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)

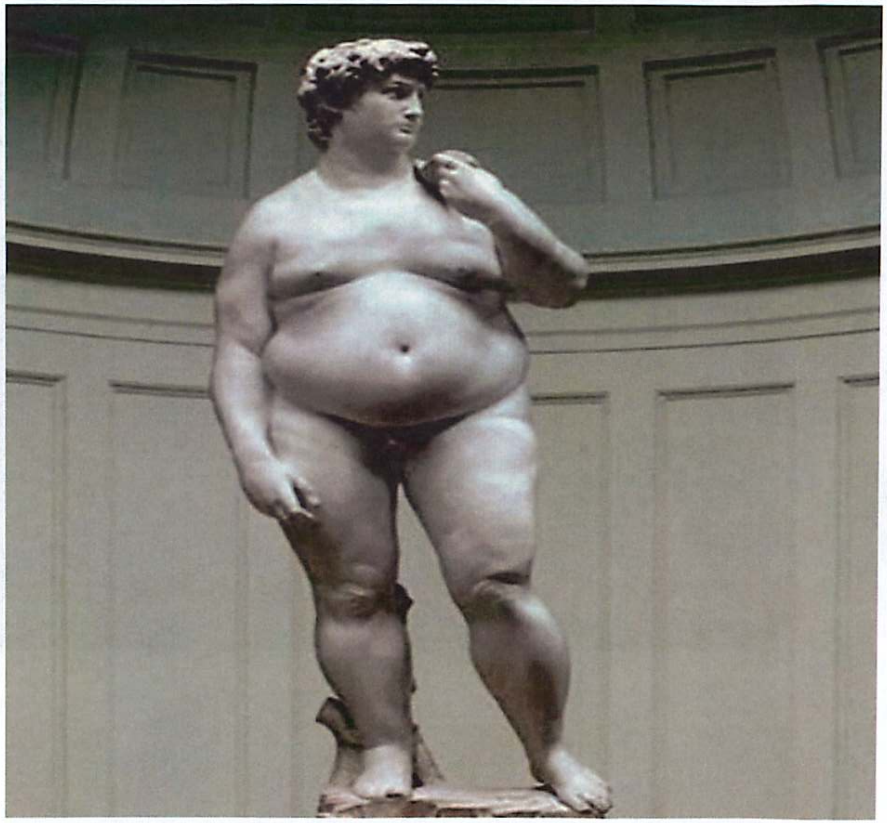
David in the ~~16~~ 21st Century:

i'm lovin' it

BURGER KING

KFC

STARBUCKS COFFEE



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

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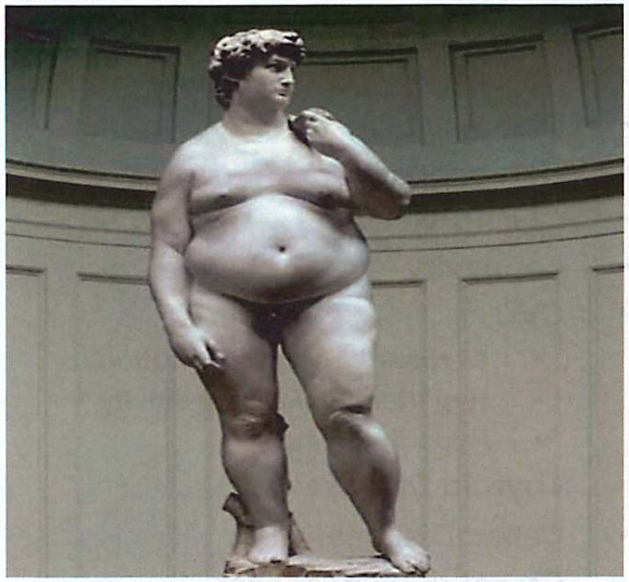
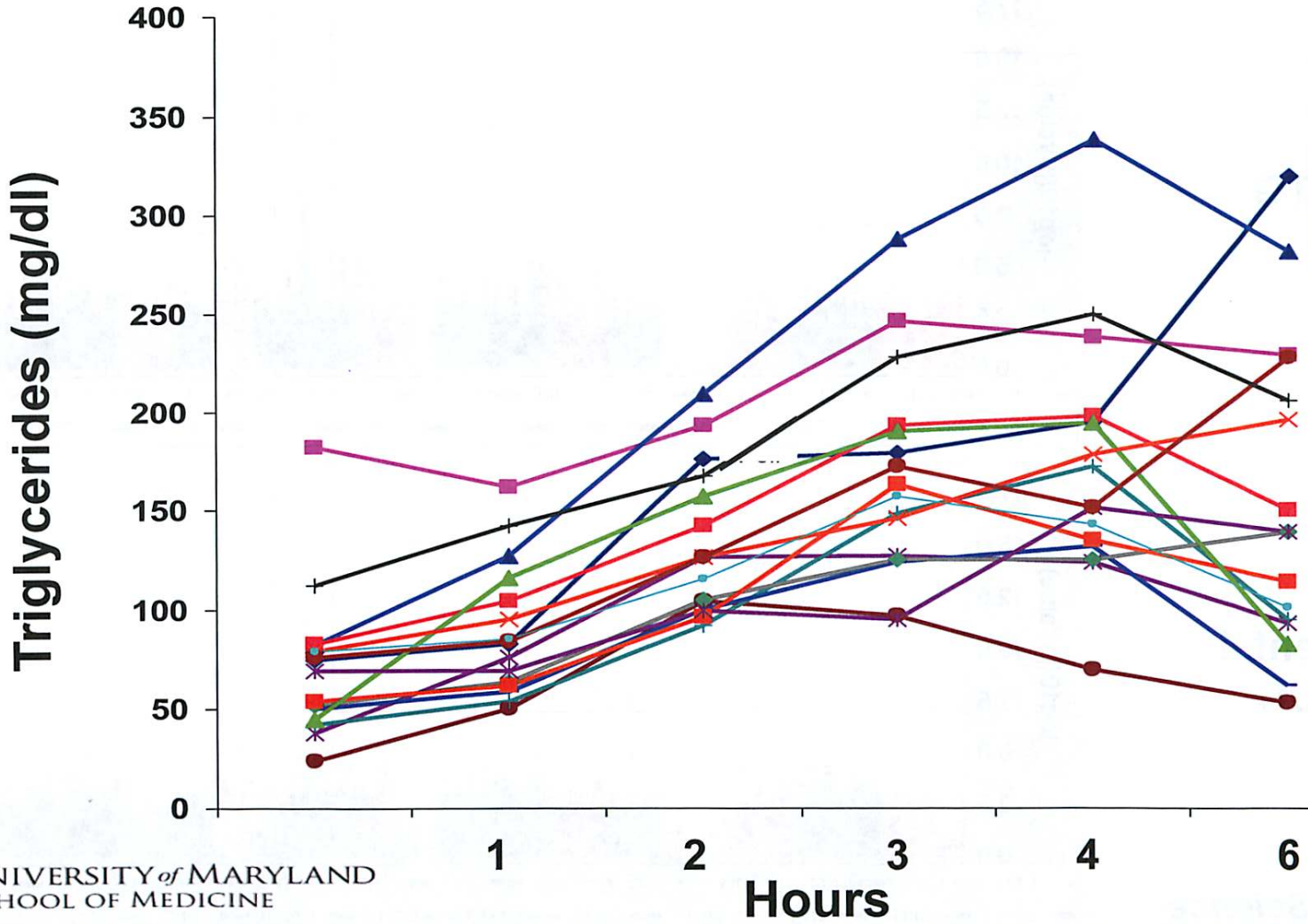
David's Lipid Profile



- 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)



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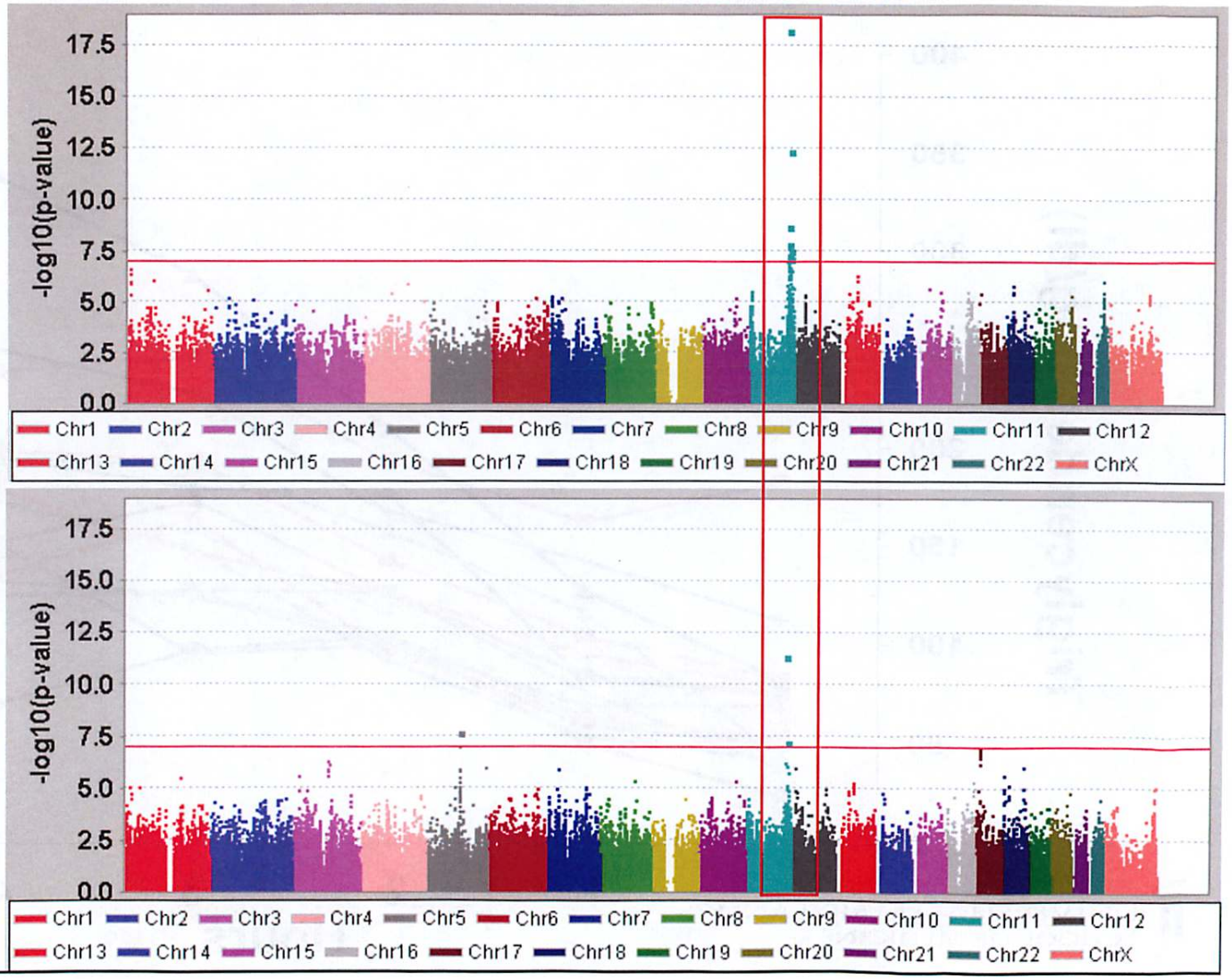
GWAS for Fasting TG and TG Excursion during a High Fat Meal

APOA1/C3/A4/A5

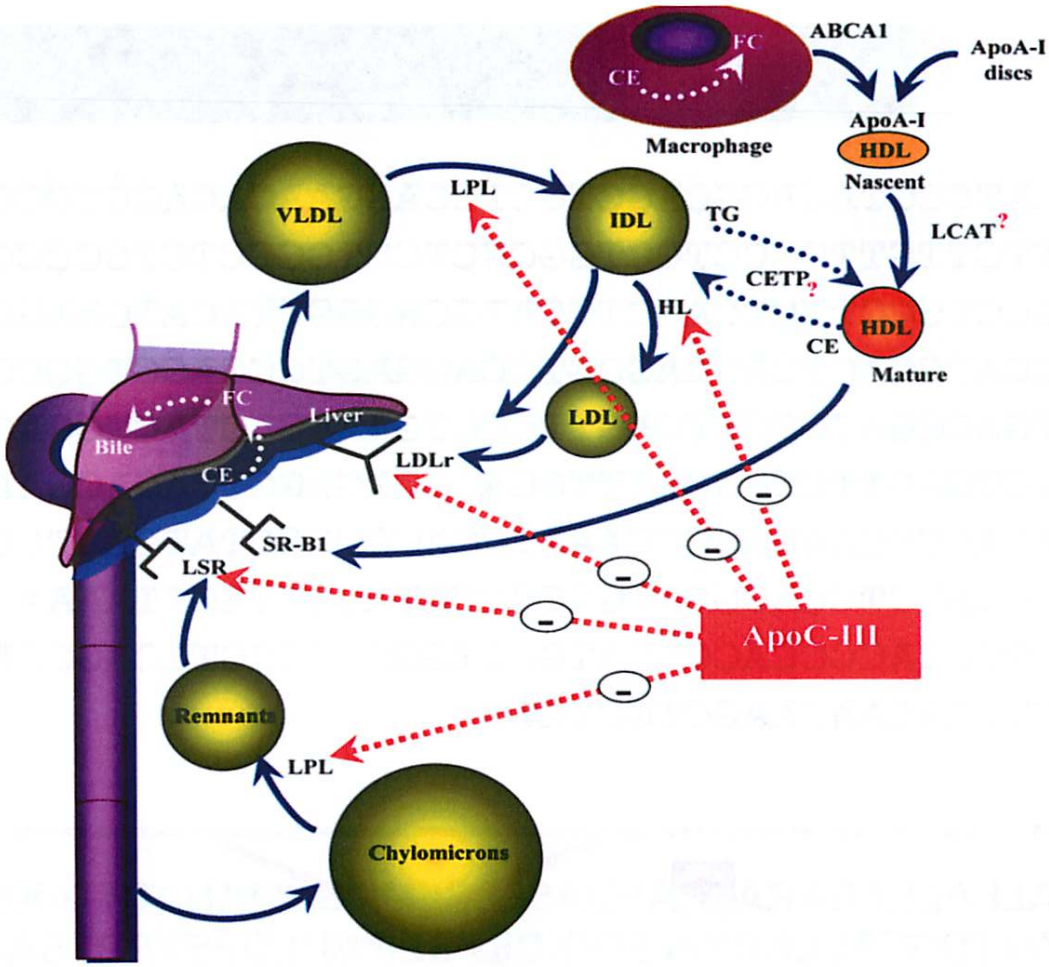
Fasting TG

Incremental TG AUC



Pollin (2008) Science

ApoC-III and Lipoprotein Metabolism



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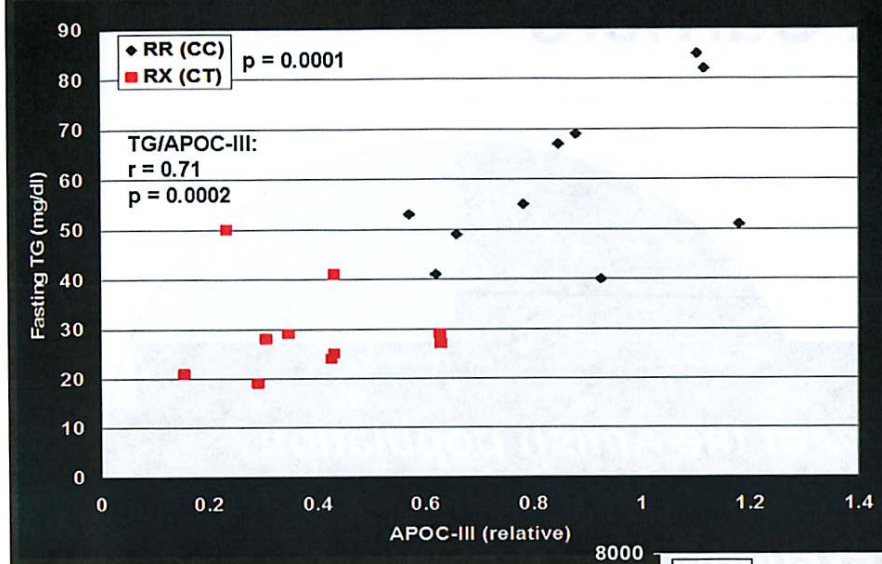
Adapted from Ooi et al, Clinical Science 114:611-624 (2008)

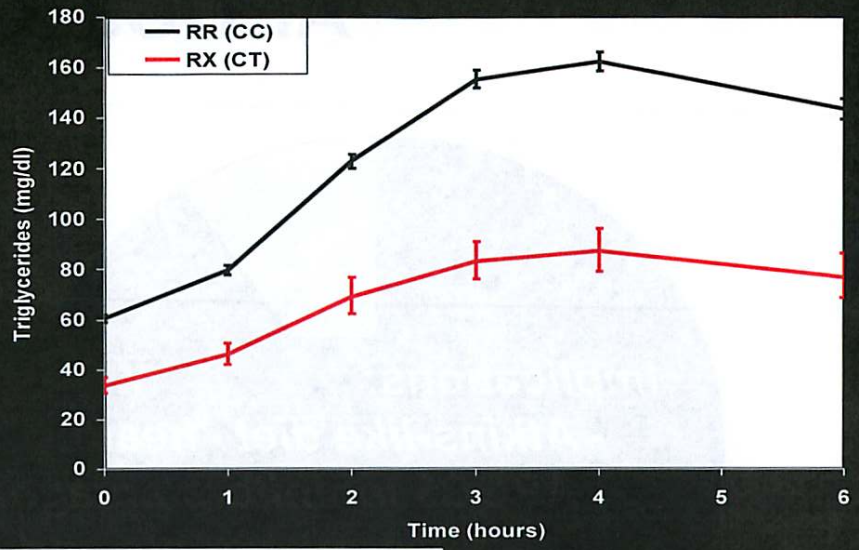
TGCTCAGTTCATCCCTAGAGGCAGCTGCTCCAGGAACAGAGGTGCCATGCAGCCCC
GGGTACTCCTTGTTGTTGCCCTCCTGGCGCTCCTGGCCTCTGCCCGAGCTTCAGAG
TGA GAGGATGCCTCCCTTCTCAGCTTCATGCAGGGTTACATGAAGCACGCCACCAA
GACCGCCAAGGATGCACTGAGCAGCGTGCAGGAGTCCCAGGTGGCCCAGCAGGCCA
GGGGCTGGGTGACCGATGGCTTCAGTTCCCTGAAAGACTACTGGAGCACCGTTAAG
GACAAGTTCTCTGAGTTCTGGGATTTGGACCCTGAGGTCAGACCAACTTCAGCCGT
GGCTGCCTGAGACCTCAATACCCCAAGTCCACCTGCCTATCCATCCTGCGAGCTCC
TTGGGTCCTGCAATCTCCAGGGCTGCCCCTGTAGGTTGCTTAAAAGGGACAGTATT
CTCAGTGCTCTCCTACCCCACCTCATGCCTGGCCCCCCTCCAGGCATGCTGGCCTC
CCAATAAAGCTGGACAAGAAGCTGCTATG

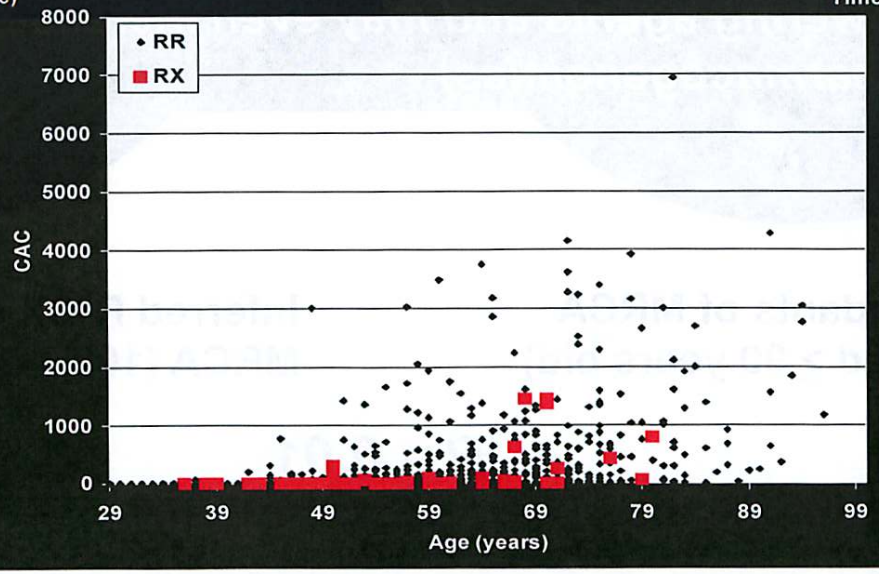
MQPRVLLVVALLALLASARA STOP AEDASLLSFMQGYMKHATKTAKDALSSVQES
QVAQQARGWVTDGFSSLKDYWSTVKDKFSEFWDLDPEVRPTSAVAA

Pollin (2008) Science

APOC3 R19X
(Carrier Frequency: 5%)







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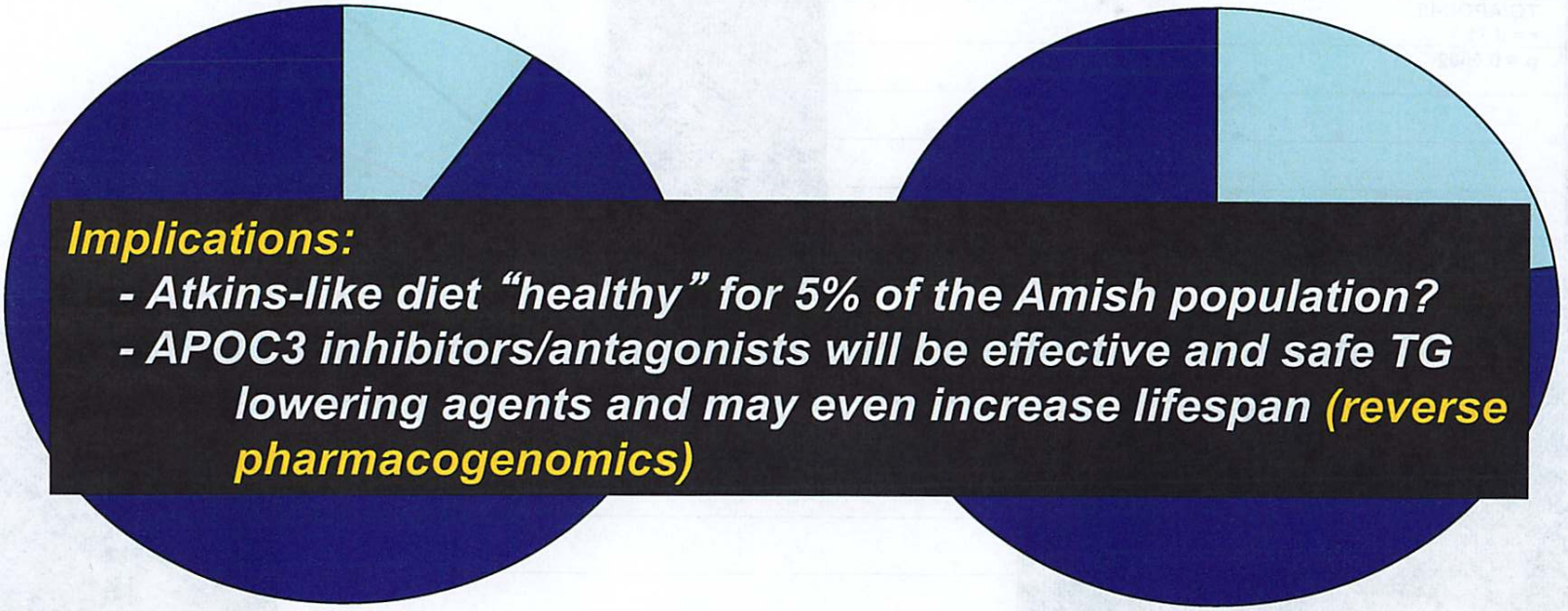
Higher Prevalence of Nonagenarians Among *APOC3* R19X Carriers



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

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Pollin, submitted

Circulation Research

JOURNAL OF THE AMERICAN HEART ASSOCIATION

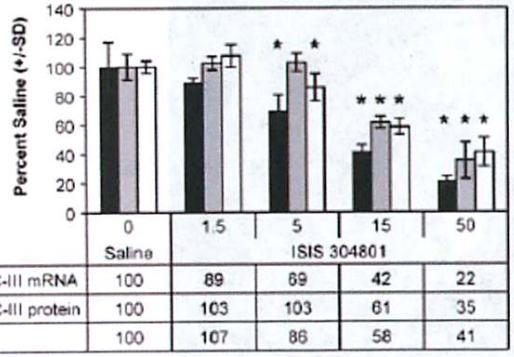
American Heart Association®

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

Mark J. Graham, Richard G. Lee, Thomas A. Bell III, Wuxia Fu, Adam Emile Mullick, Veronica J Alexander, Walter Singleton, Nick Viney, Richard Geary, John Q Su, Brenda F. Baker, Jennifer Burkey, Stanley T. Crooke and Rosanne M. Crooke

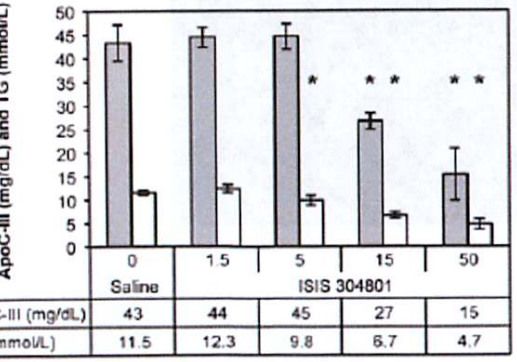
Circ Res. published online March 29, 2013;

A



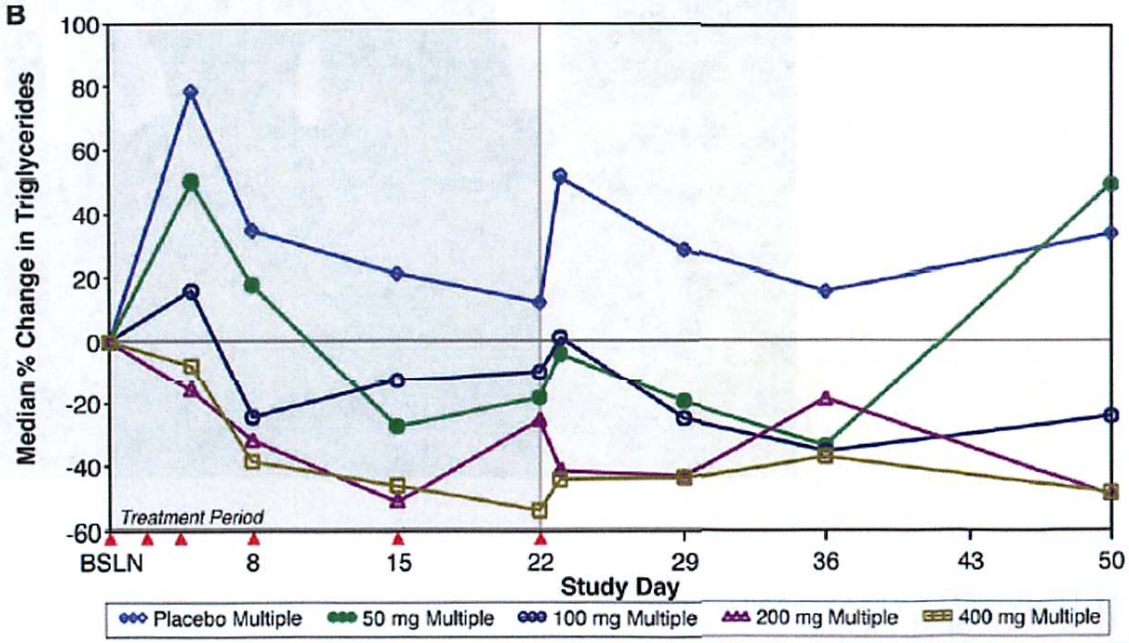
	0 Saline	1.5 ISIS 304801	5 ISIS 304801	15 ISIS 304801	50 ISIS 304801
ApoC-III mRNA	100	89	69	42	22
ApoC-III protein	100	103	103	61	35
TG	100	107	86	58	41

B

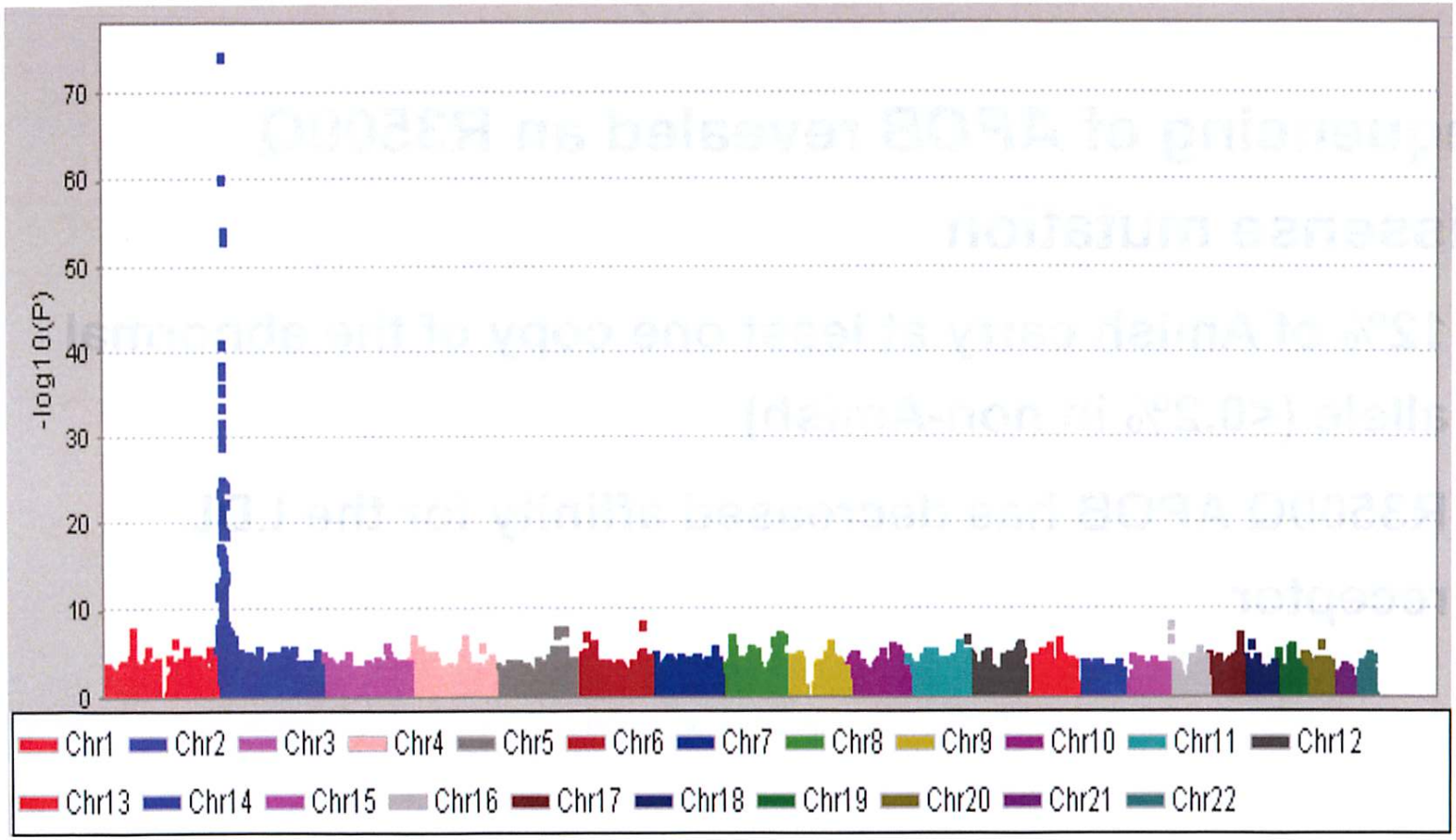


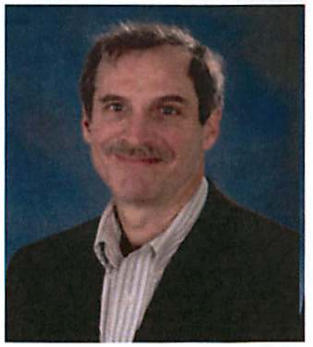
	0 Saline	1.5 ISIS 304801	5 ISIS 304801	15 ISIS 304801	50 ISIS 304801
ApoC-III (mg/dL)	43	44	45	27	15
TG (mmol/L)	11.5	12.3	9.8	6.7	4.7

B



GWAS of LDL-C in the Amish HAPI Heart Study





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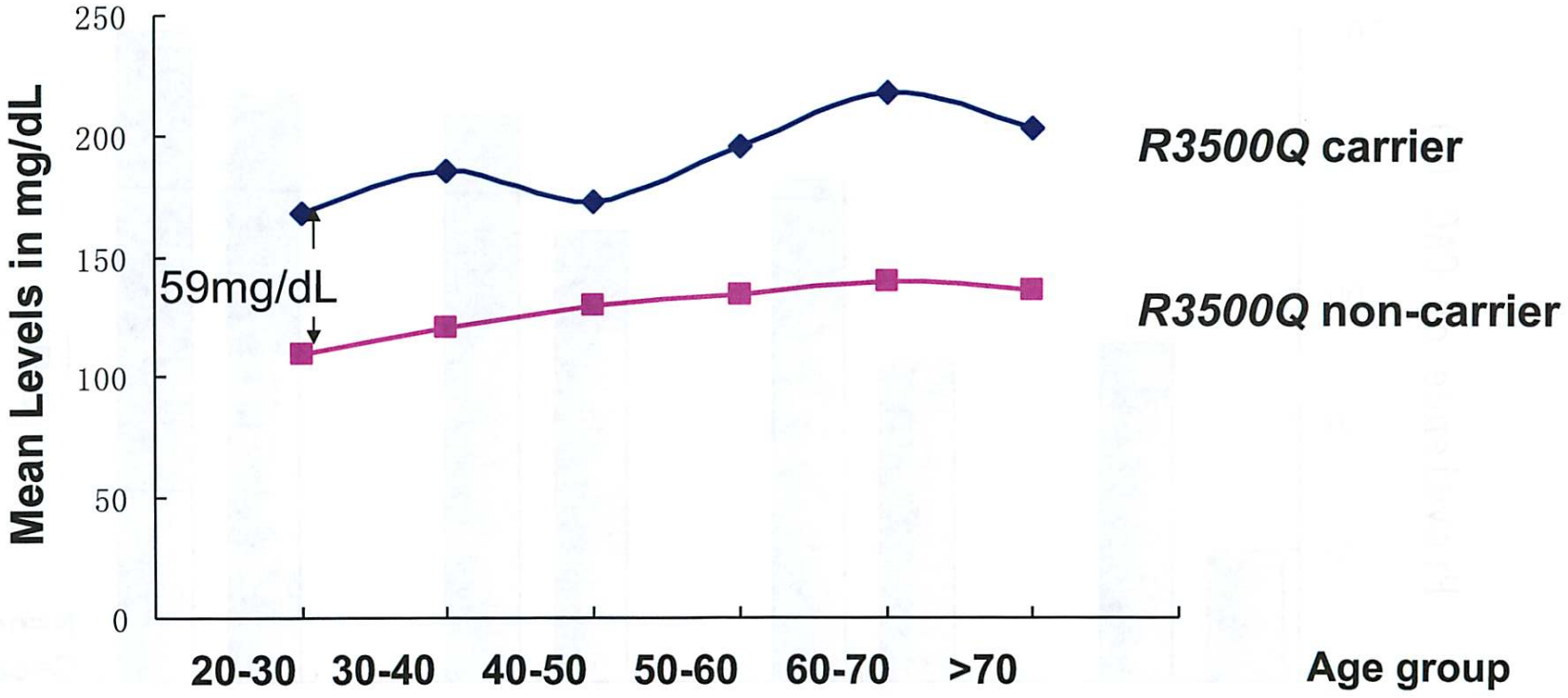
Shen, Arch Int Med, 2010.

- **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**

Shen, Arch Int Med, 2010.

Elevated LDL-C in *APOB R3500Q* Carriers Constant Across Age Groups



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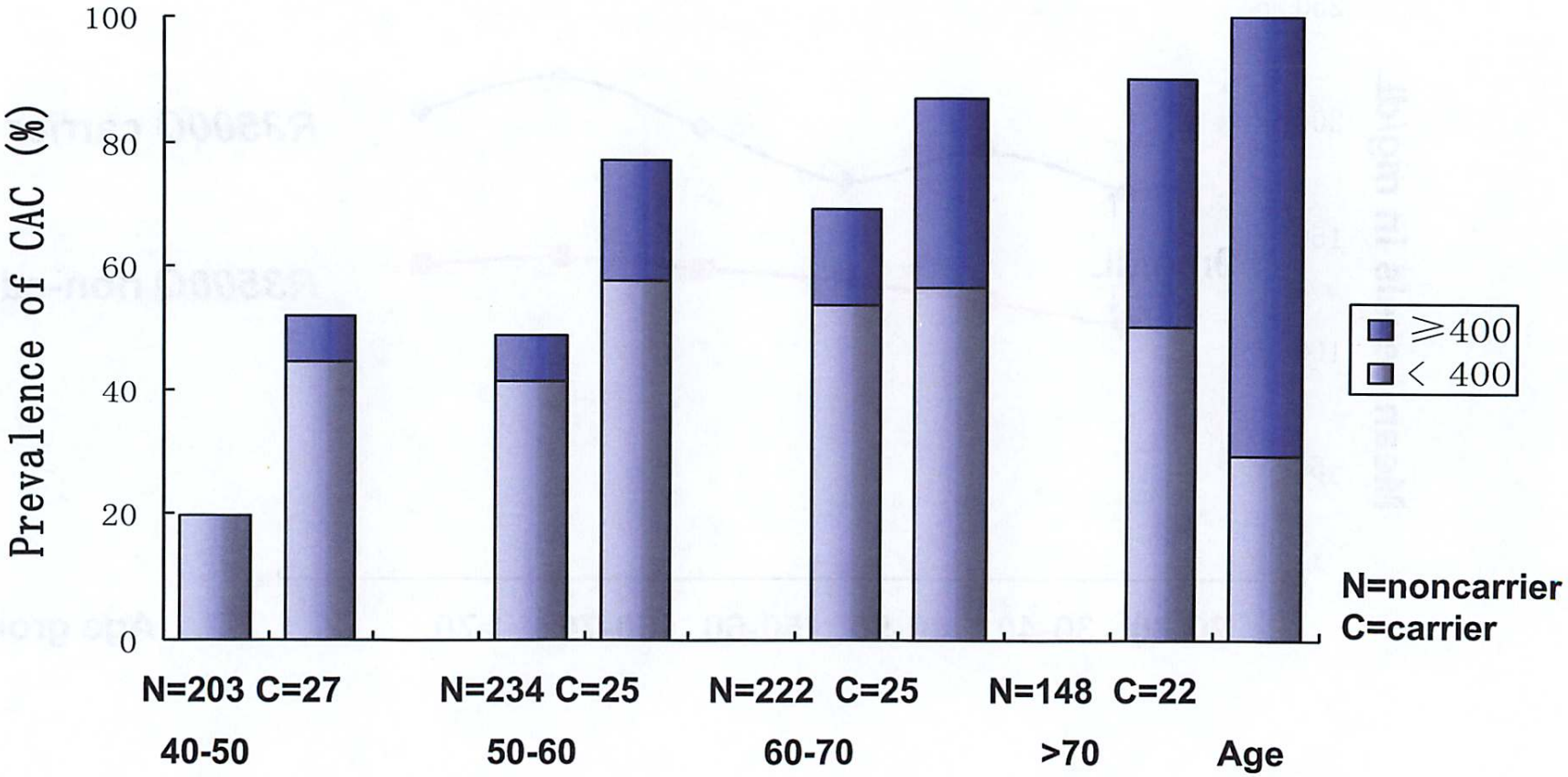
Shen, Arch Int Med, 2010.

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



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Shen, Arch Int Med, 2010.

R3500Q Carriers Have Increased Risk for CAC and Extensive CAC

	OR (95% CI) Model 1	OR (95% CI) Model1 + LDL-C
CAC	**4.65** (2.73-7.90)***	**3.26** (1.79-5.94)***
Extensive CAC	**8.54** (2.79-26.16)**	**4.75** (1.33-16.92)*

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**

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Shen, Arch Int Med, 2010.

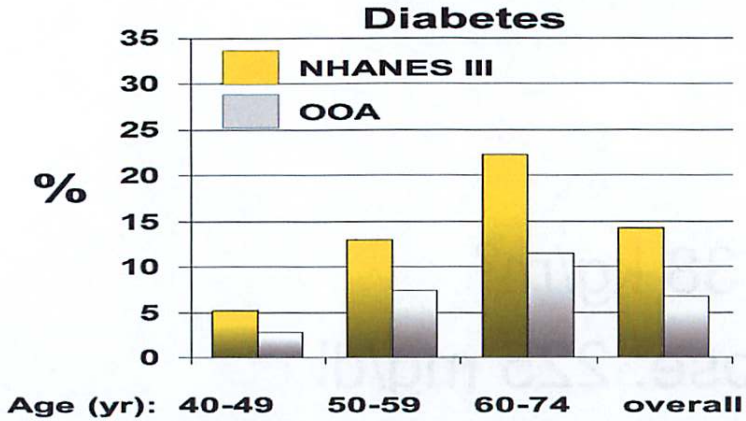
The Amish Wellness Study

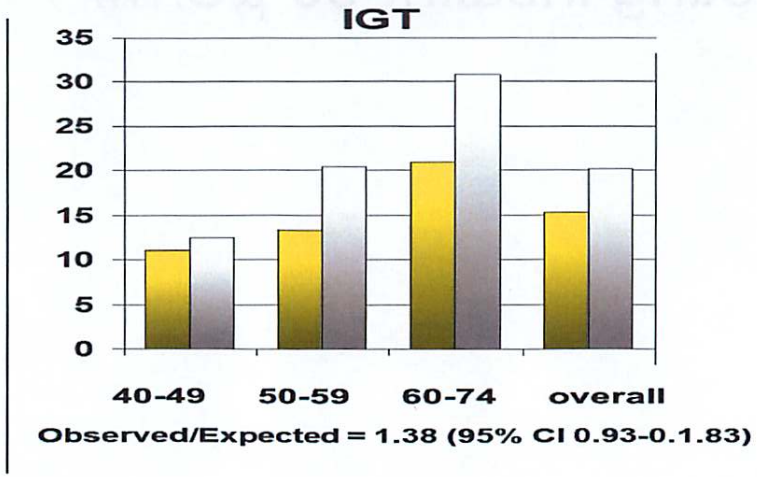
- Medical history
- Vital Signs
- CMP, CBC, Lipid panel, HgbA1c, TSH, celiac screen
- EKG
- Carotid and abdominal U/S
- Heel U/S
- APOB genotype

David is Obese and Has Diabetes

- BMI: 38 kg/m^2
- Glucose: 225 mg/dl
- Fasting insulin: 60 μU/ml

Summary of Diabetes Epidemiology in the Amish:
The Amish Family Diabetes Study (n=1400)

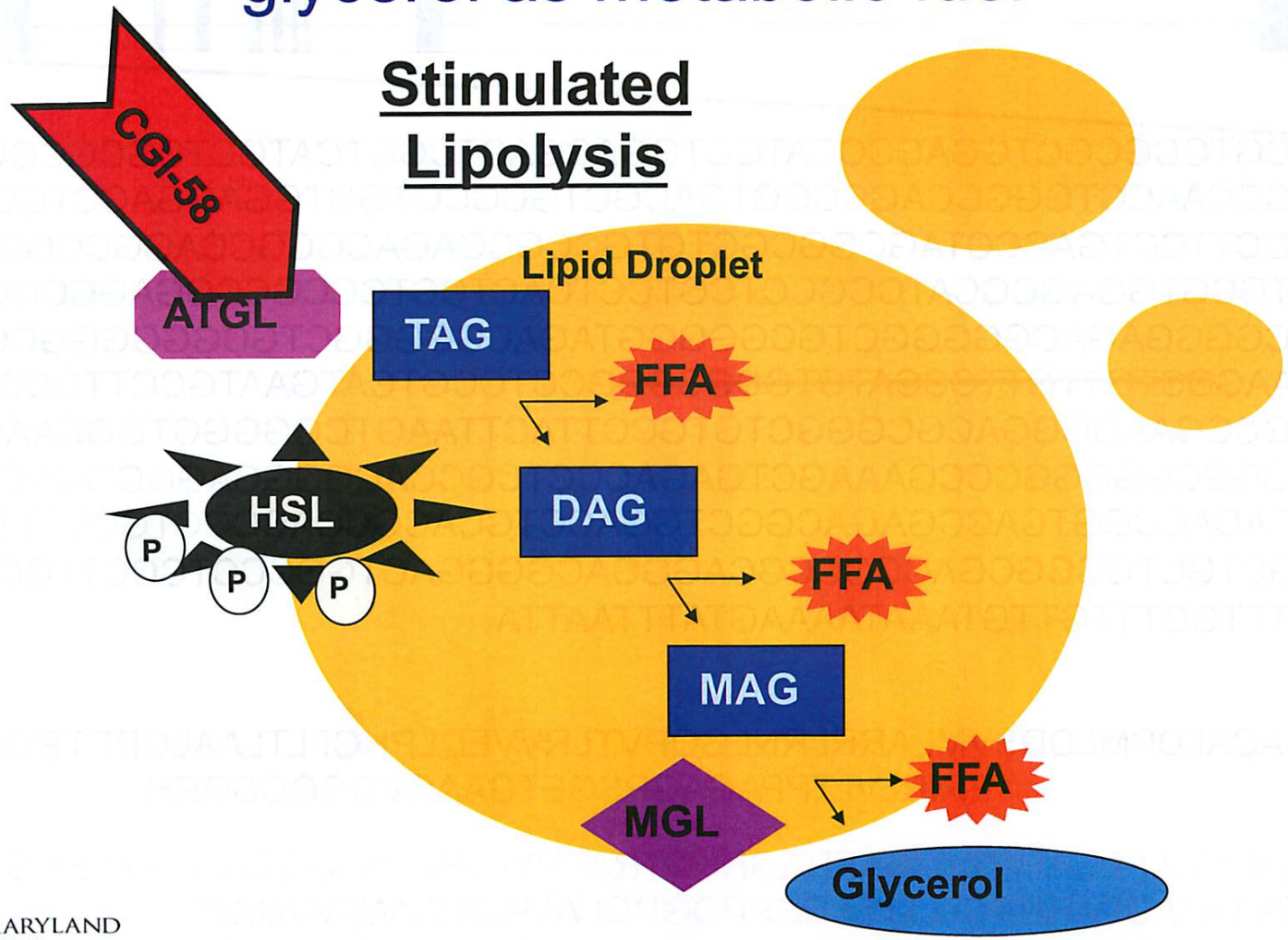




- Children: Leaner and more physically active (Hairston et al 2013)
- Adults equally overweight and obese as general US white population (Hsueh et al 2000)
  - Type 2 diabetes is <u>less</u> prevalent
  - Pre-diabetes is <u>more</u> prevalent

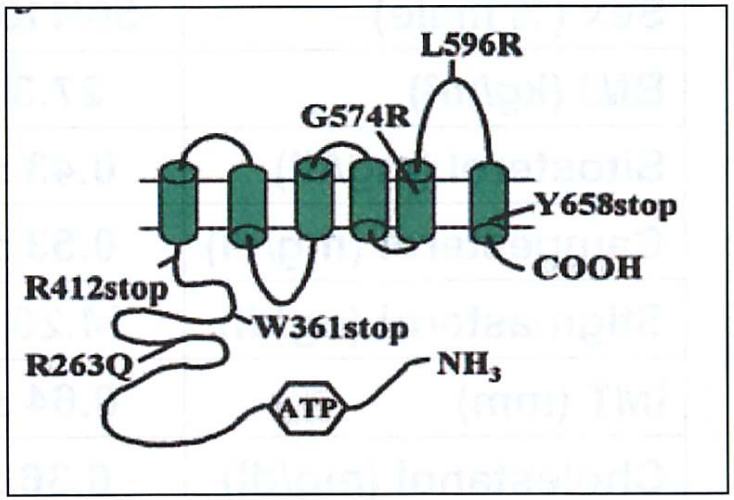
Hsueh et al, Diabetes Care 2000; Snitker et al, Lancet 2003); Hairston et al, Diabetes Care 2013

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



Sitosterolemia: *ABCG8* Mutations in Man

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

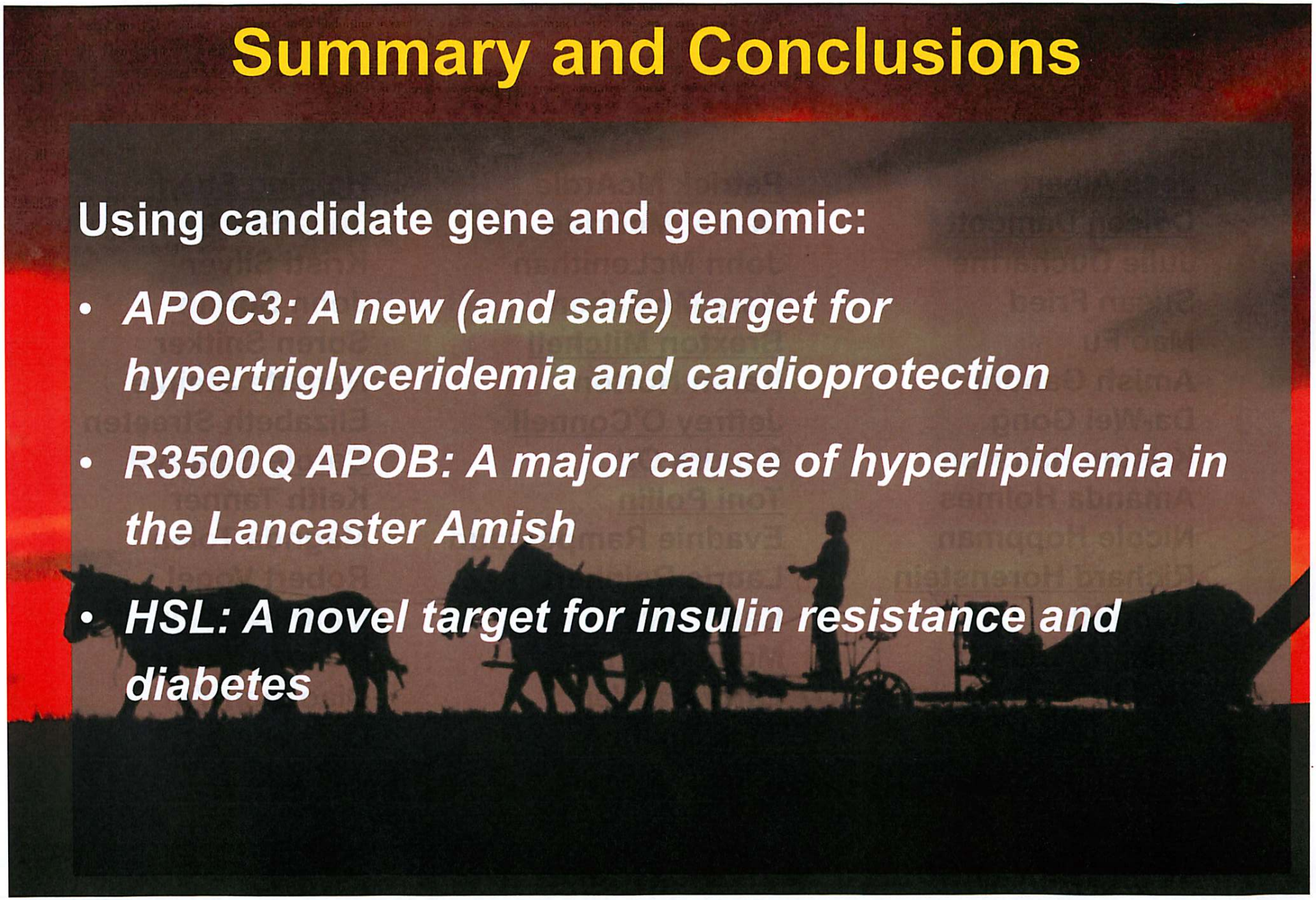


Berge et al *Science* 2000 290;1771-1775

Comparison of G574R Carriers, non-Carriers and Homozygote

	Carrier	Non-Carrier	Homozygote (n=1)
Age	44.0 ± 1.7	52.6 ± 1.4	53
Sex (% male)	50/110 (45%)	90/181 (52%)	Female
BMI (kg/m^2)	27.3 ± 1.6	30.3 ± 1.6	33.1
Sitosterol (mg/dl)	0.43 ± 0.02	0.31 ± 0.02	17.87
Campesterol (mg/dl)	0.53 ± 0.03	0.39 ± 0.03	8.80
Stigmasterol (ug/dl)	14.26 ± 0.85	10.21 ± 0.84	1290.79
IMT (mm)	0.64 ± 0.02	0.69 ± 0.02	0.97
Cholestanol (mg/dl)	0.36 ±0.01	0.30 ± 0.01	4.33
Lathosterol (mg/dl)	0.20 ± 0.01	0.23 ± 0.01	0.07

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!

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Amanda Holmes
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Richard Horenstein
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Nanette Steinle
Elizabeth Streeten
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Nauder Faraday (JHU)
Wendy Post (JHU)

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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

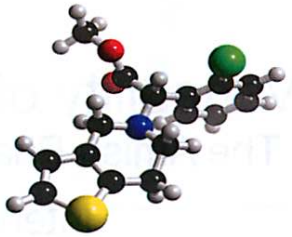
University of Maryland

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**

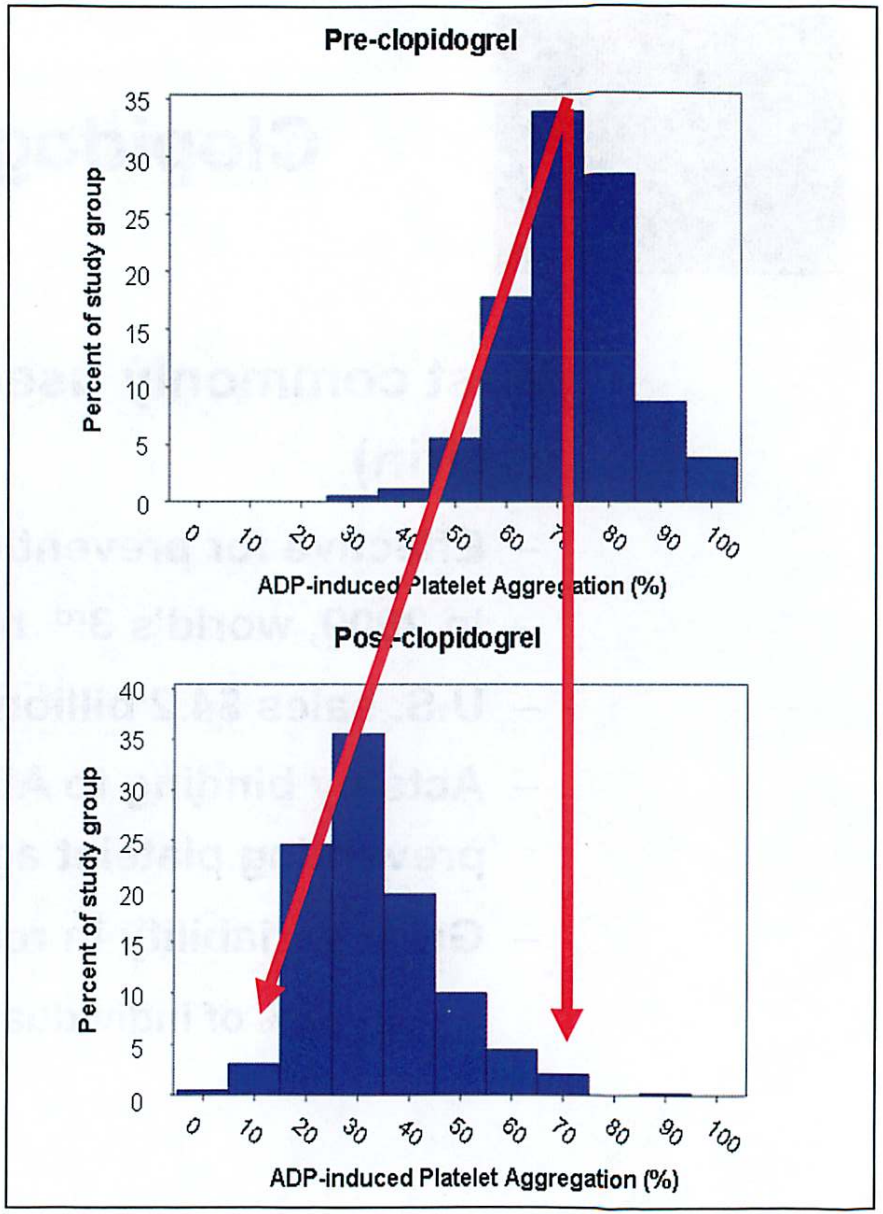
Variability of Clopidogrel Response:

The Amish Pharmacogenomics of Antiplatelet Intervention (PAPI) Study

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

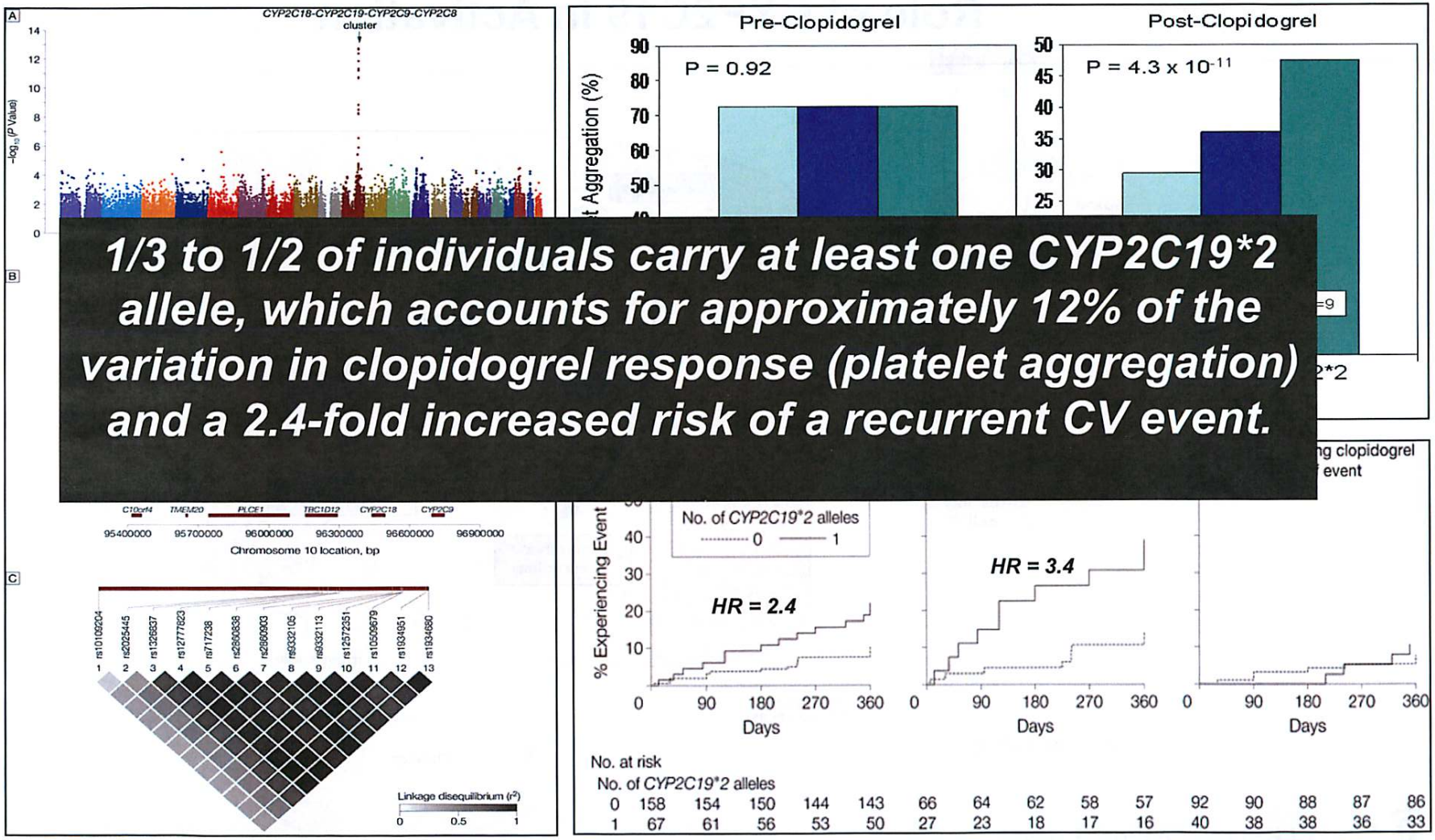
The population "responds" to clopidogrel but there is great inter-individual variation in response

Heritability of clopidogrel response = 0.7 → GENETICS !



Shuldiner et al (2009) JAMA

PAPI-1: Clopidogrel Response GWAS to Functional Variant to Clinical Outcome

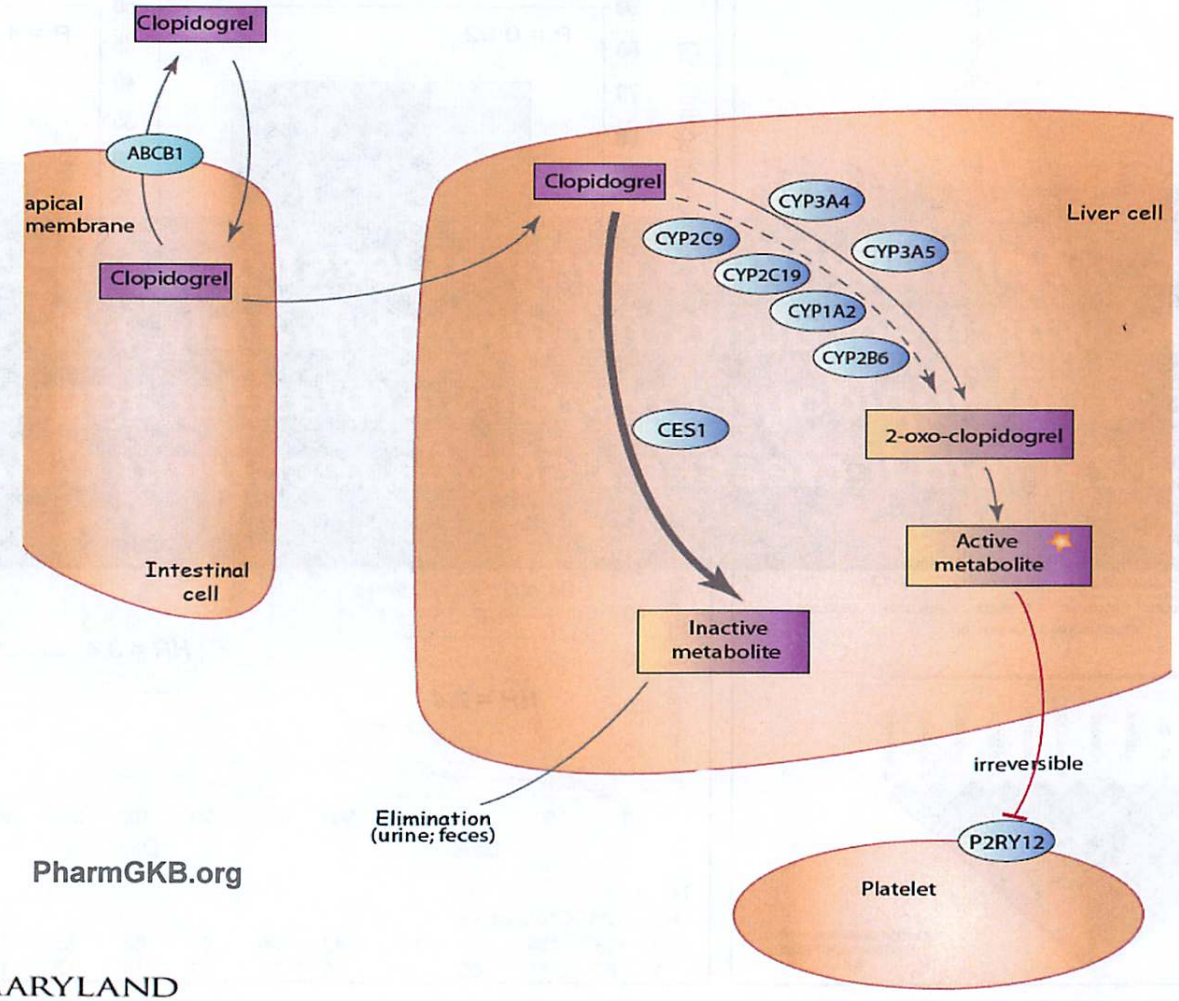


1/3 to 1/2 of individuals carry at least one CYP2C19*2 allele, which accounts for approximately 12% of the variation in clopidogrel response (platelet aggregation) and a 2.4-fold increased risk of a recurrent CV event.

Shuldiner et al (2009) JAMA

Clopidogrel Metabolism:

Role of CYP2C19 in Activation



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Genetic Determinants of Response
Clopidogrel a

The NEW ENG

Tabassome Simon, M.D
Murielle Mary-Krause, Ph.D
Nicolas Méneveau, M.D., P.
Nicolas Danchin, M.D., P
for the French Registry o
Myocardial Ir

OR

Cytochrome
and Resp

Jessica L. Mega, M.D., M.P.H.,
Lei Shen,
Jose
Willi

Cytochr
treated
a cohor

Jean-Philippe Coll
Guillaume Cayla, I

REVIEW

Reduced-Function *CYP2C19* Genotype and Risk of Adverse Clinical Outcomes Among Patients Treated With Clopidogrel Predominantly for PCI

A Meta-analysis

Jessica L. Mega, MD, MPH
Tabassome Simon, MD, PhD
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Jeffrey L. Anderson, MD
Elliott M. Antman, MD
Kevin Fliden, BS
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Lei Shen, PhD
Dirk Sibbing, MD
P. Gabriel Steg, MD
Dietmar Trenk, PhD
Stephen D. Wiviott, MD
Marc S. Sabatine, MD, MPH

Content Clopidogrel, one of the most commonly prescribed medications, is a prodrug requiring CYP450 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 EMBASE. 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 54.5% 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 5894 evaluated for such. Overall, 71.5% were noncarriers, 26.3% had 1 reduced-function *CYP2C19* allele, and 2.2% had 2 reduced-function *CYP2C19* alleles. A significantly increased risk of the composite end point was evident in both carriers of 1 (HR, 1.55; 95% CI, 1.11-2.17; $P = .01$) and 2 (HR, 1.76; 95% CI, 1.24-2.50; $P = .002$) 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, 1.69-4.22; $P < .0001$) and 2 (HR, 3.97; 95% CI, 1.75-9.02; $P = .001$) *CYP2C19* reduced-function alleles, as compared with noncarriers.

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

JAMA. 2010;304(16):1821-1830 www.jama.com

CLOPIDOGREL BLOCKS THE $P2Y_{12}$ adenosine diphosphate (ADP) receptor on platelets and has been shown to reduce cardiovascular events in patients presenting with an acute coronary syndrome (ACS), particularly in those undergoing percutaneous coronary intervention (PCI).[1,2] However, there is a large degree of interindividual variability in the pharmacodynamic response to clopidogrel.[3] One source of the variability is the metabolism of clopidogrel, which is a prodrug requiring biotransformation to generate its active metabolite. Cytochrome P450 (CYP) isoenzymes, specifically CYP2C19,[4] play 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.[5]

Based in part on a pharmacokinetic and pharmacodynamic study in 40

For editorial comment see p 1839.

Author Affiliations are listed at the end of this article.
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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

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

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