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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

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Experimental design

1. Sample size

Describe how sample size was determined.

For studies of human participants to compare APOC3 A43T carriers vs. noncarriers, the number of noncarrier samples analyzed was chosen based on a power estimate for identifying a 50% difference in plasma apoC-III levels, with a standard deviation equivalent to the difference in means. From this estimate, a minimum of 16 carriers and 16 noncarriers were required for the analysis. Given the identification of 19 A43T carriers, we searched for at least an equivalent number of matched noncarriers, and ultimately identified 76 noncarriers for the lipid and apoC-III analyses described. For mouse experiments, an average of five to six mice per group were used for all studies, which was based on prior requirements for group sample sizes for measuring 50% mean differences in plasma TGs from prior studies in our laboratory.

2. Data exclusions

Describe any data exclusions.

No human or animal samples for which measurements were performed were excluded from analyses. Individual samples for a given measure may have been excluded on the basis of sample processing error during experimental work-flow and assays but no samples were excluded from analyses after completion of sample processing and measurements.

3. Replication

Describe whether the experimental findings were reliably reproduced.

For all animal studies except for the antibody studies presented in Figure 4 L-P, experiments were independently replicated in an independent set of mice of the same strain, age and gender and under the same conditions as the original experiments. Findings and conclusions in the manuscript represent those that were reproduced across replicate experiments in independent mouse cohorts. Data in Figure 4 L-P was performed in one cohort of mice.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Mice were not randomized for any measure prior to use in AAV experiments. Human samples were not randomized for measurements.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

For animal experiments, experimenters were not blinded to group allocation for the described experiments in mice. For human sample experiments, experimenters were not blinded for measurement of lipid measures from samples (Figure 1 B-C and Figure S1), but were blinded for measurements of selective reaction monitoring-based apoC-III peptide quantification (Figure 1 D-F).

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

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6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. P values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	All human lipid phenotyping and animal measurement data presented in the manuscript was analyzed in either Excel (Microsoft) or GraphPad Prism. An open source data analysis package (University of Washington, Skyline) was used to analyze peptide fragment data from tandem mass spectrometry for targeted selective monitoring assay development. For calculation of fractional catabolic rate using multi-exponential monitoring of plasma decay curves from radioisotope tracer kinetic studies in mice, the WinSAAM curve fitting and modeling program was used. Exome chip genotyping data from human participants was analyzed using the publicly available PLINK software.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	STT505 and STT5058 antibodies are restricted from availability as they are the propriety of Staten Biotechnology BV. All other materials used in the manuscript bear no restrictions for availability or distribution.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	The anti-apoC-III antibodies used in this study were the following: Rabbit polyclonal anti-apoC-III (Abcam #ab21032), Rabbit polyclonal anti-apoC-III (Academy Biomedical, #33A-R1a), Mouse polyclonal anti-actin Santa Cruz Biotechnology, sc-981178). Antibodies against apoC-III were validated using purified commercially available human apoC-III from pools of human plasma (Academy biomedical, 33P-110). The apoC-III standard was validated by processing a small sample of the protein in solution on matrix assisted laser desorption ionization time-of-flight mass spectrometry and protein sequence identification using SkyLine as mentioned above in Software. This validated sample was then used as a standard for immunoblotting for human apoC-III in the mouse samples after expression of human APOC3 in mice, as described. Actin antibody had been validated in our laboratory previously.

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10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

ATCC was the source of the HEK293E and HepG2 cell lines used for expression and purification of recombinant monoclonal antibody to apoC-III.

b. Describe the method of cell line authentication used.

All cell lines were authenticated by ATCC by short tandem repeat profiling and karyotyping and were tested by both ATCC and our facility for mycoplasma contamination.

c. Report whether the cell lines were tested for mycoplasma contamination.

All cell lines were authenticated by ATCC by short tandem repeat profiling and karyotyping and were tested by both ATCC and our facility for mycoplasma contamination.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No cell lines used in this study are listed in the provided database.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

C57BL/6 wild-type (WT) mice were purchased from The Jackson Laboratory (Cat# 000664). Additionally, Apoc3 KO mice on a C57BL/6 background (Cat# 002057) were recovered from cryo-preservation from The Jackson Laboratory and bred at the University of Pennsylvania. Apoc3 KO mice were bred with Apobec1-deficient, human APOB transgenic mice that either were wild-type (LahB WT), heterozygous (LahB Het), or deficient (LahB KO) for the murine Ldlr gene. Mice were maintained in monitored small animal facility at the University of Pennsylvania under IACUC-approved protocols, were fed ad libitum with a standard chow diet or Western diet containing 0.21% cholesterol from OpenSource Diets (Cat# D12079B, Research Diets, New Brunswick, NJ, USA) for the indicated periods of time. All mice were provided access to water ad libitum and were maintained with a 12 hour on/off light cycle with lights off from 7:00 pm to 7:00 am daily. All blood samples from mice were collected by retro-orbital bleeding from mice anesthetized with isoflurane using EDTA-coated glass tubes under approved protocols. For all studies, male mice approximately 10-12 weeks of age were used. Mice were not randomized for any measure prior to use in AAV experiments, and experimenters were not blinded to group allocation for the described experiments. Six mice per group were used for all studies, which was based on prior requirements for group sample sizes for measuring 50% mean differences in plasma TGs from prior studies in our laboratory.
For APOC3 AAV expression experiments in mouse models, mice were initially fasted for four hours, bled, and administered a control AAV serotype 8 vector lacking a transgene (Null), APOC3 WT AAV (WT), or APOC3 A43T (A43T), all at a dose of 3x1011 genome copies of virus per mouse as determined by digital PCR based titering by the University of Pennsylvania Vector Core. All animal studies using STT505 and STT5058 were carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All procedures were approved by the Institutional Animal Care and Use Committee of Vascumab, LLC and the University of Pennsylvania. For STT505 antibody studies, 24 C57BL/6 male mice (Charles River) weighing 20-25 grams were maintained on a constant 12-hour light: 12-hour dark cycle with free access to water and ad libitum access to standard chow diet (Lab Diet; 5001). For over expression of human WT APOC3, mice were injected IP with an AAV vector expressing human APOC3 as described above. Twelve days following administration of 3x1011 Genome Copies/mouse, mice were bled via retro-orbital sinus and assigned to groups to achieve equivalent starting mean plasma apoC-III levels between groups.

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Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Human participants were recruited from two independent cohorts for ascertainment of APOC3 loss-of-function variants and subsequent phenotyping. The first cohort, the Penn High HDL Study (HHDL), is a cross-sectional study of heritable factors contributing to elevated levels of HDL-C. Participants were recruited with HDL-C levels greater than the 95th percentile for age and gender by physician referrals or through the Hospital of the University of Pennsylvania clinical lipid laboratory. Relatives of high HDL-C probands were also invited to participate in the study. Participants were consented for additional plasma lipid analysis and research genetic studies including targeted sequencing of candidate genes, exome sequencing, and SNP array genotyping under a protocol approved by the Institutional Review Board (IRB) of the University of Pennsylvania. Over 2,500 participants have been recruited for the HHDL study, with recruitment ongoing. The second cohort, the Penn Medicine BioBank, is a collection of banked plasma samples from participants who were treated at the University of Pennsylvania Health System and were consented for genetic analysis and biochemical studies of collected plasma for the study of heritable risk factors and novel biomarkers related to CHD risk. Approximately 50,000 participants have been recruited so far, with recruitment of additional participants ongoing through IRB approved protocols of the University of Pennsylvania. Demographic characteristics for carriers and noncarriers from each identification cohort were as follows: CGI cohort- A43T carriers: n=6, female n=2, male n= 4, age= 61.7 ± 11.9 years, Caucasian n=5, African American n=1, noncarriers: n=54, Female n=18, male n=36, age= 60.2 ± 6.6 years, Caucasian n=48 African American n=6. HHDL cohort- A43T carriers: n=13, female n=11, male n= 2, age=60.4 ± 9.58 years, Caucasian n=12, African American n=1, noncarriers: n=22, Female n=18, male n=4, age= 60.9 ± 10.1 years, Caucasian n=18 African American n=4. Age is expressed as mean ± standard deviation.

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