## Methods

*Subjects*. We recruited thirty-nine patients with Lp(a) above 30mg/dl (mass concentration) referred to our outpatient lipid clinic (IRB#00010649) or undergoing lipoprotein apheresis (IRB#00015117). Plasma was collected in EDTA tubes.

*Plasma analyses.* Lp(a) and LDL-C were measured in our diagnostic laboratory. Plasma PCSK9 and apoB levels were measured using commercially available ELISA kits according to manufacturer's instructions (MBL international, Woburn,MA, and Abcam, Cambridge, UK, respectively). Apolipoproteins (apoA-I, apoA-II, apoC-II, apoC-III, apoE) were quantified in blood samples using trypsin proteolysis and the subsequent analysis of proteotypic peptides by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as previously described [1, 2]. Levels of three additional apolipoproteins (apoA-IV, ApoC-I and ApoM) were determined as previously described [3, 4] Protein quantification was obtained on 3 replicates and each sample had two separate LC-MS/MS runs. The intra-assay variability of the analytical method was below 15%. The parameters used for protein detection of all the apolipoprotein are described in the table below.

| Apolipoprotein | | Proteotypic peptide | Fragment | Cone/collision | MRM transition (*m/z*) | RT (min) |
|---|---|---|---|---|---|---|
| ApoA-I | M | ATEHLSTLSEK | $y_{10}^{2+}$ | 25/15 V | 406.2 → 573.2 | 1.6 ± 0.1 |
| | IS | ATEHLSTLSE**K** | | | 408.9 → 577.2 | |
| ApoA-II | M | SPELQAEAK | $y_6^{+}$ | 30/30 V | 486.8 → 659.3 | 1.5 ± 0.1 |
| | IS | SPELQAEA**K** | | | 490.3 → 667.3 | |
| ApoA-IV | M | SELTQQLNALFQDK | $y_4^{+}$ | 40/35 V | 818.5 → 537.4 | 3.3 ± 0.1 |
| | IS | SELTQQLNALFQD**K** | | | 822.5 → 545.4 | |
| ApoC-I | M | TPDVSSALDK | $y_9^{2+}$ | 40/23 V | 517.4 → 466.8 | 2.2 ± 0.1 |
| | IS | TPDVSSALD**K** | | | 521.4 → 470.8 | |
| ApoC-II | M | TAAQNLYEK | $y_7^{+}$ | 35/20 V | 519.7 → 865.7 | 1.6 ± 0.1 |
| | IS | TAAQNLYE**K** | | | 523.7 → 873.7 | |
| ApoC-III | M | DALSSVQESQVAQQAR | $y_8^{+}$ | 40/35 V | 858.9 → 887.5 | 2.3 ± 0.1 |
| | IS | DALSSVQESQVAQQA**R** | | | 863.4 → 897.5 | |
| ApoE | M | LGPLVEQGR | $y_5^{+}$ | 25/30 V | 484.8 → 588.3 | 2.3 ± 0.1 |
| | IS | LGPLVEQG**R** | | | 489.3 → 598.3 | |
| ApoM | M | AFLLTPR | $y_5^{+}$ | 30/10 V | 409.3 → 599.5 | 2.8 ± 0.1 |
| | IS | AFLLTP**R** | | | 414.3 → 609.5 | |
| Apo(a) – Q | M | LFLEPTQADIALLK | $y_{10}^{+}$ | 55/27 V | 786.9 → 1069.7 | 3.5 ± 0.1 |
| | IS | LFLEPTQADIALL**K** | | | 790.8 → 1077.7 | |
| Apo(a) - Kr | M | GTYSTTVTGR | $y_7^{+}$ | 35/22 V | 522.0 → 721.6 | 1.5 ± 0.1 |
| | IS | GTYSTTVTG**R** | | | 527.2 → 731.4 | |

*Q = quantification, Kr = Kringle repeat*

*Lipoprotein isolation*. Lipoproteins were isolated using Optiprep™, a non-ionic density gradient medium (60% solution of iodixanol in water) of density 1.32 g/ml (Sigma) in OptiSeal tubes and a TLN100 rotor spun at 90,000 rpm for 3 hours as previously described [5]. For fraction isolation, the Optiseal tubes were cut at the visual levels corresponding to the VLDL, LDL, and Lp(a) bands using a tube slicer (Beckman Coulter, CA). Lp(a) fractions were further purified using a Sephacryl s400 column, as previously described [6]. Lipoprotein fractions purity was determined by agarose gels (HELENA Labs, Beaumont ,TX).

*Western Blots.* Samples were loaded onto a 3%-8% tris-acetate SDS or 4%-16% native gels (Invitrogen, Carlsbad, CA) for electrophoretic separation. Proteins were transferred onto nitrocellulose membrane. The indicated primary antibodies and horseradish peroxidase–conjugated secondary antibodies were used to detect target proteins. Signal was detected by use of an enhanced chemiluminescent solution made in house using, Cumaric acid, Luminol and hinydrogen peroxide .

*Immunoprecipitation*. Human Plasma or mice serum were subjected to immunoprecipitation with anti-PCSK9 antibodies (human or mouse, respectively) using BS3 as cross linker and protein G magnetic beads. Precipitated samples were blot against apoB (Santa Cruz, Biotechnology, Santa Cruz CA) and apo(a) (Abcam, Cambridge, UK) .

*Mice.* Generation of Apo(a) and Lp(a) transgenic mice was previously described [7, 8]. Mice were housed at the UCSD facility. All animal experiments were carried out in compliance with NIH guidelines and were approved by the UCSD's Institutional Animal Care and Use Committee. Blood and tissues were collected from 6- to 8-week-old mice.

*Quantitative sandwich-based ELISA.* Assays were developed to measure PCSK9-associated plasma lipoprotein (a) and apoB-100 in human plasma, as recently described [9]. Microtiter 96-well plates were coated overnight at 4°C with commercial rabbit polyclonal antibodies binding to the C-terminal region of PCSK9 (Abgent, San Diego, CA, and Thermo Scientific, Waltham, MA) or alirocumab human monoclonal antibody (each at 5 μg/ml at 40 μl/well) were bound to microtiter well plates. Conditions were established to provide a saturating and equal amount of PCSK9 captured in each well. Excess material was washed off and the plates blocked with 1% tris buffered saline/bovine serum albumin (TBS/BSA) for 45 minutes. After the plates were washed, EDTA plasma was added at 1:50 dilution (40 μL/well) for 75 minutes to bind apoB-100 and Lp(a). For the apoB-associated PCSK9 levels, after the plates were again washed, biotinylated goat anti-human apoB-100 antibody (Academy Biomedical Co., Houston, TX) at 22.5 ng/ml, 40 μl/well, was incubated for 60 minutes. For the Lp(a)-associated PCSK9 levels, biotin-labeled murine monoclonal antibody LPA4 at 1 □g/ml was added to detect Lp(a)/apo(a). After washing excess material off the plates, alkaline phosphatase-conjugated to neutravidin (Thermo Scientific, Waltham, MA) was added for 60 min. After a final wash, lumi-phos 530 (Lumigen, Inc., Southfield, Michigan) (25 μl/well) was added for 75 minutes and luminescence read on a Dynex luminometer (Chantilly Technologies, Chantilly, VA). The results are reported as relative light units (RLU) in 100 milliseconds after subtraction of background RLU (TBS/BSA).

*Statistical analyses*. Data were analyzed with multiple linear regression models adjusting for age, gender, and lipid-lowering medication status as covariates. LDL-C levels were included as a covariate where appropriate (i.e. linear regression not including LDL-C as a variant) The strength of associations between variables were assessed by estimating coefficients and Wald test p-values from the multiple regression models. When an interaction between the predictor of interest and lipid lowering medications was observed, subgroup analyses within each lipid medication group were performed and the regression slope for association between predictor and outcome was estimated and tested. Univariate Spearman's correlations were also estimated for the variables of interest.

Regression lines in the plots have slope equal to coefficient of predictor (variable on x-axis) in multiple regression, adjusted for age, sex, and lipid medication. When two lines are shown, they represent the coefficient of the predictor in multiple regression analysis within a lipid medication subgroup, adjusted for age and sex. When one line is shown, the slope represents the coefficient of the predictor, which is equivalent for both lipid medication subgroups, adjusted for age and sex. r = univariate Spearman's correlation; p = p-value from multiple (adjusted) regression; r0, p0 from model on subjects without lipid-lowering medications; r1, p1 from model on subjects with lipid-lowering medications.

A one-sample Wilcoxon signed rank test was performed on the fold changes, with the null hypothesis that the median fold increase is equal to one (dashed line). The solid horizontal line in the fold change plot the median fold increase, 1.7.

*Lipid-lowering medications by subject.*

| Subject # | Lipid-lowering medications | Lipid-lowering medications (description) |
|---|---|---|
| 1 | Yes | Atorvastatin 10mg/Ezetimibe 10mg/Niacin 2x1000mg |
| 2 | Yes | Atorvastatin 15mg |
| 3 | Yes* | Atorvastatin 20mg/Ezetimibe 10 mg/Niacin 100mg |
| 4 | Yes | Atorvastatin 40mg |
| 5 | Yes | Atorvastatin 40mg/Ezetimibe 10 mg |
| 6 | Yes | Atorvastatin 5mg |
| 7 | Yes | Rosuvastatin 20mg/Ezetimibe 10mg |
| 8 | Yes | Rosuvastatin 20mg/Ezetimibe 10mg |
| 9 | Yes* | Rosuvastatin 40mg |
| 10 | Yes | Ezetimibe 10mg |
| 11 | Yes | Rosuvastatin 20mg/Ezetimibe 10mg |
| 12 | Yes | Rosuvastatin 40mg/Ezetimibe 10mg |
| 13 | Yes | Rosuvastatin 40mg/Ezetimibe 10mg |
| 14 | Yes | Atorvastatin 80mg/Ezetimibe 10mg |
| 15 | Yes* | Lovastatin 40mg/Niacin 500mg |
| 16 | Yes | Simvastatin 80mg/Ezetimibe 10mg/Mipomersen |
| 17 | Yes | Rosuvastatin 40mg/Niacin 500mg |
| 18 | Yes* | Atorvastatin 80mg/Ezetimibe 10mg/Niacin 1000mg |
| 19 | Yes | Atorvastatin 80mg/Ezetimibe 10mg/Niacin 1500mg |
| 20 | Yes | Pitavastatin 1mg/Ezetimibe 10mg |
| 21 | Yes | Pitvastatin 1mg |
| 22 | Yes | Pravastatin 20mg/Fenofibrate 134mg |
| 23 | Yes | Rosuvastatin 5mg |
| 24 | Yes* | Simvastatin 40mg/Niacin 500mg |
| 25 | Yes* | Simvastatin 10mg/Ezetimibe 10mg |
| 26 | No | Diet +/- supplemental therapies |
| 27 | No | Diet +/- supplemental therapies |
| 28 | No | Diet +/- supplemental therapies |
| 29 | No | Diet +/- supplemental therapies |
| 30 | No | Diet +/- supplemental therapies |
| 31 | No | Diet +/- supplemental therapies |
| 32 | No | Diet +/- supplemental therapies |
| 33 | No | Diet +/- supplemental therapies |
| 34 | No | Diet +/- supplemental therapies |
| 35 | No | Diet +/- supplemental therapies |
| 36 | No | Diet +/- supplemental therapies |
| 37 | No | Diet +/- supplemental therapies |
| 38 | No | Diet +/- supplemental therapies |
| 39 | No | Diet +/- supplemental therapies |

* Subjects also undergoing Lipoprotein Apheresis (LA). Plasma samples were collected pre LA.

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