

Genetic HDL Disorders

disease	gene	frequency
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Hypoalphalipoproteinemia

ABCA1 > 100 mutations

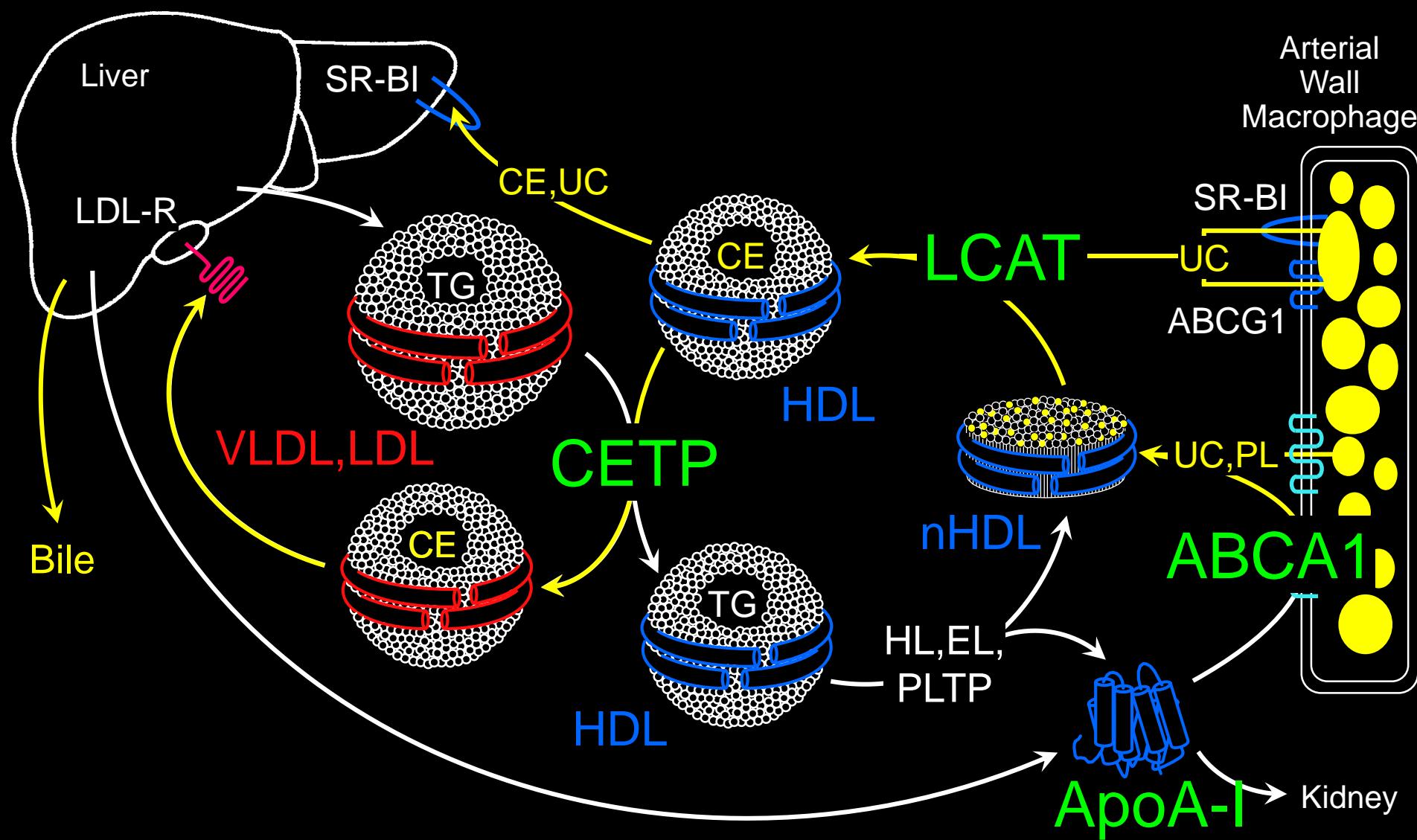
ApoA-I ~ 65 mutations

LCAT ~ 100 mutations

Hyperalphalipoproteinemia

CETP ~ 25 mutations

HDL Metabolism and Reverse Cholesterol Transport



Criteria for selection of primary hypoalphalipoproteinemia

- HDL-C <10th percentile for age and gender
- Absence of secondary causes of HA
- High likelihood of inherited low HDL-C (primary low HDL-C in at least one first-degree family member)

The causes of a low plasma HDL cholesterol are shown in the following:

Primary

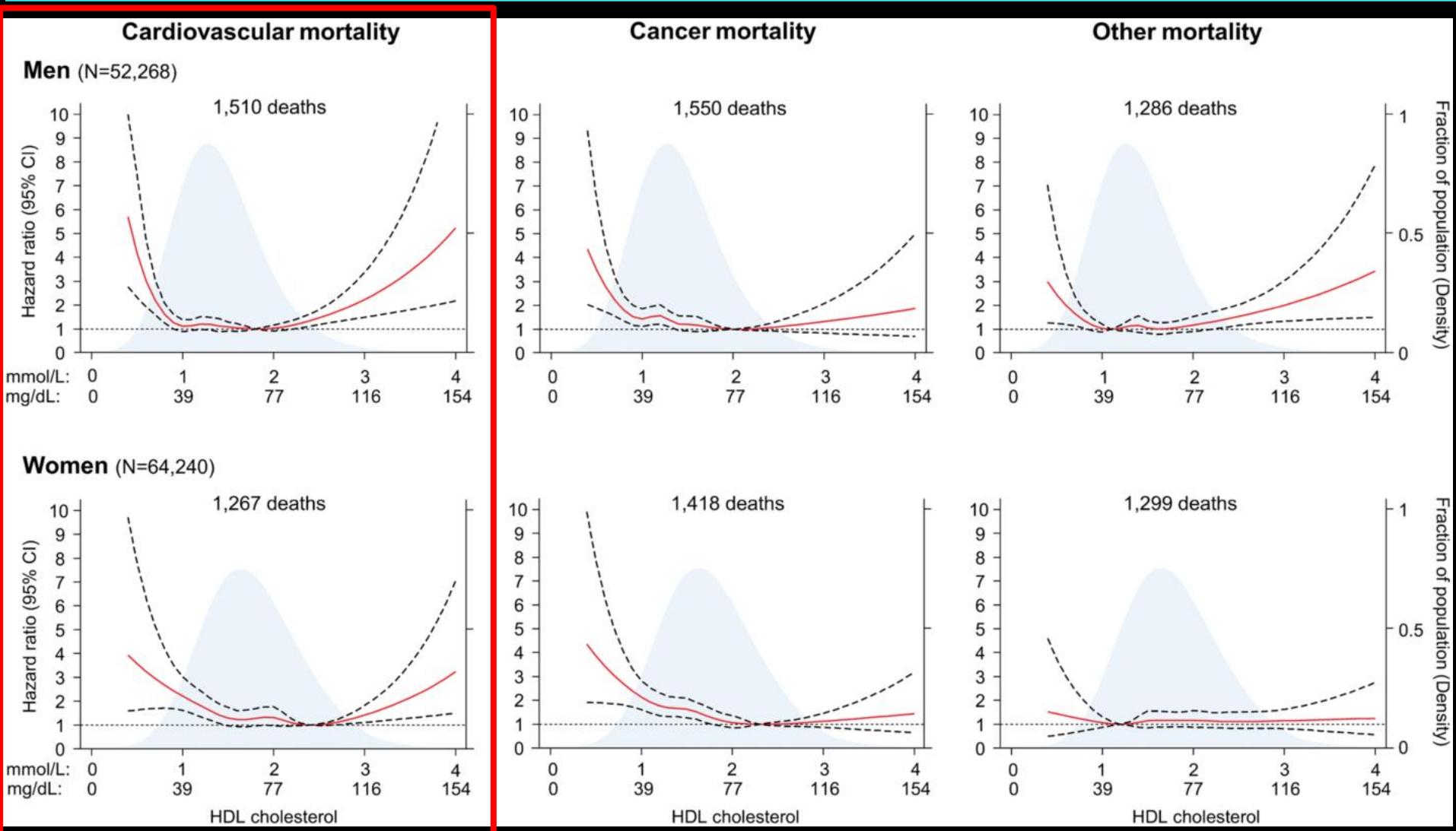
- *Familial hypoalphalipoproteinaemia*
- *ApoA abnormalities*
- *Tangier's disease*
- *Lecithin–cholesterol acyltransferase(LCAT) deficiency*
- *Fish-eye disease*

Secondary

- *Tobacco smoking*
- *Obesity*
- *Poorly controlled diabetes mellitus*
- *Insulin resistance and metabolic syndrome*
- *Chronic kidney disease*
- *Certain drugs, e.g. testosterone, probucol, β -blockers (without intrinsic sympathomimetic activity), progestogens, anabolic steroids, bexarotene.*

HDL-C and Cardiovascular Mortality

Copenhagen Studies



Monogenic HDL Disorders

Apolipoprotein A-I deficiency (OMIM 107680)

Tangier Disease (OMIM 205400)

LCAT Deficiency Syndromes

Familial LCAT deficiency (FLD) (OMIM 245900)

Fish Eye Disease (FED) (OMIM 136120)

CETP deficiency (OMIM 607322)

Apolipoprotein A-I Gene Mutations Phenotypes

No phenotype	21 mutations
Low HDL	41 mutations
Amyloidosis	16 mutations

Apolipoprotein A-I Gene Mutations Phenotypes

No phenotype

21 mutations

Low HDL

41 mutations

Amyloidosis

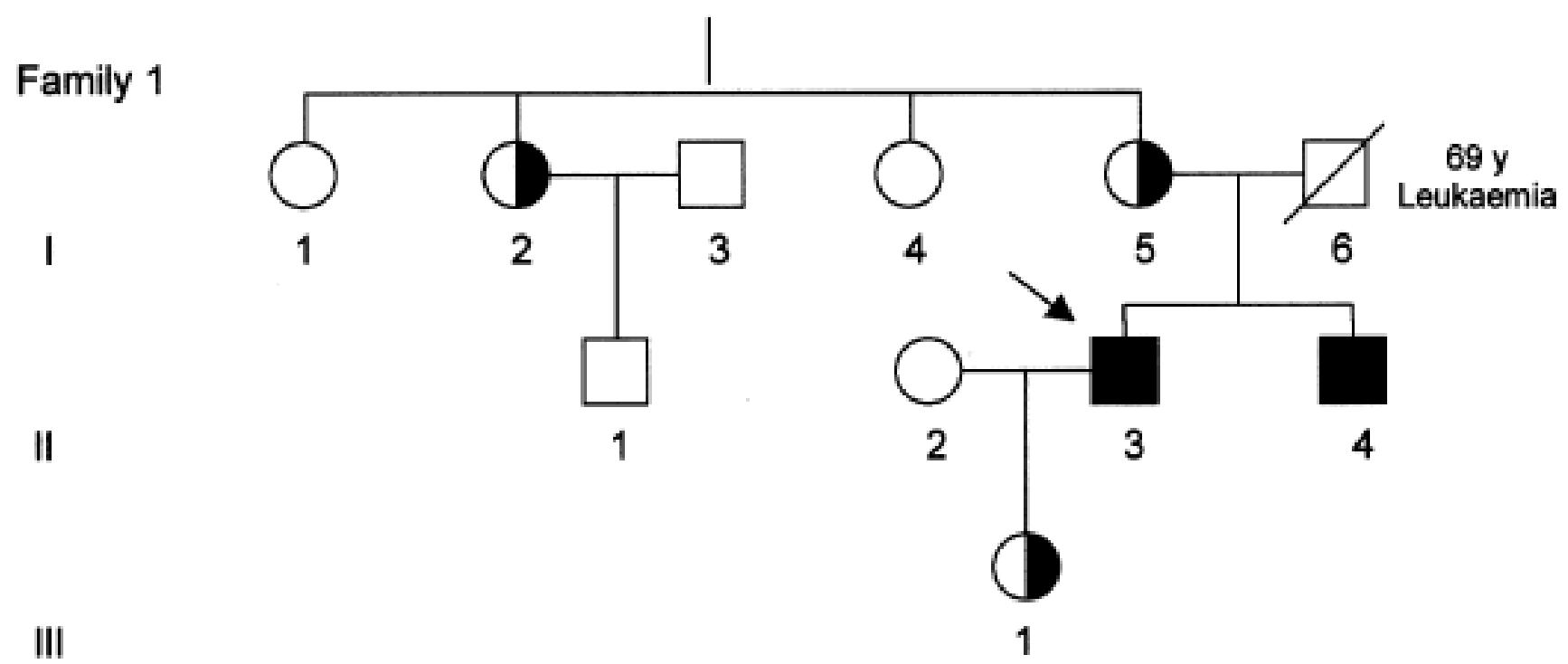
16 mutations

Apolipoprotein A-I Gene Mutations

Low-HDL Phenotype

Missense/nonsense	18
Splicing	1
Small deletions	14
Small insertions	3
Gross deletions	2
Gross insertions	1
Complex rearrangements	2
Repeat variations	0
TOTAL	41

ApoA-I Q5FsX11



II-3: planar xanthomas, corneal opacity, angina, carotid plaques
HDL-C, 8 mg/dl; apoA-I, 0 mg/dl

II-4: planar xanthomas, corneal opacity
HDL-C, 6 mg/dl; apoA-I, 0 mg/dl

Low-HDL ApoA-I Variants

D₁ EPPQSP**WDRVKDLATVYVDVLKDSGRDYVSQFEGS**₃₆

A₃₇ LGKQLNLKLL₄₇

DNWDHSVTS**TFSKLREQLG**₆₅

P₆₆ VTQE**FWDNLEKETEGLRQEMS**₈₇

K₈₈ DLEEV**KAKVQ**₉₈

P₉₉ YLDDF**QKKWQEEMELYRQKVE**₁₂₀

P₁₂₁ LRAEL**QEGARQKLHELQEKL**S₁₄₂

P₁₄₃ LGEEM**RDRARAHVDALRTHLA**₁₆₄

P₁₆₅ YSDEL**RQRLAARLEALKENG**G₁₈₆

A₁₈₇ RLAEYHAKATEHLSTLSEKAK₂₀₈

P₂₀₉ ALEDLRQG**LL**₂₁₉

P₂₂₀ VLESFKV**SFLSALEEYTKKLN**₂₄₁

TQ₂₄₃

helix 1

helix 2

helix 3

helix 4

helix 5

helix 6

helix 7

helix 8

helix 9

helix 10

Low-HDL ApoA-I Variants

D₁ EPPQSPWDRVKDLATVYVDVLKD**S**GRDYVSQFEGS₃₆
A₃₇ LGKQLNLKLL₄₇

DNWDSVTSTFSKLREQLG₆₅ helix 1

P₆₆ VTQEFWDNLEKETEGLRQEMS₈₇ helix 2

K₈₈ DLEEV**V**AKVQ₉₈ helix 3

P₉₉ YLDDFQKKWQEEMELYRQKVE₁₂₀ helix 4

P₁₂₁ LRAELQEGARQKLHEL**Q**EKLS₁₄₂ helix 5

P₁₄₃ LGEEMRDRARAHV**D**ALRTHLA₁₆₄ helix 6

P₁₆₅ YSDEL**R**Q**R**LAAR**R**EA**L**ALKENG**G**₁₈₆ helix 7

A₁₈₇ RLA**E**YHAKATEHLSTL**S**E**K**AK₂₀₈ helix 8

P₂₀₉ ALEDLRQG**LL**₂₁₉ helix 9

P₂₂₀ VLESFKV**S**FLSALEEEYT**KK**LN₂₄₁ helix 10

TQ₂₄₃

Cys

Pro

Low-HDL ApoA-I Variants

Demographics

	R173C	L178P
Males/Females	11/10	32/22
Age (y)	42.2 ±17.5	37.6 ±19.2
BMI (kg/m ²)	25.1 ± 2.7	24.9 ± 5.0
Hypertension (%)	19.0	7.4
Smokers (%)	33.3	17.6

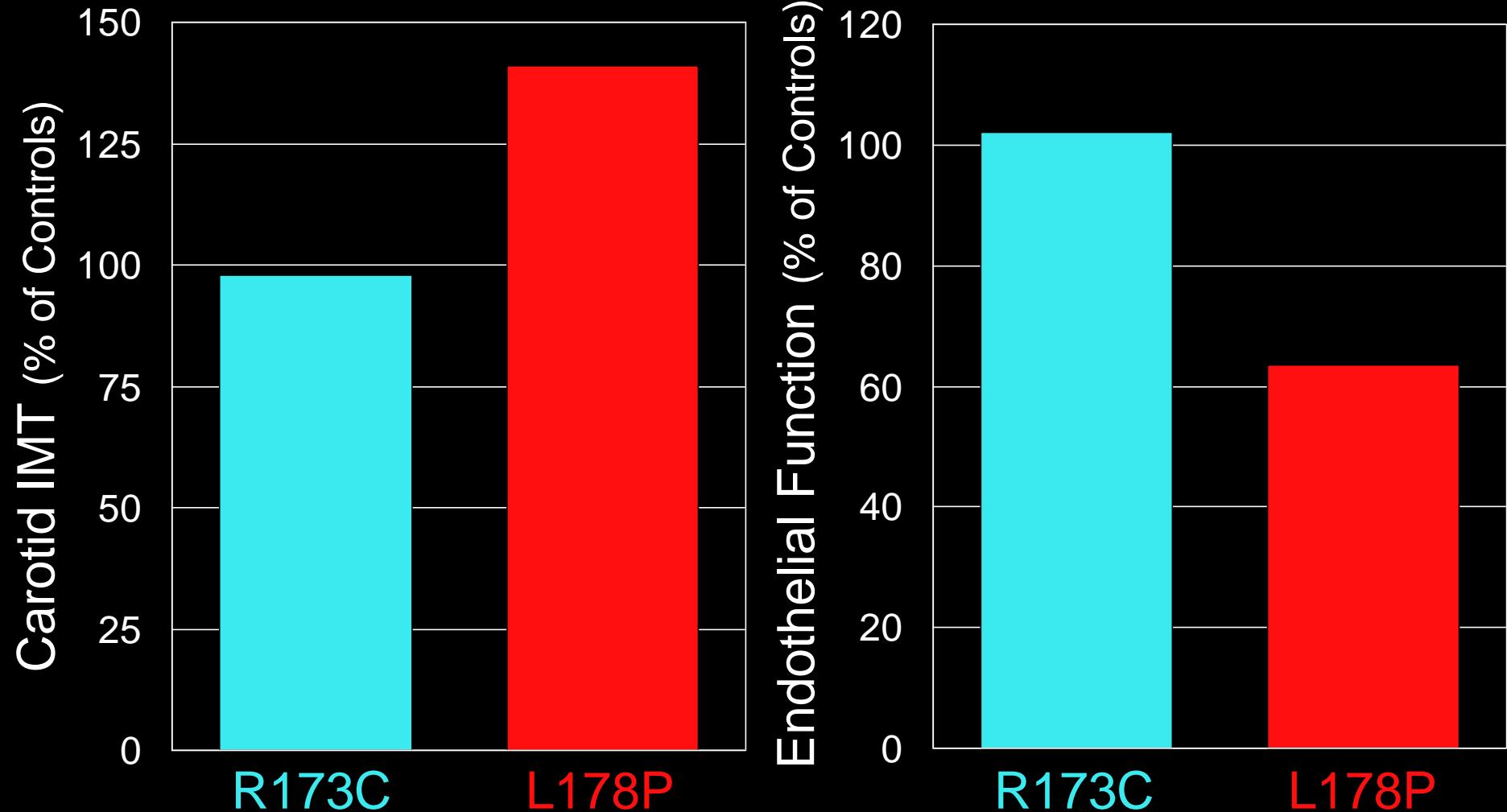
Low-HDL ApoA-I Variants

Plasma Lipids

	R173C	L178P
		mg/dl
Total Cholesterol	182 ±45	162 ±41
LDL Cholesterol	137 ±36	122 ±36
Apolipoprotein B	103 ±35	99 ±26
Triglycerides	188 ±93	111 ±75
HDL Cholesterol	18 ± 8	17 ± 8
Apolipoprotein A-I	78 ±28	70 ±33

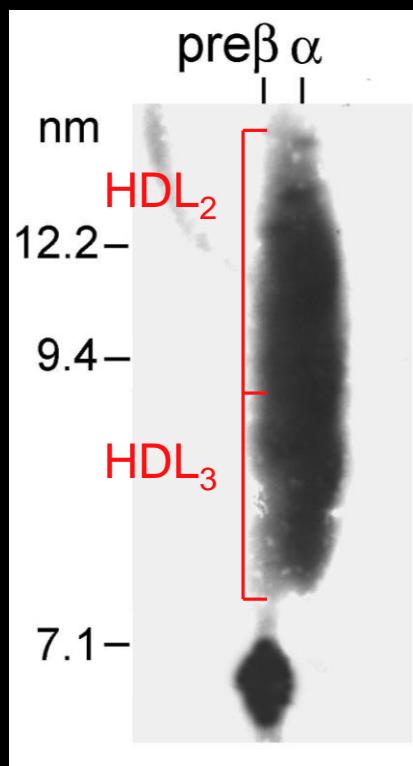
Low-HDL ApoA-I Variants

Vascular Structure and Function

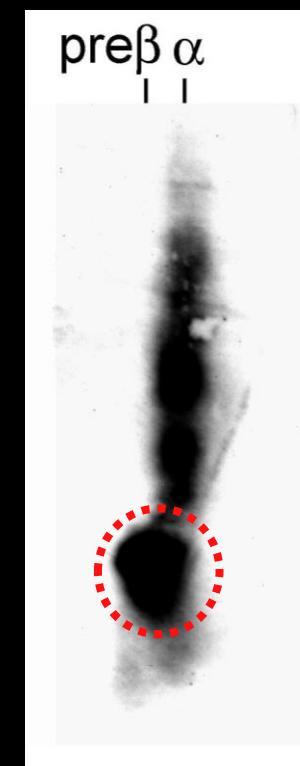
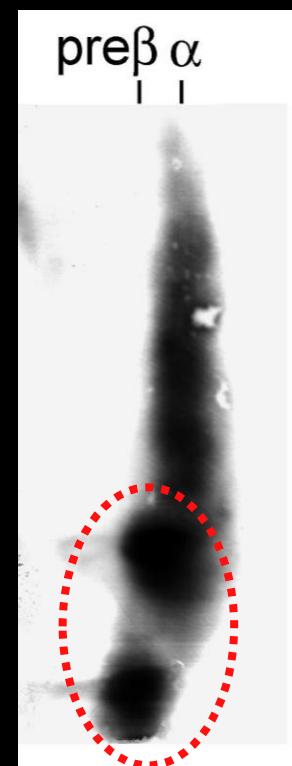


A-I_{Milano} Carriers HDL Subpopulations

Anti apoA-I



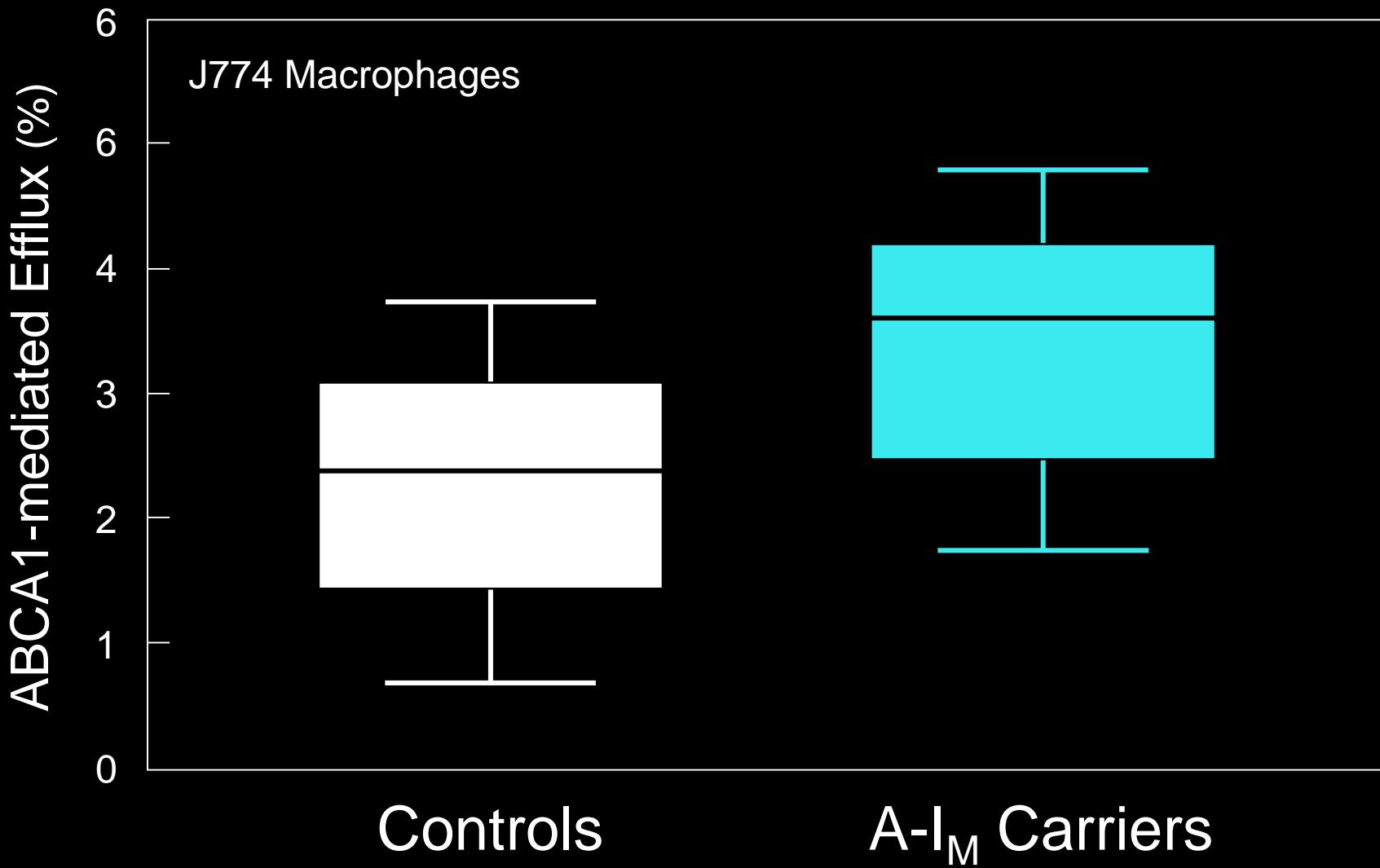
Anti apoA-I Anti AI_M-AI_M



Control

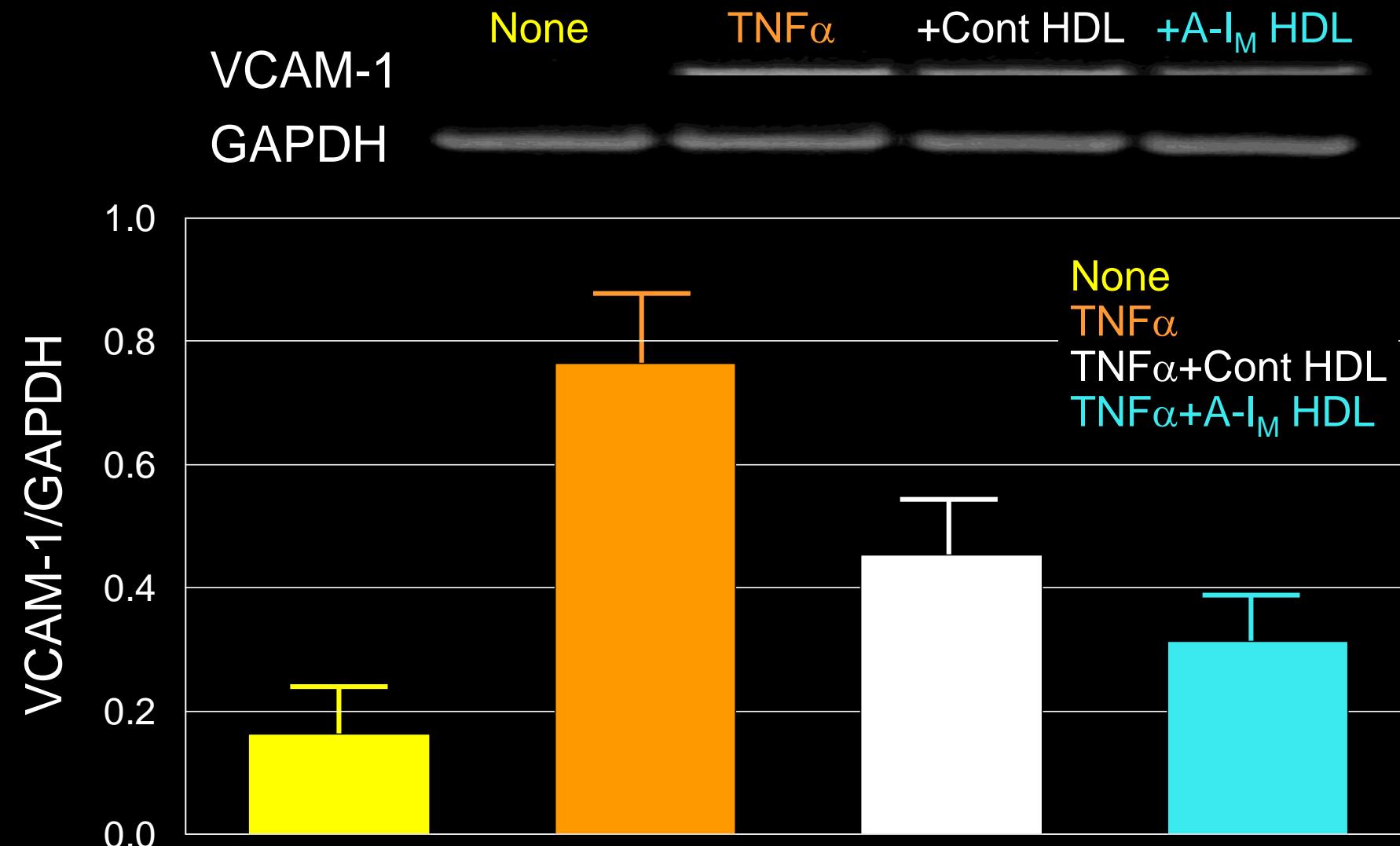
A-I_M Carrier

Cell Cholesterol Efflux to A-I_M Serum ABCA1-mediated Efflux



Vascular Function in A-I_{Milano} Carriers

Inhibition of VCAM-1 Expression by HDL



Monogenic HDL Disorders

Apolipoprotein A-I deficiency (OMIM 107680)

Tangier Disease (OMIM 205400)

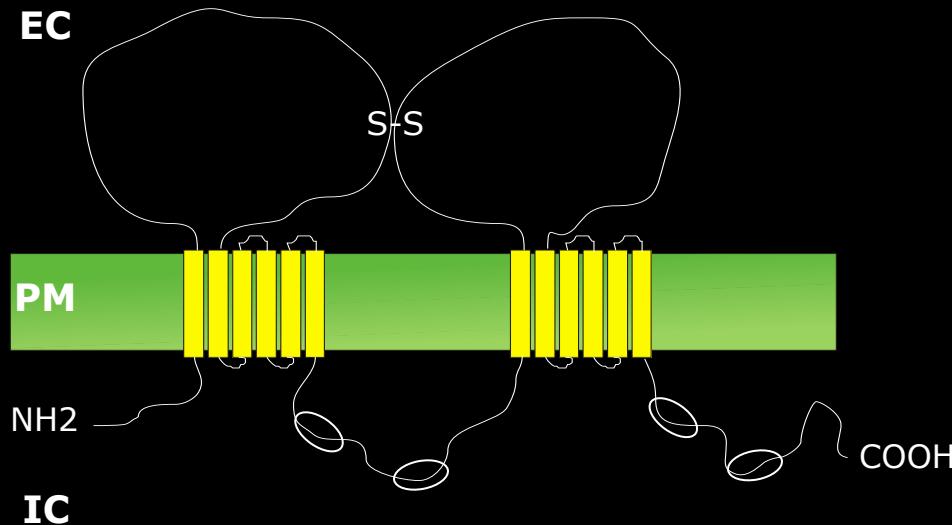
LCAT Deficiency Syndromes

Familial LCAT deficiency (FLD) (OMIM 245900)

Fish Eye Disease (FED) (OMIM 136120)

CETP deficiency (OMIM 607322)

ABCA1 Protein



- 2261 amino acids
- 2 transmembrane domains
- 2 large extracellular loops
- C-terminal and N-terminal cytoplasmic domains

Mutations in ABCA1 may cause:

- traffic defect to the PM;
- impaired binding to ApoAI;
- efflux defect;
- impaired 3D folding

Tangier Disease

Clinical manifestations

- orange tonsils
- peripheral neuropathy
- cloudy cornea
- hepatosplenomegaly
- premature CVD

Carriers of ABCA1 mutations

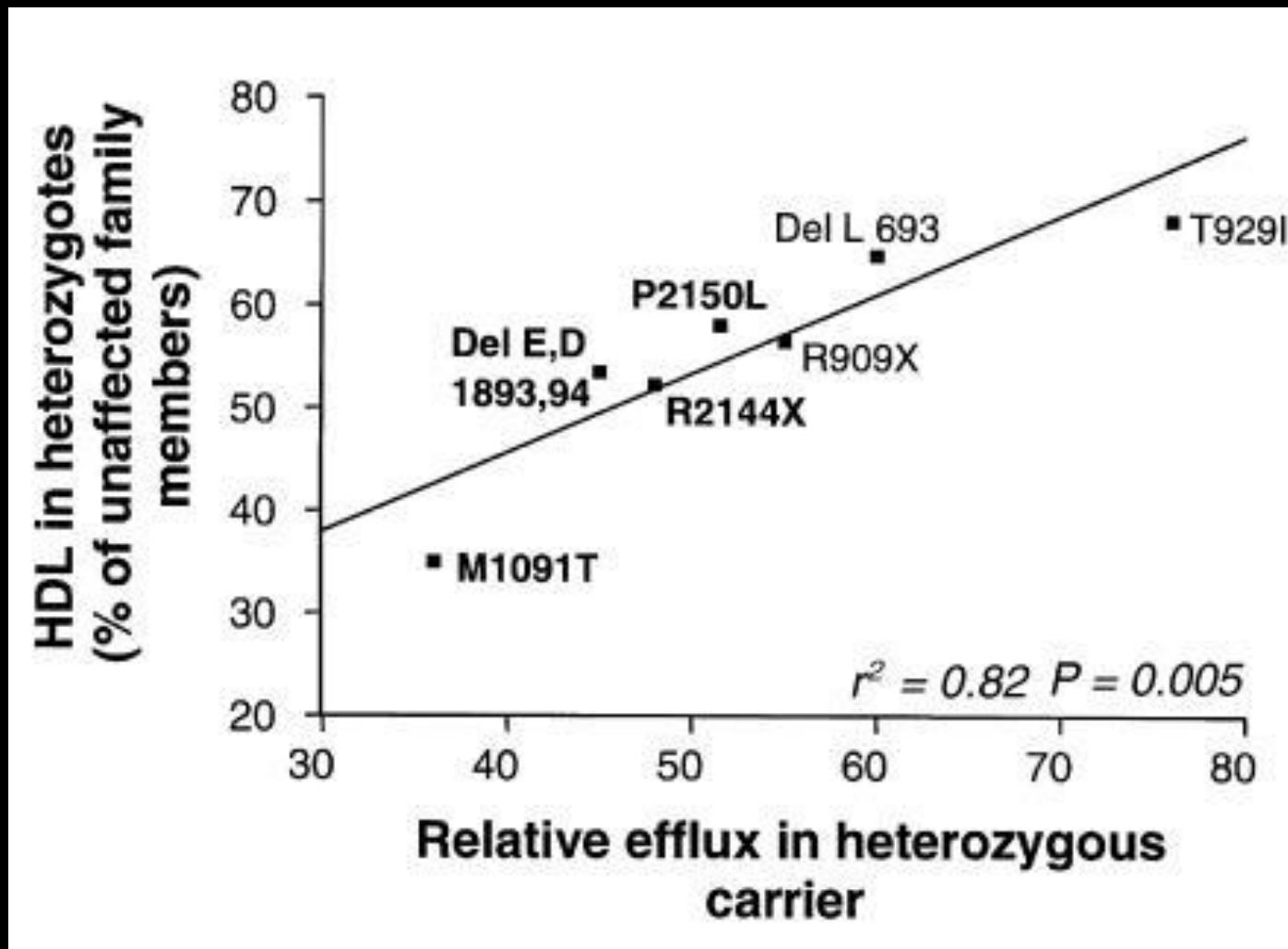
Lipid profile

	ABCA1 Heterozygotes	Normal relatives	P
N.	71M, 82F	47M, 66F	NS
Age (y)	43.7 ± 21.2	41.8 ± 20.1	NS
TC (mg/dl)	172.1±42.0	199.2±42.1	0.0001
LDL-C (mg/dl)	111.9±37.1	125.0±38.6	0.007
HDL-C (mg/dl)	29.7±10.4	51.7±13.1	0.0001
TG (mg/dl)	142.4±109.6	104.0±48.0	0.001
Apo A-I (mg/dl)	100.8 ± 24.4	135.8 ± 23.2	0.0001

Carriers of ABCA1 mutations

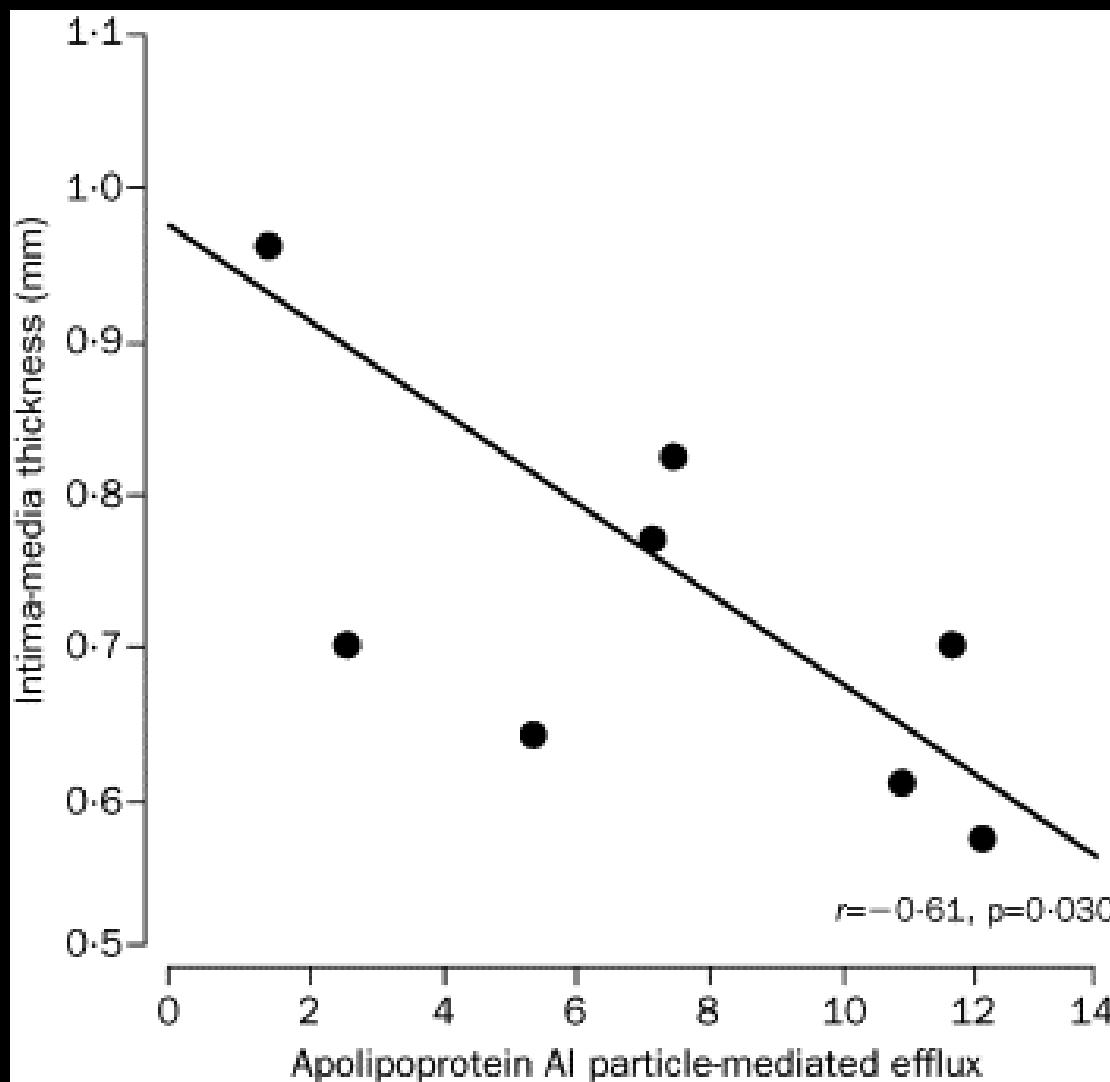
Cholesterol efflux

Carrier fibroblasts, apoA-I as acceptor



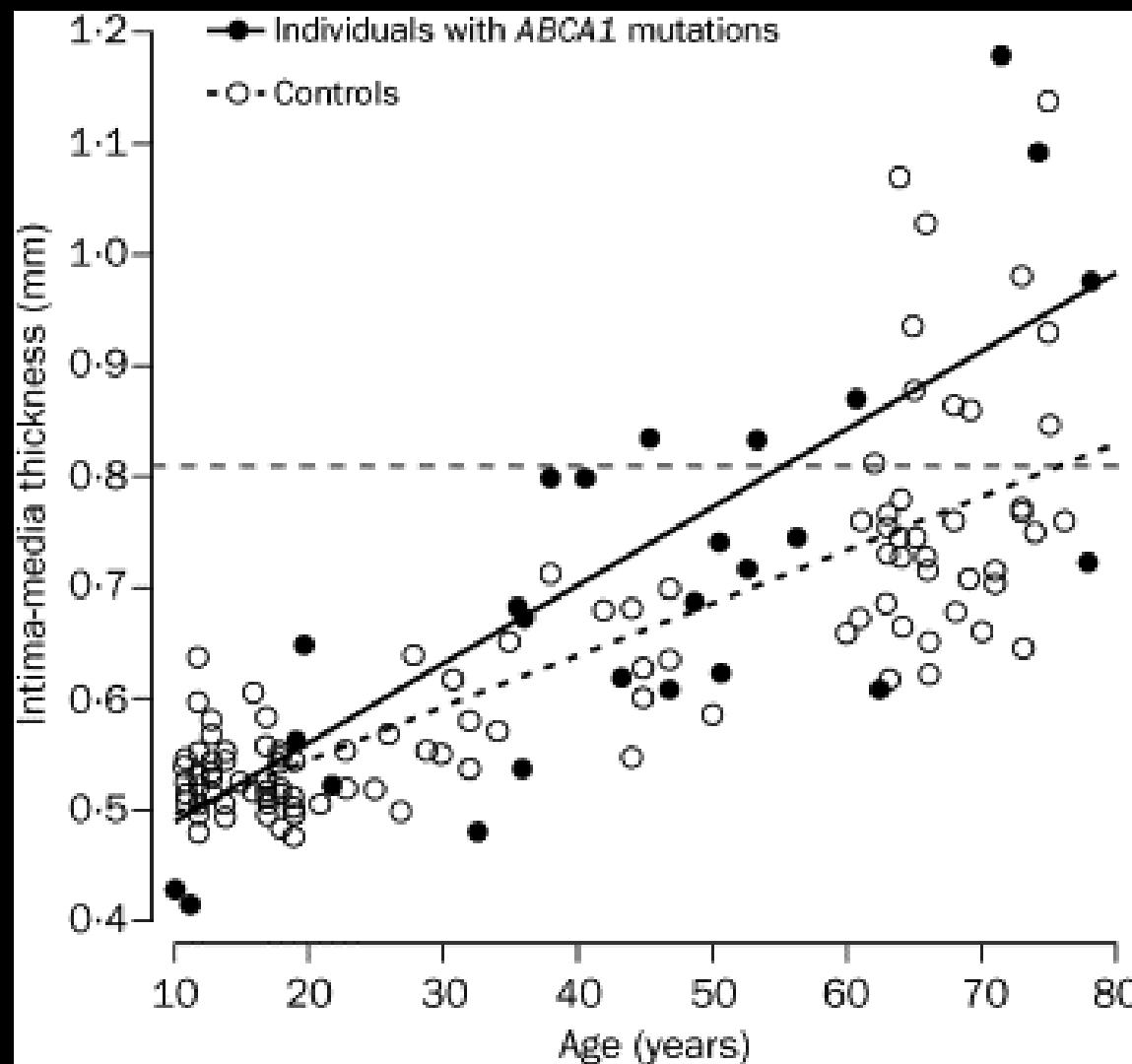
Carriers of ABCA1 mutations

Cholesterol efflux and Carotid IMT

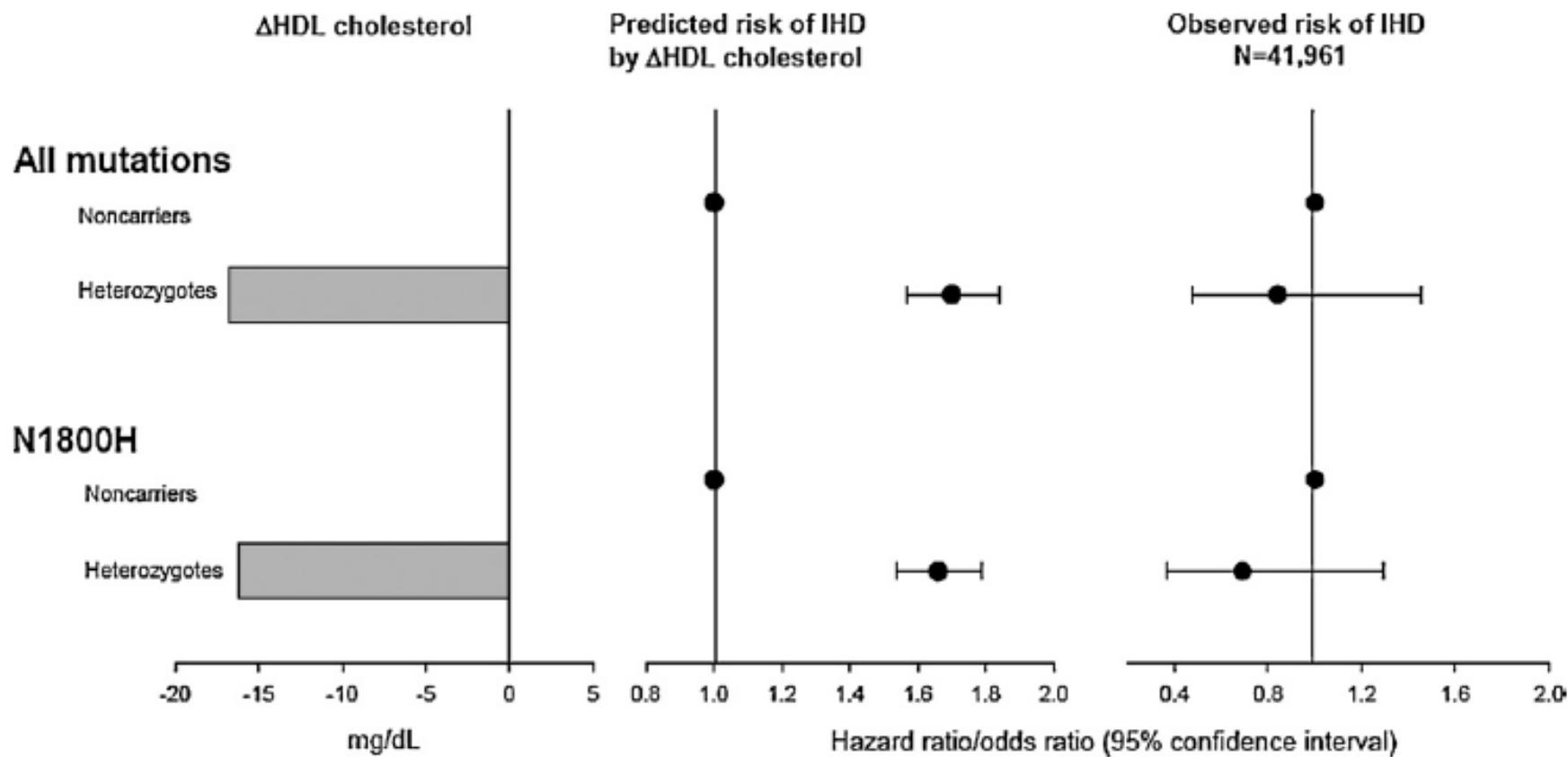


Carriers of ABCA1 mutations

Carotid IMT



ABCA1 mutations and IHD risk



Monogenic HDL Disorders

Apolipoprotein A-I deficiency (OMIM 107680)

Tangier Disease (OMIM 205400)

LCAT Deficiency Syndromes

Familial LCAT deficiency (FLD) (OMIM 245900)

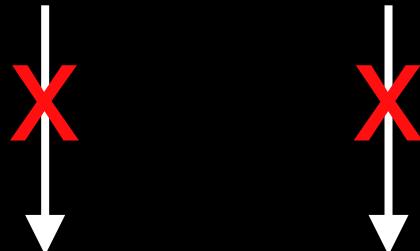
Fish Eye Disease (FED) (OMIM 136120)

CETP deficiency (OMIM 607322)

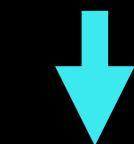
Genetic LCAT Deficiencies

Unesterified Cholesterol

HDL (LDL)



Cholesteryl Ester



FLD

(Familial LCAT Deficiency)

Unesterified Cholesterol

HDL (LDL)



Cholesteryl Ester



FED

(Fish-Eye Disease)

Genetic LCAT Deficiency in Italy

Plasma Lipids/lipoproteins

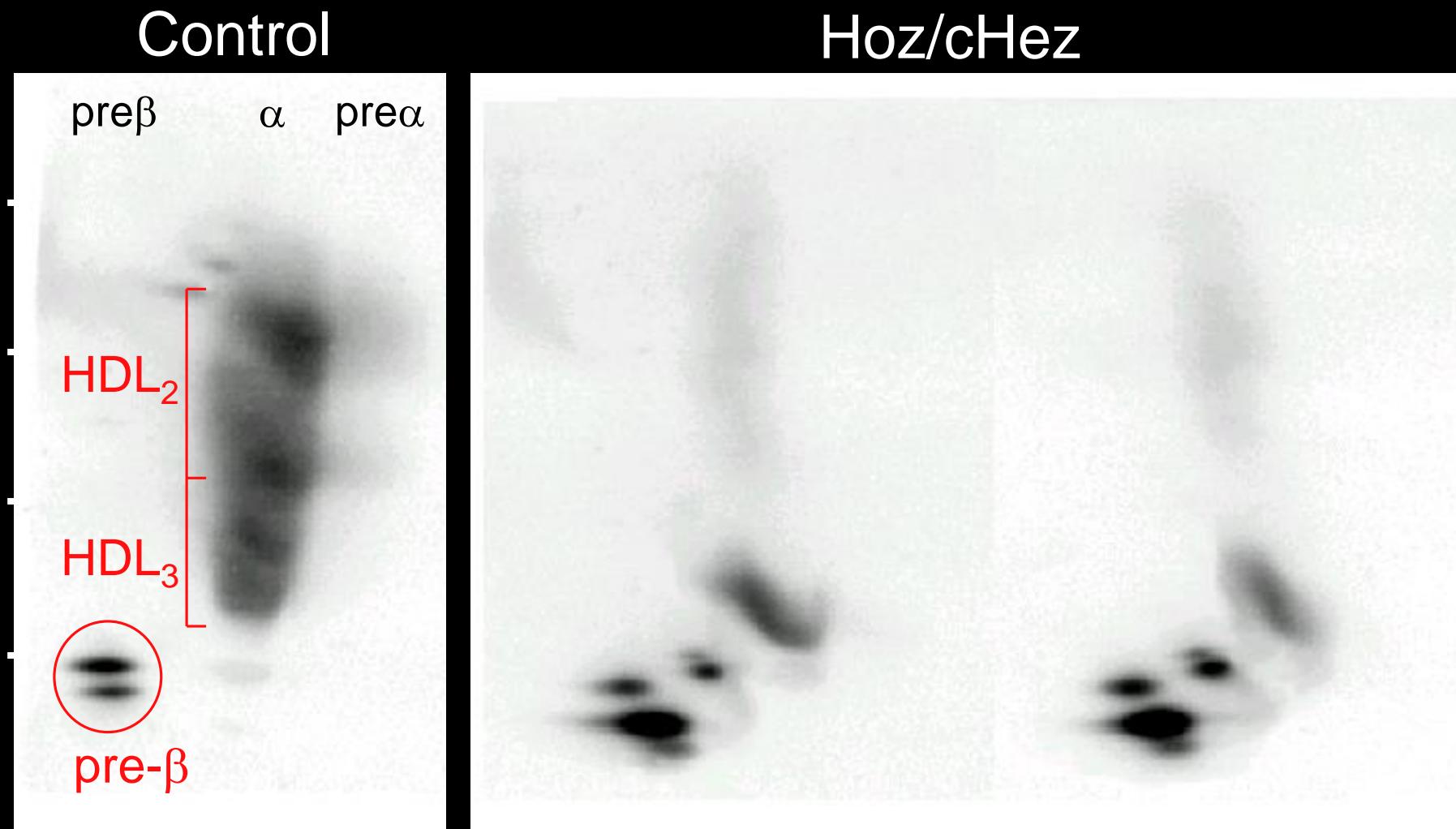
Number of mutant <i>LCAT</i> alleles	Two (n=18)	One (n=44)	Zero (n=20)	Trend <i>P</i> *
TC (mg/dl)	160.7 ±20.8	162.4 ±7.2	196.7 ±9.2	0.344
UC (mg/dl)	125.1 ±17.5	49.3 ±2.2	50.4 ±4.1	<0.001
LDL-C (mg/dl)	102.9 ±16.5	97.2 ±6.6	114.6 ±8.5	0.578
HDL-C (mg/dl)	10.4 ±1.6	40.0 ±2.0	51.4 ±2.9	<0.001
TG (mg/dl)	242.4 ±37.5	125.1 ±8.6	118.8 ±30.3	<0.001
ApoA-I (mg/dl)	44.8 ±3.8	101.8 ±3.6	131.4 ±5.0	<0.001
ApoA-II (mg/dl)	10.4 ±1.4	29.4 ±1.0	34.3 ±1.6	<0.001
ApoB (mg/dl)	60.7 ±11.7	90.0 ±4.2	93.9 ±6.1	0.004

Genetic LCAT Deficiency in Italy

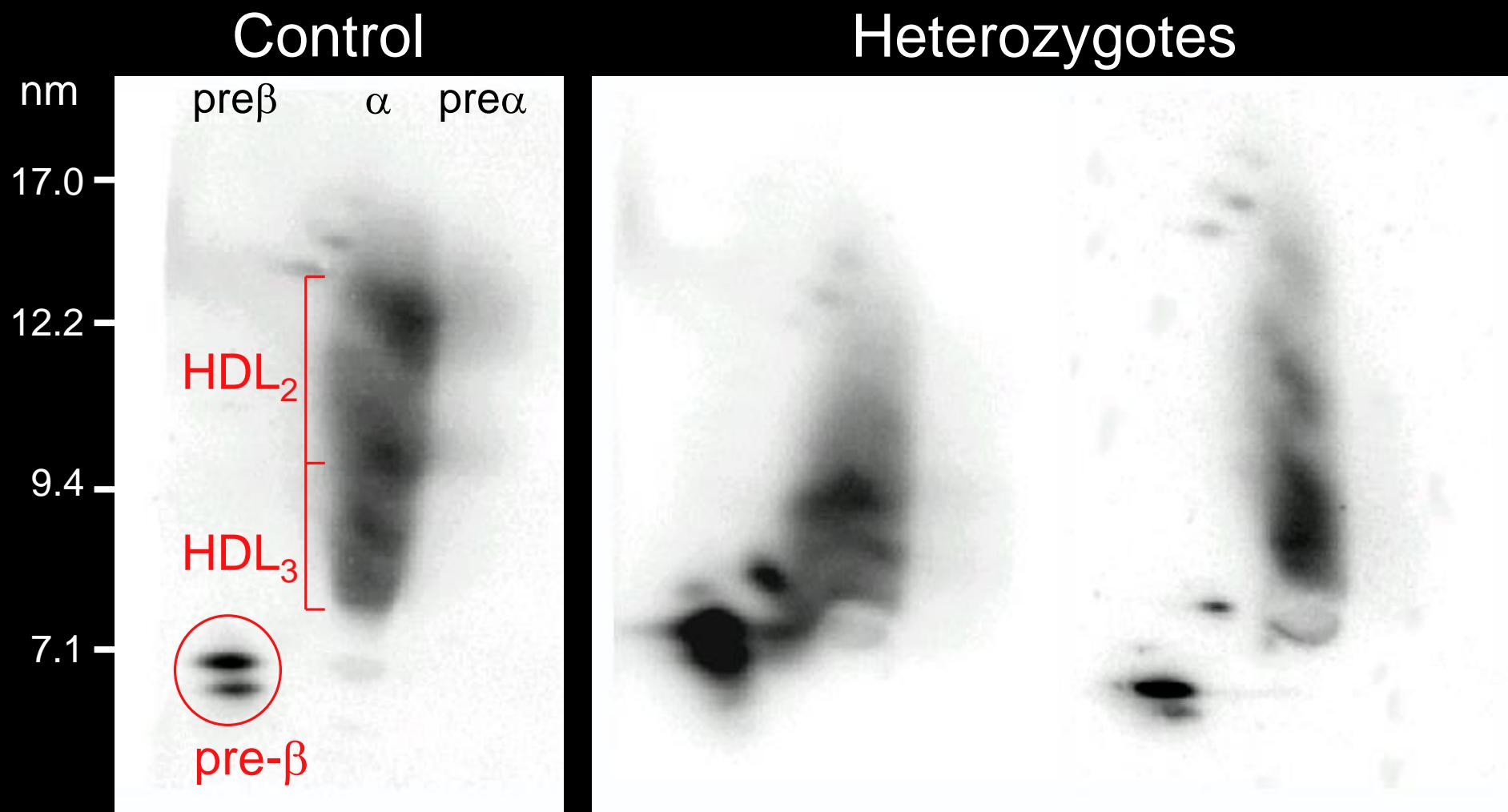
HDL Subpopulations

Number of mutant <i>LCAT</i> alleles	Two (n=17)	One (n=41)	Zero (n=13)	Trend <i>P</i> *
HDL-C (mg/ml)	10.4 ±1.6	40.0 ±2.0	51.4 ±2.9	<0.0001
LpA-I (mg/dl)	26.1 ±3.6	40.9 ±1.5	47.5 ±3.8	<0.0001
LpA-I:A-II (mg/dl)	17.4 ±3.0	59.9 ±3.6	76.6 ±6.3	<0.0001
HDL ₂ size (nm)	nd	11.3 ±0.1	11.2 ±0.1	ns
HDL ₃ size (nm)	7.4 ±0.1	8.9 ±0.1	8.9 ±0.1	<0.0001
Preβ-HDL (%)	48.2 ±6.1	19.6 ±1.1	14.7 ±1.7	0.0006

Genetic LCAT Deficiency in Italy HDL Subpopulations

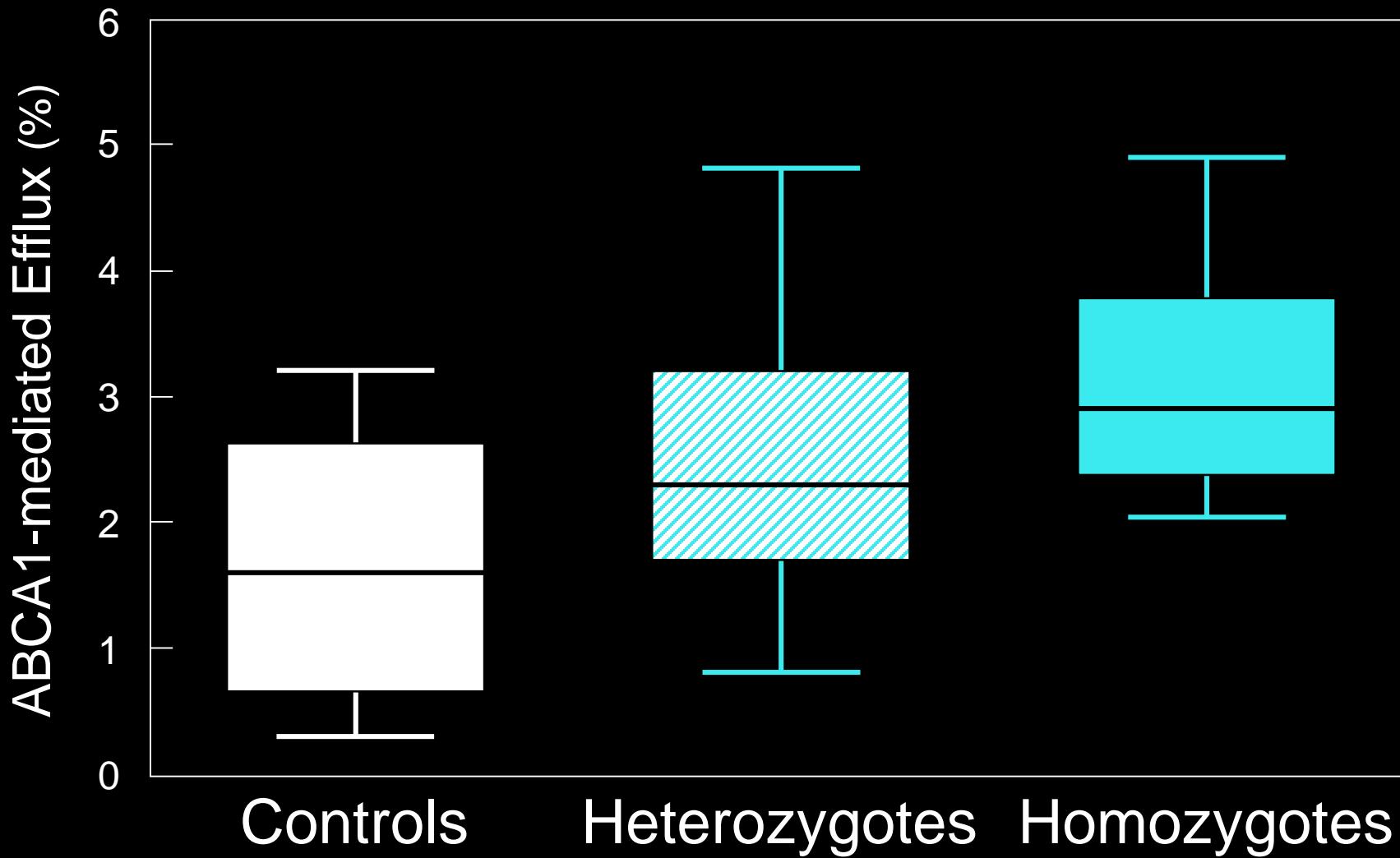


Genetic LCAT Deficiency in Italy HDL Subpopulations



Genetic LCAT Deficiency in Italy

ABCA1-mediated Cholesterol Efflux



Genetic LCAT Deficiency

The Clinical Phenotype

FLD

Corneal opacity

Anemia

Renal disease

CHD?

FED

Corneal opacity

CHD?

Genetic LCAT Deficiency in Italy

Clinical Phenotype in Hoz/cHez

	Total	FLD	FED
Corneal opacity	18/18	11/11	7/7
Anemia	12/18	10/11	2/7
Renal disease	10/18	8/11	2/7
CVD	1/18	0/11	1/7

Age: 39.2 ±3.9 y. (19-71y.)

Genetic LCAT Deficiency in Italy

Clinical Phenotype in Heterozygotes

Corneal opacity 0/64

Anemia 0/64

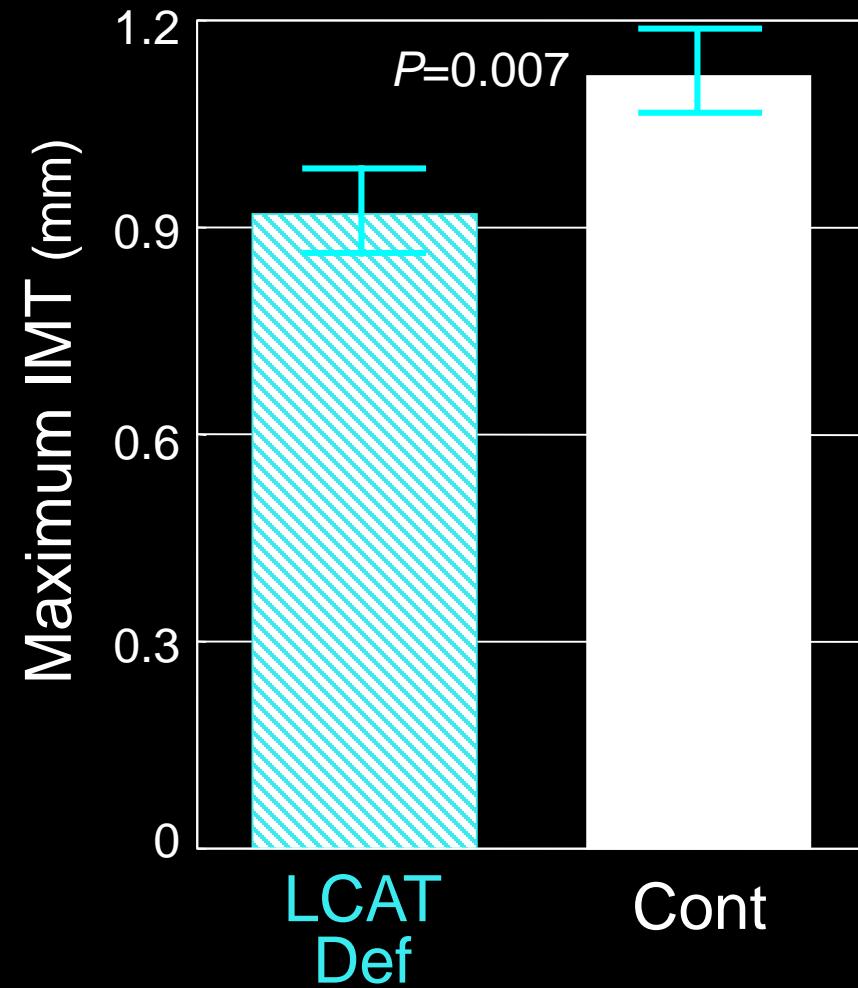
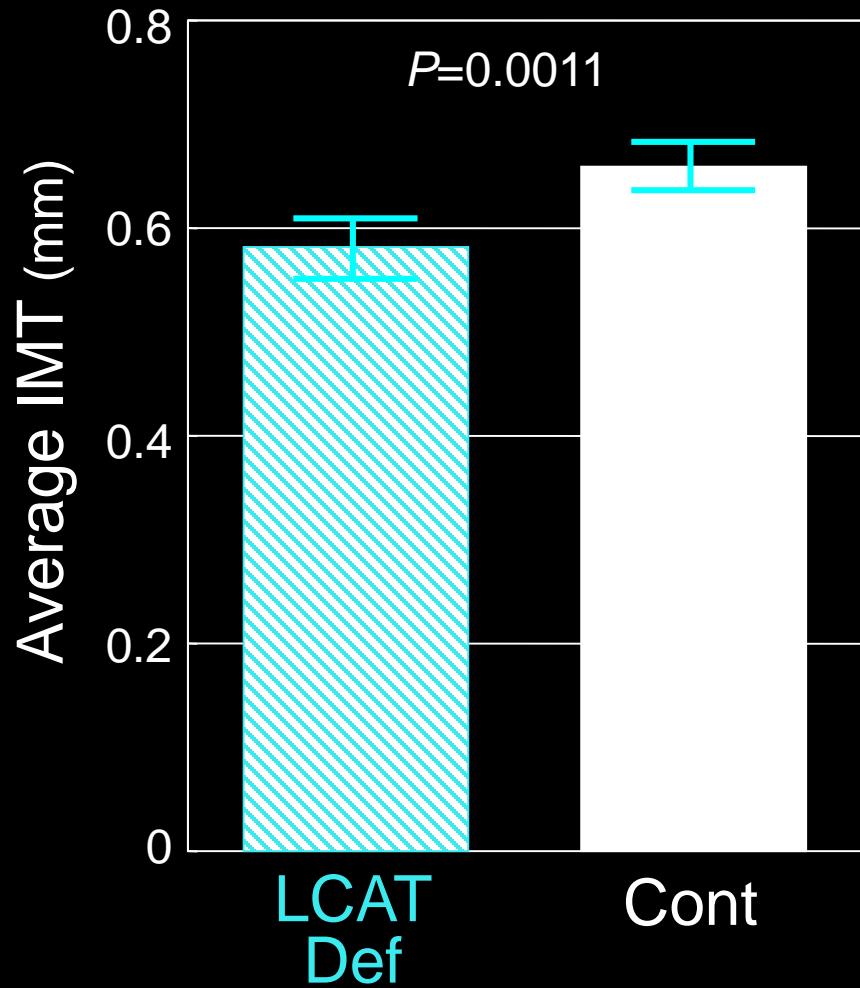
Renal disease 2/64 diabetic nephropathy,
autoimmune glomerulonephritis

CVD 2/64 stroke at 68, 70 y

Age: 48.6 ±3.4 y (4-76 y)

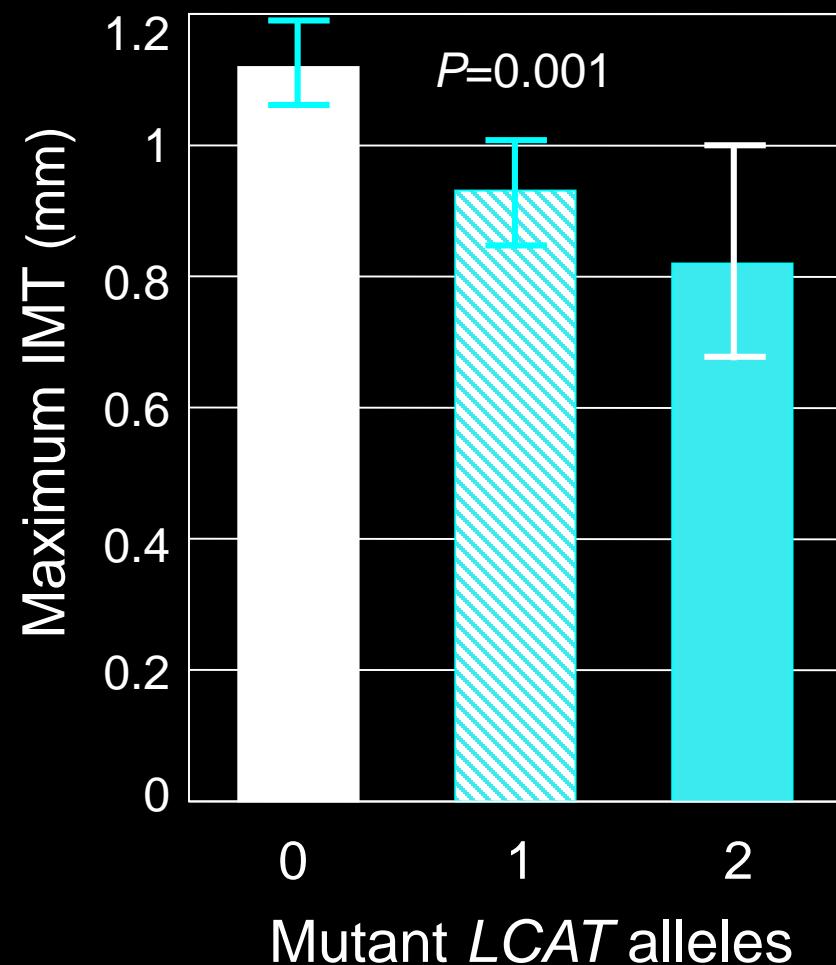
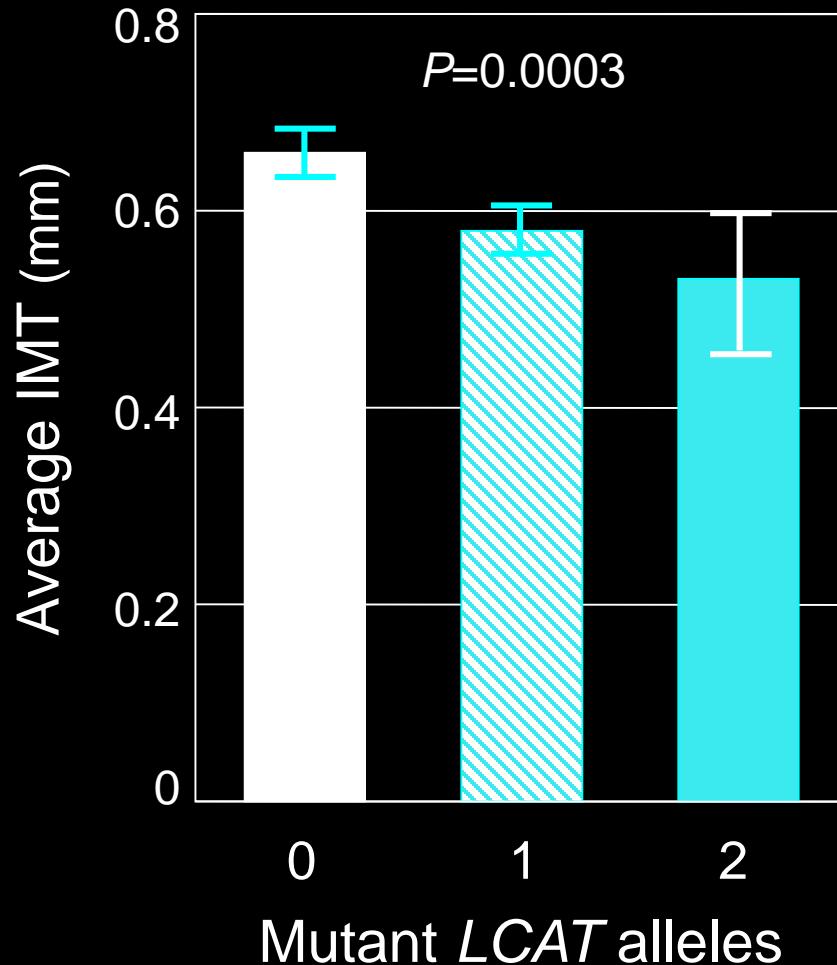
Genetic LCAT Deficiency in Italy

Carotid Intima-Media Thickness



Genetic LCAT Deficiency in Italy

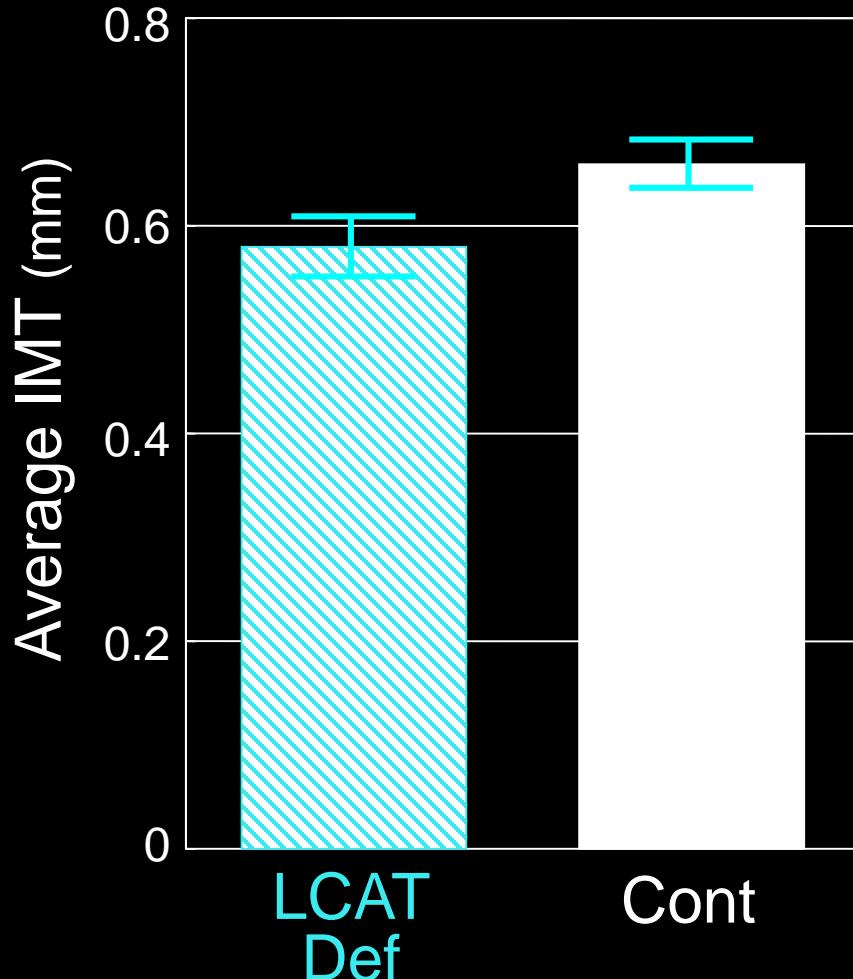
Carotid Intima-Media Thickness



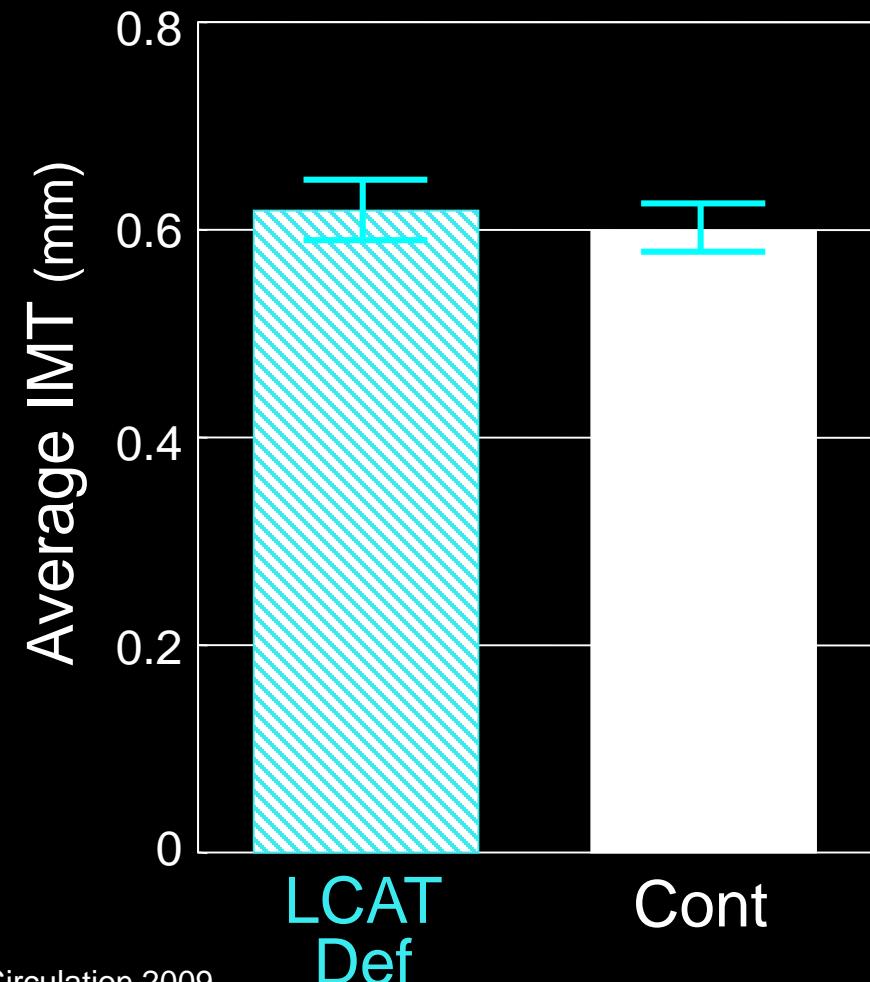
Genetic LCAT Deficiency

Carotid Intima-Media Thickness

Italians



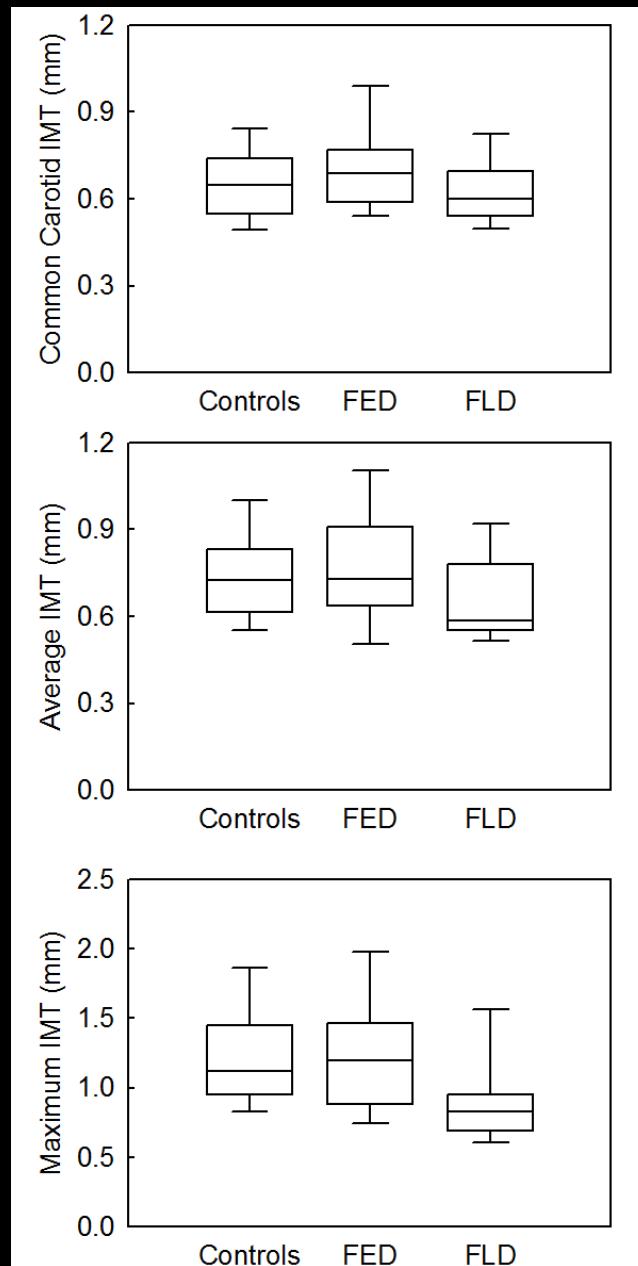
Dutch



Calabresi et al, Circulation 2009
Hovinh et al, Circulation 2005

Genetic LCAT Deficiency - IMT

	FLD Carriers	FED Carriers	Controls
n.	33	41	280
Italian/Dutch	26/7	9/32	113/167
Gender (M, %)	18 (54.5%)	27 (65.9%)	152 (54.3%)
Age (y)	44.8 ± 19.3	44.5 ± 13.8	43.9 ± 13.3
Total Cholesterol (mg/dL)	168 ± 39	186 ± 53	200 ± 39
LDL Cholesterol (mg/dL)	102 ± 35	124 ± 47	123 ± 35
HDL Cholesterol (mg/dL)	42 ± 13	38 ± 14	58 ± 16
Triglycerides (mg/dL)	97 (77; 144)	100 (81; 146)	86 (60; 123)
Non-HDL Cholesterol (mg/dL)	126 ± 41	148 ± 51	142 ± 40
Ratio LDL-C/HDL-C	2.2 (1.9; 3.4)	3.4 (2.2; 4.2)	2.1 (1.6; 2.8)



Genetic HDL Disorders

	frequency	HDL-C	CHD
<i>ABCA1</i>	~ 170 mutations	↓	↑
<i>ApoA-I</i>	~ 60 mutations	↓	↑ →
<i>LCAT</i>	~ 85 mutations	↓	↓ →
<i>CETP</i>	~ 25 mutations	↑	→

Genetic HDL defects – treatments under development

CSL-112	Synthetic HDL	TD ApoA-I Deficiency	Plaque stabilazation	Phase 2
CER-001	Synthetic HDL	TD ApoA-I Deficiency	Plaque stabilazation	Phase 2
rhLCAT	Recombinant protein	FLD	ERT	Phase 2

HDL-Therapy in Familial Hypoalphalipoproteinemia

Effect of open-label infusion of an apoA-I-containing particle (CER-001) on RCT and artery wall thickness in patients with FHA

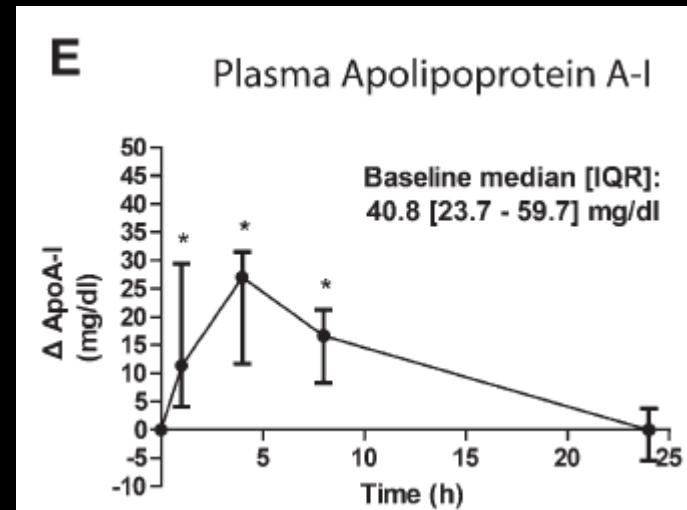
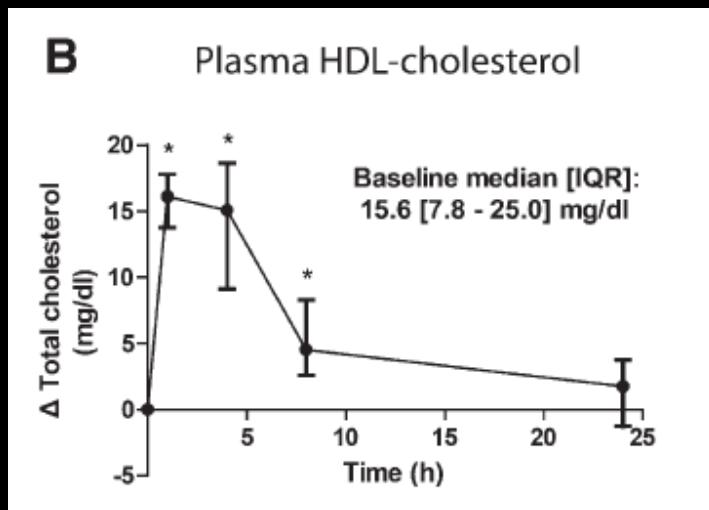
Treatment	1 infusion CER-001 8 mg/kg (day 0)	8 infusions CER-001 8 mg/kg (q3 days)	11 infusions CER-001 8 mg/kg (q2 weeks)
Outcome measures	3T MRI scan FDG-PET/CT scan Fecal sterol excretion (1)	Cholesterol efflux Fecal sterol excretion (2)	3T MRI scan (day 30) FDG-PET/CT scan (day 30)
	Pre-treatment	Initial dosing period Day 0 - 7	Induction period Day 8 - 29
			Maintenance period Week 6 - 26

HDL-Therapy in Familial Hypoalphalipoproteinemia

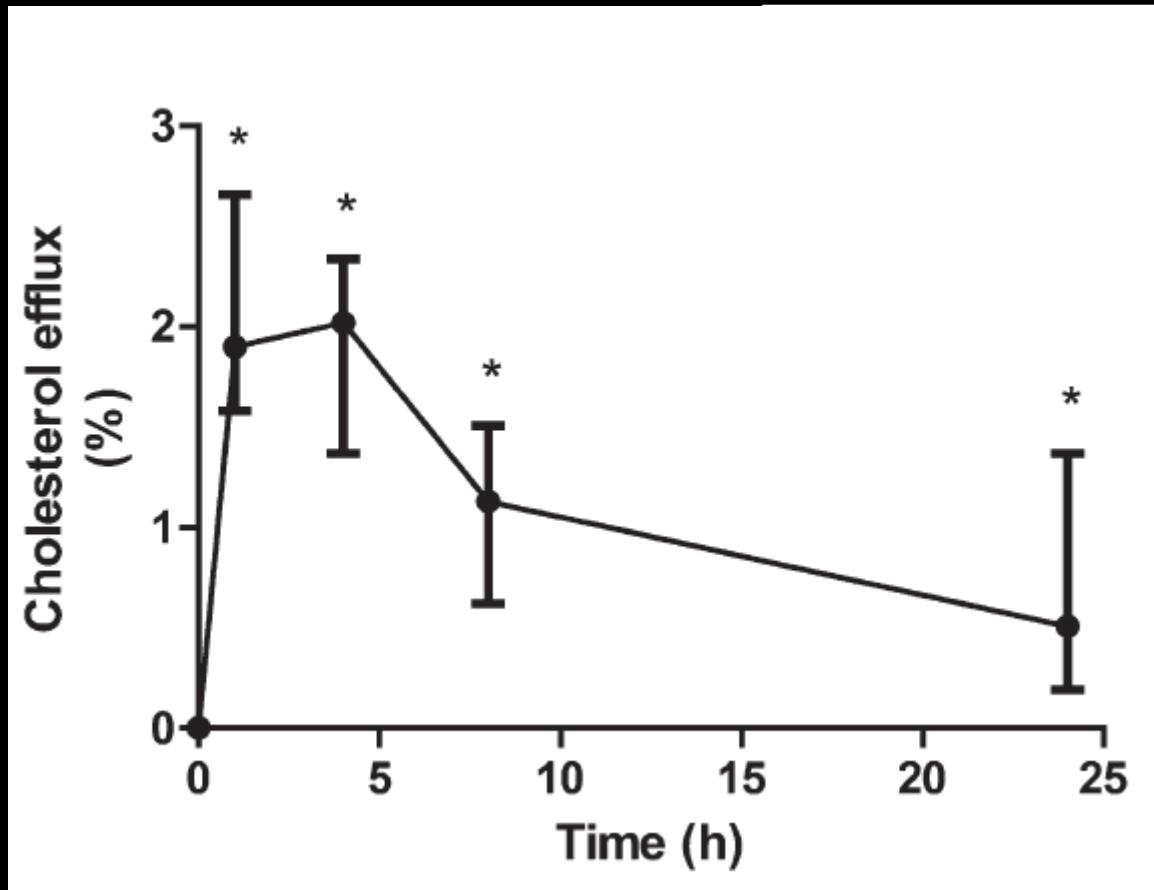
Sex (F/M)	Age (yrs.)	Genetic diagnosis	CVD	Lipid-lowering therapy	HDL-c (mg/dl)	ApoA-I (mg/dl)
M*	46	Homozygosity <i>ApoA-I</i> (Q(-2)X)	CABG	Atorvastatin 80mg, ezetimibe 10mg, nicotinic acid 500mg	1.80	0.22
M*	55	Heterozygosity <i>ABCA1</i> (6401+T2)	MIs (3)	Rosuvastatin 15mg	19.69	28.67
M*	49	Heterozygosity <i>ApoA-I</i> (391delAAG exon 4) Heterozygosity <i>ABCA1</i> (C1477R)	MIs (2) PCI (2)	Rosuvastatin 10mg	6.21	16.49
M	51	Heterozygosity <i>LCAT</i> (T147I)	-	Ezetimibe 10mg/simvastatin 40mg, fish oil capsule	29.06	59.14
M*	68	Heterozygosity <i>ABCA1</i> (N1800H)	- Hypertension	-	13.82	51.56
F	51	Heterozygosity <i>ApoA-I</i> (L202P)	-	-	37.40	70.24
F	47	Homozygosity <i>ABCA1</i> (L996P)	Angina Pectoris PCI	Rosuvastatin 10mg, ezetimibe 10mg	0.60	7.90

HDL-Therapy in Familial Hypoalphalipoproteinemia

Treatment	1 infusion CER-001 8 mg/kg (day 0)	8 infusions CER-001 8 mg/kg (q3 days)	11 infusions CER-001 8 mg/kg (q2 weeks)
Outcome measures	3T MRI scan FDG-PET/CT scan Fecal sterol excretion (1)	Cholesterol efflux Fecal sterol excretion (2) 3T MRI scan (day 30) FDG-PET/CT scan (day 30)	3T MRI scan (week 27)
Pre-treatment	Initial dosing period Day 0 - 7	Induction period Day 8 - 29	Maintenance period Week 6 - 26

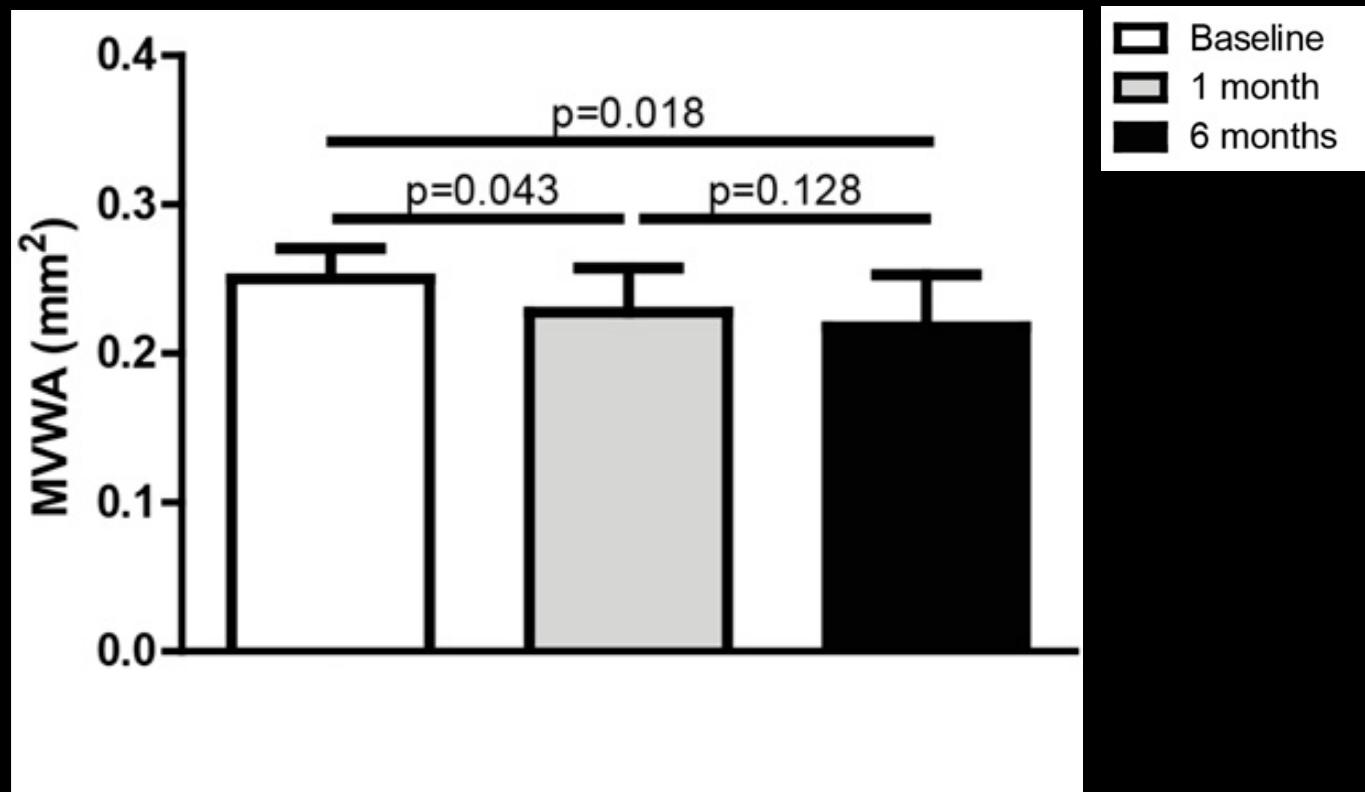


HDL-Therapy in Familial Hypoalphalipoproteinemia



J774 Macrophages

HDL-Therapy in Familial Hypoalphalipoproteinemia

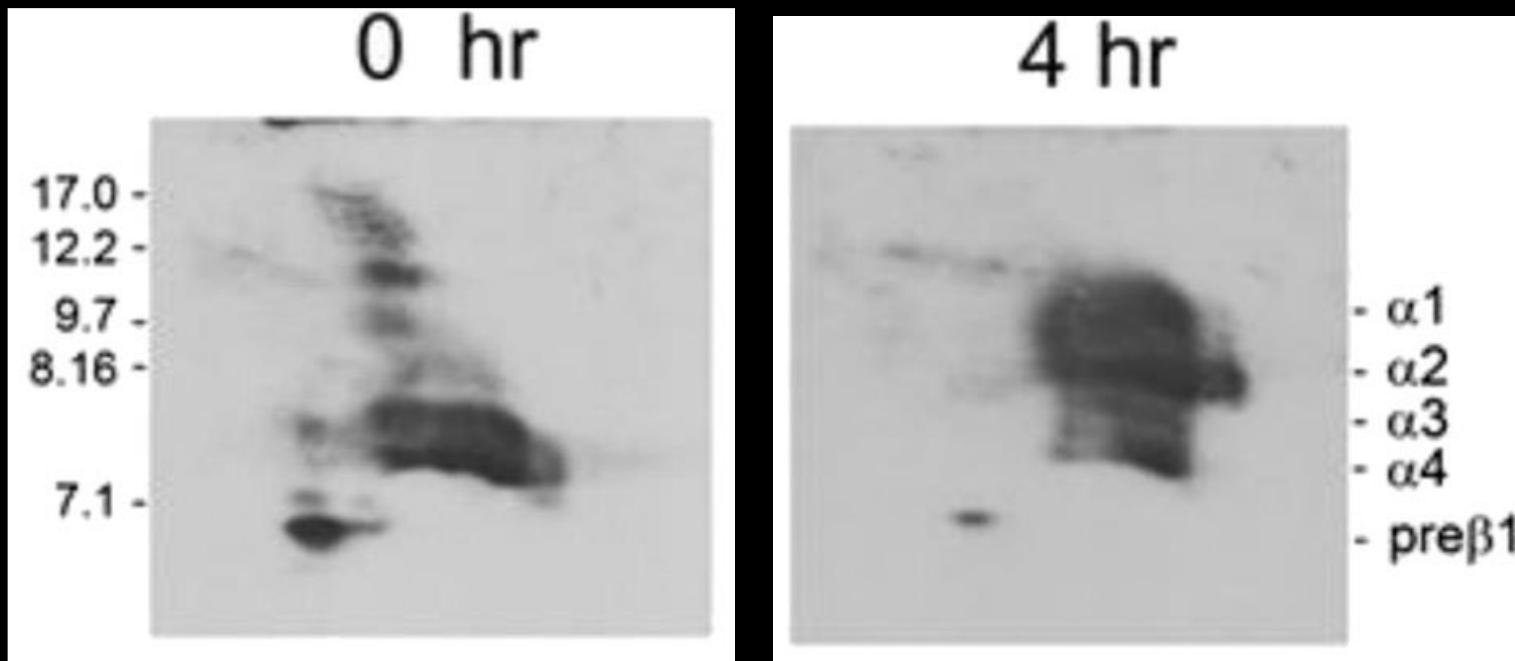


rhLCAT – Proof of concept

1 FLD carrier with end-stage renal disease

Treated with rhLCAT (9.0 mg/kg)

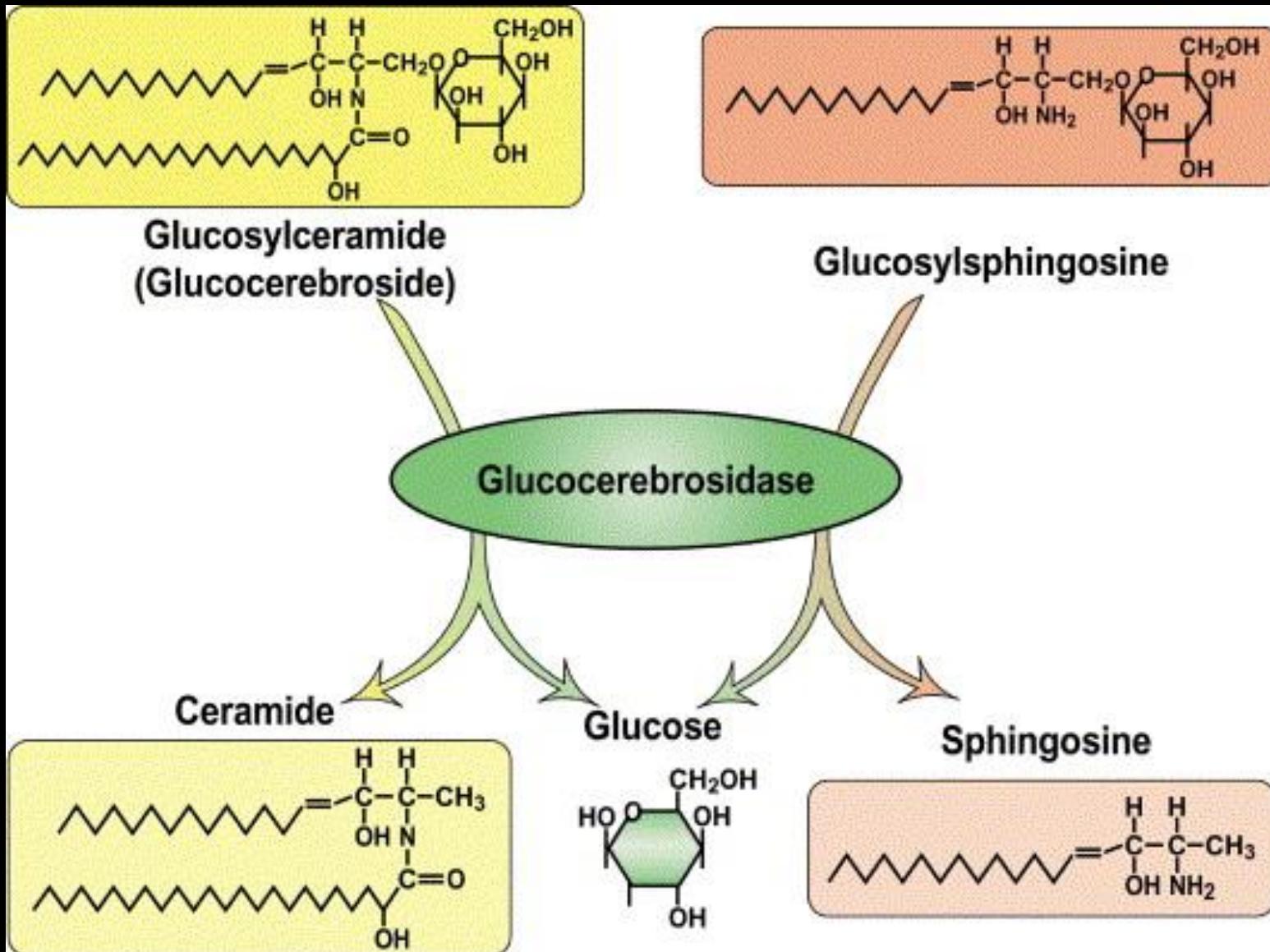
Analysis of HDL subclasses



GAUCHER'S DISEASE

- ✓ The most common lysosomal storage disorder
- ✓ Autosomal recessive disease
- ✓ Incidence 1:50000
- ✓ Mutation in the gene coding for the acid hydrolase β -glucocerebrosidase(GBA) leading to the accumulation of glucocerebroside
- ✓ First successfully managed lipid storage disease: enzyme replacement therapy for type 1 available since 1991

GAUCHER'S DISEASE



GLUCOCEREBROSIDASE

- ✓ 497 aa, associated with the lysosomal membrane
- ✓ Gene on chromosome 1
- ✓ 300 mutations described
- ✓ 3 mutations with high frequency: N370S, L444P, 84G>GG (70% cases of GD)

GAUCHER'S DISEASE

Clinical features

- Hepatomegaly
- Splenomegaly
- Anemia
- Thrombocytopenia
- Skeletal involvement

	Type 1: Adult-onset (adult)	Type 2: Neurovisceral (infantile)	Type 3: Chronic neurovisceral-macrophagic (juvenile)
Affected groups	Young adults/adults Most common in Ashkenazi Jewish population (1 in 450) 1 in 100,000 general population	Infants (rarely) No particular ethnicity 1 in 100,000 live births	Children/young adults No particular ethnicity 1 in 50,000 live births Norrbottian variant: Sweden, until early adulthood
Distinguishing symptom	Liver, spleen, and bone No nervous system problems	Early nervous system problems Brainstem abnormalities	Later onset of nervous system problems: incoordination, mental deterioration, myoclonic seizures
Effects of disease	Varies from mild to severe	Death in infancy (age <2 years)	Slowly progressive – becomes severe later in childhood
Glucocerebrosidase activity	Some activity, but much less than normal	Very little activity	Little activity

ERT for GAUCHER'S DISEASE

Ceredase (1991), from human placenta

Cerezyme (1994), hr-glucocerebrosidase (CHO cells)

Dose: 2.5-60U/kg, IV, every two weeks (depending on response)

Annual cost: 180.000\$ per patient

Abs: in 15% of patients

ERT for GAUCHER'S DISEASE

	Imiglucerase 1994	Velaglucerase alfa 2010	Taliglucerase alfa 2012
Engineered cells	CHO cells	human fibrosarcoma cells	Carrot cells
Amino acid sequence	R495H	Natural human sequence	R495H; DLLVDTM at C-terminal
Method used to produce required glycans	Post-production exoglycosidase digestion to expose mannose sugars	Co-culture with kifunesine to inhibit glycosylation process and produce high mannose structures	Targeting to vacuole to naturally produce pauci-mannose structures
Exposed mannose	40–60%	~100%	~100%
Mannose chain length	Man ₃	Man ₅ –Man ₉	Man ₃
Additional sugars	GlcNAc/Gal/SA and core (α 1-6) fucose	ND	Xylose and/or core (α 1-3) fucose