The genetic architecture of LDL cholesterol levels in a founder population

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Disclosures

Some of the data presented funded in part by Regeneron Pharmaceuticals
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

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

Amish Society

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

The Amish Lifestyle
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 prevalence

Diabetes: \( \text{FBG} \geq 126 \text{ or } \text{HbA1c} \geq 6.5 \text{ or } \text{medication use} \)

HTN prevalence

HTN: \( \text{SBP} \geq 140 \text{ or } \text{DBP} \geq 90 \text{ or } \text{medication use} \)

Unpublished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

![Graph showing prevalence of high LDL-cholesterol in Amish vs. NHANES.]

**PRR:** 0.86 (0.79-0.95)

High LDL cholesterol: ≥ 160 mg/dl or meds

![Graph showing prevalence of high LDL-cholesterol in Amish w/o APOB R3527Q vs. NHANES.]

**PRR:** 0.61 (0.55-0.68) removing APOB R3527Q carriers
Low prevalence of **obesity** in Amish men (but not women)

![Bar chart showing prevalence of obesity in Amish men and NHANES NHW compared to different age groups.](chart)

**Obesity:** BMI ≥ 30 kg/m²
Low prevalence of **obesity** in Amish men (but not women)

![Bar graph showing prevalence of obesity by age group and gender for Amish and NHANES NHW populations.](image)

**Obesity**: BMI ≥ 30 kg/m²
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

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

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

Tuomas O. Kilpeläinen¹, Lu Qi²*, Soren Brage¹, Stephen J. Sharp¹, Emily Sonestedt³, Ellen Demerath⁴.

Association of FTO rs1861868 with BMI in the Amish

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

Effect size: ~ 0.4 kg/m².
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

- **APOB**:
  - 75 mg/dl higher
  - AF: 0.065

- **APOOP1**:
  - 15 mg/dl higher
  - AF: 0.081

- **B4GALT1**:
  - 14 mg/dl lower
  - AF: 0.061

- **APOC3**:
  - 16 mg/dl lower
  - AF: 0.023

- **APOB**:
  - 75 mg/dl higher
  - AF: 0.065
Factors contributing to variation in LDL-C in the Amish

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

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

(n ~ 6,000)
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

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

Each APOB R3527Q allele associated with:

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

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

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

Associated haplotype:
- 442 kb
- 7 genes, 2 pseudogenes

Recombination mapping narrows the region
**Higher LDL-C in:**
- \textit{Idlr} MO
- Overexpressed \textit{APOOP1}

**More vascular plaques in:**
- \textit{Idlr} MO
- Overexpressed \textit{APOOP1} (high cholesterol diet only)

\textit{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 \textit{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
- \textit{APOC3} inhibits lipoprotein lipase and hepatic lipase, which break down TG-rich proteins
- Null mutation impairs \textit{APOC3}, allowing faster TG breakdown
- Cardioprotective?
APOC3 Mutations

• Identified in other isolate populations
  o Greece (Tachmazidou, Nat Commun 2013)
  o Pimas (Hsueh et al., Circ Genet 2017)
  o Pakistan: APOC3 19X homozygotes (Saleheen et al. Nature 2017)

• Reduced risk of CHD (NEJM 2014)

• Druggable target!
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