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A COMPOSITE SCORING OF GENOTYPES DISCRIMINATES CORONARY HEART DISEASE RISK BEYOND CONVENTIONAL RISK FACTORS IN THE BOSTON PUERTO RICAN HEALTH STUDY

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Abstract

Background and aims—Using a genetic predisposition score (GPS), integrating the additive associations of a set of single nucleotide polymorphisms (SNPs) with CHD, we examined the consequences of the joint presence of a high GPS and conventional risk factors (CRFs).

Methods and Results—We studied eleven SNPs at eight loci in 197 participants with prior CHD and 524 CHD-free subjects from the Boston Puerto Rican Health Study. Each polymorphism contributed 1 unit (high-risk allele homozygous), 0.5 units (heterozygous) and 0 units (low-risk allele homozygous) to the GPS. Odds ratio (OR) of CHD for those at high-risk because of GPS (>5) and simultaneous presence of CRFs were estimated, compared with subjects at low-risk, for both measurements. The mean score was higher in participants with prior CHD than those CHD-free ($P=0.015$), and the OR for CHD with a GPS>5 was 2.90 ($P<0.001$). The joint presence of a high GPS and each CRF was associated with higher risk of CHD. Compared to participants with high GPS, those with low GPS (≤ 5) were protected against CHD even if they were smokers (OR=0.44), heavy drinkers (OR=0.43), displayed low physical activity (OR=0.35), had hypertension (OR=0.52) or hyperlipidemia (OR=0.34) (P values ranging from 0.004 to 0.023).

Conclusion—A simple genetic score of eleven polymorphisms may identify those subjects at increased risk of CHD beyond conventional risk factors.

Keywords

genes; coronary heart disease; conventional risk factors; genetic predisposition score; single nucleotide polymorphism

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Coronary heart disease (CHD) is the leading cause of mortality in the United States (1) and several studies have focused on identifying risk prediction algorithms contributing to CHD. In this regard, the Framingham Study (2) developed a risk prediction equation including conventional risk factors (CRFs). Although this risk algorithm provides a logical approach to risk stratification, misclassification is frequent, as recently shown by the fact that the Framingham score underestimated the risk derived from carotid atherosclerosis measures in one-half of persons in both the low- and intermediate-risk categories, whereas overestimated the risk in one out of every three subjects in the high-risk group (3). Therefore, since CRFs fitted into these risk algorithms have been found to be incomplete predictors of CHD, additional risk factors based on molecular genetics are now being sought.

Compelling evidence from literature suggests a genetic basis for CHD (4) by which genetic data may identify those individuals who have an inherited predisposition to CHD. Although the discovery of several candidate genes (5–8), primarily through genome wide association (GWA) analyses (5,7), the optimal set of risk genotypes has yet to be identified. There is only a relatively modest risk associated with any single genotype, but in combination several risk-genotypes may improve CHD risk prediction (9).

To date, few studies have examined the utility of genetic scores to identify subjects at increased CHD risk (10–13). Yiannakouris *et al.* (10) developed a simple genetic score using eleven single nucleotide polymorphisms (SNPs) in predicting CHD risk in a Greek population. Later, these authors demonstrated that this genetic score identified subjects at increased CHD risk above CRFs (11). Moreover, the Second Northwick Park Heart (NPHSII) and Atherosclerosis Risk in Communities (ARIC) prospective studies (8,12) demonstrated that the combination of genotype data and CRFs improved CHD prediction significantly, compared with information provided by CRFs alone. On the other hand, Kathiresan *et al.* (13) described a genotype score based on nine SNPs associated with lipids that did not improve cardiovascular risk discrimination beyond CRFs. Therefore, whether the effect of genetic scores on CHD risk prediction is over-and-above CRFs remains controversial. To stimulate this line of research, our main aim was to evaluate the contribution of multiple SNPs, as a single genetic predisposition score (GPS), to discriminate CHD risk in a population-based cohort at increased risk for age-related chronic diseases. Secondly, we compared the joint presence of high GPS and established CRFs for CHD risk.

METHODS

Participants

Complete demographic, biochemical and genotype data were available in 721 participants (197 with prior CHD and 524 CHD-free). These participants aged 45–75 years, were recruited from the Boston Center for Population Health and Health Disparities to participate in the Boston Puerto Rican Health Study, a longitudinal cohort study on stress, nutrition, health, and aging. The design of the study was approved by the Institutional Review Board of Tufts Medical Center and all participants provided informed consent. The detailed design and methodology of the study have been described previously (14).

Data collection

Information on socio-demographics, health status, history, and behavior was collected by home interview administered by bilingual interviewers. CHD was defined as a positive response to the question “Have you ever been told by a physician that you had a heart attack or angina”. Anthropometric and blood pressure (BP) measurements were collected using standard methods. Weight was measured with a beam balance and height with fixed stadiometer. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. BP was measured in duplicate at three times during the interview with an oscillometric device (Dinamap Pro Series 100, GE Medical Systems) while participants were seated and had rested for at least five minutes. Hypertension was defined as systolic BP ≥ 140 mmHg or diastolic BP ≥ 90 mmHg or current use of antihypertensive medication. Smoking and alcohol intake were determined by questionnaire and defined for this analysis as never vs. ever smokers and heavy current drinkers (>2 drinks/d for men or >1 drink/d for women) vs. non-heavy current drinkers (≤ 2 drinks/d for men or ≤ 1 drink/d for women). Physical activity was estimated as a score, based on the Paffenbarger questionnaire of the Harvard Alumni Activity Survey (15). Using American Diabetes Association criteria, participants were classified as having diabetes if fasting plasma glucose concentration was ≥ 6.93 mmol/L or use of insulin or diabetes medication was reported. Because the Adult Treatment Panel III (ATP III) (16) considers diabetes as a CHD risk equivalent, we excluded individuals with self-reported diabetes from the CHD-free group.

Laboratory methods

Blood samples were drawn after an overnight fast. Total cholesterol was measured using a cholesterol esterase cholesterol oxidase reaction on an Olympus AU400e autoanalyzer (Olympus America Inc., Melville, NY). HDL-C was measured with the same reaction after precipitation of non-HDL cholesterol with magnesium/dextran and before plasma samples were frozen. Low density lipoprotein cholesterol (LDL-C) was measured by use of a homogeneous direct method (LDL Direct Liquid Select Cholesterol Reagent; Equal Diagnostics). Triglycerides (TG) were measured by a glycerol-blanked enzymatic method on the Olympus AU400e centrifugal analyzer (Olympus America Inc., Melville, NY). Additional adjustment to the lipid concentrations were calculated in those participants receiving cholesterol-lowering treatment. Taking into account the type of lipid-lowering drugs, dose, and length of treatment, the average percentage of adjustment in lipid concentrations in those participants who received lipid-lowering treatment was: +32% for total cholesterol, +41% for LDL-C, -8% for HDL-C, and +15% for TG concentrations.

SNPs selection and genotyping

DNA was extracted from blood samples and purified using commercial Puregene reagents (Gentra Systems) following manufacturer's instructions. SNPs were selected on the basis of empirical evidence and data from GWA analyses (6,10), as indicated in the Discussion section. Genotyping was performed blindly as to CHD-control status for eleven SNPs in eight genes. The following SNPs were comprised the genotyping panel (in alphabetical order): *APOB*_A618V (rs679899), *APOC3*_3238C>G (rs5128), *APOE*_C130R (rs429358), *APOE*_R176C (rs7412), *CDKN2A/B*_116191G>C (rs1333049), *CXCL12*_111738T>C

(rs501120), *IL6*_-174C>G (rs1800795), *LPL*_D9N (rs1801177), *LPL*_N291S (rs268), *LPL*_S447X (rs328), and *NOS3*_i19342A>G (rs743507). Genotyping was performed using TaqMan® assays with allele-specific probes on the ABIPrism 7900 HT Sequence Detection System (Applied Biosystems) according to routine laboratory protocols (17). The description of SNPs is presented in Supplemental Data 1.

Statistical analyses

SPSS software (version 16.0) was used for statistical analyses. Differences in mean values were assessed by analysis of variance and unpaired t-tests. Categorical variables were compared by using the Pearson chi-square or the Fisher's exact tests. The GPS was constructed according to the contribution of each polymorphism as follows: 1 unit if the participant was homozygous for the high-risk allele, 0.5 units if the participant was heterozygous and 0 if the participant was homozygous for the low-risk allele. We made no distinction among polymorphisms, that is, whether the relative risk associated with each high-risk allele was slightly or more than slightly elevated. In this regard, the relevant relative risk associated with each variant is small, generally below 2.0, and with a wide confidence interval. Therefore, it makes unrealistic to incorporate weighted allele-specific effects. With respect to recessive and dominant effects, we adopted the parsimonious view of codominance which is compatible with the way many genetic variants affect biochemical variables. Any deviation from codominance would tend to underestimate or overestimate the studied parameters through a minor misspecification of the score. High-risk alleles were considered (in alphabetical order): *APOB*_A618, *APOC3*_3238G, *APOE* ε4, *CDKN2A*/*B*_116191C, *CXCL12*_111738T, *IL6*_-174C, *LPL*_9N, *LPL*_291S, *LPL*_447S, and *NOS3*_i19342A.

Odds ratio (OR) and 95% CI of CHD risk associated with GPS (above to the median score of 5) and simultaneously as being at high or low risk on the basis of cigarette smoking (never vs. ever), current heavy drinkers (yes vs. no), physical activity score (<31 vs. ≥31), hypertension (yes vs. no), dyslipidemia (yes vs. no), and total cholesterol, LDL-C, HDL-C and TG concentrations (following ATPIII recommendations (18) were estimated, compared with subjects at low-risk, for both the GPS and each aforementioned CRF. Potential confounding factors were age, sex, BMI, physical activity, smoking habit, alcohol consumption, presence of diabetes, and prior medications (treatment for hypertension, diabetes and hyperlipidemia). All analyses were further adjusted by population admixture estimated using the program STRUCTURE 2.2 (see below). The pairwise LD between SNPs was estimated as correlation coefficient using HelixTree software package. Two-sided *P* values <0.05 were considered statistically significant.

Population admixture

Population admixture was estimated from genotypes of 100 ancestral informative markers using two programs: STRUCTURE 2.2 and IAE3CI, with reference to three ancestral populations: European settlers, native Indians, and West Africans.

RESULTS

Characteristics of participants are shown in Table 1. Participants with CHD were older, had lower HDL-C, and higher BMI, total cholesterol, and TG concentrations than CHD-free (P values ranging from <0.001 to 0.010). Participants with CHD displayed higher prevalence of diabetes and hypertension ($P<0.001$ for both) than did those who were CHD-free. CHD-free subjects were more likely to practice physical exercise, and less likely to receive treatment for diabetes, hypertension and hyperlipidemia than participants with CHD (P values <0.001). Although differences were marginally significant, participants with prior CHD were more likely to smoke than CHD-free subjects ($P=0.091$). No significant differences in other variables examined were observed. These characteristics were analyzed by sex (Supplemental Table 2). Given that women displayed higher BMI, higher total cholesterol, LDL-C, HDL-C, and lower TG concentrations than men, all performed statistical analyses were adjusted by sex.

For all polymorphisms, there was no departure from Hardy-Weinberg equilibrium ($P>0.05$). The pairwise LD in correlation coefficients of all SNPs was presented in Supplemental Table 3. Given that all pairwise LD was <0.80 , all eleven SNPs were retained for further analysis. Table 2 shows the genotype distribution and presumed high-risk allele frequencies among subjects with prior CHD and those who were CHD-free. Two polymorphisms, among the total of eleven SNPs studied, were significant (P values 0.020 and 0.009 , respectively), and four were marginally significant (P values ranging from 0.071 to 0.145).

The distribution of participants with CHD and those CHD-free, depending on the value of the GPS, was shown in Table 3. There was an over-representation of participants with CHD in the presumed high score values (>5), whereas an over-representation of CHD-free subjects was seen in the low score values (≤ 5) (P value for trend $=0.015$). From participants with CHD, 93 (47%) had a GPS >5 , whereas 345 (66%) CHD-free subjects had a GPS ≤ 5 ($P=0.001$). The OR for CHD was 2.99 with 95% CI of 1.76 to 5.09 ($P<0.001$). After further adjustment for population admixture, the OR was 2.90 with 95% CI of 1.70 to 4.99 ($P<0.001$). Further adjustment for HDL-C concentrations strengthened the OR to 2.95 with 95% CI of 1.71 to 5.08 ($P<0.001$) (data not shown).

Table 4 shown ORs and 95% CIs for CHD in relation to the joint presence of a high GPS (>5) or low GPS (≤ 5) with non-genetic established CHD risk factors. In all variables examined, the joint presence of a high GPS with CRFs was associated with a significantly higher risk for CHD than for participants with low GPS who were exposed to the corresponding CRFs (for ever smokers, OR 1 vs. 0.44; $P=0.023$, for current heavy drinkers, OR 1 vs. 0.43; $P=0.006$, for low physical activity score, OR 1 vs. 0.35; $P=0.004$, for hypertension, OR 1 vs. 0.52; $P=0.037$, and for dyslipidemia, OR 1 vs. 0.34; $P=0.014$). After further adjustment for HDL-C concentrations, results did not differ from previous findings (for ever smokers, OR 1 vs. 0.44; $P=0.023$, for current heavy drinkers, OR 1 vs. 0.42; $P=0.005$, for low physical activity score, OR 1 vs. 0.36; $P=0.007$, for hypertension, OR 1 vs. 0.31; $P=0.004$, and for dyslipidemia, OR 1 vs. 0.32; $P=0.010$) (data not shown).

Likewise, ORs and 95% CIs for CHD for those at high-risk because of the joint presence of high GPS and altered lipid concentrations (depending on ATP III criteria) compared with subjects at low-risk, for both the GPS and lipid concentrations was shown in Table 5. According to prior results, the joint presence of a high GPS and high total cholesterol (5.20 mmol/L), LDL-C (2.60 mmol/L), TG (1.65 mmol/L), or low HDL-C concentrations (<1.04 mmol/L), was associated with a significantly higher risk for CHD than those participants with low GPS who were exposed to the aforementioned lipid concentrations (for total cholesterol, OR 1 vs.0.41; $P=0.021$), for LDL-C, OR 1 vs.0.47; $P=0.021$), for HDL-C, OR 1 vs.0.41; $P=0.005$), and for TG, OR 1 vs.0.18; $P=0.001$). After further adjustment for HDL-C concentrations, the OR among participants with high GPS, compared with low GPS, and both exposed to altered lipid concentrations remained significant being 1 vs. 0.40; $P=0.022$ for total cholesterol, 1 vs. 0.47; $P=0.019$ for LDL-C, 1 vs.0.40; $P=0.005$ for HDL-C, and 1 vs.0.17; $P<0.001$ for TG concentrations (data not shown).

DISCUSSION

The current study provides a CHD risk score based on eleven genetic variants in order to identify those individuals at increased risk for CHD in a population-based cohort study. Importantly, the joint presence of high GPS and multiple CRFs was associated with higher risk of CHD compared with participants with low GPS who were exposed to those CRFs. Moreover, the combination of the GPS with each CRF individually did not add more information than provided by the GPS alone, reinforcing the importance of genetic data to identify those subjects at increased risk for CHD.

The polymorphisms included in the GPS were chosen based on empirical evidence and results from GWAs analyses (5,10). *APOB* has been implicated in LDL-C concentrations and CHD risk in several prior studies (19,20). Particularly, a significant interaction between *APOB*_A618V variant and glucose tolerance on lipid-related parameters has been described (21). The common allelic variants of the *APOE* gene have been considered important genetic markers for dyslipidemia and CHD (20). After adjustment for traditional coronary risk factors and lipid concentrations, our group found higher CHD risk in subjects carrying the $\epsilon 4$ allele than in non-carriers (22). Moreover, a recent meta-analysis concurred that the $\epsilon 4$ allele was significantly related to increased CHD risk (19). *ApoC3* is a key component modulating lipoprotein metabolism and thereby, CHD risk. The *APOC3*_3238C>G SNP, primarily affects plasma TG concentrations and carriers of the minor allele appear to display higher risk of CHD (23). Likewise, *LPL* plays a pivotal role in TG metabolism and sequence variants within this gene leading to the D9N, N291S and S447X substitutions have been shown to affect lipid concentrations, as well as CHD risk (24). An increased risk of CHD has been demonstrated for heterozygous carriers of the D9N and N291 SNPs, whereas the S447X appears to confer protection against CHD (24,25). Nitric oxide (NO) from the endothelium is an important atheroprotective mediator (10,19). The location of the *NOS3*_i19342 SNP within a conservative LD block just 1.0 to 1.8 kbp downstream of the autophagy 9-like 2 (*APG9L2*) gene has been involved in vascular disease pathogenesis (26). Genetic variations of the genes encoding the inflammatory mediators *IL-6* (27) and *CXCL12* (28) have also been associated with CHD. The *IL6*_174C>G variant has been associated with increased susceptibility to cardiovascular disease (27). On the other hand, the

*CXCL12*_111738T>C SNP has been associated with coronary artery disease in a GWA study (5). Finally, the cluster *CDKN2A/B* has also been associated with CHD, stroke, and total mortality in several studies (5,28).

Importantly, the GPS reported in the current study included seven polymorphisms (APOC3_3238C>G, APOE_C130R, APOE_R176C, IL6_-174C>G, LPL_D9N, LPL_N291S, and LPL_S447X) previously associated with prediction of coronary infarct in a Greek population (10). In addition, three of them (APOE_C130R, APOE_R176C, and LPL_D9N) have also been used in another genetic score to predict CHD in a United Kingdom population (7). Therefore, the inclusion of those polymorphisms in the current GPS reinforces the pivotal role of those SNPs to discriminate CHD risk.

Interestingly, the GPS reported in this study may identify those subjects at increased risk for CHD over-and-above than CRFs. Consistent with our findings, another study demonstrated that the joint presence of a genetic score and CRFs might improve cardiovascular risk assessment above CRFs (11). However, the utility of genetic scores to predict CHD remains controversial. Two prior prospective cohort studies (7,12) demonstrated that a GPS predicted CHD risk above CRFs. However, Kathiresan *et al* (13) identified a genetic score based on lipid concentrations that did not improve cardiovascular risk prediction.

Although the cross-sectional design of this study does not allow us to predict CHD risk, the current genetic score may identify those individuals at increased risk for CHD risk much earlier, possibly before biomarkers of disease became highly elevated, offering more effective prevention of CHD. Based on reported findings, this set of SNPs may improve the ability to identify those participants who would obtain a disproportional benefit by controlling their CRFs in order to remain CHD-free. In clinical practice, risk factor assessment guides the therapeutic strategy (2,18,29). Therefore, conservative intervention could be recommended for subjects with low GPS whereas aggressive treatment and strict surveillance of CRFs for risk reduction may be recommended to those subjects with high GPS. In this regard, each person has a unique risk for developing CHD depending on his or her levels of risk factors in close interplay with both lifestyle and with his or her genetic predisposition.

One of the main limitations is the cross-sectional design of this study, by which the existence of CHD may influence the exposure to CRFs. In this regard, prospective cohort studies are more effective in providing information on risk prediction. Secondly, the prevalence of CHD was self-reported, and this may be subject to recall bias. However, several previous large-scale epidemiological studies in the general population have used self-reported CHD to assess its association with metabolic disturbances and cardiovascular disease (14, 30). Although we did not record lipid concentrations in those participants with prior CHD before starting lipid-lowering treatment, the average imputed lipid concentrations based on dose and length of the treatment, overcame this shortcoming. Another limitation was the choice of genotypes based on previous studies and empirical evidence, without testing other loci meeting these criteria. However, the blind evaluation of genotypes and genetic score development was a strength of this study. However, further replication of our findings in other ethnic populations is clearly warranted.

In conclusion, the present study demonstrated the clinical utility of a single genetic predisposition score to identify those individuals at an increased risk for CHD. Importantly, these genetic data may discriminate those participants at high CHD risk over-and-above than CRFs. Although we believe that genetic testing is not applicable to widespread clinical use, we are confident that, in the near future, the use of genetic data will lead to a better prevention and understanding of CHD compared to information provided by CRFs alone. Therefore, our findings have wide-ranging implications for health initiatives targeted at identifying those subjects at increased CHD risk before a coronary event occurs.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Characteristics of 197 study participants with prior coronary heart disease (CHD) and 524 CHD-free.

Variable	with CHD (n=197)	CHD-free (n=524)	P
Age, years	60±7.5	56±7.1	<0.001
Men/women, n	60/137	148/376	0.580
Body mass index, kg/m ²	33.6±8.1	30.8±6.2	<0.001
Total cholesterol, mmol/L	5.40±1.41	4.99±1.00	<0.001
LDL cholesterol, mmol/L	3.17±1.16	3.01±0.84	0.084
HDL cholesterol, mmol/L	1.09±0.34	1.19±0.33	0.001
Triglycerides, mmol/L	1.70±0.01	1.51±0.01	0.010
Ever smokers, n (%)	120 (61)	279 (54)	0.091
Current heavy drinkers, n (%)	55 (28)	153 (29)	0.712
Physical activity score	30.41±3.93	32.23±4.98	<0.001
Type 2 diabetes, n (%)	115 (58)	0	<0.001
Hypertension, n (%)	161 (82)	246 (47)	<0.001
On diabetes treatment, n (%)	97 (50)	0	<0.001
On lipid lowering treatment, n (%)	122 (63)	115 (22)	<0.001
On hypertension treatment, n (%)	159 (81)	187 (36)	<0.001

Values are mean±SD.

Table 2

Genotypes and presumed high-risk allele frequencies among participants with coronary heart disease (CHD) and CHD-free.

	Genotypes				P value for trend	Presumed high-risk allele frequency
	n (%)	n (%)	n (%)	n (%)		
<i>APOB</i> _A618V	GG	GA	AA			A
With CHD	93 (47)	77 (39)	27 (14)		0.020	0.332
Controls	295 (56)	188 (36)	41 (8)			0.258
<i>APOC3</i> _3238C>G	CC	CG	GG		0.441	G
With CHD	99 (50)	49 (25)	49 (25)			0.373
Controls	268 (51)	147 (28)	109 (21)			0.348
<i>APOE</i>	ϵ 2/2+ ϵ 2/3	ϵ 3/3	ϵ 4+		0.071	ϵ 4
With CHD	14 (7)	127 (65)	194 (37)			0.769
Controls	41 (8)	289 (55)	56 (28)			0.519
<i>CDKN2A/B</i> _116191G>C	GG	CG	CC		0.084	C
With CHD	53 (27)	94 (48)	50 (25)			0.388
Controls	156 (30)	274 (52)	94 (18)			0.441
<i>CXCL12</i> _111738T>C	CC	CT	TT		0.009	T
With CHD	7 (4)	61 (31)	129 (66)			0.810
controls	44 (8)	195 (37)	285 (54)			0.730
<i>IL6</i> _174C>G	GG	GC	CC		0.087	C
With CHD	12 (6)	70 (36)	115 (58)			0.761
Controls	24 (5)	148 (28)	352 (67)			0.813
<i>LPL</i> _D9N	DD	DN	NN		0.560	N
With CHD	184 (93)	13 (7)	0			0.033
Controls	494 (94)	28 (5)	2 (0.4)			0.031
<i>LPL</i> _N291S	NN	NS	SS		0.371	S
With CHD	190 (96)	7 (4)	0			0.018
Controls	511 (98)	11 (2)	2 (0.4)			0.014
<i>LPL</i> _S447X	SS	SX	XX		0.713	S
With CHD	158 (80)	38 (19)	1 (0.5)			0.898

	Genotypes			P value for trend	Presumed high-risk allele frequency
	n (%)	n (%)	n (%)		
Controls	428 (82)	91 (17)	5 (1)		0.904
NOS3_i19342A>G	GG	GA	AA	0.145	A
With CHD	8 (4)	60 (31)	129 (66)		0.807
controls	25 (5)	198 (38)	301 (57)		0.763

Table 3

Distribution of study participants depending on presence of coronary heart disease and score in the genetic predisposition scale (GPS).

GPS	with CHD n (%)	CHD-free n (%)
0.0–1.5	0	0
2	0	1 (0.2)
2.5	2 (1.0)	0
3	3 (1.5)	10 (1.9)
3.5	13 (6.6)	35 (6.7)
4	19 (9.6)	65 (12.4)
4.5	30 (15.2)	109 (20.8)
5	37 (18.8)	125 (23.9)
5.5	39 (19.8)	86 (16.4)
6	27 (13.7)	50 (9.5)
6.5	22 (11.2)	26 (5.0)
7	3 (1.5)	14 (2.7)
7.5	2 (1)	3 (0.6)
8.0–11.0	0	0
Total	197 (100.0)	524 (100.0)

Table 4

OR for coronary heart disease in relation to the joint presence of a high (>5) or low GPS (≤5) and non-genetic CHD risk factors.

GPS	CHD risk factor	With CHD, n	CHD-free, n	OR (95%CI)	P value*	OR (95%CI)	P value†
5	never smoker	42	169	0.22(0.10–0.50)	<0.001	0.21(0.09–0.48)	<0.001
	ever smoker	62	175	0.43(0.21–0.87)	0.019	0.44(0.21–0.89)	0.023
	never smoker	35	76	0.91(0.43–1.89)	0.792	0.83(0.39–1.78)	0.632
	ever smoker	58	104	1(Reference)		1(Reference)	
5	non-heavy drinkers	73	242	0.09(0.03–0.33)	<0.001	0.10(0.03–0.34)	<0.001
	heavy drinkers	30	103	0.40(0.22–0.72)	0.002	0.43(0.24–0.79)	0.006
	non-heavy drinkers	69	129	0.75(0.32–1.74)	0.500	0.83(0.35–2.00)	0.681
	heavy drinkers	25	50	1(Reference)		1(Reference)	
5	physical activity score ≥31	31	178	0.36(0.14–0.95)	0.039	0.30(0.12–0.77)	0.012
	physical activity score <31	73	167	0.37(0.18–0.76)	0.007	0.35(0.17–0.71)	0.004
	physical activity score ≥31	33	96	1.13(0.44–2.92)	0.801	0.95(0.38–2.36)	0.911
	physical activity score <31	60	83	1(Reference)		1(Reference)	
5	no hypertension	14	185	0.09(0.03–0.25)	<0.001	0.09(0.03–0.27)	<0.001
	presence hypertension	90	160	0.49(0.27–0.90)	0.021	0.52(0.28–0.96)	0.037
	no hypertension	22	93	0.58(0.28–1.22)	0.151	0.64(0.30–1.36)	0.242
	presence hypertension	71	86	1(Reference)		1(Reference)	
5	no dyslipidemia	39	268	0.19(0.09–0.40)	<0.001	0.18(0.08–0.40)	<0.001
	presence dyslipidemia	65	76	0.34(0.15–0.80)	0.013	0.34(0.14–0.81)	0.014
	no dyslipidemia	36	40	0.50(0.24–1.07)	0.074	0.46(0.21–1.02)	0.057
	presence dyslipidemia	57	40	1(Reference)		1(Reference)	

* Adjusted for age, sex, body mass index, physical activity, smoking habit, alcohol consumption, diabetes, and prior medications.

† Further adjustment for population admixture.

Table 5

OR for coronary heart disease in relation to the joint presence of a high (>5) or low GPS (<5) and lipids.

GPS	CHD risk factor	With CHD, n	CHD-free, n	OR (95%CI)	P value*	OR (95%CI)	P value†
5	Total cholesterol<5.20 mmol/L	59	179	0.51(0.24–1.10)	0.086	0.49(0.23–1.06)	0.068
	Total cholesterol ≥5.20 mmol/L	45	166	0.43(0.20–0.92)	0.029	0.41(0.19–0.87)	0.021
	Total cholesterol<5.20 mmol/L	56	87	1.89(0.89–4.01)	0.097	1.59(0.73–3.43)	0.241
	Total cholesterol ≥5.20 mmol/L	37	92	1(Reference)		1(Reference)	
5	LDL cholesterol<2.60 mmol/L	33	123	0.23(0.09–0.56)	0.001	0.24(0.10–0.59)	0.002
	LDL cholesterol ≥2.60 mmol/L	72	220	0.47(0.25–0.87)	0.017	0.47(0.25–0.89)	0.021
	LDL cholesterol<2.60 mmol/L	35	50	1.15(0.50–2.62)	0.745	1.06(0.45–2.49)	0.902
	LDL cholesterol ≥2.60 mmol/L	57	131	1(Reference)		1(Reference)	
5	HDL cholesterol 1.04 mmol/L	50	116	0.17(0.07–0.43)	<0.001	0.18(0.07–0.45)	<0.001
	HDL cholesterol<1.04 mmol/L	54	229	0.39(0.21–0.72)	0.003	0.40(0.21–0.76)	0.005
	HDL cholesterol 1.04 mmol/L	42	65	0.69(0.32–1.52)	0.362	0.69(0.31–1.57)	0.381
	HDL cholesterol<1.04 mmol/L	51	114	1(Reference)		1(Reference)	
5	Triglycerides<1.65 mmol/L	56	207	0.63(0.30–1.31)	0.212	0.57(0.27–1.20)	0.140
	Triglycerides ≥1.65 mmol/L	48	138	0.19(0.07–0.50)	0.001	0.18(0.07–0.47)	0.001
	Triglycerides<1.65 mmol/L	42	104	1.38(0.66–2.91)	0.394	1.15(0.54–2.45)	0.720
	Triglycerides ≥1.65 mmol/L	51	75	1(Reference)		1(Reference)	

* Adjusted for age, sex, body mass index, physical activity, smoking habit, alcohol consumption, diabetes, and prior medications.

† Further adjustment for population admixture.