Induction of Cardiovascular Calcification in Non-transgenic Mice via a Single Injection of Pcsk9 Adeno-associated Viral Vector

Claudia Goettsch, Joshua Hutcheson, Sumihiko Hagita, Maximillian Rogers, Jung Choi, Michael Creager, Tan Pham, Andrew Mlynarchik, Brigham and Women's Hosp, Harvard Medical Sch, Boston, MA; Mads Fuglsang Kjolby, Aarhus Univ, Aarhus, Denmark; Masanori Aikawa, Elena Aikawa, Brigham and Women's Hosp, Harvard Medical Sch, Boston, MA

Background: Studying atherosclerotic calcification *in vivo* requires mouse models with genetic deletion of low-density lipoprotein receptor (Ldlr) or apolipoprotein E. A previous study showed a rapid induction of atherosclerosis by proprotein convertase subtilisin/kexin type 9 (PCSK9) in mice. Here, we hypothesize that this method is a useful *in vivo* tool to study cardiovascular calcification in non-genetically modified C57BL/6 mice. **Results:** 10 week old C57BL/6 mice received a single tail vein injection of recombinant adeno-associated viral vector (AAV) encoding PCSK9 (rAAV8/D377Y-mPCSK9). *Ldlr*$^{-/-}$ and saline injected C57BL/6 mice served as controls. Mice consumed a high-fat, high-cholesterol (HF/HC) diet for 15-20 weeks. PCSK9 and total cholesterol serum levels were significantly increased within one week after injection and maintained for 20 weeks (cholesterol: 82 mg/dL to 820 mg/dL, $p<0.01$; PCSK9: 0.14 µg/ml to 20 µg/ml, $p<0.01$). Total cholesterol levels remained 20-30% lower than those of of *Ldlr*$^{-/-}$ mice. Atherosclerotic lesion size was similar between PSCK9 and *Ldlr*$^{-/-}$ mice. Saline injected mice did not show any lesions. Plaque collagen content was 31.9%±6.6 in PCSK9 mice and 62.9%±16.6 in *Ldlr*$^{-/-}$ mice at 15 weeks of HF/HC diet ($p=0.01$). However, by 20 weeks, the PCSK9 mice had 57.9%±18.6 plaque collagen, suggesting a different stage of plaque progression. Fluorescence reflectance imaging of a near infrared calcium tracer in intact arteries detected 0.4%±0.4 aortic calcification in PCSK9 mice and 9.7%±1.6 in *Ldlr*$^{-/-}$ mice at 15 weeks of HF/HC diet ($p=0.01$); by 20 weeks, the PCSK9 mice had 5.3%±1.0 aortic calcification. Tissue non-specific alkaline phosphatase activity positive lesion area was 7.9%±4.0 and 8.3%±2.6 in PCSK9 mice and 10.8%±2.5 and 12.7%±1.7in *Ldlr*$^{-/-}$ mice at 15 and 20 weeks, respectively. Immunofluorescence analysis demonstrated accumulation of CD68 and RUNX2-positive cells in the plaques of PCSK9 mice similar to *Ldlr*$^{-/-}$. **Conclusion:** While injection of recombinant AAV encoding PCSK9 into C57BL/6 mice induces atherosclerotic calcification with slower sclerotic plaque remodeling compared to *Ldlr*$^{-/-}$ mice, it may serve as a useful tool to study cardiovascular calcification in mice independent of their genetic background.

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