UNIVERSITY of MARYLAND
SCHOOL OF MEDICINE

The genetic architecture of LDL cholesterol levels in a founder population

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Disclosures

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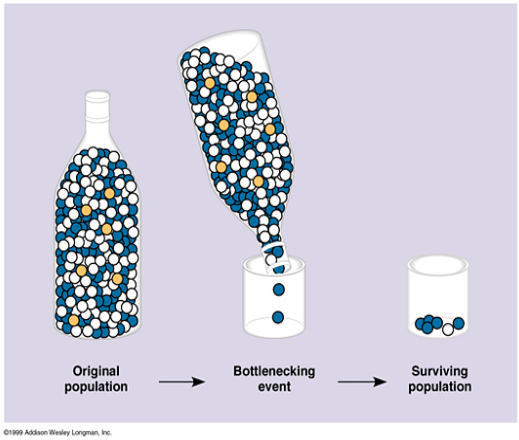
Outline

- Origins of the Amish and the Amish lifestyle
- The health of the Amish
- Founder populations as a source for genetic discovery (LDL-C)

The Lancaster County Old Order Amish as a Genetic Isolate

~ 35,000 present day Lancaster County Amish

~ 400-500 'founding' immigrants



Traditional/unique culture

Unique genetics

Less genetic diversity

Some core characteristics of the Old Order Amish

Amish Society

- Adult baptism (anabaptist)
- Church, community, family (high social cohesiveness)
- Education through 8th grade
- Technological conservatism
- Excellent genealogical records

The Amish Lifestyle

- High levels of physical activity
- Low smoking and alcohol consumption
- Home grown and prepared foods
- Limited access to health care systems

THE AMISH GENETICS PROGRAM

University of Maryland School of Medicine

Amish of Lancaster, PA

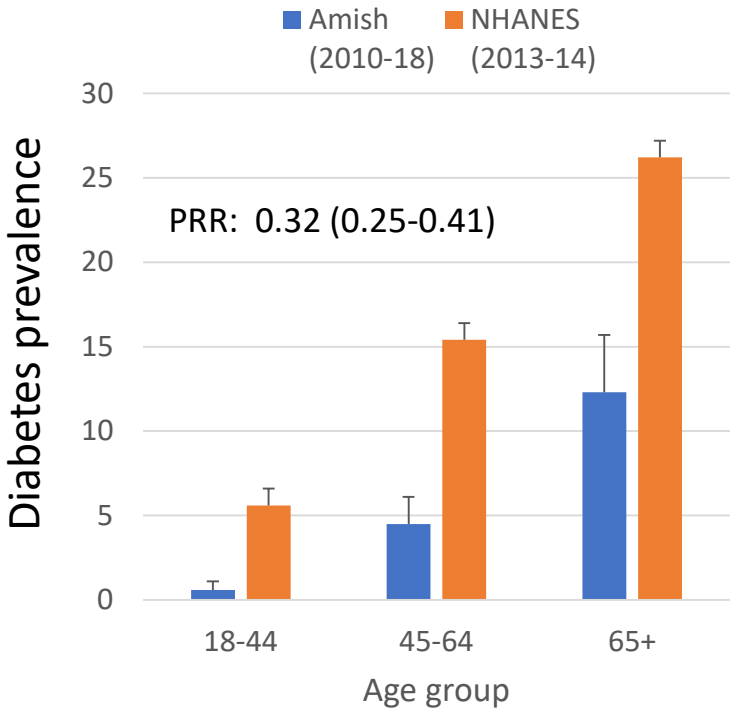
- Community of 38,000 Amish
- > 7,000 Amish enrolled with whole exome sequencing & blood biobanking

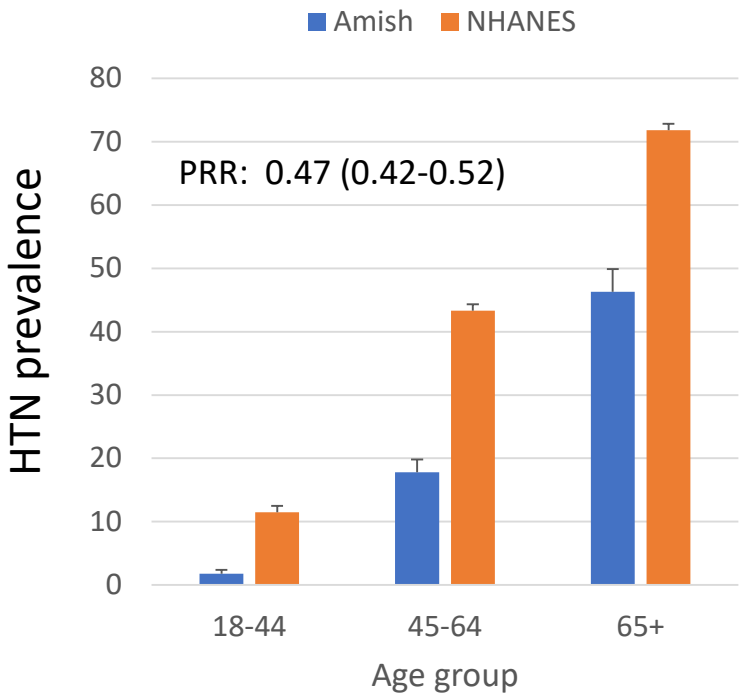
Unique Genetics Epidemiology Resource

- Enrichment of causal mutations that provide insights into human biology
- OASIS, state-of-the-art data mining

The health of the Amish

Low prevalence of **diabetes and hypertension** in the Amish



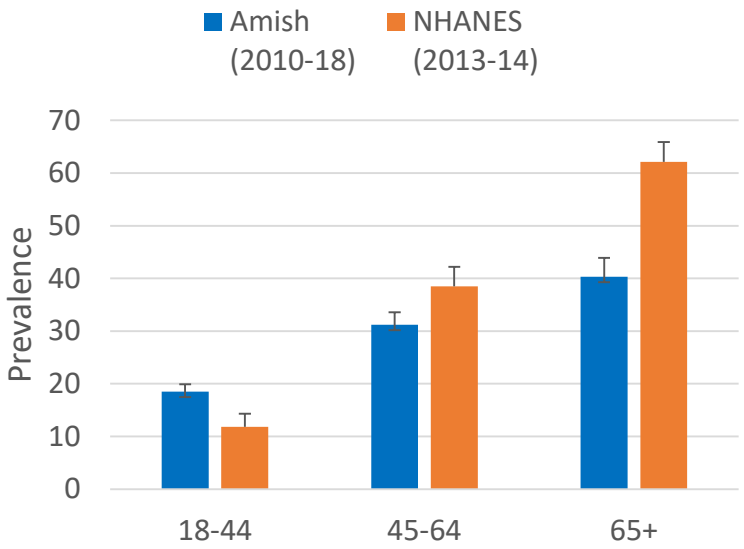


Diabetes: FBG ≥ 126 or HbA1c ≥ 6.5 or medication use

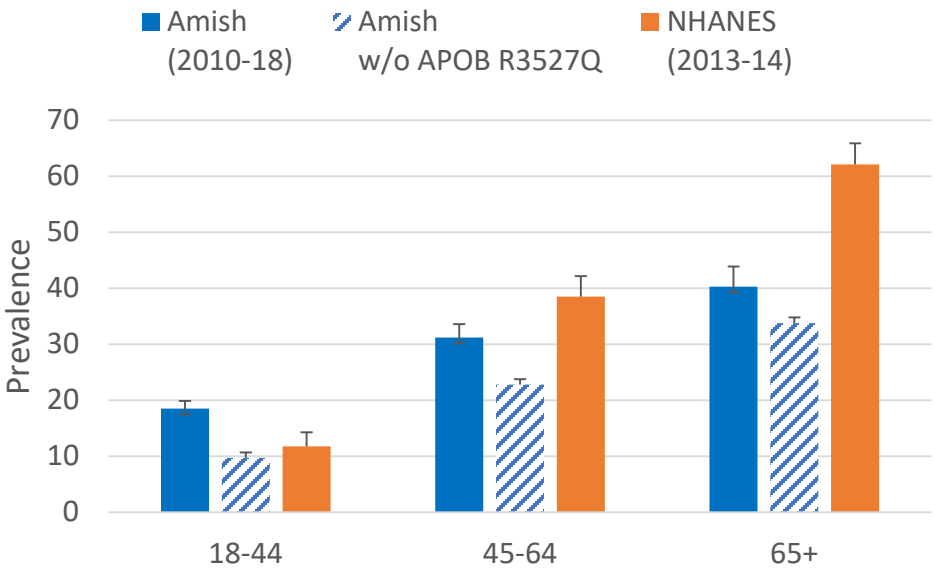
HTN: SBP ≥ 140 or DBP ≥ 90 or medication use

Unpublished

Low prevalence of **high LDL-cholesterol** in the Amish



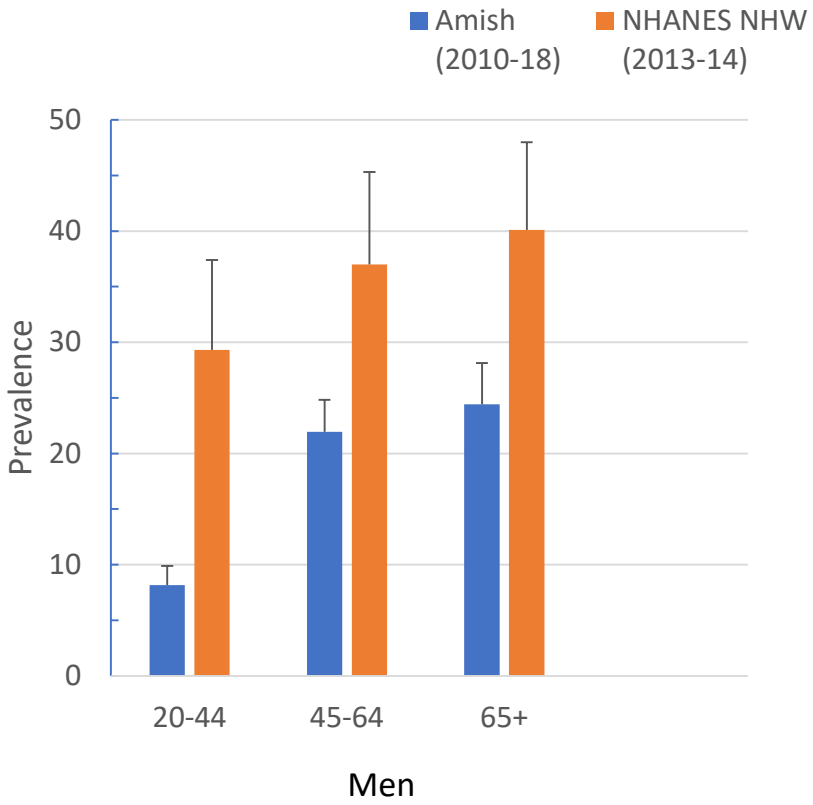
PRR: 0.86 (0.79-0.95)



PRR: 0.61 (0.55-0.68) removing APOB R3527Q carriers

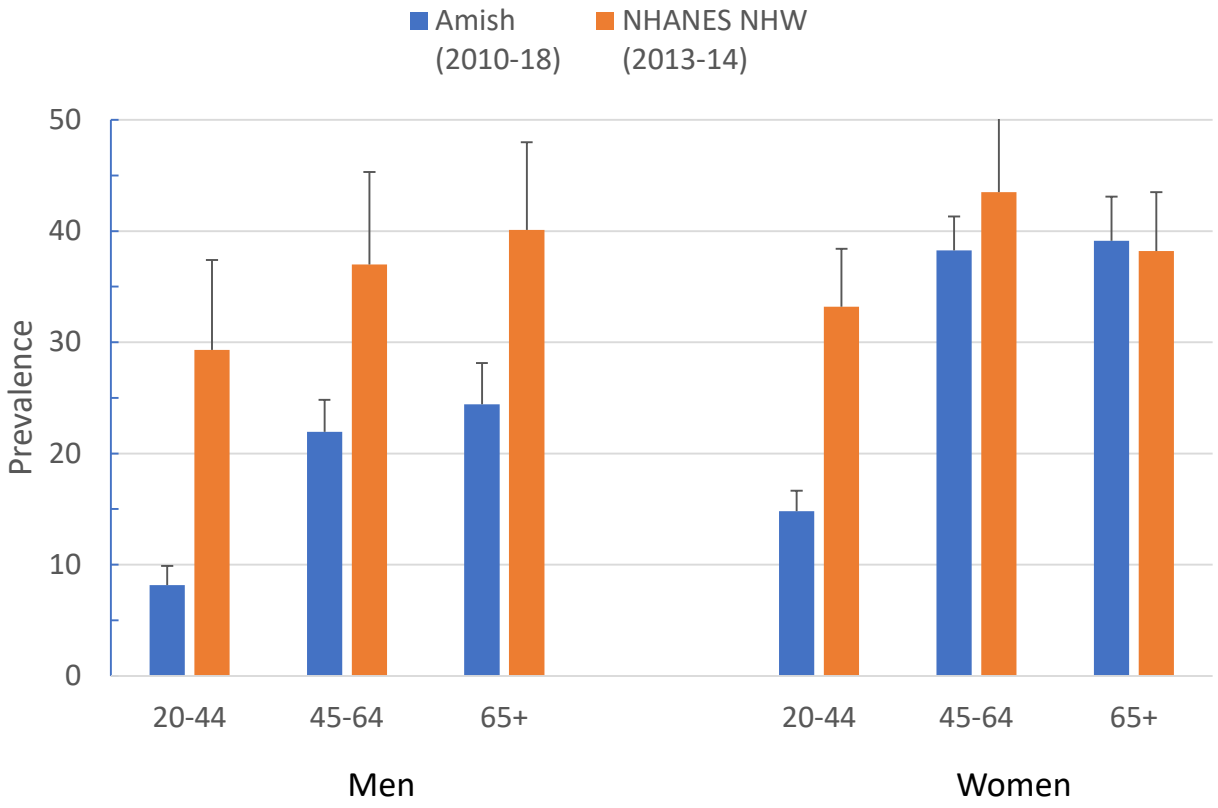
high LDL cholesterol: ≥ 160 mg/dl or meds

Low prevalence of **obesity i**n Amish men (but not women)



Obesity: BMI ≥ 30 kg/m^2

Low prevalence of **obesity i**n Amish men (but not women)



Obesity: BMI ≥ 30 kg/m^2

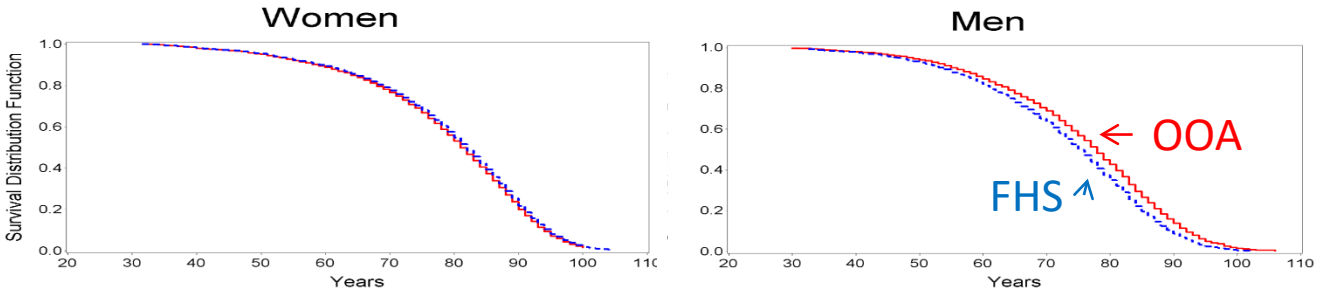
Cardiovascular health in Amish and non-Amish Caucasians

Compared to non-Amish Caucasians, Amish have:

- Less diabetes, hypertension, high cholesterol
- Lower BMI (men)
- Less Rx medication use and access to medical care:
- Less smoking: (20% of Amish men)
- Higher physical activity/lower TGs

Hsueh et al, Diab Care 2000; 23:595;
Bielak et al., Atherosclerosis 2008; 196:888;
Mitchell et al., Am Heart J 2008; 155:823.

Lifespan in Amish vs than Framingham Heart Study: (cohorts born 1886 - 1922)



Mitchell et al., PLoS One 2012; 7:e51560.

A Common Variant

Effect size:
~ 0.4 kg/m²

OPEN ACCESS Freely available online

PLoS MEDICINE

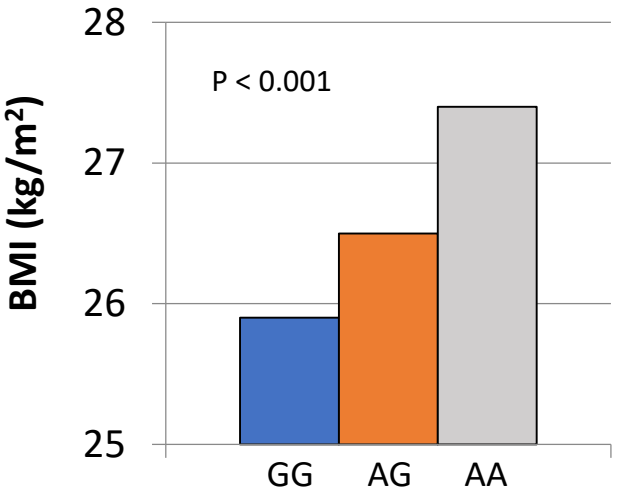
ıt the
BMI?

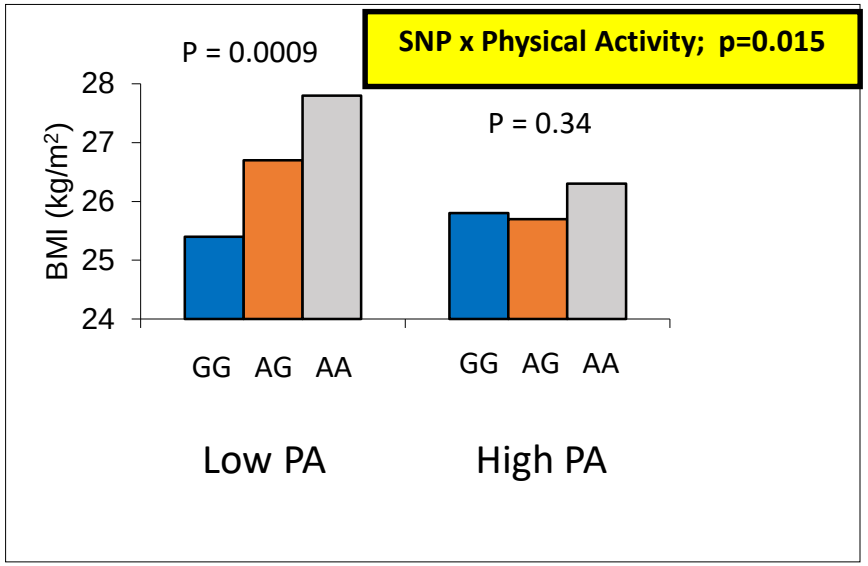
Physical Activity Attenuates the Influence of *FTO* Variants on Obesity Risk: A Meta-Analysis of 218,166 Adults and 19,268 Children

2011

Tuomas O. Kilpeläinen[1], Lu Qi[2]*, Soren Brage[1], Stephen J. Sharp[1], Emily Sonestedt[3], Ellen Demerath[4],

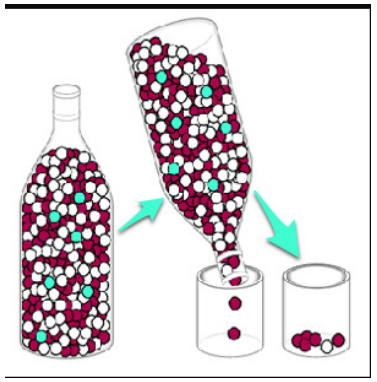
Association of *FTO* rs1861868 with BMI in the Amish



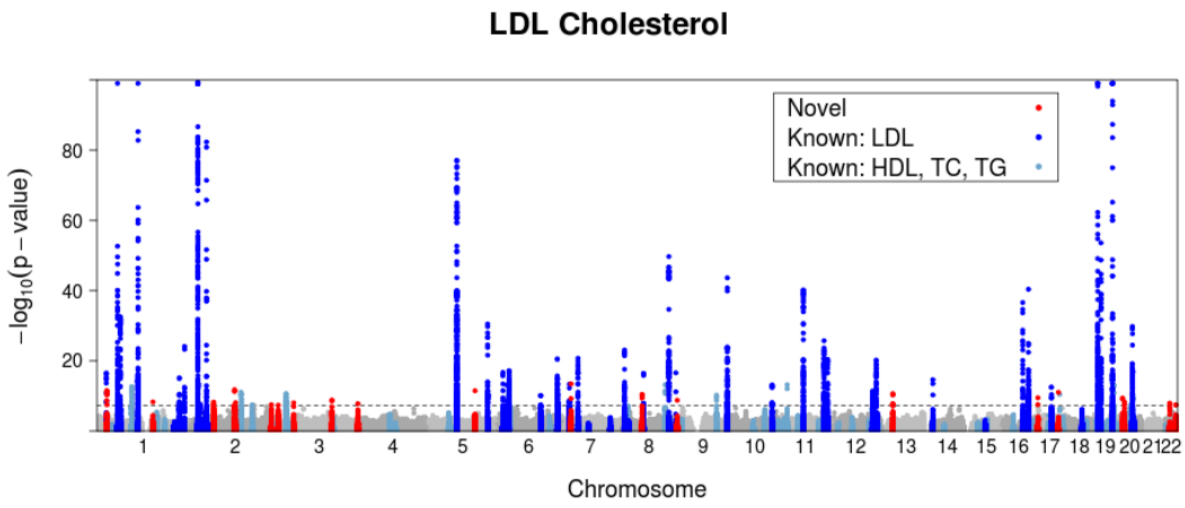


Rampersaud et al., Arch Intern Med 168:1791, 2008

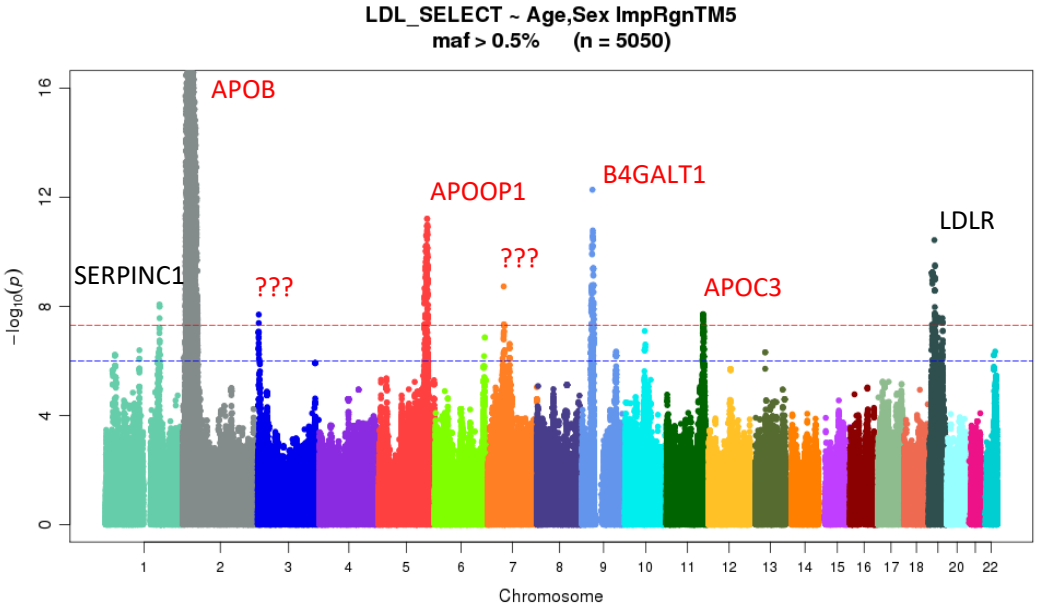
The strength of founder populations for genetic discovery



- Enrichment of high penetrance, rare variants
- Easier to find – entered population on single haplotype
- Many in coding parts of genes
- Call-back studies to find more copies and deeper phenotyping

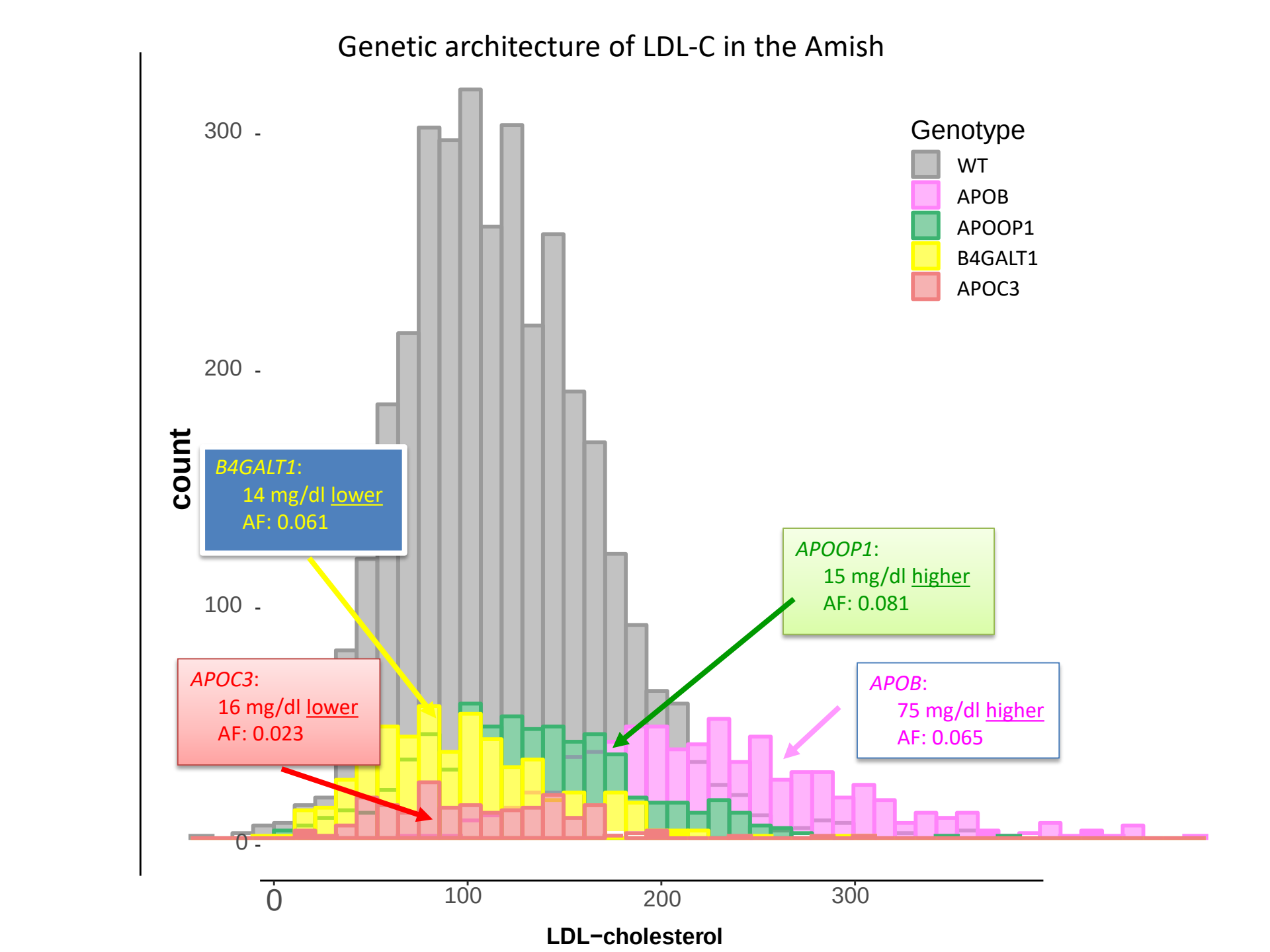


Global Lipids Consortium
N = 188,500
Willer et al., Nat Genet, 2013



Amish
N ~ 5,000

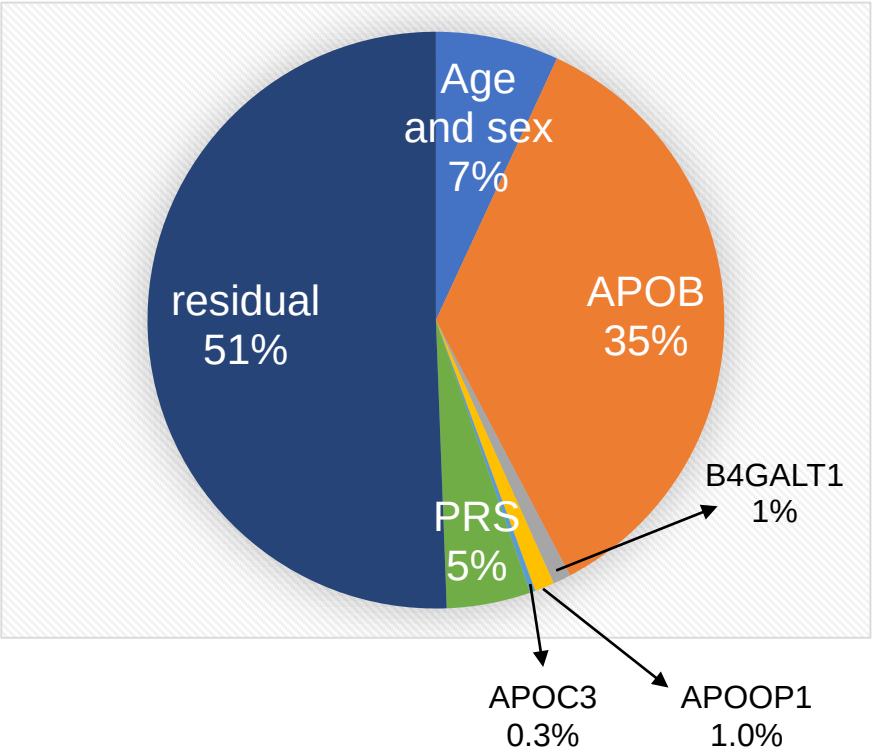
Red = Highly enriched in Amish


Genetic architecture of LDL-C in the Amish
Genotype
WT
APOB
APOOP1
B4GALT1
APOC3
count
300
200
100
0
B4GALT1:
14 mg/dl lower
AF: 0.061
APOOP1:
15 mg/dl higher
AF: 0.081
APOC3:
16 mg/dl lower
AF: 0.023
APOB:
75 mg/dl higher
AF: 0.065
0
100
200
300
LDL−cholesterol

Factors contributing to variation in LDL-C in the Amish

Known genetic variants and age account for ~50% of variation in LDL-C

Variable	MAF	Beta (mg/dl)	Partial r2
Age (10 yr)		8.4	6.8%
Sex			0.1%
APOB	0.067	77.7	35.4%
APOOP1	0.076	10.1	1.0%
B4GALT1	0.061	-14.5	1.1%
APOC3	0.024	-15.0	0.3%
LDL PRS (1 SD unit)		10.1	4.7%



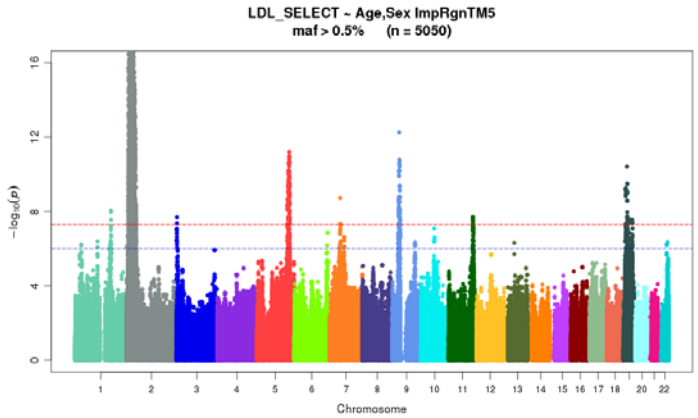
(n ~ 6,000)

ORIGINAL INVESTIGATION

Familial Defective Apolipoprotein B-100 and Increased Low-Density Lipoprotein Cholesterol and Coronary Artery Calcification in the Old Order Amish

Shen et al., Arch Intern Med. 2010;170(20):1850-1855

APOB



Associated with brain health?

Familial Hypercholesterolemia and Type 2 Diabetes in the Old Order Amish

Xu et al., Diabetes 2017;66:2054–2058

JCEM ONLINE

Advances in Genetics—Endocrine Research

Decreased Bone Mineral Density in Subjects Carrying Familial Defective Apolipoprotein B-100

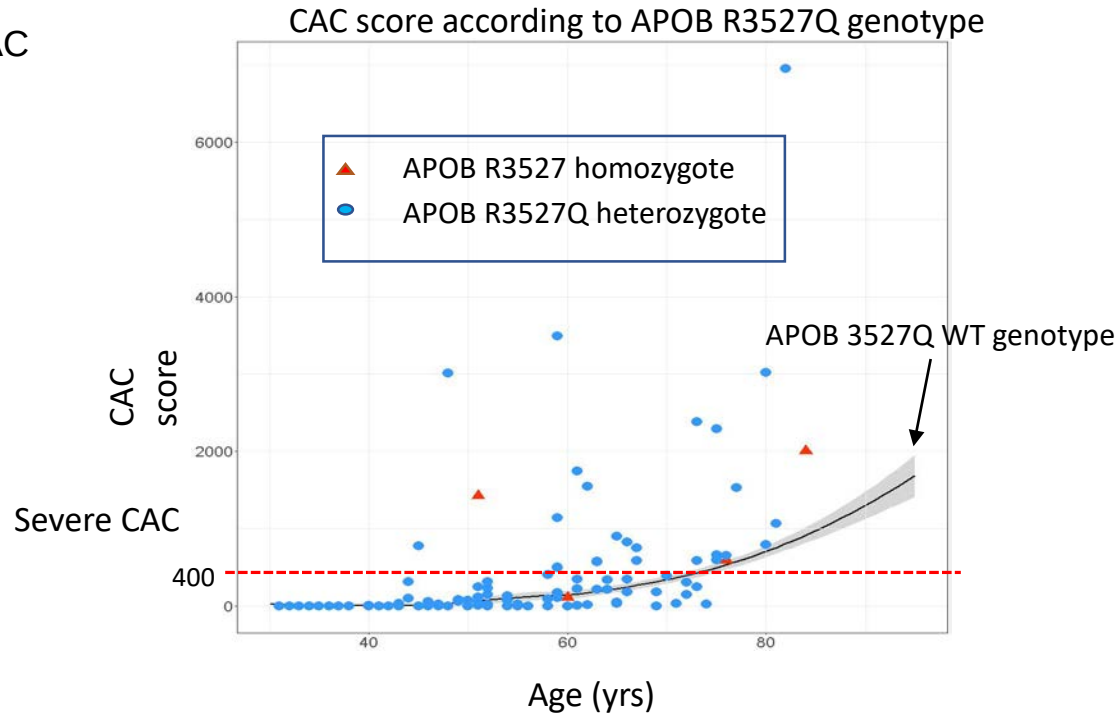
Yerges-Armstrong et al J Clin Endocrinol Metab, December 2013, 98(12):E1999–E2005

APOB R3527Q and Atherosclerosis

Many individuals with FH do not get severe CAC even age 60 (including some homozygotes

Each APOB R3527Q allele associated with:

- 75 mg/dl increase in LDL-C
- 9-fold increase in odds of severe CAC



Circulation

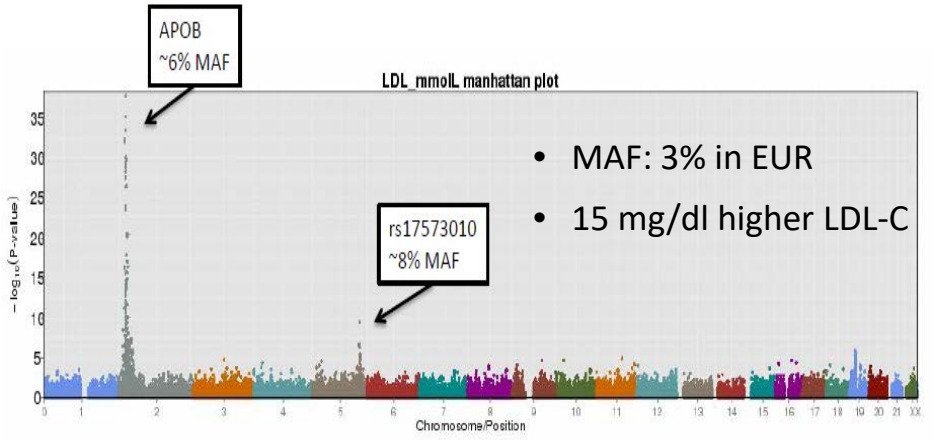
ORIGINAL RESEARCH ARTICLE

An *APOO* Pseudogene on Chromosome 5q Is Associated With Low-Density Lipoprotein Cholesterol Levels

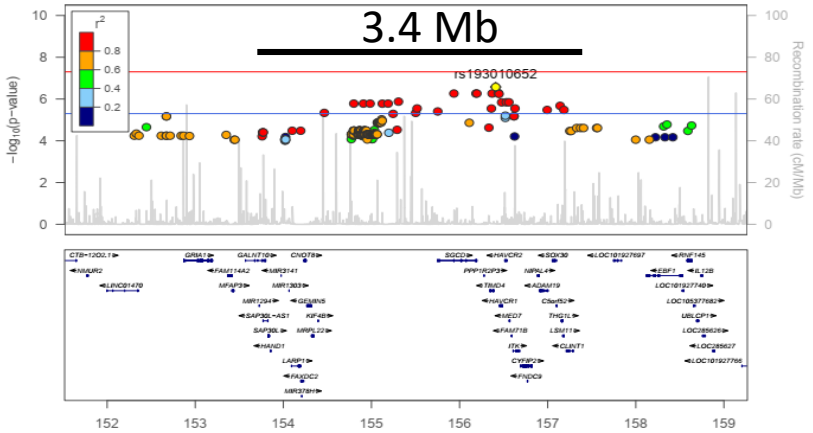
Montasser et al., Circulation, 2018

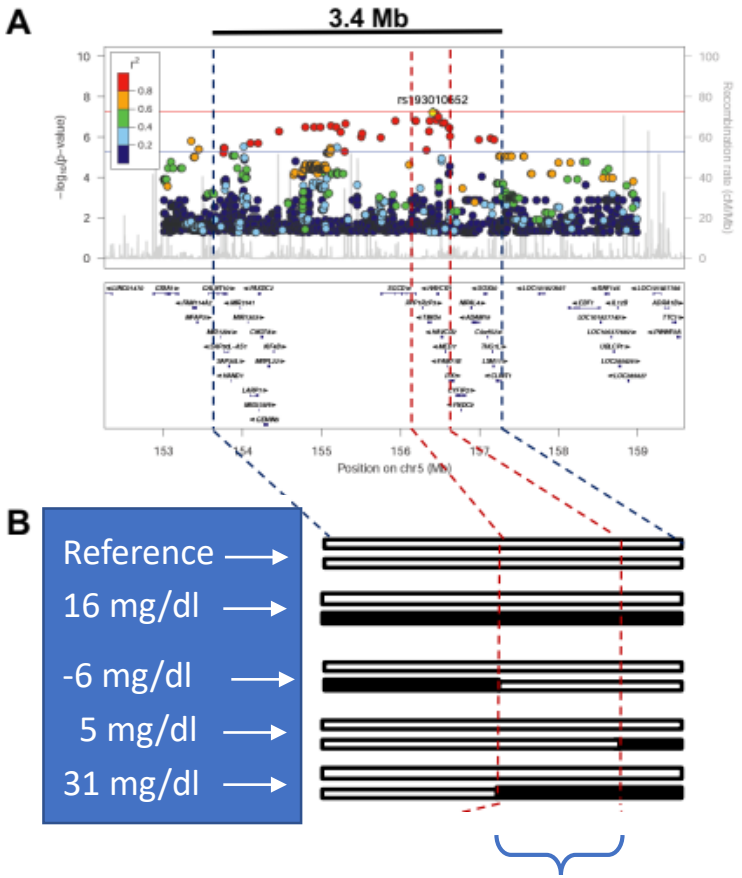
Discovery of the APOOP1 locus and LDL-C in the Amish

Recombination mapping narrows the region



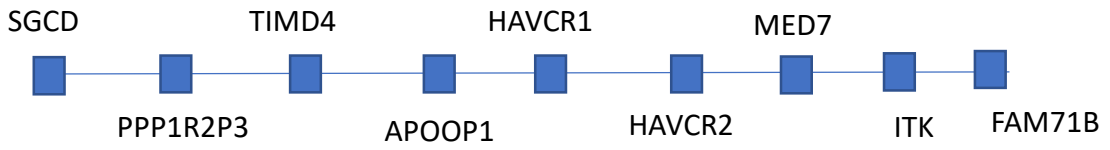
- MAF: 3% in EUR
- 15 mg/dl higher LDL-C

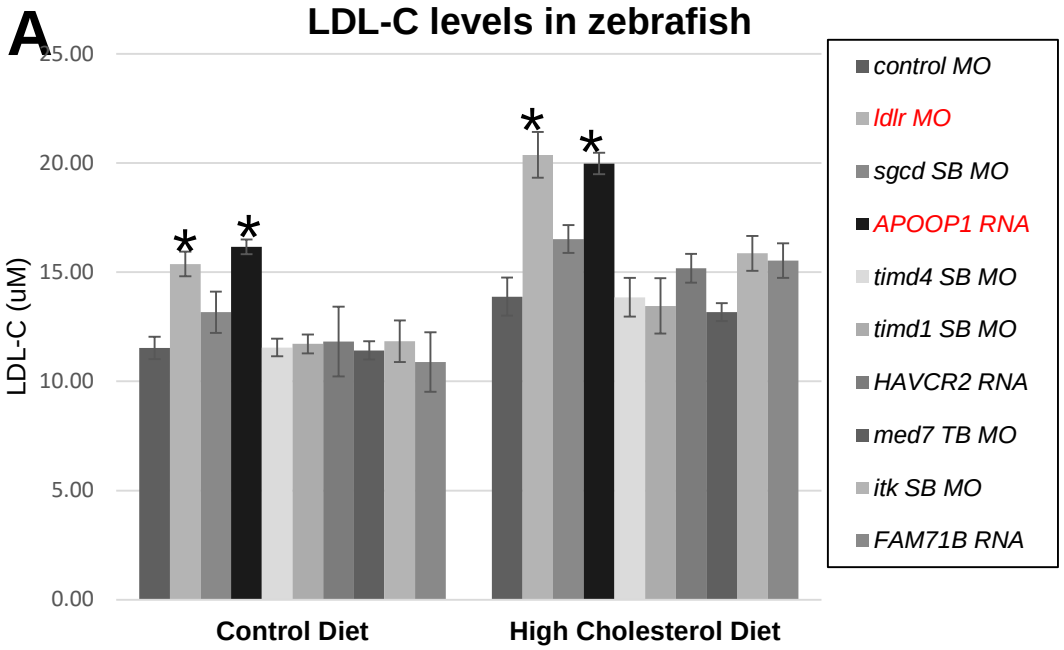


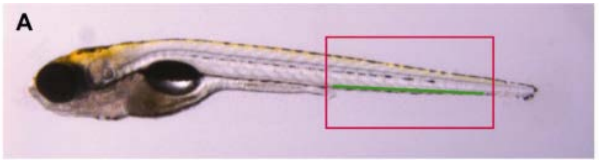


Associated haplotype:

- 442 kb
- 7 genes, 2 pseudogenes

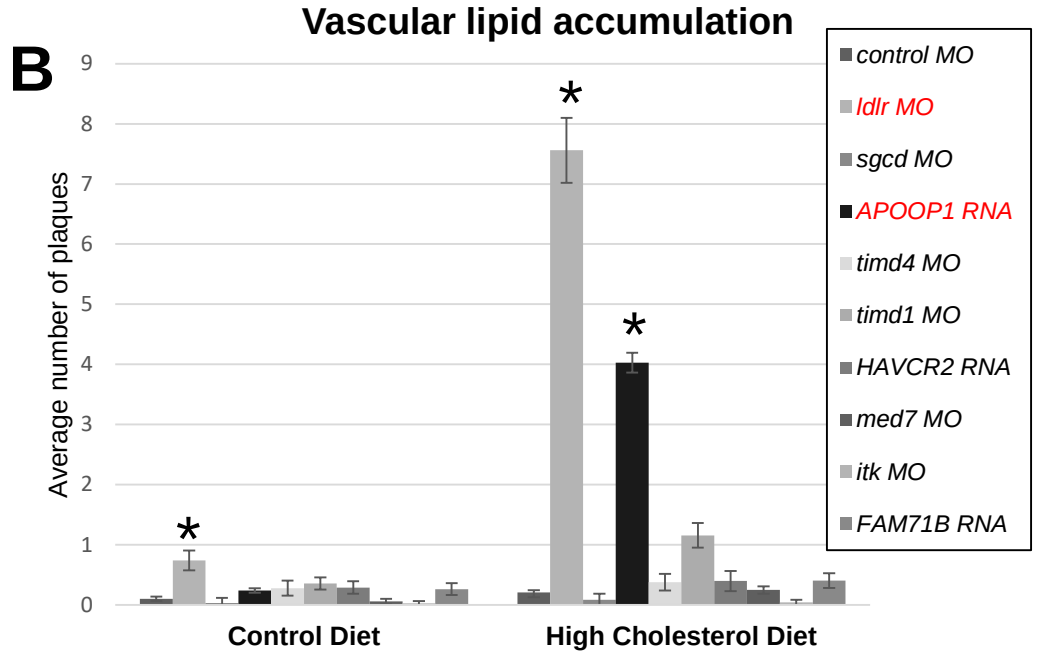






Higher LDL-C in:

- *ldlr* MO
- Overexpressed *APOOP1*



More vascular plaques in:

- *ldlr* MO
- Overexpressed *APOOP1* (high cholesterol diet only)

Montasser et al., Circ, 2018

How does *APOOP1* affect LDL-C?
(ongoing work)

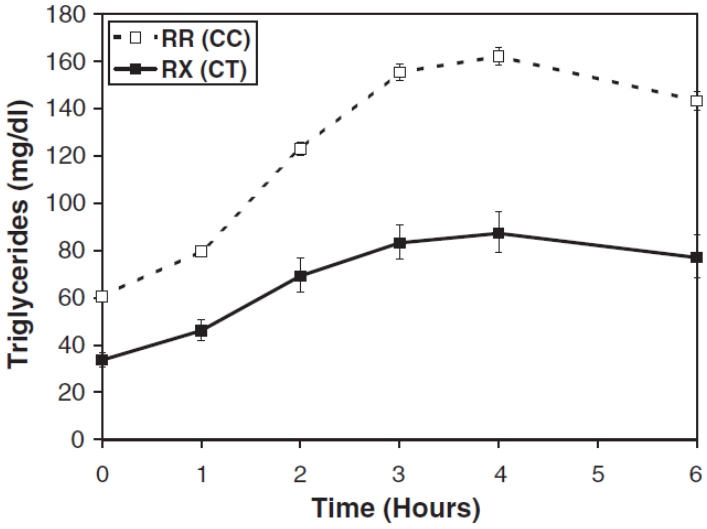
- *APOOP1* is a tissue-specific, transcribed pseudogene
- *APOOP1* transcript contains binding sites for 3 related microRNAs (miR429, 200b, 200c) that regulate multiple genes involved in cholesterol metabolism.
- We hypothesize: Amish-specific haplotype drives expression of *APOOP1*

  → which competes for availability of microRNAs

  → which disturbs the expression of other microRNA target transcripts, many of which are involved in cholesterol metabolism (e.g., *SORT1*, *VLDLR*, *ANGPTL3*, etc.)
- Can deletion of the miRNA binding site in *APOOP1* abolish its biological effect?

A Null Mutation in Human *APOC3* Confers a Favorable Plasma Lipid Profile and Apparent Cardioprotection

Pollin et al., 12 DECEMBER 2008 VOL 322 SCIENCE

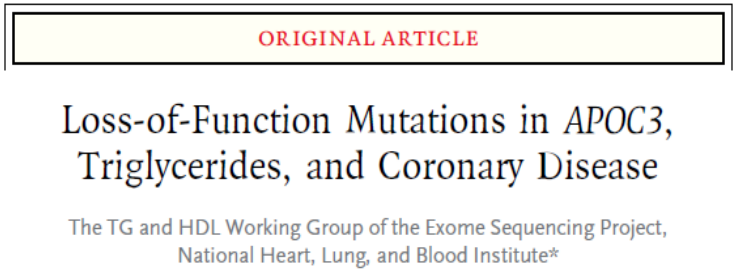


- Associated with fasting TG and TG excursion following oral fat tolerance test
- APOC3 inhibits lipoprotein lipase and hepatic lipase, which break down TG-rich proteins
- Null mutation impairs APOC3 , allowing faster TG breakdown
- Cardioprotective ?

APOC3 Mutations

- Identified in other isolate populations
  - Greece (Tachmazidou, Nat Commun 2013)
  - Pimas (Hsueh et al., Circ Genet 2017)
  - Pakistan: APOC3 19X homozygotes (Saleheen et al. Nature 2017)
- Reduced risk of CHD (NEJM 2014)
- Druggable target!

NEJM 2014


ORIGINAL ARTICLE

Loss-of-Function Mutations in *APOC3*, Triglycerides, and Coronary Disease

The TG and HDL Working Group of the Exome Sequencing Project, National Heart, Lung, and Blood Institute*

Summary and Conclusions

- Amish, like many founder populations, are unique in terms of their lifestyle and genetics
- More 'traditional' lifestyle
  - Less diabetes, hypertension, hypercholesterolemia, and obesity
  - Protective influence of physical activity (social support?)
- Founder populations enriched for rare variants with high penetrance (e.g., LDL cholesterol)
  - Opportunities for gene discovery: novel variants/new genes

University of Maryland Amish Investigators

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Patrick McArdle
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