SUPPLEMENTARY INFORMATION

Assessment of the value of a genetic risk score in improving the estimation of coronary risk

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SUPPLEMENTARY METHODS

S1. **Genetic variant selection, genotyping, quality controls and generation of the multi-locus risk score.**

S1.1. Genetic variant selection: SNP-selection was carried out as described previously [1]. Briefly, we searched the NHGRI GWAS catalog [2] (August, 2010) for the terms 'Myocardial Infarction/Coronary disease (MI/CAD)' and related phenotypes. This search returned 21 genetic variants. Those variants that reported an association p-value >$1x10^{-6}$ were excluded for the present analysis. In order to minimize redundant information in the genetic risk score (GRS), we computed the linkage equilibrium between variants using data from the HapMap CEU sample, and from those variants that presented high correlation (LD r^2>0.3), one was randomly selected. We evaluated the evidence in the NHGRI GWAS catalogue for each of the 14 remaining variants, and excluded those that had been reported to be associated with classical cardiovascular risk factors (CVRF), such as total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, diabetes, hypertension and smoking. Moreover, we excluded 2 of the remaining SNPs because literature-based evidence strongly suggested an association between those loci and CVRF. From this list we also excluded variants that were not associated with MI/CAD in the CARDIoGRAM study [3]. We added the rs10455872 variant in *LPA* because it has since been reported to be strongly association with MI/CAD [3,4]. See the flow chart of the selection process in *S.F2.*

S1.2. Generation of multi-locus genetic risk score: The GRS was weighted by the estimated effect size reported for each variant in the CARDIoGRAM study [3] using the following formula:

$$GRS = \sum_{i=1}^{8} \beta_i \cdot SNP_i$$

Where:

- β_i is the estimated effect size reported for each variant in the CARDIoGRAM study;

- SNP_i is the number of copies of each individual SNP evaluated (can have values of 0, 1 or 2 for genotyped SNPs and values ranging from 0 to 2 for imputed SNPs)

S1.3. Genotyping and genotyping quality control: REGICOR participants' DNA was obtained from buffy coat using standardized methods [5] (L'ARS services, Barcelona, Spain) and samples were genotyped by Centro Nacional de Investigación Oncológica (CNIO, Madrid, Spain) using the Cardio inCode chip (Ferrer inCode, Barcelona, Spain) based on Veracode (Illumina, San Diego, USA) and KASPar (KBioscience , Hoddesdon, United Kingdom) technologies. The overall percentage of agreement of the chip with reference technology is 99.9% and the analytical sensitivity and specificity are greater than 98.6%. For the Framingham participants, the genotypes for genotyped SNPs were obtained using the Affymetrix 500K and 50K chips, and for additional SNPs by imputation into the HapMapII CEU haplotype panel (build 36, release 22), using MACH version 1.00.15.

S1.4. Quality control: Various quality control measures were applied at both participant and SNP levels to the data from both cohorts: Individuals with low call rates or sex mismatches were excluded before imputation in the Framingham cohort database. Moreover, high levels of missingness ($p<10^{-9}$), highly significant departures from Hardy-Weinberg equilibrium ($p<10^{-6}$), or Mendelian errors (>100) were used to determine which SNPs to use for the imputation step, and were also applied as quality control criteria for the SNPs selected.

S2. Follow-up and phenotype definition

All REGICOR participants were periodically contacted by telephone or by mail to ascertain whether they had presented any cardiovascular event up until the end of 2009. Fatal events were identified from regional and national mortality registers. All the reported events were reviewed with hospital records or primary care records. An event committee classified the suspected cardiovascular (CVD) events after review of all medical records and physician notes using standardized criteria [6]. This study was approved by the local Ethics Committee and all participants gave written informed consent.

All Framingham participants were analyzed for onset of cardiovascular events during follow-up until the end of 2007. Repeated examinations and clinic visits were carried out approximately every 2 and 4 years, respectively. Suspected cardiovascular events were reviewed and adjudicated by a panel of three Framingham physician investigators after review of all available examination records, hospitalization records and physician notes using standardized criteria [7].

Methodology for laboratory determinations has been described elsewhere [7,8].

Myocardial infarction was defined on the basis of the classical WHO definition by the presence of 2 out of 3 clinical criteria: new diagnostic Q-waves on ECG, prolonged ischemic chest discomfort and elevation of serum biomarkers of myocardial necrosis. Angina was defined by the presence of ischemic chest discomfort with signs of ischemia in the ECG. Coronary artery by-pass grafting or percutaneous coronary interventions were considered as revascularization procedures. CHD death was considered after reviewing the mortality register when the most likely cause of death was CHD and no other cause could be ascribed.

Atherothrombotic stroke was defined as a non-embolic acute-onset focal neurological deficit of vascular origin that persisted for more than 24 hours or an ischemic infarction that was

documented at autopsy. Peripheral artery disease was defined by the presence of symptoms of claudication and an objective diagnostic test such as a pathological ankle-brachial index (<0.9) or a pathological arteriography or revascularization procedure.

S3. Ten-year cardiovascular risk estimation

All cardiovascular risk factors required for the risk functions were measured using standard methods [9,10]. Participants were considered to be diabetic if they had been diagnosed with diabetes or treated with oral hypoglycemic agents or insulin or presented a glycemia higher or equal to 126 mg/dL. Those who reported smoking ≥1 cigarette/day in the preceding year were considered smokers. All necessary baseline lipid and blood pressure measurements were collected and used to estimate the risk of each participant.

S4. Statistical analysis

To account for family structure in the Framingham cohort we also adjusted for the first five genetic principal components (computed using PLINK) [11] as covariates in the models [12,13].

All other analyses were performed using R version 2.11 (packages and functions indicated below by <*package*>::<*function*>).

The proportional hazards assumption was tested using *survival::cox.zph*.

The meta-analysis was computed using the *rmeta::meta.DSL* function [14].

We used three different statistics to assess the potential value of including the GRS in risk prediction:

a) to assess the goodness-of-fit of the models we used a version of the Hosmer-Lemeshow test that takes right censoring of the data into account [15];

b) to evaluate the improvement in the discriminative capacity of the model that included the genetic score with respect to a model without the score, we computed the concordance index (c-statistic) using the *Hmisc::rcorr.cens* function [16];

c) to assess the reclassification we calculated the net reclassification improvement (NRI) [17] and integrated discrimination improvement (IDI) [18] in the whole sample and in the subgroup of individuals considered to have intermediate coronary risk according to the classical risk function. To calculate the 10-year expected number of events in each risk category and in each cohort we used the Kaplan-Meier estimates as proposed by Steyerberg and Pencina [15,18]. A bootstrapping method was used to construct confidence intervals for IDI and NRI to take into account the uncertainty of the Kaplan-Meier estimates.

The estimated risk for each individual was computed under the Proportional Hazards assumption (Cox Model)

$$Risk = 1 - S_{\bar{X}}^{\exp\eta},$$

where:

a) $S_{\bar{X}}$ is survival value for the population average. This value depends on gender and has been taken from Framingham equation [19] for the Framingham cohort, and from REGICOR calibrated equation [20] for the REGICOR cohort.

b) $\exp$: exponential value (or anti-logarithm function).

c) η is the linear predictor, i.e, the product of coefficients and factors, and differs for each cohort:

a) For REGICOR $\eta = \sum_{j=1}^{p} \beta_j^F \left(F_j - \overline{F}_j\right) + \beta^G \left(G - \overline{G}\right)$

b) For Framingham $\eta = \sum_{j=1}^{p} \beta_j^F \left(F_j - \overline{F}_j\right) + \beta^G \left(G - \overline{G}\right) + \sum_{k=1}^{5} \beta_k^C \left(C_k - \overline{C}_k\right)$,

where,

- β_j^F : log-hazard-ratios of each of the classical risk factors. These coefficients have not been estimated but taken from the Framingham equation [7].
- F_j : individual value of each classical risk factor.
- $\overline{F}_j$: population average value of each classical risk factor. This value has been taken from Framingham equation [7] for the Framingham cohort, and from REGICOR calibrated equation [20] for the REGICOR cohort.
- β^G : log-hazard-ratios of genetic score, estimated from the data
- G : individual value of genetic score
- $\overline{G}$: average value of genetic score in the sample
- β_k^C : log-hazard-ratios of each of the first five principal components, estimated from the data.
- C_k : individual value of each of the first five principal components.
- $\overline{C}_k$: sample average value of each of the first five principal components.

NOTE: In Framingham cohort, computation of goodness-of-fit (Hosmer-Lemeshow), discrimination (c index), NRI and IDI was performed after adjustment for the first five principal components, in order to allow for the familial nature of the data.

S5. Power calculations

We performed a post-hoc calculation of our analyses' power to detect significant associations. In these power calculations, the variant's effect on disease risk was taken as the beta obtained from each study. All power computations were based on an alpha value (Type I error rate) equivalent to 0.05. Within each analysis we performed the following steps:

i. The minimum effect size (beta) the analysis had high (~80%) or moderate (~50%) power to detect. The definitions of high and moderate power were selected arbitrarily to indicate where our

analysis was well powered to detect risk effects (high power), but also to allow for the fact that, if multiple independent but more subtle effects were present, at least some proportion of these could also be detected (e.g. 50%, moderate power).

ii. The power of the analysis to detect each of a series of effect sizes (betas, corresponding to the following hazard ratios: 1.05, 1.09, 1.10, 1.12, 1.14, 1.18, 1.29 and 1.35). These data were computed to help indicate the circumstances under which our study was unable to provide conclusive information, e.g. for rarer variants or for more subtle effect sizes. These hazard ratios were in part selected because are the ones reported in the CARDIoGRAM study for the values we include in this analysis, and therefore we can observe the specific power that we have to achieve each reported HR.

iii. These two computations described were also computed for the GRS and the risk of coronary or cardiovascular disease to evaluate the study power.

The results of these power calculations are shown in *S.T4*.

SUPPLEMENTARY TABLES

S.T1. Clinical characteristics of individuals included in the analysis or not, based on the availability of genetic information.

	Not included	Included	P-value
REGICOR			
Individuals	698	2,351	--
Age (years) *	54.6 (11.0)	53.9 (11.2)	0.128
Gender (male) †	343 (49.1%)	1,123 (47.8%)	0.552
Systolic Blood Pressure (mmHg) *	133 (21.0)	132 (20.8)	0.346
Diastolic Blood Pressure (mmHg) *	79.1 (10.2)	79.5 (10.4)	0.414
Hypertension †	274 (39.5%)	938 (40.1%)	0.843
Smoking †	123 (18.1%)	511 (22.0%)	0.034
Total cholesterol (mg/dL)*	223 (40.7)	225 (42.4)	0.357
LDL cholesterol (mg/dL)*	152 (36.3)	152 (37.9)	0.886
HDL cholesterol (mg/dL)*	50.2 (13.3)	51.7 (13.3)	0.017
Triglycerides (mg/dL)‡	95.0 (69.0-131)	92.0 (70.0-127)	0.523
Cholesterol treatment †	48 (6.91%)	157 (6.71%)	0.926
Diabetic status †	111 (17.2%)	316 (13.8%)	0.036
Diabetes treatment †	35 (5.04%)	96 (4.11%)	0.337
Body mass index (kg/m^2)*	27.6 (4.24)	27.4 (4.47)	0.436
Obesity (BMI≥30 kg/m^2) †	177 (25.8%)	596 (25.6%)	0.962
Estimated 10-y coronary risk §	3.7 (1.9-6.8)	3.3 (1.7-6.2)	0.061
FRAMINGHAM			
Individuals	1,699	3,537	--
Age (years) *	65.8 (12.1)	56.0 (9.26)	<0.001
Gender (male) †	675 (39.7%)	1,540 (43.5%)	0.009
Systolic Blood Pressure (mmHg) *	135 (19.9)	127 (18.3)	<0.001
Diastolic Blood Pressure (mmHg) *	75.3 (10.5)	75.0 (9.79)	0.249
Hypertension †	861 (50.9%)	1,121 (31.7%)	<0.001
Smoking †	449 (26.5%)	713 (20.2%)	<0.001
Total cholesterol (mg/dL)*	222 (43.1)	210 (38.6)	<0.001
LDL cholesterol (mg/dL)*	125 (32.9)	125 (34.1)	0.911
HDL cholesterol (mg/dL)*	50.2 (15.4)	51.0 (15.2)	0.087
Triglycerides (mg/dL)‡	120 (84.0-179)	116 (83.0-172)	0.224
Cholesterol treatment †	55 (3.25%)	166 (4.69%)	0.015
Diabetic status †	164 (10.1%)	226 (6.39%)	<0.001
Diabetes treatment †	72 (4.25%)	90 (2.54%)	0.001
Body mass index (kg/m^2)*	26.7 (4.77)	27.1 (4.78)	0.001
Obesity (BMI≥30 kg/m^2) †	332 (20.2%)	780 (22.1%)	0.126
Estimated 10-y coronary risk §	12.3 (6.9-20.4)	7.79 (4.5-14.1)	<0.001

The 'not included' group includes individuals who were not between 35 and 74 years of age, who had had a previous event, or were missing values for classical risk factors or SNP.
* mean (standard deviation); † n (proportion (%)); ‡ median (25 and 75 percentiles); § mean (95% confidence interval).

S.T2. Effects of classical risk factors on risk of a coronary event.

	HR [95%CI]	P-value
REGICOR		
Age (10 years)	2.05 [1.69-2.49]	<0.001
Gender (men)	2.56 [1.69-3.85]	<0.001
Total cholesterol (10 mg/dL)	1.04 [1.00-1.09]	0.092
HDL cholesterol (10 mg/dL)	0.60 [0.50-0.72]	<0.001
Systolic BP (10 mmHg)	1.38 [1.27-1.49]	<0.001
Diastolic BP (10 mmHg)	1.37 [1.15-1.64]	0.001
Diabetes	2.55 [1.66-3.91]	<0.001
Smoker	1.21 [0.78-1.87]	0.392
Family history of CVD*	1.58 [0.96-2.60]	0.068
Estimated 10-y coronary risk†	1.15 [1.12-1.18]	<0.001
FRAMINGHAM		
Age (10 years)	1.60 [1.42-1.81]	<0.001
Gender (men)	2.22 [1.82-2.70]	<0.001
Total cholesterol (10 mg/dL)	1.07 [1.04-1.09]	<0.001
HDL cholesterol (10 mg/dL)	0.74 [0.69-0.80]	<0.001
Systolic BP (10 mmHg)	1.25 [1.19-1.31]	<0.001
Diastolic BP (10 mmHg)	1.33 [1.21-1.47]	<0.001
Diabetes	2.66 [2.02-3.49]	<0.001
Smoker	1.32 [1.07-1.65]	0.011
Family history of CVD‡	1.50 [1.09-2.07]	0.013
Estimated 10-y coronary risk†	1.06 [1.05-1.06]	<0.001

* CVD: Cardiovascular disease.
† Coronary risk was calculated using the original Framingham risk function for the Framingham cohort, and the calibrated function for the REGICOR cohort.
‡ Only in the Offspring sample.

S.T3. SNPs included in the genetic risk score, including genotype quality control.

							REGICOR					Framingham					Meta-analysis	
SNP	Chr	Gene	Position	Risk allele	Minor Allele	Weight (OR)	N total	MAF	p-HWE	HR[95%CI]	p-val	N total	MAF	p-HWE	HR[95%CI]	p-val	HR[95%CI]	p-val
rs17465637	1	*MIA3*	220890152	C	A	1.14	2,351	0.290	0.3409	0.99 [0.74-1.33]	0.482	3,537	0.305	0.9592	0.95 [0.82-1.09]	0.454	0.96 [0.84-1.09]	0.506
rs6725887	2	*WDR12*	203454130	C	C	1.14	2,351	0.144	0.9334	1.10 [0.76-1.60]	0.307	3,537	0.123	0.0572	1.11 [0.91-1.34]	0.299	1.11 [0.93-1.32]	0.242
rs9818870	3	*MRAS*	139604812	T	T	1.12	2,351	0.127	0.0634	1.00 [0.67-1.51]	0.496	3,537	0.142	0.1418	1.15 [0.96-1.37]	0.127	1.12 [0.96-1.32]	0.158
rs12526453	6	*PHACTR1*	13035530	C	G	1.10	2,351	0.353	0.9281	1.19 [0.89-1.59]	0.119	3,537	0.358	0.0098	0.97 [0.84-1.12]	0.656	1.03 [0.86-1.24]	0.739
rs1333049	9	*CDKN2A/2B*	22115503	C	G	1.29	2,351	0.484	0.2006	1.22 [0.93-1.60]	0.077	3,537	0.467	1.0000	1.18 [1.03-1.35]	0.020	1.19 [1.05-1.34]	0.005
rs1746048	10	*CXCL12*	44095830	C	T	1.09	2,351	0.134	0.9291	1.01 [0.68-1.50]	0.475	3,537	0.143	0.0488	0.99 [0.81-1.21]	0.931	0.99 [0.83-1.19]	0.948
rs9982601	21	*SCL5A3*	34520998	T	T	1.18	2,351	0.124	1.0000	1.14 [0.78-1.67]	0.250	3,537	0.147	Imputed	1.15 [0.96-1.39]	0.137	1.15 [0.97-1.36]	0.104
rs10455872	6	*LPA*	160930108	G	G	1.35	2,351	0.078	0.8856	2.26 [1.56-3.29]	<0.001	3,537	0.076	Imputed	1.09 [0.76-1.55]	0.638	1.57 [0.77-3.20]	0.219

Chr: Chromosome; p-HWE: p-value for the Hardy-Weinberg equilibrium; MAF: Minor allele frequency; N total: number of individuals with available genotype (or imputed value) for each variant. P-val: p-value. Weight (OR): odds ratio reported in the CARDIoGRAM study; analyses were weighted by the ln(OR); HR [95%CI]: Hazard ratio [95% confidence interval].

S.T4. Power calculations.

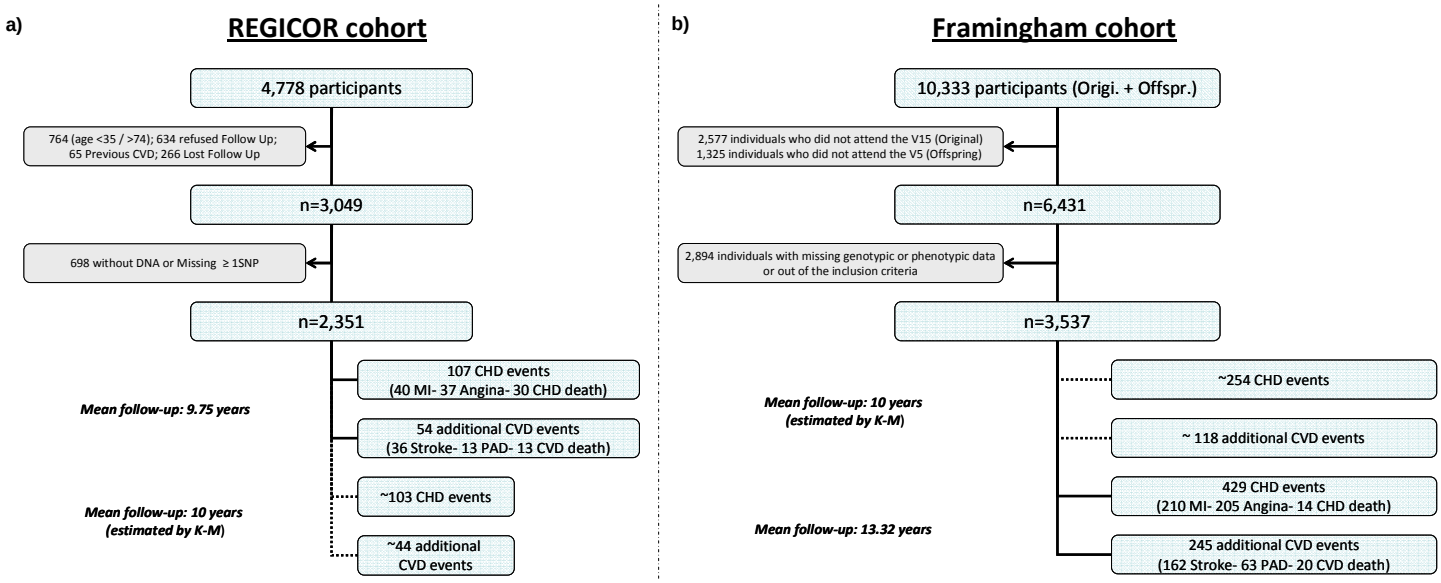
			Minimum HR detectable with high or moderate power		Power to detect a specific HR							
	SNP	se	0.8	0.5	1.05	1.09	1.10	1.12	1.14	1.18	1.29	1.35
REGICOR	rs17465637	0.150	1.52	1.34	0.062	0.089	0.098	0.118	0.141	0.198	0.399	0.519
	rs6725887	0.190	1.70	1.45	0.058	0.074	0.079	0.092	0.106	0.141	0.268	0.352
	rs9818870	0.207	1.79	1.50	0.056	0.070	0.075	0.085	0.097	0.126	0.233	0.305
	rs12526453	0.148	1.51	1.34	0.063	0.090	0.099	0.119	0.143	0.201	0.405	0.527
	rs1333049	0.138	1.47	1.31	0.064	0.095	0.106	0.130	0.157	0.223	0.452	0.582
	rs1746048	0.202	1.76	1.49	0.057	0.071	0.076	0.087	0.100	0.130	0.243	0.318
	rs9982601	0.194	1.72	1.46	0.057	0.073	0.078	0.090	0.104	0.136	0.259	0.339
	rs10455872	0.190	1.70	1.45	0.058	0.074	0.079	0.091	0.106	0.140	0.267	0.351
Framingham	rs17465637	0.073	1.23	1.15	0.103	0.221	0.259	0.345	0.438	0.625	0.939	0.985
	rs6725887	0.099	1.32	1.21	0.078	0.141	0.162	0.209	0.264	0.389	0.732	0.860
	rs9818870	0.091	1.29	1.19	0.084	0.158	0.183	0.239	0.303	0.446	0.801	0.911
	rs12526453	0.073	1.23	1.15	0.102	0.217	0.255	0.339	0.431	0.616	0.934	0.983
	rs1333049	0.069	1.21	1.14	0.109	0.239	0.282	0.375	0.476	0.669	0.958	0.992
	rs1746048	0.102	1.33	1.22	0.076	0.134	0.154	0.198	0.249	0.366	0.701	0.834
	rs9982601	0.094	1.30	1.20	0.081	0.150	0.172	0.225	0.284	0.418	0.769	0.888
	rs10455872	0.182	1.66	1.43	0.058	0.076	0.082	0.096	0.111	0.149	0.288	0.379
Meta-analysis	rs17465637	0.066	1.20	1.14	0.114	0.254	0.300	0.400	0.505	0.702	0.969	0.995
	rs6725887	0.089	1.28	1.19	0.085	0.162	0.187	0.245	0.311	0.457	0.813	0.919
	rs9818870	0.081	1.26	1.17	0.092	0.186	0.217	0.286	0.364	0.531	0.880	0.959
	rs12526453	0.093	1.30	1.20	0.082	0.152	0.175	0.229	0.289	0.426	0.779	0.895
	rs1333049	0.062	1.19	1.13	0.123	0.283	0.335	0.445	0.558	0.758	0.984	0.998
	rs1746048	0.092	1.29	1.20	0.083	0.155	0.179	0.234	0.297	0.437	0.791	0.904
	rs9982601	0.086	1.27	1.18	0.087	0.170	0.198	0.260	0.330	0.484	0.840	0.936
	rs10455872	0.363	2.77	2.04	0.052	0.056	0.058	0.061	0.065	0.074	0.108	0.131

			Minimum HR detectable with high or moderate power		Power to detect a specific HR							
	GRS	se	0.8	0.5	1.05	1.10	1.15	1.20	1.25	1.30	1.40	1.50
REGICOR	Linear	0.056	1.17	1.12	0.139	0.393	0.697	0.898	0.977	0.996	1.000	1.000
	Q2	0.362	2.76	2.03	0.052	0.058	0.067	0.080	0.095	0.112	0.153	0.201
	Q3	0.320	2.45	1.87	0.053	0.060	0.072	0.088	0.107	0.130	0.183	0.244
	Q4	0.294	2.28	1.78	0.053	0.062	0.076	0.095	0.118	0.145	0.209	0.281
	Q5	0.277	2.17	1.72	0.054	0.064	0.080	0.101	0.127	0.157	0.229	0.310
Framingham	Linear	0.031	1.09	1.06	0.352	0.870	0.995	1.000	1.000	1.000	1.000	1.000
	Q2	0.158	1.56	1.36	0.061	0.093	0.143	0.211	0.292	0.382	0.566	0.727
	Q3	0.156	1.55	1.36	0.061	0.094	0.146	0.215	0.298	0.390	0.577	0.738
	Q4	0.153	1.53	1.35	0.062	0.096	0.150	0.223	0.310	0.405	0.597	0.758
	Q5	0.156	1.55	1.36	0.061	0.094	0.146	0.216	0.299	0.391	0.579	0.739
Meta-analysis	Linear	0.058	1.18	1.12	0.133	0.371	0.667	0.877	0.968	0.994	1.000	1.000
	Q2	0.145	1.50	1.33	0.063	0.101	0.162	0.243	0.338	0.442	0.643	0.800
	Q3	0.154	1.54	1.35	0.062	0.095	0.148	0.219	0.304	0.398	0.587	0.748
	Q4	0.134	1.45	1.30	0.065	0.110	0.181	0.275	0.385	0.500	0.710	0.857
	Q5	0.168	1.60	1.39	0.060	0.088	0.132	0.192	0.264	0.345	0.517	0.674

GRS: Genetic risk score; Se: Standard error; 'HR detectable' indicates the minimum risk effect detectable (expressed as the exponent of the beta from the meta-analysis) with high or moderate power. 'Power' indicates the study's power to detect the effects sizes (hazard ratios) shown. In the computation of power for given effect size, scenarios with high power (≥80%) are shaded dark grey, those with moderate power (≥50% and <80%) are shaded light grey, and those with power lower than 50% are unshaded.

SUPPLEMENTARY FIGURES

S.F1. Process of sample inclusion.

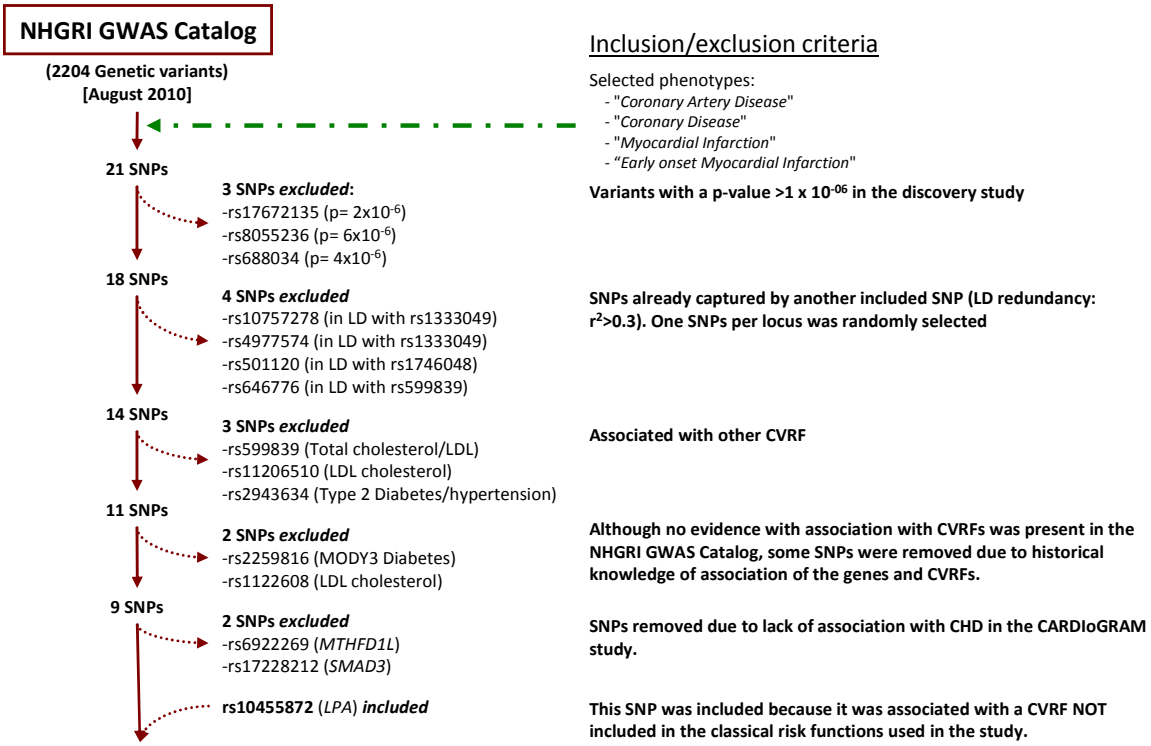


CHD: coronary heart disease; CVD: Cardiovascular disease; n: number of individuals; Origi: individuals from the Framingham Original cohort; Offspr: individuals from the Framingham Offspring cohort.

The values for the 10-year follow up in both cohorts have been estimated by Kaplan-Meyer (in REGICOR extending the results from 9.75 years of follow up to 10 years and in Framingham censoring the events from 13.32 to 10 years).

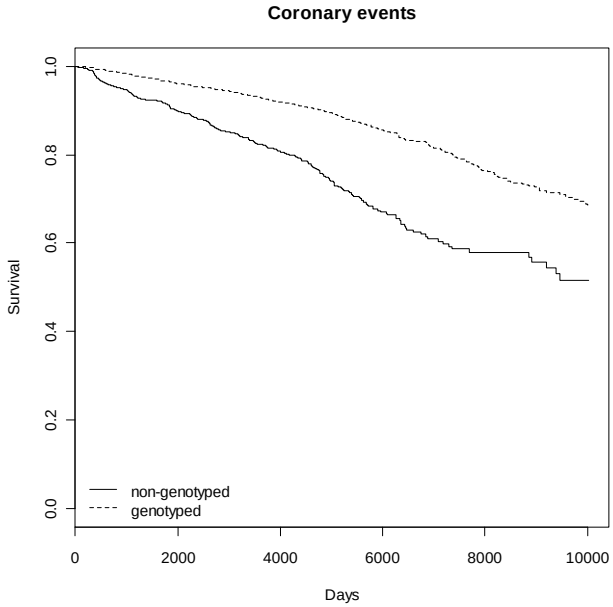
In the REGICOR cohort, the events estimated by Kaplan-Meyer were lower than in the observed sample at a median of 9.75 years because some of the observed events occur at a later stage (>10 years of follow up), and therefore the estimation obtained considers those individuals as event-free. By contrast, some individuals with a follow up <10 years who have not presented an event are considered as event by the estimator. By the same principle, a reduction of ~41% and ~52% of CHD and CVD events from the Framingham cohort can be due to the high number of individuals with unavailability of genetic data (although they were eligible for the present study).

S.F2. Process of SNP selection.



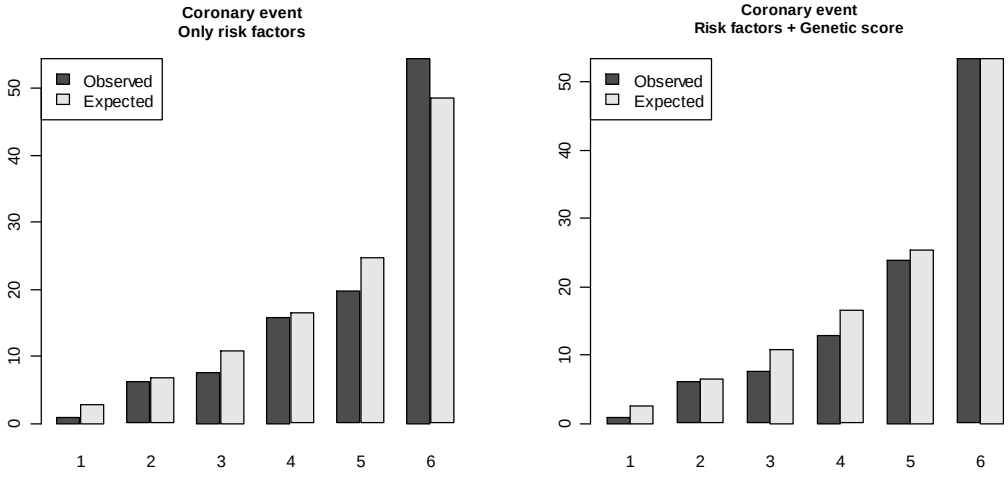
SNP	Chromosome	Gene	Position	Minor Allele
rs17465637	1	*MIA3*	220890152	A
rs6725887	2	*WDR12*	203454130	C
rs9818870	3	*MRAS*	139604812	T
rs12526453	6	*PHACTR1*	13035530	G
rs1333049	9	*CDKN2A/2B*	22115503	G
rs1746048	10	*CXCL12*	44095830	T
rs9982601	21	*SCL5A3*	34520998	T
rs10455872	6	*LPA*	160930108	G

S.F3. Kaplan-Meier curves for those individuals who were included in the analysis or not, based on the availability of phenotypic or genotypic information from the Framingham Heart Study.



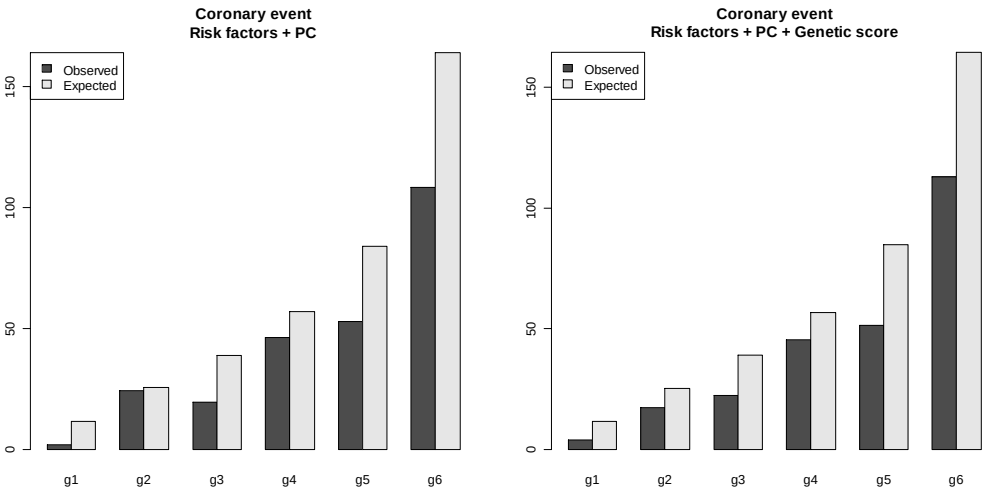
***S.F4*.** Analysis of the goodness-of-fit of the models with and without the genetic risk score, for coronary heart disease events both in REGICOR (a) and Framingham (b) cohorts using the Hosmer-Lemeshow test.

a) REGICOR



REGICOR risk function	Chi-square = 4.20 (df = 4), p-value = 0.383
REGICOR risk function + genetic risk score	Chi-square = 3.00 (df = 4), p-value = 0.557

b) FRAMINGHAM



Framinghan risk function	Chi-square = 60.38 (df = 4), p-value <0.001
Framinghan risk function + genetic risk score	Chi-square = 55.37 (df = 4), p-value <0.001

SUPPLEMENTARY ANALYSES

Supplementary Analysis 1

Predictive capacity of a coronary risk function improved by including a genetic score – extension of main analysis to CVD

1. INTRODUCTION

In 1994 the European Atherosclerosis Society and the European Society of Hypertension published a set of recommendations for CHD prevention [21]. The main reason for separating CHD and total cardiovascular risk (CVD), which are similar but distinct outcomes, was an attempt to simplify the estimation of CVD risk. However, by 2003 the Third Joint Task Force Guidelines proposed a change from CHD to CVD prevention, to reflect the fact that atherosclerosis may affect any part of the vascular tree [22,23], and because some of the clinical manifestations of CVD are thought to share a common etio-pathogenesis with CHD.

Although a population based strategy is critical to reducing the overall incidence of CVD [23], primary prevention in high risk groups is also widely implemented and an improvement of the risk functions for a significant reduction of incidence of the disease is warranted.

The aims of the current analyses were also to address steps 2 and 3 of the AHA recommendations for the same GRS. First, we assessed the association between the multi-locus GRS and incident CVD events in two prospective cohort studies with low and high CVD mortality (AHA, step 2). Second, we assessed whether the inclusion of this GRS improves the predictive capacity of the Framingham risk function (AHA, step 3). In addition, we evaluated the hypothesis that the improvement in predictive capacity provided by the GRS is greater among individuals with intermediate risk.

2. METHODS

Follow-up and phenotype definition

All REGICOR participants were periodically contacted to ascertain whether they had presented any CVD event up until the end of 2009, and events were reviewed using hospital or primary care records. Fatal events were identified from regional and national mortality registers. After reviewing all medical records and physician notes, suspected CVD events were classified in committee according to standardized criteria [6].

Among Framingham participants, a record was made of all CHD events that occurred during follow-up until the end of 2007. Suspected cardiovascular events were reviewed by a panel of

Framingham physician investigators after reviewing all available medical records and physician notes using standardized criteria [7].

CVD events included myocardial infarction (MI), angina, coronary revascularization and death due to CHD, plus atherothrombotic stroke and peripheral artery disease.

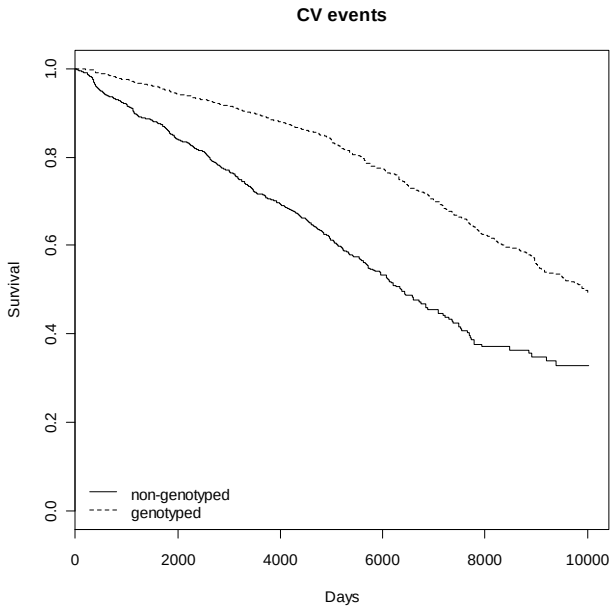
3. RESULTS

Sample selection and sample characteristics

The number of participants included was 2,351 from the REGICOR cohort and 3,537 from the Framingham cohort, and the number of observed CVD events was 161 in a mean follow-up of 9.75 years, and 674 in a mean follow-up of 13.32 years, respectively (*S.F2*).

As observed for CHD, in the Framingham sample, there was a difference in survival rates between individuals who had DNA sample available and those who did not and those included presented a better cardiovascular risk profile (*S.T1*) and a lower incidence of CVD events than those not included (*S.A1.Figure 1*)

S.A1.Figure 1. Kaplan-Meier curves for those individuals who were included in the analysis or not, based on the availability of phenotypic or genotypic information from the Framingham Heart Study.



The characteristics of the participants included in the present analyses stratified by cohort, and by the presence/absence of CVD events are shown in *S.A1.Table 1*. The effect of each cardiovascular risk factor on risk of CVD (hazard ratio) is presented in *S.A1.Table 2*.

S.A1.Table 1. Description of the phenotypic characteristics of the individuals included in the analysis from the REGICOR and from the Framingham Heart Study cohorts.

	All	None	CVD	p-value
REGICOR				
N	2,351	2,19	161	-
Age (years)[a]	53.9 (11.2)	53.3 (11.1)	61.5 (9.52)	<0.001
Gender (male)[b]	1123 (47.8)	1,016 (46.4)	72 (66.5)	<0.001
SBP (mmHg)[a]	132 (20.8)	131 (20.5)	147 (20.1)	<0.001
DBP (mmHg)[a]	79.5 (10.4)	79.3 (10.3)	82.4 (11.5)	0.001
Hypertension[b]	938 (40.1)	822 (37.7)	116 (72.0)	<0.001
Smoking[b]	511 (22.0)	476 (22.0)	35 (21.9)	0.947
Total cholesterol (mg/dL)[a]	225 (42.4)	224 (42.0)	235 (47.3)	0.011
LDL cholesterol (mg/dL)[a]	152 (37.9)	151 (37.7)	161 (40.6)	0.011
HDL cholesterol (mg/dL)[a]	51.7 (13.3)	52.1 (13.2)	46.4 (12.4)	<0.001
Triglycerides (mg/dL)[c]	92 (70-127)	91 (69-125)	116 (82-164)	<0.001
Cholesterol treatment[b]	157 (6.7)	136 (6.2)	21 (13.2)	0.001
Diabetes[b]	316 (13.8)	280 (13.1)	36 (22.9)	0.001
Diabetes treatment[b]	96 (4.11)	74 (3.4)	22 (13.7)	<0.001
Body mass index (kg/m^2)[a]	27.4 (4.47)	27.3 (4.46)	28.8 (4.28)	<0.001
Obesity (BMI≥30 kg/m^2)[b]	596 (25.6)	540 (24.9)	56 (35.2)	0.005
Family history of CHD[b]	272 (11.7)	301 (11.5)	29 (18.1)	0.012
Framingham				
N	3,537	2,863	674	-
Age (years)[a]	56.0 (9.3)	54.8 (9.2)	61.2 (7.4)	<0.001
Gender (male)[b]	1,540 (43.5)	1,190 (41.6)	350 (51.9)	<0.001
SBP (mmHg)[a]	127 (18.3)	125 (17.9)	134 (18.0)	<0.001
DBP (mmHg)[a]	75.0 (9.8)	74.6 (9.8)	76.6 (9.7)	<0.001
Hypertension[b]	1121 (31.7)	802 (28.0)	319 (47.5)	<0.001
Smoking[b]	713 (20.2)	531 (18.5)	182 (27.0)	<0.001
Total cholesterol (mg/dL)[a]	210 (38.6)	207 (37.4)	226 (39.3)	<0.001
LDL cholesterol (mg/dL)[a]	126 (34.0)	124 (33.3)	135 (37.3)	<0.001
HDL cholesterol (mg/dL)[a]	51 (15.2)	52 (15.3)	47 (14.1)	<0.001
Triglycerides (mg/dL)[c]	116 (83-172)	112 (80-164)	157 (107-217)	<0.001
Cholesterol treatment[b]	166 (4.7)	118 (4.1)	48 (7.1)	0.001
Diabetes[b]	226 (6.4)	138 (4.8)	88 (13.1)	<0.001
Diabetes treatment[b]	90 (2.5)	48 (1.7)	42 (6.2)	<0.001
Body mass index (kg/m^2)[a]	27.1 (4.8)	27.0 (4.8)	27.8 (4.5)	<0.001
Obesity (BMI≥30 kg/m^2)[b]	780 (22.1)	604 (21.2)	176 (26.2)	0.005
Family history of CHD[b]	551 (24.8)	478 (24.3)	73 (29.2)	0.089

CVD: individuals who presented a cardiovascular event (includes those with a coronary event); SBP: systolic blood pressure; DBP: diastolic blood pressure; LDL: low density lipoprotein; HDL: high density lipoprotein; BMI: body mass index; CI: confidence interval.

[a] mean (standard deviation); [b] n (proportion, %); [c] median (25 and 75 percentiles); [d] mean (95% confidence interval).

S.A1.Table 2. Effects (hazard ratio) of classical risk factors on risk of cardiovascular events.

	HR [95%CI]	P-value
REGICOR		
Age (10 years)	2.11 [1.79-2.47]	<0.001
Gender (men)	2.27 [1.64-3.23]	<0.001
Total cholesterol (10 mg/dL)	1.05 [1.01-1.09]	0.033
HDL cholesterol (10 mg/dL)	0.69 [0.60-0.79]	<0.001
Systolic BP (10 mmHg)	1.37 [1.29-1.46]	<0.001
Diastolic BP (10 mmHg)	1.37 [1.18-1.58]	<0.001
Diabetes	2.02 [1.39-2.93]	<0.001
Smoker	0.99 [0.68-1.44]	0.957
Family history of CVD[a]	1.59 [1.06-2.37]	0.024
Estimated 10-y CVD risk[b]	1.14 [1.12-1.16]	<0.001
FRAMINGHAM		
Age (10 years)	1.78 [1.61-1.96]	<0.001
Gender (men)	1.75 [1.52-2.04]	<0.001
Total cholesterol (10 mg/dL)	1.07 [1.05-1.09]	<0.001
HDL cholesterol (10 mg/dL)	0.79 [0.75-0.84]	<0.001
Systolic BP (10 mmHg)	1.24 [1.19-1.28]	<0.001
Diastolic BP (10 mmHg)	1.19 [1.10-1.29]	<0.001
Diabetes	2.53 [2.02-3.16]	<0.001
Smoker	1.42 [1.20-1.68]	<0.001
Family history of CVD[c]	1.29 [0.98-1.69]	0.067
Estimated 10-y CVD risk[b]	1.06 [1.05-1.06]	<0.001

[a] CVD: Cardiovascular disease. [b] Coronary risk was calculated using the original Framingham risk function for the Framingham cohort, and the calibrated function for the REGICOR cohort; [c] Only in the Offspring sample.

Validation of the association between the GRS and risk of CVD

The results of the test for association between the genetic variants included in the GRS and incidence of CVD events is shown in *S.A1.Table 3*. The variants nominally associated with CVD events were rs1333049 in *CDKN2A/2B* and rs10455872 in *LPA*. The minimum hazard ratio (HR) we were able to detect with 80% power for each individual variant ranged from 1.36 to 1.64, in REGICOR, from 1.17 to 1.48 in Framingham, and from 1.15 to 1.74 in the meta-analysis (*S.A1.Table 4*).

S.A1.Table 3. Characteristics of the genetic variants included in the multi-locus genetic risk score, magnitude of the association for coronary events in both cohorts and meta-analyses results of the observed effect sizes.

SNP	REGICOR		FRAMINGHAM		Meta-analysis	
	HR[95%CI]	p-value	HR[95%CI]	p-value	HR[95%CI]	p-value
rs17465637	1.03 [0.80-1.31]	0.420	0.99 [0.88-1.11]	0.825	1.00 [0.90-1.11]	0.957
rs6725887	1.30 [0.98-1.74]	0.037	1.07 [0.92-1.25]	0.402	1.13 [0.95-1.35]	0.158
rs9818870	0.99 [0.71-1.39]	0.478	1.13 [0.98-1.30]	0.097	1.11 [0.97-1.26]	0.124
rs12526453	1.02 [0.82-1.29]	0.418	0.95 [0.85-1.07]	0.394	0.96 [0.87-1.07]	0.483
rs1333049	1.12 [0.90-1.39]	0.161	1.23 [1.10-1.37]	<0.001	1.21 [1.09-1.33]	<0.001
rs1746048	1.30 [0.92-1.84]	0.070	0.93 [0.80-1.09]	0.375	1.06 [0.77-1.46]	0.725
rs9982601	1.06 [0.77-1.46]	0.357	1.15 [0.98-1.33]	0.083	1.13 [0.99-1.30]	0.076
rs10455872	1.85 [1.33-2.57]	<0.001	1.25 [0.95-1.64]	0.113	1.50 [1.02-2.21]	0.037

MAF: Minor allele frequency obtained from CEU samples from HapMap; Weight (OR): weight assigned to each genetic variant; HR [95%CI]: Hazard ratio [95% confidence interval].

S.A1.Table 4. Power calculations for cardiovascular disease.

Individual SNPs

			Minimum HR detectable with high or moderate power		Power to detect a specific HR							
	SNP	se	0.8	0.5	1.05	1.09	1.10	1.12	1.14	1.18	1.29	1.35
REGICOR	rs17465637	0.126	1.42	1.28	0.067	0.105	0.118	0.147	0.181	0.260	0.526	0.665
	rs6725887	0.146	1.51	1.33	0.063	0.091	0.100	0.121	0.146	0.204	0.413	0.536
	rs9818870	0.171	1.62	1.40	0.059	0.079	0.086	0.101	0.119	0.162	0.318	0.417
	rs12526453	0.116	1.38	1.25	0.071	0.116	0.131	0.165	0.205	0.299	0.596	0.738
	rs1333049	0.111	1.36	1.24	0.072	0.122	0.138	0.176	0.219	0.320	0.632	0.772
	rs1746048	0.177	1.64	1.41	0.059	0.078	0.084	0.098	0.115	0.155	0.302	0.396
	rs9982601	0.163	1.58	1.38	0.060	0.083	0.090	0.107	0.126	0.174	0.345	0.452
	rs10455872	0.168	1.60	1.39	0.060	0.081	0.088	0.104	0.122	0.166	0.329	0.431
Framingham	rs17465637	0.059	1.18	1.12	0.131	0.307	0.363	0.481	0.600	0.798	0.990	0.999
	rs6725887	0.078	1.24	1.17	0.096	0.197	0.230	0.305	0.388	0.562	0.903	0.970
	rs9818870	0.072	1.22	1.15	0.104	0.223	0.262	0.349	0.444	0.632	0.942	0.986
	rs12526453	0.059	1.18	1.12	0.132	0.312	0.368	0.488	0.607	0.805	0.991	0.999
	rs1333049	0.056	1.17	1.12	0.140	0.337	0.398	0.526	0.648	0.840	0.995	1.000
	rs1746048	0.079	1.25	1.17	0.095	0.194	0.227	0.301	0.382	0.555	0.897	0.967
	rs9982601	0.078	1.24	1.17	0.096	0.198	0.231	0.307	0.391	0.565	0.905	0.971
	rs10455872	0.139	1.48	1.31	0.064	0.095	0.105	0.129	0.156	0.221	0.448	0.577
Meta-analysis	rs17465637	0.054	1.16	1.11	0.149	0.364	0.429	0.563	0.688	0.872	0.997	1.000
	rs6725887	0.090	1.29	1.19	0.085	0.161	0.186	0.244	0.309	0.455	0.811	0.917
	rs9818870	0.067	1.21	1.14	0.113	0.252	0.298	0.397	0.502	0.699	0.968	0.994
	rs12526453	0.053	1.16	1.11	0.152	0.372	0.439	0.574	0.699	0.880	0.998	1.000
	rs1333049	0.051	1.15	1.11	0.161	0.397	0.467	0.607	0.733	0.903	0.999	1.000
	rs1746048	0.163	1.58	1.38	0.060	0.083	0.090	0.107	0.126	0.174	0.345	0.452
	rs9982601	0.069	1.22	1.15	0.108	0.236	0.279	0.371	0.470	0.663	0.956	0.991
	rs10455872	0.197	1.74	1.47	0.057	0.072	0.077	0.089	0.102	0.134	0.252	0.331

GRS

			Minimum HR detectable with high or moderate power		Power to detect a specific HR							
	GRS	se	0.8	0.5	1.05	1.10	1.15	1.20	1.25	1.30	1.40	1.50
REGICOR	Linear	0.046	1.14	1.09	0.185	0.543	0.858	0.977	0.998	1.000	1.000	1.000
	Q2	0.272	2.14	1.70	0.054	0.064	0.081	0.103	0.130	0.161	0.235	0.319
	Q3	0.261	2.08	1.67	0.054	0.065	0.083	0.107	0.137	0.171	0.252	0.342
	Q4	0.244	1.98	1.61	0.055	0.068	0.089	0.116	0.150	0.190	0.282	0.384
	Q5	0.237	1.94	1.59	0.055	0.069	0.091	0.120	0.156	0.197	0.294	0.400
Framingham	Linear	0.023	1.07	1.05	0.549	0.983	1.000	1.000	1.000	1.000	1.000	1.000
	Q2	0.127	1.43	1.28	0.067	0.117	0.196	0.300	0.419	0.542	0.754	0.891
	Q3	0.124	1.42	1.28	0.068	0.120	0.203	0.311	0.435	0.560	0.773	0.904
	Q4	0.123	1.41	1.27	0.068	0.121	0.205	0.315	0.439	0.566	0.778	0.907
	Q5	0.122	1.40	1.27	0.069	0.123	0.210	0.323	0.451	0.579	0.791	0.916
Meta-analysis	Linear	0.028	1.08	1.06	0.424	0.932	0.999	1.000	1.000	1.000	1.000	1.000
	Q2	0.114	1.37	1.25	0.071	0.134	0.234	0.361	0.502	0.637	0.842	0.946
	Q3	0.113	1.37	1.25	0.072	0.134	0.234	0.363	0.504	0.639	0.844	0.947
	Q4	0.110	1.36	1.24	0.073	0.139	0.246	0.381	0.527	0.665	0.864	0.958
	Q5	0.108	1.35	1.24	0.074	0.143	0.253	0.392	0.541	0.679	0.875	0.963

Se: Standard error; 'HR detectable' indicates the minimum risk effect detectable (expressed as the exponent of the beta from the meta-analysis) with high or moderate power. 'Power' indicates the study's power to detect the effects sizes (hazard ratios) shown. In the computation of power for given effect size, scenarios with high power (≥80%) are shaded dark grey, those with moderate power (≥50% and <80%) are shaded light grey, and those with power lower than 50% are unshaded.

The characteristics of the participants within each quintile of the GRS are shown in *S.A1.Table 5*. The GRS was not associated with classical CVRFs but was associated with gender in Framingham.

S.A1.Table 5. Description of the characteristics of the participants across quintiles of the genetic risk score in both cohorts.

	Quintiles of genetic score						
Variables	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p-value**	**p-trend**
REGICOR							
N	524	416	473	471	467		
Age (years)[a]	54.1 (11.1)	52.9 (11.0)	54.6 (11.4)	54.2 (11.0)	53.6 (11.3)	0.170	0.998
Gender (men)[b]	243 (46.4)	205 (49.3)	217 (45.9)	234 (49.7)	224 (48.0)	0.705	0.581
Total cholesterol (mg/dL)[a]	221 (42.8)	225 (41.8)	227 (42.5)	228 (42.0)	225 (42.8)	0.072	0.049
HDL cholesterol (mg/dL)[a]	51.1 (12.9)	52.4 (13.5)	52.5 (13.4)	51.0 (13.0)	51.5 (13.4)	0.304	0.866
SBP (mmHg)[a]	132.0 (22.0)	131.0 (20.4)	132.0 (20.4)	134.0 (21.5)	132.0 (19.5)	0.278	0.749
DBP (mmHg)[a]	78.9 (10.2)	79.5 (10.8)	79.0 (10.2)	80.2 (10.6)	79.8 (10.0)	0.257	0.099
Diabetes[b]	62 (12.1)	71 (17.5)	66 (14.3)	61 (13.3)	56 (12.3)	0.137	0.590
Smoking[b]	107 (20.7)	87 (21.0)	98 (20.8)	107 (23.1)	112 (24.3)	0.577	0.128
Family history of CHD[b]	46 (8.88)	51 (12.4)	55 (11.6)	63 (13.5)	57 (12.4)	0.207	0.064
Incidence of CVD events[c]	6.46	6.10	5.72	8.42	8.35	0.200	0.028
FRAMINGHAM							
N	743	712	681	711	690		
Age (years)[a]	56.6 (9.10)	56.1 (9.12)	55.6 (9.58)	56.1 (9.12)	55.6 (9.41)	0.172	0.060
Gender (men)[b]	351 (47.2)	321 (45.1)	305 (44.8)	299 (42.1)	264 (38.3)	0.008	<0.001
Total cholesterol (mg/dL)[a]	208 (37.1)	209 (37.6)	213 (39.0)	211 (39.3)	210 (39.8)	0.151	0.242
HDL cholesterol (mg/dL)[a]	50.5 (14.7)	50.2 (14.9)	51.1 (15.2)	52.0 (15.8)	51.3 (15.2)	0.151	0.048
SBP (mmHg)[a]	127 (18.4)	126 (17.0)	127 (18.8)	126 (18.2)	127 (18.9)	0.938	0.647
DBP (mmHg)[a]	75.2 (10.2)	75.1 (9.54)	74.8 (9.81)	75.0 (9.65)	74.7 (9.73)	0.872	0.329
Diabetes[b]	47 (6.33)	59 (8.29)	32 (4.70)	39 (5.49)	49 (7.10)	0.059	0.658
Smoking[b]	132 (17.8)	146 (20.5)	146 (21.4)	140 (19.7)	149 (21.6)	0.358	0.144
Family history of CHD[b]	113 (24.6)	112 (24.7)	105 (24.7)	109 (24.8)	112 (25.3)	0.999	0.763
Incidence of CVD events[c]	8.36	8.99	11.5	10.7	12.8	0.013	0.001

HDL: high density lipoprotein; SBP: systolic blood pressure; DBP: diastolic blood pressure; CHD: coronary heart disease; CVD: cardiovascular disease.

[a] mean (standard deviation); [b] n (proportion, %); [c] number of cases/100 individuals in 10 years.

For the GRS, we estimated that our study had 80% power to detect a HR of 1.14, 1.07 and 1.08 per unit increase in REGICOR, Framingham, and the meta-analysis, respectively (*S.A1.Table 4*). The GRS was linearly associated with incidence of CHD in both cohorts (p=0.002 in REGICOR and p<0.001 in Framingham; *S.A1.Table 6*), and in the meta-analysis, with a ~11% increase in risk of having a CVD event per unit of the GRS (p<0.001; *S.A1.Table 6*). This association remained statistically significant after further adjustment for family history of CHD (HR=1.15; 95% CI: 1.08-1.22). Participants in the top quintile of the GRS had 1.54 times greater risk of CHD, compared to those in the bottom quintile (p-value for linear trend <0.001) (*S.A1.Table 6*)). In both cohorts the

distribution of the GRS was slightly shifted to the right in individuals who had had an event, compared to those who had not (*S.A1.Figure 2*).

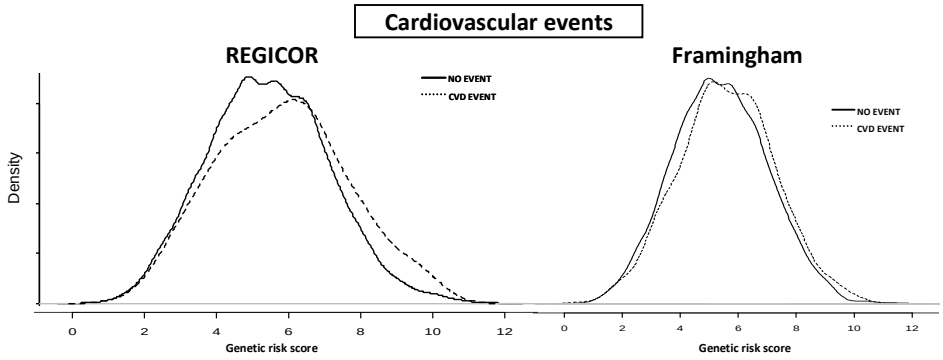
S.A1.Table 6. Multivariate adjusted association between risk of cardiovascular events and the genetic risk score, or quintiles thereof, in both cohorts and meta-analyses results of the observed effect sizes.

Genetic risk score	REGICOR		Framingham		Meta-analysis	
	HR [95%CI][a]	P-value	HR [95%CI][a]	P-value	HR [95%CI][a]	P-value
Linear	1.16 [1.06-1.27]	0.002	1.09 [1.04-1.14]	<0.001	1.11 [1.05-1.17]	<0.001
Quintiles	P-trend	0.018	P-trend	<0.001	P-trend	<0.001
Q1	1	---	1	---	1	---
Q2	1.09 [0.64-1.86]	0.749	1.01 [0.79-1.30]	0.916	1.02 [0.82-1.28]	0.838
Q3	1.00 [0.60-1.67]	0.993	1.20 [0.94-1.53]	0.143	1.16 [0.93-1.45]	0.185
Q4	1.32 [0.82-2.13]	0.255	1.25 [0.98-1.59]	0.075	1.26 [1.02-1.57]	0.033
Q5	1.72 [1.08-2.74]	0.023	1.50 [1.18-1.90]	0.001	1.54 [1.25-1.91]	<0.001

All models were adjusted for the sum of the products of the coefficient for each classical risk factor estimated in the Framingham original and calibrated risk functions and the difference between the participant's value and the population mean of that risk factor (see main text for formula). To account for family structure in the Framingham cohort we also adjusted for the first five genetic principal components.

[a] HR [95%CI]: Hazard ratio [95% confidence interval].

S.A1.Figure 2. Density distribution of genetic risk score in REGICOR and Framingham participants according to the incidence of cardiovascular events during the follow-up. The GRS is represented on the x-axis and is computed as a cumulative sum of all the risk alleles that a person carries, weighted by the effect of each SNP.



	REGICOR			Framingham		
	NO EVENT	CVD EVENT		NO EVENT	CVD EVENT	
	N=2,190	N=161	P-value	N=2,863	N=674	P-value
Weighted genetic score (95%CI)	5.46 [1.67]	5.84 [1.81]	0.010	5.38 [1.62]	5.60 [1.65]	0.002
Coronary risk (95%CI)	3.21 (1.65-5.89)	7.33 (4.40-12.1)	<0.001	7.07 (3.65-12.4)	13.9 (8.08-21.5)	<0.001
Coronary risk + genetic score (95%CI)	3.23 (1.59-5.92)	7.59 (4.50-12.1)	<0.001	6.82 (3.67-12.2)	14.7 (8.39-23.7)	<0.001

Improvement in predictive capacity: discrimination and reclassification

The addition of the GRS to the basic risk function improved its capacity to predict CVD in the Framingham cohort (c-statistic, 73.18 vs. 72.65, p-value=0.005) but not in the REGICOR cohort (76.09 vs. 76.10, p-value=0.621).

We observed a general tendency for both measures of reclassification improvement, the NRI and IDI, to increase after addition of the GRS to the basic risk function, although this improvement was not statistically significant for IDI index in the meta-analysis of the two cohorts. Overall, the NRI index in the meta-analysis was 3.67, 95%CI 0.04-7.31. However, reclassification improvement was more marked in the group with intermediate risk, and was statistically significant for both measures (NRI: 13.52, 95%CI 5.47-21.57; IDI: 0.29, 95%CI 0.06-0.52). Raw reclassification data and NRI and IDI for each cohort are shown in *S.A1.Figure3*.

S.A1.Figure 3. Reclassification of individuals based on the predicted 10-year risk of cardiovascular heart disease with and without the genetic risk score. Four risk categories (low, intermediate-low, intermediate-high and high), with cut-off points defined in each cohort, were defined according to current guidelines in each country (REGICOR: [0-5)%, [5-10)%, [10-15)%, ≥15%; Framingham: [0-10)%, [10-15)%, [15-20)%, ≥20%, respectively). Light grey cells represent an improvement in reclassification and dark grey cells represent the opposite.

REGICOR

Cardiovascular events	Classical risk factors	Classical risk factors + Genetic Score: Low risk	Intermediate-low risk	Intermediate-high risk	High risk
Cases					
	Low risk	42	5	0	0
	Intermediate-low risk	2	35	12	0
	Intermediate-high risk	0	5	15	4
	High risk	0	0	5	25
Non-cases					
	Low risk	1428	77	0	0
	Intermediate-low risk	79	383	46	2
	Intermediate-high risk	0	30	80	20
	High risk	0	1	18	38

Framingham

Classical risk factors	Classical risk factors + Genetic Score: Low risk	Intermediate-low risk	Intermediate-high risk	High risk
Cases				
Low risk	92	12	0	0
Moderate risk	8	52	13	0
Moderate risk	0	5	38	9
High risk	0	0	11	132
Non-cases				
Low risk	1953	76	0	0
Moderate risk	83	367	70	2
Moderate risk	0	61	180	34
High risk	0	1	41	297

		REGICOR		Framingham		Meta-analysis	
		All	Intermediate risk	All	Intermediate risk	All	Intermediate risk
NRI	Cardiovascular event	5.89 [-2.44;14.21]	18.76 [4.12;33.41]	3.15 [-0.89;7.20]	11.25 [1.61;20.89]	3.67 [0.04;7.31]	13.52 [5.47;21.57]
IDI	Cardiovascular event	0.81 [0.34;1.29]	0.39 [-0.12;0.90]	0.24 [0.05;0.43]	0.26 [-0.07;0.45]	0.48 [-0.07;1.03]	0.29 [0.06;0.52]

4. DISCUSSION

As for CHD events (main manuscript) and in accordance with the AHA statement regarding assessment of the value of novel risk biomarkers s [24], we have validated the association between a multi-locus GRS and incidence of CVD events in two prospective cohort studies, and have shown that this GRS improves the capacity of the Framingham risk function to predict CVD events. In addition, we have also observed greater improvement in risk reclassification among individuals with intermediate risk.

Prospective validation of the association between a novel multi-locus genetic risk score and CHD events

As in the case of CHD, the GRS is linearly and directly associated with the incidence of CVD events in two cohorts with different basal 10-year coronary risks with a ~11% increased risk per unit of the GRS. The association GRS results were similar in both populations and independent of familial history of CHD. As observed for CHD events, this result is mainly driven by the effect size in the Framingham cohort and we believe that the effect size per unit of the GRS could be slightly underestimated.

The 1.54-times increased risk observed for CVD is also very similar to the 1.44-times risk increase in CHD between the extreme quintiles of the GRS.

Incremental value of the genetic risk score for CHD risk prediction

The inclusion of the GRS improved the classification of the individuals in the different risk categories, especially in those individuals with intermediate risk.

The discriminative capacity of the classical risk function was improved by inclusion of the GRS in the Framingham cohort but not the REGICOR.

Risk estimation including information for the GRS in risk functions in individuals with intermediate risk

We observed that the GRS improved the classification of individuals mainly in the intermediate risk group. The results of the NRI for CVD events observed in our study was 13.52%.

Supplementary Analysis 2
Four SNP analysis.

1. METHODS

We sought to evaluate the reclassification of individuals based on the 10-year predicted risk of coronary heart disease, with and without the genetic risk score (GRS), using a GRS composed of the 4 SNPs (rs6725887 [*WDR12*], rs9982601 [*SCL5A3*], rs1333049 [*CDKN2A/2B*], rs10455872 [*LPA*]) that presented consistent effects in the direction of the association in the two cohorts and in the meta-analysis (see *table 2* in the main article).

2. RESULTS

S.A2.Table 1. Comparison of the Net Reclassification Index (NRI) results for the analyses using the 4-SNP and 8-SNP scores for the entire sample and separately for the intermediate risk group.

	NRI results obtained using 4-SNP GRS		NRI results obtained using 8-SNP GRS	
	Cardiovascular event	Coronary event	Cardiovascular event	Coronary event
All events				
REGICOR	5.35 [-3.57;14.27]	5.54 [-7.78;18.86]	5.89 [-2.44;14.21]	12.17 [1.99;22.34]
Framingham	2.28 [-2.54;7.11]	3.75 [-1.45;8.95]	3.15 [-0.89;7.20]	11.25 [1.61;20.89]
Meta-analysis	2.97 [-1.27;7.22]	3.99 [-0.86;8.83]	3.67 [0.04;7.31]	13.52 [5.47;21.57]
Intermediate risk				
REGICOR	21.36 [5.05;39.91]	17.71 [-4.49;39.91]	18.76 [4.12;33.41]	24.76 [7.62;41.91]
Framingham	15.10 [4.72;25.47]	18.04 [6.23;29.85]	2.56 [-2.89;8.01]	14.30 [3.08;25.51]
Meta-analysis	16.77 [7.76;25.78]	17.97 [7.54;28.39]	6.37 [-2.85;15.58]	17.44 [8.04;26.83]

Columns 3 and 4 show the NRI results for the 8-SNP GRS from *Figure 2* in the main manuscript.
Cell shaded in yellow indicate the results for the score that provided the greatest improvement in reclassification.

S.A2.Figure 1. Reclassification of individuals based on the predicted 10-year risk of coronary heart disease with and without the genetic risk score. Four risk categories (low, intermediate-low, intermediate-high and high), with cut-off points defined in each cohort, were defined according to current guidelines in each country (REGICOR: [0-5)%, [5-10)%, [10-15)%, ≥15%; Framingham: [0-10)%, [10-15)%, [15-20)%, ≥20%, respectively). Light grey cells represent an improvement in reclassification and dark grey cells represent the opposite.

REGICOR

Coronary events

Classical risk factors	Classical risk factors + Genetic Score: Low risk	Intermediate-low risk	Intermediate-high risk	High risk
Cases				
Low risk	22	7	2	0
Intermediate-low risk	7	13	8	5
Intermediate-high risk	1	5	8	6
High risk	0	1	3	19
Non-cases				
Low risk	1352	153	13	3
Intermediate-low risk	155	258	77	35
Intermediate-high risk	2	44	49	41
High risk	0	16	9	39

Framingham

Coronary events

Classical risk factors	Classical risk factors + Genetic Score: Low risk	Intermediate-low risk	Intermediate-high risk	High risk
Cases				
Low risk	61	10	0	0
Intermediate-low risk	6	35	7	0
Intermediate-high risk	0	3	30	10
High risk	0	0	8	83
Non-cases				
Low risk	1995	70	0	0
Intermediate-low risk	66	421	62	2
Intermediate-high risk	0	63	180	42
High risk	0	0	45	340

REGICOR

Cardiovascular events

Classical risk factors	Classical risk factors + Genetic Score: Low risk	Intermediate-low risk	Intermediate-high risk	High risk
Cases				
Low risk	40	7	0	0
Intermediate-low risk	5	29	11	4
Intermediate-high risk	0	7	12	6
High risk	0	3	2	25
Non-cases				
Low risk	1384	118	2	0
Intermediate-low risk	126	308	66	10
Intermediate-high risk	0	36	63	32
High risk	0	7	14	36

Framingham

Cardiovascular events

Classical risk factors	Classical risk factors + Genetic Score: Low risk	Intermediate-low risk	Intermediate-high risk	High risk
Cases				
Low risk	93	11	0	0
Intermediate-low risk	11	44	17	1
Intermediate-high risk	0	6	34	12
High risk	0	0	16	127
Non-cases				
Low risk	1937	95	1	0
Intermediate-low risk	88	349	76	5
Intermediate-high risk	0	77	154	45
High risk	0	0	42	296

3. DISCUSSION

The results obtained for the NRI using only the 4 SNPs that presented the same direction of effect both in the REGICOR and Framingham studies, showed that although the SNPs were selected on the basis on the results they have in both cohorts, we still gain more information from the full set of SNPs independent from CVRFs.

Supplementary Analysis 3

Predictive capacity analysis without CDKN2A-2B variant

1. INTRODUCTION

Genetic variants in the chromosomal region 9p21.3, specifically between the genes *CDKN2A* and *CDKN2B*, have been identified by GWAS studies as being associated with several complex diseases, including *Abdominal aortic aneurysm, Breast cancer, Coronary heart disease, Glioma, Intracranial aneurysm, Melanoma, Myocardial infarction* and *Type 2 diabetes* (NHGRI GWAS catalog, accessed in 17th November 2011). Although some variants in this region are known to be associated with T2D, we included in our GRS a variant from chromosomal region 9p21 that is known to be associated with MI/CHD risk independently of T2D risk [25].

In the present analysis we evaluated the sensitivity of our analysis to the inclusion of this variant, not only to avoid the possibility of including a variant that could have some undetected association with T2D, but also because this variant has the largest effect on risk (OR=1.29, according to the CARDIoGRAM study). Our aim was to evaluate if the results in the main analyses are mainly driven variant.

2. RESULTS

S.A3.Table 1. Description of the characteristics of the participants across genetic risk score quintiles in both cohorts.

	Quintiles of genetic score						
Variables	**Q1**	**Q2**	**Q3**	**Q4**	**Q5**	**p-value**	**p-trend**
REGICOR							
N	511	439	502	438	461		
Age (years)[a]	54.7 (11.2)	52.5 (11.1)	53.6 (11.2)	53.5 (11.2)	55.1 (11.1)	0.005	0.343
Gender (men)[b]	247 (48.3)	207 (47.2)	231 (46.0)	204 (46.6)	234 (50.8)	0.617	0.577
TC (mg/dL)[a]	223 (41.8)	224 (40.6)	226 (43.4)	227 (44.9)	226 (41.6)	0.608	0.135
HDLc (mg/dL)[a]	50.8 (12.6)	52.9 (13.4)	52.5 (13.8)	51.1 (13.2)	51.2 (13.2)	0.058	0.695
SBP (mmHg)[a]	133 (21.9)	132 (21.4)	130 (20.2)	132 (20.3)	134 (20.0)	0.139	0.753
DBP (mmHg)[a]	79.3 (10.5)	80.0 (10.5)	78.9 (10.4)	79.0 (10.2)	80.3 (10.2)	0.151	0.444
Diabetes[b]	73 (14.7)	61 (14.3)	61 (12.3)	67 (15.8)	54 (11.9)	0.404	0.400
Smoking[b]	102 (20.2)	98 (22.4)	106 (21.4)	93 (21.6)	112 (24.4)	0.621	0.202
CHD Family hist [b]	55 (10.8)	39 (9.01)	53 (10.7)	68 (15.7)	57 (12.5)	0.028	0.038
Estimated 10-y coronary risk[c]	3.6 (1.9-6.6)	3.1 (1.4-5.5)	3.1 (1.7-5.9)	3.2 (1.6-6.5)	3.6 (1.9-6.3)	0.015	0.299
Incidence of CVD events[d]	6.23	5.98	5.94	6.82	10.3	0.004	0.004
Incidence of coronary events[d]	4.43	3.93	3.84	4.95	7.95	0.004	0.002
FRAMINGHAM							
N	743	712	681	711	690		
Age (years)[a]	56.3 (9.18)	56.4 (9.12)	55.6 (9.44)	56.0 (9.32)	55.7 (9.27)	0.389	0.145
Gender (men)[b]	371 (50.2)	299 (42.2)	316 (46.2)	282 (41.0)	272 (37.9)	<0.001	<0.001

TC (mg/dL)[a]	209 (37.5)	211 (37.7)	209 (38.5)	209 (38.6)	213 (40.4)	0.158	0.233
HDLc (mg/dL)[a]	50.4 (14.5)	51.0 (14.8)	50.9 (15.7)	51.1 (15.4)	51.8 (15.4)	0.532	0.103
SBP (mmHg)[a]	126 (17.4)	127 (18.3)	127 (19.2)	126 (17.9)	127 (18.6)	0.785	0.941
DBP (mmHg)[a]	75.0 (9.61)	75.3 (9.70)	75.5 (10.3)	74.6 (9.82)	74.4 (9.55)	0.230	0.131
Diabetes[b]	48 (6.50)	53 (7.49)	40 (5.85)	39 (5.67)	46 (6.41)	0.668	0.499
Smoking[b]	135 (18.3)	140 (19.8)	138 (20.2)	135 (19.6)	165 (23.0)	0.250	0.048
CHD Family hist [b]	113 (24.6)	112 (24.7)	105 (24.7)	109 (24.8)	112 (25.3)	0.999	0.763
Estimated 10-y coronary risk[c]	8.6 (4.7-14.5)	8.1 (4.6-14.1)	8.1 (4.4-14.3)	7.5 (4.5-13.3)	7.8 (4.1-14.1)	0.342	0.041
Incidence of CVD events[d]	10.40	11.10	10.70	8.06	12.50	0.200	0.369
Incidence of coronary events[d]	7.20	7.38	7.34	5.43	8.72	0.210	0.672

HDLc: high density lipoprotein cholesterol; SBP: systolic blood pressure; DBP: diastolic blood pressure; CHD: coronary heart disease; CVD: cardiovascular disease; TC: Total cholesterol; CHD Family hist: CHD Family history.

[a] mean (standard deviation); [b] n (proportion, %); [c] mean (95% confidence interval); [d] number of cases/100 individuals in 10 years.

S.A3.Table 2. Multivariate adjusted association of the genetic risk score with cardiovascular and coronary events as a linear variable and across quintiles in both cohorts and meta-analyses results of the observed effect sizes.

	Genetic risk score	REGICOR		Framingham		Meta-analysis	
		HR [95%CI][a]	P-value	HR [95%CI][a]	P-value	HR [95%CI][a]	P-value
Cardiovascular events	**Linear**	1.21 [1.08-1.35]	0.001	1.05 [0.99-1.12]	0.099	1.12 [0.97-1.28]	0.113
	Quintiles	P-trend	0.0050	P-trend	0.452	P-trend	0.235
	Q1	1	---	1	---	1	---
	Q2	1.02 [0.60-1.73]	0.944	0.92 [0.73-1.17]	0.515	0.94 [0.75-1.16]	0.546
	Q3	0.86 [0.50-1.45]	0.566	1.03 [0.81-1.31]	0.801	1.00 [0.80-1.24]	0.993
	Q4	1.19 [0.73-1.94]	0.487	0.87 [0.68-1.12]	0.278	0.95 [0.72-1.24]	0.685
	Q5	1.87 [1.19-2.91]	0.006	1.13 [0.89-1.42]	0.316	1.40 [0.86-2.28]	0.177
Coronary events	**Linear**	1.26 [1.10-1.43]	0.001	1.05 [0.97-1.13]	0.247	1.14 [0.95-1.36]	0.147
	Quintiles	P-trend	0.0024	P-trend	0.781	P-trend	0.318
	Q1	1	---	1	---	1	---
	Q2	0.88 [0.44-1.77]	0.718	0.98 [0.73-1.31]	0.874	0.96 [0.74-1.26]	0.792
	Q3	0.90 [0.47-1.74]	0.760	1.00 [0.74;1.35]	0.995	0.98 [0.75-1.29]	0.895
	Q4	1.36 [0.75-2.48]	0.311	0.80 [0.59-1.11]	0.179	0.98 [0.59-1.62]	0.935
	Q5	2.10 [1.21-3.64]	0.008	1.13 [0.85-1.51]	0.412	1.47 [0.81-2.68]	0.208

All models were adjusted for the sum of the products of the coefficient for each classical risk factor estimated in the Framingham original and calibrated risk functions and the difference between the participant's value and the population mean of that risk factor (see main text for formula). To account for family structure in the Framingham cohort we also adjusted for the first five genetic principal components. [a] HR [95%CI]: Hazard ratio [95% confidence interval].

Cell shaded in yellow indicate the results for the score that provided a more significant association between the GRS and risk of CVD or CHD events.

S.A3.Table 3. Comparison of the Net Reclassification Index (NRI) results for the 7-SNP score (GRS of the main analysis without the variant of Chromosome 9: *CDKN2A-2B*) and 8-SNP score analyses, for the entire sample and separately for the intermediate risk group.

	NRI results obtained with 7 SNPs GRS		NRI results obtained with 8 SNPs GRS	
	Cardiovascular event	Coronary event	Cardiovascular event	Coronary event
All individuals				
REGICOR	6.76 [-1.60;15.11]	11.02 [-0.78;22.82]	5.89 [-2.44;14.21]	12.17 [1.99;22.34]

Framingham	3.15 [-1.02;7.32]	2.56 [-2.89;8.01]	3.15 [-0.89;7.20]	11.25 [1.61;20.89]
Meta-analysis	3.87 [0.14;7.60]	5.10 [-2.50;12.71]	3.67 [0.04;7.31]	13.52 [5.47;21.57]
Intermediate risk				
REGICOR	21.80 [6.82;36.79]	21.91 [2.25;41.56]	18.76 [4.12;33.41]	24.76 [7.62;41.91]
Framingham	11.25 [1.60;20.90]	14.30 [3.82;24.77]	2.56 [-2.89;8.01]	14.30 [3.08;25.51]
Meta-analysis	14.90 [5.07;27.74]	15.98 [6.74;25.23]	6.37 [-2.85;15.58]	17.44 [8.04;26.83]

The two columns presented for NRI results obtained with a GRS composed of 8 SNPs are the ones presented in the main document.
Cell shaded in yellow indicate the results for the score that provided the greatest improvement in reclassification.

3. DISCUSSION

The results shown in *S.A3.Table 2* and *S.A3.Table 3* suggest that, although the results do not change markedly after excluding the variant on 9p21, it is mainly in the Framingham Heart study that this variant evaluated has a greater effect on the GRS, and in some cases it can drive the meta-analyses to a significant result. This is consistent with the effect sizes observed for the individual SNPs in each cohort, because this variant presents a HR lower than the average in the REGICOR study, and the opposite scenario for both the Framingham and meta-analysis (see *table 2* in the main article).

Supplementary Analysis 4

Predictive capacity analysis with a 12-SNP based GRS in the Framingham cohort

1. METHODS

We sought to evaluate the reclassification of individuals based on the 10-year predicted risk of coronary heart disease, with and without the genetic risk score (GRS), using a GRS composed of the 12 SNPs (rs17465637 [*MIA3*]; rs6725887 [*WDR12*]; rs9818870 [*MRAS*]; rs12526453 [*PHACTR1*]; rs1333049 [*CDKN2A/2B*]; rs1746048 [*CXCL12*]; rs9982601 [*SCL5A3*]; rs10455872 [*LPA*];) representing the addition of 4 additional SNPs obtained from refs [3,26].

2. RESULTS

S.A4.Table 1. Multivariate adjusted association between the genetic risk score and risk of coronary events as a continuous variable and between quintiles.

	Coronary event		Cardiovascular event	
Genetic risk score	HR (95% CI)	p-value	HR (95% CI)	p-value
Continuous	1.06 (1.01-1.11)	0.013	1.08 (1.04-1.12)	<0.001
Quintiles	p-trend	0.017	p-trend	<0.001
Q1	1	--	1	--
Q2	1.08 (0.80-1.46)	0.628	1.08 (0.84-1.39)	0.531
Q3	1.05 (0.78-1.43)	0.737	1.17 (0.91-1.50)	0.221
Q4	1.28 (0.95-1.71)	0.104	1.33 (1.05-1.70)	0.020
Q5	1.36 (1.02-1.81)	0.039	1.52 (1.20-1.93)	0.001

S.A4.Table 2. Reclassification of individuals based on the 10-year predicted risk of coronary heart disease with and without the genetic risk score. Risk categories were defined using national recommendations. Cut-off points: low [0-10)%, intermediate-low [10-15)%, intermediate-high [15-20)% and high =20% risk.

		ALL	Intermediate risk
NRI	Coronary event	0.91 [-4.38;6.21]	7.80 [-1.76;17.36]
	Cardiovascular event	1.30 [-3.16;5.76]	10.55 [0.40;20.70]
IDI	Coronary event	0.22 [0.04; 0.41]	0.22 [-0.06; 0.49]
	Cardiovascular event	0.27 [0.09; 0.46]	0.25 [-0.03; 0.54]

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