

# Replication of Linkage to High Density Lipoprotein Cholesterol in the NHLBI Family Heart Study (FHS)

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# Background

- High-density lipoprotein cholesterol (HDL-c) is a strong independent predictor of Coronary Heart Disease (CHD)
- HDL-c is also a predictor of asymptomatic atherosclerosis
- Environmental predictors do not fully explain the total phenotypic variance of HDL-c

# Heritability

- Heritability of HDL-c estimated to be near 50%
- $h^2$  = proportion of variance due to familial resemblance
- Approx. 30 studies since 1977
  - $n = 210-5096$  individuals
  - $h^2$  estimates range .24-.83
- Mostly male, Caucasian
- Some covariate adjustment
- Largest studies  $h^2$  estimates range .40-.60

# Candidate Genes

- Major Genetic Components
  - Apolipoprotein A-I
  - Apolipoprotein A-II
  - LCAT (Lecithin-Cholesterol Acyltransferase)
  - CETP (Cholesteryl ester transfer protein)
  - Lipoprotein Lipase
  - ABC-1 (ATP Binding Cassette)
  - Hepatic Lipase

\* Explain less than 2% of variation in HDL-c

# Genetic + Environmental

- HDL-c is a complex trait and is likely determined by multiple environmental and genetic factors
- Genetic component of variance appears to contribute most to total phenotypic variance

# Locating Genes

- Anonymous Marker Linkage

- Localize genomic regions linked to genetic variability of HDL-c
- Hypothesis-generating exercise
- Our published results – Caucasians (ATVB, 11 /2001)
  - Chromosome 5p (39.9 cM, LOD = 3.64)
  - Chromosome 13p (27.5 cM, LOD = 2.36)
- 3 other Genome-wide scans of HDL-c
  - Arya et al. (2002) – Mexican-Americans, 9p, LOD = 3.40
  - Klos et al. (2001) – Caucasians, no strong evidence
  - Newman et al. (2000) – Hutterites, 5p & 20p,  $p = 0.002$

# Objective

- To replicate findings from a previous genome-wide anonymous marker scan for quantitative trait loci influencing variation in HDL-cholesterol

# Subjects

- Family Heart Study (FHS)
  - Multicenter Study with participants recruited from 4 established population-based cohorts
    - Framingham Heart Study (MA)
    - Utah Health Family Tree Study
    - Atherosclerosis Risk in Communities Study
      - Minneapolis, MN & Forsyth County, NC
  - All spouses, siblings, parents, and children over age 25 asked to participate

# Phenotyping

- Clinical Examination
  - Anthropometrics, blood pressure, pulmonary function, carotid artery ultrasound, electrocardiogram, blood draw (299 blind replicates, HDL-c assay reliability = .98)
  - Questionnaires assessed lifestyle, medication use, exercise, food frequency intake, and psychosocial factors
  - 5975 participants

# Genotyping

NHLBI Mammalian Genotyping Service (MGS)

383 autosomal markers (CHLC10), ~ 9.5 cM apart

Heterozygosity = 76%

Largest FHS pedigrees genotyped

Original Sample (1,027 individuals, 101 pedigrees)

Replication Sample (1,986 individuals, 300 pedigrees)

# Methods

- HDL-c adjustment
  - Standardized residual HDL-c phenotype
  - Sex-specific models
  - Age, age<sup>2</sup>, field center, BMI

# Analysis Package

- GENEHUNTER version 2
  - Multipoint IBD
  - 4 estimates between markers
  - Phenotypic variance decomposition
    - Quantitative trait locus (QTL)
    - Residual polygenic
    - Residual environmental
    - Additive and dominance variance estimation
  - LOD Score calculated as the difference of the ML estimates when 1 ) modelling a QTL and 2) constraining the QTL to zero

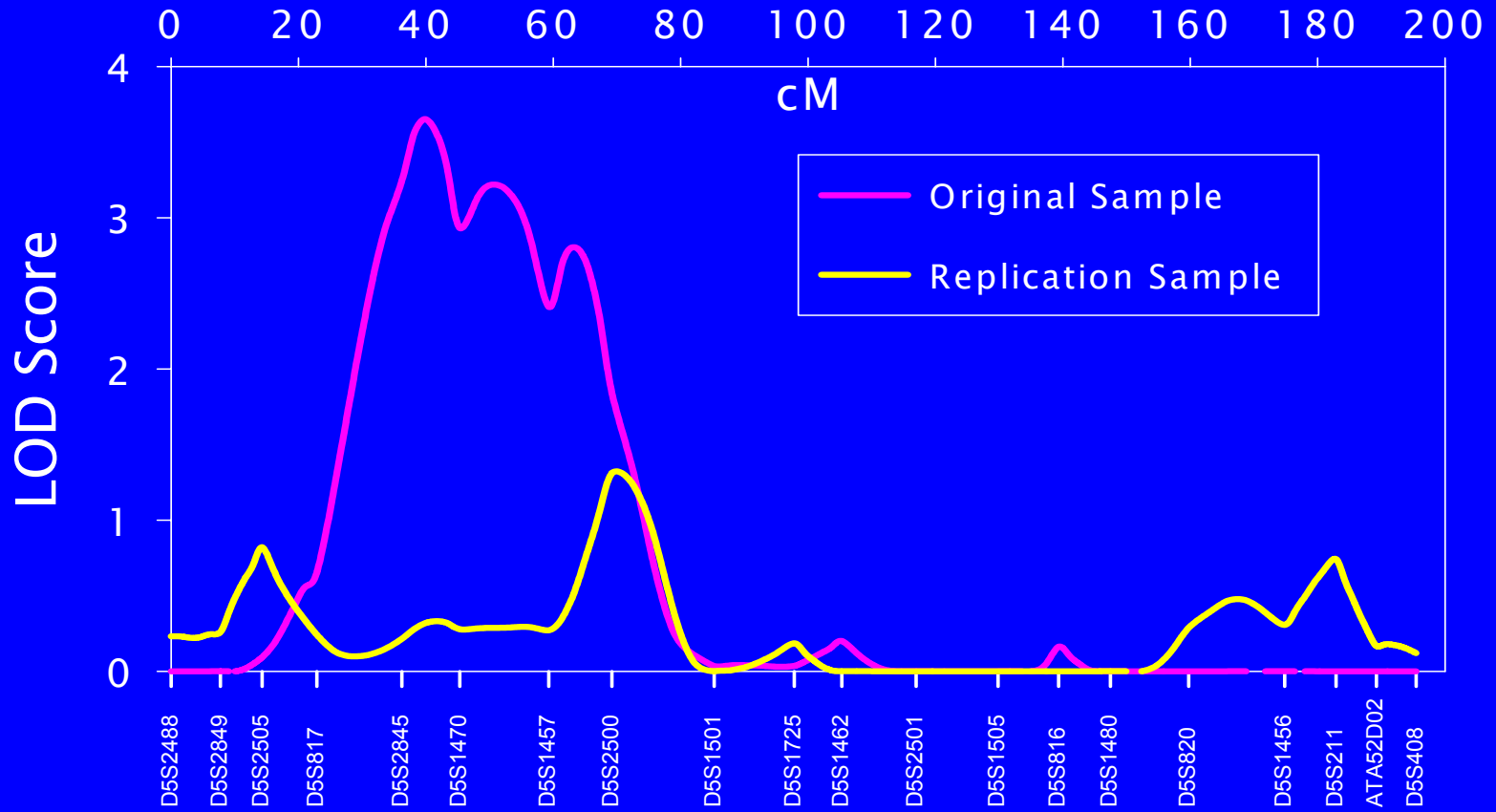
# Results

## Distribution of Environmental Risk Factors

<b>Characteristic</b>	<b>Men (n=803)</b>	<b>Women (n=966)</b>
HDL-c (mg/dL)	43.1 (11.1)	55.9 (14.6)
Age (yrs)	51.5 (14.4)	52.4 (13.8)
BMI (kg/m <sup>2</sup> )	27.9 (4.6)	26.8 (5.7)

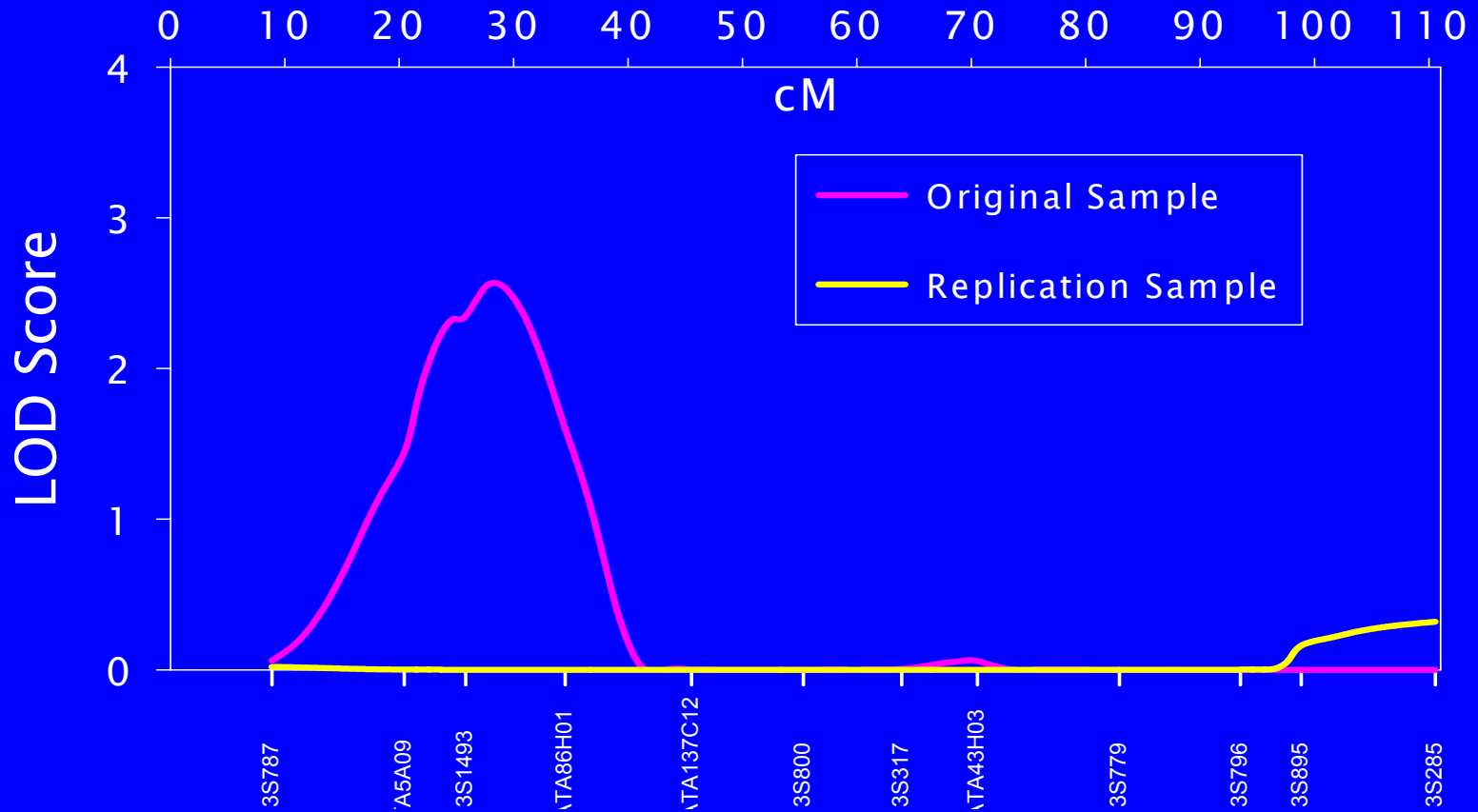
# MGS Scan Results

## Chromosome 5



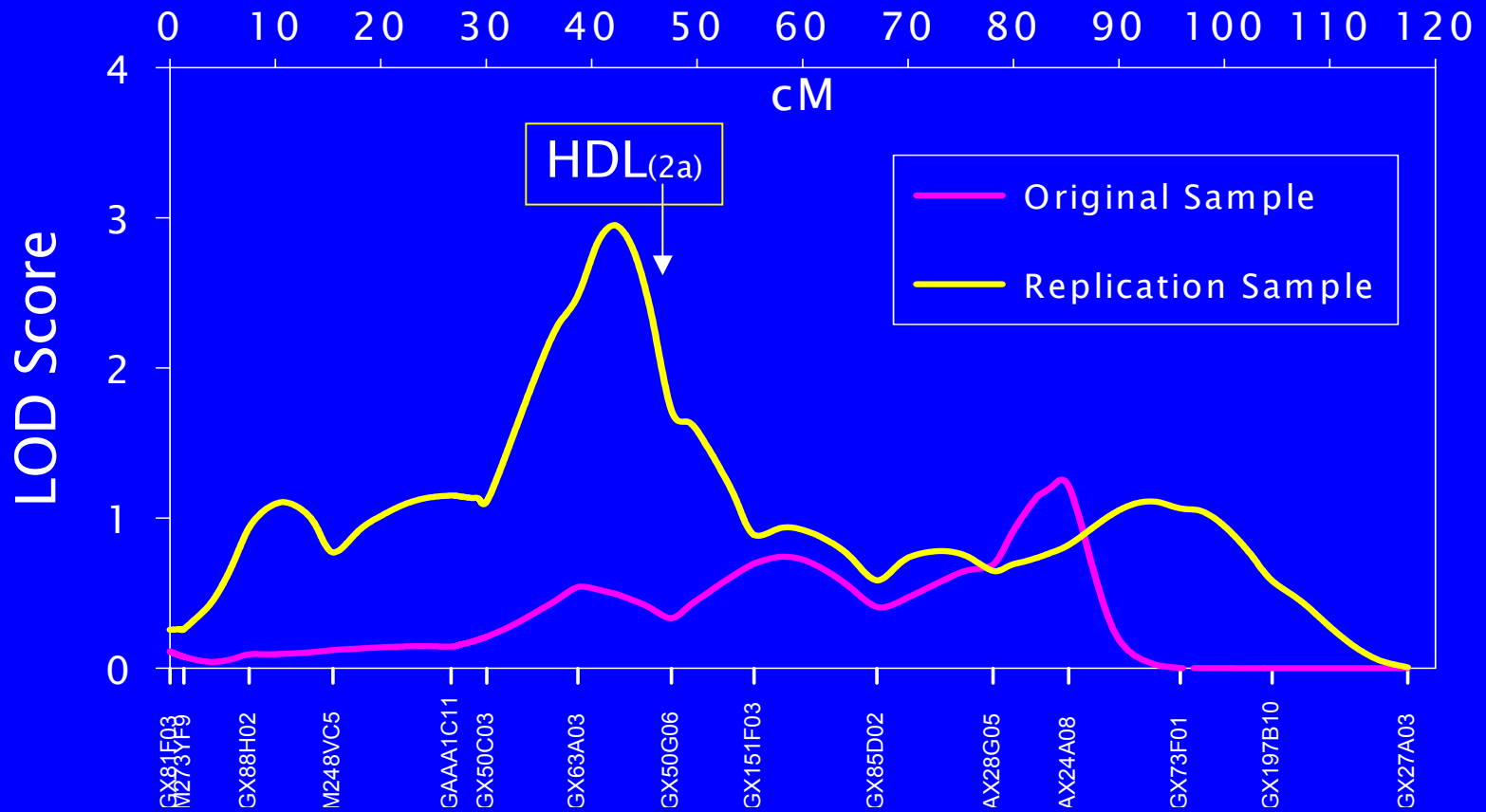
# MGS Scan Results

## Chromosome 13



# MGS Scan Results

## Chromosome 15



# Summary

Chromosomal locations suggestive of linkage to HDL-c

Original Sample		Replication Sample	
Location (cM)	LOD Score	Location (cM)	LOD Score (p-value)
1 - 108	1.59	1 - 98	0.12 (.75)
4 - 181	1.33	4 - 182	0.52 (.30)
<b>5 - 40*</b>	<b>3.64</b>	<b>5 - 70</b>	<b>1.31 (&lt;.05)</b>
6 - 127	1.49	6 - 141	0.62 (.24)
8 - 78	1.33	8 - 82	0.75 (.18)
13 - 28	2.36	13 p arm	0
		12 - 109*	1.38
		<b>15 - 47*</b>	<b>2.95</b>

\*Evidence from other studies

# Conclusions (1)

- At least one genomic region may harbor a gene that significantly influences variation in HDL-c levels (LOD = 3.64, D5S1470, 40 cM)
- Confirmation of linkage on Chromosome 5p with Replication Sample scan *and* overlap with a region found in Alberta Hutterites
- No overlap with other published reports
- Suggestive linkage (LOD = 2.95) from Replication Sample on Chromosome 15p close to a report of significant linkage to HDL<sub>2a</sub> in Mexican-Americans

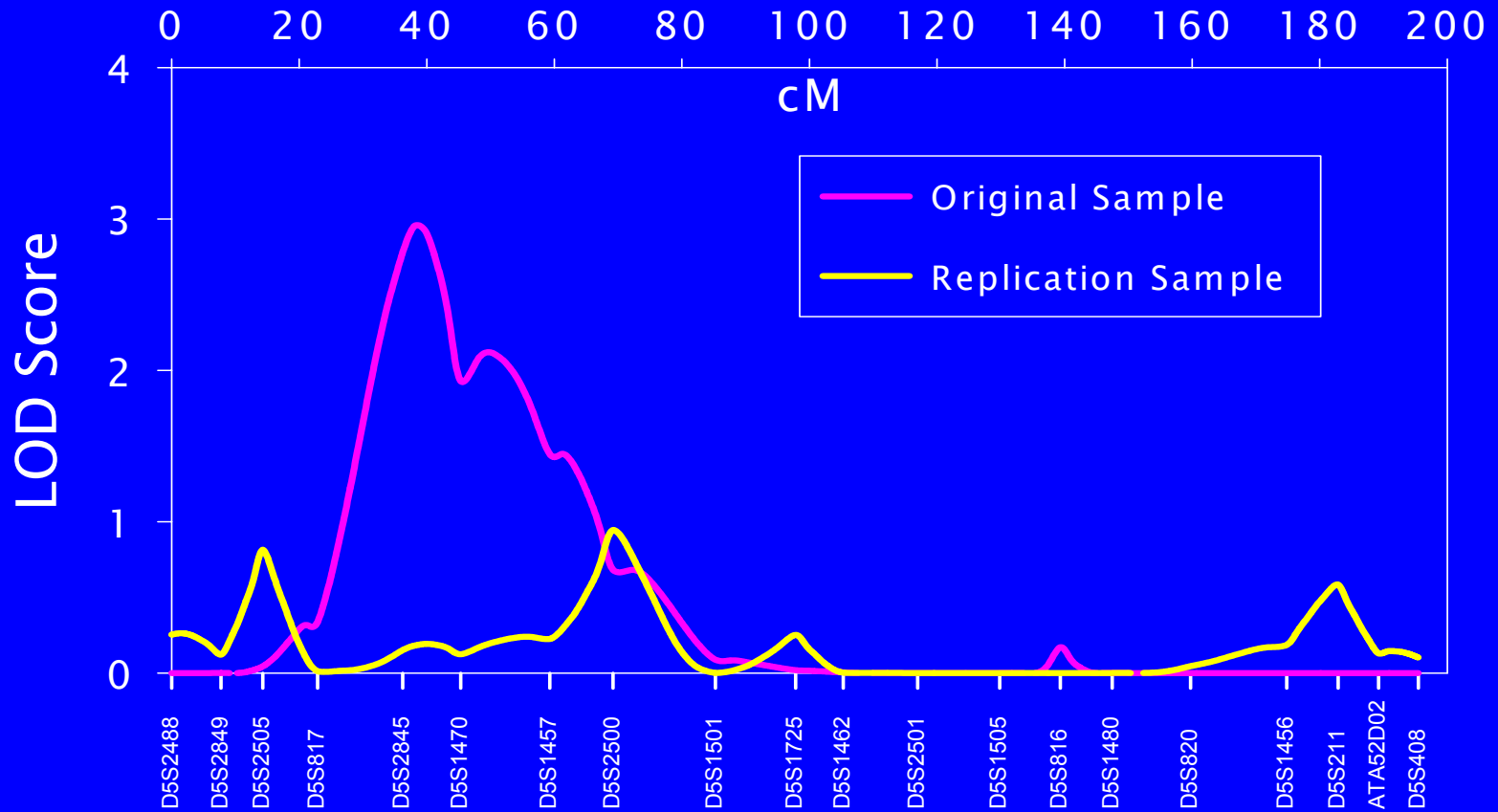
# Conclusions (2)

- Other 'suggestive' LOD scores indicate multiple regions that may influence HDL-c
- No candidate genes related to lipid metabolism in this region on Chromosome 5 have been identified
- Replication of results in different populations needed
- Bivariate linkage analysis may provide insight into biologic mechanisms influencing HDL-c variability

# Supplementary Figures

# MGS Scan Results – Additive

Chromosome 5



# MGS Combined Results

## Chromosome 5

