Peripheral vascular atherosclerosis in a novel PCSK9 gain-of-function mutant Ossabaw miniature pig model

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Hypercholesterolemia is a major risk factor for atherosclerosis. Remaining challenges in the management of atherosclerosis necessitate development of animal models that mimic human pathophysiology. We characterized a novel mutant pig model with DNA transposition of D374Y gain-of-function (GOF) cDNA of chimp proprotein convertase subtilisin/kexin type-9 *(PCSK9)*, and tested the hypothesis that it would develop peripheral vascular remodeling and target organ injury in the kidney. Wild-type or PCSK9-GOF Ossabaw miniature pigs fed a standard or atherogenic diet (AD) (n = 7 each) were studied in vivo after 3 and 6 months of diet. Single-kidney hemodynamics and function were studied using multidetector computed tomography and kidney oxygenation by blood oxygen level-dependent magnetic resonance imaging. The renal artery was evaluated by intravascular ultrasound, aortic stiffness by multidetector computed tomography, and kidney stiffness by magnetic resonance elastography. Subsequent ex vivo studies included the renal artery endothelial function and morphology of abdominal aorta, renal, and femoral arteries by histology. Compared with wild type, PCSK9-GOF pigs had elevated cholesterol, triglyceride, and blood pressure levels at 3 and 6 months. Kidney stiffness increased in GOF groups, but aortic stiffness only in GOF-AD. Hypoxia, intrarenal fat deposition, oxidative stress, and fibrosis were observed in both GOF groups, whereas kidney function remained unchanged. Peripheral arteries in GOF groups showed medial thickening and development of atheromatous plaques. Renal endothelial function was impaired only in GOF-AD. Therefore, the PCSK9-GOF mutation induces rapid development of atherosclerosis in peripheral vessels of Ossabaw pigs, which is exacerbated by a high-cholesterol diet. This model may be useful for preclinical studies of atherosclerosis.

Abbreviations: GOF = gain of function; PCSK9 = proprotein convertase subtilisin/kexin type-9; WT = wild-type; AD = atherogenic diet; MDCT = multidetector computed tomography; LDL-C = low-density lipoprotein cholesterol; ND = normal diet; IVUS = intravascular ultrasound; BOLD-MRI = blood oxygen level-dependent magnetic resonance imaging; TUNEL = terminal deoxynucleotidyl transferase dUTP nick-end labeling; DHE = dihydroethidium; MAP = mean

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arterial pressure; HDL-C = high-density lipoprotein cholesterol; ROI = region of interest; RBF = renal blood flow; GFR = glomerular filtration rate; CTA = computed tomography angiography; MPR = multiplanar-reformatted; MRE = magnetic resonance elastography; VLDL-C = very low-density lipoprotein cholesterol

INTRODUCTION

Atherosclerosis is responsible for coronary and peripheral artery disease, which can remain asymptomatic for decades.[1] Despite advances in medical, interventional, and surgical treatment, atherosclerotic disease is still the leading cause of death in both developed and developing countries.[2] Animal studies have revealed fundamental mechanisms by which low-density lipoprotein cholesterol (LDL-C) causes atherosclerosis, but numerous challenges remain regarding optimal management and understanding of the disease.[3]

Using animal models for understanding the pathophysiology of atherosclerosis and developing treatment or diagnostic strategies has proven somewhat challenging. Genetically modified mouse models have increased the understanding of the mechanisms and role of signaling pathways and genetic factors, which are important contributors to coronary artery disease and development of vascular disease. However, many models do not adequately replicate human disease, and their small size restricts clinically applicable intravascular interventions and noninvasive imaging.[4,5] Additionally, many key features of human atherosclerosis are rarely seen, particularly development of peripheral vascular disease. These limitations necessitate development of novel models to assess atherosclerotic features and management, as well as validating new treatments and devices.

Pigs can develop spontaneous atherosclerosis, which similar to humans is accelerated by an atherogenic diet (AD),[6,7] yet in normal pigs the lesions are generally minimal.[8] However, recent progress in genetic engineering has paved the way for the creation of new models by genetic manipulations. Proprotein convertase subtilisin/kexin type-9 (PCSK9) plays a key role in the clearance of LDL-C via its interaction with, and subsequent degradation of, LDL receptor (LDLR).[9] Following its discovery in 2003, a quest was started for both new gain of function (GOF) and loss-of-function mutations including D374Y PCSK9 and the heterozygote African nonsense C679X variants, respectively.[10,11] GOF mutations result in greater binding of PCSK9 with LDL-R and increased LDL-C blood level. In addition to the PCSK9 enzyme, cholesteryl ester transfer protein (CETP), HMG-CoA reductase, peroxisome proliferator-activated receptor-α protein, and microsomal triglyceride transfer protein play an important role in the metabolism of lipids. In particular, the PCSK9 and CETP genes are both regulated by the SREBP transcription factor family.[12,13] CETP facilitates the transport of cholesteryl ester from high-density lipoprotein (HDL) to apolipoprotein B-containing lipoproteins, such as very low-density lipoprotein (VLDL) and LDL. CETP decreases the concentration of LDL-C and increases the concentration of HDL-C. PCSK9 may also affect plasma triglyceride via metabolism of VLDL-C and their remnants,[14] but this overall effect is considered modest. Yet given the rapid developments in PCSK9 inhibition, analogous PCSK9-related approaches might be discovered for inhibition of triglyceride as well.[15]

Most of the genetically modified pig models have been developed by gene editing in porcine somatic cells followed by animal cloning.[16,17] Al-Mashhadi et al. described development of hypercholesterolemia and atherosclerosis in PCSK9-GOF Yucatan minipigs.[18] Recently, we generated a novel D374Y human PCSK9-GOF Ossabaw pig model created by transposition of chimp DNA. Ossabaw pigs have the highest levels of total body lipid of any mammal even in the absence of a high-fat diet, and are naturally prone for spontaneous development of vascular disease. Furthermore, Ossabaw pigs fed a high-fat cholesterol diet develop hypertriglyceridemia and increased LDL-to-HDL cholesterol ratio, mild hypertension, and coronary artery disease.[19] Therefore, a combination of the Ossabaw pig genetic background with PCSK9-GOF could constitute a particularly promising model for vascular translational research.

The kidney is a common target organ in cardiovascular disease. Atherosclerotic changes in the renal artery are evident in 50% of patients with atherosclerotic disease elsewhere.[20] In addition to vascular remodeling, a growing body of evidence suggests that atherosclerosis has direct effects on the kidney, eliciting intrarenal microvascular and glomerular disease, positioning the kidney as an important target organ in peripheral vascular disease.[21] In the present study, we characterized the PCSK9 Ossabaw pig model and tested the hypothesis that pigs with PCSK9-GOF mutation fed with a high-fat diet would develop peripheral vascular remodeling and renal injury.

MATERIALS AND METHODS

Study protocols. This study was approved by the Mayo Clinic Institutional Animal Care and Use Committee. Twenty-one 3-month-old female Ossabaw pigs (Recombinetics Inc., St. Paul, MN) were studied in 3 groups (n = 7 each), including wild-type (WT), fed a