Supplemental material

Methods

Reagents - The pcDNA3.1 Directional TOPO Expression Kit, Glutathione-agarose column and precast (4-12%) NuPage™ Bis-Tris gels were purchased from Life Technologies (Grand Island, NY). HEK293T cells were purchased from ATCC (Manassas, VA). Sheep polyclonal antibody toward human PCSK9 was purchased from R&D systems (Minneapolis, MN). Rabbit antibody toward apoB was purchased from Abcam (Cambridge, MA). An ELISA kit for detecting human PCSK9 was purchased from MBL International Corp. (Woburn, MA). OptiPrep (natural gradient solution), Donkey anti-sheep HRP IgG antibody and goat anti-rabbit HRP were purchased from Sigma (St. Louis, MO). The Mammalian Transfection System was purchased from Promega (Madison, WI). OptiSeal tubes were purchased from Beckman-Coulter (Brea, CA). Commercial-grade dextran-sulfate cellulose beads (from an unused Liposorber® column) were a kind gift from Kaneka Pharma (New York, NY).

Collection of pre- and post-LDL apheresis plasma samples - Plasma from pre- and post-LDL-A treatments was used to measure lipids and proteins. In the apheresis procedure, the patient's blood is drawn via venous access to flow in the plasma separator. As blood flows through the hollow fibers of the plasma separator, the plasma (pre-apheresis) is separated and pumped into one of two LDL absorption columns, where the apoB-containing lipoproteins are selectively absorbed by the dextran-sulfate cellulose beads. The LDL-depleted plasma (post-apheresis) exits the column and is recombined with the blood cells exiting the separator, all of which is returned to the patient via a separate venous access. Once one column is full the system automatically moves to the second column, while the first column is washed and then eluted into a waste bag using lactated Ringer's solution (0.6% Sodium chloride, 0.31% sodium lactate, 0.03% potassium chloride and 0.02% calcium chloride) and 5% NaCl (column eluate).

Western Blotting - Samples were loaded onto NuPage™ 4–12% Bis-Tris precast gels for electrophoresis. The size-separated proteins were then transferred to nitrocellulose membranes. The indicated primary antibodies and HRP-conjugated secondary antibodies were used to detect target PCSK9 and apoB. Signal was detected by enhanced chemoluminescence

(ECL) using a mixture of luminol, p-coumaric acid and hydrogen peroxide in 100 mM Tris pH 8.5.

Experimental dextran sulfate column - Using commercial-grade dextran-sulfate cellulose beads (from an unused Liposorber® column), we gravity-packed 1 mL of beads into a vertical column for 30 minutes, followed by washing with 5 volumes of 0.9% NaCl. Six mL of plasma were loaded on the column at 0.2 mL/minute; 6 mL of plasma eluate were collected. Sequentially, using 2 volumes of Ringer's solution, 2 volumes of 5% NaCl and 2 volumes of Ringer's solution (total volume of 6 mL) were used to elute LDL and proteins from the column.

Plasma PCSK9 levels - The levels of human PCSK9 in pre- and post-apheresis plasma, column eluate, ultracentrifuge fractions and purified GST-tag protein were determined by ELISA kits as recommended by the manufacturer (MBL-International).

Recombinant PCSK9 with Glutathione S-transferase (GST) affinity tag - We previously generated a pcDNA-PCSK9-GST construct in which a GST tag was placed in the C-terminal region of the protein[1]. Using the Mammalian Transfection System purchased from Promega (Madison, WI), HEK293T cells were transfected with pcDNA-PCSK9-GST as previously described[1]. The full-length PCSK9 protein with a C-terminal GST tag was purified from the culture medium of transfected HEK293T cells using a glutathione-agarose column and protein concentrations were then determined.

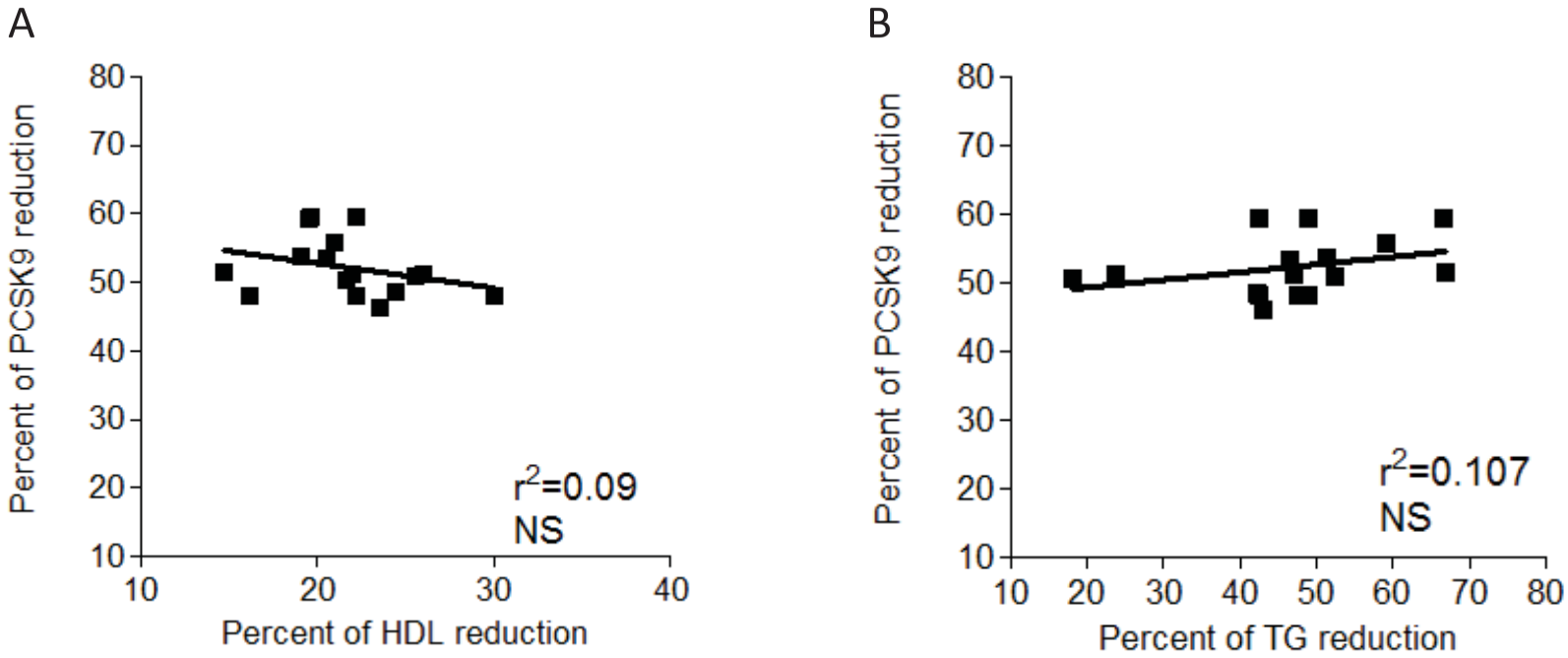
Plasma lipoprotein separation - Plasma lipoproteins from pre- and post-apheresis plasma were isolated by natural gradient media (Optiprep) using a TLN100 rotor and OptiSeal tubes at 90,000 rpm for 2.5 hours, as previously described[2].

Statistical Analyses - GraphPad Prism 4 software was used to carry out statistical analyses. Pairwise t-test was used to compare pre- and post-apheresis values. Student's paired t-test was used to compare the means of two groups. Results are presented as means ± SD or as percent ± CV. (*p<0.05, **p<0.01, ***p<0.001).

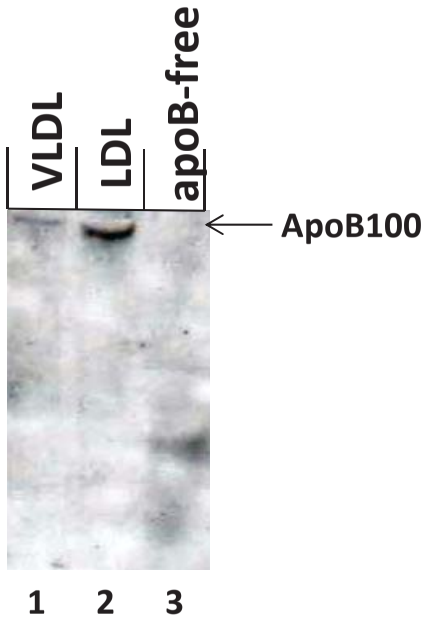
References:

1. Du F, Hui Y, Zhang M, Linton MF, Fazio S, Fan D. Novel domain interaction regulates secretion of proprotein convertase subtilisin/kexin type 9 (pcsk9) protein. *J Biol Chem*. 2011;286:43054-43061
2. Tavori H, Fan D, Blakemore JL, Yancey PG, Ding L, Linton MF, Fazio S. Serum proprotein convertase subtilisin/kexin type 9 and cell surface low-density lipoprotein receptor: Evidence for a reciprocal regulation. *Circulation*. 2013;127:2403-2413

Online Figures



Online Figure I: (A) Correlation between HDL-C reduction and PCSK9 reduction after LDL-apheresis treatment (B) Correlation between triglyceride reduction and PCSK9 reduction after LDL-apheresis treatment. (NS = Not Significant).



Online Figure II: Representative immunoblot of apoB in pre-apheresis plasma fractions isolated by ultracentrifugation, lane 1=VLDL, lane 2= LDL, lane 3=apoB-free.

	Age	Sex	Height (cm)	Weight (Kg)	BMI	Race	List of Medication
Patient #1	17	Male	183	63.5	19.0	Caucasian	Zetia, Metoprolol succinate, Aspirin, Crestor, Niaspan, Cozaar
Patient #2	61	Female	170	90.2	31.2	Caucasian	Aspirin , Plavix, Atenolol, Furosemide , Advair Diskus, Cetirizine, Fluticasone, Potassium chloride, Prevacid, Cyanocobalamin, Alamast Ophthalmic Soln, Medtronic insulin pump, INSPRA, Gabapentin, Ketoconazole, Diclofenac sodium, Amlodipine, Lunesta, Humalog, Fish Oil, Astepro, Docusate sodium.
Patient #3	62	Female	145	69.4	33.0	Caucasian	Ambien, Sertraline, Hydrocodone-acetaminophen, Buspirone, Amlodipine, Livalo.
Patient #4	21	Female	160	99.3	38.8	Caucasian	Lopressor, Niaspan, WelChol, Zetia, Amlodipine, Aspirin, Claritin, Crestor, Mirena, Senna, Warfarin Furosemide, Spironolactone, Oxycodone-acetaminophen.
Patient #5	56	Female	157	42.1	17.1	Caucasian	Premarin patch, Aspirin, Atorvastatin, Hyoscyamine sulfate, Lactobacillus acidoph & bulgar, Lactulose, Levofloxacin, Levothyroxine, Lisinopril, Metoprolol tartrate, Nitroglycerin, Nystatin, Omeprazole, Sennosides, Simethicone, Warfarin, Zolpidem, Plavix, Protonix, Donnatal TID, Zetia, Crestor, Carvedilol.
Patient #6	57	Male	168	114.8	40.7	Caucasian	Endocet, Patanol, Fluticasone, Flovent HFA, Vimovo, Miralax, Diazepam, Nexium, Metoprolol tartrate, Crestor, Simethicone, Aspirin, Soma, Nitroglycerin.

Online Table I: Patients characteristic of the six subjects with Familial Hypercholesterolemia (FH) included in the study.