

Supplemental Material

Method

Genotyping

Cardiovascular Gene-Centric 50K SNP Array in INVEST-GENES Case-control

We obtained genotype data for 153 SNPs in the Chromosome 9p21 region (position 21900000- 22200000, as defined by Samani et al.¹) on INVEST-GENES case-control samples (n = 1,460) from the gene-centric Human CVD 50K SNP array (Illumina), a custom chip with approximately 49,094 SNPs from ~2,100 loci related to cardiovascular, inflammatory and metabolic phenotypes with most loci captured at a density equal or greater than that afforded by genome-wide genotyping products (hereafter referred to as the CVD SNP array).²

Quality control (QC) procedures were performed using GenomeStudio software (Illumina Inc. San Diego, CA). The QC criteria were determined *a priori* as: GenTrain clustering algorithm score of > 0.3; SNP call rate > 95%; sample call rate > 90%; minor allele frequency of > 0.01; Hardy Weinberg Equilibrium p value within race/ethnicity >10⁻⁵.

Genotyping for rs2383207 and rs10757278 in INVEST-GENES cohort

Since the risk allele we observed was opposite from the literature in this cohort based on the CVD SNP array data, we sought to independently genotype 2 additional SNPs that have been commonly published to confirm that we found the same directional

findings. Two of the 9p21 SNPs: rs2383207 and rs10757278 (in high LD ($r^2 > 0.8$) with rs1333049, rs10757274 and rs2891168 in Caucasians), were either not on the CVD SNP array or did not pass the CVD SNP array QC procedure (deviated from Hardy Weinberg Equilibrium in African Americans). We therefore genotyped these 2 SNPs in the entire INVEST-GENES cohort of 5,979. DNA samples from INVEST-GENES cohort were genotyped for these 2 variants using TaqMan allelic discrimination (Applied Biosystems, Foster City, CA) at University of Florida (INVEST-GENES). The PCR primers and probes for these 2 SNPs (rs2383207, Assay ID C__15789010_10, and rs10757278, Assay ID C__11841860) were purchased from Applied Biosystems. Genotype accuracy was verified by genotyping 5-10% randomly selected duplicate samples for each SNP and 36 samples (12 random samples for each genotype for each SNP) were also genotyped by pyrosequencing³ (Biotage, Uppsala, Sweden) using a PSQ HS96A SNP reagent kit (Biotage AB, Uppsala, Sweden) to confirm genotyping results from TaqMan (primers available upon request).

Genotyping for rs2383207, rs10757278, rs7049105, rs2157719 in INFORM

To replicate the findings in INVEST, DNA samples from INFORM were genotyped for 4 SNPs: rs2383207, 10757278, rs7049105 and rs2157719, using TaqMan allelic discrimination (Applied Biosystems, Foster City, CA) or pyrosequencing at Washington University. The PCR primers and probes for the first 2 SNPs (rs2383207, Assay ID C__15789010_10, and rs10757278, Assay ID C__11841860) were purchased from Applied Biosystems. Genotyping of rs7049105 and 2157719 were performed by pyrosequencing³ (Biotage, Uppsala, Sweden) using a PSQ HS96A SNP reagent kit (Biotage AB, Uppsala, Sweden) (primers available upon request).

Table S1: Baseline demographics and medical history of INVEST participants

	INVEST Case Control (n = 1460)						INVEST Cohort (n=5907)					
	Caucasians (n=858)		African Americans (n = 212)		Hispanics (n = 390)		Caucasians (n = 2364)		African Americans (n = 736)		Hispanics (n = 2770)	
	case (n=178)	control (n=680)	case (n=43)	control (n= 169)	case (n=77)	control (n=313)	Event (n=178)	No event (2186)	Event (n=43)	No event (n=693)	Event (n=77)	No Event (n=2693)
Demographic												
Age, year, mean (SD)	71.4 (9.5)	70.6 (9.1)	68.8 (11.9)	68.3 (9.4)	72.8 (9.0)	71.2 (8.9)	71.4 (9.5)	68.2 (9.4)	68.8 (11.9)	63.0 (9.4)	72.8 (9.0)	64.7 (9.5)
sex, % male	92 (51.7%)	363 (53.4%)	19 (44.2%)	70 (41.2%)	39 (50.7%)	130 (41.5%)	92 (51.7%)	1223 (56.0%)	19 (44.2%)	256 (36.9%)	39 (50.7%)	954 (35.4%)
Body Mass Index, kg/m ²	27.3 (5.2)	28.8 (5.3)	28.6 (4.5)	31.0 (6.2)	27.7 (4.3)	28.0 (4.9)	27.3 (5.2)	29.2 (5.5)	28.6 (4.5)	31.7 (6.4)	27.7 (4.3)	29.2 (5.3)
Baseline SBP (mmHg)	151.3 (18.2)	148.4 (17.5)	153.9 (17.5)	149.0 (20.2)	146.8 (19.7)	147.2 (18.7)	151.3 (18.2)	148.7 (18.1)	153.9 (17.5)	149.2 (19.0)	146.8 (19.7)	146.8 (18.4)
Baseline DBP (mmHg)	81.8 (10.5)	82.3 (10.5)	88.0 (11.1)	86.1 (9.2)	83.8 (11.1)	85.0 (9.7)	81.8 (10.5)	83.1 (10.8)	88.0 (11.1)	88.2 (10.7)	83.8 (11.1)	86.9 (10.1)
Baseline Heart Rate (1/min)	76.0 (9.1)	75.1 (9.3)	76.3 (8.6)	76.3 (9.7)	76.0 (9.3)	73.0 (9.1)	76.0 (9.1)	74.9 (9.4)	76.3 (8.6)	76.8 (9.5)	76.0 (9.3)	74.1 (9.9)
Medical History, No. (%)												
History of MI	69 (38.8%)	226 (33.2%)	16 (37.2%)	37 (21.8%)	17 (22.1%)	27 (8.6%)	69 (38.8%)	865 (40.0%)	16 (37.2%)	146 (21.1%)	17 (22.1%)	237 (8.8%)
Heart Failure (NYHA class I-III)	20 (11.2%)	34 (5.0%)	5 (11.6%)	12 (7.1%)	8 (10.4%)	7 (2.2%)	20 (11.2%)	102 (4.7%)	5 (11.6%)	31 (4.5%)	8 (10.4%)	35 (1.3%)
Chronic stable angina	98 (55.1%)	326 (47.9%)	26 (60.5%)	134 (78.8%)	64 (83.1%)	302 (96.5%)	98 (55.1%)	1082 (49.5%)	26 (60.5%)	546 (78.8%)	64 (83.1%)	2560 (95.1%)
Unstable angina	32 (18.0%)	100 (14.7%)	5 (11.6%)	22 (12.9%)	9 (11.7%)	17 (5.4%)	32 (18.0%)	320 (14.6%)	5 (11.6%)	106 (15.3%)	9 (11.7%)	109 (4.1%)
Diabetes*	53 (30.0%)	122 (17.9%)	26 (60.5%)	51 (30.0%)	34 (44.2%)	52 (16.6%)	53 (30.0%)	526 (24.1%)	26 (60.5%)	233 (33.6%)	34 (44.2%)	789 (29.3%)

*History of and/or currently taking anti-diabetic medications; Abbreviations: SD: standard deviation; SBP: systolic blood pressure; DBP: diastolic blood pressure; MI: myocardial infarction;

Table S2. Baseline demographics and medical history of INFORM participants

	INFORM (n=714)			
	Caucasians (n =573)		African Americans (n = 141)	
	Event (n=142)	No Event (n=431)	Event (n=35)	No Event (n=106)
Demographic				
Age, year, mean (SD)	63.6 (12.8)	61.5 (12.4)	55.6 (9.9)	53.4 (10.6)
sex, % male	83 (58.5%)	289 (67.1%)	16 (45.7%)	61 (57.6%)
Body Mass Index, kg/m ²	29.5 (5.6)	29.4 (5.9)	31.0 (7.6)	31.0 (8.1)
Baseline SBP (mmHg)	132.8 (23.7)	135.0 (24.8)	150.0 (37.2)	146.5 (29.1)
Baseline DBP (mmHg)	71.4 (14.6)	72.7 (14.9)	80.3 (19.8)	83.0 (20.0)
Baseline Heart Rate (1/min)	78.7 (18.0)	76.2 (18.2)	86.2 (23.1)	81.5 (19.7)
ACS type, No. (%)				
ST-elevation MI	35 (24.7%)	141 (32.7%)	7 (20.0%)	16 (15.1%)
Non-ST elevation MI	44 (31.0%)	138 (32.0%)	11 (31.4%)	23 (21.7%)
Unstable angina	62 (43.7%)	151 (35.0%)	17 (48.6%)	67 (63.2%)
Old LBBB/Unknown	1 (0.7%)	1 (0.2%)		
Medical History, No. (%)				
History of MI	114 (80.3%)	348 (80.7%)	21 (60.0%)	68 (64.2%)
Heart Failure (NYHA class I-III)	9 (6.3%)	22 (5.1%)	4 (11.4%)	20 (18.9%)
Chronic stable angina	n/a	n/a	n/a	n/a
Unstable angina	n/a	n/a	n/a	n/a
Diabetes*	53 (37.3%)	85 (19.7%)	20 (57.1%)	40 (37.7%)

*History of and/or currently taking anti-diabetic medications; Abbreviations: SD: standard deviation; SBP: systolic blood pressure; DBP: diastolic blood pressure; MI: myocardial infarction; LBBB: left bundle branch block

Table S3. Genotype and Allele frequencies of 9p21 SNPs in the INVEST-GENES and INFORM

		INVEST GENES				INFORM			
	Race/Ethnicity	N	Genotype: n	Allele frequency	HWE p	N	Genotype: n	Allele frequency	HWE p
rs2383207	Caucasians	2307	AA/AG/GG: 444/1170/693	A: 45%	0.21	530	AA/AG/GG: 111/249/170	A: 44%	0.26
	African Americans	715	AA/AG/GG: 13/153/549	A: 13%	0.54	121	AA/AG/GG: 13/15/93	A: 17%	<0.0001*
	Hispanics	2717	AA/AG/GG: 269/1182/1266	A: 32%	0.78				
rs10757278	Caucasians	2297	AA/AG/GG: 588/1142/567	A: 50.5%	0.79	530	AA/AG/GG: 119/259/152	A: 47%	0.66
	African Americans	718	AA/AG/GG: 461/214/43	A: 79%	0.008	121	AA/AG/GG: 69/46/6	A: 76%	0.64
	Hispanics	2721	AA/AG/GG: 787/1320/614	A: 53.2%	0.18				
rs7049105	Caucasians	858	AA/AG/GG: 217/409/232	A: 49%	0.17	526	AA/AG/GG: 121/261/144	A: 48%	0.9
	African Americans	211	AA/AG/GG: 21/90/100	A: 31%	0.91	115	AA/AG/GG: 9/51/55	A: 30%	0.55
	Hispanics	388	AA/AG/GG: 52/156/180	A: 34%	0.05				
rs2157719	Caucasians	869	AA/AG/GG: 314/421/134	G: 40%	0.72	548	AA/AG/GG: 205/266/77	G: 38%	0.53
	African Americans	213	AA/AG/GG: 184/28/1	G: 7%	0.95	105	AA/AG/GG: 88/17/0	G: 8%	0.37
	Hispanics	390	AA/AG/GG: 217/409/232	G: 24%	0.47				
rs10757274	Caucasians	867	AA/AG/GG: 214/440/213	A: 50%	0.66				
	African Americans	214	AA/AG/GG: 116/73/25	A: 71%	0.015				
	Hispanics	390	AA/AG/GG: 115/192/83	A: 54%	0.86				
rs2891168	Caucasians	859	AA/AG/GG: 214/433/212	A: 50.1%	0.81				
	African Americans	211	AA/AG/GG: 111/78/22	A: 71%	0.14				
	Hispanics	386	AA/AG/GG: 114/192/80	A: 54%	0.96				
rs1333049	Caucasians	860	GG/CG/CC: 227/424/209	C: 49%	0.69				
	African Americans	212	GG/CG/CC: 114/73/25	C: 29%	0.02				
	Hispanics	389	GG/CG/CC: 110/192/87	C: 47%	0.85				

*p<0.0017 (0.05/29), therefore considered deviated from HWE in INFORM African Americans.

References:

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