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Genetic predisposition to coronary heart disease and stroke using an additive genetic risk score: A population-based study in Greece

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Abstract

Objective—To determine the extent to which the risk for incident coronary heart disease (CHD) increases in relation to a genetic risk score (GRS) that additively integrates the influence of high-risk alleles in nine documented single nucleotide polymorphisms (SNPs) for CHD, and to examine whether this GRS also predicts incident stroke.

Methods—Genotypes at nine CHD-relevant SNPs were determined in 494 cases of incident CHD, 320 cases of incident stroke and 1345 unaffected controls drawn from the population-based Greek component of the European Prospective Investigation into Cancer and nutrition (EPIC) cohort. An additive GRS was calculated for each study participant by adding one unit for the presence of each high-risk allele multiplied by the estimated effect size of that allele in the discovery samples. Statistical analysis was performed using logistic regression.

Results—The GRS was significantly associated with the incidence of CHD where the odds of CHD incidence in the highest quintile of the GRS were 1.74 times higher (95% confidence interval [CI] = 1.25–2.43, p for trend = 0.0004), compared to the lowest quintile. With respect to stroke, a weaker and non-significant positive association with GRS was apparent as the odds of stroke incidence in the highest quintile of the GRS were 1.36 times higher (95% CI = 0.90–2.06, p for trend = 0.188), compared to the lowest quintile.

Conclusion—A GRS relying on nine documented “CHD-specific” SNPs is significantly predictive of CHD but it was not found to be statistically significantly associated with incident stroke.

Keywords

Genetic risk score; Coronary heart disease; Stroke; Myocardial infarction; Cardiovascular disease; Greek-EPIC

1. Introduction

Coronary heart disease (CHD) and stroke are the two major manifestations of atherosclerotic processes. Although risk factors, notably high low-density lipoprotein cholesterol, tobacco smoking, hypertension and diabetes, play a role in the pathogenesis of both disease entities, the strengths of the respective associations differ, indicating that the two diseases are partly distinct [1,2]. Genome-wide association studies (GWAS) of CHD [3–10] and stroke [11–13] also have identified different chromosomal loci as underlying genetic predisposition to these diseases, although at least for the chromosome 9p21.3 locus significant associations have been observed with both CHD and stroke [14,15].

Despite the discovery of several candidate genes, primarily through GWAS, the optimal and clinically relevant sets of risk genotypes have yet to be identified. In addition, although most genetic variants identified have shown modest effects on cardiovascular risk, an aggregate of many variants combined to a score might substantially influence risk of CHD [16,17]. To date, few studies have examined the utility of genetic risk scores to identify subjects at increased CHD risk [16,18–21]. Importantly, those studies have not examined the association of these genetic scores with stroke risk.

We have sought to examine whether, and to what extent, integrated genetic predisposition to CHD, as captured through a CHD-specific genetic risk score (GRS), also accounts for any extent of genetic predisposition to stroke. We have used resources generated in the Greek-EPIC cohort in which medically documented incident cases of CHD and stroke are recorded during an extended follow-up of this population-based cohort.

2. Methods

2.1. Study population

The European Prospective Investigation into Cancer and nutrition (EPIC) is a cohort study conducted in 23 research centers in 10 European countries, with the purpose of investigating the role of biologic, dietary, lifestyle, and environmental factors in the etiology of cancer and other chronic diseases. Enrollment of participants in the Greek component of EPIC occurred between 1994 and 1999. A total of 28572 participants aged 20–86 years were recruited from all regions of Greece. All procedures were in accord with the Helsinki Declaration and all participants provided written informed consent. The study protocol was approved by the ethics committees of the International Agency for Research on Cancer and the Medical School of the University of Athens.

By June 2008, 742 of the study participants, diagnosed with a medically confirmed incident CHD or stroke event, had entered the study. For each case, an attempt was made to choose two control subjects matched for sex, age (± 2 years), and date of recruitment (± 6 months). All subjects (cases and controls) were free of CHD and stroke at baseline and controls must have not developed CHD or stroke by the date of the diagnosis of the disease in the corresponding case (incidence density sampling). For each study participant, a buffy coat

sample was drawn from the Greek-EPIC bio-repository, where biological specimens are preserved under liquid nitrogen since collection. However, until December 2009, a number of the control subjects had developed cardiovascular disease and therefore the statistical analysis was run with a total of 788 cases (494 CHD, 320 stroke, 26 both diseases) and 1345 controls. Cases were confirmed using standardized procedures through hospital discharge data, in patient medical records or death certificates if they met the criteria of the Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (MONICA) Project [22] and/or if substantiated by the treating physician diagnosis [23]. The International Statistical Classification of Diseases and Health-Related Problems (Tenth Revision) [24] was used to classify incident events of CHD (I20-I25, I46, Z95.1, Z95.5, Z95.8 and Z95.9) and stroke (I60-I69, G45 and G46).

2.2. SNP selection and genotyping

For the present study, we used nine previously reported SNPs associated with myocardial infarction (MI) or CHD from GWAS with convincing replication evidence in populations with European ancestry [4,8,20,25,26], including rs11206510 at 1p32 near *PCSK9*, rs646776 at 1p13 near *CELSR2-PSRC1-SORT1*, rs17465637 at 1q41 in *MIA3*, rs6725887 at 2q33 in *WDR12*, rs9349379 at 6p24 in *PHACTR1*, rs1746048 at 10q11 near *CXCL12*, rs1122608 at 19p13 near *LDLR*, rs9982601 at 21q22 near *SLC5A3-MRPS6-KCNE2*, and the lead variant (rs1333049) at locus 9p21 near *CDKN2A/2B* identified by the Wellcome Trust Case Control Consortium [5].

Genomic DNA was extracted from the buffy coat fraction of centrifuged blood, collected at enrollment and preserved at -196°C , with the NucleoSpin Blood Kit (Macherey-Nagel GmbH & Co. KG, Germany) according to the vendor's recommended protocol. Genotyping was performed blindly as to case-control status with the TaqMan allelic discrimination system on the ABI 7900HT platform using custom genotyping assays and probes designed by Applied Biosystems, Inc (Foster City, CA). Replicate quality control samples yielded 100% concordance and call rates exceeded 98%. All genotypes were analysed in the Nutrition and Genomics Laboratory, Jean Mayer US Department of Agriculture, Human Nutrition Research Center on Aging at Tufts University, Boston, Massachusetts, USA.

2.3. Calculation of genetic risk score

Using previously described methods to construct genetic risk scores (GRS) for CHD [16,20,21], we created a simple a priori GRS per participant by summing the number of risk alleles (0, 1 or 2) at each locus (resulting in a possible score ranging from 0 to 18). In additional analyses, we also calculated a weighted GRS by multiplying the number of risk alleles at each locus by their estimated effect sizes in the discovery samples (SNP specific weights are shown in Table 2). The determination of risk alleles in this study was based on the literature which first reported the SNP. We assumed that each SNP was independently associated with the risk of CHD according to an additive genetic model, which performs well even when the true genetic model may not be known or may be incorrectly specified [27]. Placing the proteins encoded by genes near these nine loci into a network based on known protein-protein interactions produces a network with very weak connectivity, underscoring a lack of interdependence from a functional standpoint. Furthermore, there is no evidence in published reports of gene-gene interactions involving any pair from these nine loci.

2.4. Statistical Analysis

A chi-square test was used to assess whether the SNPs were in Hardy-Weinberg equilibrium and to determine differences in genotype frequencies between CHD or stroke cases and controls. We tested whether CHD and stroke incidence is related to: (a) each SNP separately

and (b) the unweighted and weighted GRS divided into control-based quintiles, using unconditional logistic regression analysis adjusting for age and sex. Because each of the nine reported SNPs previously has been associated with CHD at significance levels exceeding a stringent genome-wide threshold, in this report we regarded an association to be significant if a two-sided p -value was less than 0.05 (for the same risk allele in the same direction as in the original report). Statistical analyses were performed using the Stata/SE 11.0 for Windows statistical package (Stata Corp LP Lakeway Drive College Station, Texas, USA). The Power Analysis and Sample Size software (PASS, version 11) was used for power calculations for the detected odds ratios (OR) from the logistic regressions.

3. Results

Four-hundred and ninety-four cases of incident CHD (345 men and 149 women) and 320 incident cases of stroke (166 men and 154 women) contributed to this study. For these cases of incident cardiovascular diseases a total of 1345 control participants were chosen, matched for sex and age. However, because matching was not always successful, all analyses were adjusted for age and sex. Table 1 shows distribution of incident CHD and stroke cases and controls by age at enrolment and sex.

Table 2 shows the association between SNPs chosen on the basis of their documented association with CHD in relation to incident CHD and stroke in the Greek-EPIC cohort. For all polymorphisms, genotype distribution in controls was compatible with Hardy-Weinberg proportions. With respect to CHD, only two associations were significant (for rs1333049 at 9p21 near *CDKN2A-CDKN2B* and for rs6725887 at 2p33 in *WDR12*), whereas the associations for another six were in the expected direction but statistically not significant. With respect to stroke, associations were not significant except for rs9982601 at 21q22 for which an inverse association was noted, possibly due to chance.

The association of the genetic risk score (GRS) with incident CHD and stroke in the study sample is indicated in Table 3. In this study, the minimum and maximum weighted GRS values were, respectively, 4.62 and 17.72 in control subjects, 5.74 and 18.82 in CHD cases, and 7.05 and 17.67 in stroke cases. With respect to CHD, there was a monotonic increase in OR's with increasing GRS value, reaching a statistically significant 74% and 87% increase of the odds of CHD incidence in the top quintile of the weighted and unweighted GRS, respectively, compared to the first quintile. The p -value for the linear trend across quintiles of the weighted GRS was 0.0004 (Table 3). The OR for incident CHD per one standard deviation increment in the weighted GRS was 1.21 (95% CI = 1.09 to 1.35, $p = 0.0004$). We had 95% power to detect an OR of 1.21 for incident CHD at a 5% level of significance. With respect to incident stroke, there was weak and statistically not significant evidence that participants in the first quintile of the weighted GRS values were at lower risk in comparison to that of participants in the four higher quintiles (for score 10.57 or higher vs. lower, OR = 1.35, 95% CI = 0.97–1.88, $p = 0.076$), but further increases in score values were not associated in a dose response pattern with increasing stroke risk (overall p for trend = 0.188). The OR for incident stroke per 1 standard deviation increment in the weighted GRS was 1.06 (95% CI = 0.93–1.20, $p = 0.365$; 15% power at a 5% level of significance to detect OR of 1.06). The pattern of results was not different when the change in OR for CHD and stroke across a wider range of the weighted GRS values was evaluated (Supplementary Table 1).

4. Discussion

In a case-control study of incident CHD and incident stroke nested within the population-based Greek-EPIC cohort we evaluated the associations of these diseases with nine SNPs documented as associated with CHD in large GWAS, as well as with a composite genetic

risk score (GRS) integrating the additive impact of the high-risk alleles. The GRS was significantly associated with CHD risk (p -value for trend = 0.0004) but not with incident stroke (p = 0.188).

In the past few years, GWAS in populations of European ancestry have led to the identification of at least 13 chromosomal loci at which common variants modestly but reproducibly influence the risk of MI or CHD [3,5–10], the majority of which modulate disease risk via hitherto unknown mechanisms [28,29]. The Coronary Artery Disease Genome wide Replication and Meta-analysis (CARDIoGRAM) consortium [26] in a recent meta-analysis of 14 GWAS comprising more than 22,000 cases of coronary artery disease (CAD) and over 64,000 controls of European descent, with follow up genotyping of top association signals in 56682 additional individuals, confirmed the association of 10 of 12 previously reported CAD loci, including those investigated here, and identified 13 new susceptibility loci [4]. That work thus expanded the number of independent loci showing genome-wide significant associations with coronary disease beyond 25. In the present study, we were able to replicate in the Greek population associations with incident CHD of two of the nine previously documented CAD loci (rs1333049 at 9p21 near *CDKN2A-CDKN2B* and rs6725887 at 2p33 in *WDR12*), whereas the associations for another six were in the expected direction but statistically not significant, probably on account of statistical power limitations. In addition to CHD risk, some chromosomal loci appear to affect several other seemingly unrelated disease phenotypes [28]. For example, the risk allele on chromosome 9p21.3, with the strongest and most replicated effect on the risk of MI known today [25,26,30], appears to also increase the susceptibility of stroke, as well as peripheral arterial disease and also to induce aneurysmal disease of the aorta and cerebral vessels [14,31,32]. However, no association of 9p21.3 with incident stroke was observed in our study, although the power to detect a significant association with stroke may be limited due to the relatively smaller sample size of stroke cases.

To combine the relatively small effects of individual genes and to better capture the complex relationship between genetics and CHD, genotypes at multiple SNPs are often combined into scores calculated according to the number of risk alleles carried [16,17]. Kathiresan et al. [18] used such an approach to predict the risk of cardiovascular events on the basis of nine SNPs associated with cholesterol levels. That score was strongly associated with the risk of cardiovascular disease even after adjustment for conventional risk factors, including family history, but it did not improve cardiovascular risk discrimination beyond conventional risk factors. Paynter et al. [19] used literature-based genetic risk scores in an attempt to predict cardiovascular risk in a prospective cohort of more than 19,000 white women, using either a large set of 101 SNPs reported to be associated with cardiovascular disease risk or intermediate phenotypes, or a panel of 12 SNPs robustly associated with cardiovascular disease risk. They showed that both genetic risk scores were associated with increased risk of total cardiovascular disease but did not evaluate association with stroke alone.

Using samples independent from the discovery samples, Ripatti et al. [20] showed that a GRS based on 13 SNPs from GWAS for MI or CHD, including the nine SNPs used in this investigation, was associated with risk of CHD, and that the upper quintile of individuals of European ancestry who carried the most risk alleles had a roughly 1.7-times increased risk of CHD when compared with those in the lowest quintile of GRS. Furthermore, the GRS improved risk reclassification in participants who were at intermediate risk on the basis of traditional risk factors. Interestingly, in a recent study among 4022 middle-aged subjects from the general Swedish population the same GRS was independently associated with carotid bulb intima-media thickness and carotid plaques, providing evidence of an association with early markers of atherosclerosis [33]. Schunkert et al., relying on a

weighted score based on 23 CAD risk variant studied in the CARDIoGRAM investigation, observed a threefold difference in CAD risk between the top and bottom 10% of the risk scores [4]. In the present study, we have found, as expected, that an additive GRS integrating the risk-imparting effects on CHD of nine SNPs with documented influence was significantly associated with incident CHD ($p = 0.0004$) notwithstanding the modest size of the investigation.

Strengths of this investigation are reliance on a population-based sample in a small country with little concern of possible population stratification and the use of SNPs with documented association with CHD. The main limitation of this study stems from the modest numbers of incident CHD and stroke cases even though the study was nested in a fairly large cohort of 28,572 people followed for approximately ten years. In addition, due to insufficient availability of data on important conventional risk factors of CHD (i.e. blood cholesterol levels) we were not able to examine at this stage if the GRS improves cardiovascular risk prediction over and above that of traditional CHD risk factors. Moreover, several important risk factors for cardiovascular diseases, including blood cholesterol, hypertension and diabetes, are under strong genetic influences and as such should be considered as intermediates rather than confounders and do not have to be controlled in the analysis [34]. This analysis was also limited by a lack of distinction between ischemic and hemorrhagic stroke, which did not allow examining whether the “CHD-specific” risk alleles were more relevant to ischemic than hemorrhagic stroke. Finally, it would have been better a full cohort analysis with direct calculation of hazard ratios, but we could not undertake this analysis because we did not have information on genotypes on the nine SNPs for the total Greek-EPIC cohort of more than 28,000 participants.

In conclusion, we have attempted to replicate in a Greek population associations with CHD of nine previously documented SNPs from GWAS and the results were largely in the expected direction. Moreover, we have shown that a composite genetic risk score integrating the additive impact of high-risk alleles reveals that the coexistence of these alleles may convey a substantial increase of CHD risk by about 70%.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CHD	coronary heart disease
CAD	coronary artery disease
MI	myocardial infarction
GRS	genetic risk score
OR	odds ratio
CI	confidence interval
SNP	single nucleotide polymorphism

<i>MIA3</i>	melanoma inhibitory activity family, member 3
<i>PCSK9</i>	proprotein convertase subtilisin/kexin type 9
<i>CELSR2</i>	cadherin EGF LAG seven-pass G-type receptor 2
<i>PSRC1</i>	proline/serine-rich coiled-coil 1
<i>SORT1</i>	sortilin 1
<i>WDR12</i>	WD repeat domain 12
<i>PHACTR1</i>	phosphatase and actin regulator 1
<i>CDKN2A/2B</i>	cyclin-dependent kinase inhibitor 2A/2B
<i>CXCL12</i>	chemokine (C-X-C motif) ligand 12
<i>LDLR</i>	low density lipoprotein receptor
<i>SLC5A3</i>	solute carrier family 5 (sodium/myo-inositol cotransporter), member 3
<i>MRPS6</i>	mitochondrial ribosomal protein S6
<i>KCNE2</i>	potassium voltage-gated channel, Isk-related family, member 2
<i>EPIC</i>	European Investigation into Cancer and nutrition
<i>CARDIoGRAM</i>	coronary artery disease genome wide replication and meta-analysis consortium

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.atherosclerosis.2012.02.033.

Table 1

Distribution of incident CHD and stroke cases and controls by age at enrollment and sex in the Greek-EPIC cohort.

Age (years)	CHD (n = 494)		Stroke (n = 320)		Controls (n = 1345)	
	Men	Women	Men	Women	Men	Women
<50	80 (23.2)	5 (3.4)	15 (9.0)	8 (5.2)	196 (23.3)	27 (5.3)
50–59	72 (20.9)	23 (15.4)	17 (10.2)	15 (9.7)	163 (19.4)	71 (14.1)
60–69	128 (37.1)	78 (52.3)	67 (40.4)	80 (52.0)	321 (38.2)	265 (52.6)
70	165 (18.8)	43 (28.9)	67 (40.4)	51 (33.1)	161 (19.1)	141 (28.0)
Total	345 (100.0)	149 (100.0)	166 (100.0)	154 (100.0)	841 (100.0)	504 (100.0)
Mean (SD)	59.8 (11.5)	66.0 (7.2)	66.3 (9.2)	66.6 (8.0)	60.1 (11.2)	65.6 (7.6)
Median	62.2	67.3	68.8	68.0	62.6	66.6

Values are numbers (percentages). Abbreviations: CHD = coronary heart disease; EPIC = European prospective investigation into cancer and nutrition.

Table 2

Association between SNPs and incident CHD and stroke in the Greek-EPIC cohort*.

SNP	Locus	Gene(s) in region	Weight**	Alleles risk/other	High-risk allele frequency in controls (n = 1345)	CHD (n = 494)		Stroke (n = 320)	
						OR (95% CI)	p-value	OR (95% CI)	p-value
rs11206510	1p32	<i>PCSK9</i>	1.15	T/C	0.79	1.02 (0.85–1.22)	0.836	1.03 (0.83–1.28)	0.799
rs646776	1p13	<i>CELSR2-PSRC1-SORT1</i>	1.19	T/C	0.81	1.10 (0.91–1.33)	0.388	1.16 (0.92–1.47)	0.258
rs17465637	1q41	<i>MIA3</i>	1.14	C/A	0.71	1.09 (0.93–1.29)	0.290	1.13 (0.93–1.38)	0.228
rs6725887	2q33	<i>WDR12</i>	1.17	C/T	0.14	1.26 (1.04–1.54)	0.021	1.00 (0.78–1.28)	0.979
rs12526453	6p24	<i>PHACTR1</i>	1.12	C/G	0.61	1.09 (0.93–1.26)	0.287	1.05 (0.88–1.26)	0.570
rs1333049	9p21	<i>CDKN2A-CDKN2B</i>	1.29	C/G	0.51	1.30 (1.12–1.51)	0.001	1.05 (0.88–1.26)	0.575
rs1746048	10q11	<i>CXCL12</i>	1.17	C/T	0.82	0.95 (0.79–1.15)	0.613	0.89 (0.71–1.11)	0.312
rs1122608	19p13	<i>LDLR</i>	1.15	G/T	0.72	1.05 (0.89–1.24)	0.558	1.12 (0.92–1.36)	0.273
rs9982601	21q22	<i>SLC5A3-MRPS6-KCNE2</i>	1.20	T/C	0.12	1.15 (0.92–1.42)	0.213	0.71 (0.52–0.96)	0.026

Abbreviations: SNP = single nucleotide polymorphism; OR = odds ratio parallel; CI = confidence interval; CHD = coronary heart disease; EPIC = European prospective investigation into cancer and nutrition.

* Association tested with unconditional logistic regression adjusted for age and sex.

** SNP specific weights used for genetic risk score calculation; weights are effect sizes for the SNPs from Ref. [20] except for rs1333049 at 9p21 for which Ref. [26] was used.

Table 3

Association of genetic risk score (GRS) in quintiles with incident CHD and stroke in the Greek-EPIC cohort^{*}.

	GRS quintile					<i>p</i> -value for trend
	1st (ref)	2nd	3rd	4th	5th	
Weighted GRS values	<10.57	10.57–11.79	11.80–12.93	12.94–14.13	>14.13	
Cases/Controls (<i>n</i>)	77/273	88/280	98/258	108/281	123/253	
OR (95%CI)	1.00	1.10 (0.78–1.57)	1.36 (0.96–1.92)	1.37 (0.98–1.92)	1.74 (1.25–2.43)	0.0004
			CHD			
Cases/Controls (<i>n</i>)	51/273	71/280	63/258	73/281	62/253	
OR (95% CI)	1.00	1.37 (0.91–2.05)	1.28 (0.85–1.93)	1.39 (0.93–2.08)	1.36 (0.90–2.06)	0.188
Unweighted GRS values	9	10	11	12	13	
			CHD			
Cases/Controls, (<i>n</i>)	101/371	105/282	115/296	91/232	82/164	
OR (95% CI)	1.00	1.37 (1.00–1.88)	1.44 (1.06–1.96)	1.46 (1.05–2.03)	1.87 (1.32–2.64)	0.001
			Stroke			
Cases/Controls, (<i>n</i>)	84/371	64/282	79/296	52/232	41/164	
OR (95% CI)	1.00	1.01 (0.70–1.45)	1.16 (0.82–1.64)	0.97 (0.66–1.44)	1.16 (0.76–1.78)	0.576

Abbreviations: OR = odds ratio; CI = confidence interval; CHD = coronary heart disease; EPIC =European prospective investigation into cancer and nutrition.

^{*} Association tested with unconditional logistic regression adjusted for age and sex.