# Genetic Protection from Coronary Atherosclerosis: From Genes to Public Health

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**4/20/06**

*This is to acknowledge that Helen Hobbs has no financial interests or other relationships with commercial concerns related directly to this program. She will not be discussing off-label uses in her presentation.*

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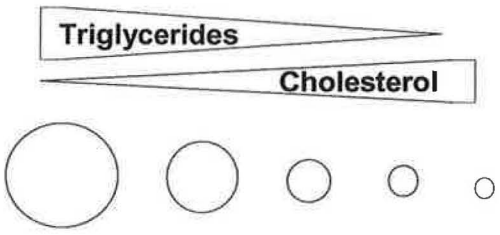
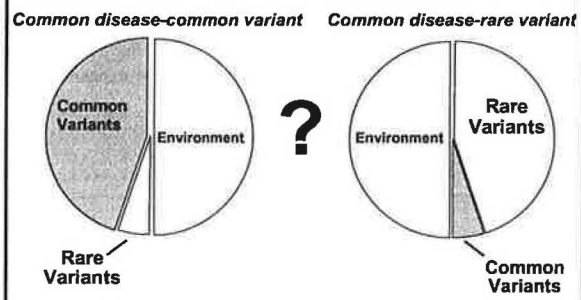
Professor
Medical Genetics
Internal Medicine Grand Rounds
4/20/06

## Classes of Lipoproteins




Chylo-micron VLDL IDL LDL HDL

## What is the Genetic Architecture of Lipoprotein Levels?



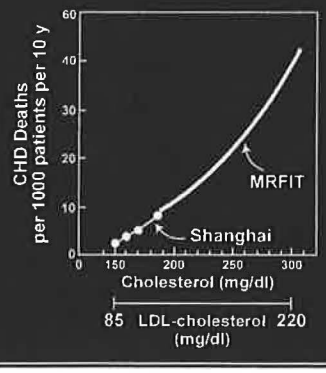

## General Characteristics of Single Gene & Complex Traits

| | *Single Gene* | *Complex* |
|---|---|---|
| HISTORY | Recent | Ancient |
| EFFECT | Large | Small |
| NO. OF ALLELES | Many, rare | Few, common |
| DETECTION | Sequencing | SNP association |

## Plasma Levels of LDL as a Model Complex Quantitative Trait

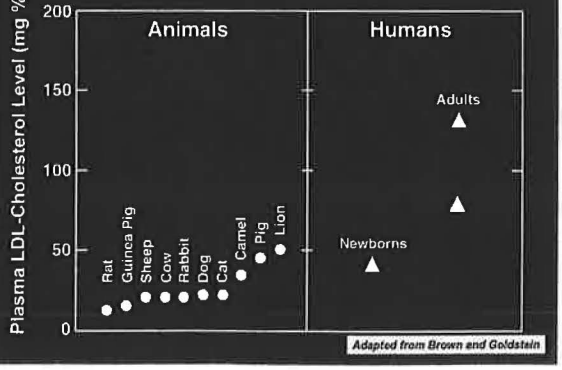
- Easy to measure
- Small *intra*-individual variation (~10%)
- Large *inter*-individual variation (3-fold)
- Variation ~ 50% genetic
- Well characterized metabolic pathway
- Clinically important

## Plasma LDL-Cholesterol & CHD Deaths



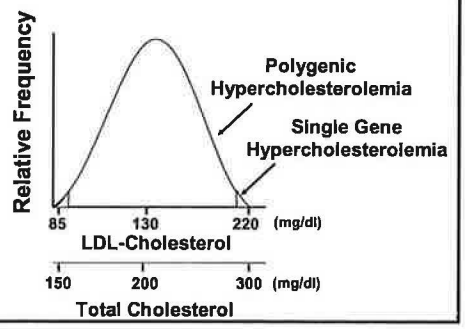

J. Dietschy

## PLASMA LDL LEVELS IN MAMMALS

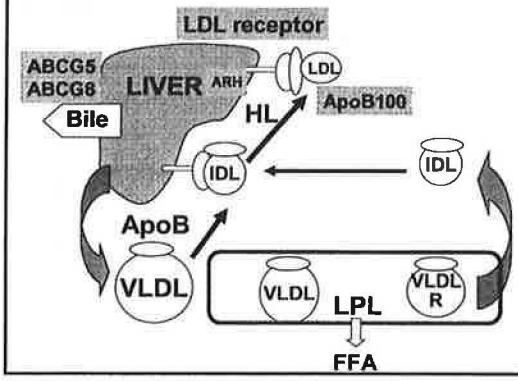



Adapted from Brown and Goldstein

## LDL-Cholesterol Levels In Industrialized Societies




## LDL Metabolism

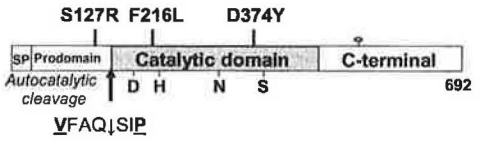



## Single Gene Disorders: Hypercholesterolemia

| Disease | Gene | Prevalence | Defect |
|---|---|---|---|
| **Autosomal Dominant** | | | |
| Familial Hypercholesterolemia (AD) | *LDLR* | 1: 500 | LDL Clearance |
| Familial Defective ApoB (AD) | *APOB* | 1: 1,000 | LDL Clearance |
| FH3 | *PCSK9* | ? | ? |
| **Autosomal Recessive** | | | |
| ARH (AR) | *ARH* | <1: 1 X $10^6$ | LDL Clearance |
| Sitosterolemia (AR) | *ABCG5* *ABCG8* | <1: 1 X $10^6$ | Chol. excretion |

## Missense Mutations in *PCSK9* Cause Dominant Hypercholesterolemia

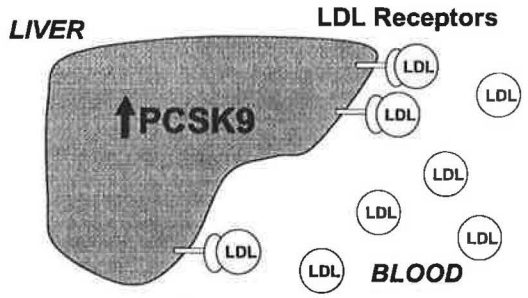
***PCSK9:*** **proprotein convertase subtilisin/kexin type 9 serine protease**




- *Embryo*: liver, cerebrum, renal cortex
  *Adult*: liver, small intestine, cerebellum, renal medulla
- Regulated by SREBP2, the master regulator of cholesterol metabolism

Abifadel *et al.* (2003) *Nat. Genet.* 34:154
Seidah *et al.* (2003) *PNAS* 100:928

## Hepatic LDLR Number is Inversely Related to Plasma LDL Levels

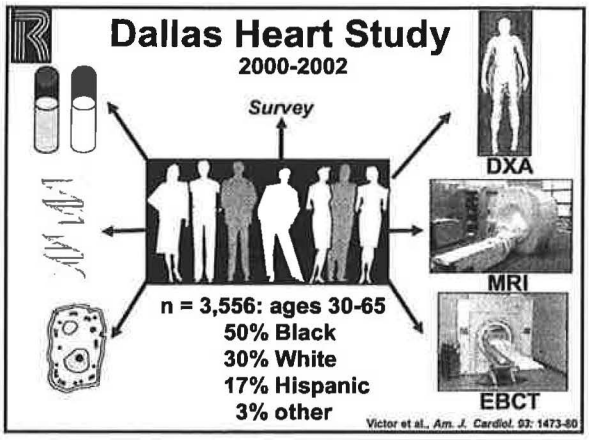


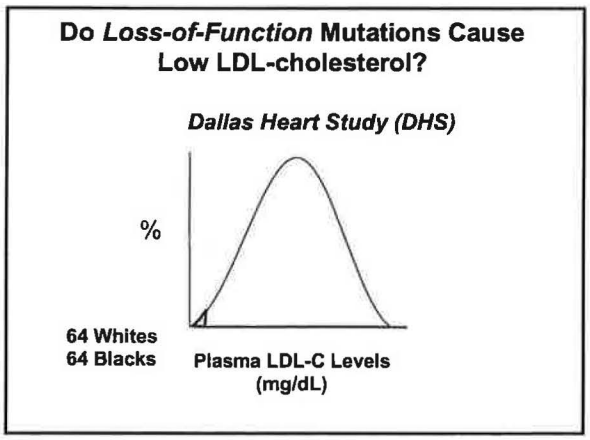

Maxwell et al. Proc Natl Acad Sci (2004) 101:7100
Horton et al. *J Biol Chem.* (2004) 279:50630

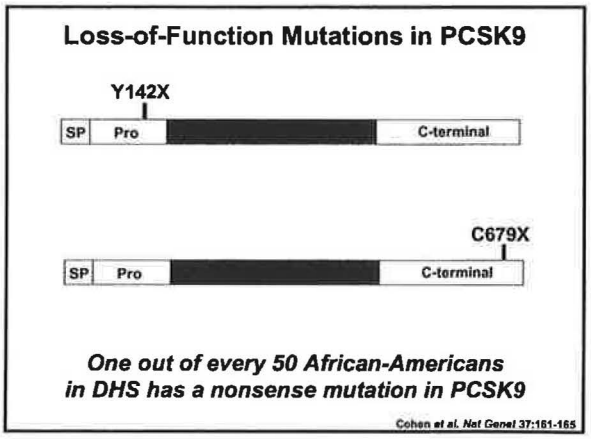
## PCSK9 Promotes Degradation of LDLR
**(no change in LDLR mRNA)**


*Missense mutations → Gain-of-function*

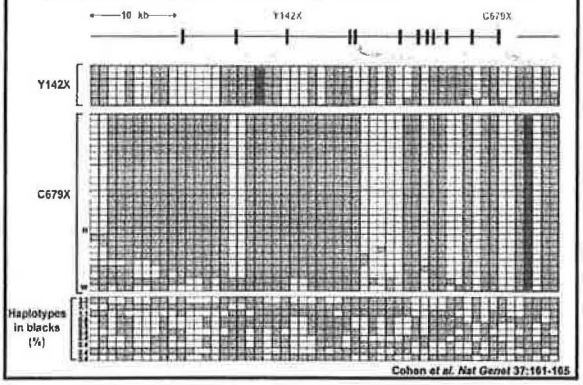

*Nonsense mutations → Loss-of-function*


Dallas Heart Study
2000-2002
Survey
DXA
MRI
EBCT
n = 3,556: ages 30-65
50% Black
30% White
17% Hispanic
3% other
Victor et al., Am. J. Cardiol. 93: 1473-80


Do Loss-of-Function Mutations Cause Low LDL-cholesterol?
Dallas Heart Study (DHS)
%
64 Whites
64 Blacks
Plasma LDL-C Levels (mg/dL)

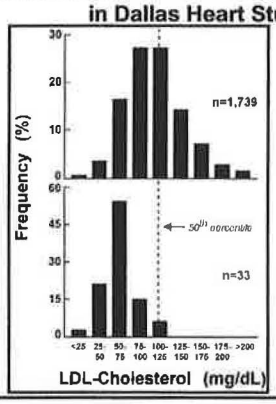

Loss-of-Function Mutations in PCSK9
Y142X
SP
Pro
C-terminal
C679X
SP
Pro
C-terminal
One out of every 50 African-Americans in DHS has a nonsense mutation in PCSK9
Cohen et al. Nat Genet 37:161-165

## Haplotype Analysis of PCSK9




Cohen *et al. Nat Genet* 37:161-165

## Distribution of LDL-C in African-Americans in Dallas Heart Study




**No nonsense mutation in *PCSK9***

**Y142X or C679X**

Cohen *et al. Nat Genet* 37:161-165

## LDL Size and Metabolism in African-Americans with *PCSK9* Nonsense Mutations

| | Normal | Y142X+ C679X |
|---|---|---|
| n | 1,769 | **33** |
| LDL-C | 105+/-37 | 63+/-23* |
| Campesterol:C** | 1.5+/-0.9 | 1.8+/-1.0 |
| Lathosterol:C** | 0.7+/-0.4 | 0.7+/-0.5 |
| LDL diameter (nm)¶ | 21.1+/-0.7 | 21.2+/-0.6 |
| Hepatic TG (%)€ | 3.1% | 2.0% |

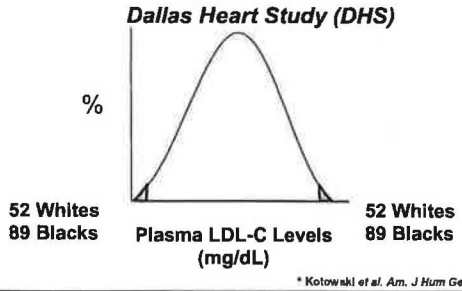
** Gas chromatography
€ Proton magnetic resonance spectroscopy
¶ Nuclear magnetic resonance

* P<0.001

* Kotowski *et al. Am. J Hum Genet.* 2006

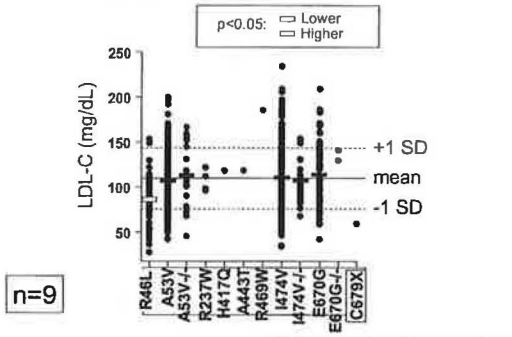
## Sequencing the Extremes: Dallas Heart Study

**High LDL: > 95th percentile (B & W)**
**Low LDL: < 5th percentile (B & W)**
**Blacks (n=89), Whites (52)**




* Kotowski *et al. Am. J Hum Genet.* 2006

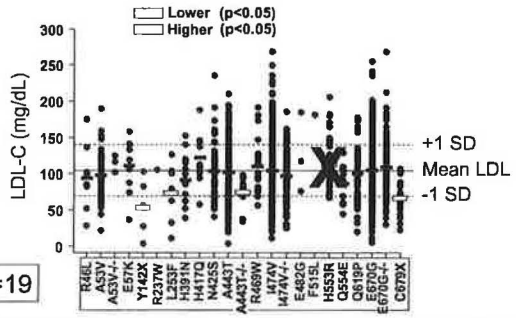
## Distribution of LDL-C Levels in DHS: Untreated Caucasians




n=9

*LDL-C levels adjusted for age and sex*

## Distribution of LDL-C Levels in DHS: Untreated African-Americans (n=1,711)

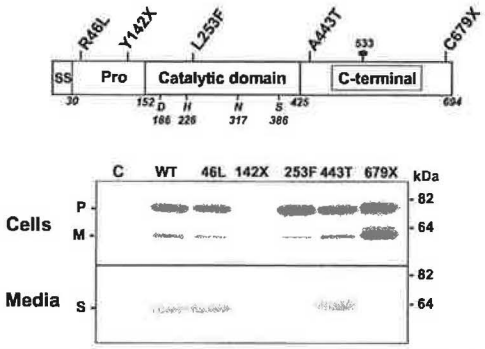



n=19

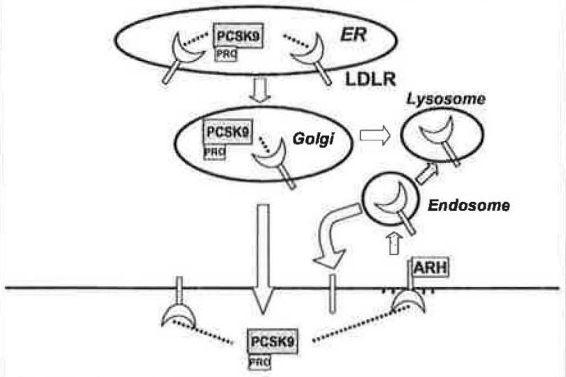
**X – Not confirmed in Maywood**

*LDL-C levels adjusted for age and sex*

## Expression of Loss-of-Function Mutations




## Mechanism of Action of PCSK9



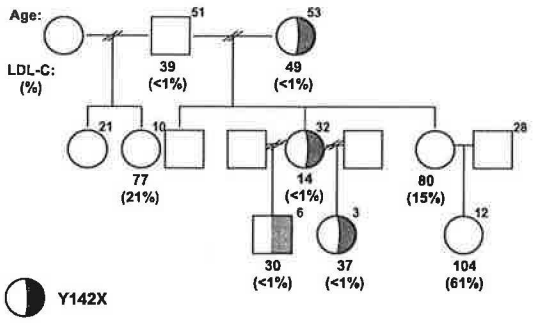

## The Dallas Heart Study

*The Human Biology Laboratory*

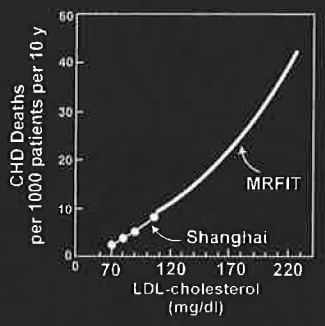
**Exquisite Phenotyping**
**+**
**Extensive Genotyping**
**+**
**Genetic Diversity**
**+**
**Family/Mechanistic Studies**

***FOCUS ON LOCAL SCIENTIFIC EXPERTISE***

## Family DHS20 Contains PCSK9 Y142X

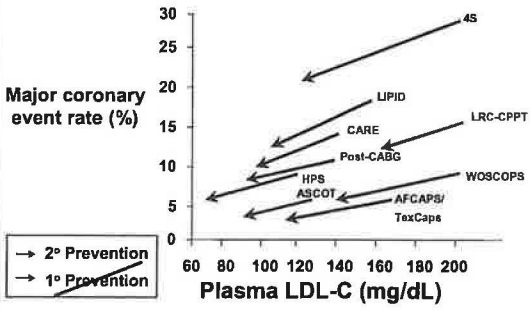



## Plasma LDL-Cholesterol & CHD Deaths

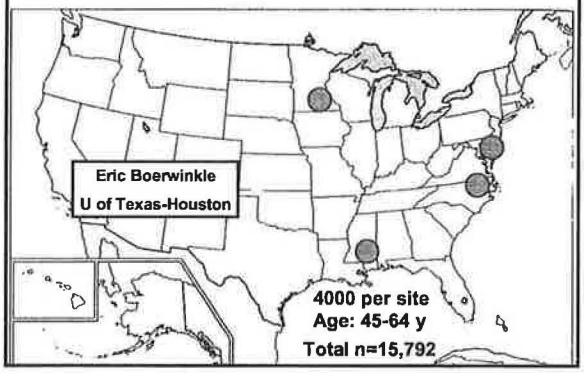



*J. Dietschy*

## Major LDL-Lowering Trials: ~20-30% Reduction in Coronary Events



## Relationship to CAD: Atherosclerosis Risk in Communities (ARIC): Initiated 1987




## Relationship to CAD: Atherosclerosis Risk in Communities (ARIC)

- Eligible: African-Americans: n=3,716
  Caucasians: n=10,045
- Mean age at baseline: 53 y
- Mean follow-up: 15 y
- Endpoints: Incident MI, CHD death, coronary revascularization

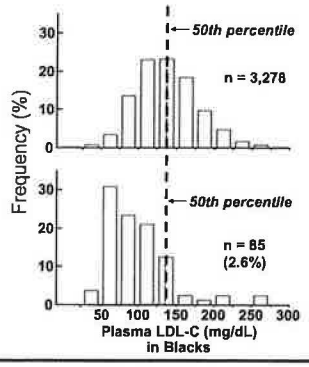
*Eric Boerwinkle, UT Houston*

## Cardiovascular Risk Factors in African-Americans in ARIC

| | Non-carriers | Carriers | P-value* | |
|---|---|---|---|---|
| n (%) | 3278 (97.4%) | 85 (2.6%) | | |
| Age† – yr | 53 ± 6 | 54 ± 6 | 0.61 | |
| Sex – % men | 37 | 31 | 0.22 | |
| BMI – kg/m² | 29.6 ± 6.1 | 29.5 ± 5.2 | 0.88 | |
| TC – mg/dL | 215 ± 44 | 173 ± 44 | <0.001 | |
| TG – mg/dL | 113 ± 81 | 94 ± 38 | 0.04 | |
| LDL-C–mg/dL | 138 ± 42 | 100 ± 43 | <0.001 | *28%* ↓ |
| HDL-C–mg/dL | 55 ± 17 | 55 ± 16 | 0.716 | |
| HTN (%) | 55 | 37 | 0.001 | |
| Diabetes (%) | 18 | 13 | 0.256 | |
| Smoking (%) | 30 | 27 | 0.625 | |

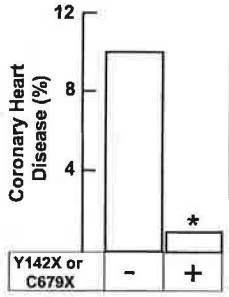
Hypertension: SBP>140; DBP>90, medication
Diabetes: FBS>126 mg/dL; NFBS>200 mg/dL

## Nonsense Mutations in PCSK9 is Associated with 28% Reduction in LDL-C in ARIC




## Cardiac Events in ARIC

HTN - 55%
Diabetes - 18%
Smoking - 30%




68 y old obese, hypertensive male smoker with a family history of CHD
LDL-C=53 mg/dL
Lp(a)>95th %

* P = 0.008

Hazards ratio = 0.11 (CI: 0.02-0.8, P=0.03)

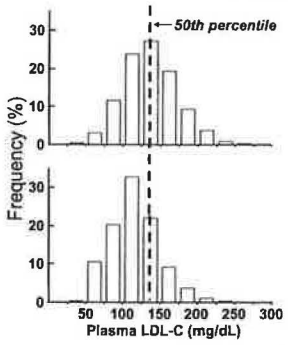
## Cardiovascular Risk Factors in Caucasians in ARIC

| | Non-carriers | Carriers | P-value* |
|---|---|---|---|
| n (%) | 9,223 | 301 (3.2%) | — |
| Age† – yr | 54±6 | 54±6 | 0.563 |
| Sex – % men | 45 | 46 | 0.839 |
| BMI – kg/m² | 26.9±4.9 | 26.8±4.5 | 0.509 |
| Cholesterol–mg/dL | 214±40 | 194±37 | <0.0001 |
| Triglyceride–mg/dL | 133±87 | 135±89 | 0.791 |
| LDL-C – mg/dL | 137±37 | 116±33 | <0.0001 |
| HDL-C – mg/dL | 51±17 | 52±17 | 0.636 |
| Hypertension‡ (%) | 25.0 | 24.6 | 0.872 |
| Diabetes§ (%) | 8.0 | 7.3 | 0.681 |
| Smoking¶ (%) | 24.6 | 25.2 | 0.804 |

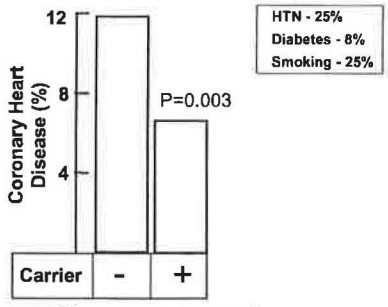
15%↓

Hypertension: SBP>140; DBP>90, medication
Diabetes: FBS>126 mg/dL; NFBS>200 mg/dL

## PCSK9-R46L Associated with 15% Reduction in LDL-C in Caucasians in ARIC




## Cardiac Events Reduced 2-fold in Caucasians with a Missense Mutation in *PCSK9*



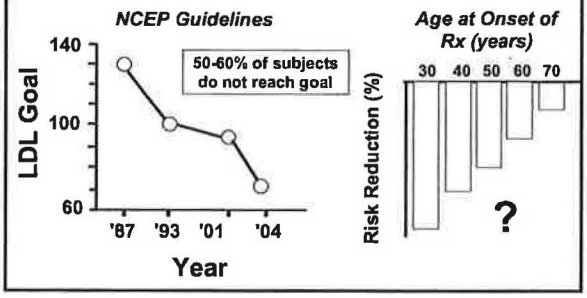

HR=0.5 (CI: 0.32-0.79, P=0.003)

## Mutations in *PCSK9* Associated with Reduced Carotid Atherosclerosis in *ARIC*

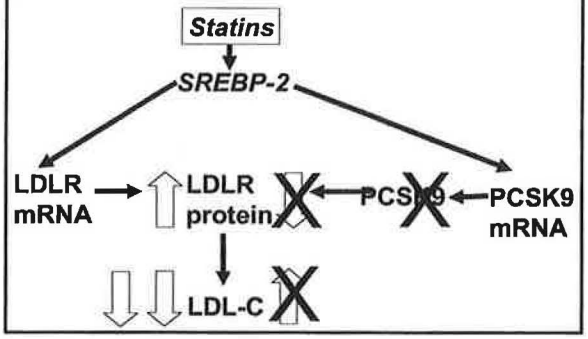
| | Non-carriers | Carriers | P value |
|---|---|---|---|
| **African-Americans:** | | | |
| Number | 3278 | 85 | |
| Percentage (%) | 97.4% | 2.6% | |
| Carotid IMT (mm) | 0.73±0.16 | 0.70±0.13 | **P<0.04** |
| **Caucasians:** | | | |
| Number | 9,223 | 301 | |
| Percentage (%) | 96.7% | 3.3% | |
| Carotid IMT (mm) | 0.73 ± 0.18 | 0.71 ± 0.16 | **P<0.005** |

## LDL Lowering Therapy

*A little too little* *A little too late*

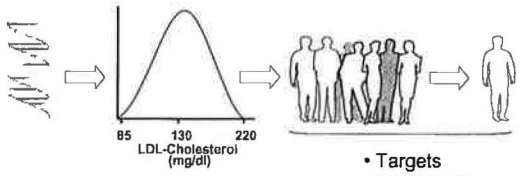



## PCSK9 Inhibitors: Magnifying the LDL-Lowering Effect of Statins




## Overview of General Strategy

GENES TRAIT PUBLIC HEALTH




- Targets
- Stratification
- Treatment

**HHMI - UT Southwestern**

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***Jay Horton***

Tom Legace

***Dallas Heart Study Investigators***

**ARIC**

Eric Boerwinkle

## BIBLIOGRAPHY


1. Abifadel M *et al.* Mutations in PCSK9 cause autosomal dominant hypercholesterolemia. *Nat. Genet.* **34**:154-6 (2003)
2. Benjannet, S *et al.* NARC-1/PCSK9 and its natural mutants: zymogen cleavage and effects on the LDLR and LDL-cholesterol. *J. Biol. Chem.* (2004).
3. Berge KE, Ose L, Leren TP. Missense mutations in the *PCSK9* gene are associated with hypocholesterolemia and possibly increased response to statin therapy. *Arterioscler. Thromb. Vasc. Biol., in press* (2006)
4. Brown MS, Goldstein JL. Biomedicine. Lowering LDL--not only how low, but how long? *Science* **311**:1721-3 (2006).
5. Brown SA *et al.* Plasma lipid, lipoprotein cholesterol, and apoprotein distributions in selected U.S. communities. *Arteriscler. Thromb.* **13:** 1139-1158 (1993).
6. Cameron J, Holla OL, Ranheim T, Kulseth MA, Berge KE, Leren TP. Effect of mutations in the *PCSK9* gene on the cell surface LDL receptors. *Hum. Mol. Genet.*, in press (2006)
7. Chen Z. *et al.* Serum cholesterol concentration and coronary heart disease in population with low cholesterol concentrations. *B.M.J.* **303:** 276-82 (1991).
8. Cohen JC, Pertsemlidis A, Marcel YL, McPherson R, Hobbs HH. Multiple rare alleles contribute to low plasma levels of HDL cholesterol. *Science* **305**: 869-872 (2004).
9. Cohen JC, Pertsemlidis A, Kotowski IK, Graham R, Garcia CK, Hobbs HH. Low LDL cholesterol in individuals of African descent resulting from frequent nonsense mutations in PCSK9. *Nat. Genet.* **37**: 161-165 (2005).
10. Cohen JC, Boerwinkle E, Mosley TH & Hobbs HH. Sequence variations in PCSK9, low LDL, and protection against coronary heart disease. *N. Engl. J. Med.* **354**: 34-42 (2006).
11. Dubuc G, Chamberland A, Wassef H, Davignon J, Seidah NG, Bernier L, Prat A. Statins upregulate PCSK9, the gene encoding the proprotein convertase neural apoptosis-regulated convertase-1 implicated in familial hypercholesterolemia. *Arterioscler. Thromb. Vasc. Biol.* **24**: 1454-9 (2004).
12. Kotowski IK, Pertsemlidis A, Luke A, Cooper RS, Vega GL, Cohen JC, Hobbs HH. A spectrum of PCSK9 alleles contributes to plasma levels of low-density lipoprotein cholesterol. *Am. J.Hum. Genet.* **78:** 410-422 (2006).
13. Lalanne F. *et al.* Wild-type PCSK9 inhibits LDL clearance but does not affect apoB-containing lipoprotein production in mouse and cultured cells. *J. Lipid Res.* **46**: 1312-9 (2005).
14. Law MR, Wald NJ & Rudnicka AR. Quantifying effect of statins on low density lipoprotein cholesterol, ischaemic heart disease, and stroke: systematic review and meta-analysis. *B.M.J.* **326**: 1423 (2003).

15. Leren TP. Mutations in the PCSK9 gene in Norwegian subjects with autosomal dominant hypercholesterolemia. *Clin. Genet.* **65**:419-22. (2004)

16. Maxwell KN & Breslow JL. Adenoviral-mediated expression of Pcsk9 in mice results in a low-density lipoprotein receptor knockout phenotype. *Proc. Natl. Acad. Sci. U.S.A* **101**: 7100-5 (2004).

17. Maxwell KN, Soccio RE, Duncan EM, Sehayek E & Breslow JL Novel putative SREBP and LXR target genes identified by microarray analysis in liver of cholesterol-fed mice. *J. Lipid Res.* **44**: 2109-19 (2003).

18. McGill HC, Jr. & McMahan CA. Determinants of atherosclerosis in the young. Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. *Am. J. Cardiol.* **82**: 30T-36T (1998).

19. Naoumova RP, Tosi I, Patel D, Neuwirth C, Horswell SD, Marais AD, van Heyningen C, Soutar AK. Severe hypercholesterolemia in four British families with the D374Y mutation in the PCSK9 gene: long-term follow-up and treatment response. *Arterioscler. Thromb. Vasc. Biol.* **25**:2654-60 (2005)

20. Park SW, Moon YA & Horton JD. Post-transcriptional regulation of LDL receptor protein by proprotein convertase subtilisin/kexin type 9a (PCSK9) in mouse liver. *J. Biol. Chem.* **279**: 50630-38 (2004).

21. Rashid S, Curtis DE, Garuti R, Anderson NN, Bashmakov Y, Ho YK, Hammer RE, Moon YA, Horton JD. Decreased plasma cholesterol and hypersensitivity to statins in mice lacking Pcsk9. *Proc. Natl. Acad. Sci. U.S.A.* **102**: 5374-9 (2005).

22. Seidah NG, Benjannet S, Wickham L, Marcinkiewicz J, Jasmin SB, Stifani S, Basak A, Prat A, Chretien M. The secretory proprotein convertase neural apoptosis-regulated convertase 1 (NARC-1): Liver regeneration and neuronal differentiation. *Proc. Natl. Acad. Sci. U. S. A.* **100**: 928-33 (2003).

23. Stamler J, Wentworth D & Neaton JD. Is relationship between serum cholesterol and risk of premature death from coronary heart disease continuous and graded? Findings in 356,222 primary screenees of the Multiple Risk Factor Intervention Trial (MRFIT). *J.A.M.A.* **256:** 2823-8 (1986).

24. Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* **106**: 3143-421 (2002).

25. Timms KM, Wagner S, Samuels ME, Forbey K, Goldfine H, Jammulapati S, Skolnick MH, Hopkins PN, Hunt SC, Shattuck DM. A mutation in PCSK9 causing autosomal-dominant hypercholesterolemia in a Utah pedigree. *Hum. Genet.* **114**:349-53 (2004)