatan minipigs.<sup>21</sup> Given that PCSK9 is highly conserved among species, the rAAV8-D374Y-hPCSK9 approach may be generally applicable in several animal models.

## Disclosures

Dr N. Zelcer is supported by a European Research Council Consolidator Grant 617376. Dr N. Zelcer and Dr M. Westerterp are supported by The Netherlands Organization of Scientific Research (NWO) VIDI grant 17.106.355 and VENI grant 916.11.072, respectively.

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KEY WORDS: Editorials ■ atherosclerosis ■ liver ■ receptors, LDL