GENETIC SCORE BASED ON HIGH-RISK GENETIC POLYMORPHISMS AND EARLY ONSET OF ISCHEMIC HEART DISEASE IN AN ITALIAN COHORT OF ISCHEMIC PATIENTS

Maria Grazia Andreassi[1], Daniel Adlerstein[2], Michele Coceani[3], Erlet Shehi[2], Cecilia Vecoli[4], Tiziana Sampietro[3], Danilo Neglia[3], Antonio L'Abbate[4], Clara Carpeggiani[1]

[1]Institute of Clinical Physiology of CNR, Pisa, Italy; [2] DiaSorin SpA, Saluggia, Italy; [3]Gabriele Monasterio Foundation-Tuscany Region, Pisa, Italy; [4]High School Sant'Anna, Pisa, Italy

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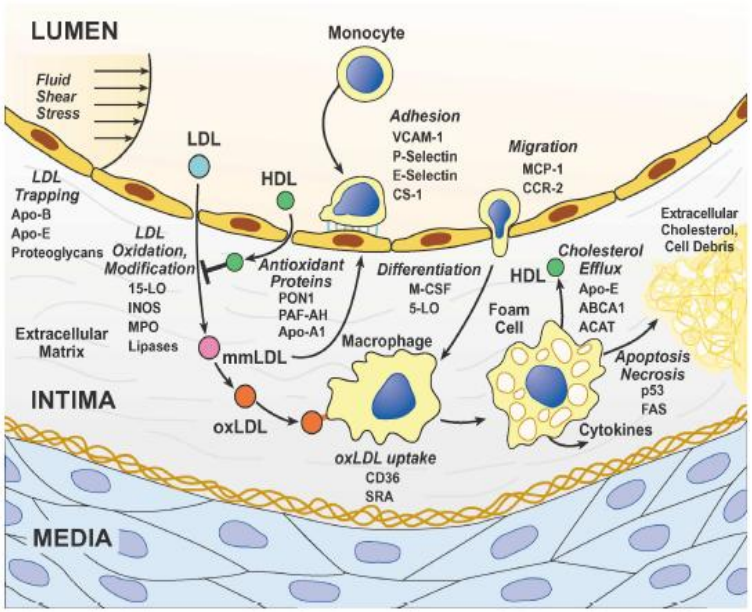
- There are no other relationship to declare

Genetics of CAD: the most complex disorder

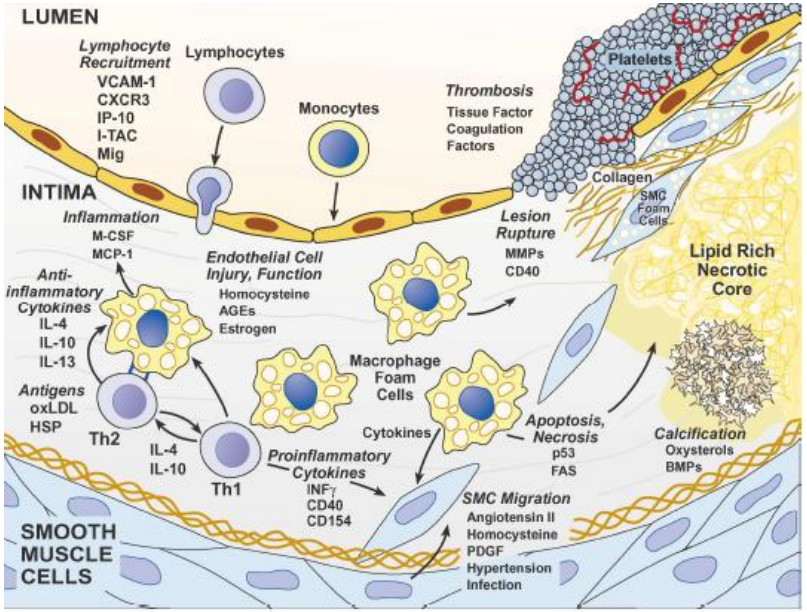
CAD is not a monogenic trait

-Rare exceptions involving mutation of genes LDL receptor, apolipoprotein B

Early stages of atherosclerosis



Late stages of atherosclerosis



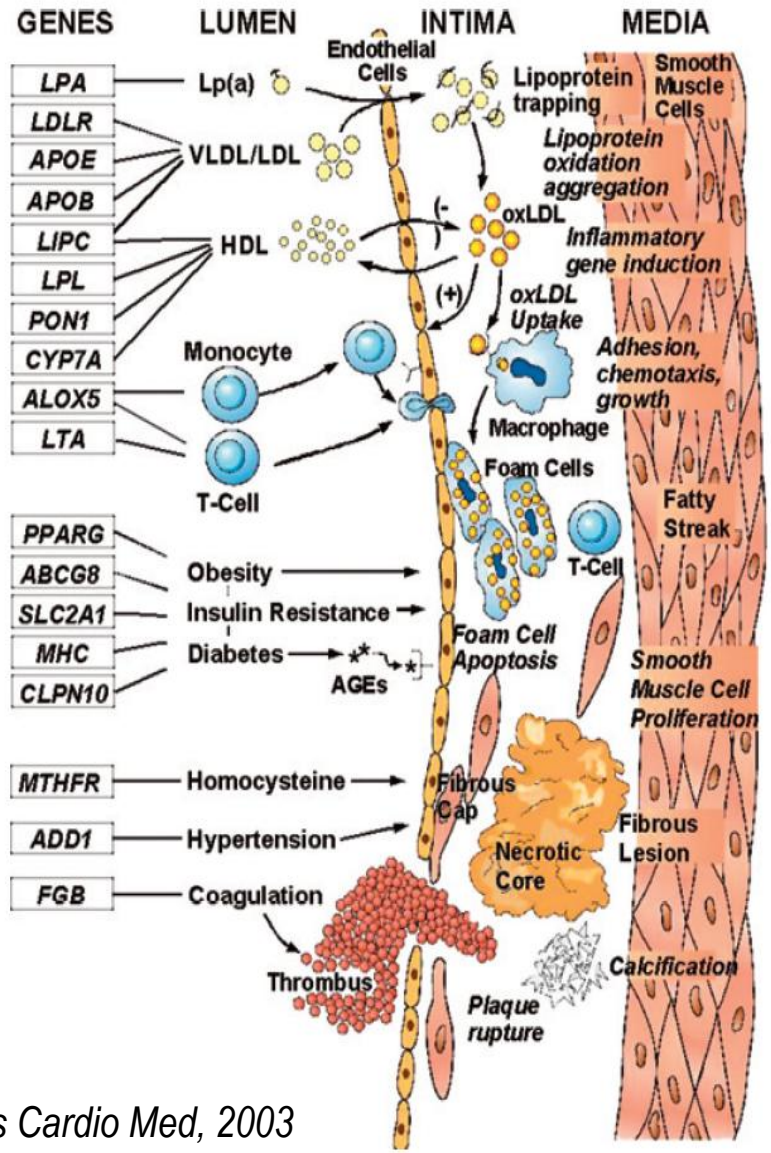
- Experiments with transgenic and gene-targeted mice have revealed >100 genes that can influence the development of atherosclerotic lesions

Lusis et al. Genetic Basis of Atherosclerosis: Part I. New Genes and Pathways: Circulation. 2004;

Genes contributing to CAD susceptibility

Genetic and Environmental Risk Factors for CHD

- Risk factors with a significant genetic component (heritability)
  - Elevated LDL and VLDL cholesterol (40%–60%)
  - Low HDL cholesterol (45%–75%)
  - Elevated triglycerides (40%–80%)
  - Increased body mass index (25%–60%)
  - Elevated systolic blood pressure (50%–70%)
  - Elevated diastolic blood pressure (50%–65%)
  - Elevated lipoprotein(a) levels (90%)
  - Elevated homocysteine levels (≈45%)
  - Type 2 diabetes mellitus (40%–80%)
  - Elevated fibrinogen (20%–50%)
  - Elevated C-reactive protein (≈40%)
  - Gender
  - Age
  - Family history
- Environmental risk factors
  - Smoking
  - Diet
  - Exercise
  - Infection
  - Fetal environment
  - Air pollution (particulates)



Lusis A, Trends Cardio Med, 2003

The Genetic Basis of Coronary Artery Disease: From Candidate Genes to Whole Genome Analysis

Massimo Franchini, Flora Peyvandi, and Pier Mannuccio Mannucci*

(Trends Cardiovasc Med 2008;18:157–162)

Table 3. Whole genome association studies and replication studies in patients with coronary artery disease

First author, year	Population screened	No. of cases/ controls	Clinical event	Genes/chromosomal loci associated with coronary artery disease	OR (95% CI)
Ozaki, 2002, 2004	Japanese	1133/1006	MI	*LTA*	1.78 (1.39-2.27)
Shiffman, 2005	American	1345/1843	MI	*Palladin*	1.40 (NA)
				ROS1	1.75 (NA)
				TAS2R50	1.58 (NA)
				OR13G1	1.40 (NA)
Iakoubova, 2006[a]	European, American	2903/1080	MI	*FCAR*	1.68 (1.10-2.57)
Shiffman, 2006[a]	American	1200/262	MI	*VAMP8*	1.75 (1.17-2.62)
				HNRPUL1	1.92 (1.28-2.86)
Helgadottir, 2007	European	4587/12,767	MI	9p21	2.02 (1.72-2.36)
McPherson, 2007	European	2326/10,427	CAD	9p21	1.20 (1.02-1.42)
WTCCC, 2007	European	2000/3000	CAD	9p21	1.37 (1.26-1.48)
Samani, 2007	European	2801/4582	CAD	9p21	1.33 (1.18-1.51)
				6q25	1.24 (1.09-1.41)
				2q36	1.20 (1.06-1.35)
Larson, 2007	American	1345	CVD	9p21	2.11 (NA)
Luke, 2007[a]	American	1806/1274	CAD	*LPA*	3.14 (1.51-6.56)
Iakoubova, 2008[a]	European, American	3394/1080	MI, CAD	*KIF6*	1.50 (1.05-2.15)
Shiffman, 2008[a]	American	4522	MI	*KIF6*	1.29 (1.1-1.52)
				VAMP8	1.2 (1.02-1.41)
				TAS2R50	1.13 (1-1.27)
				LPA	1.62 (1.09-2.42)

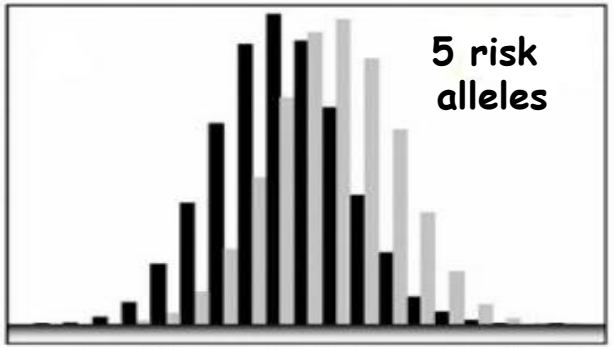
WTCCC indicates the Wellcome Trust Case Control Consortium; CVD, cardiovascular disease.

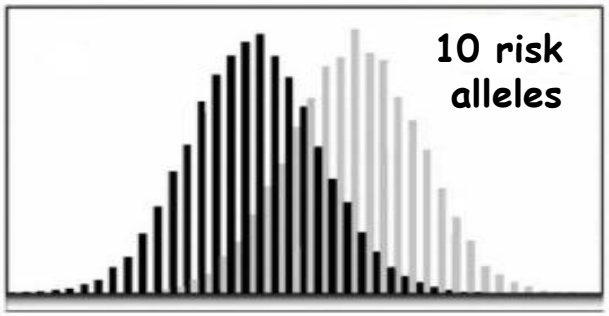
[a] Replications of whole genome association studies.

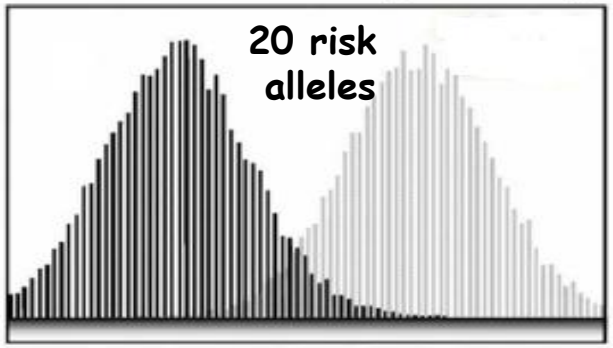
Current Atherosclerosis Reports 2009, 11:175–181

Creating a Genetic Risk Score for Coronary Artery Disease

Sonny Dandona, MD, FRCPC, and Robert Roberts, MD, FRCPC







Ortlepp et al. EJIM 2002

THE LANCET

Lancet 2010; 376: 1393–400

A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses

Samuli Ripatti, Emmi Tikkanen, Marju Orho-Melander, Aki S Havulinna, Kaisa Silander, Amitabh Sharma, Candace Guiducci, Markus Perola, Antti Jula, Juha Sinisalo, Marja-Liisa Lokki, Markku S Nieminen, Olle Melander, Veikko Salomaa, Leena Peltonen, Sekar Kathiresan*

AHJ
American Heart Journal
October 2008 2010;

Joint effects of common genetic variants from multiple genes and pathways on the risk of premature coronary artery disease

Jeffrey L. Anderson, MD,[a,b] Benjamin D. Horne, PhD, MPH,[a,c] Nicola J. Camp, PhD,[c] Joseph B. Muhlestein, MD,[a,b] Paul N. Hopkins, MD, MPH,[b] Lisa A. Cannon-Albright, PhD,[c] Chrissa P. Mower, BS,[a] James J. Park, BA,[a] Jessica L. Clarke, BS,[a] Zachary P. Nicholas, BS,[a] Jason T. McKinney, PhD,[d] and John F. Carlquist, PhD[a,b]
Murray and Salt Lake City, UT

Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION
American Heart Association.
Learn and Live.
Genetics

Genetic Risk Score and Risk of Myocardial Infarction in Hispanics

Lu Qi, MD, PhD; Jiantao Ma, MD; Qibin Qi, PhD; Jaana Hartiala, MS; Hooman Allayee, PhD; Hannia Campos, PhD

(*Circulation*. 2011;123:374-380.)

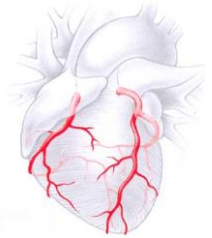
AIM

To investigate the impact of high-risk SNPs and their joint effects as a genetic score on early onset of IHD

Methods

Patient Population

In the GENOCOR (Genetic Mapping for Assessment of Cardiovascular Risk) study, we enrolled 114 patients with early onset IHD (age ≤50 years for men and age ≤ 60 years for women) and 384 patients with late onset IHD (age>50 years for men and age > 60 years for women).

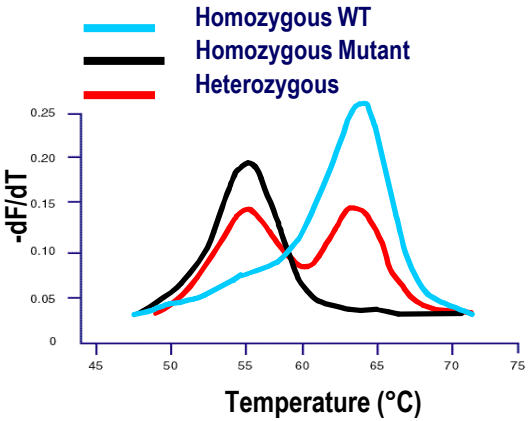


Inclusion Criteria

- Coronary angiography
- Complete history of traditional risk factors

Genetic analysis

High-throughput genotyping of SNPs was performed by using high-resolution melting curve analysis based on post-PCR (Idaho Technology LightScanner).



Selection of Polymorphisms

Locus	Gene	Symbol	SNP name	rs number
3q27	Adipocyte, C1Q, and collagen domain containing	ADIPOQ/ACDC	-11377 C>G	266729
4p16.3	Adducin 1 (alfa)	ADD1	Gly460Trp	4961
9q31.1	ATP-binding cassette, sub-family A (ABC1), member 1	ABCA1	Gln597Arg	2853578
17q23.3	Angiotensin I converting enzyme (peptidyl-dipeptidase A) 1	ACE	Thr776Thr	4343
6p21.3	Advanced glycosylation end product-specific receptor	AGER	Gly82Ser	2070600
17p11.1	A-kinase (PRKA) ancoring protein 10	AKAP10	Ile646Val	203462
11q23.1	Apolipoprotein C III	APOC3	-482C>T	2854117
11q23.1	Apolipoprotein C III	APOC3	-455T>C	2854116
19q13.2	Apolipoprotein E	ApoE	T3932C	429358
19q13.2	Apolipoprotein E	ApoE	C4070T	7412
16q21	Cholesteryl ester transfer protein, plasma Taq1B	CETP Taq1B	G>A	708272
16q21	Cholesteryl ester transfer protein, plasma, CETP,	CETP	-629C>A	1800775
1p35.1	Gap junction protein, alpha 4/Connexin 37	GJA4/Cx37	C1019T	1764391
7q36	Endothelial nitric oxide synthase/ nitric oxide synthase 3	ENOS/NOS3	-786T>C	2070744
4q28-31	Fatty acid binding protein II	FABP II	G2445A	1799883
4q28	Fibrinogen beta chain 1	FGB 1	Ser189Ser	6056
4q28	Fibrinogen beta chain 2	FGB 2	Arg448Lys	4220
4q28	Fibrinogen beta chain 3	FGB 3	-148C>T	1800787
13q34	Factor VII	FVII	Arg353Gln	6046
17pter-p12	Glycoprotein IB platelet, alpha polypeptide	GP1BA	Thr145Met	6065
15q21-23	Hepatic lipase	LIPC	-514C>T	1800588
19p13.3-2	Intercellular adhesion molecule I	ICAM I	Lys469Glu	5498
2q14.2	Interleukin 1 receptor antagonist	IL1RN 3	T13760C	419598
7p21	Interleukin 6	IL 6	-572G>C	1800796
2q36	Insulin receptor substrate 1	IRS1	G3494A	1801278
13q12.1	Insulin promoter factor 1	IPF1	-108 3G>4G	S82168
4q32.3	Palladin, cytoskeletal associated protein	PALLD/ KIAA0992	A>G	12510359
22q12-q13	Lectin, garactoside-binding, soluble, 2	LGALS2	C3279T	7291467
8p22	Lipoprotein lipase	LPL	Ser447Stop	328
6p21.3	Limphotoxin-alpha gene	LTA	Thr26Asn	1041981
1p36.3	Methylentetrahydrofolate reductase	MTHFR	C677T	1801133
16q24	Cytochrome b-245, alpha polipeptide, NADH(P)H oxidase	NADHPH p22-PHOX	C242T	4673
1q44	Olfactory receptor, family 13, subfamily G, member 1	OR13G1	Ile132Val	1151640
7q21.3	Paraoxonase II	PON2	Ser311Cys	7493
6q22	ROS I c-ros oncogene 1 , receptor tyrosine	ROS 1	Asp2213Asn	529038
1q23-q25	Selectin E	SELE	G98T	1805193
1q23-q25	Selectin E	SELE	A561C	5361
1q23-q25	Selectin P	SELP	Thr715Pro	6131
11q22.3	Matrix metalloproteinase 3 stromelysine I	MMP3	1171 5A>6A	3025058
12p13.2	Taste receptor, type 2, member 50	TAS2R50	Cys203Tyr	1376251
6q27	Trombospondine 2	THBS2	T3949G	8089
5q13	Trombospondine 4	THBS4	Ala387Pro	1866389
3q26.3-q27	Trombopoietine	THPO	A5713G	6141
6p21.3	Tumor Necrosis Factor α	TNFα	-850C>T	1799724
1q25	Tumor Necrosis Factor α ligand superfamily, member 4	TNFSF4	A/G	3850641
1q23	Coagulation factor V	FV	G1691A	6025
9p21.3	Locus 9p21.3	9p21.3	C>G	1333049
12q13-q14	Low density lipoprotein-related protein 1	LRP1	C>T	1800156

48 high-risk SNPs previously identified to be independently associated with CAD and/or MI from large-scale association studies and GWA studies

Clinical characteristics of the study population

Characteristic	Late IHD	Early IHD	p value
Mean age (years)	60.7±5.9	46.2±5.1	0.0000
Gender male, n (%)	339 (88)	93 (82)	0.06
Smoking, n (%)	234 (61)	83 (73)	0.02
Diabetes, n (%)	57 (15)	8 (7)	0.03
Cholesterolemia, n (%)	268 (70)	80 (70)	0.9
Triglyceridemia, n (%)	99 (26)	44 (39)	0.008
Obesity (%)	111 (29)	36 (31)	0.6
Hypertension, n (%)	183 (48)	41 (36)	0.03
Family history of CAD (%)	200 (52)	70 (61)	0.06
Ejection fraction(%)	51.2±10.2	52.9±7.8	0.1
N° of narrowed coronary arteries			0.0000
0 (%)	32 (8)	23 (20)	
1 (%)	139 (36)	47(41)	
2 (%)	133 (35)	36 (32)	
3 (%)	80 (21)	8 (7)	

There was a higher prevalence of family history in patients with early-onset IHD.

Association between SNPs and early IHD

Gene symbol (SNP)	Late IHD			Early IHD			p-value
	1/1 n(%)	1/2 n (%)	2/2 n (%)	1/1 n (%)	1/2 n (%)	2/2 n (%)	
ADIPOQ/ACDC (-11377 C>G)	233 (60.7)	127 (33.1)	24 (6.3)	62 (54.4)	46 (40.4)	6 (5.3)	0.3
ADD1 (Gly460Trp)*	264 (68.8)	104 (27.1)	16 (4.1)	83 (72.8)	24 (21.0)	7 (6.2)	0.3
ABCA1 (Gln597Arg)	36(9.4)	14 (36.7)	207 (53.9)	11 (9.6)	53 (46.4)	50 (44.0)	0.1
ACE (Thr776Th)	329 (86.7)	8 (2.0)	47 (12.3)	95 (83.3)	4 (3.6)	15 (13.2)	0.6
AGER (Gly82Ser)	374 (97.4)	10 (2.6)	/	110 (96.5)	4 (3.5)	/	0.6
AKAP10 (Ile646Val)	157 (40.8)	168 (43.8)	59 (15.4)	50 (43.8)	51 (44.8)	13 (11.4)	0.6
APOC3 (-482C>T)	181 (47.1)	170 (44.3)	33 (8.6)	39 (34.2)	57 (50.0)	18(15.8)	0.02
APOC3 (-455T>C)	128 (33.3)	190 (49.5)	66 (17.2)	30 (26.3)	64 (56.1)	20 (17.5)	0.07
ApoE (C4070T)*	346 (90.1)	19 (5.0)	19 (4.9)	98 (86.0)	4 (3.5)	12 (10.5)	0.08
ApoE (T3932C)*	308 (80.2)	68 (17.8)	8 (2.0)	94 (82.5)	18 (15.8)	2 (1.7)	0.8
CETP Taq1B (G>A)	131 (34.1)	184 (48.0)	69 (17.9)	37 (32.5)	65 (57.0)	12 (10.5)	0.1
CETP (-629C>A)	106 (27.6)	187 (48.7)	91 (23.7)	28 (24.5)	65 (57.0)	21 (18.4)	0.3
GJA4/Cx37 (C1019T)	184 (47.9)	153 (39.9)	47 (12.3)	54 (47.4)	53 (46.5)	7 (6.1)	0.1
ENOS/NOS3 (-786T>C)	101 (26.3)	202 (52.6)	81 (21.1)	32 (28.1)	60 (52.6)	22 (19.3)	0.9
FGB 1 (Ser189Ser)	257 (67)	121 (31.5)	6 (1.5)	85 (74.6)	24 (21.1)	5 (4.3)	0.03
FABP II (G2445A)	197 (51.3)	162 (42.2)	25 (6.5)	62 (54.4)	43 (37.7)	9 (7.9)	0.6
FGB 2 (Arg448Lys)	256 (66.7)	122 (31.7)	6 (1.6)	85 (74.6)	25 (21.9)	4 (3.5)	0.07
FGB 3 (-148C>T)	232 (60.4)	140 (36.5)	12 (3.1)	77 (67.5)	32 (28.1)	5 (4.4)	0.2
FVII(Arg353Gln)	270 (70.3)	107 (27.8)	7 (1.9)	78 (68.4)	35 (30.7)	1 (0.9)	0.7
GP1BA (Thr145Met)*	308 (80.2)	76 (19.8)	/	93 (81.6)	21 (18.4)	/	0.7
ICAM I (Lys469Glu)	122 (31.8)	189 (49.2)	73 (19.0)	33 (28.9)	58 (51.0)	23 (20.1)	0.8
IL1RN 3 (T13760C)	209 (54.4)	146 (38.0)	29 (7.6)	64 (56.1)	43 (37.7)	7 (6.1)	0.8
IL 6 (G-572C)	352 (91.7)	32 (8.3)	/	98 (86.0)	14 (12.3)	2 (1.7)	0.01
IPF1 (-108 3G>4G)*	230 (59.9)	142 (37.0)	12 (3.1)	65 (57.0)	46 (40.3)	3 (2.7)	0.8
LGALS2 (C3279T)	89 (23.1)	208 (54.2)	87 (22.7)	32 (28.1)	55 (48.3)	27 (23.6)	0.5
LPL (Ser447Stop)	289 (75.3)	91 (23.7)	4 (1.0)	91 (79.8)	20 (17.5)	3 (2.6)	0.2
LTA (Thr26Asn)*	189 (49.2)	147 (38.2)	48 (12.6)	54 (47.4)	47 (41.2)	13 (11.4)	0.8
MTHFR (C677T)	106 (27.6)	188 (48.9)	90 (23.5)	40 (35.1)	44 (38.6)	30 (26.3)	0.1
NADHPH p22-PHOX (C242T)	139 (36.2)	184 (47.9)	61 (15.0)	40 (35.1)	57 (50.0)	17 (14.9)	0.9
PON2 (Ser311Cys)	230 (59.9)	136 (35.4)	18 (4.7)	63 (55.3)	43 (37.7)	8 (7.0)	0.5
OR13G1 (Ile132Val)	148 (38.5)	168 (43.7)	68 (17.8)	35 (30.7)	63 (55.3)	16 (14.0)	0.09
ROS I (Asp2213Asn)*	232 (60.5)	4 (1.0)	148 (38.5)	67 (58.8)	2 (1.7)	45 (39.5)	0.8
SELE (G98T)	312 (81.3)	49 (12.7)	23 (6.0)	93 (81.6)	8 (7.0)	13 (11.4)	0.04
SELE (A561C)	312 (81.3)	67 (17.5)	5 (1.3)	93 (81.6)	17 (14.9)	4 (3.4)	0.2
SELP (Thr715Pro)	250 (65.1)	119 (31.0)	15 (3.9)	80 (70.2)	30 (26.3)	4 (3.4)	0.6
MMP3 (1171 5A>6)	115 (29.9)	197 (51.3)	72 (18.8)	31 (27.2)	48 (42.1)	35 (30.7)	0.02
TAS2R50 (Cys203Tyr)	153 (39.8)	171 (44.5)	60 (15.6)	38 (33.3)	58 (50.8)	18 (15.9)	0.4
THBS2, (T3949G)	205 (53.4)	147 (38.3)	32 (8.3)	63 (55.3)	43 (37.3)	8 (7.0)	0.8
THPO, (A5713G)	137 (35.7)	194 (50.5)	53 (13.8)	43 (37.7)	52 (45.6)	19 (16.7)	0.6
TNFα (C-850T)*	255 (66.4)	103 (26.8)	26 (6.8)	82 (71.9)	22 (19.3)	10 (8.8)	0.2
TNFSF4 (A/G)	270 (70.3)	99 (25.8)	15 (3.9)	83 (72.8)	28 (24.6)	3 (2.6)	0.8
FV, G1691A	377 (98.2)	7 (1.8)	/	112 (98.3)	2 (1.7)	/	0.9
Locus 9p21.3 (C/G)	120 (31.2)	180 (46.9)	84 (21.9)	36 (31.6)	65 (57.0)	13 (11.4)	0.03
LRP1 (C/T)	216 (56.3)	144 (37.5)	24 (6.2)	59 (51.8)	45 (39.5)	10 (8.8)	0.5

1 refers to ancestral allele (usually the commonest allele) and 2 to variant allele, 1/1 refers to baseline homozygote, 1/2 to heterozygote and 2/2 to variant homozygote.
*HWE deviation (P < 0.05)

- Six SNPs violated HWE at the $p<0.05$ level
- Six SNPs were significant ($p<0.05$), and four were marginally significant (p values ranging from 0.06 to 0.1)

Multivariate regression analysis by genotypes

Gene Polymorphism	Unadjusted OR(95%CI)	p value	Adjusted OR (95%CI)	p value
APOC3 -482C>T (rs2854117)	1.6 (1.0-2.6)	0.03	1.7 (1.1-2.6)	0.03
FGB 2 Arg448Lys (rs4220)	1.6 (1.1-2.5)	0.04	1.6 (1.0-2.7)	0.046
MMP311715A/6A (rs3025058)	1.3 (1.1-3.1)	0.04	1.3(1.1-2.9)	0.047
Locus 9p21.3 C/G (rs 1333049)	2.0(1.2-4.0)	0.01	1.8 (1.2-3.9)	0.01

Adjusted for gender, family history and atherogenic risk factors

On multivariate regression analysis 4 APOC3 (C-482T ; rs2854117), FGB-2 (Arg448Lys; rs4220), MMP3 (5A/6A; rs3025058) and locus9p21.3 (rs1333049) variants were independently related to early IHD

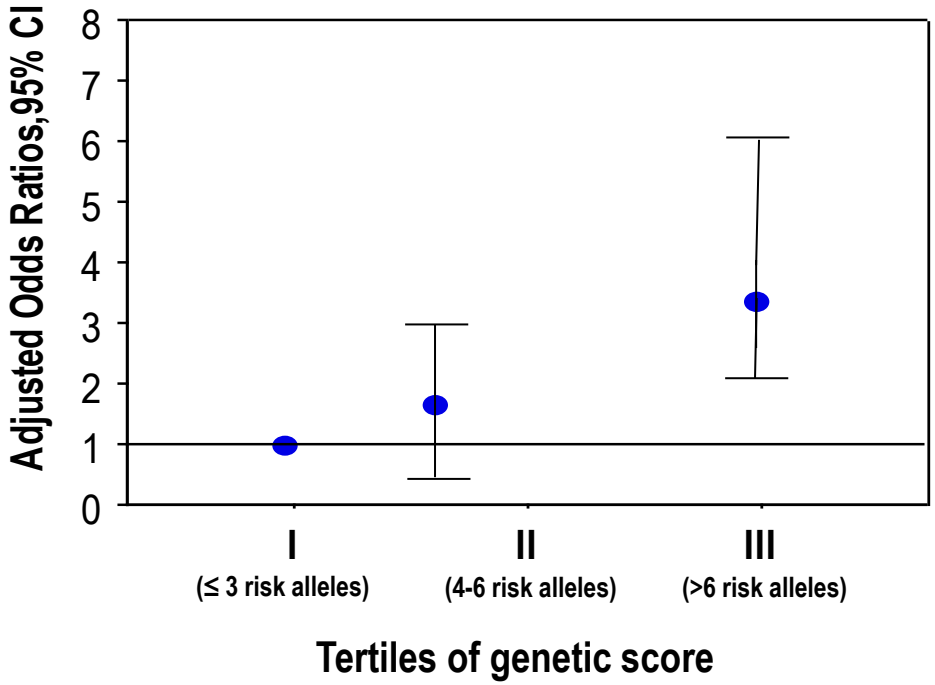
Genetic score risk modeling

- 2 units=homozygous for the high-risk allele
- 1 unit =heterozygous for the high-risk allele
- 0 unit =homozygous for the low-risk allele

Association Between Genetic score and early IHD

Genetic score	Late IHD (n=384)	Early IHD (n=114)
2	12 (3.1)	1 (0.9)
3	39 (10.2)	9 (7.9)
4	76 (19.8)	16 (14.0)
5	116 (30.2)	29 (25.4)
6	94 (24.5)	31 (27.2)
7	38 (9.9)	23 (20.2)
8	9 (2.3)	5 (4.4)
OR (95% CI) per Allele	1.3 (95% CI 1.1-1.6)	
p for Trend	0.001	

OR= 1.3 (95% CI 1.1-1.6, p= 0.001) per risk allele adjusted for sex and traditional risk factors



Patients in the top tertile of the genetic risk score were estimated to have a 3.1-fold (95% CI 1.6-5.9 p= 0.001) increased risk of early IHD compared with those in the bottom tertile.

Conclusion

Our findings showed that *APOC3 (C-482T)*, *FGB-2 (Arg448Lys)*, *MMP3 (5A/6A)* and *locus9p21.3 (rs1333049)* variants are genetic risk factors of early IHD.

Genetic risk score comprising these variants may allow better risk stratification that any single variant, improving the assessment of an individual's genetic predisposition .