Lecture Outline

- Obesity and related complications
- The University of Maryland Amish Research Clinic
- Bench to Bedside Lessons
  - Triglycerides
  - LDL-cholesterol
  - New monogenic diabetes
  - (Sitosterolemia)
  - (Anti-platelet pgx)
David in the 18th Century:

21st Century:

McDonald's
Burger King
KFC
Starbucks
Health Consequences of Obesity

- Cardiovascular disease
- Type 2 diabetes
- Hypertension
- Dyslipidemia
- Stroke
- Sleep apnea
- Degenerative joint disease
- Some types of cancer
- Gallstones
- Gynecologic irregularities
- Psychosocial
Genetics of Obesity-Related Medical Problems

A complex interaction between genetic susceptibility, the environment, and time

How do variants in genes interact with the environment to influence risk of disease?
The Old Order Amish:
Ongoing Genetic Studies of Complex Phenotypes
at the University of Maryland Amish Research Clinic
N > 6000 (50% of adult population)

- Diabetes/Obesity
- Osteoporosis
- OI /OPPG
- Longevity
- CVD
  - Coronary artery calcification
  - Hypertension/salt sensitivity
  - Hyperlipidemia
- Thyroid Disease
- Celiac Disease
- Breast density/cancer
- Pharmacogenomics (CVD, HTN, T2D)
- Nutrigenomics
- Gut microbiome/Metabolic syndrome
- Amish Wellness Program
- Mental Health
- Pain
David’s Lipid Profile

- Total cholesterol: 260 mg/dl
- LDL-C: 194 mg/dl
- HDL-C: 25 mg/dl
- TG: 300 mg/dl
TG Excursion During High Fat Load is Highly Variable Among HAPI Heart Study Subjects (n=868)
GWAS for Fasting TG and TG Excursion during a High Fat Meal

Fasting TG

Incremental TG AUC

Pollin (2008) Science
ApoC-III and Lipoprotein Metabolism

Adapted from Ooi et al, Clinical Science 114:611-624 (2008)
APOP3 R19X
(Carrier Frequency: 5%)

Pollin (2008) Science
Higher Prevalence of Nonagenarians Among APOC3 R19X Carriers

Implications:
- Atkins-like diet "healthy" for 5% of the Amish population?
- APOC3 inhibitors/antagonists will be effective and safe TG lowering agents and may even increase lifespan (reverse pharmacogenomics)

All descendants of MRCA (38/409 died ≥ 90 years old)  Inferred R19X descendants of MRCA (10/44 died ≥ 90 years old)

P = 0.01

Pollin, submitted
Antisense Oligonucleotide Inhibition of Apolipoprotein C-III Reduces Plasma Triglycerides in Rodents, Nonhuman Primates, and Humans


_Circ Res._ published online March 29, 2013;
GWAS of LDL-C in the Amish HAPI Heart Study

• Sequencing of *APOB* revealed an R3500Q missense mutation
  – 12% of Amish carry at least one copy of the abnormal allele (<0.2% in non-Amish)
  – R3500Q *APOB* has decreased affinity for the LDL receptor
Elevated LDL-C in APOB R3500Q Carriers Constant Across Age Groups

Mean Levels in mg/dL

20-30 30-40 40-50 50-60 60-70 >70

R3500Q carrier
R3500Q non-carrier

59mg/dL

CAC More Common and More Extensive (CAC score ≥ 400) in APOB R3500Q Carriers

R3500Q Carriers Have Increased Risk for CAC and Extensive CAC

<table>
<thead>
<tr>
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<th>OR (95% CI) Model 1</th>
<th>OR (95% CI) Model 1 + LDL-C</th>
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<tbody>
<tr>
<td>CAC</td>
<td>4.65 (2.73-7.90)***</td>
<td>3.26 (1.79-5.94)***</td>
</tr>
<tr>
<td>Extensive CAC</td>
<td>8.54 (2.79-26.16)**</td>
<td>4.75 (1.33-16.92)*</td>
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</tbody>
</table>

Model 1: covariates include age, sex, BMI, smoking, SBP, DBP, HDL-C, lipid-lowering medication, and sibships
CAC: CAC≥1 vs CAC<1; Extensive CAC: CAC≥400 vs CAC <1

***P< Implication: APOB genotype (in addition to lipid profile) might identify patients earlier and who should be managed more aggressively with lipid lowering agents

The Amish Wellness Study

- Medical history
- Vital Signs
- CMP, CBC, Lipid panel, HgbA1c, TSH, celiac screen
- EKG
- Carotid and abdominal U/S
- Heel U/S
- APOB genotype
David is Obese and Has Diabetes

- BMI: 38 kg/m²
- Glucose: 225 mg/dl
- Fasting insulin: 60 μU/ml
Summary of Diabetes Epidemiology in the Amish:
The Amish Family Diabetes Study (n=1400)

- Children: Leaner and more physically active (Hairston et al 2013)
- Adults equally overweight and obese as general US white population (Hsueh et al 2000)
  - Type 2 diabetes is less prevalent
  - Pre-diabetes is more prevalent

Lipolysis releases free fatty acids (FFA) and glycerol as metabolic fuel

**Stimulated Lipolysis**

- CGI-58
- ATGL
- HSL
- TAG
- DAG
- MAG
- MGL
- Lipid Droplet
- FFA
- Glycerol
Sitosterolemia: 
*ABCG8* Mutations in Man

- Autosomal recessive
- Excessive plant sterols
- Xanthomas
- Premature atherosclerosis
- Caused by mutations in *ABCG5/8*
- 4% Frequency in Lancaster Amish

*Berge et al Science 2000 290;1771-1775*
## Comparison of G574R Carriers, non-Carriers and Homozygote

<table>
<thead>
<tr>
<th></th>
<th>Carrier</th>
<th>Non-Carrier</th>
<th>Homozygote (n=1)</th>
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</thead>
<tbody>
<tr>
<td><strong>Age</strong></td>
<td>44.0 ± 1.7</td>
<td>52.6 ± 1.4</td>
<td>53</td>
</tr>
<tr>
<td><strong>Sex (% male)</strong></td>
<td>50/110 (45%)</td>
<td>90/181 (52%)</td>
<td>Female</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>27.3 ± 1.6</td>
<td>30.3 ± 1.6</td>
<td>33.1</td>
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<tr>
<td><strong>Sitosterol (mg/dl)</strong></td>
<td>0.43 ± 0.02</td>
<td>0.31 ± 0.02</td>
<td>17.87</td>
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<tr>
<td><strong>Campesterol (mg/dl)</strong></td>
<td>0.53 ± 0.03</td>
<td>0.39 ± 0.03</td>
<td>8.80</td>
</tr>
<tr>
<td><strong>Stigmasterol (ug/dl)</strong></td>
<td>14.26 ± 0.85</td>
<td>10.21 ± 0.84</td>
<td>1290.79</td>
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<tr>
<td><strong>IMT (mm)</strong></td>
<td>0.64 ± 0.02</td>
<td>0.69 ± 0.02</td>
<td>0.97</td>
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<tr>
<td><strong>Cholestanol (mg/dl)</strong></td>
<td>0.36 ±0.01</td>
<td>0.30 ± 0.01</td>
<td>4.33</td>
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<tr>
<td><strong>Lathosterol (mg/dl)</strong></td>
<td>0.20 ± 0.01</td>
<td>0.23 ± 0.01</td>
<td>0.07</td>
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Summary and Conclusions

Using candidate gene and genomic:

- **APOC3**: A new (and safe) target for hypertriglyceridemia and cardioprotection
- **R3500Q APOB**: A major cause of hyperlipidemia in the Lancaster Amish
- **HSL**: A novel target for insulin resistance and diabetes
## Team Science!

<table>
<thead>
<tr>
<th>Jess Albert</th>
<th>Patrick McArdle</th>
<th>Haiqing Shen</th>
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<tbody>
<tr>
<td>Coleen Damcott</td>
<td>Daniel McBride</td>
<td>Julia Shi</td>
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<td>Julie Ducharme</td>
<td>John McLenithan</td>
<td>Kristi Silver</td>
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<td>Susan Fried</td>
<td>Cary McMahon</td>
<td>John Sorkin</td>
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<td>Mao Fu</td>
<td>Braxton Mitchell</td>
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<td>Amish Gandhi</td>
<td>Karen Norton</td>
<td>Nanette Steinle</td>
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<td>Da-Wei Gong</td>
<td>Jeffrey O'Connell</td>
<td>Elizabeth Streeten</td>
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<td>Kristen Hairston</td>
<td>Sandra Ott</td>
<td>Carole Sztalryd</td>
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<td>Amanda Holmes</td>
<td>Toni Pollin</td>
<td>Keith Tanner</td>
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<td>Nicole Hoppman</td>
<td>Evadnie Rampersaud</td>
<td>Magnda Tolea</td>
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<td>Richard Horenstein</td>
<td>Laurie Reinhart</td>
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<td>Hong Hu</td>
<td>Kathy Ryan</td>
<td>Matthew Weir</td>
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<td>Jie Liu</td>
<td>Larry Sauder</td>
<td>Diaozhan Yu</td>
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<td></td>
<td>Jack Shelton</td>
<td>Li Zhang</td>
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<td>Joe Zhao</td>
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**Paul Gurbel (Sinai Hospital)**

**Nauder Faraday (JHU)**

**Wendy Post (JHU)**
Support

NIH: R01 DK54261; R01 DK073490; K24 DK02673; P30 DK072488; P60 DK079637; R01 HL104193; R01 HL69313; U01 HL105198; U01 HL072515; R01 HL076768; R01 AG18728; R01 AR046838; U01 GM074518

ADA; AHA; AFAR/Beeson

University of Maryland

The Amish Community
David Has an MI

- David developed crushing chest pain and was brought urgently to the catheterization lab for PCI.
Clopidogrel (Plavix)

- Most commonly used anti-platelet therapy (with aspirin)
  - Effective for prevention of MI and stroke
  - In 2009, world’s 3rd highest selling drug
  - U.S. sales $4.2 billion
  - Acts by binding to ADP receptors on platelets, preventing platelet aggregation and thrombosis
  - Great variability in response to clopidogrel
    - 4 - 32% of individuals are resistant
Variability of Clopidogrel Response: The Amish Pharmacogenomics of Antiplatelet Intervention (PAPI) Study

- 668 healthy subjects treated with clopidogrel for 1 week
- Platelet aggregation measured before and after therapy

The population “responds” to clopidogrel but there is great inter-individual variation in response

Heritability of clopidogrel response = 0.7 → GENETICS!

Shuldiner et al (2009) JAMA
1/3 to 1/2 of individuals carry at least one CYP2C19*2 allele, which accounts for approximately 12% of the variation in clopidogrel response (platelet aggregation) and a 2.4-fold increased risk of a recurrent CV event.

Shuldiner et al (2009) JAMA
Clopidogrel Metabolism:
Role of CYP2C19 in Activation

PharmGKB.org
Reduced-Function CYP2C19 Genotype and Risk of Adverse Clinical Outcomes Among Patients Treated With Clopidogrel Predominantly for PCI: A Meta-analysis

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Jeffrey L. Anderson, M.D.
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Marc S. Sabatino, M.D., M.P.H.

Cytochrome and Resp

Cytochrome treated a cohort

Content: Clopidogrel, one of the most commonly prescribed medications, is a prodrug requiring CYP2C19 biotransformation. Data suggest its pharmacologic effect varies based on CYP2C19 genotype, but there is uncertainty regarding the clinical risk imparted by specific genotypes.

Objective: To define the risk of major adverse cardiovascular outcomes among carriers of 1 (≈26% prevalence in whites) and carriers of 2 (≈2%, prevalence in whites) reduced-function CYP2C19 genetic variants in patients treated with clopidogrel.

Data Sources and Study Selection: A literature search was conducted (January 2000-August 2010) in MEDLINE, Cochrane Database of Systematic Reviews, and EBASE. Genetic studies were included in which clopidogrel was initiated in predominantly invasively managed patients in a manner consistent with the current guideline recommendations and in which clinical outcomes were ascertained.

Data Extraction: Investigators from 9 studies evaluating CYP2C19 genotype and clinical outcomes in patients treated with clopidogrel contributed the relevant hazard ratios (HRs) and 95% confidence intervals (CIs) for specific cardiovascular outcomes by genotype.

Results: Among 9685 patients (91.3% who underwent percutaneous coronary intervention and 84.5% who had an acute coronary syndrome), 863 experienced the composite end point of cardiovascular death, myocardial infarction, or stroke, and 84 patients had stent thrombosis among the 5844 evaluated for such. Overall, 17.6% were noncarriers, 26.3% had 1 reduced-function CYP2C19 allele, and 22.2% had 2 reduced-function CYP2C19 alleles. A significantly increased risk of the composite end point was evident in both carriers of 1 (HR, 1.56; 95% CI, 1.11-2.17; P = .01) and 2 (HR, 1.36; 95% CI, 1.24-2.30; P < .001) reduced-function CYP2C19 alleles, as compared with noncarriers. Similarly, there was a significantly increased risk of stent thrombosis in both carriers of 1 (HR, 2.67; 95% CI, 1.60-4.23; P < .001) and 2 (HR, 3.89; 95% CI, 1.78-9.02; P = .001) CYP2C19 reduced-function alleles, as compared with noncarriers.

Conclusion: Among patients treated with clopidogrel for percutaneous coronary intervention, carriers of even 1 reduced-function CYP2C19 allele appears to be associated with a significantly increased risk of major adverse cardiovascular events, particularly stent thrombosis.

CYP2C19 is a member of the cytochrome P450 (CYP) superfamily of enzymes involved in drug metabolism, specifically CYP2C19, a key role in clopidogrel metabolism, and carriers of reduced-function genetic variants in the CYP2C19 gene have lower active clopidogrel metabolite levels and diminished platelet inhibition. Based in part on a pharmacokinetic and pharmacodynamic study in 607 patients treated with clopidogrel, 75% of patients treated with the 10-mg dose of clopidogrel achieved a therapeutic level of active metabolite (median, 52 ng/mL) later than patients treated with the 300-mg dose of clopidogrel (median, 43 ng/mL).

For editorial comment see p 1839.

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FDA Boxed warning: Plavix (3/20/2010):

WARNING: DIMINISHED EFFECTIVENESS IN POOR METABOLIZERS
See full prescribing information for complete boxed warning.

- Effectiveness of Plavix depends on activation to an active metabolite by the cytochrome P450 (CYP) system, principally CYP2C19. (5.1)
- Poor metabolizers treated with Plavix at recommended doses exhibit higher cardiovascular event rates following acute coronary syndrome (ACS) or percutaneous coronary intervention (PCI) than patients with normal CYP2C19 function. (12.5)
- Tests are available to identify a patient's CYP2C19 genotype and can be used as an aid in determining therapeutic strategy. (12.5)
- Consider alternative treatment or treatment strategies in patients identified as CYP2C19 poor metabolizers. (2.3, 5.1)

http://www.plavix.com/plavix-videos.aspx
# CYP2C19 Enrollment

<table>
<thead>
<tr>
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<th>Total (%)</th>
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<tbody>
<tr>
<td><strong>As of 7/10/13</strong></td>
<td></td>
</tr>
<tr>
<td>Start Date</td>
<td>2/17/13</td>
</tr>
<tr>
<td>No. Screened</td>
<td>93</td>
</tr>
<tr>
<td>No. Enrolled</td>
<td>68 (73)</td>
</tr>
<tr>
<td>No. of IM/PM</td>
<td>19 (28)</td>
</tr>
<tr>
<td>No. Actionable Genotypes (IM/PM w/PCI)</td>
<td>14 (21)</td>
</tr>
<tr>
<td>No. of Patients w/ Actionable Genotypes Prescribed Alternate Tx</td>
<td>10 (71)</td>
</tr>
</tbody>
</table>

4 patients w/actionable genotypes not prescribed alt. tx d/c prior to result return