RNA Editing: A Modality for Precise Introduction of Gain-of-Function Variants Identified by Human Genetics

#1426

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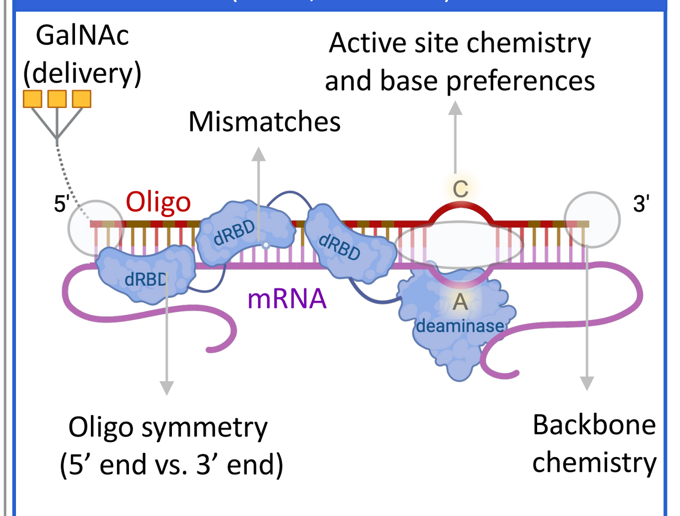
Introduction

- Large-scale human genetics studies have identified numerous **protective variants** linked to reduced disease risk
- These genetic insights remain **unexploited**, especially those involving **gain-of-function mechanisms** like increased protein expression or altered protein function
- **Endogenous ADAR-mediated RNA editing** offers a promising strategy to introduce protective variants at the transcript level, enabling precise, reversible modulation of protein expression or function without altering the underlying DNA sequence
- In these studies, we applied RNA editing to unlock previously "undruggable targets" in **lipid metabolism**, leveraging population-scale genetics to identify protective variants in **LDLR and APOB** that existing therapies fail to address — targeting **atherosclerotic cardiovascular disease (ASCVD)**, the leading cause of mortality worldwide

Site-directed RNA editing with RESTORE+ oligonucleotides

Features of ADAR-oligo RNA editing complex (A-to-I, read as G)



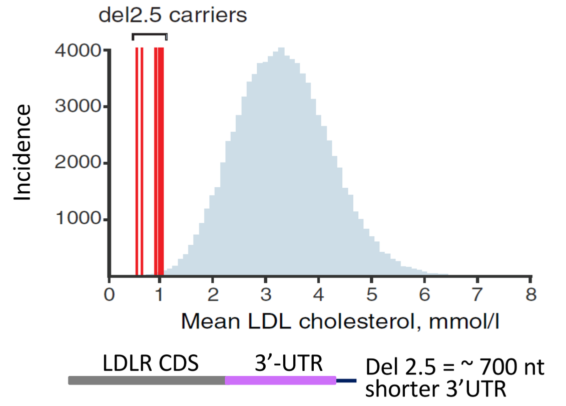
Adapted from Aquino-Jarquin, Mol. Ther. Nucleic Acids, 2020

Key elements of RESTORE+ platform

1. **Backbone and base modifications** — Enhance stability and reduce non-specific protein binding
2. **Optimized GalNAc** — Maximize delivery to liver, without inhibiting ADAR potency
3. **Structure-based design** — Maximize ADAR1 p110 isoform engagement, minimize degradation

LDLR human genetics: Identified population in Iceland with heterozygous mutation in LDLR associated with 74% lower LDL-C

Gain-of-function mutation in LDLR 3'UTR in Icelandic population[1] (heterozygous 2.kb del)



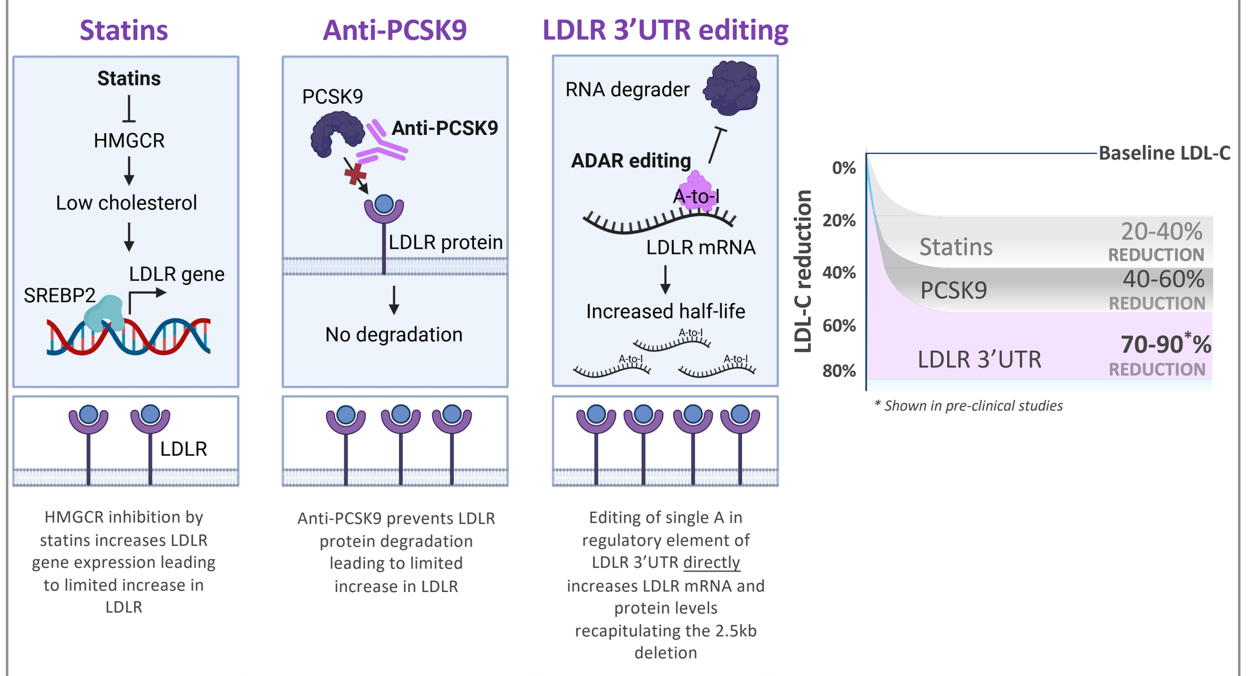
Detailed characteristics of identified heterozygous carriers (vs >100k genotyped individuals in Iceland)

LDL-C reduction	74% (1.79x LDLR increase)
Lp(a) reduction	40-80%[4]
Non-HDL-C reduction	63%
APOB reduction	54%
Triglyceride reduction	8%
Safety	No evidence for impairment

Comparison with PCSK9 loss-of-function carriers
Heterozygous PCSK9 loss-of-function carriers have a 10-28% mean LDL-C reduction[2,3] *which is significantly less than heterozygous* LDLR del 2.5 carriers
→ **Editing of LDLR may enable more significant lowering of LDL-C levels**

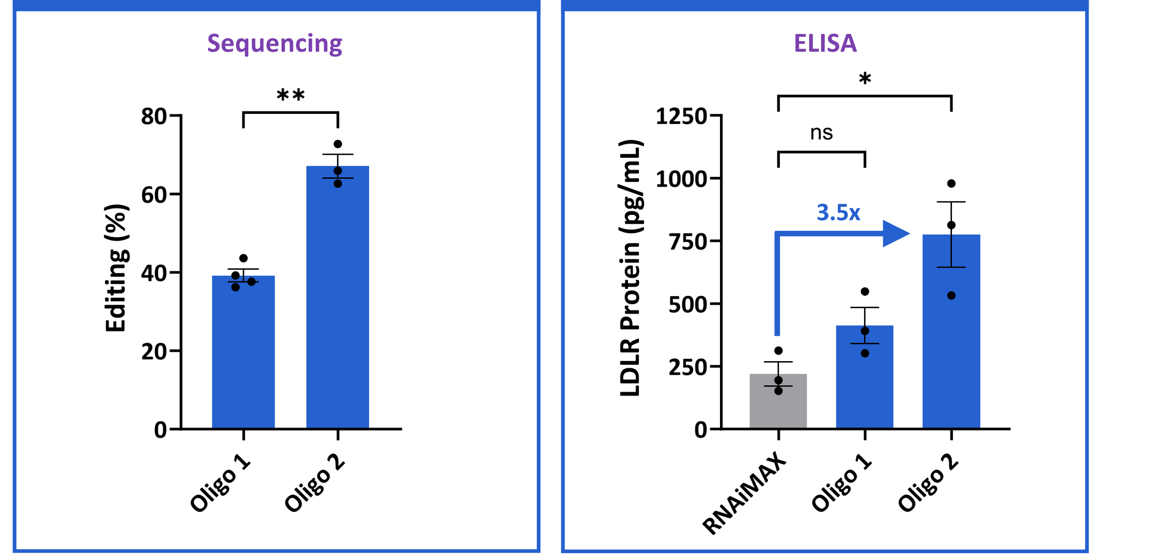
[1] Bjornsson et al., Circ Genom Precis Med, 2021; [2] UK Biobank analysis based on 12535 heterozygous R46L carriers; [3] Y124X and C678X carriers described in Cohen et al., New Engl J Med, 2006; [4] Lp(a) reduction varies due to strong differences in mean and medium values in noncarriers

Harnessing human genetics: RNA editing strategy designed to directly upregulate LDLR



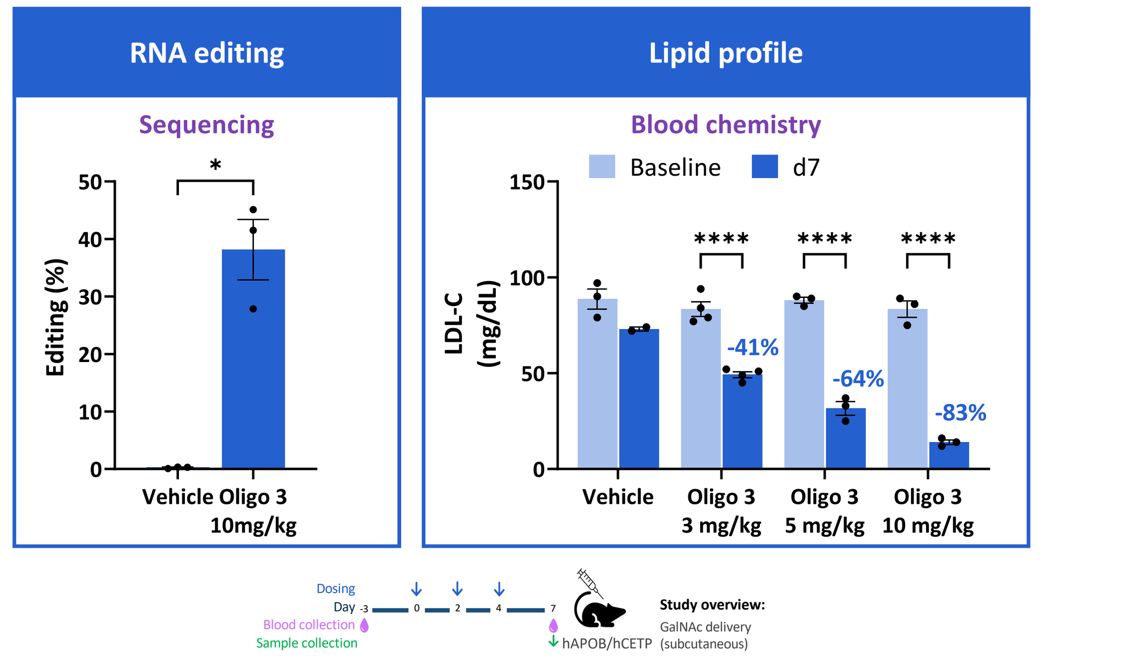
Direct LDLR 3'UTR RNA editing is differentiated and offers stronger LDL-C lowering than anti-PCSK9 and statins

3'UTR of LDLR: AIRNA's RNA editing oligos demonstrate >60% editing and 3.5-fold increase in hLDLR expression in primary human hepatocytes



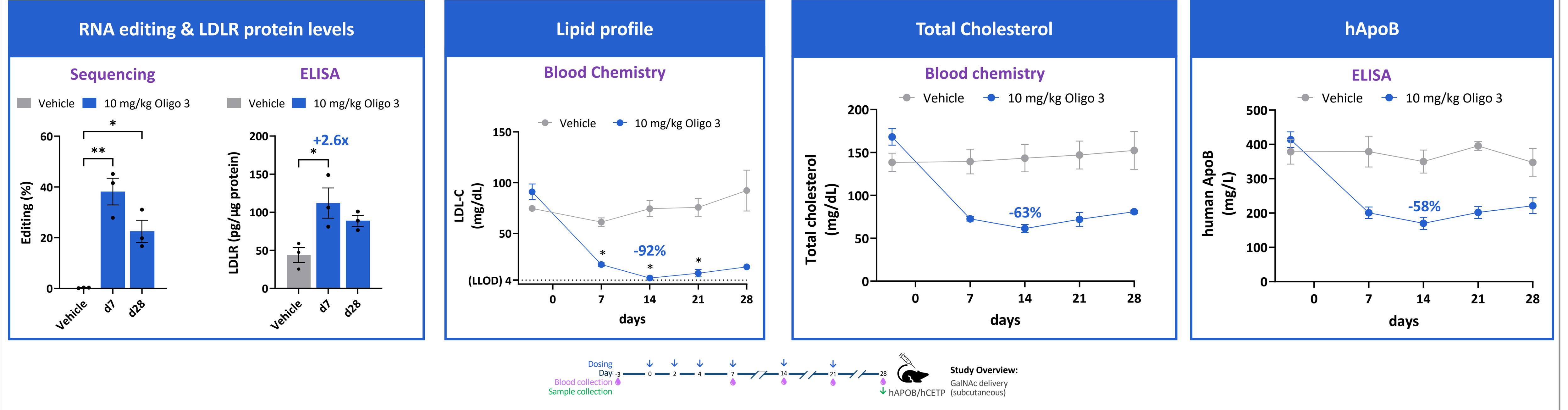
Primary human hepatocytes were transfected with 20 nM oligos, and samples were harvested 48 hours post-transfection. Left: RNA editing levels determined by NGS. Right: LDLR protein levels measured by ELISA in cell lysates. Data are presented as mean ± SEM. Statistics performed by two-tailed t-test (left) or one-way ANOVA with Tukey post-test (right). ns: nonsignificant,*p<0.05, **p<0.01.

3'UTR of LDLR: AIRNA's GalNAc-RNA editing oligo results in 38% editing and 83% LDL-C reduction in a short-term study in hAPOB/hCETP mouse model



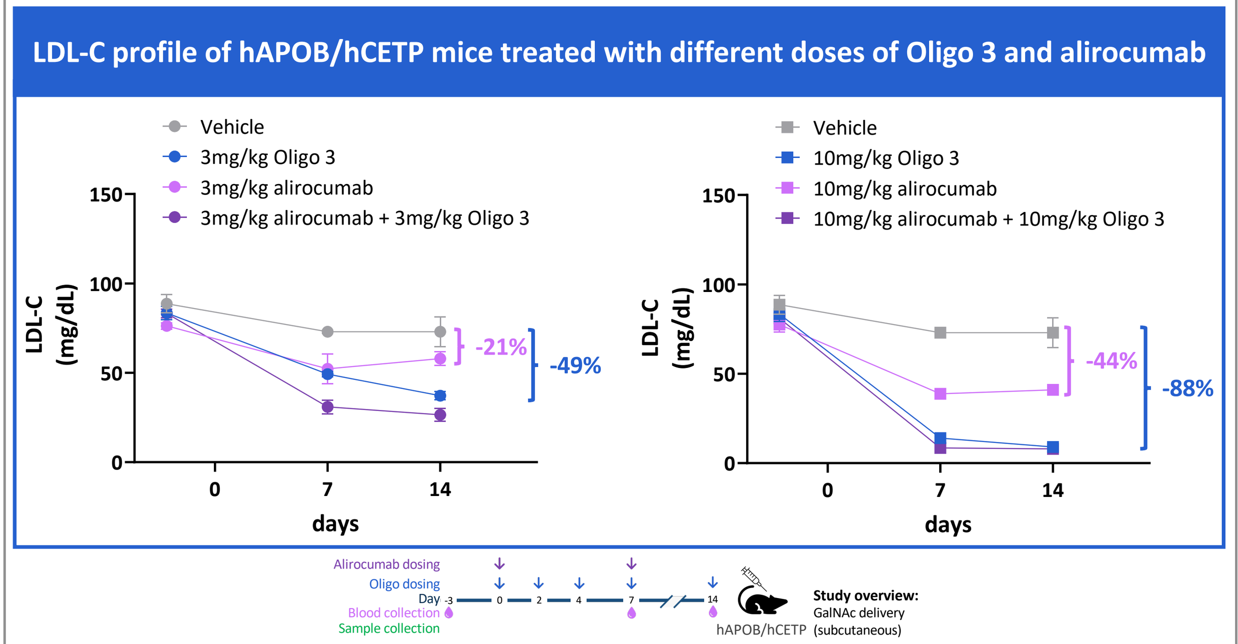
hAPOB/hCETP mice (n = 3-4/group) were treated subcutaneously with 3 doses of DPBS or 3, 5 or 10 mg/kg of GalNAc-oligo. Left: Day-7 liver RNA editing levels determined by NGS. Right: Serum LDL-C measured by automated analyzer. Data are present as mean ± SEM. Statistics performed by two-tailed t-test (left) or two-way ANOVA with Fischer's LSD post-test (right); *p<0.05 ****p<0.0001.

3'UTR of LDLR: AIRNA's GalNAc-RNA editing oligo demonstrated durable 92% LDL-C reduction and >50% decrease in cholesterol and ApoB in a one-month study in hAPOB/hCETP mouse model



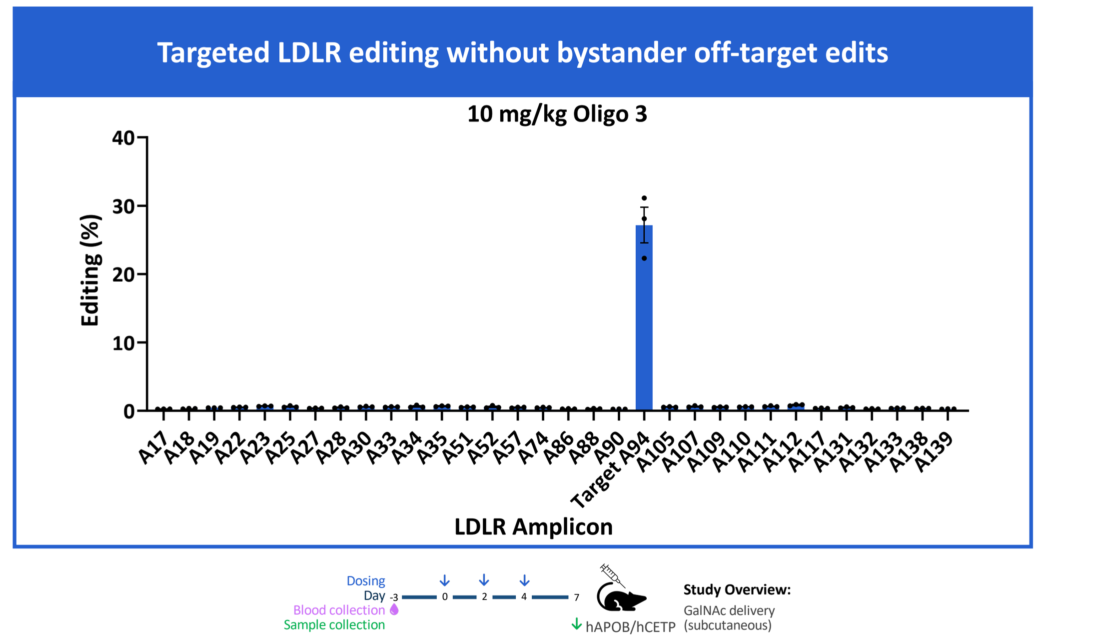
hAPOB/hCETP mice were treated subcutaneously with 3 loading doses of DPBS or 10 mg/kg of GalNAc-oligo, followed by 3 weekly maintenance doses (n = 3/group). Far left: Day-7 liver RNA editing levels determined by NGS and LDLR protein level measured by ELISA. Center left: Serum LDL-C levels over time measured by automated analyzer. The dashed line indicates the LLOQ of 4 mg/dL. Center right: Serum total cholesterol measured by automated analyzer. Far right: Serum human ApoB levels quantified by ELISA. Data are present as mean ± SEM. Statistics performed by one-way ANOVA with Tukey post-test (center left) or repeated measures two-way ANOVA with Sidak's post-test; *p<0.05, **p<0.01.

3'UTR of LDLR: AIRNA's GalNAc-RNA editing oligo demonstrated greater reduction in LDL-C as compared to alirocumab (anti-PCSK9 mAb)



hAPOB/hCETP mice (n = 3-4/group) were treated subcutaneously with GalNAc-oligo (3, 5, or 10 mg/kg on days 0, 2, 4, and 7) or alirocumab (3 or 10 mg/kg weekly on days 0 and 7). Combination groups received both treatments according to their respective dosing schedules. Serum LDL-C was quantified by automated analyzer. Data are present as mean ± SEM.

3'UTR of LDLR: No detectable bystander off-target editing with LDLR GalNAc-RNA editing molecule in vivo



hAPOB/hCETP mice were treated subcutaneously with 3 doses of 10 mg/kg GalNAc-oligo, and liver samples were collected on day 7. On-target LDLR editing and bystander editing of adenosines within the LDLR amplicon were evaluated by NGS. All surrounding adenosines exhibited RNA editing levels below 1%. Data are present as mean ± SEM from n = 3 mice.

APOB: Validated therapeutic cardiovascular target, but a pure knock-down approach (mipomersen) had dose-limiting hepatotoxicity

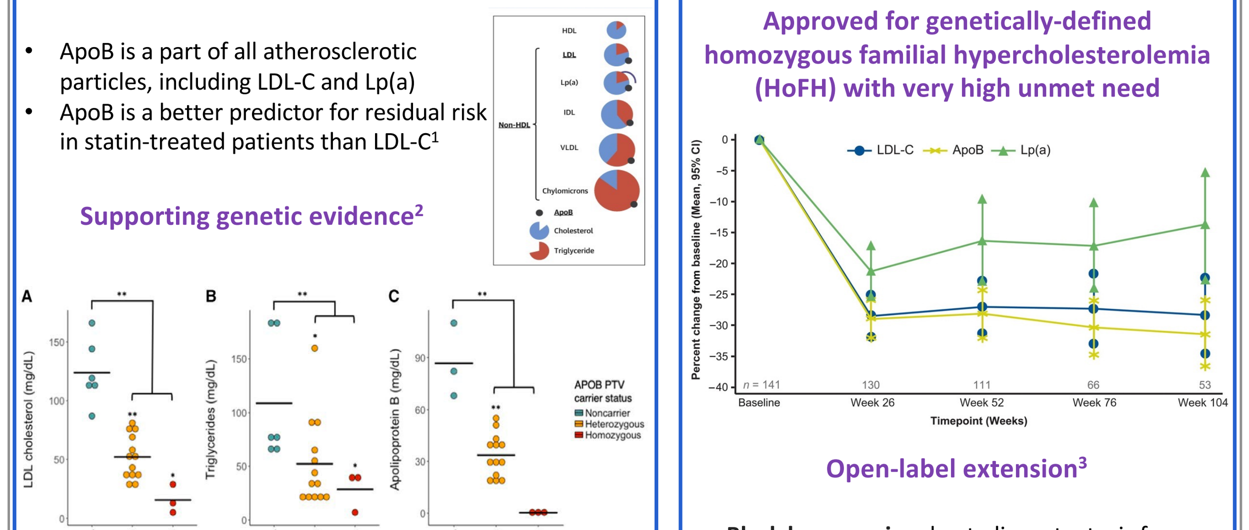
Human genetics across multiple populations show APOB as high-potential target

- ApoB is a part of all atherosclerotic particles, including LDL-C and Lp(a)
- ApoB is a better predictor for residual risk in statin-treated patients than LDL-C[1]

Supporting genetic evidence[2]

Mipomersen (IONIS) approved with positive efficacy, but black box warning

Approved for genetically-defined homozygous familial hypercholesterolemia (HoFH) with very high unmet need



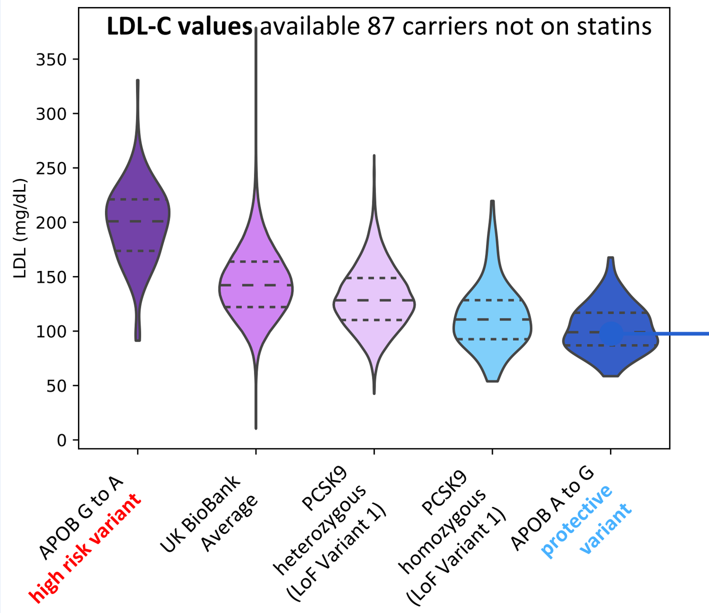
Open-label extension[3]

Black box warning due to liver steatosis from ApoB knock-down

[1] Johanneson et al. J Am Coll Cardiol, 2021; [2] Peloso et al., Circ Genom Precis Med, 2019; [3] Santos et al. Eur Heart J, 2015

APOB: >100 people in UK BioBank have a variant of APOB associated with lower LDL-C and an absence of hepatotoxicity

>100 people in the UK BioBank share a single A-to-G mutation (heterozygous)

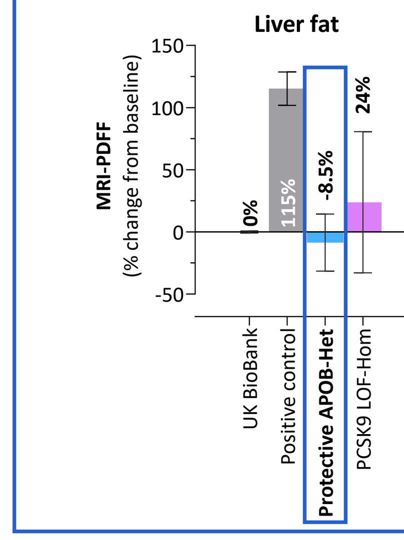


APOB: Average values from UKBB

	% change	P-value
LDL-C	-29.0%	<<1e-6
Total cholesterol	-21.8%	<<1e-6
APOB	-29.9%	<<1e-6
Triglycerides	-15.9%	0.08
Lp(a)	-21.4%	0.53
ALT	-4.6%	0.96
GGT	-14.4%	0.75

APOB: UK Biobank cohort with APOB protective variant showed no increase in liver fat or other liver-related health issues

No steatosis observed in protective APOB variant carriers



No-measurable steatosis observed in MRI

Liver health parameters of heterozygous carriers

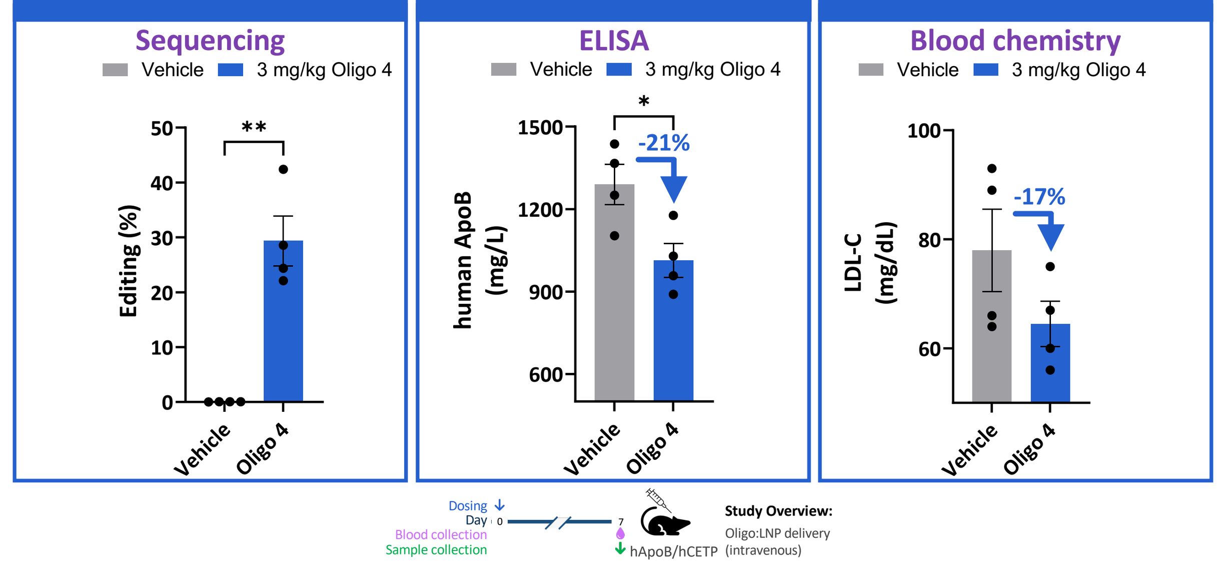
Liver enzymes:
- No measurable increase in ALT, AST, GGT

Steatosis:
- No increase in liver fat (MRI-PDFF)

Fibrosis:
- No increase in fibrosis markers (cT1 and Fib4)

UK BioBank n=28745, Positive Control N=170, Protective APOB N=10, PCSK9 LOF N=11, Het = Heterozygous, Hom = Homozygous

APOB: Introduction of protective variant by AIRNA's oligos demonstrate 29% editing leading to ~17% LDL-C reduction in hAPOB/hCETP mouse model



hAPOB/hCETP mice (n = 4/group) were treated intravenously (IV) with a single dose of 3 mg/kg oligo formulated in MC3-LNP. Liver and serum samples were collected on day 7. Left: RNA editing levels determined by NGS. Middle: Serum human ApoB levels quantified by ELISA Right: Serum LDL-C measured by automated analyzer. Data are expressed as mean ± SEM. Statistics performed by two-tailed t-test; *p<0.05.

Summary

- **LDLR**: RNA editing oligonucleotides targeting the 3'UTR achieved up to **70% editing in primary human hepatocytes**, a **3.5-fold increase in LDLR expression**, and a striking **92% reduction in LDL-C** in vivo in a human APOB/CETP mouse model. We also demonstrated an 88% LDL-C reduction compared to a 44% LDL-C reduction with alirocumab, an approved anti-PCSK9 antibody. Combination of alirocumab and our LDLR RNA editing oligo demonstrated improved LDL-C reduction at lower doses.
- **APOB**: a human genetics-identified protective variant associated with lower LDL-C was introduced in vivo, achieving **30% editing**, a **21% decrease in APOB**, and a **17% reduction in LDL-C**— without the hepatotoxicity seen with prior knockdown based oligonucleotide approaches.
- These results demonstrate AIRNA's **RNA editing platform's unique ability to unlock previously undruggable targets**, bringing the benefits of protective variants to patients and advancing RNA editing therapeutics with **transformational potential**.

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