Socioeconomic Status Interacts with the Genetic Effect of a Chromosome 9p21.3 Common Variant to Influence Coronary Artery Calcification and Incident Coronary Events in the Heinz Nixdorf Recall Study (Risk Factors, Evaluation of Coronary Calcium, and Lifestyle)

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Background—Genetic variants of a locus within the chromosome 9p21.3 region are consistently associated with coronary artery disease and coronary artery calcification (CAC). The aim of this study was to examine whether a 9p21.3 common variant interacts with socioeconomic status (SES) to influence CAC and incident coronary events in a population-based cohort.

Methods and Results—9p21.3 single nucleotide polymorphism rs2891168 was genotyped in 4116 participants of the Heinz Nixdorf Recall study. SES indicators (education and income) and CAC were assessed at baseline. Incident coronary events were ascertained over a median follow-up of 9.3 years. Multiple regression models were fitted to estimate genetic effects on $\log_e$(CAC+1) and incident coronary events. Genetic effects were highest in the lower income tertile with a 53.1% (95% confidence interval, 30.6%–79.6%; P=1.8×10^{-7}) increase in CAC and a hazard ratio of 1.44 (95% confidence interval, 1.01–2.07; P=0.049) for incident coronary events per additional risk allele. After including genotype×SES interaction terms in the regression models, genotype×income interactions were observed for CAC (exp[$\beta_{g\times income}$]=0.85 [95% confidence interval, 0.74–0.98; $P_{g\times income}$=0.02] per 1000€/mo increase and additional risk allele) and for incident coronary events (hazard ratio$_{g\times income}$=0.69 [95% confidence interval, 0.48–0.98; $P_{g\times income}$=0.04] per 1000€/mo increase and additional risk allele). No interaction was observed using education as SES indicator.

Conclusions—A 9p21.3 common variant seems to interact with SES to influence CAC and incident coronary events in a population-based cohort. This supports the hypothesis that better material, psychosocial, and lifestyle conditions enable higher SES groups to reduce the expression of their genetic susceptibility to coronary artery disease. **(*Circ Cardiovasc Genet*. 2017;10:e001441. DOI: 10.1161/CIRCGENETICS.116.001441.)**

Key Words: alleles ■ chromosomes ■ coronary artery disease ■ genetic predisposition to disease ■ polymorphism, single nucleotide ■ socioeconomic status

Common genetic variants of a locus within the chromosome 9p21.3 region are robustly associated with coronary artery disease (CAD) showing the strongest effect size estimates for CAD risk obtained by genome-wide association studies.[1–7] Effects of 9p21.3 genetic variants on coronary artery calcification (CAC)—a marker for subclinical coronary atherosclerosis—have also been detected in different study populations.[8–10] As no associations have been observed between 9p21.3 genetic variants and established cardiovascular risk factors (CVRFs), such as hypertension, diabetes mellitus, and hyperlipidemia, the impact on CAD seems to arise because of an independent mechanism.[3,4,11] Recent studies suggest that 9p21 genetic variants regulate the expression of the antisense noncoding RNA in the INK4 locus (*ANRIL*), a long intergenic noncoding RNA that plays a role in the expression of the cyclin-dependent kinase inhibitor 2A/2B (*CDKN2A/2B*) and cell growth, potentially contributing to atherogenesis.[12] However, the exact biological mechanisms have not been identified to date.

See Clinical Perspective

Complex diseases such as CAD are supposed to be influenced by a wide range of environmental and genetic risk factors,[13] and it is widely accepted that the interaction between

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genes and the environment contribute to the cause of complex diseases. In case of 9p21.3 genetic variants, little consistent findings exist with reports of interaction by single risk factors such as age,[14,15] blood pressure,[16] smoking,[17] dietary intake,[18,19] glycemic control,[20] and abdominal obesity[21] in some study populations, but lack replication in others.[21–23] In most of these studies indicators of socioeconomic status (SES) have not been included although strong associations of SES with CAD and CAC have been reported that are partly being explained by socioeconomic differences in CVRF frequency.[24–28] SES strata can serve as context-defining categories that may be better suited for describing differences in risk-associated environments that are not limited to single risk factors. This may be crucial for defining subgroups for which genetic effects show stronger signals than for the average population.[29]

Twin studies have suggested that genetic effects on disease may be lower in groups of higher SES.[30,31] It has been concluded that those effects reflect better material, behavioral, and psychosocial conditions for higher SES groups to manage their environments, thus reducing the expression of their genetic susceptibility to disease. If this was true for CAD, genetic effects of single genetic markers would—at least partly—been modified by indicators of SES such as education and income. The aim of this study was to investigate whether such SES differences in genetic effects can be observed for a single nucleotide polymorphism (SNP) of a locus within the 9p21.3 region and its association with CAC and incident coronary events (ie, coronary death and nonfatal myocardial infarction) in a population-based cohort, where strong inverse associations between indicators of SES and CAC have already been shown.[27] To explore whether any detected SES interactions are because of underlying genotype by CVRF interactions, established CVRFs were included in the analysis.

Methods

Study Population

The present study used data of the Heinz Nixdorf Recall study (Risk Factors, Evaluation of Coronary Calcium, and Lifestyle), a prospective, population-based cohort. The design and rationale of the study have been described in detail elsewhere.[32] Briefly, 4814 women and men aged 45 to 74 years were recruited using a random sample of individuals derived from mandatory citizen registries of 3 large cities (Bochum, Essen, and Mülheim/Ruhr) in an urban region in the Western part of Germany. The recruitment took place from 2000 to 2003, and the baseline response proportion was 55.8%.[33] The study was approved by the local ethics committee and comprises extended quality management procedures including a certification according to DIN ISO 9001:2000. Informed consent was obtained from all participants.

CAC and Incident Coronary Events

CAC was quantified at the baseline examination by noncontrast-enhanced electron beam computed tomography using a C-150 scanner (GE Imatron, South San Francisco, CA). Details have been described elsewhere.[32,34] The Agatston score was computed as a measure of total CAC defined as the sum of the area (in mm^2) of each detectable focus in the epicardial coronary system multiplied by its computed tomography density.[35] A value of zero indicates nondetectable calcification.

Incident coronary events were assessed during follow-up by using annual postal questionnaires including questions on current health status (ie, medication, hospital admission, and outpatient diagnosis of coronary events). Validation of reported incident coronary and fatal events was performed by reviewing hospital records, records of the participants' attending physicians, and death certificates and by obtaining as much information as possible to verify causes of death. The end point for the present study was unequivocally documented incident coronary death or nonfatal myocardial infarction that met the predefined study criteria.[32,36] These study criteria considered symptoms, electrocardiographic signs, and enzymes (levels of creatinine kinase), as well as troponin T or I, and necropsy. End points were ascertained over a median follow-up period of 9.3 (interquartile range, 9.0–10.2) years and were validated by an independent expert committee. For sensitivity analyses, incident cases of coronary revascularization were additionally considered as study end points.

Genetic Data

The SNP rs2891168 was selected as a marker for genetic variation at a locus in the 9p21.3 region representing a ≈30-kb haplotype block with high levels of linkage disequilibrium (LD) robustly associated with CAD and CAC in different study populations.[1–6,8–10] In a recent genome-wide association studies meta-analysis for CAD, rs2891168 showed the strongest association among SNPs considered within the 9p21.3 region with G coded as the risk effect allele.[7] Genotyping was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry-based iPLEX Gold assay at the Department of Genomics, Life and Brain Center, Bonn, Germany. No deviation from Hardy–Weinberg equilibrium was detected for rs2891168 genotypes using an exact 2-sided test (P=0.69). The risk allele frequency in the study population was 0.47.

Indicators of SES

Information on education and income was collected by standardized interviews at baseline examination. Education was defined as total years of formal education by combining school and vocational training according to the International Standard Classification of Education.[37] For stratified analyses, education was categorized into 3 groups with the lowest educational group of ≤10 years (equivalent to a basic school degree with no vocational training) and the highest educational group of ≥14 years of education (equivalent to vocational training including additional qualification or a university degree). Income was measured as the monthly household equivalent income calculated by dividing the participants' total household net income by a weighting factor for each household member.[38] For stratified analyses, income was divided into 3 groups using sex-specific tertiles. Both SES indicators were analyzed separately to account for their different mechanisms in causing health inequalities.[39,40]

Cardiovascular Risk Factors

Levels of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and triglycerides were derived from blood serum samples by using standardized enzymatic methods. High-sensitivity C-reactive protein was measured using a standardized assay (Roche Diagnostics, Basel, Switzerland). For all measurements, blood samples were analyzed within 12 hours after collection at the central laboratory of the University Hospital of Essen, Germany. Diabetes mellitus was defined as either of the following criteria: reported history of diabetes mellitus, taking glucose-lowering drugs, having fasting blood glucose levels of >125 mg/dL, or having nonfasting glucose levels of ≥200 mg/dL. Current smoking was defined as smoking cigarettes during the last 12 months. Body mass index was computed from standardized measurements of height and weight (kg/m^2). Hypertension was defined as having a systolic value of ≥140 mm Hg or a diastolic value of ≥90 mm Hg or taking regular antihypertensive medication. Blood pressure was assessed using an automated oscillometric device (Omron HEM-705-CP) and calculated as the mean of the second and third values of 3 measurements.[41] Physical activity was defined as no exercise versus exercise 1 and more times per week. Dietary intake was assessed by a validated 21-item food frequency questionnaire.[42] Based on the food frequency questionnaire, a dietary pattern index was calculated to determine the quality of the participants' diet (ie, the higher the dietary

pattern index score, the healthier the diet).[43] In addition, food frequency questionnaire–based information on wine intake and intake of raw and cooked vegetables was defined as low intake (ie, consumption frequency ≤3× per month) versus high intake (ie, consumption frequency >3× per month).

Statistical Analyses

For the cross-sectional analyses of CAC at study baseline, 4116 participants who had nonmissing information on CAC and genetic variation at rs2891168 were included (Figure 1). Sensitivity analysis was conducted excluding participants reporting physician-diagnosed CAD at baseline (ie, history of myocardial infarction or coronary revascularization). Linear regression models were fitted to detect associations with CAC. To normalize the distribution of the CAC score and to avoid numeric errors a $\log_e$-transformation of (CAC+1) was applied. Effect size estimates were presented back-transformed (exp[β]) or as percent change per unit increase unless stated otherwise. For the longitudinal analyses of incident coronary events, 3843 participants free of physician-diagnosed CAD at study baseline were included (Figure 1). Sensitivity analysis was conducted additionally including incident cases of coronary revascularization as study end points (n=117). Hazard ratios derived by Cox proportional hazards regression models were calculated for estimating effects on incident coronary events. As there were some observations missing on education (n=7) and income (n=249), as well as on some of the CVRFs, participants were excluded from the respective analyses. Participants with missing information on income had slightly lower rates of incident coronary events (P=0.16), lower CAC (P=0.01), and lower education (P<0.001). All analyses were performed using the R statistical package version 3.0.2[44] and PLINK (v1.07) for Windows.[45] All P values reported are unadjusted for multiple testing. The selected SNP was investigated assuming a (log-) additive genetic model, as suggested in previous studies.[7]

First, regression models adjusted for sex and age were fitted to calculate effect size estimates and their corresponding 95% confidence intervals (95% CIs) for the association of the SES indicators and for the genetic association of rs2891168 risk alleles with CAC and incident coronary events. Education and income were entered separately as continuous variables in the regression models.

Second, regression models adjusted for sex and age were fitted including the main effects of rs2891168 risk alleles and the respective SES indicator in addition to an interaction term between them (base model). It has been proposed that interaction described as departure from additivity of effects on disease events is better suited for indicating biological interaction.[46] However, the regression coefficient of the interaction term in Cox proportional hazard regression models reflects interaction on a multiplicative scale. The relative excess risk due to interaction (RERI) and the corresponding 95% CI was calculated to additionally assess additive interaction effects on incident coronary events.[47] Association analysis was also conducted stratified by SES groups separately for income tertiles and education categories, as well as stratified by 9p21.3 genotype. Within each of the strata, sex- and age-adjusted effect size estimates and 95% CIs were calculated. To calculate joint effects of 9p21.3 genotype and SES indicators, all possible combinations of the number of risk alleles and SES groups were entered in the regression models separately for income and education using the group with no risk alleles and the highest SES as reference.

Third, to explore whether any interactions between rs2891168 risk alleles and SES are because of underlying interactions incorporating CVRFs that may be connected to SES, CVRF main effects and the respective genotype×CVRF and SES×CVRF interaction terms were included in the base model separately for each risk factor.[48] A full regression model was fitted also (ie, the base model additionally including all CVRFs except: low-density lipoprotein because of its correlation to total cholesterol; HbA1c (hemoglobin A1c) and blood glucose because of their correlation to diabetes mellitus; vegetable and wine intake because of their correlation to the dietary pattern index). Each of the fitted regression models was then checked for substantial changes (ie, >|10%|) in the magnitude of the genotype by SES interaction effect size estimate ($\beta_{g\times SES}$). Genotype by CVRF interaction was also assessed in sex- and age-adjusted regression models not including SES indicators and SES interaction terms.

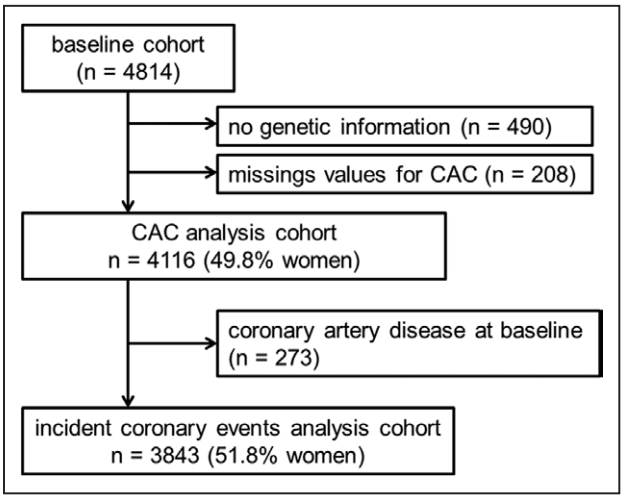

Figure 1. Flowchart of participants out of the entire study cohort included in the analysis. CAC indicates coronary artery calcification.

Results

The median CAC score was 17.6 (interquartile range, 0.0–166.2) for the study population (Table 1) with women having a lower median score than men (2.1 [interquartile range, 0.0–44.4] versus 74.0 [interquartile range, 5.7–340.9]; P<0.0001). In participants free of CAD at baseline, the overall number of incident coronary events per 10 000 person years was 39.4 (95% CI, 33.3–46.7). As expected, women and men also showed differences in the number of coronary events per 10 000 person years (19.9 [95% CI, 14.4–27.6] versus 60.9 [95% CI, 50.1–74.1]; P<0.0001). For CAC and the number of coronary events per 10 000 person years, increasing values were observed for each added risk allele (Table 1). Differences in study population characteristics across income tertiles and educational groups are presented in Tables I and II in the Data Supplement.

There were marked health inequalities regarding CAC in the study population. A decrease in average CAC was observed with increasing household income and years of education, whereas a similar decreasing trend in the occurrence of coronary events was seen but was not statistically significant (Table 2). As expected, an increase in CAC was observed per rs2891168 risk allele. Effect size estimates for the occurrence of coronary events were again lacking statistical significance but were directionally consistent (Table 2). No associations were detected between number of risk alleles and SES indicators (P>0.25).

A 9p21.3 genotype by income interaction was observed for CAC (exp[$\beta_{g\times income}$]=0.86 [95% CI, 0.74–0.98; $P_{g\times income}$=0.02] per 1000€/mo increase and additional risk allele) and for incident coronary events (hazard ratio$_{g\times income}$=0.69 [95% CI, 0.48–0.98; $P_{g\times income}$=0.04] per 1000€/mo increase and additional risk allele). No interaction was observed using education as SES indicator (CAC: exp[$\beta_{g\times education}$]=0.97 [95% CI, 0.93–1.00; $P_{g\times education}$=0.08] per year of education and additional risk allele; incident coronary events: hazard ratio$_{g\times education}$=0.97

Table 1. Characteristics of Study Participants (n=4116) Stratified by Chromosome 9p21.3 Genotype (rs2891168; risk effect allele G).

	All	Genotype A/A	Genotype A/G	Genotype G/G
n	4116	1172	2059	884
Age, y*	59.6 (±7.8)	59.7 (±7.8)	59.4 (±7.8)	59.9 (±8.0)
Women†	2050 (49.8%)	592 (50.5%)	1013 (49.2%)	444 (50.2%)
CAC score‡	17.6 (0.0–166.2)	11.0 (0.0–111.6)	19.9 (0.0–170.1)	28.4 (0.0–235.0)
Log_e(CAC score+1)‡	2.9 (0.0–5.1)	2.5 (0.0–4.7)	3.0 (0.0–5.1)	3.4 (0.0–5.5)
CAD at baseline†	273 (6.6%)	66 (5.6%)	134 (6.5%)	73 (8.3%)
Coronary events† (only for participants without CAD at baseline (n=3843)	136 (3.5%)	33 (3.0%)	71 (3.7%)	32 (3.9%)
Coronary events per 10 000 person years§	39.4 (33.3–46.7)	33.0 (23.5–46.5)	40.9 (32.4–51.6)	44.8 (31.7–63.4)
Education (years of training)†				
≤10	464 (11.3%)	135 (11.6%)	231 (11.2%)	98 (11.1%)
11–13	2288 (55.7%)	624 (53.3%)	1165 (56.7%)	499 (56.5%)
≥14	1358 (33.0%)	411 (35.1%)	660 (32.1%)	286 (32.4%)
Income, €/mo‡	1449 (1108–1875)	1449 (1108–1875)	1449 (1108–1875)	1449 (1108–1875)
10-y Framingham Risk Score*	11.8 (± 8.7)	12.1 (±8.6)	11.6 (±8.5)	11.8 (±9.1)
Total cholesterol, mg/dL*	229.4 (±39.0)	230.2 (±38.5)	229.5 (±39.3)	227.9 (±39.0)
Low-density lipoprotein, mg/dL*	145.3 (±36.2)	147.4 (±36.2)	144.6 (±36.7)	144.1 (±34.9)
High-density lipoprotein, mg/dL*	58.1 (±17.1)	57.1 (±16.2)	58.5 (±17.3)	58.7 (±17.6)
Triglycerides, mg/dL*	149.4 (±99.9)	149.2 (±94.3)	151.8 (±108.3)	144.2 (±85.6)
Hypertension†	2347 (57.2%)	670 (57.4%)	1178 (57.3%)	498 (56.6%)
Diabetes mellitus†	556 (13.5%)	163 (13.9%)	272 (13.2%)	121 (13.7%)
Blood glucose, mg/dL*	111.6 (±27.9)	111.6 (±26.0)	111.7 (±28.2)	111.3 (±29.8)
HbA1c, %*	5.5 (±0.9)	5.5 (±0.8)	5.5 (±0.9)	5.6 (±0.9)
Current smoker†	972 (23.6%)	265 (22.6%)	504 (24.5%)	203 (23.0%)
Low physical activity†	1979 (48.1%)	607 (51.8%)	1003 (48.7%)	411 (46.5%)
Dietary pattern index*	12.7 (±3.1)	12.7 (±3.2)	12.6 (±3.1)	12.7 (±3.1)
Low vegetable intake†	1007 (24.8%)	294 (25.5%)	503 (24.8%)	210 (23.9%)
Low wine intake†	327 (8.2%)	99 (8.8%)	163 (8.3%)	65 (7.6%)
Body mass index, kg/m^2*	27.9 (±4.5)	27.9 (±4.5)	28.0 (±4.6)	27.6 (±4.4)
hs-CRP, mg/L‡	1.5 (0.7–3.2)	1.4 (0.7–3.3)	1.5 (0.7–3.1)	1.4 (0.7–3.3)

CAC indicates coronary artery calcification; CAD, coronary artery disease; and hs-CRP, high-sensitivity C-reactive protein.
*Mean (±SD).
†n (%).
‡Median (interquartile range).
§Rate (95% confidence interval).

[95% CI, 0.88–1.08; $P_{g\times education}$=0.62] per year of education and additional risk allele). The RERI for the additive genotype by income interaction effect on incident coronary events was −0.59 (95% CI, −1.27 to 0.09; *P*=0.09) per 1000€/mo and additional risk allele. For the genotype by education interaction effect, the RERI was −0.05 (95% CI, −0.24 to 0.14; *P*=0.61) per year of education and additional risk allele. The effects size estimates observed for CAC in the sensitivity analyses excluding participants with CAD at study baseline were slightly reduced (Tables III and IV in the Data Supplement) showing a genotype by income interaction effect (exp[$\beta_{g\times income}$]) of 0.88 (95% CI, 0.77–1.01; $P_{g\times income}$=0.07). The effect size estimate for the genotype by income interaction in the sensitivity analysis additionally considering cases of coronary revascularization as incident coronary events was also less strong (hazard ratio$_{g\times income}$=0.81 [95% CI, 0.63–1.05; $P_{g\times income}$=0.11]; Tables V and VI in the Data Supplement) compared with the results not including cases of coronary revascularization.

For all SES groups, the estimated genetic effects indicated an increase in CAC and incident coronary events per additional rs2891168 risk allele, except for the occurrence of

Table 2. Sex- and Age-Adjusted Effects (% Change or HR) of Chromosome 9p21.3 (rs2891168) Risk Alleles and Socioeconomic Status Indicators on CAC and Incident Coronary Events

n		% Change	95% CI	*P* Value
CAC				
Income (per 1000€/mo)				
3867		−12.3	−20.4 to −3.3	0.009
Education (per year)				
4109		−5.2	−8.0 to −2.3	0.0004
Chromosome 9p21.3 (per risk allele)				
4116		31.2	19.5 to 44.1	1.37×10^{-8}
n	n Case	HR	95% CI	*P* Value
Incident coronary events				
Income (per 1000€/mo)				
3608	131	0.82	0.63 to 1.06	0.14
Education (per year)				
3837	136	0.96	0.89 to 1.04	0.34
Chromosome 9p21.3 (per risk allele)				
3843	136	1.17	0.92 to 1.49	0.19

CAC indicates coronary artery calcification; CI, confidence interval; and HR, hazard ratio.

coronary events in the highest income tertile (Figure 2). The effect size estimates for lower SES groups were generally stronger than for higher SES groups after an inverse gradient.

Associations of SES indicators with CAC and incident coronary events stratified by genotype also revealed differences in the magnitude of the obtained effect size estimates for the relationship between income and CAC, education and CAC, and income and incident coronary events, showing the strongest effects in the G/G genotype stratum (Tables VII and VIII in the Data Supplement).

Participants in the lower income tertile having 2 copies of the rs2891168 risk allele showed on average a 125.9% (95% CI, 63.4%–212.4%; $P=8.47\times10^{-7}$) higher CAC score when compared with the reference group of participants in the highest income tertile having 2 protective alleles (Table 3). Effect size estimates for CAC were stronger the lower the income tertile and number of protective alleles. Similar results were obtained for the joint effects of education and rs2891168 risk alleles on CAC (Table 3), whereas results for the joint effects on incident coronary events did not show clear patterns (Table 4).

Tables 5 and 6 show the effect size estimates for the multiplicative genotype by income interaction in addition to the genotype by CVRF interaction effect size estimates derived from the same regression model. With regard to CAC, substantial changes in the estimated $\beta_{g\times income}$ were observed for hypertension and physical activity leading to stronger genotype by income interaction effect size estimates (Table 5). Some indication for genotype by CVRF interaction was observed for physical activity, but not for hypertension. In the full model, the estimated $\beta_{g\times income}$ was slightly pronounced. Compared with the interaction effect size estimate derived from the regression model including

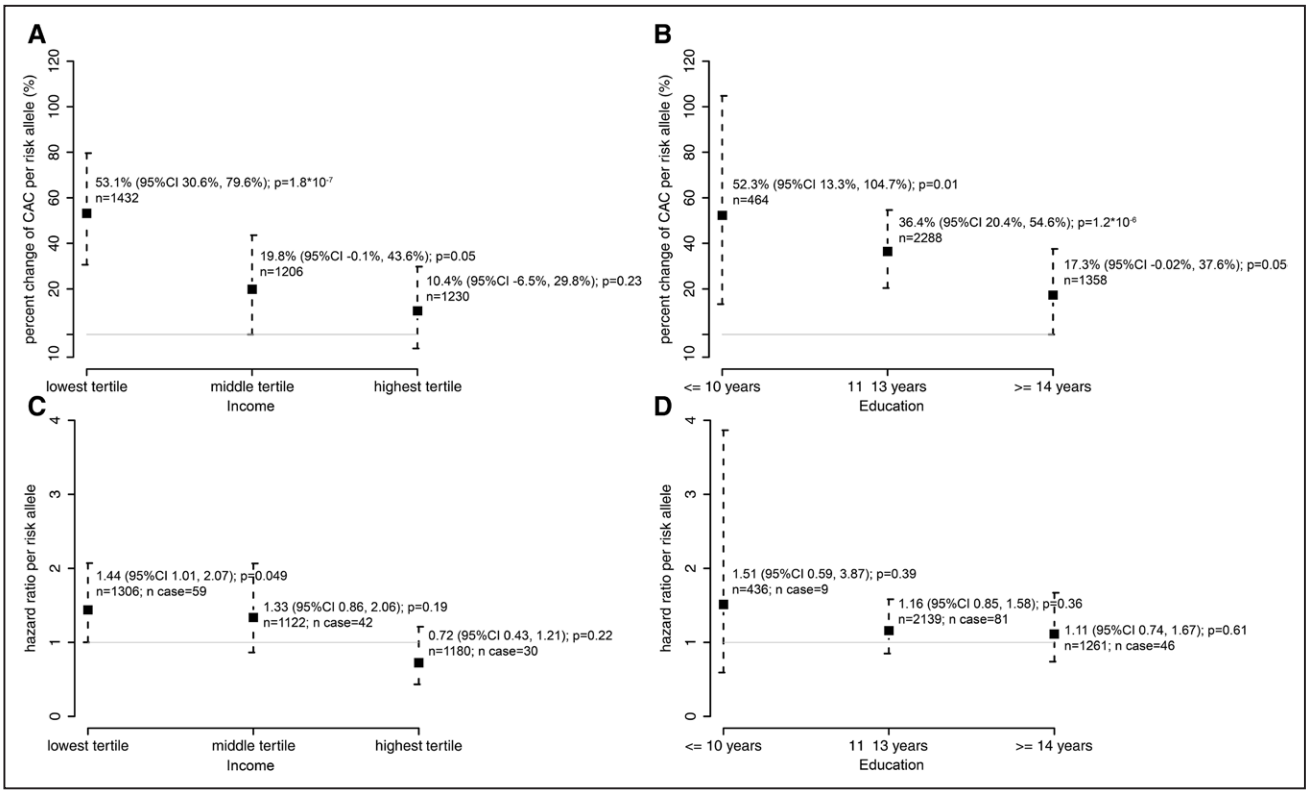

Figure 2. Sex- and age-adjusted genetic effects on coronary artery calcification (CAC) per chromosome 9p21.3 (rs2891168) risk allele stratified by income tertiles (**A**) and education groups (**B**), as well as on incident coronary events stratified by income tertiles (**C**) and education groups (**D**) in a (log-) additive genetic model.

Table 3. Sex- and Age-Adjusted Joint Effects (% Change) of Chromosome 9p21.3 (rs2891168) Genotype and Socioeconomic Status Indicator on Coronary Artery Calcification Calculated Separately for Income and Education With the Group of Having 2 Protective Alleles and the Highest Socioeconomic Status as Reference

Genotype	n	% Change	95% CI	*P* Value
Income				
Lower tertile				
G/G	322	125.9	63.4–212.4	8.47×10^{-7}
A/G	713	47.3	12.1–93.5	0.01
A/A	397	-3.8	29.3–30.8	0.80
Middle tertile				
G/G	246	43.4	1.1–103.3	0.04
A/G	619	33.1	0.7–76.1	0.04
A/A	340	1.5	26.3–39.6	0.93
Highest tertile				
G/G	265	19.2	15.2–67.6	0.31
A/G	603	27.3	3.8–68.5	0.09
A/A	362	Ref.	…	…
Education				
≤10 y				
G/G	98	184.5	75.9–360.3	2.07×10^{-5}
A/G	231	75.1	22.5–150.3	2.14×10^{-3}
A/A	135	25.0	18.2–91.0	0.30
11–13 y				
G/G	499	107.1	55.9–175.0	5.08×10^{-7}
A/G	1165	57.9	23.6–101.6	2.56×10^{-4}
A/A	624	11.5	15.0–46.3	0.43
≥14 y				
G/G	286	32.0	4.6–82.6	0.09
A/G	660	43.2	9.8–86.6	0.01
A/A	411	Ref.	…	…

CI indicates confidence interval.

Table 4. Sex- and Age-Adjusted Joint Effects (HR) of Chromosome 9p21.3 (rs2891168) Genotype and Socioeconomic Status Indicator on Incident Coronary Events Calculated Separately for Income and Education With the Group of Having 2 Protective Alleles and the Highest Socioeconomic Status as Reference

Genotype	n	n Case	HR	95% CI	*P* Value
Income					
Lower tertile					
G/G	288	20	1.82	0.87–3.82	0.11
A/G	649	25	1.00	0.49–2.03	0.99
A/A	369	14	0.92	0.42–2.03	0.83
Middle tertile					
G/G	220	8	0.92	0.37–2.30	0.86
A/G	583	28	1.21	0.60–2.43	0.60
A/A	319	6	0.46	0.17–1.25	0.13
Highest tertile					
G/G	256	4	0.48	0.15–1.51	0.21
A/G	574	15	0.82	0.38–1.79	0.62
A/A	350	11	Ref.	…	…
Education					
≤10 y					
G/G	89	2	0.99	0.22–4.50	0.99
A/G	218	6	1.20	0.44–3.25	0.72
A/A	129	1	0.31	0.04–2.35	0.26
11–13 y					
G/G	459	19	1.58	0.78–3.23	0.21
A/G	1090	43	1.48	0.79–2.77	0.22
A/A	590	19	1.18	0.58–2.40	0.65
≥14 y					
G/G	262	11	1.24	0.56–2.78	0.60
A/G	614	22	1.05	0.53–2.08	0.89
A/A	385	13	Ref.	…	…

CI indicates confidence interval; and HR, hazard ratio.

income, the effect size estimate for the genotype by physical activity interaction was considerably less strong in a linear regression model not including SES indicators (Table IX in the Data Supplement). With regard to incident coronary events, the estimated genotype by income interaction effect size estimate was diminished after including sex, the dietary pattern index, and vegetable intake in the analyses (Table 6). This led also to a diminished effect size estimate in the full model. Indication for interaction was observed for vegetable intake, also in a regression model not including income (Table X in the Data Supplement). In addition, there was some indication for genotype by physical activity interaction with observing a slightly stronger interaction effect size estimate in a regression model not including income (Table X in the Data Supplement).

Discussion

In the present study, a common variant at a locus within the chromosome 9p21.3 region interacts with income as an indicator of SES to influence CAC levels and the incidence of coronary events in a population-based cohort although evidence was less strong for incident coronary events than for CAC. Directions of the interaction effect size estimates on the multiplicative scale indicate that the estimated joint effects of income and each additional 9p21.3 risk allele together is less than the product of the estimated effects of both factors separately, that is, indicating negative interaction on the multiplicative scale. This is supported by the results obtained in stratified analyses where the highest genetic effects were observed in the lowest income tertile and by comparing joint effects of all possible combinations of the number of 9p21.3 risk alleles and income tertiles. The RERI estimate

Table 5. Changes in the Magnitude of the Chromosome 9p21.3 (rs2891168) Genotype by Income Interaction Effect (exp[$\beta_{g\times income}$]) on coronary Artery Calcification in Sex- and Age-Adjusted Regression Models Additionally Including CVRF Main Effects and the Respective Interaction Terms Separately for Each CVRF (*Changes of the $\beta_{g\times income}$ Estimate >|10%|)

CVRF	N	exp($\beta_{g\times income}$) Per 1000€/mo	95% CI	$P_{g\times income}$	% Change of $\beta_{g\times income}$	exp($\beta_{g\times CVRF}$)	95% CI	$P_{g\times CVRF}$
Base model	3867	0.85	0.74–0.98	0.02	…	…	…	…
Age, y	3867	0.85	0.74–0.97	0.02	–1.56	1.00	0.99–1.01	0.71
Sex	3867	0.84	0.74–0.97	0.01	–5.91	0.91	0.75–1.10	0.34
Framingham Risk Score	3845	0.84	0.73–0.96	0.01	–8.65	1.00	1.00–1.00	0.94
Total cholesterol, mg/dL	3865	0.85	0.74–0.97	0.02	–1.24	1.00	1.00–1.00	0.17
Low-density lipoprotein, mg/dL	3852	0.85	0.75–0.98	0.02	1.62	1.00	1.00–1.00	0.75
High-density lipoprotein, mg/dL	3864	0.86	0.75–0.98	0.03	4.36	1.00	0.99–1.00	0.16
Triglycerides, mg/dL	3862	0.85	0.75–0.98	0.02	1.74	1.00	1.00–1.00	0.86
Hypertension	3858	0.83	0.72–0.94	0.01	–19.29*	1.00	0.83–1.22	0.96
Diabetes mellitus	3867	0.85	0.74–0.97	0.01	–4.60	1.23	0.93–1.61	0.15
Glucose, mg/dL	3862	0.85	0.74–0.97	0.02	–2.05	1.00	1.00–1.00	0.38
HbA1c, %	3836	0.84	0.74–0.97	0.01	–4.98	1.02	0.91–1.14	0.77
Current smoker	3867	0.85	0.75–0.98	0.02	1.37	0.96	0.76–1.21	0.72
Low physical activity	3867	0.83	0.72–0.95	0.01	–16.99*	0.75	0.62–0.91	0.003
Dietary pattern index	3768	0.86	0.74–0.98	0.03	2.68	1.02	0.99–1.05	0.31
Low vegetable intake	3824	0.85	0.74–0.97	0.02	–0.89	0.98	0.78 –1.22	0.83
Low wine intake	3736	0.84	0.73 – 0.96	0.01	–9.98	1.19	0.82–1.72	0.36
Body mass index, kg/m^2	3852	0.86	0.75–0.98	0.03	4.17	0.99	0.97–1.01	0.24
hs-CRP, mg/L	3855	0.85	0.75–0.97	0.02	–0.56	0.99	0.98–1.01	0.56
Full model	3728	0.84	0.74–0.96	0.01	–8.52	…	…	…

CI indicates confidence interval; CVRF, cardiovascular risk factor; and hs-CRP, high-sensitivity C-reactive protein.

gave some indication that the estimated joint effect on incident coronary events was less than the sum of the estimated effects of income and each additional 9p21.3 risk allele separately, that is, suggesting negative interaction on the additive scale. For using education as SES indicator, the results obtained were not that conclusive although decreasing effect size estimates were also observed for increasing educational levels in the stratified analyses suggesting modification of the genetic effect. As education and income are highly correlated aspects of SES, considerably deviant results have not been anticipated.

Reports on 9p21.3 genotype by environment interactions have been inconclusive in the past. Only a few studies have included indicators of SES in their analyses even if the environmental parameters under investigation were likely to be associated with SES. In the study of Hamrefors et al,[17] multiplicative 9p21.3 genotype by education interaction influencing incident CAD has been investigated showing nonsignificant results (P=0.08). Effect size estimates for interaction and direction of effects have not been reported although the reported P value suggests that in the study population under investigation the effect size estimate has not been equal to zero. However, compared with education, the results obtained in the present study indicate that income better reflects the SES-related aspects that seem to modify 9p21.3 genetic effects.

As formal genetic studies have suggested that favorable environmental conditions associated with higher SES enable higher SES groups to reduce the expression of their genetic susceptibility to disease,[30,31] it might be argued that genotype by environment interactions reported for 9p21.3 risk alleles may be partly explained by underlying interactions with SES acting as a proxy marker for a range of SES-associated environmental conditions, which in sum modify the genetic effect. SES may then be interpreted as a context-defining variable not limited to single environmental factors and thus more suitable to identify subgroups for which the genetic effect is stronger than the average effect at the population level.[29]

In the present study, however, the $\beta_{g\times income}$ estimate for interaction between 9p21.3 risk alleles and income on CAC was even slightly stronger while adjusting for hypertension and physical activity. All other CVRFs included in the models did not affect the magnitude of the $\beta_{g\times income}$ estimate, suggesting that the 9p21.3 genotype by income interaction on CAC operates independently. Hypertension did not show any indication for interaction with 9p21.3 risk alleles. This is consistent with the results of several studies that have investigated SNPs of the chromosome 9p21.3 region being in perfect LD (r^2=1.0) to rs2891168 in the 1000 Genomes CEU population.[21–23] In a recent study, interaction with diastolic blood pressure on CAC has been reported for 9p21 risk alleles. However, the SNP

Table 6. Changes in the Magnitude of the Chromosome 9p21.3 (rs2891168) Genotype by Income Interaction Effect (HR) on Incident Coronary Events in Sex- and Age-Adjusted Regression Models Additionally Including CVRF Main Effects and the Respective Interaction Terms Separately for Each CVRF (*Changes of the $\beta_{g\times income}$ Estimate >|10%|)

CVRF	n	n Case	$HR_{g\times income}$ Per 1000€/mo	95% CI	$P_{g\times income}$	% Change of $\beta_{g\times income}$	$HR_{g\times CVRF}$	95% CI	$P_{g\times CVRF}$
Base model	3608	131	0.69	0.48–0.98	0.04	…	…	…	…
Age, y	3608	131	0.68	0.48–0.98	0.04	–0.53	1.00	0.97–1.03	0.99
Sex	3608	131	0.72	0.50–1.03	0.07	12.17*	1.23	0.69–2.18	0.48
CAC	3608	131	0.68	0.48–0.98	0.04	–0.32	1.00	1.00–1.00	0.23
Framingham Risk Score	3588	131	0.66	0.46–0.94	0.02	–11.37*	1.00	0.98–1.02	0.64
Total cholesterol, mg/dL	3606	131	0.68	0.48–0.98	0.04	–1.70	1.00	0.99–1.00	0.78
Low-density lipoprotein, mg/dL	3594	131	0.68	0.48–0.97	0.03	–2.07	1.00	0.99–1.00	0.93
High-density lipoprotein, mg/dL	3605	131	0.68	0.48–0.97	0.04	–0.93	1.00	0.99–1.02	0.72
Triglycerides, mg/dL	3603	131	0.68	0.48–0.97	0.04	–1.83	1.00	1.00–1.00	0.28
Hypertension	3605	131	0.67	0.47–0.96	0.03	–4.72	1.31	0.76–2.25	0.33
Diabetes mellitus	3608	131	0.69	0.48–0.98	0.04	0.34	0.72	0.41–1.27	0.26
Glucose, mg/dL	3603	130	0.68	0.47–0.98	0.04	–1.35	1.00	0.99–1.00	0.25
HbA1c, %	3578	129	0.70	0.49–0.99	0.04	3.79	0.84	0.68–1.06	0.14
Current smoker	3608	131	0.68	0.47–0.96	0.03	–3.95	0.67	0.38–1.19	0.17
Low physical activity	3608	131	0.67	0.47–0.96	0.03	–5.04	0.62	0.37–1.02	0.06
Dietary pattern index	3515	126	0.71	0.49–1.04	0.08	10.60*	0.99	0.91–1.07	0.80
Low vegetable intake	3566	127	0.74	0.51–1.08	0.12	19.59*	0.47	0.26–0.87	0.02*
Low wine intake	3493	119	0.71	0.49–1.02	0.07	8.31	1.38	0.53–3.59	0.51
Body mass index, kg/m^2	3594	130	0.69	0.49–1.00	0.05	3.55	0.98	0.93–1.04	0.56
hs-CRP, mg/L	3596	131	0.69	0.48–0.98	0.04	–0.24	0.99	0.95–1.04	0.82
Full model	3482	125	0.72	0.50–1.04	0.08	12.30*	…	…	…

CI indicates confidence interval; CVRF, cardiovascular risk factor; HR, hazard ratio; and hs-CRP, high-sensitivity C-reactive protein.

under investigation (rs2069416) has been selected from a different 9p21 region only in low LD (r^2<0.5) to SNPs from the 9p21.3 region.[16] This is in line with the study of Lusk et al[29] in which the genetic effect of a SNP in low LD to rs2891168 (rs3217992; r^2=0.49) has shown much stronger heterogeneity between hypertensives and normotensives than observed for an SNP in perfect LD (rs1333049; r^2=0.98).

Physical activity showed indication for interaction with 9p21.3 risk alleles in the regression model that considered income and income interaction terms. This would not have been detected in an analysis unadjusted for SES, as the respective effect size estimate for interaction was considerably less strong. It may serve as an example for the importance of proper SES-adjustment in gene by environment interaction analysis (ie, including SES interaction terms in the model next to SES main effects) where confounding by SES is present. It may also explain why multiplicative interaction between 9p21.3 risk alleles and physical activity has not been detected in a previous study.[17]

For incident coronary events, the magnitude of the $\beta_{g\times income}$ estimate indicating multiplicative interaction was reduced while controlling for sex, the dietary pattern index, and vegetable intake main effects and interaction terms. Indication for multiplicative interaction was observed only for vegetable intake. Dietary intake has been reported previously as a promising environmental factor modifying the genetic effect of 9p21.3 risk alleles on myocardial infarction and incident cardiovascular disease,[18,19] indicating that this interaction is robust against different approaches of measuring dietary factors. However, as the magnitude of the $\beta_{g\times income}$ estimate is only partly reduced while controlling for the dietary pattern index or vegetable intake, other SES-related factors not included in the present study are also suggested to mediate the observed genotype by income interaction effect on incident coronary events.

The strengths of the present study were its stringent predefined end point criteria, the inclusion of 2 different SES indicators, and the wide range of CVRFs available for analysis. A limitation of the study was its sample size, as for the detection of small interaction effect size estimates large samples are required. Power calculations were performed using QUANTO (version 1.2.3 software, http://hydra.usc.edu/gxe) considering the given minor allele frequency and a 2-sided α=0.05 under a (log-) additive genetic model. Power was ≈53% to detect the obtained multiplicative genotype by income interaction effect size estimate for using CAC as study outcome and ≈54% for using incident coronary events based on the given sample size. However, deriving evidence for interaction

was not solely based on testing interaction terms but also on stratified analysis and calculating joint effects of 9p21.3 genotype and SES indicators. That indication for interaction was less strong for incident coronary events may reflect not only power limitations but also a less strong association of 9p21.3 genotype with myocardial infarction compared with coronary atherosclerotic burden.[49]

In conclusion, the present study indicates that a common variant of a locus within the chromosome 9p21.3 region interacts with income as an indicator of SES to influence CAC levels and the incidence of coronary events. This supports the hypothesis that better material, psychosocial, and lifestyle conditions enable higher SES groups to reduce the expression of their genetic susceptibility to CAD. Hypertension, physical activity, sex, and diet may play a part in the complex interplay of 9p21.3 genetic variation, SES, and its impact on CAD. Further studies on the interaction between common genetic variants and SES indicators as surrogate markers for a range of unequally distributed risk factors are needed to identify population groups at increased risk for developing complex diseases and to further explain how social and environmental factors get under the skin to produce health inequalities.

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Disclosures

None.

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CLINICAL PERSPECTIVE

In the present study, a common variant at a locus within the chromosome 9p21.3 region interacts with income as an indicator of socioeconomic status (SES) to influence levels of coronary artery calcification and the incidence of coronary events in a population-based cohort. The strongest genetic effect was observed in the group of low SES. Although the study results gave some indication that hypertension, physical activity and diet may play a part in the complex relation of 9p21.3 genetic variation, SES and coronary artery disease, the risk factors included in the analysis only partly explained the observed genotype by income interaction effect. Thus, other SES-related factors not included in the analysis are likely to be involved. Overall, the study results support the hypothesis that better material, psychosocial, and lifestyle conditions enable higher SES groups to reduce the expression of their genetic susceptibility to coronary artery disease. Further investigating interaction between common genetic variants and lifestyle-related health indicators may help to identify subgroups at increased risk for developing complex diseases and to further explain how social and environmental factors get under the skin to produce health inequalities. The present findings underline the importance for coronary artery disease prevention and clinical intervention in terms of lifestyle changes especially in subgroups of high vulnerability.

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Socioeconomic Status Interacts with the Genetic Effect of a Chromosome 9p21.3 Common Variant to Influence Coronary Artery Calcification and Incident Coronary Events in the Heinz Nixdorf Recall Study (Risk Factors, Evaluation of Coronary Calcium, and Lifestyle)

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SUPPLEMENTAL MATERIAL

Table S1: Characteristics of study participants (N=4116) stratified by income tertiles.

	All	Low income	Middle income	High income
N	4116	1431	1206	1230
age (years) *	59.6 (±7.8)	60.8 (±7.8)	59.8 (±8.1)	57.9 (±7.4)
Women †	2050 (49.8%)	688 (48.1%)	516 (42.8%)	678 (55.1%)
CAC score ‡	17.6 (0.0-166.2)	27.3 (1.0-212.2)	25.8 (0.0-197.3)	8.4 (0.0-101.2)
Log_e(CAC score + 1) ‡	2.9 (0.0-5.12)	3.3 (0.7-5.4)	3.3 (0.0-5.3)	2.2 (0.0-4.6)
CAD at baseline †	273 (6.6 %)	126 (8.8%)	83 (6.9%)	50 (4.1%)
Coronary events † (only for participants without CAD at baseline; n=3843)	136 (3.5%)	59 (4.5%)	42 (3.7%)	30 (2.5%)
Coronary events per 10,000 person years §	39.4 (33.3-46.7)	51.7 (40.1-66.7)	41.4 (30.6-56.0)	27.6 (19.3-39.5)
education (years of training) †				
≤10 y	464 (11.3%)	268 (18.7%)	119 (9.9%)	47 (3.8%)
11–13 y	2288 (55.7%)	912 (63.7%)	681 (56.5%)	534 (43.4%)
≥14 y	1358 (33.0%)	251 (17.6%)	406 (33.6%)	649 (52.8%)
income (€/month) ‡	1449 (1108-1875)	937 (767-1108)	1449 (1313-1619)	2216 (1917-2740)
10-years Framingham Risk Score *	11.8 (±8.7)	13.1 (±9.5)	12.3 (±8.8)	9.9 (±7.2)
Total cholesterol (mg/dl) *	229.4 (±39.0)	230.6 (±39.0)	227.4 (±37.6)	228.9 (±39.3)
Low-density lipoprotein (mg/dl) *	145.3 (±36.2)	146.9 (±35.8)	144.5 (±35.4)	143.4(±36.9)
High-density lipoprotein (mg/dl) *	58.1 (±17.1)	57.7 (±17.2)	56.2 (±15.9)	59.9 (±17.7)
Triglycerides (mg/dL) *	149.4 (±99.9)	151.6 (±88.3)	151.8 (±101.9)	146.3 (±113.9)
Hypertension †	2347 (57.2%)	894 (62.6%)	709 (58.9%)	602 (49.0%)
Diabetes mellitus †	556 (13.5%)	220 (15.4%)	177 (14.7%)	133 (10.8%)
Blood glucose (mg/dL) *	111.6 (±27.9)	113.5 (±30.3)	112.2 (±27.8)	109.1 (±24.5)
HbA1c (%)*	5.5 (±0.9)	5.6 (±0.9)	5.5 (±0.9)	5.5 (±0.8)
Current smoker †	972 (23.6%)	373 (26.1%)	257 (21.3%)	273 (22.2%)
Low physical activity †	1979 (48.1%)	814 (56.9%)	562 (46.6%)	483 (39.3%)
Dietary pattern index *	12.7 (±3.1)	12.7 (±3.1)	12.5 (±3.1)	12.7 (±3.2)
Low vegetable intake †	1007 (25.1%)	401 (28.4%)	288 (24.2%)	268 (21.9%)
Low wine intake †	327 (8.2%)	62 (4.6%)	74 (6.3%)	169 (13.9%)
Body mass index (kg/m^2) *	27.9 (±4.5)	28.5 (±4.6)	27.9 (±4.5)	27.2 (±4.4)
hs-CRP (mg/L) ‡	1.5 (0.7-3.2)	1.8 (±6.6)	1.4 (0.7-3.0)	1.2 (0.7-2.6)

* mean (±sd); † number (%);‡ median (interquartile range); § rate (95% confidence interval); CAD, coronary artery disease; CAC, coronary artery calcification

Table S2: Characteristics of study participants (N=4116) stratified by educational groups.

	All	Education ≤10 years	Education 11-13 years	Education ≥14 years
N	4116	464	2288	1358
age (years) *	59.6 +/- 7.8	63.2 (± 7.6)	59.6 (±7.7)	58.3 (±7.7)
Women †	2050 (49.8%)	366 (78.9%)	1298 (56.8%)	386 (28.4%)
CAC score ‡	17.6 (0.0-166.2)	19.5 (0.0-136.7)	14.7 (0.0-149.9)	24.8 (0.0-197.0)
Log_e(CAC score + 1) ‡	2.9 (0.0-5.12)	3.0 (0.0-4.9)	2.8 (0.0-5.0)	3.3 (0.0-5.3)
CAD at baseline †	273 (6.6 %)	28 (6.0%)	149 (6.5%)	96 (7.1%)
Coronary events † (only for participants without CAD at baseline; n=3843)	136 (3.5%)	9 (2.1%)	81 (3.8%)	46 (3.6%)
Coronary events per 10,000 person years §	39.4 (33.3-46.7)	24.3 (12.6-46.7)	42.1 (33.8-52.3)	40.1 (30.0-53.5)
income (€/month) ‡	1449 (1108-1875)	1108 (895-1278)	1278 (937-1662)	1875 (1420-2557)
10-years Framingham Risk Score *	11.8 +/- 8.7	11.5 (±8.5)	11.7 (±8.8)	12.1 (±8.5)
Total cholesterol (mg/dl) *	229.4 +/- 39.0	235.8 (±40.4)	230.0 (±39.4)	226.0 (±37.7)
Low-density lipoprotein (mg/dl) *	145.3 +/- 36.2	60.39 (±36.8)	146.2 (±36.3)	142.7 (±35.7)
High-density lipoprotein (mg/dl) *	58.1 +/- 17.1	148.4 (±17.0)	58.7 (±17.1)	56.6 (±16.9)
Triglycerides (mg/dL) *	149.4 +/- 99.9	153.31 (±86.3)	148.5 (±94.8)	149.4 (±111.9)
Hypertension †	2347 (57.2%)	314 (67.8%)	991 (43.4%)	737 (54.4%)
Diabetes mellitus †	556 (13.5%)	88 (19.0%)	297 (13.0%)	171 (12.6%)
Blood glucose (mg/dL) *	111.6 +/- 27.9	114.8 (±33.4)	111.3 (±28.4)	110.8 (±25.0)
HbA1c (%)*	5.5 +/- 0.9	5.7 (±1.0)	5.5 (±0.8)	5.5 (±0.8)
Current smoker †	972 (23.6%)	96 (20.7%)	607 (26.5%)	268 (19.7%)
Low physical activity †	1979 (48.1%)	280 (60.3%)	1162 (50.1%)	534 (39.3%)
Dietary pattern index *	12.7 +/- 3.1	13.1 (±3.1)	12.7 (±3.1)	12.5 (±3.1)
Low vegetable intake †	1007 (25.1%)	154 (34.0%)	559 (24.8%)	291 (21.6%)
Low wine intake †	327 (8.2%)	14 (3.3%)	124 (5.6%)	188 (14.1%)
Body mass index (kg/m^2) *	27.9 +/- 4.5	29.0 (±5.4)	28.0 (±4.5)	27.3 (±4.1)
hs-CRP (mg/L) ‡	1.5 (0.7-3.2)	2.0 (1.0-4.2)	1.6 (0.8-3.4)	1.2 (0.6-2.5)

* mean (±sd); † number (%);‡ median (interquartile range); § rate (95% confidence interval); CAD, coronary artery disease; CAC, coronary artery calcification

Table S3: Sex- and age-adjusted effects (% change) of chromosome 9p21.3 (rs2891168) risk alleles and socioeconomic status indicators on coronary artery calcification (CAC) excluding participants with coronary artery disease at study baseline.

CAC		
n	**% Change (95% CI)**	***p***
Income (per 1000€/month)		
3609	-8.4 (--16.9 - 1.0)	0.08
Education (per year)		
3837	-4.4 (-7.2 - -1.5)	0.003
Chromosome 9p21.3 (per risk allele)		
3843	26.1 (14.8 - 38.6)	$4.60*10^{-6}$

Table S4: Chromosome 9p21.3 (rs2891168) genotype by income interaction effect (exp[$\beta_{g\times income}$]) and genotype by education interaction effect (exp[$\beta_{g\times education}$]) on coronary artery calcification (CAC) excluding participants with coronary artery disease at study baseline.

income	**n**	**exp($\beta_{g\times income}$)**	**(95% CI)**	$p_{g\times income}$
base model	3609	0.88	(0.77 - 1.01)	0.07
full model	3483	0.87	(0.76 - 0.99)	0.04
education	**n**	**exp($\beta_{g\times education}$)**	**(95% CI)**	$p_{g\times education}$
base model	3837	0.97	(0.94 - 1.01)	0.18
full model	3692	0.97	(0.93 - 1.01)	0.14

Table S5: Sex- and age-adjusted effects (hazard ratios; HR) of chromosome 9p21.3 (rs2891168) risk alleles and socioeconomic status indicators on incident coronary events including incident cases of coronary revascularization.

Incident Coronary Events (incl. coronary revascularization)				
n	**n Case**	**HR**	**(95% CI)**	***p***
Income (per 1000€/month)				
3608	245	0.92	(0.76 - 1.10)	0.36
Education (per year)				
3836	253	0.98	(0.93 - 1.04)	0.50
Chromosome 9p21.3 (per risk allele)				
3841	253	1.24	(0.81 - 1.04)	0.02

Table S6: Chromosome 9p21.3 (rs2891168) genotype by income interaction effect (hazard ratio; $HR_{g\times income}$) and genotype by education interaction effect ($HR_{g\times education}$) on incident coronary events including incident cases of coronary revascularization.

income	**n**	**n Case**	**$HR_{g\times income}$**	**(95% CI)**	**$p_{g\times income}$**
base model	3608	245	0.81	(0.63 - 1.05)	0.11
full model	3482	229	0.82	(0.63 - 1.07)	0.14
education	**n**	**n Case**	**$HR_{g\times education}$**	**(95% CI)**	**$p_{g\times education}$**
base model	3836	253	0.95	(0.88 - 1.02)	0.16
full model	3691	236	0.94	(0.87 - 1.02)	0.13

Table S7: Sex- and age-adjusted effects (% change) of socioeconomic status indicators on coronary artery calcification (CAC) stratified by chromosome 9p21.3 (rs2891168) genotype separately for income (per 1000€) and education (per year of education).

Income				
Genotype	**n**	**% Change**	**(95% CI)**	***p***
G/G	833	-30.1	(-43.9 - -13.0)	$1.43*10^{-3}$
A/G	1935	-9.9	(-21.5 - 3.5)	0.14
A/A	1099	-0.28	(-16.1 - 18.6)	0.98
Education				
Genotype	**n**	**% Change**	**(95% CI)**	***p***
G/G	883	-11.5	(-17.2 - -5.4)	$3.37*10^{-4}$
A/G	2056	-2.9	(-6.9 - 1.4)	0.18
A/A	1170	-3.3	(-8.2 - 1.8)	0.20

Table S8: Sex- and age-adjusted effects (hazard ratio; HR) of socioeconomic status indicators on incident coronary events stratified by chromosome 9p21.3 (rs2891168) genotype separately for income (per 1000€) and education (per year of education).

Income					
Genotype	**n**	**n Case**	**HR**	**(95% CI)**	***p***
G/G	764	32	0.47	(0.25 - 0.87)	0.02
A/G	1806	68	0.90	(0.63 - 1.29)	0.58
A/A	1038	31	1.05	(0.64 - 1.72)	0.85
Education					
Genotype	**n**	**n Case**	**HR**	**(95% CI)**	***p***
G/G	810	32	1.00	(0.86 - 1.17)	0.95
A/G	1922	71	0.92	(0.83 - 1.02)	0.13
A/A	1104	33	1.02	(0.87 - 1.18)	0.83

Table S9: Chromosome 9p21.3 (rs2891168) genotype by coronary risk factor (CRF) interaction effects (exp[$\beta_{g\times CRF}$]) on coronary artery calcification (CAC) separately for each CRF in sex- and age-adjusted regression models not adjusted for indicators of socioeconomic status.

Coronary Risk Factor	n	exp($\beta_{g\times CRF}$) (95% CI)		$p_{g\times CRF}$
Age (years)	4116	1.00	(0.99 - 1.01)	0.69
Sex	4116	0.97	(0.80 - 1.17)	0.75
Framingham Risk Score	4088	1.00	(0.99 – 1.01)	0.98
Total cholesterol (mg/dl)	4113	1.00	(1.00 - 1.00)	0.52
Low-density lipoprotein (mg/dl)	4099	1.00	(1.00 - 1.00)	0.96
High-density lipoprotein (mg/dl)	4112	1.00	(0.99 - 1.00)	0.38
Triglycerides (mg/dL)	4110	1.00	(1.00 - 1.00)	0.43
Hypertension	4105	1.00	(0.83 - 1.20)	0.98
Diabetes mellitus	4115	1.18	(0.90 - 1.54)	0.23
Glucose (mg/dL)	4110	1.00	(1.00 - 1.00)	0.57
HbA1c (%)	4082	1.01	(0.91 - 1.13)	0.83
Current smoker	4103	0.95	(0.76 - 1.18)	0.63
Low physical activity	4114	0.89	(0.74 - 1.08)	0.23
Dietary pattern index	3997	1.02	(0.99 - 1.05)	0.15
Low vegetable intake	4057	0.95	(0.76 – 1.18)	0.64
Low wine intake	3965	1.10	(0.78 - 1.56)	0.59
Body mass index (kg/m^2)	4098	0.99	(0.97 - 1.01)	0.27
hs-CRP (mg/L)	4102	1.00	(0.98 - 1.02)	0.97

Table S10: Chromosome 9p21.3 (rs2891168) genotype by coronary risk factor (CRF) interaction effects (hazard ratio; HR) on incident coronary events separately for each CRF in sex- and age-adjusted regression models not adjusted for indicators of socioeconomic status.

Coronary Risk Factor	n	n Case	$HR_{g\times CRF}$	(95% CI)	$p_{g\times CRF}$
Age (years)	3843	136	1.00	(0.97 - 1.04)	0.83
Sex	3843	136	1.32	(0.77 - 2.27)	0.31
CAC	3843	136	1.00	(1.00 - 1.00)	0.15
Framingham Risk Score	3816	136	1.00	(0.98 – 1.02)	0.75
Total cholesterol (mg/dl)	3839	136	1.00	(0.99 - 1.01)	0.86
Low-density lipoprotein (mg/dl)	3826	136	1.00	(0.99 - 1.01)	0.67
High-density lipoprotein (mg/dl)	3838	136	1.00	(0.98 - 1.02)	0.95
Triglycerides (mg/dL)	3836	136	1.00	(1.00 - 1.00)	0.44
Hypertension	3837	136	1.19	(0.70 - 2.01)	0.52
Diabetes mellitus	3843	136	0.63	(0.37 - 1.09)	0.10
Glucose (mg/dL)	3836	135	0.99	(0.99 - 1.00)	0.11
HbA1c (%)	3809	134	0.82	(0.66 - 1.02)	0.08
Current smoker	3839	136	0.72	(0.41 - 1.25)	0.24
Low physical activity	3840	136	0.58	(0.35 - 0.95)	0.03
Dietary pattern index	3732	131	1.00	(0.92 - 1.08)	0.90
Body mass index (kg/m^2)	3825	135	0.98	(0.93 - 1.04)	0.48
Low vegetable intake	3787	132	0.48	(0.26 - 0.88)	0.02
Low wine intake	3710	123	1.28	(0.50 - 3.31)	0.61
hs-CRP (mg/L)	3828	136	1.00	(0.96 - 1.04)	0.93