Deficiency of Cholesteryl Ester Transfer Protein Protects Against Atherosclerosis in Rabbits

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Objective—CETP (cholesteryl ester transfer protein) plays an important role in lipoprotein metabolism; however, whether inhibition of CETP activity can prevent cardiovascular disease remains controversial.

Approach and Results—We generated CETP knockout (KO) rabbits by zinc finger nuclease gene editing and compared their susceptibility to cholesterol diet–induced atherosclerosis to that of wild-type (WT) rabbits. On a chow diet, KO rabbits showed higher plasma levels of high-density lipoprotein (HDL) cholesterol than WT controls, and HDL particles of KO rabbits were essentially rich in apolipoprotein AI and apolipoprotein E contents. When challenged with a cholesterol-rich diet for 18 weeks, KO rabbits not only had higher HDL cholesterol levels but also lower total cholesterol levels than WT rabbits. Analysis of plasma lipoproteins revealed that reduced plasma total cholesterol in KO rabbits was attributable to decreased apolipoprotein B–containing particles, while HDLs remained higher than that in WT rabbits. Both aortic and coronary atherosclerosis was significantly reduced in KO rabbits compared with WT rabbits. Apolipoprotein B–depleted plasma isolated from CETP KO rabbits showed significantly higher capacity for cholesterol efflux from macrophages than that from WT rabbits. Furthermore, HDLs isolated from CETP KO rabbits suppressed tumor necrosis factor-α–induced vascular cell adhesion molecule 1 and E-selectin expression in cultured endothelial cells.

Conclusions—These results provide evidence that genetic ablation of CETP activity protects against cholesterol diet–induced atherosclerosis in rabbits.

Visual Overview—An online visual overview is available for this article. **(*Arterioscler Thromb Vasc Biol*. 2017;37:1068-1075. DOI: 10.1161/ATVBAHA.117.309114.)**

Key Words: apolipoprotein ■ atherosclerosis ■ cholesterol reduction ■ cholesteryl ester transfer protein genetics ■ high-density lipoprotein

It is well known that high levels of plasma high-density lipoprotein (HDL) cholesterol (HDL-C) are inversely correlated with low risk of cardiovascular disease.[1] Elevation of plasma HDL-C has been considered a new strategy for the prevention and treatment of cardiovascular disease.[2] One of the therapeutic strategies to raise plasma HDL-C is the inhibition of plasma CETP (cholesteryl ester transfer protein).[3,4] CETP is a hydrophobic glycoprotein synthesized mainly in the liver and circulates in blood in association with HDL. CETP transfers cholesteryl esters, triglycerides, and phospholipids among lipoproteins and, therefore, playing an important role in the metabolism of lipoproteins and the reverse cholesterol transport from the peripheral tissues to the liver.[3] Patients genetically deficient for the *CETP* gene showed low or no CETP activity along with hyper-HDL-cholesterolemia.[5,6] Patients deficient for CETP have a low incidence of coronary heart disease if their plasma HDL-C levels are >80 mg/dL,[7,8] whereas those carrying CETP mutations, such as *D442G*, are found to have low HDL-C and high levels of triglycerides, which are associated with high prevalence of coronary heart disease.[9] In spite of this complexity, inhibition of CETP was considered a promising way to treat cardiovascular disease through elevation of plasma HDL-C.[10] This notion was initially supported by the finding that inhibition of CETP activity by vaccine,[11] antisense,[12] or therapeutic inhibitors[13–15] in cholesterol-fed rabbits raised plasma HDL-C and attenuated atherosclerosis. Unfortunately, other studies failed to demonstrate atheroprotective effects from inhibiting CETP (either by vaccine or CETP inhibitors) in cholesterol-fed rabbits.[16,17] Transgenic

Received on: January 25, 2017; final version accepted on: April 5, 2017.

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The online-only Data Supplement is available with this article at http://atvb.ahajournals.org/lookup/suppl/doi:10.1161/ATVBAHA.117.309114/-/DC1.

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Nonstandard Abbreviations and Acronyms	
Apo	apolipoprotein
CETP	cholesteryl ester transfer protein
HDL	high-density lipoprotein
HDL-C	high-density lipoprotein cholesterol
KO	knockout
LDL	low-density lipoprotein
VLDL	very-low–density lipoprotein
WT	wild-type
ZFN	zinc finger nuclease

expression of the simian *CETP* gene in mice, a species without endogenous *CETP* gene, resulted in severe atherosclerosis.[18] In the meantime, human clinical trials of CETP inhibitors were generally unsuccessful because of off-target side effects (torcetrapib) or lack of efficacy (dalcetrapib and evacetrapib).[19–22] Currently, Merck's anacetrapib is ongoing a Phase III clinical trial, which is expected to be completed by 2017. This conflicting landscape underscores the need for further studies on CETP roles and its value as a therapeutic target. In this study, we generated CETP knockout (KO) rabbits by zinc finger nuclease (ZFN)–mediated gene targeting to clarify the pathophysiological functions of CETP in atherosclerosis. Rabbits are sensitive to cholesterol diet challenge and have been widely used as a classical model system to study hypercholesterolemia and atherosclerosis.[23] Importantly, wild-type (WT) rabbits have the *CETP* gene and show high plasma CETP activity. Our current study showed that deletion of the *CETP* gene in rabbits protects against cholesterol diet–induced atherosclerosis.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Generation and Characterization of CETP KO Rabbits

We used 3 ZFN pairs designed by the SAGE Labs (St Louis, MO) for rabbit CETP gene targeting. ZFN activity was confirmed by the yeast MEL-1 (melibiase 1) reporter assay.[24] Based on the results, we choose the ZFN pair 1 for CETP gene targeting in rabbit embryos. The ZFN pair 1 target sequence is ctgtccatcgccagcagcCAGGTGgagctggtggacgccaag and is located in exon 3 of the rabbit *CETP* gene. ZFN-induced double-strand breaks stimulate error-prone nonhomologous end joining or homology-directed repair at specific genomic locations. Nonhomologous end joining typically leads to the introduction of small insertions or deletions (indels) at the site of the break, often inducing frameshifts that knock out target gene functions. A total of 188 embryos were injected with ZFN pair 1 mRNA and transferred to 11 pseudopregnant recipient rabbits (16–18 embryos per recipient). After 1-month gestation, 6 (54.5%) recipients gave birth to 30 live kits (5 kits/litter), out of which 3 were identified as positive KO founders after an initial T7 endonuclease assay and final confirmation by polymerase chain reaction and sequencing. One founder rabbit had a 4-bp deletion that introduced an early stop codon into the *CETP* gene, and this rabbit was used for breeding heterozygous and homozygous KO rabbits (Figure 1A) for this study. Genotyping of the CETP KO heterozygous and homozygous rabbits was conducted by polymerase chain reaction, and homozygous KO rabbits showed no CETP proteins in the plasma, while CETP activity was almost undetectable compared with the WT littermates (Figure 1B through 1D).

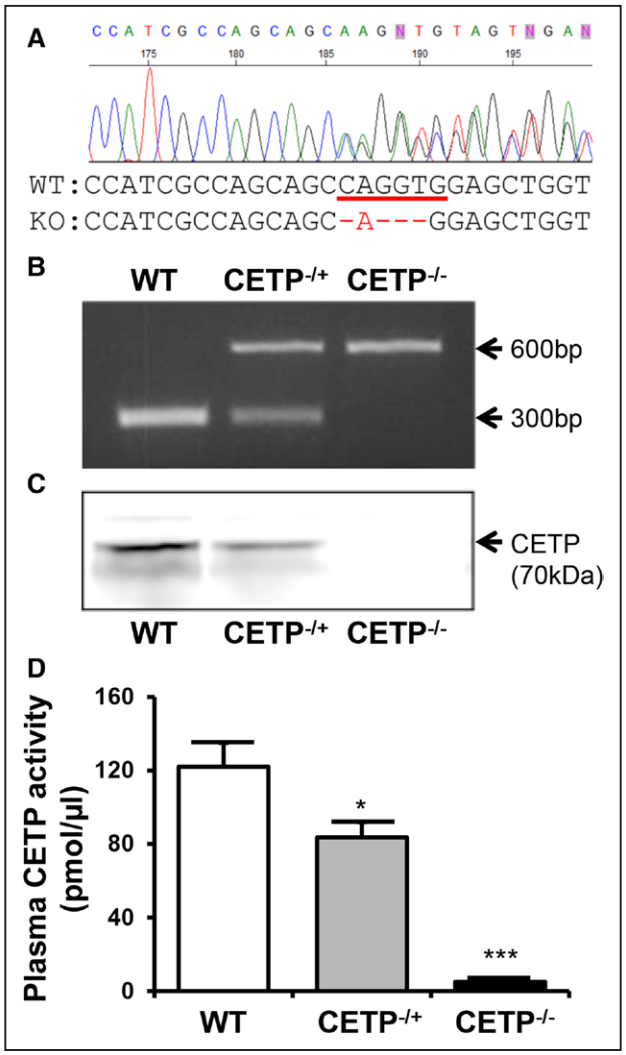

Figure 1. Generation of CETP (cholesteryl ester transfer protein) knockout (KO) rabbits by zinc finger nuclease (ZFN) genome editing. **A**, Sequencing analysis revealed 4-bp deletion that introduces an early stop codon into the *CETP* gene. The 4-bp deletion also causes the loss of an XcmI restriction enzyme recognition site. **B**, Polymerase chain reaction (PCR) for the genotyping of rabbits wild-type (WT), heterozygous, and homozygous for the *CETP* gene was conducted with the primers caccgccagcaccccgcacacc (forward) and tcaaccccagaagccccgaggacact (reverse), which result in a 608-bp and 604-bp amplicon from WT and homozygous CETP knockout allele, respectively. Subsequent XcmI enzyme digestion brings the WT amplicon to 293 and 315 bp, as evidenced by agarose gel electrophoresis. **C**, Plasma CETP protein was detected by Western blotting using a monoclonal antibody against rabbit CETP. One microliter of plasma was loaded in each lane. **D**, Plasma CETP activity was measured by Roar CETP activity assay kit as described in the Materials and Methods. N=8 to 14 for each group. Data are expressed as the mean±SEM. **P*<0.05, ****P*<0.001 vs the WT control group.

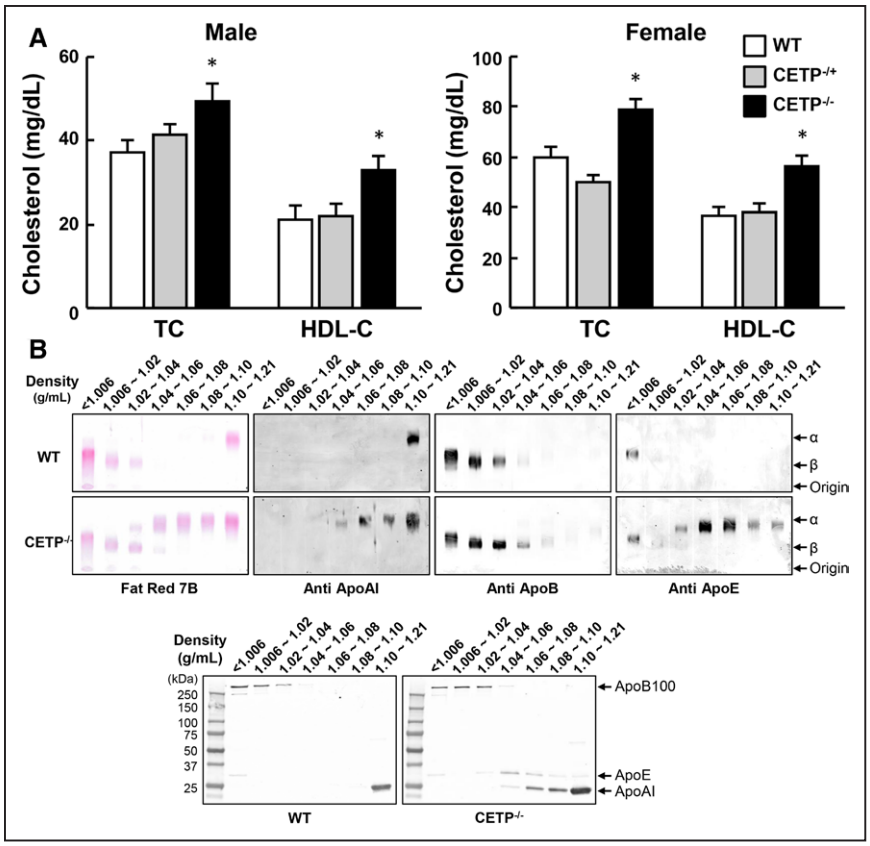

Figure 2. Analysis of plasma lipid profiles from rabbits fed a chow diet. **A**, Total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) levels in plasma from CETP (cholesteryl ester transfer protein) KO and wild-type (WT) rabbits. **B**, Plasma lipoproteins of rabbits fed a chow diet. **Top**, Plasma lipoproteins were separated by sequential density ultracentrifugation according to the density ranges shown above the gels. An equal volume of each fraction was resolved by electrophoresis in a 1% agarose gel. Lipoproteins were visualized using Fat red 7B staining, and apolipoproteins (Apo) were identified by immunoblotting with specific antibodies against apoB, apoE, and apoAI. α and β indicate electrophoretic mobility. **Bottom**, These fractions were further analyzed using 5%–20% SDS-PAGE and probed with antibodies against apoB, apoE, and apoAI. N=8 to 14 for each group. Data are expressed as the mean±SEM. All rabbits are in the range between 3 and 4 months old. *P<0.05 vs the WT control group.

CETP KO rabbits showed no apparent abnormalities in terms of body weight, and autopsy examination did not reveal any changes in lung, heart, kidneys, liver, and other organs (data not shown). On a chow diet, both male and female homozygous (but not heterozygous) KO rabbits showed significantly higher levels of plasma total cholesterol (34% and 32% increase over the control, respectively), which is mainly because of increased HDL-C levels (52% and 54% increase over the control, respectively; Figure 2A), while triglyceride levels were unchanged (data not shown). Analysis of plasma lipoproteins showed that all HDL particles (HDL_1–HDL_3, with a density range from 1.04 to 1.21 g/mL) in homozygous KO rabbits were increased along with enrichment of apolipoprotein (apo)AI and apoE contents, while apoB-containing particles were unchanged compared with control rabbits (Figure 2B).

Next we examined the response of CETP KO rabbits to a cholesterol-rich diet (Figure 3). At baseline (week 0), total cholesterol levels were as follows: for the male rabbits, 37±3, 41±3, and 50±4 mg/dL (mean±SEM) for WT, heterozygous, and homozygous CETP KO rabbits, respectively; for the female rabbits, they were 60±4, 30±3, and 79±5 mg/dL (mean±SEM) for WT, heterozygous, and homozygous KO rabbits, respectively. As shown in Figure 3A, both heterozygous and homozygous CETP KO rabbits exhibited lower hypercholesterolemia compared with WT rabbits throughout the experimental period. This trend was seen in both male and female rabbits carrying the KO allele, but statistical significance was only achieved in homozygous KO rabbits. In spite of this, HDL-C levels in both heterozygous and homozygous KO rabbits remained consistently higher than in WT rabbits. On the other hand, cholesterol feeding did not significantly change triglyceride levels, and there was no significant difference between KO and WT rabbits (data not shown). Analysis of plasma lipoproteins showed that there were 2 striking features in cholesterol-fed KO rabbits compared with controls. First, apoB-containing particles (β-very-low–density lipoprotein [β-VLDL]) were remarkably reduced in KO rabbits (Figure 3B and 3C). Second, $HDL_{1–3}$ was increased with enrichment of apoAI and apoE in KO rabbits compared with that in WT rabbits (Figure 3B and 3C).

Quantification of Aortic and Coronary Atherosclerosis

Analysis of en face aortic lesion area revealed that KO rabbits had significantly smaller aortic atherosclerotic lesions than did WT rabbits (Figure 4A). In KO males, there was a 43% reduction (P<0.05) in heterozygous and a 75% reduction (P<0.01) in homozygous KO rabbits over the WT controls. In KO females, there was a 52% reduction (P<0.05) in heterozygous and a 64% reduction (P<0.01) in homozygous KO rabbits over the respective WT controls. Microscopic examination of lesions also showed prominent reduction of intimal lesion size, with 82% and 81% reduction in male and female homozygous KO rabbits, respectively, compared with WT littermate controls. The aortic lesions of KO rabbits were characterized by reduced macrophage staining by 88% in males and 86% in females over the respective WT controls and smooth muscle cells by 90% in males and 88% in females over the respective WT controls (Figure 4B). We also measured coronary atherosclerosis and found that coronary stenosis was significantly reduced by 74% in male KO rabbits and

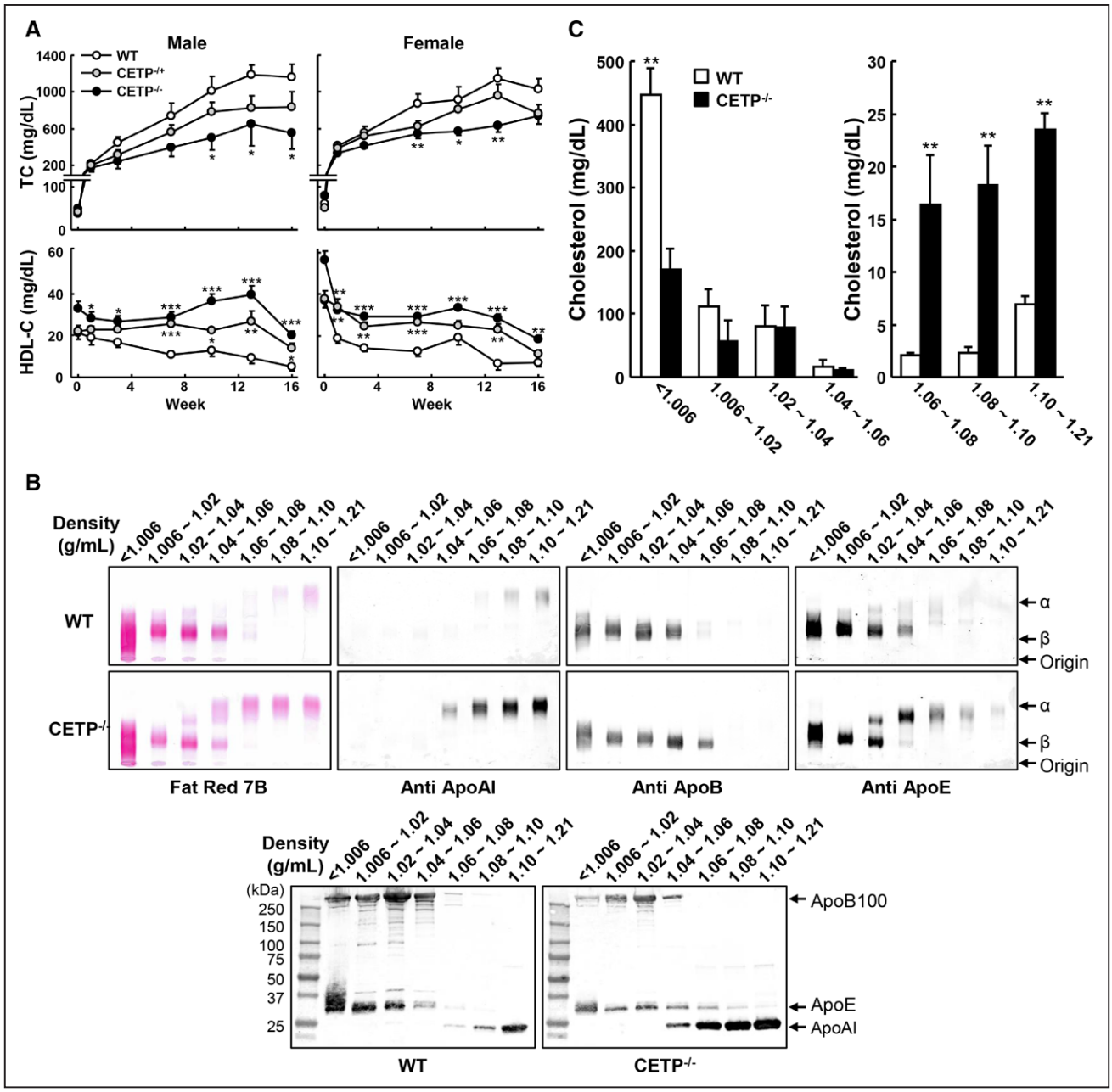

Figure 3. Analysis of plasma lipid profiles from rabbits fed a cholesterol-rich diet. **A**, The plasma lipid profile was monitored during the 16 weeks of cholesterol-rich diet treatment. N=8 to 14 for each group. **B**, Plasma lipoproteins were separated by sequential density ultracentrifugation and analyzed as in Figure 2B. **C**, Cholesterol contents of each lipoprotein fraction were quantified using the Wako total cholesterol assay kit. The combined recovery for each animal averaged ≈80% of the total amount in plasma. N=3 for each group. Data are expressed as the mean±SEM. ***P<0.001, **P<0.01, or *P<0.05 vs WT control group. Apo indicates apolipoprotein; CETP, cholesteryl ester transfer protein; HDL-C, high-density lipoprotein cholesterol; TC, total cholesterol; and WT, wild-type.

47% in female KO rabbits compared with the corresponding WT controls (Figure 5A and 5B).

Cholesterol Efflux Capacity Assay

We examined whether there was any functional difference between HDLs of WT and CETP KO rabbits by cholesterol efflux assay. As shown in Figure 6A, apoB-depleted plasma from chow-fed female (but not male) CETP KO rabbits exhibited a significant increase in cholesterol efflux activity. After being fed a cholesterol-rich diet for 10 weeks, we repeated the cholesterol efflux assay and found that ApoB-depleted plasma from both male and female KO rabbits shows significant increase in cholesterol efflux capacity compared with that of the corresponding WT rabbit controls.

Anti-Inflammatory Effects of HDL

Our finding that CETP KO rabbits had higher HDL levels, and those HDLs were enriched in apoAI and apoE, prompted us to examine whether these changes in HDLs affected their anti-inflammatory function. Human umbilical vein endothelial

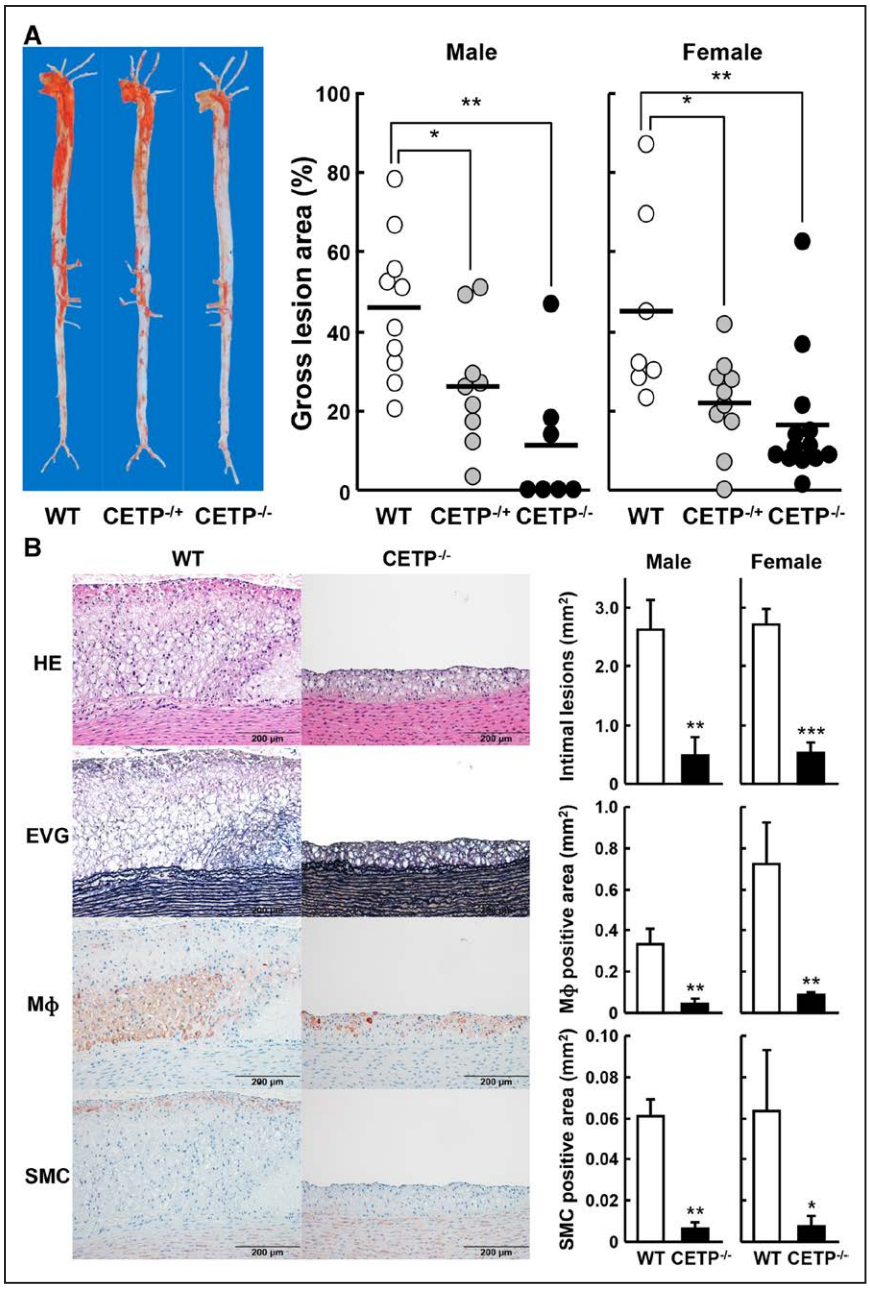

Figure 4. Quantification of aortic atherosclerosis at 16 weeks of cholesterol-rich diet feeding. **A**, Representative pictures of aortas stained with Sudan IV are shown on the **left**. The lesion area (defined by sudanophilic staining as red) was quantified using an image analysis system (**right**). Each dot represents the lesion area of an individual animal. Horizontal bar represents the mean for each genotype. N=7 to 13 for each group. **B**, Representative micrographs of the aortic arch lesions from each group. Serial cuts from paraffin sections were stained with hematoxylin–eosin (HE) and elastica van Gieson (EVG) or immunohistochemically stained with monoclonal antibodies against macrophages (Mϕ) or α-smooth muscle actin for smooth muscle cells (SMCs). The lesions are characterized by intimal accumulation of macrophage-derived foam cells intermingled with smooth muscle cells (**left**). Scale bars represent 200 μm. **Right**, The quantification of the lesions of different parts of aortas. The intimal lesion area and the area with positive immunostaining for macrophages and SMCs were quantified using an image analysis system as described in the Materials and Methods. N=5 to 14 for each group. Data are expressed as the mean±SEM. ***P<0.001, **P<0.01, or *P<0.05 vs WT control group. CETP indicates cholesteryl ester transfer protein; and WT, wild-type.

cells were pretreated with HDL_3 isolated from either WT or CETP KO rabbits at the same concentration (5 μg protein/mL) for 1 hour and then stimulated by tumor necrosis factor-α for an additional 4 hours. We found that although both HDLs suppressed the expression of vascular cell adhesion molecule 1 and E-selectin induced by tumor necrosis factor-α at both mRNA and protein levels (Figure 6B; Figure I in the online-only Data Supplement), the HDLs isolated from CETP KO rabbits showed a tendency to exert stronger inhibitory effects than HDLs isolated from WT rabbits. These findings suggest that an improvement in the anti-inflammatory effect of HDL may also contribute to reduce atherogenesis in CETP KO rabbits.

Discussion

In the current study, we generated CETP KO rabbits by gene editing using a ZFN approach and characterized their lipid and lipoprotein profiles and their susceptibility to cholesterol-rich diet–induced atherosclerosis. On a chow diet, and at 4 months of age, only homozygous (but not in heterozygous) KO rabbits showed elevated plasma levels of HDL-C compared with control rabbits. This may suggest that half the amount of CETP protein and activity (as in the heterozygous KO rabbits; Figure 1) is sufficient to maintain plasma HDL-C homeostasis. CETP KO rabbits, with significantly elevated HDL-C, appeared overall healthy and did not show any signs of other abnormalities.

It is well known that rabbits develop hypercholesterolemia and atherosclerosis rapidly when fed a cholesterol-rich diet.[23] The major lipoproteins in cholesterol-fed rabbits were those of hepatic- and intestinal-derived remnant lipoproteins, called β-VLDLs. β-VLDLs are atherogenic lipoproteins because they are rich in cholesteryl esters along with apoB-100 and apoE.

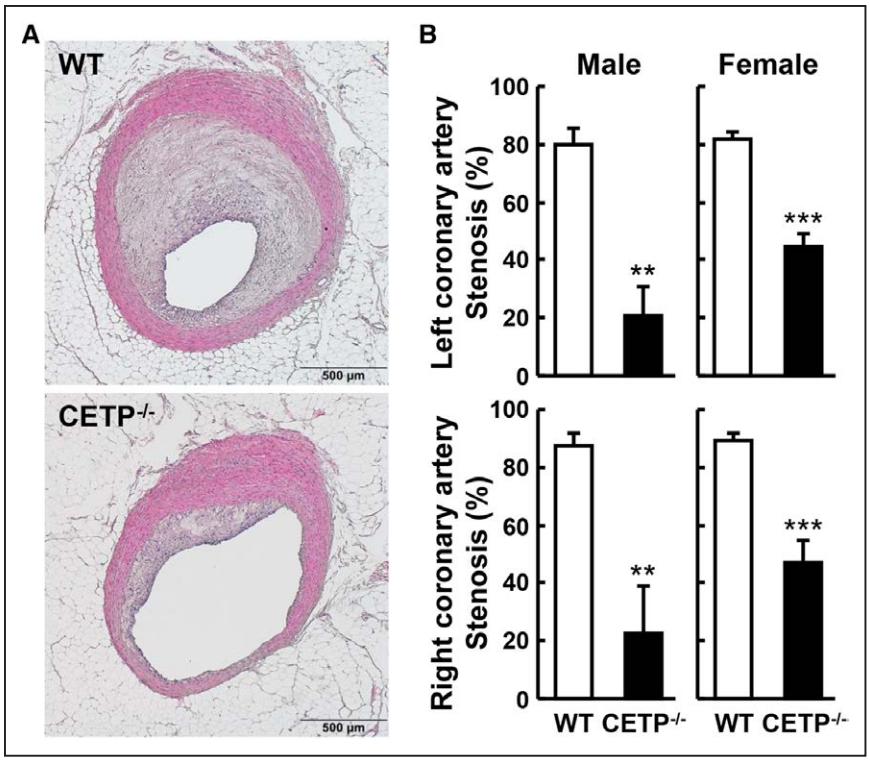

Figure 5. Quantification of coronary atherosclerotic lesions. **A**, Representative micrographs of the left coronary atherosclerotic lesions from each group (hematoxylin–eosin [HE] staining). **B**, The lesion size (expressed as stenosis %) of both right and left coronary arteries is shown. N=5 to 12 for each group. Data are expressed as the mean±SEM. $^{**}P<0.01$ or $^{*}P<0.05$ vs WT control group. CETP indicates cholesteryl ester transfer protein; and WT, wild-type.

Both heterozygous and homozygous CETP KO rabbits showed lower hypercholesterolemia when compared with WT littermate controls fed a cholesterol-rich diet, suggesting that deficiency of CETP attenuates hypercholesterolemia induced by cholesterol feeding. The molecular mechanisms for this phenomenon remain unknown, but it is possible that with lower CETP (as in heterozygous KO rabbits) or absence of CETP activity (as in homozygous KO rabbits), the exchange of cholesteryl esters in HDLs and triglyceride in apoB-containing particles was diminished, thus, resulting in less apoB-containing particles or that the apoB-containing particles with less cholesteryl esters could be catabolized faster by the liver. It is not clear whether CETP deficiency has any effects on hepatic low-density lipoprotein (LDL) receptor activity because hepatic LDL receptor function in cholesterol-fed rabbits is saturated.[25,26] In the current study, we did not find significant changes in the expression of LDL receptor and scavenger receptor class B member 1 in the CETP KO rabbit liver tissue after cholesterol-rich diet feeding for 16 weeks (Figure II in the online-only Data Supplement). In a recent report, Miyosawa et al[15] showed that the CETP inhibitor K-312 mediates LDL cholesterol metabolism by reducing PCSK9 (proprotein convertase subtilisin/kexin type 9) expression. In human studies, CETP deficiency is associated with low LDL-C levels[8] but is insufficient to prevent coronary heart disease in patients with familial hypercholesterolemia because of LDL receptor deficiency.[27] In addition, CETP inhibitors could also lower the plasma LDL-C in human patients in a recent clinical trial.[28] Whether this LDL-C-lowering effect leads to a reduction of cardiovascular events in humans should be answered in follow-up outcome studies.

As predicted, CETP KO rabbits showed significantly higher plasma HDL-C levels than the control rabbits. There is a clear correlation between CETP activity and plasma levels of HDL-C: homozygous KO rabbits>heterozygous KO rabbits>WT rabbits. This finding is consistent with the reports using a CETP vaccine,[11] antisense,[12] or inhibitors[13–15] in rabbits.

The key question is whether increased HDLs levels in CETP KO rabbits show normal or improved functions. As shown in Figure 6, apoB-depleted plasma isolated from cholesterol-fed CETP KO rabbits exhibited increased cholesterol efflux capacity from cholesterol-loaded macrophages than the plasma from WT rabbits in vitro, which is consistent with CETP inhibitor studies.[14,15] Increased HDL particle number in CETP KO rabbits may be responsible for enhancement of cholesterol efflux. In addition, HDL_3 isolated from CETP KO rabbits showed potent anti-inflammatory activity. Therefore, increased HDL particles caused by CETP genetic deficiency result in enhancement of HDL functions, with no noticeable abnormalities in rabbits.

Both heterozygous and homozygous CETP KO rabbits are protected against aortic and coronary atherosclerosis compared with WT control rabbits, albeit the reduction in median lesion area was more prominent in homozygous KO rabbits than in heterozygous KO rabbits (even though there was no statistical significance between those 2 genotypes). Increased resistance to atherosclerosis may result from lower plasma β-VLDL and higher HDL-C levels in the homozygous KO rabbits. It should be noted that the lesions of KO rabbits were characterized by having less macrophages and smooth muscle cells than those of control rabbits. Although CETP deficiency did not affect the specific cell types in the lesions, it is not known whether CETP may have other direct roles in atherosclerotic plaque initiation or progression because lesion macrophages also express CETP.[29]

In conclusion, we have successfully generated CETP KO rabbits using ZFN genome editing. CETP KO rabbits are

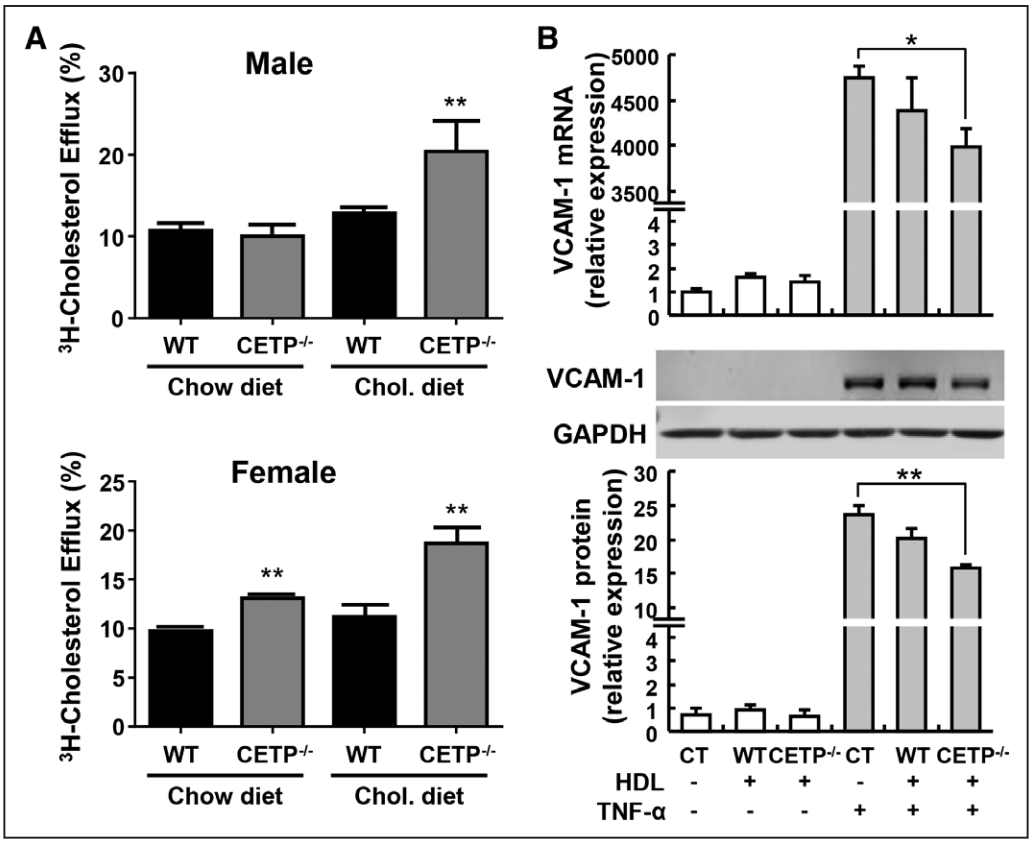

Figure 6. Improved high-density lipoprotein (HDL) function in CETP (cholesteryl ester transfer protein) knockout (KO) rabbits. **A**, Cholesterol efflux capacity assay. **A**, Apolipoprotein (Apo)B–depleted plasma from CETP KO rabbits shows significantly higher cholesterol efflux capacity compared with plasma from wild-type (WT) rabbits, especially after cholesterol-rich diet feeding. Plasma was collected from male and female rabbits, respectively, fed with normal chow or cholesterol-rich diet for 10 weeks. ApoB-depleted plasma was obtained by PEG (polyethylene glycol) precipitation as described in the Materials and Methods section. **B**, Anti-inflammatory activity of HDL. HDL_3 isolated from CETP KO rabbits shows increased anti-inflammatory in cultured endothelial cells effect than that from WT rabbits. HDL_3 was isolated by sequential density ultracentrifugation as described in Figure 2B. Human umbilical vein endothelial cells (HUVECs) were pretreated with rabbit HDL_3 (5 μg protein/mL, N=6) for 1 h and then stimulated with tumor necrosis factor (TNF)-α (1 ng/mL) for 4 h. The expression of the proinflammatory adhesion molecule vascular cell adhesion molecule 1 (VCAM-1) was determined by quantitative reverse transcriptase polymerase chain reaction (qRT-PCR; **upper**). The protein levels of VCAM-1 were detected by Western blotting (**middle** and **lower**). Quantitative data were generated with Image Studio (LI-COR) from 3 independent Western blot experiments. Data are expressed as the mean±SEM **P*<0.05 vs WT group.

protected against cholesterol-rich diet–induced atherosclerosis, likely because of lower β-VLDL and higher HDL levels and functionality. These results support the need for continuing efforts to inhibit CETP for the treatment of hypercholesterolemia and atherosclerosis and warrant future studies to dissect the roles of CETP in a tissue-specific context.

Acknowledgments

We thank Dr Xiancheng Jiang, SUNY Downstate Medical Center, for providing the CETP (cholesteryl ester transfer protein) monoclonal antibody and Nakagawa Y for her technical assistance in processing pathological specimens.

Sources of Funding

This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports and Technology, Japan (22390068, 25670190, and 15H04718 to J. Fan) and National Institutes of Health (NIH) grant (R01HL117491 and R01HL129778 to Y.E. Chen).

Disclosures

None.

References

1. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. *Am J Med*. 1977;62:707–714.
2. Degoma EM, Rader DJ. Novel HDL-directed pharmacotherapeutic strategies. *Nat Rev Cardiol*. 2011;8:266–277. doi: 10.1038/nrcardio.2010.200.
3. Tall AR. Plasma cholesteryl ester transfer protein. *J Lipid Res*. 1993;34:1255–1274.
4. Barter PJ, Nicholls SJ, Kastelein JJ, Rye KA. Is cholesteryl ester transfer protein inhibition an effective strategy to reduce cardiovascular risk? CETP inhibition as a strategy to reduce cardiovascular risk: The Pro Case. *Circulation*. 2015;132:423–432. doi: 10.1161/CIRCULATIONAHA.114.014025.
5. Brown ML, Inazu A, Hesler CB, Agellon LB, Mann C, Whitlock ME, Marcel YL, Milne RW, Koizumi J, Mabuchi H. Molecular basis of lipid transfer protein deficiency in a family with increased high-density lipoproteins. *Nature*. 1989;342:448–451. doi: 10.1038/342448a0.
6. Yamashita S, Sprecher DL, Sakai N, Matsuzawa Y, Tarui S, Hui DY. Accumulation of apolipoprotein E-rich high density lipoproteins in hyperalphalipoproteinemic human subjects with plasma cholesteryl ester transfer protein deficiency. *J Clin Invest*. 1990;86:688–695. doi: 10.1172/JCI114764.
7. Moriyama Y, Okamura T, Inazu A, Doi M, Iso H, Mouri Y, Ishikawa Y, Suzuki H, Iida M, Koizumi J, Mabuchi H, Komachi Y. A low prevalence of coronary heart disease among subjects with increased high-density lipoprotein cholesterol levels, including those with plasma cholesteryl ester transfer protein deficiency. *Prev Med*. 1998;27(5 pt 1):659–667. doi: 10.1006/pmed.1998.0340.

8. Inazu A, Brown ML, Hesler CB, Agellon LB, Koizumi J, Takata K, Maruhama Y, Mabuchi H, Tall AR. Increased high-density lipoprotein levels caused by a common cholesteryl-ester transfer protein gene mutation. *N Engl J Med*. 1990;323:1234–1238. doi: 10.1056/NEJM199011013231803.
9. Zhong S, Sharp DS, Grove JS, Bruce C, Yano K, Curb JD, Tall AR. Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. *J Clin Invest*. 1996;97:2917–2923. doi: 10.1172/JCI118751.
10. Barter P. CETP and atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2000;20:2029–2031.
11. Rittershaus CW, Miller DP, Thomas LJ, Picard MD, Honan CM, Emmett CD, Pettey CL, Adari H, Hammond RA, Beattie DT, Callow AD, Marsh HC, Ryan US. Vaccine-induced antibodies inhibit CETP activity *in vivo* and reduce aortic lesions in a rabbit model of atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2000;20:2106–2112.
12. Sugano M, Makino N, Sawada S, Otsuka S, Watanabe M, Okamoto H, Kamada M, Mizushima A. Effect of antisense oligonucleotides against cholesteryl ester transfer protein on the development of atherosclerosis in cholesterol-fed rabbits. *J Biol Chem*. 1998;273:5033–5036.
13. Okamoto H, Yonemori F, Wakitani K, Minowa T, Maeda K, Shinkai H. A cholesteryl ester transfer protein inhibitor attenuates atherosclerosis in rabbits. *Nature*. 2000;406:203–207. doi: 10.1038/35018119.
14. Morehouse LA, Sugarman ED, Bourassa PA, Sand TM, Zimetti F, Gao F, Rothblat GH, Milici AJ. Inhibition of CETP activity by torcetrapib reduces susceptibility to diet-induced atherosclerosis in new zealand white rabbits. *J Lipid Res*. 2007;48:1263–1272
15. Miyosawa K, Watanabe Y, Murakami K, Murakami T, Shibata H, Iwashita M, Yamazaki H, Yamazaki K, Ohgiya T, Shibuya K, Mizuno K, Tanabe S, Singh SA, Aikawa M. New CETP inhibitor K-312 reduces PCSK9 expression: a potential effect on LDL cholesterol metabolism. *Am J Physiol Endocrinol Metab*. 2015;309:E177–E190. doi: 10.1152/ajpendo.00528.2014.
16. Aghebati T, Badiee A, Mohammadpour AH, Afshar M, Jaafari MR, Abnous K, Issazadeh S, Hashemzadeh S, Zareh M, Hashemizadeh H, Nazemi S. Anti-atherosclerosis effect of different doses of CETP vaccine in rabbit model of atherosclerosis. *Biomed Pharmacother*. 2016;81:468–473. doi: 10.1016/j.biopha.2016.04.035.
17. Huang Z, Inazu A, Nohara A, Higashikata T, Mabuchi H. Cholesteryl ester transfer protein inhibitor (JTT-705) and the development of atherosclerosis in rabbits with severe hypercholesterolaemia. *Clin Sci (Lond)*. 2002;103:587–594. doi: 10.1042/.
18. Marotti KR, Castle CK, Boyle TP, Lin AH, Murray RW, Melchior GW. Severe atherosclerosis in transgenic mice expressing simian cholesteryl ester transfer protein. *Nature*. 1993;364:73–75. doi: 10.1038/364073a0.
19. Barter PJ, Caulfield M, Eriksson M, et al.; ILLUMINATE Investigators. Effects of torcetrapib in patients at high risk for coronary events. *N Engl J Med*. 2007;357:2109–2122. doi: 10.1056/NEJMoa0706628.
20. Nissen SE, Tardif JC, Nicholls SJ, Revkin JH, Shear CL, Duggan WT, Ruzyllo W, Bachinsky WB, Lasala GP, Lasala GP, Tuzcu EM; ILLUSTRATE Investigators. Effect of torcetrapib on the progression of coronary atherosclerosis. *N Engl J Med*. 2007;356:1304–1316. doi: 10.1056/NEJMoa070635.
21. Schwartz GG, Olsson AG, Abt M, et al.; dal-OUTCOMES Investigators. Effects of dalcetrapib in patients with a recent acute coronary syndrome. *N Engl J Med*. 2012;367:2089–2099. doi: 10.1056/NEJMoa1206797.
22. McLain JH, Alsterda AJ, Arora RR. Cholesteryl ester transfer protein inhibitors: Trials and tribulations. *J Cardiovasc Pharmacol Ther*. 2016: pii: 1074248416662349.
23. Fan J, Kitajima S, Watanabe T, Xu J, Zhang J, Liu E, Chen YE. Rabbit models for the study of human atherosclerosis: from pathophysiological mechanisms to translational medicine. *Pharmacol Ther*. 2015;146:104–119. doi: 10.1016/j.pharmthera.2014.09.009.
24. Doyon Y, McCammon JM, Miller JC, Faraji F, Ngo C, Katibah GE, Amora R, Hocking TD, Zhang L, Rebar EJ, Gregory PD, Urnov FD, Amacher SL. Heritable targeted gene disruption in zebrafish using designed zinc-finger nucleases. *Nat Biotechnol*. 2008;26:702–708. doi: 10.1038/nbt1409.
25. Kovanen PT, Brown MS, Basu SK, Bilheimer DW, Goldstein JL. Saturation and suppression of hepatic lipoprotein receptors: A mechanism for the hypercholesterolemia of cholesterol-fed rabbits. *Proc Natl Acad Sci USA*. 1981;78:1396–1400.
26. Xu G, Salen G, Shefer S, Ness GC, Nguyen LB, Parker TS, Chen TS, Zhao Z, Donnelly TM, Tint GS. Unexpected inhibition of cholesterol 7 alpha-hydroxylase by cholesterol in New Zealand white and Watanabe heritable hyperlipidemic rabbits. *J Clin Invest*. 1995;95:1497–1504. doi: 10.1172/JCI117821.
27. Haraki T, Inazu A, Yagi K, Kajinami K, Koizumi J, Mabuchi H. Clinical characteristics of double heterozygotes with familial hypercholesterolemia and cholesteryl ester transfer protein deficiency. *Atherosclerosis*. 1997;132:229–236.
28. Kastelein JJ, Besseling J, Shah S, Bergeron J, Langslet G, Hovingh GK, Al-Saady N, Koeijvoets M, Hunter J, Johnson-Levonas AO, Fable J, Sapre A, Mitchel Y. Anacetrapib as lipid-modifying therapy in patients with heterozygous familial hypercholesterolaemia (REALIZE): a randomised, double-blind, placebo-controlled, phase 3 study. *Lancet*. 2015;385:2153–2161. doi: 10.1016/S0140-6736(14)62115-2.
29. Zhang Z, Yamashita S, Hirano K, Nakagawa-Toyama Y, Matsuyama A, Nishida M, Sakai N, Fukasawa M, Arai H, Miyagawa J, Matsuzawa Y. Expression of cholesteryl ester transfer protein in human atherosclerotic lesions and its implication in reverse cholesterol transport. *Atherosclerosis*. 2001;159:67–75.

Highlights

- We generated a CETP (cholesteryl ester transfer protein) knockout rabbit model by zinc finger nuclease genome editing technology.
- CETP knockout rabbits show reduced plasma total cholesterol with increased high-density lipoprotein cholesterol levels and functionality compared with wild-type rabbits.
- Both aortic and coronary atherosclerosis was significantly reduced in CETP knockout rabbits compared with wild-type controls.
- These results indicate that genetic ablation of CETP protects against cholesterol diet–induced atherogenesis.