BMJ open

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Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-004387
Article Type:	Research
Date Submitted by the Author:	03-Nov-2013
Complete List of Authors:	Yiannakouris, N.; Hellenic Health Foundation, ; Harokopio University, Nutrition and Dietetics Katsoulis, Michail; Hellenic Health Foundation, Trichopoulou, Antonia; Hellenic Health Foundation, Ordovas, Jose; Human Nutrition Research Center on Aging (HNRCA) at Tufts University, Nutrition and Genomics Laboratory, Jean Mayer–US Department of Agriculture Trichopoulos, Dimitrios; Harvard School of Public Health, Department of Epidemiology
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Epidemiology, Genetics and genomics
Keywords:	Coronary heart disease < CARDIOLOGY, EPIDEMIOLOGY, GENETICS

Research report

Additive influence of genetic predisposition and conventional risk factors in the incidence of coronary heart disease: a population-based study in Greece

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Key words: genetic risk score; risk factors; coronary heart disease; gene-environment interaction; relative excess risk

Running title: Genetic and other risk factors in coronary heart disease

Abstract word count: 251

Text word count: 2782

Number of Tables: 4

ABSTRACT

Background and Objectives: An additive genetic risk score (GRS) for coronary heart disease (CHD) has previously been associated with incident CHD in the population-based Greek European Prospective Investigation into Cancer and nutrition (EPIC) cohort. In this study we explore GRS-"environment" joint actions on CHD for several conventional cardiovascular risk factors (ConvRFs), including smoking, hypertension, body mass index (BMI), physical activity and adherence to the Mediterranean diet.

Design: A case-control study.

Setting: The general Greek population of the EPIC study.

Participants and Outcome measures: Subjects were 477 patients with medically confirmed incident CHD and 1271 controls. We estimated the odds ratios for CHD by dividing participants at higher or lower GRS and, alternatively, at higher or lower ConvRF, and calculated the relative excess risk due to interaction (RERI) as a measure of deviation from additivity.

Results: The joint presence of higher GRS and higher-risk ConvRF was in all instances associated with an increased risk of CHD, compared to the joint presence of lower GRS and lower-risk ConvRF. The odds ratio (95% confidence interval) was 1.7 (1.2-2.4) for smoking, 2.7 (1.9-3.8) for hypertension, 1.9 (1.4-2.5) for lower physical activity, 2.0 (1.3-3.2) for high BMI and 1.5 (1.1-2.1) for poor adherence to the Mediterranean diet. In all instances RERI values were fairly small and not statistically significant suggesting that the GRS and the ConvRFs do not have effects beyond additivity.

Conclusion: Genetic predisposition to CHD, operationalised through a multi-locus genetic risk score, and conventional cardiovascular risk factors have essentially additive effects on CHD risk.

ARTICLE SUMMARY

Strengths and limitations of this study

- Strengths of the study are the population based prospective cohort design of the underlying study and the minimal concern for population stratification

- The main limitation of this study stems from the modest numbers of incident CHD cases, not withstanding the fact that the underlying cohort was large and was followed for approximately ten years

INTRODUCTION

Coronary heart disease (CHD) is a leading cause of death and disability worldwide.[1] Lifestyle and environmental factors, such as cigarette smoking, physical inactivity, chronodisruption and unhealthy diets, play a significant role in its development and are largely responsible for increased risk of this disease.[2, 3] In addition, compelling evidence from the literature suggest a genetic basis for CHD [4] so that genetic data may identify individuals who have an inherited predisposition to develop CHD.

During the past few years, genome-wide association studies (GWAS) have successfully identified a large number of chromosomal loci and genetic variants that are robustly associated with CHD,[5-11] although their effects on risk are generally fairly small. To combine the relatively small effects of individual genes and to better capture the complex relationship between genetics and CHD, genotypes at multiple genetic variants have previously been combined into scores calculated according to the number of risk alleles carried.[12, 13] To date, several studies have examined the utility of different genetic risk scores to identify subjects at increased CHD risk.[14-18] Ripatti et al.[16] reported that a genetic risk score (GRS) based on a series of genetic variants from GWAS for myocardial infarction or CHD 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. Using a similar approach, we have shown that a GRS based on nine documented genetic variants from GWAS is associated with incident CHD in the population-based Greek European Prospective Investigation into Cancer and nutrition (EPIC) cohort.[19]

Despite the success of GWAS in identifying novel genetic contributors to CHD, the heritability of common disorders cannot be adequately explained by the genes that have been discovered; moreover, for the most part, we don't know how these recently discovered loci interact with the environment and what role such interactions play in the development of the disease.[20, 21] Testing such interactions is thus a new frontier for large scale GWAS of CHD [22] and some initial findings support the important role of environmental exposures in influencing the magnitude of the genetic associations with cardiovascular disease [23] or other common diseases and traits.[24, 25]

The aim of the current study was to explore potential GRS-"environment" interaction effects on CHD for several important conventional cardiovascular risk factors, including smoking, hypertension, body mass index (BMI), physical activity and adherence to the Mediterranean diet (MedDiet). We have used resources generated in the Greek-EPIC cohort in which medically documented incident cases of CHD [26] are recorded during an extended follow-up of this population-based cohort.

METHODS

Study population

The European Prospective Investigation into Cancer and nutrition (EPIC) is a longitudinal study aimed at investigating the role of biologic, nutritional, lifestyle, and environmental factors in the etiology of cancer and other chronic diseases. The study has been described in detail elsewhere.[27, 28] The recruitment of Greek-EPIC participants was from 1994 to 1999. The active follow-up of study participants is repeated every two to four years. In each round, the focus of follow-up is on the update of information related to health status of the participants. For this analysis, exposure data at enrolment and follow-up data until the end of 2009 for outcomes are considered.

By December 2009, 788 subjects were diagnosed with an incident, medically confirmed, CHD or stroke event and were considered eligible for a study also evaluating genetic predisposition.[19] For each case, an attempt was made to choose two control subjects matched for sex, age (±2 years), and date of recruitment (±6 months). Both cases and controls were free of CHD and stroke at baseline; the final study sample consisted of 788 cases (494 CHD, 320 stroke, 26 both diseases) and 1345 controls. For each study participant, a buffy coat sample was drawn from the Greek-EPIC bio-repository and genomic DNA was extracted. CHD events included myocardial infarction, angina and other ischemic heart disease (cardiac arrest, presence of cardiac and vascular implants and grafts), with several cases following in more than one categories.[26,28] All procedures were in accordance 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.

Selection of genetic variants, genotyping and genetic risk score calculation

We constructed a multi-locus genetic risk score (GRS) by using nine previously reported genetic variants associated with myocardial infarction or CHD from GWAS, with convincing replication evidence in populations with European ancestry,[6, 10, 16, 29, 30] as previously described.[19] The variants used were: 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.[7]

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.

A GRS was computed for each individual as the sum of the number of risk alleles across all nine variants, after weighting each one by its estimated effect size in the discovery samples [5, 10] as generally used [16-18] and previously described.[19] In this study, the minimum and maximum weighted GRS values were, respectively, 4.6 and 17.7 in control subjects and 5.7 and 18.8 in CHD cases.

Conventional risk factors for CHD

We evaluated GRS-"environment" interaction effects on CHD for several important conventional cardiovascular risk factors (ConvRFs) for which information was collected at enrolment. These factors

were: smoking status, hypertension, BMI, waist-to-hip ratio, physical activity, energy intake and adherence to the MedDiet. Participants were characterized as current, former or never smokers and were considered as hypertensive if they met one of the following criteria: i) their measured arterial blood pressure was 140 mmHg or higher systolic, or 90 mmHg or higher diastolic, and ii) self-reported intake of an antihypertensive treatment. Weight, height, waist and hip circumference were measured using standard procedures, and BMI was calculated in kg/m^2. With respect to physical activity, we used a metabolic equivalent index (MET-value) that expresses the amount of energy per kilogram of body weight expended during an average day.[31] Dietary information of the participants was measured at baseline using a validated interviewer-administered food frequency questionnaire (FFQ).[32] The frequency of consumption of about 200 foods and recipes that are common in Greece was reflected at the FFQ. The daily energy intake was assessed by recording participants' energy intake (in kcal). Adherence to the MedDiet was assessed with a MedDiet-score that incorporates the salient characteristics of this diet, that is, high intake of plant foods and olive oil, low intake of meat and dairy products, and moderate intake of alcohol. This score, with values from 0 to 9 (higher scores indicate greater adherence to the MedDiet), is associated with death from CHD, with lower values predicting higher incidence of death from CHD.[28, 33]

Statistical analysis

For this study we have used all incident CHD cases and all available control subjects and we have proceeded through unconditional logistic regression.

Mean values of quantitative characteristics, as well as percentages for qualitative ones, by sex and case-control status were calculated for descriptive purposes. We evaluated whether CHD incidence is related to the aforementioned ConvRFs using logistic regression, adjusting for age, sex and GRS. We evaluated odds ratios (ORs) for CHD, as estimates of the incidence rate ratios, in relation to age, sex and higher or lower risk with respect to GRS (above or equal to *vs.* below the sex-specific median score in controls) and, alternatively, on the basis of smoking status (current *vs.* never/former smoker),

hypertension (yes *vs.* no), physical activity (below *vs.* above or equal to the sex-specific median), energy intake (below *vs.* above or equal to the sex-specific median), MedDiet-score (below *vs.* above or equal to the median score of 4.0), BMI (above or equal *vs.* below 25 kg/m^2) or waist-to-hip ratio (above or equal to *vs.* below the sex-specific median).

In order to access the nature of the joint effects of GRS and ConvRFs, we calculated the relative excess risk due to interaction (RERI), as defined by Rothman.[34] RERI is an estimate of excess or deficit risk that is attributable to the interaction between 2 exposures, in this case GRS and each one of the ConvRFs; it measures deviation from additivity of effects independently of the risk scale of the outcome. From the ORs of the logistic regression we computed the RERIs between GRS and ConRFs, as follows;[35] we let X+ and Y+ denote the presence of the risk factors X (GRS in our analysis) and Y (conventional factor) and X- and Y- denote the absence of these risk factors. Then, by considering that the OR estimates the relative risk (RR) we have that:

$$\mathrm{RERI}(X,Y) = (RR_{X+Y+} - RR_{X-Y-}) - (RR_{X+Y-} - RR_{X-Y-}) - (RR_{X-Y+} - RR_{X-Y-})$$

$$\text{i.e., } \mathrm{RERI}(X,Y) = (OR_{X+Y+} - 1) - (OR_{X+Y-} - 1) - (OR_{X-Y+} - 1)$$

The necessary variance estimators of RERI for the construction of 95% confidence intervals (CI) were derived using the standard delta method.[35] All statistical analyses were conducted using the Stata Statistical Software, release 11 (StataCorp. 2009, StataCorp LP).

RESULTS

Of the 1839 study participants with genotype data (494 patients with incident CHD only and 1345 controls), 91 subjects had missing data for one or more of the conventional cardiovascular risk factors; thus, our analyses were restricted to 477 CHD cases and 1271 controls with complete datasets. **Table 1** gives characteristics at enrolment for the study participants according to sex and case-control status.

The association of ConvRFs with CHD incidence in this prospective cohort study is illustrated in **Table 2**. As expected, smoking, hypertension and an increased BMI and waist-to-hip ratio were all associated with a substantial increase in the risk of CHD, whereas higher levels of physical activity and

energy expenditure (as reflected in an increased energy intake) [36] were associated with a decrease in risk. Greater adherence to the MedDiet was also associated with an 11% decreased risk of CHD, although this association was not statistically significant.

We then examined the impact on CHD risk of the joint presence of genetic predisposition and conventional cardiovascular risk factors by modelling the data through unconditional logistic regression, adjusting for age and sex. Specifically, we estimated ORs for CHD incidence depending on subjects having a higher or lower GRS and simultaneously as being at higher or lower risk on the basis of a conventional risk factor. **Table 3** gives the distribution of CHD cases and controls by GRS and each ConvRF (lower *vs.* higher risk for CHD) in men and women. As shown in **Table 4**, in all instances the joint presence of higher GRS and higher-risk ConvRF is associated with a substantial increase in the risk of CHD, compared to the joint presence of lower GRS and lower-risk ConvRF. In addition, subjects with higher GRS values (high-risk genetic predisposition) and simultaneously at higher risk because of a ConvRF are characterized by an OR for CHD that is higher than the OR among individuals with high-risk genetic predisposition who belong to the lower risk category of the respective ConvRF (smoking status, OR 1.70 *vs.* 1.49; hypertension, OR 2.72 *vs.* 1.21; physical activity, OR 1.86 *vs.* 1.25; energy intake, OR 1.75 *vs.* 1.43; MedDiet-score, OR 1.51 *vs.* 1.24; BMI, OR 2.01 *vs.* 1.47; waist-to-hip ratio, OR 1.88 *vs.* 1.25).

Relative excess risks due to interaction (RERIs) between the GRS and each one of the conventional cardiovascular risk factors are presented in the last column of Table 4. There is some evidence for superadditivity with respect to hypertension and on the contrary some evidence for subadditivity with respect to smoking. Nevertheless, in all instances, RERI values are fairly small and the 95% confidence intervals cover the null values of RERI, suggesting that the genetic risk score and the conventional risk factors do not have effects beyond additivity.

DISCUSSION

In a sizable case-control study nested in the population based Greek-EPIC cohort, we have found that genetic predisposition to CHD, operationalized through a multi-locus genetic risk score (the sum of high-risk alleles in nine genetic variants), and conventional cardiovascular risk factors have essentially additive influence on CHD risk. In other words, people at high risk for CHD because of genetic susceptibility tend to have additively increased relative risk when also exposed to any of the investigated conventional risk factors. This is highlighted by the fact that, whereas among people with low genetic risk only four out of the seven investigated conventional cardiovascular risk factors were documentable as "statistically significant", all seven were documentable as such among people at high genetic risk.

Evaluation of joint effects in a multiplicative scale through interaction terms in logistic regression and other models that rely on similar principles are very valuable on account of their flexibility and provision of insights on causal pathways. Additive models (and deviations from additivity), however, as evaluated in this paper, convey straightforward answers to questions of preventive and clinical importance by pointing to individual change of risk in relation to values of conventional risk factors and specified genetic risk background.[34, 37] The results of the present study indicate that persons at high genetic risk for CHD increase this risk when they move into a high-risk category of a conventional cardiovascular risk factor no more than persons at low genetic risk, although they end up with a higher overall risk on account of the joint presence of high-risk genetic predisposition and ConvRF. Our results are not incompatible with those of previous investigations focusing on joint effects of genetic predisposition, assessed in variable ways, and selected ConvRF for CHD.[38]

In the present investigation we found no evidence of superadditive or subadditive effect of the GRS in conjunction with several conventional cardiovascular risk factors. This does not preclude that such interactions does not exist between ConvRFs not studied in the present investigation and genetic variants not included in the GRS, over and beyond issues related to statistical power.[21, 39, 40] It does appear, however, that the joint effects of genetic and non-genetic risk factors tend, generally, to be additive.

Strengths of the present nested case-control investigation are the population based prospective cohort design of the underlying study, the minimal concern for population stratification and the use of

SNPs with documented association with CHD. In this investigation, the effect estimates for the ConvRFs used (smoking, hypertension, etc) as well as the genetic factors which were components of the GRS were comparable to those reported in the literature that argues for the validity of the database used.[10, 16] The main limitation of this study stems from the modest numbers of incident CHD cases, not withstanding the fact that the underlying cohort was large and was followed for approximately ten years. In addition, due to lack of available data on certain conventional risk factors of CHD, such as blood cholesterol levels, we were not able to examine in this study their joint relations with the GRS used.

In conclusion, this study provides evidence that genetic and conventional cardiovascular risk factors tend to have additive consequences on CHD, an issue that may be of preventive importance even when genetic predisposition is not assessed through an ad-hoc genetic risk sore but simply through a positive family history.

Author Contributions:

Study concept and design: Yiannakouris, Trichopoulou, Ordovas and Trichopoulos.
Acquisition of data: Yiannakouris, Trichopoulou and Trichopoulos.
Analysis and interpretation of data: Yiannakouris, Katsoulis, Trichopoulou, Ordovas, and Trichopoulos.
Drafting and critical revision of the manuscript for important intellectual content: Yiannakouris, Katsoulis, Trichopoulou, Ordovas, and Trichopoulos.
Statistical analysis: Katsoulis, Yiannakouris and Trichopoulos.
Obtained funding: Trichopoulou and Ordovas.
Administrative, technical, and material support: Yiannakouris, Trichopoulou and Ordovas.
Study supervision: Trichopoulou and Yiannakouris

Ethics: All procedures were in accordance 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.

Funding: This study was supported by the Hellenic Health Foundation and the Stavros Niarchros Foundation; and by contracts 53-K06-5-10 and 58-1950-9-001 from the US Department of Agriculture Research.

Data sharing: There is no additional data available.

Competing interests: The authors declare that they have no conflict of interest.

REFERENCES

1. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;**380**:2095-128.
2. Yusuf S, Hawken S, Ounpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;**364**:937-52.
3. Hu FB. Diet and lifestyle influences on risk of coronary heart disease. Curr Atheroscler Rep 2009;**11**:257-63.
4. Vaidya D, Yanek LR, Moy TF, Pearson TA, Becker LC, Becker DM. Incidence of coronary artery disease in siblings of patients with premature coronary artery disease: 10 years of follow-up. Am J Cardiol 2007;**100**:1410-1415.
5. WTCCC. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;**447**:661-78.
6. Schunkert H, Konig IR, Kathiresan S, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet 2011;**43**:333-38.
7. Samani NJ, Erdmann J, Hall AS, et al. Genomewide association analysis of coronary artery disease. N Engl J Med 2007;**357**:443-53.
8. Samani NJ, Deloukas P, Erdmann J, et al. Large scale association analysis of novel genetic loci for coronary artery disease. Arterioscler Thromb Vasc Biol 2009;**29**:774-80.
9. McPherson R, Pertsemlidis A, Kavaslar N, et al. A common allele on chromosome 9 associated with coronary heart disease. Science 2007;**316**:1488-91.
10. Kathiresan S, Voight BF, Purcell S, et al. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet 2009;**41**:334-41.

11. Helgadottir A, Thorleifsson G, Manolescu A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science 2007;**316**:1491-93.

12. Yiannakouris N, Trichopoulou A, Benetou V, Psaltopoulou T, Ordovas JM, Trichopoulos D. A direct assessment of genetic contribution to the incidence of coronary infarct in the general population Greek EPIC cohort. Eur J Epidemiol 2006;**21**:859-67.

13. Humphries SE, Drenos F, Ken-Dror G, Talmud PJ. Coronary heart disease risk prediction in the era of genome-wide association studies: current status and what the future holds. Circulation 2010;**121**:2235-48.

14. Kathiresan S, Melander O, Anevski D, et al. Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med 2008;**358**:1240-49.

15. Paynter NP, Chasman DI, Pare G, et al. Association between a literature-based genetic risk score and cardiovascular events in women. JAMA 2010;**303**:631-7.

16. Ripatti S, Tikkanen E, Orho-Melander M, et al. A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses. Lancet 2010;**376**:1393-400.

17. Davies RW, Dandona S, Stewart AF, et al. Improved prediction of cardiovascular disease based on a panel of single nucleotide polymorphisms identified through genome-wide association studies. Circ Cardiovasc Genet 2010;**3**:468-74.

18. Thanassoulis G, Peloso GM, Pencina MJ, et al. A genetic risk score is associated with incident cardiovascular disease and coronary artery calcium: the Framingham Heart Study. Circ Cardiovasc Genet 2012;**5**:113-21.

19. Yiannakouris N, Katsoulis M, Dilis V, et al. Genetic predisposition to coronary heart disease and stroke using an additive genetic risk score: a population-based study in Greece. Atherosclerosis 2012;**222**:175-9.

20. Manolio TA. Cohort studies and the genetics of complex disease. Nat Genet 2009;**41**:5-6.

21. Ordovas JM, Tai ES. Why study gene-environment interactions? Curr Opin Lipidol 2008;**19**:158-67.

22. Lanktree MB, Hegele RA. Gene-gene and gene-environment interactions: new insights into the prevention, detection and management of coronary artery disease. Genome Med 2009;**1**:28.

23. Do R, Xie C, Zhang X, Mannisto S, et al. The effect of chromosome 9p21 variants on cardiovascular disease may be modified by dietary intake: evidence from a case/control and a prospective study. PLoS Med 2011;**8**:e1001106.

24. Hamza TH, Chen H, Hill-Burns EM, et al. Genome-wide gene-environment study identifies glutamate receptor gene GRIN2A as a Parkinson's disease modifier gene via interaction with coffee. PLoS Genet 2011;**7**:e1002237.

25. Surakka I, Isaacs A, Karssen LC, et al. A genome-wide screen for interactions reveals a new locus on 4p15 modifying the effect of waist-to-hip ratio on total cholesterol. PLoS Genet 2011;**7**:e1002333.

26. Misirli G, Bamia C, Dilis V, Benetou V, Zilis D, Trichopoulou A. Validation of self-reported incident cardiovascular disease events in the Greek EPIC cohort study. Italian Journal of Public Health 2012;**9**:e7538.

27. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;**5**:1113-24.

28. Dilis V, Katsoulis M, Lagiou P, Trichopoulos D, Naska A, Trichopoulou A. Mediterranean diet and CHD: the Greek European Prospective Investigation into Cancer and Nutrition cohort. Br J Nutr 2012;**108**:699-709.

29. Schunkert H, Gotz A, Braund P, et al. Repeated replication and a prospective meta-analysis of the association between chromosome 9p21.3 and coronary artery disease. Circulation 2008;**117**:1675-84.

30. Preuss M, Konig IR, Thompson JR, et al. A Genome-wide association meta-analysis involving more than 22 000 cases and 60 000 controls. Circ Cardiovasc Genet 2010;**3**:475-83.

31. Trichopoulou A, Gnardellis C, Lagiou A, Benetou V, Trichopoulos D. Body mass index in relation to energy intake and expenditure among adults in Greece. Epidemiology 2000;**11**:333-36.

32. Gnardellis C, Trichopoulou A, Katsouyanni K, Polychronopoulos E, Rimm EB, Trichopoulos D. Reproducibility and validity of an extensive semiquantitative food frequency questionnaire among Greek school teachers. Epidemiology 1995;**6**:74-7.

33. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;**348**:2599-608.

34. Rothman, K.J. Modern Epidemiology. Boston, Toronto: Little Brown and Co.; 1986.

35. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. Epidemiology 1992;**3**:452-6.

36. Morris JN, Marr JW, Clayton DG. Diet and heart: a postscript. Br Med J 1977;**2**:1307-14.

37. de Mutsert R, de Jager DJ, Jager KJ, Zoccali C, Dekker FW. Interaction on an additive scale. Nephron Clin Pract 2011;**119**:c154-7.

38. Lee YC, Lai CQ, Ordovas JM, Parnell LD. A Database of Gene-Environment Interactions Pertaining to Blood Lipid Traits, Cardiovascular Disease and Type 2 Diabetes. J Data Mining Genomics Proteomics 2011;**2**:pii:106 doi:10.4172/2153-0602.1000106.

39. Talmud PJ. Gene-environment interaction and its impact on coronary heart disease risk. Nutr Metab Cardiovasc Dis 2007;**17**:148-52.

40. Carty CL, Heagerty P, Heckbert SR, et al. Interaction between fibrinogen and IL-6 genetic variants and associations with cardiovascular disease risk in the Cardiovascular Health Study. Ann Hum Genet 2010;**74**:1-10.

What is already known on this subject?

Several non-genetic risk factors for coronary heart disease have been established and several common genetic variants have been documented as affecting the risk of this disease; however, we don't know how the genetic and non-genetic risk factors interact and what role such interactions play in the development of coronary heart disease.

What this study adds?

We provide evidence that genetic predisposition to coronary heart disease and conventional cardiovascular risk factors, including smoking, hypertension, body mass index, physical activity and adherence to the Mediterranean diet, tend to have additive impact on coronary heart disease. In other words, people at high risk for coronary heart disease because of genetic susceptibility tend to have additively increased relative risk when also exposed to the aforementioned conventional risk factors. These findings have considerable public health consequences.

Table 1. Characteristics of conventional cardiovascular risk factors and genetic risk score for incident CHD cases and controls in the Greek-EPIC cohort.

	Cases (n=477)		Controls (n= 1271)	
	Men (n=331)	Women (n=146)	Men (n=784)	Women (n=487)
Age (yrs)	60.1 (11.4)	66.2 (6.9)	60.6 (10.9)	65.6 (7.3)
Body mass index (kg/m^2)	28.7 (3.8)	31.1 (5.5)	28.0 (3.9)	29.8 (4.9)
Waist-to-hip ratio	0.97 (0.06)	0.87 (0.07)	0.96 (0.07)	0.85 (0.09)
Physical activity (MET-h/d)	33.8 (5.6)	33.6 (3.7)	34.7 (6.0)	34.5 (4.5)
Energy intake (kJ)	9250.8 (3000.8)	6733.7 (2021.7)	9370.9 (2700.4)	7028.7 (2330.5)
MedDiet-score [a]	4.4 (1.7)	4.1 (1.6)	4.4 (1.7)	4.2 (1.6)
Hypertensive, n (%) [b]	224 (67.7)	131 (89.7)	452 (57.7)	318 (65.3)
Current smokers, n (%)	138 (41.7)	13 (8.9)	269 (34.3)	34 (7.0)
Weighted GRS [c]	12.6 (2.0)	12.9 (2.1)	12.3 (2.1)	12.3 (2.1)

Data are expressed as mean (SD) unless otherwise indicated.

[a] The range of the MedDiet-score is from 0 to 9, with higher values indicating greater adherence to the Mediterranean diet.[33]

[b] Defined as a systolic blood pressure of 140 mmHg or higher or a diastolic blood pressure of 90 mmHg or higher, or self reported receipt of an antihypertensive treatment.

[c] The minimum and maximum weighted GRS values were 4.6 and 18.8.

Abbreviations: CHD=Coronary heart disease; GRS=Genetic risk score; MET-h/d=Metabolic equivalent–hours/day; MedDiet=Mediterranean diet; EPIC=European prospective investigation into cancer and nutrition.

Table 2. Odds Ratios for CHD incidence by conventional risk factors in the Greek-EPIC cohort.[a]

	OR (95% CI)	p-value
Smoking status (current *vs.* never/former smokers)	1.39 (1.08 to 1.80)	0.012
Hypertension (yes *vs.* no)	2.16 (1.68 to 2.78)	<0.001
Physical activity (≥ sex-specific median *vs.* < sex-specific median)	0.70 (0.56 to 0.87)	0.002
Energy intake (≥ sex-specific median *vs.* < sex-specific median)	0.75 (0.60 to 0.93)	0.011
MedDiet score (≥ sex-specific median *vs.*< sex-specific median)	0.89 (0.71 to 1.11)	0.299
Body mass index (≥ 25 kg/m^2 *vs.* < 25 kg/m^2)	1.45 (1.08 to 1.96)	0.015
Waist-to-hip ratio (≥ sex-specific median *vs.* < sex-specific median)	1.46 (1.17 to 1.81)	0.001

[a] Association tested with unconditional logistic regression adjusted for age, sex and genetic risk score; median values according to the overall sample (cases and controls combined)

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

Table 3. Distribution of CHD cases and controls by genetic risk score and conventional cardiovascular risk factors (lower/higher risk for CHD), in men and women.

	Men (n=1115)		Women (n=663)	
	Cases (n=331)	**Controls (n= 784)**	**Cases (n=146)**	**Controls (n= 487)**
	lower/higher risk	lower/higher risk	lower/higher risk	lower/higher risk
GRS (lower risk: < sex-specific median of controls; higher risk: ≥ sex-specific median of controls)	150/181 (45/55)	400/384 (51/49)	60/86 (41/59)	243/244 (50/50)
Smoking status (lower risk: never/former smokers; higher risk: current smokers)	193/138 (58/42)	515/269 (66/34)	133/13 (91/9)	453/34 (93/7)
Hypertension (lower risk: no; higher risk: yes)	107/224 (32/68)	332/452 (42/58)	15/131 (10/90)	169/318 (35/65)
Physical activity (lower risk: ≥ sex-specific median; higher risk: < sex-specific median)	148/183 (45/55)	410/374 (52/48)	64/82 (44/56)	254/233 (52/48)
Energy intake (lower risk: ≥ sex-specific median; higher risk: < sex-specific median)	153/178 (46/54)	405/379 (52/48)	63/83 (43/57)	254/233 (52/48)
MedDiet-score (lower risk: ≥4.0; higher risk: < 4.0)	224/107 (68/32)	545/239 (69/31)	93/53 (64/36)	328/159 (67/33)
Body mass index (lower risk: < 25 kg/m^2; higher risk: ≥ 25 kg/m^2)	51/280 (15/85)	160/624 (20/80)	14/132 (10/90)	70/417 (14/86)
Waist-to-hip ratio (lower risk: < sex-specific median; higher risk: ≥ sex-specific median)	147/184 (44/56)	410/374 (52/48)	60/86 (41/59)	255/232 (52/48)

Data are numbers (% in parenthesis). Median values for GRS are based on controls only [19] whereas for conventional risk factors median values are based on cases and controls combined.

Abbreviations: CHD=Coronary heart disease; GRS=Genetic risk score; MedDiet=Mediterranean diet

Table 4. Odds Ratios for CHD occurrence by both genetic risk score and, alternatively, the indicated conventional cardiovascular risk factors in the Greek-EPIC cohort (CHD cases: n=477; controls: n=1271) [a]

	1st (reference) GRS: lower risk ConvRF: lower risk	**2nd** GRS: lower risk ConvRF: higher risk		**3rd** GRS: higher risk ConvRF: lower risk		**4th** GRS: higher risk ConvRF: higher risk		**Relative Excess Risk due to Interaction (RERI)**	
	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	Estimate (95% CI)	p
Smoking status (lower risk: never/former smokers higher risk: current smokers)	630	**1.75** (1.22 to 2.49)	223	**1.49** (1.15 to 1.92)	664	**1.70** (1.19 to 2.41)	231	-0.54 (-1.31 to 0.24)	0.18
Hypertension (lower risk: no; higher risk: yes)	318	**2.07** (1.45 to 2.94)	535	1.21 (0.81 to 1.80)	305	**2.72** (1.92 to 3.83)	590	0.44 (-0.27 to 1.16)	0.22
Physical activity (lower risk: ≥ sex-specific median; higher risk: < sex-specific median)	425	1.36 (0.99 to 1.88)	428	1.25 (0.92 to 1.71)	451	**1.86** (1.36 to 2.54)	444	0.25 (-0.32 to 0.81)	0.39
Energy intake (lower risk: ≥ sex-specific median; higher risk: < sex-specific median)	439	**1.47** (1.07 to 2.03)	414	**1.43** (1.05 to 1.94)	436	**1.75** (1.29 to 2.39)	459	-0.14 (-0.76 to 0.47)	0.65
MedDiet score (lower risk: ≥4.0; higher risk: < 4.0)	574	1.03 (0.73 to 1.43)	279	1.24 (0.95 to 1.60)	616	**1.51** (1.10 to 2.08)	279	0.25 (-0.29 to 0.79)	0.36
Body mass index (lower risk: < 25 kg/m^2; higher risk: ≥ 25 kg/m^2)	143	1.56 (0.99 to 2.46)	710	1.47 (0.84 to 2.56)	152	**2.01** (1.28 to 3.15)	743	-0.02 (-0.82 to 0.78)	0.96
Waist-to-hip ratio (lower risk: < sex-specific median; higher risk: ≥ sex-specific median)	433	**1.40** (1.02 to 1.93)	420	1.25 (0.92 to 1.71)	439	**1.88** (1.39 to 2.55)	456	0.23 (-0.35 to 0.80)	0.44

[a] Association tested with unconditional logistic regression adjusted for age and sex. Statistically significant results (p≤0.05) are in bolded fonts.

Abbreviations: OR= odds ratio; CI=confidence interval; CHD =coronary heart disease; GRS=genetic risk score; ConvRF=conventional cardiovascular risk factor; MedDiet=Mediterranean diet; EPIC=European prospective investigation into cancer and nutrition.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No		Recommendation
Title and abstract	1	OK	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		OK	(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction			
Background/rationale	2	OK	Explain the scientific background and rationale for the investigation being reported
Objectives	3	OK	State specific objectives, including any prespecified hypotheses
Methods			
Study design	4	OK	Present key elements of study design early in the paper
Setting	5	OK	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6		(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		OK	*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
			Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants
			(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed
		OK	*Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	OK	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	OK	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	OK	Describe any efforts to address potential sources of bias
Study size	10	OK	Explain how the study size was arrived at
Quantitative variables	11	OK	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	OK	(*a*) Describe all statistical methods, including those used to control for confounding
		OK	(*b*) Describe any methods used to examine subgroups and interactions
		OK	(*c*) Explain how missing data were addressed
			(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed
		OK	*Case-control study*—If applicable, explain how matching of cases and controls was addressed
			Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy
		OK	(*e*) Describe any sensitivity analyses

Continued on next page

Results			
Participants	13*	**OK**	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		OK	(b) Give reasons for non-participation at each stage
			(c) Consider use of a flow diagram
Descriptive data	14*	**OK**	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		OK	(b) Indicate number of participants with missing data for each variable of interest
		OK	(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*		*Cohort study*—Report numbers of outcome events or summary measures over time
		OK	*Case-control study*—Report numbers in each exposure category, or summary measures of exposure
			Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	**OK**	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		OK	(*b*) Report category boundaries when continuous variables were categorized
		OK	(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	**OK**	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion			
Key results	18	**OK**	Summarise key results with reference to study objectives
Limitations	19	**OK**	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	**OK**	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	**OK**	Discuss the generalisability (external validity) of the study results
Other information			
Funding	22	**OK**	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Additive influence of genetic predisposition and conventional risk factors in the incidence of coronary heart disease: a population-based study in Greece

Journal:	*BMJ Open*
Manuscript ID:	bmjopen-2013-004387.R1
Article Type:	Research
Date Submitted by the Author:	23-Dec-2013
Complete List of Authors:	Yiannakouris, N.; Hellenic Health Foundation, ; Harokopio University, Nutrition and Dietetics Katsoulis, Michail; Hellenic Health Foundation, Trichopoulou, Antonia; Hellenic Health Foundation, Ordovas, Jose; Human Nutrition Research Center on Aging (HNRCA) at Tufts University, Nutrition and Genomics Laboratory, Jean Mayer–US Department of Agriculture Trichopoulos, Dimitrios; Harvard School of Public Health, Department of Epidemiology
Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Epidemiology, Genetics and genomics
Keywords:	Coronary heart disease < CARDIOLOGY, EPIDEMIOLOGY, GENETICS

Research report

Additive influence of genetic predisposition and conventional risk factors in the incidence of coronary heart disease: a population-based study in Greece

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Key words: genetic risk score; risk factors; coronary heart disease; gene-environment interaction; relative excess risk

Running title: Genetic and other risk factors in coronary heart disease

Abstract word count: 259

Text word count: 2820

Number of Tables: 4

ABSTRACT

Objectives: An additive genetic risk score (GRS) for coronary heart disease (CHD) has previously been associated with incident CHD in the population-based Greek European Prospective Investigation into Cancer and nutrition (EPIC) cohort. In this study we explore GRS-"environment" joint actions on CHD for several conventional cardiovascular risk factors (ConvRFs), including smoking, hypertension, type-2 diabetes mellitus (T2DM), body mass index (BMI), physical activity and adherence to the Mediterranean diet.

Design: A case-control study.

Setting: The general Greek population of the EPIC study.

Participants and Outcome measures: Subjects were 477 patients with medically confirmed incident CHD and 1271 controls. We estimated the odds ratios for CHD by dividing participants at higher or lower GRS and, alternatively, at higher or lower ConvRF, and calculated the relative excess risk due to interaction (RERI) as a measure of deviation from additivity.

Results: The joint presence of higher GRS and higher-risk ConvRF was in all instances associated with an increased risk of CHD, compared to the joint presence of lower GRS and lower-risk ConvRF. The odds ratio (95% confidence interval) was 1.7 (1.2-2.4) for smoking, 2.7 (1.9-3.8) for hypertension, 4.1 (2.8-6.1) for T2DM, 1.9 (1.4-2.5) for lower physical activity, 2.0 (1.3-3.2) for high BMI and 1.5 (1.1-2.1) for poor adherence to the Mediterranean diet. In all instances RERI values were fairly small and not statistically significant suggesting that the GRS and the ConvRFs do not have effects beyond additivity.

Conclusion: Genetic predisposition to CHD, operationalised through a multi-locus genetic risk score, and conventional cardiovascular risk factors have essentially additive effects on CHD risk.

ARTICLE SUMMARY

Strengths and limitations of this study

- Strengths of the study are the population based prospective cohort design of the underlying study and the minimal concern for population stratification
- The main limitation of this study stems from the modest numbers of incident CHD cases, not withstanding the fact that the underlying cohort was large and was followed for approximately ten years

INTRODUCTION

Coronary heart disease (CHD) is a leading cause of death and disability worldwide.[1] Lifestyle and environmental factors, such as cigarette smoking, physical inactivity, chronodisruption and unhealthy diets, play a significant role in its development and are largely responsible for increased risk of this disease.[2,3] In addition, compelling evidence from the literature suggest a genetic basis for CHD [4] so that genetic data may identify individuals who have an inherited predisposition to develop CHD.

During the past few years, genome-wide association studies (GWAS) have successfully identified a large number of chromosomal loci and genetic variants that are robustly associated with CHD,[5-11] although their effects on risk are generally fairly small. To combine the relatively small effects of individual genes and to better capture the complex relationship between genetics and CHD, genotypes at multiple genetic variants have previously been combined into scores calculated according to the number of risk alleles carried.[12, 13] To date, several studies have examined the utility of different genetic risk scores to identify subjects at increased CHD risk.[14-18] Ripatti et al. [16] reported that a genetic risk score (GRS) based on a series of genetic variants from GWAS for myocardial infarction or CHD 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. Using a similar approach, we have shown that a GRS based on nine documented genetic variants from GWAS is associated with incident CHD in the population-based Greek European Prospective Investigation into Cancer and nutrition (EPIC) cohort.[19]

Despite the success of GWAS in identifying novel genetic contributors to CHD, the heritability of common disorders cannot be adequately explained by the genes that have been discovered; moreover, for the most part, we don't know how these recently discovered loci interact with the environment and what role such interactions play in the development of the disease.[20, 21] Testing such interactions is thus a new frontier for large scale GWAS of CHD [22] and some initial findings support the important role of environmental exposures in influencing the magnitude of the genetic associations with cardiovascular disease [23] or other common diseases and traits.[24, 25]

The aim of the current study was to explore potential GRS-"environment" interaction effects on CHD for several important conventional cardiovascular risk factors, including smoking, hypertension, type-2 diabetes mellitus (T2DM), body mass index (BMI), physical activity and adherence to the Mediterranean diet (MedDiet). We have used resources generated in the Greek-EPIC cohort in which medically documented incident cases of CHD [26] are recorded during an extended follow-up of this population-based cohort.

METHODS

Study population

The European Prospective Investigation into Cancer and nutrition (EPIC) is a longitudinal study aimed at investigating the role of biologic, nutritional, lifestyle, and environmental factors in the etiology of cancer and other chronic diseases. The study has been described in detail elsewhere.[27, 28] The recruitment of Greek-EPIC participants was from 1994 to 1999. The active follow-up of study participants is repeated every two to four years. In each round, the focus of follow-up is on the update of information related to health status of the participants. For this analysis, exposure data at enrolment and follow-up data until the end of 2009 for outcomes are considered.

By December 2009, 788 subjects were diagnosed with an incident, medically confirmed, CHD or stroke event and were considered eligible for a study also evaluating genetic predisposition.[19] For each case, an attempt was made to choose two control subjects matched for sex, age (±2 years), and date of recruitment (±6 months). Both cases and controls were free of CHD and stroke at baseline; the final study sample consisted of 788 cases (494 CHD, 320 stroke, 26 both diseases) and 1345 controls. For each study participant, a buffy coat sample was drawn from the Greek-EPIC bio-repository and genomic DNA was extracted. CHD events included myocardial infarction, angina and other ischemic heart disease (cardiac arrest, presence of cardiac and vascular implants and grafts), with several cases following in more than one categories.[26, 28] All procedures were in accordance 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.

Selection of genetic variants, genotyping and genetic risk score calculation

We constructed a multi-locus genetic risk score (GRS) by using nine previously reported genetic variants associated with myocardial infarction or CHD from GWAS, with convincing replication evidence in populations with European ancestry,[6, 10, 16, 29, 30] as previously described.[19] The variants used were: 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.[7]

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.

A GRS was computed for each individual as the sum of the number of risk alleles across all nine variants, after weighting each one by its estimated effect size in the discovery samples [5, 10] as generally used [16-18] and previously described.[19] In this study, the minimum and maximum weighted GRS values were, respectively, 4.6 and 17.7 in control subjects and 5.7 and 18.8 in CHD cases.

Conventional risk factors for CHD

We evaluated GRS-"environment" interaction effects on CHD for several important conventional cardiovascular risk factors (ConvRFs) for which information was collected at enrolment. These factors

were: smoking status, hypertension, T2DM, BMI, waist-to-hip ratio, physical activity, energy intake and adherence to the MedDiet. Participants were characterized as current, former or never smokers and were considered as hypertensive if they met one of the following criteria: i) their measured arterial blood pressure was 140 mmHg or higher systolic, or 90 mmHg or higher diastolic, and ii) self-reported intake of an antihypertensive treatment. Type-2 diabetes was identified through self-reported T2DM-spesific medication use or self-reported medical diagnosis of T2DM. Weight, height, waist and hip circumference were measured using standard procedures, and BMI was calculated in kg/m^2. With respect to physical activity, we used a metabolic equivalent index (MET-value) that expresses the amount of energy per kilogram of body weight expended during an average day.[31] Dietary information of the participants was measured at baseline using a validated interviewer-administered food frequency questionnaire (FFQ).[32] The frequency of consumption of about 200 foods and recipes that are common in Greece was reflected at the FFQ. The daily energy intake was assessed by recording participants' energy intake (in kcal). Adherence to the MedDiet was assessed with a MedDiet-score that incorporates the salient characteristics of this diet, that is, high intake of plant foods and olive oil, low intake of meat and dairy products, and moderate intake of alcohol. This score, with values from 0 to 9 (higher scores indicate greater adherence to the MedDiet), is associated with death from CHD, with lower values predicting higher incidence of death from CHD.[28, 33]

Statistical analysis

For this study we have used all incident CHD cases and all available control subjects and we have proceeded through unconditional logistic regression.

Mean values of quantitative characteristics, as well as percentages for qualitative ones, by sex and case-control status were calculated for descriptive purposes. We evaluated whether CHD incidence is related to the aforementioned ConvRFs using logistic regression, adjusting for age, sex and GRS. We evaluated odds ratios (ORs) for CHD, as estimates of the incidence rate ratios, in relation to age, sex and higher or lower risk with respect to GRS (above or equal to *vs.* below the sex-specific median score in

controls) and, alternatively, on the basis of smoking status (current *vs.* never/former smoker), hypertension (yes *vs.* no), T2DM (yes *vs.* no), physical activity (below *vs.* above or equal to the sex-specific median), energy intake (below *vs.* above or equal to the sex-specific median), MedDiet-score (below *vs.* above or equal to the median score of 4.0), BMI (above or equal *vs.* below 25 kg/m^2) or waist-to-hip ratio (above or equal to *vs.* below the sex-specific median).

In order to access the nature of the joint effects of GRS and ConvRFs, we calculated the relative excess risk due to interaction (RERI), as defined by Rothman.[34] RERI is an estimate of excess or deficit risk that is attributable to the interaction between 2 exposures, in this case GRS and each one of the ConvRFs; it measures deviation from additivity of effects independently of the risk scale of the outcome. From the ORs of the logistic regression we computed the RERIs between GRS and ConRFs, as follows;[35] we let X+ and Y+ denote the presence of the risk factors X (GRS in our analysis) and Y (conventional factor) and X- and Y- denote the absence of these risk factors. Then, by considering that the OR estimates the relative risk (RR) we have that:

$$\mathrm{RERI}(X,Y) = (RR_{X+Y+} - RR_{X-Y-}) - (RR_{X+Y-} - RR_{X-Y-}) - (RR_{X-Y+} - RR_{X-Y-})$$

$$\text{i.e., } \mathrm{RERI}(X,Y) = (OR_{X+Y+} - 1) - (OR_{X+Y-} - 1) - (OR_{X-Y+} - 1)$$

The necessary variance estimators of RERI for the construction of 95% confidence intervals (CI) were derived using the standard delta method.[35] All statistical analyses were conducted using the Stata Statistical Software, release 11 (StataCorp. 2009, StataCorp LP).

RESULTS

Of the 1839 study participants with genotype data (494 patients with incident CHD only and 1345 controls), 91 subjects had missing data for one or more of the conventional cardiovascular risk factors; thus, our analyses were restricted to 477 CHD cases and 1271 controls with complete datasets. **Table 1** gives characteristics at enrolment for the study participants according to sex and case-control status.

The association of ConvRFs with CHD incidence in this prospective cohort study is illustrated in **Table 2**. As expected, smoking, hypertension, type-2 diabetes mellitus, and an increased BMI and waist-

to-hip ratio were all associated with a substantial increase in the risk of CHD, whereas higher levels of physical activity and energy expenditure (as reflected in an increased energy intake) [36] were associated with a decrease in risk. Greater adherence to the MedDiet was also associated with an 11% decreased risk of CHD, although this association was not statistically significant.

We then examined the impact on CHD risk of the joint presence of genetic predisposition and conventional cardiovascular risk factors by modelling the data through unconditional logistic regression, adjusting for age and sex. Specifically, we estimated ORs for CHD incidence depending on subjects having a higher or lower GRS and simultaneously as being at higher or lower risk on the basis of a conventional risk factor. **Table 3** gives the distribution of CHD cases and controls by GRS and each ConvRF (lower *vs.* higher risk for CHD) in men and women. As shown in **Table 4**, in all instances the joint presence of higher GRS and higher-risk ConvRF is associated with a substantial increase in the risk of CHD, compared to the joint presence of lower GRS and lower-risk ConvRF. In addition, subjects with higher GRS values (high-risk genetic predisposition) and simultaneously at higher risk because of a ConvRF are characterized by an OR for CHD that is higher than the OR among individuals with high-risk genetic predisposition who belong to the lower risk category of the respective ConvRF (smoking status, OR 1.70 *vs.* 1.49; hypertension, OR 2.72 *vs.* 1.21; T2DM, OR 4.13 *vs.* 1.34; physical activity, OR 1.86 *vs.* 1.25; energy intake, OR 1.75 *vs.* 1.43; MedDiet-score, OR 1.51 *vs.* 1.24; BMI, OR 2.01 *vs.* 1.47; waist-to-hip ratio, OR 1.88 *vs.* 1.25).

Relative excess risks due to interaction (RERIs) between the GRS and each one of the conventional cardiovascular risk factors are presented in the last column of Table 4. There is some evidence for superadditivity with respect to hypertension and on the contrary some evidence for subadditivity with respect to smoking. Nevertheless, in all instances, RERI values are fairly small and the 95% confidence intervals cover the null values of RERI, suggesting that the genetic risk score and the conventional risk factors do not have effects beyond additivity.

DISCUSSION

In a sizable case-control study nested in the population based Greek-EPIC cohort, we have found that genetic predisposition to CHD, operationalized through a multi-locus genetic risk score (the sum of high-risk alleles in nine genetic variants), and conventional cardiovascular risk factors have essentially additive influence on CHD risk. In other words, people at high risk for CHD because of genetic susceptibility tend to have additively increased relative risk when also exposed to any of the investigated conventional risk factors. This is highlighted by the fact that, whereas among people with low genetic risk only five out of the eight investigated conventional cardiovascular risk factors were documentable as "statistically significant", all eight were documentable as such among people at high genetic risk.

Evaluation of joint effects in a multiplicative scale through interaction terms in logistic regression and other models that rely on similar principles are very valuable on account of their flexibility and provision of insights on causal pathways. Additive models (and deviations from additivity), however, as evaluated in this paper, convey straightforward answers to questions of preventive and clinical importance by pointing to individual change of risk in relation to values of conventional risk factors and specified genetic risk background.[34,37] The results of the present study indicate that persons at high genetic risk for CHD increase this risk when they move into a high-risk category of a conventional cardiovascular risk factor no more than persons at low genetic risk, although they end up with a higher overall risk on account of the joint presence of high-risk genetic predisposition and ConvRF. Our results are not incompatible with those of previous investigations focusing on joint effects of genetic predisposition, assessed in variable ways, and selected ConvRF for CHD.[38] In this respect, Tavani et al.[39] have previously examined the joint effect of a family history of heart disease, taken as a proxy for genetically determined predisposition to the disease, and selected adult life risk factors on the risk of the disease and have shown that a substantial increase in heart disease is evident when both a family history and the environmental risk factors are present.

In the present investigation we found no evidence of superadditive or subadditive effect of the GRS in conjunction with several conventional cardiovascular risk factors. This does not preclude that such

interactions does not exist between ConvRFs not studied in the present investigation and genetic variants not included in the GRS, over and beyond issues related to statistical power.[21, 40, 41] It does appear, however, that the joint effects of genetic and non-genetic risk factors tend, generally, to be additive.

Strengths of the present nested case-control investigation are the population based prospective cohort design of the underlying study, the minimal concern for population stratification and the use of SNPs with documented association with CHD. In this investigation, the effect estimates for the ConvRFs used (smoking, hypertension, etc) as well as the genetic factors which were components of the GRS were comparable to those reported in the literature that argues for the validity of the database used.[10, 16] Nevertheless, the use of single baseline measurements of ConvRFs can lead to underestimation of associations with CHD risk (through regression dilution bias). [42] For example, the association between smoking and cardiovascular disease is intrinsically underestimated in cohort studies, since a proportion of smokers stop after data collection, and the relative risk falls rapidly after stopping. Correcting for within-person variation in lifestyle factors over time may result in more informative estimates of CHD risk associated with these factors, particularly for the risks associated with continued smoking and the benefits of regular physical activity,[43], and therefore, future studies should take these influences into account. The main limitation of this study stems from the modest numbers of incident CHD cases, not withstanding the fact that the underlying cohort was large and was followed for approximately ten years. In addition, due to lack of available data on certain conventional risk factors of CHD, such as blood cholesterol levels, we were not able to examine in this study their joint relations with the GRS used.

In conclusion, this study provides evidence that genetic and conventional cardiovascular risk factors tend to have additive consequences on CHD, an issue that may be of preventive importance even when genetic predisposition is not assessed through an ad-hoc genetic risk sore but simply through a positive family history.

Author Contributions:

Study concept and design: Yiannakouris, Trichopoulou, Ordovas and Trichopoulos.

Acquisition of data: Yiannakouris, Trichopoulou and Trichopoulos.
Analysis and interpretation of data: Yiannakouris, Katsoulis, Trichopoulou, Ordovas, and Trichopoulos.
Drafting and critical revision of the manuscript for important intellectual content: Yiannakouris, Katsoulis, Trichopoulou, Ordovas, and Trichopoulos.
Statistical analysis: Katsoulis, Yiannakouris and Trichopoulos.
Obtained funding: Trichopoulou and Ordovas.
Administrative, technical, and material support: Yiannakouris, Trichopoulou and Ordovas.
Study supervision: Trichopoulou and Yiannakouris

Ethics: All procedures were in accordance 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.

Funding: This study was supported by the Hellenic Health Foundation and the Stavros Niarchros Foundation; and by contracts 53-K06-5-10 and 58-1950-9-001 from the US Department of Agriculture Research.

Data sharing: No additional data available.

Competing interests: The authors declare that they have no conflict of interest.

REFERENCES

1. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;**380**:2095-128.
2. Yusuf S, Hawken S, Ounpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;**364**:937-52.
3. Hu FB. Diet and lifestyle influences on risk of coronary heart disease. Curr Atheroscler Rep 2009;**11**:257-63.
4. Vaidya D, Yanek LR, Moy TF, et al. Incidence of coronary artery disease in siblings of patients with premature coronary artery disease: 10 years of follow-up. Am J Cardiol 2007;**100**:1410-1415.
5. WTCCC. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;**447**:661-78.
6. Schunkert H, Konig IR, Kathiresan S, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet 2011;**43**:333-38.
7. Samani NJ, Erdmann J, Hall AS, et al. Genomewide association analysis of coronary artery disease. N Engl J Med 2007;**357**:443-53.
8. Samani NJ, Deloukas P, Erdmann J, et al. Large scale association analysis of novel genetic loci for coronary artery disease. Arterioscler Thromb Vasc Biol 2009;**29**:774-80.
9. McPherson R, Pertsemlidis A, Kavaslar N, et al. A common allele on chromosome 9 associated with coronary heart disease. Science 2007;**316**:1488-91.
10. Kathiresan S, Voight BF, Purcell S, et al. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet 2009;**41**:334-41.

11. Helgadottir A, Thorleifsson G, Manolescu A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science 2007;**316**:1491-93.

12. Yiannakouris N, Trichopoulou A, Benetou V, et al. A direct assessment of genetic contribution to the incidence of coronary infarct in the general population Greek EPIC cohort. Eur J Epidemiol 2006;**21**:859-67.

13. Humphries SE, Drenos F, Ken-Dror G, et al. Coronary heart disease risk prediction in the era of genome-wide association studies: current status and what the future holds. Circulation 2010;**121**:2235-48.

14. Kathiresan S, Melander O, Anevski D, et al. Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med 2008;**358**:1240-49.

15. Paynter NP, Chasman DI, Pare G, et al. Association between a literature-based genetic risk score and cardiovascular events in women. JAMA 2010;**303**:631-7.

16. Ripatti S, Tikkanen E, Orho-Melander M, et al. A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses. Lancet 2010;**376**:1393-400.

17. Davies RW, Dandona S, Stewart AF, et al. Improved prediction of cardiovascular disease based on a panel of single nucleotide polymorphisms identified through genome-wide association studies. Circ Cardiovasc Genet 2010;**3**:468-74.

18. Thanassoulis G, Peloso GM, Pencina MJ, et al. A genetic risk score is associated with incident cardiovascular disease and coronary artery calcium: the Framingham Heart Study. Circ Cardiovasc Genet 2012;**5**:113-21.

19. Yiannakouris N, Katsoulis M, Dilis V, et al. Genetic predisposition to coronary heart disease and stroke using an additive genetic risk score: a population-based study in Greece. Atherosclerosis 2012;**222**:175-9.

20. Manolio TA. Cohort studies and the genetics of complex disease. Nat Genet 2009;**41**:5-6.

21. Ordovas JM, Tai ES. Why study gene-environment interactions? Curr Opin Lipidol 2008;**19**:158-67.

22. Lanktree MB, Hegele RA. Gene-gene and gene-environment interactions: new insights into the prevention, detection and management of coronary artery disease. Genome Med 2009;**1**:28.

23. Do R, Xie C, Zhang X, Mannisto S, et al. The effect of chromosome 9p21 variants on cardiovascular disease may be modified by dietary intake: evidence from a case/control and a prospective study. PLoS Med 2011;**8**:e1001106.

24. Hamza TH, Chen H, Hill-Burns EM, et al. Genome-wide gene-environment study identifies glutamate receptor gene GRIN2A as a Parkinson's disease modifier gene via interaction with coffee. PLoS Genet 2011;**7**:e1002237.

25. Surakka I, Isaacs A, Karssen LC, et al. A genome-wide screen for interactions reveals a new locus on 4p15 modifying the effect of waist-to-hip ratio on total cholesterol. PLoS Genet 2011;**7**:e1002333.

26. Misirli G, Bamia C, Dilis V, et al. Validation of self-reported incident cardiovascular disease events in the Greek EPIC cohort study. Italian Journal of Public Health 2012;**9**:e7538.

27. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;**5**:1113-24.

28. Dilis V, Katsoulis M, Lagiou P, et al. Mediterranean diet and CHD: the Greek European Prospective Investigation into Cancer and Nutrition cohort. Br J Nutr 2012;**108**:699-709.

29. Schunkert H, Gotz A, Braund P, et al. Repeated replication and a prospective meta-analysis of the association between chromosome 9p21.3 and coronary artery disease. Circulation 2008;**117**:1675-84.

30. Preuss M, Konig IR, Thompson JR, et al. A Genome-wide association meta-analysis involving more than 22 000 cases and 60 000 controls. Circ Cardiovasc Genet 2010;**3**:475-83.

31. Trichopoulou A, Gnardellis C, Lagiou A, et al. Body mass index in relation to energy intake and expenditure among adults in Greece. Epidemiology 2000;**11**:333-36.

32. Gnardellis C, Trichopoulou A, Katsouyanni K, et al. Reproducibility and validity of an extensive semiquantitative food frequency questionnaire among Greek school teachers. Epidemiology 1995;**6**:74-7.

33. Trichopoulou A, Costacou T, Bamia C, et al. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;**348**:2599-608.

34. Rothman, K.J. Modern Epidemiology. Boston, Toronto: Little Brown and Co.; 1986.

35. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. Epidemiology 1992;**3**:452-6.

36. Morris JN, Marr JW, Clayton DG. Diet and heart: a postscript. Br Med J 1977;**2**:1307-14.

37. de Mutsert R, de Jager DJ, Jager KJ, et al. Interaction on an additive scale. Nephron Clin Pract 2011;**119**:c154-7.

38. Lee YC, Lai CQ, Ordovas JM, et al. A Database of Gene-Environment Interactions Pertaining to Blood Lipid Traits, Cardiovascular Disease and Type 2 Diabetes. J Data Mining Genomics Proteomics 2011;**2**:pii:106 doi:10.4172/2153-0602.1000106.

39. Tavani A, Augustin L, Bosetti C, et al. Influence of selected lifestyle factors on risk of acute myocardial infarction in subjects with familial predisposition for the disease. Prev Med 2004;**38**:468-72.

40. Talmud PJ. Gene-environment interaction and its impact on coronary heart disease risk. Nutr Metab Cardiovasc Dis 2007;**17**:148-52.

41. Carty CL, Heagerty P, Heckbert SR, et al. Interaction between fibrinogen and IL-6 genetic variants and associations with cardiovascular disease risk in the Cardiovascular Health Study. Ann Hum Genet 2010;**74**:1-10.

42. Emberson JR, Whincup PH, Morris RW, et al. Extent of regression dilution for established and novel coronary risk factors: results from the British Regional Heart Study. Eur J Cardiovasc Prev Rehabil 2004;**11**:125-34.

43. Emberson JR, Whincup PH, Morris RW, et al. Lifestyle and cardiovascular disease in middle-aged British men: the effect of adjusting for within-person variation. Eur Heart J 2005;**26**:1774-82.

What is already known on this subject?

Several non-genetic risk factors for coronary heart disease have been established and several common genetic variants have been documented as affecting the risk of this disease; however, we don't know how the genetic and non-genetic risk factors interact and what role such interactions play in the development of coronary heart disease.

What this study adds?

We provide evidence that genetic predisposition to coronary heart disease and conventional cardiovascular risk factors, including smoking, hypertension, body mass index, physical activity and adherence to the Mediterranean diet, tend to have additive impact on coronary heart disease. In other words, people at high risk for coronary heart disease because of genetic susceptibility tend to have additively increased relative risk when also exposed to the aforementioned conventional risk factors. These findings have considerable public health consequences.

Table 1. Characteristics of conventional cardiovascular risk factors and genetic risk score for incident CHD cases and controls in the Greek-EPIC cohort.

	Cases (n=477)				Controls (n= 1271)			
	Men (n=331)		Women (n=146)		Men (n=784)		Women (n=487)	
Age (yrs)	60.1	(11.4)	66.2	(6.9)	60.6	(10.9)	65.6	(7.3)
Body mass index (kg/m^2)	28.7	(3.8)	31.1	(5.5)	28.0	(3.9)	29.8	(4.9)
Waist-to-hip ratio	0.97	(0.06)	0.87	(0.07)	0.96	(0.07)	0.85	(0.09)
Physical activity (MET-h/d)	33.8	(5.6)	33.6	(3.7)	34.7	(6.0)	34.5	(4.5)
Energy intake (kJ)	9250.8	(3000.8)	6733.7	(2021.7)	9370.9	(2700.4)	7028.7	(2330.5)
MedDiet-score [a]	4.4	(1.7)	4.1	(1.6)	4.4	(1.7)	4.2	(1.6)
Hypertensive, n (%) [b]	224	(67.7)	131	(89.7)	452	(57.7)	318	(65.3)
Type-2 diabetics , n (%) [c]	68	(20.5)	51	(34.9)	66	(8.4)	58	(11.9)
Current smokers, n (%)	138	(41.7)	13	(8.9)	269	(34.3)	34	(7.0)
Weighted GRS [d]	12.6	(2.0)	12.9	(2.1)	12.3	(2.1)	12.3	(2.1)

Data are expressed as mean (SD) unless otherwise indicated.

[a] The range of the MedDiet-score is from 0 to 9, with higher values indicating greater adherence to the Mediterranean diet.[33]

[b] Defined as a systolic blood pressure of 140 mmHg or higher or a diastolic blood pressure of 90 mmHg or higher, or self reported receipt of an antihypertensive treatment.

[c] Identified through self-reported T2DM-spesific medication use or self-reported medical diagnosis of T2DM

[d] The minimum and maximum weighted GRS values were 4.6 and 18.8.

Abbreviations: CHD=Coronary heart disease; GRS=Genetic risk score; MET-h/d=Metabolic equivalent–hours/day; MedDiet=Mediterranean diet; EPIC=European prospective investigation into cancer and nutrition.

Table 2. Odds Ratios for CHD incidence by conventional risk factors in the Greek-EPIC cohort.[a]

	OR (95% CI)	p-value
Smoking status (current *vs.* never/former smokers)	1.39 (1.08 to 1.80)	0.012
Hypertension (yes *vs.* no)	2.16 (1.68 to 2.78)	<0.001
Type-2 diabetes mellitus (yes *vs.* no)	3.36 (2.52 to 4.47)	<0.001
Physical activity (≥ sex-specific median *vs.* < sex-specific median)	0.70 (0.56 to 0.87)	0.002
Energy intake (≥ sex-specific median *vs.* < sex-specific median)	0.75 (0.60 to 0.93)	0.011
MedDiet score (≥ sex-specific median *vs.*< sex-specific median)	0.89 (0.71 to 1.11)	0.299
Body mass index (≥ 25 kg/m^2 *vs.* < 25 kg/m^2)	1.45 (1.08 to 1.96)	0.015
Waist-to-hip ratio (≥ sex-specific median *vs.* < sex-specific median)	1.46 (1.17 to 1.81)	0.001

[a] Association tested with unconditional logistic regression adjusted for age, sex and genetic risk score; median values according to the overall sample (cases and controls combined)

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

Table 3. Distribution of CHD cases and controls by genetic risk score and conventional cardiovascular risk factors (lower/higher risk for CHD), in men and women.

	Men (n=1115)		Women (n=663)	
	Cases (n=331)	**Controls (n= 784)**	**Cases (n=146)**	**Controls (n= 487)**
	lower/higher risk	lower/higher risk	lower/higher risk	lower/higher risk
GRS (lower risk: < sex-specific median of controls; higher risk: ≥ sex-specific median of controls)	150/181 (45/55)	400/384 (51/49)	60/86 (41/59)	243/244 (50/50)
Smoking status (lower risk: never/former smokers; higher risk: current smokers)	193/138 (58/42)	515/269 (66/34)	133/13 (91/9)	453/34 (93/7)
Hypertension (lower risk: no; higher risk: yes)	107/224 (32/68)	332/452 (42/58)	15/131 (10/90)	169/318 (35/65)
Type-2 diabetes mellitus (lower risk: no; higher risk: yes)	263/68 (79/21)	718/66 (92/8)	95/51 (65/35)	429/58 (88/12)
Physical activity (lower risk: ≥ sex-specific median; higher risk: < sex-specific median)	148/183 (45/55)	410/374 (52/48)	64/82 (44/56)	254/233 (52/48)
Energy intake (lower risk: ≥ sex-specific median; higher risk: < sex-specific median)	153/178 (46/54)	405/379 (52/48)	63/83 (43/57)	254/233 (52/48)
MedDiet-score (lower risk: ≥ 4; higher risk: < 4)	224/107 (68/32)	545/239 (69/31)	93/53 (64/36)	328/159 (67/33)
Body mass index (lower risk: < 25 kg/m^2; higher risk: ≥ 25 kg/m^2)	51/280 (15/85)	160/624 (20/80)	14/132 (10/90)	70/417 (14/86)
Waist-to-hip ratio (lower risk: < sex-specific median; higher risk: ≥ sex-specific median)	147/184 (44/56)	410/374 (52/48)	60/86 (41/59)	255/232 (52/48)

Data are numbers (% in parenthesis). Median values for GRS are based on controls only [19] whereas for conventional risk factors median values are based on cases and controls combined.

Abbreviations: CHD=Coronary heart disease; GRS=Genetic risk score; MedDiet=Mediterranean diet

Table 4. Odds Ratios for CHD occurrence by both genetic risk score and, alternatively, the indicated conventional cardiovascular risk factors in the Greek-EPIC cohort (CHD cases: n=477; controls: n=1271) [a]

	1st (reference) GRS: lower risk ConvRF: lower risk	**2nd** GRS: lower risk ConvRF: higher risk		**3rd** GRS: higher risk ConvRF: lower risk		**4th** GRS: higher risk ConvRF: higher risk		**Relative Excess Risk due to Interaction (RERI)**	
	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	Estimate (95% CI)	p
Smoking status (lower risk: never/former smokers higher risk: current smokers)	630	**1.75** (1.22 to 2.49)	223	**1.49** (1.15 to 1.92)	664	**1.70** (1.19 to 2.41)	231	-0.54 (-1.31 to 0.24)	0.18
Hypertension (lower risk: no; higher risk: yes)	318	**2.07** (1.45 to 2.94)	535	1.21 (0.81 to 1.80)	305	**2.72** (1.92 to 3.83)	590	0.44 (-0.27 to 1.16)	0.22
Type-2 diabetes mellitus (lower risk: no; higher risk: yes)	740	**3.72** (2.45 to 5.63)	113	**1.34** (1.05 to 1.71)	765	**4.13** (2.79 to 6.12)	130	0.07 (-1.94 to 2.07)	0.95
Physical activity (lower risk: ≥ sex-specific median; higher risk: < sex-specific median)	425	1.36 (0.99 to 1.88)	428	1.25 (0.92 to 1.71)	451	**1.86** (1.36 to 2.54)	444	0.25 (-0.32 to 0.81)	0.39
Energy intake (lower risk: ≥ sex-specific median; higher risk: < sex-specific median)	439	**1.47** (1.07 to 2.03)	414	**1.43** (1.05 to 1.94)	436	**1.75** (1.29 to 2.39)	459	-0.14 (-0.76 to 0.47)	0.65
MedDiet score (lower risk: ≥4.0; higher risk: < 4.0)	574	1.03 (0.73 to 1.43)	279	1.24 (0.95 to 1.60)	616	**1.51** (1.10 to 2.08)	279	0.25 (-0.29 to 0.79)	0.36
Body mass index (lower risk: < 25 kg/m^2; higher risk: ≥ 25 kg/m^2)	143	1.56 (0.99 to 2.46)	710	1.47 (0.84 to 2.56)	152	**2.01** (1.28 to 3.15)	743	-0.02 (-0.82 to 0.78)	0.96
Waist-to-hip ratio (lower risk: < sex-specific median; higher risk: ≥ sex-specific median)	433	**1.40** (1.02 to 1.93)	420	1.25 (0.92 to 1.71)	439	**1.88** (1.39 to 2.55)	456	0.23 (-0.35 to 0.80)	0.44

[a] Association tested with unconditional logistic regression adjusted for age and sex. Statistically significant results (p≤0.05) are in bolded fonts.

Abbreviations: OR= odds ratio; CI=confidence interval; CHD =coronary heart disease; GRS=genetic risk score; ConvRF=conventional cardiovascular risk factor; MedDiet=Mediterranean diet; EPIC=European prospective investigation into cancer and nutrition.

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No		Recommendation
Title and abstract	1	OK	(*a*) Indicate the study's design with a commonly used term in the title or the abstract
		OK	(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found
Introduction			
Background/rationale	2	OK	Explain the scientific background and rationale for the investigation being reported
Objectives	3	OK	State specific objectives, including any prespecified hypotheses
Methods			
Study design	4	OK	Present key elements of study design early in the paper
Setting	5	OK	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection
Participants	6		(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up
		OK	*Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls
			Cross-sectional study—Give the eligibility criteria, and the sources and methods of selection of participants
			(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed
		OK	*Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	OK	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable
Data sources/ measurement	8*	OK	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group
Bias	9	OK	Describe any efforts to address potential sources of bias
Study size	10	OK	Explain how the study size was arrived at
Quantitative variables	11	OK	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	OK	(*a*) Describe all statistical methods, including those used to control for confounding
		OK	(*b*) Describe any methods used to examine subgroups and interactions
		OK	(*c*) Explain how missing data were addressed
			(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed
		OK	*Case-control study*—If applicable, explain how matching of cases and controls was addressed
			Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy
		OK	(*e*) Describe any sensitivity analyses

Continued on next page

Results			
Participants	13*	**OK**	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed
		OK	(b) Give reasons for non-participation at each stage
			(c) Consider use of a flow diagram
Descriptive data	14*	**OK**	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders
		OK	(b) Indicate number of participants with missing data for each variable of interest
		OK	(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*		*Cohort study*—Report numbers of outcome events or summary measures over time
		OK	*Case-control study*—Report numbers in each exposure category, or summary measures of exposure
			Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	**OK**	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included
		OK	(*b*) Report category boundaries when continuous variables were categorized
		OK	(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	**OK**	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses
Discussion			
Key results	18	**OK**	Summarise key results with reference to study objectives
Limitations	19	**OK**	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	**OK**	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence
Generalisability	21	**OK**	Discuss the generalisability (external validity) of the study results
Other information			
Funding	22	**OK**	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

Research report

Additive influence of genetic predisposition and conventional risk factors in the incidence of coronary heart disease: a population-based study in Greece

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Key words: genetic risk score; risk factors; coronary heart disease; gene-environment interaction; relative excess risk

Running title: Genetic and other risk factors in coronary heart disease

Abstract word count: 259

Text word count: 2820

Number of Tables: 4

ABSTRACT

Background and Objectives: An additive genetic risk score (GRS) for coronary heart disease (CHD) has previously been associated with incident CHD in the population-based Greek European Prospective Investigation into Cancer and nutrition (EPIC) cohort. In this study we explore GRS-"environment" joint actions on CHD for several conventional cardiovascular risk factors (ConvRFs), including smoking, hypertension, type-2 diabetes mellitus (T2DM), body mass index (BMI), physical activity and adherence to the Mediterranean diet.

Design: A case-control study.

Setting: The general Greek population of the EPIC study.

Participants and Outcome measures: Subjects were 477 patients with medically confirmed incident CHD and 1271 controls. We estimated the odds ratios for CHD by dividing participants at higher or lower GRS and, alternatively, at higher or lower ConvRF, and calculated the relative excess risk due to interaction (RERI) as a measure of deviation from additivity.

Results: The joint presence of higher GRS and higher-risk ConvRF was in all instances associated with an increased risk of CHD, compared to the joint presence of lower GRS and lower-risk ConvRF. The odds ratio (95% confidence interval) was 1.7 (1.2-2.4) for smoking, 2.7 (1.9-3.8) for hypertension, 4.1 (2.8-6.1) for T2DM, 1.9 (1.4-2.5) for lower physical activity, 2.0 (1.3-3.2) for high BMI and 1.5 (1.1-2.1) for poor adherence to the Mediterranean diet. In all instances RERI values were fairly small and not statistically significant suggesting that the GRS and the ConvRFs do not have effects beyond additivity.

Conclusion: Genetic predisposition to CHD, operationalised through a multi-locus genetic risk score, and conventional cardiovascular risk factors have essentially additive effects on CHD risk.

ARTICLE SUMMARY

Strengths and limitations of this study

- Strengths of the study are the population based prospective cohort design of the underlying study and the minimal concern for population stratification
- The main limitation of this study stems from the modest numbers of incident CHD cases, not withstanding the fact that the underlying cohort was large and was followed for approximately ten years

INTRODUCTION

Coronary heart disease (CHD) is a leading cause of death and disability worldwide.[1] Lifestyle and environmental factors, such as cigarette smoking, physical inactivity, chronodisruption and unhealthy diets, play a significant role in its development and are largely responsible for increased risk of this disease.[2,3] In addition, compelling evidence from the literature suggest a genetic basis for CHD [4] so that genetic data may identify individuals who have an inherited predisposition to develop CHD.

During the past few years, genome-wide association studies (GWAS) have successfully identified a large number of chromosomal loci and genetic variants that are robustly associated with CHD,[5-11] although their effects on risk are generally fairly small. To combine the relatively small effects of individual genes and to better capture the complex relationship between genetics and CHD, genotypes at multiple genetic variants have previously been combined into scores calculated according to the number of risk alleles carried.[12, 13] To date, several studies have examined the utility of different genetic risk scores to identify subjects at increased CHD risk.[14-18] Ripatti et al. [16] reported that a genetic risk score (GRS) based on a series of genetic variants from GWAS for myocardial infarction or CHD 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. Using a similar approach, we have shown that a GRS based on nine documented genetic variants from GWAS is associated with incident CHD in the population-based Greek European Prospective Investigation into Cancer and nutrition (EPIC) cohort.[19]

Despite the success of GWAS in identifying novel genetic contributors to CHD, the heritability of common disorders cannot be adequately explained by the genes that have been discovered; moreover, for the most part, we don't know how these recently discovered loci interact with the environment and what role such interactions play in the development of the disease.[20, 21] Testing such interactions is thus a new frontier for large scale GWAS of CHD [22] and some initial findings support the important role of environmental exposures in influencing the magnitude of the genetic associations with cardiovascular disease [23] or other common diseases and traits.[24, 25]

The aim of the current study was to explore potential GRS-"environment" interaction effects on CHD for several important conventional cardiovascular risk factors, including smoking, hypertension, type-2 diabetes mellitus (T2DM), body mass index (BMI), physical activity and adherence to the Mediterranean diet (MedDiet). We have used resources generated in the Greek-EPIC cohort in which medically documented incident cases of CHD [26] are recorded during an extended follow-up of this population-based cohort.

METHODS

Study population

The European Prospective Investigation into Cancer and nutrition (EPIC) is a longitudinal study aimed at investigating the role of biologic, nutritional, lifestyle, and environmental factors in the etiology of cancer and other chronic diseases. The study has been described in detail elsewhere.[27, 28] The recruitment of Greek-EPIC participants was from 1994 to 1999. The active follow-up of study participants is repeated every two to four years. In each round, the focus of follow-up is on the update of information related to health status of the participants. For this analysis, exposure data at enrolment and follow-up data until the end of 2009 for outcomes are considered.

By December 2009, 788 subjects were diagnosed with an incident, medically confirmed, CHD or stroke event and were considered eligible for a study also evaluating genetic predisposition.[19] For each case, an attempt was made to choose two control subjects matched for sex, age (±2 years), and date of recruitment (±6 months). Both cases and controls were free of CHD and stroke at baseline; the final study sample consisted of 788 cases (494 CHD, 320 stroke, 26 both diseases) and 1345 controls. For each study participant, a buffy coat sample was drawn from the Greek-EPIC bio-repository and genomic DNA was extracted. CHD events included myocardial infarction, angina and other ischemic heart disease (cardiac arrest, presence of cardiac and vascular implants and grafts), with several cases following in more than one categories.[26, 28] All procedures were in accordance 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.

Selection of genetic variants, genotyping and genetic risk score calculation

We constructed a multi-locus genetic risk score (GRS) by using nine previously reported genetic variants associated with myocardial infarction or CHD from GWAS, with convincing replication evidence in populations with European ancestry,[6, 10, 16, 29, 30] as previously described.[19] The variants used were: 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.[7]

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.

A GRS was computed for each individual as the sum of the number of risk alleles across all nine variants, after weighting each one by its estimated effect size in the discovery samples [5, 10] as generally used [16-18] and previously described.[19] In this study, the minimum and maximum weighted GRS values were, respectively, 4.6 and 17.7 in control subjects and 5.7 and 18.8 in CHD cases.

Conventional risk factors for CHD

We evaluated GRS-"environment" interaction effects on CHD for several important conventional cardiovascular risk factors (ConvRFs) for which information was collected at enrolment. These factors

were: smoking status, hypertension, T2DM, BMI, waist-to-hip ratio, physical activity, energy intake and adherence to the MedDiet. Participants were characterized as current, former or never smokers and were considered as hypertensive if they met one of the following criteria: i) their measured arterial blood pressure was 140 mmHg or higher systolic, or 90 mmHg or higher diastolic, and ii) self-reported intake of an antihypertensive treatment. Type-2 diabetes was identified through self-reported T2DM-spesific medication use or self-reported medical diagnosis of T2DM. Weight, height, waist and hip circumference were measured using standard procedures, and BMI was calculated in kg/m^2. With respect to physical activity, we used a metabolic equivalent index (MET-value) that expresses the amount of energy per kilogram of body weight expended during an average day.[31] Dietary information of the participants was measured at baseline using a validated interviewer-administered food frequency questionnaire (FFQ).[32] The frequency of consumption of about 200 foods and recipes that are common in Greece was reflected at the FFQ. The daily energy intake was assessed by recording participants' energy intake (in kcal). Adherence to the MedDiet was assessed with a MedDiet-score that incorporates the salient characteristics of this diet, that is, high intake of plant foods and olive oil, low intake of meat and dairy products, and moderate intake of alcohol. This score, with values from 0 to 9 (higher scores indicate greater adherence to the MedDiet), is associated with death from CHD, with lower values predicting higher incidence of death from CHD.[28, 33]

Statistical analysis

For this study we have used all incident CHD cases and all available control subjects and we have proceeded through unconditional logistic regression.

Mean values of quantitative characteristics, as well as percentages for qualitative ones, by sex and case-control status were calculated for descriptive purposes. We evaluated whether CHD incidence is related to the aforementioned ConvRFs using logistic regression, adjusting for age, sex and GRS. We evaluated odds ratios (ORs) for CHD, as estimates of the incidence rate ratios, in relation to age, sex and higher or lower risk with respect to GRS (above or equal to *vs.* below the sex-specific median score in

controls) and, alternatively, on the basis of smoking status (current *vs.* never/former smoker), hypertension (yes *vs.* no), T2DM (yes *vs.* no), physical activity (below *vs.* above or equal to the sex-specific median), energy intake (below *vs.* above or equal to the sex-specific median), MedDiet-score (below *vs.* above or equal to the median score of 4.0), BMI (above or equal *vs.* below 25 kg/m^2) or waist-to-hip ratio (above or equal to *vs.* below the sex-specific median).

In order to access the nature of the joint effects of GRS and ConvRFs, we calculated the relative excess risk due to interaction (RERI), as defined by Rothman.[34] RERI is an estimate of excess or deficit risk that is attributable to the interaction between 2 exposures, in this case GRS and each one of the ConvRFs; it measures deviation from additivity of effects independently of the risk scale of the outcome. From the ORs of the logistic regression we computed the RERIs between GRS and ConRFs, as follows;[35] we let X+ and Y+ denote the presence of the risk factors X (GRS in our analysis) and Y (conventional factor) and X- and Y- denote the absence of these risk factors. Then, by considering that the OR estimates the relative risk (RR) we have that:

$$\mathrm{RERI}(X,Y) = (RR_{X+Y+} - RR_{X-Y-}) - (RR_{X+Y-} - RR_{X-Y-}) - (RR_{X-Y+} - RR_{X-Y-})$$

$$\text{i.e., } \mathrm{RERI}(X,Y) = (OR_{X+Y+} - 1) - (OR_{X+Y-} - 1) - (OR_{X-Y+} - 1)$$

The necessary variance estimators of RERI for the construction of 95% confidence intervals (CI) were derived using the standard delta method.[35] All statistical analyses were conducted using the Stata Statistical Software, release 11 (StataCorp. 2009, StataCorp LP).

RESULTS

Of the 1839 study participants with genotype data (494 patients with incident CHD only and 1345 controls), 91 subjects had missing data for one or more of the conventional cardiovascular risk factors; thus, our analyses were restricted to 477 CHD cases and 1271 controls with complete datasets. **Table 1** gives characteristics at enrolment for the study participants according to sex and case-control status.

The association of ConvRFs with CHD incidence in this prospective cohort study is illustrated in **Table 2**. As expected, smoking, hypertension, type-2 diabetes mellitus, and an increased BMI and waist-

to-hip ratio were all associated with a substantial increase in the risk of CHD, whereas higher levels of physical activity and energy expenditure (as reflected in an increased energy intake) [36] were associated with a decrease in risk. Greater adherence to the MedDiet was also associated with an 11% decreased risk of CHD, although this association was not statistically significant.

We then examined the impact on CHD risk of the joint presence of genetic predisposition and conventional cardiovascular risk factors by modelling the data through unconditional logistic regression, adjusting for age and sex. Specifically, we estimated ORs for CHD incidence depending on subjects having a higher or lower GRS and simultaneously as being at higher or lower risk on the basis of a conventional risk factor. **Table 3** gives the distribution of CHD cases and controls by GRS and each ConvRF (lower *vs.* higher risk for CHD) in men and women. As shown in **Table 4**, in all instances the joint presence of higher GRS and higher-risk ConvRF is associated with a substantial increase in the risk of CHD, compared to the joint presence of lower GRS and lower-risk ConvRF. In addition, subjects with higher GRS values (high-risk genetic predisposition) and simultaneously at higher risk because of a ConvRF are characterized by an OR for CHD that is higher than the OR among individuals with high-risk genetic predisposition who belong to the lower risk category of the respective ConvRF (smoking status, OR 1.70 *vs.* 1.49; hypertension, OR 2.72 *vs.* 1.21; T2DM, OR 4.13 *vs.* 1.34; physical activity, OR 1.86 *vs.* 1.25; energy intake, OR 1.75 *vs.* 1.43; MedDiet-score, OR 1.51 *vs.* 1.24; BMI, OR 2.01 *vs.* 1.47; waist-to-hip ratio, OR 1.88 *vs.* 1.25).

Relative excess risks due to interaction (RERIs) between the GRS and each one of the conventional cardiovascular risk factors are presented in the last column of Table 4. There is some evidence for superadditivity with respect to hypertension and on the contrary some evidence for subadditivity with respect to smoking. Nevertheless, in all instances, RERI values are fairly small and the 95% confidence intervals cover the null values of RERI, suggesting that the genetic risk score and the conventional risk factors do not have effects beyond additivity.

DISCUSSION

In a sizable case-control study nested in the population based Greek-EPIC cohort, we have found that genetic predisposition to CHD, operationalized through a multi-locus genetic risk score (the sum of high-risk alleles in nine genetic variants), and conventional cardiovascular risk factors have essentially additive influence on CHD risk. In other words, people at high risk for CHD because of genetic susceptibility tend to have additively increased relative risk when also exposed to any of the investigated conventional risk factors. This is highlighted by the fact that, whereas among people with low genetic risk only five out of the eight investigated conventional cardiovascular risk factors were documentable as "statistically significant", all eight were documentable as such among people at high genetic risk.

Evaluation of joint effects in a multiplicative scale through interaction terms in logistic regression and other models that rely on similar principles are very valuable on account of their flexibility and provision of insights on causal pathways. Additive models (and deviations from additivity), however, as evaluated in this paper, convey straightforward answers to questions of preventive and clinical importance by pointing to individual change of risk in relation to values of conventional risk factors and specified genetic risk background.[34,37] The results of the present study indicate that persons at high genetic risk for CHD increase this risk when they move into a high-risk category of a conventional cardiovascular risk factor no more than persons at low genetic risk, although they end up with a higher overall risk on account of the joint presence of high-risk genetic predisposition and ConvRF. Our results are not incompatible with those of previous investigations focusing on joint effects of genetic predisposition, assessed in variable ways, and selected ConvRF for CHD.[38] In this respect, Tavani et al.[39] have previously examined the joint effect of a family history of heart disease, taken as a proxy for genetically determined predisposition to the disease, and selected adult life risk factors on the risk of the disease and have shown that a substantial increase in heart disease is evident when both a family history and the environmental risk factors are present.

In the present investigation we found no evidence of superadditive or subadditive effect of the GRS in conjunction with several conventional cardiovascular risk factors. This does not preclude that such

interactions does not exist between ConvRFs not studied in the present investigation and genetic variants not included in the GRS, over and beyond issues related to statistical power.[21, 40, 41] It does appear, however, that the joint effects of genetic and non-genetic risk factors tend, generally, to be additive.

Strengths of the present nested case-control investigation are the population based prospective cohort design of the underlying study, the minimal concern for population stratification and the use of SNPs with documented association with CHD. In this investigation, the effect estimates for the ConvRFs used (smoking, hypertension, etc) as well as the genetic factors which were components of the GRS were comparable to those reported in the literature that argues for the validity of the database used.[10, 16] Nevertheless, the use of single baseline measurements of ConvRFs can lead to underestimation of associations with CHD risk (through regression dilution bias). [42] For example, the association between smoking and cardiovascular disease is intrinsically underestimated in cohort studies, since a proportion of smokers stop after data collection, and the relative risk falls rapidly after stopping. Correcting for within-person variation in lifestyle factors over time may result in more informative estimates of CHD risk associated with these factors, particularly for the risks associated with continued smoking and the benefits of regular physical activity,[43], and therefore, future studies should take these influences into account. The main limitation of this study stems from the modest numbers of incident CHD cases, not withstanding the fact that the underlying cohort was large and was followed for approximately ten years. In addition, due to lack of available data on certain conventional risk factors of CHD, such as blood cholesterol levels, we were not able to examine in this study their joint relations with the GRS used.

In conclusion, this study provides evidence that genetic and conventional cardiovascular risk factors tend to have additive consequences on CHD, an issue that may be of preventive importance even when genetic predisposition is not assessed through an ad-hoc genetic risk sore but simply through a positive family history.

Author Contributions:

Study concept and design: Yiannakouris, Trichopoulou, Ordovas and Trichopoulos.

Acquisition of data: Yiannakouris, Trichopoulou and Trichopoulos.
Analysis and interpretation of data: Yiannakouris, Katsoulis, Trichopoulou, Ordovas, and Trichopoulos.
Drafting and critical revision of the manuscript for important intellectual content: Yiannakouris, Katsoulis, Trichopoulou, Ordovas, and Trichopoulos.
Statistical analysis: Katsoulis, Yiannakouris and Trichopoulos.
Obtained funding: Trichopoulou and Ordovas.
Administrative, technical, and material support: Yiannakouris, Trichopoulou and Ordovas.
Study supervision: Trichopoulou and Yiannakouris

Ethics: All procedures were in accordance 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.

Funding: This study was supported by the Hellenic Health Foundation and the Stavros Niarchros Foundation; and by contracts 53-K06-5-10 and 58-1950-9-001 from the US Department of Agriculture Research.

Data sharing: There is no additional data available.

Competing interests: The authors declare that they have no conflict of interest.

REFERENCES

1. Lozano R, Naghavi M, Foreman K, et al. Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;**380**:2095-128.
2. Yusuf S, Hawken S, Ounpuu S, et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. Lancet 2004;**364**:937-52.
3. Hu FB. Diet and lifestyle influences on risk of coronary heart disease. Curr Atheroscler Rep 2009;**11**:257-63.
4. Vaidya D, Yanek LR, Moy TF, Pearson TA, Becker LC, Becker DM. Incidence of coronary artery disease in siblings of patients with premature coronary artery disease: 10 years of follow-up. Am J Cardiol 2007;**100**:1410-1415.
5. WTCCC. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. Nature 2007;**447**:661-78.
6. Schunkert H, Konig IR, Kathiresan S, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet 2011;**43**:333-38.
7. Samani NJ, Erdmann J, Hall AS, et al. Genomewide association analysis of coronary artery disease. N Engl J Med 2007;**357**:443-53.
8. Samani NJ, Deloukas P, Erdmann J, et al. Large scale association analysis of novel genetic loci for coronary artery disease. Arterioscler Thromb Vasc Biol 2009;**29**:774-80.
9. McPherson R, Pertsemlidis A, Kavaslar N, et al. A common allele on chromosome 9 associated with coronary heart disease. Science 2007;**316**:1488-91.
10. Kathiresan S, Voight BF, Purcell S, et al. Genome-wide association of early-onset myocardial infarction with single nucleotide polymorphisms and copy number variants. Nat Genet 2009;**41**:334-41.

11. Helgadottir A, Thorleifsson G, Manolescu A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. Science 2007;**316**:1491-93.

12. Yiannakouris N, Trichopoulou A, Benetou V, Psaltopoulou T, Ordovas JM, Trichopoulos D. A direct assessment of genetic contribution to the incidence of coronary infarct in the general population Greek EPIC cohort. Eur J Epidemiol 2006;**21**:859-67.

13. Humphries SE, Drenos F, Ken-Dror G, Talmud PJ. Coronary heart disease risk prediction in the era of genome-wide association studies: current status and what the future holds. Circulation 2010;**121**:2235-48.

14. Kathiresan S, Melander O, Anevski D, et al. Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med 2008;**358**:1240-49.

15. Paynter NP, Chasman DI, Pare G, et al. Association between a literature-based genetic risk score and cardiovascular events in women. JAMA 2010;**303**:631-7.

16. Ripatti S, Tikkanen E, Orho-Melander M, et al. A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses. Lancet 2010;**376**:1393-400.

17. Davies RW, Dandona S, Stewart AF, et al. Improved prediction of cardiovascular disease based on a panel of single nucleotide polymorphisms identified through genome-wide association studies. Circ Cardiovasc Genet 2010;**3**:468-74.

18. Thanassoulis G, Peloso GM, Pencina MJ, et al. A genetic risk score is associated with incident cardiovascular disease and coronary artery calcium: the Framingham Heart Study. Circ Cardiovasc Genet 2012;**5**:113-21.

19. Yiannakouris N, Katsoulis M, Dilis V, et al. Genetic predisposition to coronary heart disease and stroke using an additive genetic risk score: a population-based study in Greece. Atherosclerosis 2012;**222**:175-9.

20. Manolio TA. Cohort studies and the genetics of complex disease. Nat Genet 2009;**41**:5-6.

21. Ordovas JM, Tai ES. Why study gene-environment interactions? Curr Opin Lipidol 2008;**19**:158-67.

22. Lanktree MB, Hegele RA. Gene-gene and gene-environment interactions: new insights into the prevention, detection and management of coronary artery disease. Genome Med 2009;**1**:28.

23. Do R, Xie C, Zhang X, Mannisto S, et al. The effect of chromosome 9p21 variants on cardiovascular disease may be modified by dietary intake: evidence from a case/control and a prospective study. PLoS Med 2011;**8**:e1001106.

24. Hamza TH, Chen H, Hill-Burns EM, et al. Genome-wide gene-environment study identifies glutamate receptor gene GRIN2A as a Parkinson's disease modifier gene via interaction with coffee. PLoS Genet 2011;**7**:e1002237.

25. Surakka I, Isaacs A, Karssen LC, et al. A genome-wide screen for interactions reveals a new locus on 4p15 modifying the effect of waist-to-hip ratio on total cholesterol. PLoS Genet 2011;**7**:e1002333.

26. Misirli G, Bamia C, Dilis V, Benetou V, Zilis D, Trichopoulou A. Validation of self-reported incident cardiovascular disease events in the Greek EPIC cohort study. Italian Journal of Public Health 2012;**9**:e7538.

27. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. Public Health Nutr 2002;**5**:1113-24.

28. Dilis V, Katsoulis M, Lagiou P, Trichopoulos D, Naska A, Trichopoulou A. Mediterranean diet and CHD: the Greek European Prospective Investigation into Cancer and Nutrition cohort. Br J Nutr 2012;**108**:699-709.

29. Schunkert H, Gotz A, Braund P, et al. Repeated replication and a prospective meta-analysis of the association between chromosome 9p21.3 and coronary artery disease. Circulation 2008;**117**:1675-84.

30. Preuss M, Konig IR, Thompson JR, et al. A Genome-wide association meta-analysis involving more than 22 000 cases and 60 000 controls. Circ Cardiovasc Genet 2010;**3**:475-83.

31. Trichopoulou A, Gnardellis C, Lagiou A, Benetou V, Trichopoulos D. Body mass index in relation to energy intake and expenditure among adults in Greece. Epidemiology 2000;**11**:333-36.

32. Gnardellis C, Trichopoulou A, Katsouyanni K, Polychronopoulos E, Rimm EB, Trichopoulos D. Reproducibility and validity of an extensive semiquantitative food frequency questionnaire among Greek school teachers. Epidemiology 1995;**6**:74-7.

33. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. N Engl J Med 2003;**348**:2599-608.

34. Rothman, K.J. Modern Epidemiology. Boston, Toronto: Little Brown and Co.; 1986.

35. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. Epidemiology 1992;**3**:452-6.

36. Morris JN, Marr JW, Clayton DG. Diet and heart: a postscript. Br Med J 1977;**2**:1307-14.

37. de Mutsert R, de Jager DJ, Jager KJ, Zoccali C, Dekker FW. Interaction on an additive scale. Nephron Clin Pract 2011;**119**:c154-7.

38. Lee YC, Lai CQ, Ordovas JM, Parnell LD. A Database of Gene-Environment Interactions Pertaining to Blood Lipid Traits, Cardiovascular Disease and Type 2 Diabetes. J Data Mining Genomics Proteomics 2011;**2**:pii:106 doi:10.4172/2153-0602.1000106.

39. Tavani A, Augustin L, Bosetti C, et al. Influence of selected lifestyle factors on risk of acute myocardial infarction in subjects with familial predisposition for the disease. Prev Med 2004;**38**:468-72.

40. Talmud PJ. Gene-environment interaction and its impact on coronary heart disease risk. Nutr Metab Cardiovasc Dis 2007;**17**:148-52.

41. Carty CL, Heagerty P, Heckbert SR, et al. Interaction between fibrinogen and IL-6 genetic variants and associations with cardiovascular disease risk in the Cardiovascular Health Study. Ann Hum Genet 2010;**74**:1-10.

42. Emberson JR, Whincup PH, Morris RW, Walker M, Lowe GD, Rumley A. Extent of regression dilution for established and novel coronary risk factors: results from the British Regional Heart Study. Eur J Cardiovasc Prev Rehabil 2004;**11**:125-34.

43. Emberson JR, Whincup PH, Morris RW, Wannamethee SG, Shaper AG: Lifestyle and cardiovascular disease in middle-aged British men: the effect of adjusting for within-person variation. Eur Heart J 2005;**26**:1774-82.

What is already known on this subject?

Several non-genetic risk factors for coronary heart disease have been established and several common genetic variants have been documented as affecting the risk of this disease; however, we don't know how the genetic and non-genetic risk factors interact and what role such interactions play in the development of coronary heart disease.

What this study adds?

We provide evidence that genetic predisposition to coronary heart disease and conventional cardiovascular risk factors, including smoking, hypertension, body mass index, physical activity and adherence to the Mediterranean diet, tend to have additive impact on coronary heart disease. In other words, people at high risk for coronary heart disease because of genetic susceptibility tend to have additively increased relative risk when also exposed to the aforementioned conventional risk factors. These findings have considerable public health consequences.

Table 1. Characteristics of conventional cardiovascular risk factors and genetic risk score for incident CHD cases and controls in the Greek-EPIC cohort.

	Cases (n=477)				Controls (n= 1271)			
	Men (n=331)		Women (n=146)		Men (n=784)		Women (n=487)	
Age (yrs)	60.1	(11.4)	66.2	(6.9)	60.6	(10.9)	65.6	(7.3)
Body mass index (kg/m^2)	28.7	(3.8)	31.1	(5.5)	28.0	(3.9)	29.8	(4.9)
Waist-to-hip ratio	0.97	(0.06)	0.87	(0.07)	0.96	(0.07)	0.85	(0.09)
Physical activity (MET-h/d)	33.8	(5.6)	33.6	(3.7)	34.7	(6.0)	34.5	(4.5)
Energy intake (kJ)	9250.8	(3000.8)	6733.7	(2021.7)	9370.9	(2700.4)	7028.7	(2330.5)
MedDiet-score [a]	4.4	(1.7)	4.1	(1.6)	4.4	(1.7)	4.2	(1.6)
Hypertensive, n (%) [b]	224	(67.7)	131	(89.7)	452	(57.7)	318	(65.3)
Type-2 diabetics , n (%) [c]	68	(20.5)	51	(34.9)	66	(8.4)	58	(11.9)
Current smokers, n (%)	138	(41.7)	13	(8.9)	269	(34.3)	34	(7.0)
Weighted GRS [d]	12.6	(2.0)	12.9	(2.1)	12.3	(2.1)	12.3	(2.1)

Data are expressed as mean (SD) unless otherwise indicated.

[a] The range of the MedDiet-score is from 0 to 9, with higher values indicating greater adherence to the Mediterranean diet.[33]

[b] Defined as a systolic blood pressure of 140 mmHg or higher or a diastolic blood pressure of 90 mmHg or higher, or self reported receipt of an antihypertensive treatment.

[c] Identified through self-reported T2DM-spesific medication use or self-reported medical diagnosis of T2DM

[d] The minimum and maximum weighted GRS values were 4.6 and 18.8.

Abbreviations: CHD=Coronary heart disease; GRS=Genetic risk score; MET-h/d=Metabolic equivalent–hours/day; MedDiet=Mediterranean diet; EPIC=European prospective investigation into cancer and nutrition.

Table 2. Odds Ratios for CHD incidence by conventional risk factors in the Greek-EPIC cohort.[a]

	OR (95% CI)	p-value
Smoking status (current *vs.* never/former smokers)	1.39 (1.08 to 1.80)	0.012
Hypertension (yes *vs.* no)	2.16 (1.68 to 2.78)	<0.001
Type-2 diabetes mellitus (yes *vs.* no)	3.36 (2.52 to 4.47)	<0.001
Physical activity (≥ sex-specific median *vs.* < sex-specific median)	0.70 (0.56 to 0.87)	0.002
Energy intake (≥ sex-specific median *vs.* < sex-specific median)	0.75 (0.60 to 0.93)	0.011
MedDiet score (≥ sex-specific median *vs.*< sex-specific median)	0.89 (0.71 to 1.11)	0.299
Body mass index (≥ 25 kg/m^2 *vs.* < 25 kg/m^2)	1.45 (1.08 to 1.96)	0.015
Waist-to-hip ratio (≥ sex-specific median *vs.* < sex-specific median)	1.46 (1.17 to 1.81)	0.001

[a] Association tested with unconditional logistic regression adjusted for age, sex and genetic risk score; median values according to the overall sample (cases and controls combined)

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

Table 3. Distribution of CHD cases and controls by genetic risk score and conventional cardiovascular risk factors (lower/higher risk for CHD), in men and women.

	Men (n=1115)		Women (n=663)	
	Cases (n=331)	**Controls (n= 784)**	**Cases (n=146)**	**Controls (n= 487)**
	lower/higher risk	lower/higher risk	lower/higher risk	lower/higher risk
GRS (lower risk: < sex-specific median of controls; higher risk: ≥ sex-specific median of controls)	150/181 (45/55)	400/384 (51/49)	60/86 (41/59)	243/244 (50/50)
Smoking status (lower risk: never/former smokers; higher risk: current smokers)	193/138 (58/42)	515/269 (66/34)	133/13 (91/9)	453/34 (93/7)
Hypertension (lower risk: no; higher risk: yes)	107/224 (32/68)	332/452 (42/58)	15/131 (10/90)	169/318 (35/65)
Type-2 diabetes mellitus (lower risk: no; higher risk: yes)	263/68 (79/21)	718/66 (92/8)	95/51 (65/35)	429/58 (88/12)
Physical activity (lower risk: ≥ sex-specific median; higher risk: < sex-specific median)	148/183 (45/55)	410/374 (52/48)	64/82 (44/56)	254/233 (52/48)
Energy intake (lower risk: ≥ sex-specific median; higher risk: < sex-specific median)	153/178 (46/54)	405/379 (52/48)	63/83 (43/57)	254/233 (52/48)
MedDiet-score (lower risk: ≥ 4; higher risk: < 4)	224/107 (68/32)	545/239 (69/31)	93/53 (64/36)	328/159 (67/33)
Body mass index (lower risk: < 25 kg/m^2; higher risk: ≥ 25 kg/m^2)	51/280 (15/85)	160/624 (20/80)	14/132 (10/90)	70/417 (14/86)
Waist-to-hip ratio (lower risk: < sex-specific median; higher risk: ≥ sex-specific median)	147/184 (44/56)	410/374 (52/48)	60/86 (41/59)	255/232 (52/48)

Data are numbers (% in parenthesis). Median values for GRS are based on controls only [19] whereas for conventional risk factors median values are based on cases and controls combined.

Abbreviations: CHD=Coronary heart disease; GRS=Genetic risk score; MedDiet=Mediterranean diet

Table 4. Odds Ratios for CHD occurrence by both genetic risk score and, alternatively, the indicated conventional cardiovascular risk factors in the Greek-EPIC cohort (CHD cases: n=477; controls: n=1271) [a]

	1st (reference) GRS: lower risk ConvRF: lower risk	**2nd** GRS: lower risk ConvRF: higher risk		**3rd** GRS: higher risk ConvRF: lower risk		**4th** GRS: higher risk ConvRF: higher risk		**Relative Excess Risk due to Interaction (RERI)**	
	n	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)	n	Estimate (95% CI)	p
Smoking status (lower risk: never/former smokers higher risk: current smokers)	630	**1.75** (1.22 to 2.49)	223	**1.49** (1.15 to 1.92)	664	**1.70** (1.19 to 2.41)	231	-0.54 (-1.31 to 0.24)	0.18
Hypertension (lower risk: no; higher risk: yes)	318	**2.07** (1.45 to 2.94)	535	1.21 (0.81 to 1.80)	305	**2.72** (1.92 to 3.83)	590	0.44 (-0.27 to 1.16)	0.22
Type-2 diabetes mellitus (lower risk: no; higher risk: yes)	740	**3.72** (2.45 to 5.63)	113	**1.34** (1.05 to 1.71)	765	**4.13** (2.79 to 6.12)	130	0.07 (-1.94 to 2.07)	0.95
Physical activity (lower risk: ≥ sex-specific median; higher risk: < sex-specific median)	425	1.36 (0.99 to 1.88)	428	1.25 (0.92 to 1.71)	451	**1.86** (1.36 to 2.54)	444	0.25 (-0.32 to 0.81)	0.39
Energy intake (lower risk: ≥ sex-specific median; higher risk: < sex-specific median)	439	**1.47** (1.07 to 2.03)	414	**1.43** (1.05 to 1.94)	436	**1.75** (1.29 to 2.39)	459	-0.14 (-0.76 to 0.47)	0.65
MedDiet score (lower risk: ≥4.0; higher risk: < 4.0)	574	1.03 (0.73 to 1.43)	279	1.24 (0.95 to 1.60)	616	**1.51** (1.10 to 2.08)	279	0.25 (-0.29 to 0.79)	0.36
Body mass index (lower risk: < 25 kg/m^2; higher risk: ≥ 25 kg/m^2)	143	1.56 (0.99 to 2.46)	710	1.47 (0.84 to 2.56)	152	**2.01** (1.28 to 3.15)	743	-0.02 (-0.82 to 0.78)	0.96
Waist-to-hip ratio (lower risk: < sex-specific median; higher risk: ≥ sex-specific median)	433	**1.40** (1.02 to 1.93)	420	1.25 (0.92 to 1.71)	439	**1.88** (1.39 to 2.55)	456	0.23 (-0.35 to 0.80)	0.44

[a] Association tested with unconditional logistic regression adjusted for age and sex. Statistically significant results (p≤0.05) are in bolded fonts.

Abbreviations: OR= odds ratio; CI=confidence interval; CHD =coronary heart disease; GRS=genetic risk score; ConvRF=conventional cardiovascular risk factor; MedDiet=Mediterranean diet; EPIC=European prospective investigation into cancer and nutrition.