Genetic Predisposition, Nongenetic Risk Factors, and Coronary Infarct

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Background: Using a genetic predisposition score (GPS), additively integrating the associations of 11 polymorphisms with coronary heart disease (CHD), we examined the consequences of the joint presence of a high GPS and nongenetic CHD risk factors.

Methods: Within the European Prospective Investigation Into Cancer and Nutrition, 202 case patients with medically confirmed incident coronary infarct and 197 control subjects were identified in Greece. Each polymorphism contributed 1 unit (high-risk homozygous), one-half unit (heterozygous), or no units (low-risk homozygous) to the GPS. Odds ratios of coronary infarction for those at high risk because of genetic predisposition and simultaneous presence of an established CHD risk factor were estimated, compared with subjects at low risk, for both GPS and each CHD risk factor.

Results: The joint presence of a high GPS (≥3.5) and each studied CHD risk factor was in all instances associated with a significantly increased risk of coronary infarction. The odds ratio (95% confidence interval) was 2.62 (1.14-6.02) for ever smoking, 2.88 (1.33-6.24) for hypertension, 3.50 (1.67-7.33) for low high-density lipoprotein (HDL) level, 3.05 (1.53-6.08) for high non-HDL level, and 3.66 (1.75-7.65) for poor adherence to the Mediterranean diet. The odds ratios were always lower and nonsignificant when the GPS was low. There was suggestive evidence for interaction of a high GPS with hypertension (P = .05) and non-HDL cholesterol level (P = .13).

Conclusions: Genetic predisposition may interact with hypertension and, perhaps, also with the level of non-HDL cholesterol, in the causation of CHD. Genetic predisposition and the other studied exposures seem to have converging effects. Thus, the GPS may identify individuals who could realize disproportional benefits by controlling their hypertension and, possibly, their non-HDL cholesterol level.

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Research in medical genetics and genetic epidemiology has been focused on identification of either highly penetrant gene mutations that, when present, are likely to cause disease or on low-penetration predisposing genetic polymorphisms that are thought to interact with other polymorphisms or environmental factors to cause disease. Several major gene mutations have been discovered, primarily through genetic linkage studies or more recently through genomewide association studies. In contrast to major gene mutations, which are frequently associated with high risk of disease, low-penetration genetic polymorphisms are generally associated with modest or minimal increases in risk. The consequences of the modest or minimal increases in risk associated with low-penetration genetic polymorphisms are the limited repeatability of findings reported in the literature and resultant difficulty to investigate gene environment interactions, although several successful attempts have been reported. Following the paradigm of a priori scores (ie, scores that rely on previous collective evidence rather than on the data at hand), which have been successfully used in nutritional epidemiology, intensive care, and other fields, we developed a simple a priori genetic predisposition score (GPS) for coronary heart disease (CHD), relying on a set of genetic polymorphisms considered to affect the risk of CHD. This score has been successfully validated in predicting coronary infarction in a general population cohort study in Greece, whereas the individual genetic polymorphisms that were components of the score had limited predictive ability. In the present study, we studied the joint action of the GPS and established nongenetic risk factors for CHD. This was an incidence
density case-control study nested within the Greek cohort of the European Prospective Investigation Into Cancer and Nutrition.

**STUDY SUBJECTS**

Enrollment of participants in the Greek component of the European Prospective Investigation Into Cancer and Nutrition occurred primarily between 1994 and 1999. A total of 28,572 participants aged 20 to 86 years were recruited from all regions of Greece. The European Prospective Investigation Into Cancer and Nutrition is conducted in 23 research centers in 10 European countries, with the purpose of investigating the role of biologic, dietary, lifestyle, and environmental factors in the etiology of cancer. Each center may undertake studies that do not focus on cancer. All procedures were in accord with the Helsinki Declaration, and all participants provided written informed consent.

By March 2005, 847 patients were recorded as having a medically confirmed new coronary infarct (International Classification of Diseases, Ninth Revision, code 410, and International Statistical Classification of Diseases and Health-Related Problems, Tenth Revision, code 121). Of those patients, 6 were excluded because of missing information. Among the remaining 841 patients with a coronary infarct during follow-up, 202 were randomly chosen (the target number was 200, but in the process 202 patients were selected). All 202 patients were confirmed through medical records that met the criteria of the Multinational Monitoring of Trends and Determinants in Cardiovascular Disease Project. For each patient, an attempt was made to choose 1 control subject matched for sex, age (exact year), and date of recruitment (±6 months). Control subjects had to have not developed a new coronary infarct by the date of the development of the infarct in the corresponding patient (incidence density sampling). Eventually, 197 eligible control subjects were identified.

**GENETIC ANALYSES AND THE GPS**

For each study subject, buffy coat samples, collected at enrollment and preserved at ~196°C, were sent to the Nutrition and Genomics Laboratory, Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston, Massachusetts, where a total of 11 polymorphisms in 9 genes, presumed not to be in linkage disequilibrium among each other, were determined. Genotyping was performed blindly as to case-control status for these polymorphisms on the basis of previously reported associations concerning their likely involvement in CHD occurrence through pathways of lipid metabolism (apolipoprotein [APO] E2/ε3/ε4 polymorphism [GenBank M10065; rs49398 and rs7412] and APOA5 268, and APOE [GenBank M10065; rs429358 and rs7412] and LPL [GenBank AY422951; rs5128], and lipoprotein lipase [LPL] D9N, N291S, and S447X [GenBank M10065; rs7801177, rs268, and rs328, respectively]), homocysteine metabolism (methylene tetrahydrofolate reductase [MTHFR] 677C→T [GenBank AY383223; rs1801133]), endothelial cell integrity (endothelial nitric oxide synthase [NOS3] E280D [GenBank AF519768; rs1799983]), and inflammation (interleukin [IL]-1β 886G→A [GenBank AY137079; rs16946], and IL6–174G→C [GenBank AF372214; rs1800795], and tumor necrosis factor α [TNF-α] 308G→A [GenBank AB103618; rs1800629]). For the construction of the a priori GPS, each polymorphism contributed 1 unit if the subject was homozygous for the presumed high-risk allele, one-half unit if the subject was heterozygous, and no units if the subject was homozygous for the low-risk allele. High-risk alleles were considered alphabetically, as follows: APOA5 1131C, APOC3 3238G, APOE 4/4, IL1β–511C, IL6–174C, LPL 9N, LPL 497S, MTHFR 677T, NOS3 298D, and TNF-α 308G. Accordingly, individuals could, in theory, have GPS values between 0 (very low genetic risk) and 11 (very high genetic risk). We made no distinction among polymorphisms by likely size of effect because the relevant relative risk estimates are generally less than 2.0, with wide confidence intervals, making it unrealistic to attempt incorporating quantifiable allele-specific effects. For recessive and dominant effects, we adopted the parsimonious view of codominance. Any deviation from codominance would only tend to underestimate effect parameters through minor misspecification of the score used.

**OTHER RISK FACTORS FOR CHD**

We evaluated the association with myocardial infarction of the joint action of GPS and, alternatively, important CHD risk factors for which information was collected at enrollment. These factors were smoking, hypertension, high-density lipoprotein cholesterol (HDL-C) level, non-HDL-C level, and diet. At enrollment, study participants were characterized as ever smokers or never smokers and as having hypertension if they were receiving antihypertensive treatment or their measured arterial blood pressure was ≥140 mm Hg or higher systolic or ≥90 mm Hg or higher diastolic. Blood levels of HDL-C and non−HDL-C were determined in 193 case patients and 178 control subjects, as previously described. Diet was assessed with a Mediterranean diet 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 wine. This score, with values from 0 to 9 (higher scores indicate greater adherence to the Mediterranean diet), is strongly associated with death from coronary disease, with lower values predicting higher incidence of death from coronary disease.

**STATISTICAL ANALYSIS**

Mean values of quantitative characteristics or percentages for qualitative characteristics, by sex and case-control status, as well as frequency distributions of case patients and control subjects by GPS, were calculated for descriptive purposes. Odds ratios for being a patient with CHD rather than a control subject were estimated by means of logistic regression depending on subjects having a low or high GPS (above or equal to vs below the approximate median score of 3.5) and simultaneously as being at high or low risk on the basis of smoking status (ever vs never), hypertension (yes vs no), HDL-C level (low vs high on the basis of the corresponding sex-specific median), non−HDL-C level (high vs low on the basis of the corresponding sex-specific median), or Mediterranean diet score (below vs above or equal to the median score of 3.0). In each analysis, the indicated factors were controlled for, as were diabetes at enrollment, body mass index, physical activity (in total metabolic equivalent of the task hours per day), and total energy intake. Data were analyzed with both conditional and unconditional logistic regression. At conditional regression analysis, 187 case patients and 176 control subjects were studied, that is, all subjects for whom cholesterol measurements were available and who were matched during the process of selection or could be matched after selection. At unconditional logistic regression, age and sex were also controlled for. A total of 193 case patients and 178 control subjects were studied, that is, all subjects but those for whom cholesterol measurements were unavailable.
In a prospective cohort study based on the general population of Greece, we evaluated the association with coronary infarct and the joint presence of high-risk GPS (above or equal to vs below the median score of 3.5) and, alternatively, smoking, hypertension, HDL-C and non–HDL-C levels, and adherence to the Mediterranean diet. For the nongenetic factors, categorical contrasts (yes vs no) or median values were used as cutoff points on the basis of data at enrollment. We found that the joint presence of a high-risk GPS and a high-risk nongenetic factor (ie, smoking, hypertension, low HDL-C level, high non–HDL-C level, or poor adherence to the Mediterranean diet) is associated with a statistically significant increase in the risk of coronary infarct. Moreover, the joint presence of high-risk GPS and high-risk nongenetic factor is characterized by an odds ratio (OR) of 8 or higher (ie, smoking, hypertension, low HDL-C level, high non–HDL-C level, or poor adherence to the Mediterranean diet).

A total of 11 polymorphisms in 9 genes, presumably not in linkage disequilibrium among each other, were analyzed. For each polymorphism, subjects homozgyous for the allele presumed to confer a higher risk for coronary heart disease were assigned a value of 1, whereas individuals with the heterozygous genotype were given a value of 0.5, and subjects homozgyous for the more “favorable” alleles, a value of 0.

Due to rounding percentages do not total 100.

P value for trend=.001.
characterized by an OR for coronary infarction that is considerably higher than the OR among individuals with a low-risk GPS who are exposed to the corresponding nongenetic risk factor. For hypertension and non–HDL-C level, there was suggestive evidence for statistical interaction, which was significant \((P < .05)\) in the unconditional analysis. Evaluation of these data under an alternative conceptualization of synergy\(^{16,17}\) that relies on deviation of the OR of the joint effect from additivity of the ORs of the component risk factors leads to similar conclusions. Thus, our data suggest that genetic predisposition may interact with hypertension and perhaps non–HDL-C in the causation of CHD, whereas genetic predisposition and either poor adherence to the Mediterranean diet, smoking, or low HDL-C level seem to have congruent effects. In other words, our results indicate that genetic predisposition, at least as ascertained with the GPS, identifies individuals who may realize disproportional benefits by controlling their arterial blood pressure and non–HDL-C levels.

### Table 3. Odds Ratios of Being a Patient With Coronary Infarct in Relation to the Joint Presence of a High (\(\geq 3.5\)) or Low GPS and CHD Risk Factors\(^a\)

<table>
<thead>
<tr>
<th>GPS</th>
<th>CHD Risk Factor</th>
<th>Case Patients</th>
<th>Control Subjects</th>
<th>Conditional Regression for 187 Case Patients, 176 Control Subjects</th>
<th>Unconditional Regression for 193 Case Patients, 178 Control Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OR(^b) (95% CI)</td>
<td>(P) Value</td>
<td>OR(^c) (95% CI)</td>
<td>(P) Value</td>
</tr>
<tr>
<td>(&lt; 3.5)</td>
<td>Smoking status</td>
<td>Never</td>
<td>46</td>
<td>66</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ever</td>
<td>60</td>
<td>70</td>
<td>1.20 (0.54-2.66)</td>
</tr>
<tr>
<td>(\geq 3.5)</td>
<td></td>
<td>Never</td>
<td>42</td>
<td>27</td>
<td>2.11 (1.03-4.33)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ever</td>
<td>54</td>
<td>34</td>
<td>2.62 (1.14-6.02)</td>
</tr>
<tr>
<td></td>
<td>(P) value for interaction</td>
<td>.95</td>
<td>.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt; 3.5)</td>
<td>Hypertension</td>
<td>No</td>
<td>40</td>
<td>46</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>66</td>
<td>90</td>
<td>1.08 (0.54-2.18)</td>
</tr>
<tr>
<td>(\geq 3.5)</td>
<td></td>
<td>No</td>
<td>20</td>
<td>27</td>
<td>0.94 (0.37-2.40)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yes</td>
<td>76</td>
<td>34</td>
<td>2.88 (1.33-6.24)</td>
</tr>
<tr>
<td></td>
<td>(P) value for interaction</td>
<td>.05</td>
<td>.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt; 3.5)</td>
<td>HDL-C level</td>
<td>Equal to or greater than sex-specific median</td>
<td>42</td>
<td>70</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Less than sex-specific median</td>
<td>57</td>
<td>51</td>
<td>1.69 (0.88-3.24)</td>
</tr>
<tr>
<td>(\geq 3.5)</td>
<td></td>
<td>Equal to or greater than sex-specific median</td>
<td>40</td>
<td>34</td>
<td>1.97 (0.98-3.97)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Less than sex-specific median</td>
<td>54</td>
<td>23</td>
<td>3.52 (1.67-7.33)</td>
</tr>
<tr>
<td></td>
<td>(P) value for interaction</td>
<td>.91</td>
<td>.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt; 3.5)</td>
<td>Non-HDL-C level</td>
<td>Less than sex-specific median</td>
<td>53</td>
<td>66</td>
<td>1 [Reference]</td>
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<tr>
<td></td>
<td></td>
<td>Equal to or greater than sex-specific median</td>
<td>46</td>
<td>55</td>
<td>1.01 (0.55-1.86)</td>
</tr>
<tr>
<td>(\geq 3.5)</td>
<td></td>
<td>Less than sex-specific median</td>
<td>34</td>
<td>31</td>
<td>1.39 (0.69-2.78)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Equal to or greater than sex-specific median</td>
<td>60</td>
<td>26</td>
<td>3.05 (1.53-6.08)</td>
</tr>
<tr>
<td></td>
<td>(P) value for interaction</td>
<td>.13</td>
<td>.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&lt; 3.5)</td>
<td>Mediterranean diet score</td>
<td>(\geq 5)</td>
<td>42</td>
<td>75</td>
<td>1 [Reference]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&lt; 5)</td>
<td>64</td>
<td>61</td>
<td>1.74 (0.87-3.49)</td>
</tr>
<tr>
<td>(\geq 5)</td>
<td></td>
<td>(\geq 5)</td>
<td>45</td>
<td>34</td>
<td>1.82 (0.88-3.76)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(&lt; 5)</td>
<td>51</td>
<td>27</td>
<td>3.66 (1.75-7.65)</td>
</tr>
<tr>
<td>(P) value for interaction</td>
<td>.78</td>
<td>.56</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CHD, coronary heart disease; CI, confidence interval; GPS, genetic predisposition score; HDL-C, high-density lipoprotein cholesterol; MET, metabolic equivalent; OR, odds ratio.

\(^a\)Case-control study nested within the Greek European Prospective Investigation Into Cancer and Nutrition cohort.

\(^b\)Adjusted for diabetes (yes/no, categorically), hypertension (yes/no, categorically), HDL-C (continuously), non–HDL-C (continuously), smoking status (ever/never, categorically), Mediterranean diet score (continuously), educational achievement level (none and elementary/higher/university, categorically), body mass index (in quintiles, ordered), physical activity (MET hours per day in quintiles, ordered), and total energy intake (continuously).

\(^c\)As at conditional analysis and also adjusted for sex and age (continuously).
els of fasting plasma triglycerides, lower HDL-C levels, and an increased risk of CHD have been demonstrated for carriers of the LPL D9N and N291S polymorphisms, whereas the LPL S447X variant has been reported to confer some protection against CHD.21 The functional MTHFR 677C→T polymorphism correlates highly with plasma homocysteine levels, and a recent meta-analysis indicates an association of the 677T allele with increased CHD risk.22 Another meta-analysis indicates an increased risk of ischemic heart disease associated with the NOS3 298D allele, presumably through effects on nitric oxide availability. The common IL1B–511 C→T polymorphism confers some protection against myocardial infarction, possibly by modulating the response of mononuclear cells to inflammatory stimulation. The IL–6–174C allele is associated with higher plasma levels of IL-6 and C-reactive protein and increased susceptibility to cardiovascular disease.23 The TNF-α–308G>A polymorphism is associated with increased susceptibility to several diseases in which inflammation has a role, and carriers of the minor allele seem to be at increased risk of CHD.24 The GPS will retain its predictive ability for CHD even if 1 or a few of its 11 components are found to be unrelated to the disease. Several genetic association studies of CHD, including genomewide association studies, have been undertaken.25 Considerably less information, however, exists about the joint effects of genetic and classic epidemiologic risk factors for the disease, and much of the data are derived using family history as a proxy of genetic predisposition.26 To a certain extent, the relative scarcity of genetic association studies evaluating the joint effects of genetic and classic epidemiologic risk factors is the result of the difficulty in documenting statistical interactions when the excess risk associated with genetic factors is low. The main advantage of a simple additive score such as the GPS is that it decreases sample size requirements because it captures extreme risks, even if some of its components are found to have little or even no effect.27 This is because the score accumulates excess risks imparted or likely to be imparted by 11 component polymorphisms rather than by any of these separately. In addition to the use of the GPS, other strengths of this investigation include the blind evaluation, insofar as case-control status, of polymorphisms and score development, and the prospective nature of the underlying study base, which minimizes selection and information bias, although it should be acknowledged that prospective investigations cannot always adequately evaluate events and processes proximal to the study outcome.

A limitation of the study is its small sample size, which is mitigated by the statistical power-enhancing properties of an additive linear score.28 Another limitation is the choice of polymorphisms to be included in the GPS, which was constrained by the existing knowledge and the orientation of the major laboratory in which these were determined. Indeed, even if the use of a GPS were to be more broadly adopted, it should be expected that its components would change on the basis of updated scientific data on the role of the various polymorphisms. A limitation of the GPS score is that it does not account for the possibly different magnitude of the effect of the high-risk component polymorphisms or their mode of action. These 2 limitations, however, are likely to attenuate the associations.

In conclusion, we found evidence that a high GPS relying on 11 polymorphisms that have been shown to be related to CHD tends to affect myocardial infarction risk disproportionately when blood pressure and non–HDL-C level are also elevated, whereas the joint presence of high GPS and, alternatively, poor adherence to the Mediterranean diet, smoking, or low levels of HDL-C have converging effects on risk of coronary infarction. Thus, individuals with increased genetic predisposition to CHD have additional reasons to avoid or reduce exposure to nongenetic factors that are established component causes of the disease.

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Author Contributions: Dr Trichopoulos had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Trichopoulos, Yiannakouris, Bamia, Benetou, and Trichopoulos. Acquisition of data: Trichopoulos, Yiannakouris, Benetou, Trichopoulos, and Ordovas. Analysis and interpretation of data: Trichopoulos, Yiannakouris, Bamia, Benetou, Trichopoulos, and Ordovas. Drafting of the manuscript: Trichopoulos, Yiannakouris, Bamia, Benetou, Trichopoulos, and Ordovas. Critical revision of the manuscript for important intellectual content: Trichopoulos, Yiannakouris, Bamia, Benetou, Trichopoulos, and Ordovas. Statistical analysis: Bamia and Trichopoulos. Obtained funding: Trichopoulos and Ordovas. Administrative, technical, and material support: Trichopoulos, Yiannakouris, Trichopoulos, and Ordovas. Study supervision: Trichopoulos, Benetou, and Trichopoulos.

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