**Genetic Susceptibility to Coronary Heart Disease in Type 2 Diabetes**

3 Independent Studies

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**Objectives**

The aim of this study was to evaluate whether coronary heart disease (CHD)-susceptibility loci identified by genome-wide association studies of the general population also contribute to CHD in type 2 diabetes.

**Background**

No study has examined the effects of these genetic variants on CHD in diabetic patients.

**Methods**

We genotyped 15 genetic markers of 12 loci in 3 studies of diabetic patients: the prospective Nurses’ Health Study (309 CHD cases, and 544 control subjects) and Health Professional Follow-up Study (345 CHD cases, and 451 control subjects) and the cross-sectional Joslin Heart Study (422 CHD cases, and 435 control subjects).

**Results**

Five single-nucleotide polymorphisms, rs4977574 (CDKN2A/2B), rs12526453 (PHACTR1), rs646776 (CELSR2-PSRC1-SORT1), rs2259816 (HNF1A), and rs11206510 (PCSK9) showed directionally consistent associations with CHD in the 3 studies, with combined odds ratios (ORs) ranging from 1.17 to 1.25 (p = 0.03 to 0.0002).

None of the other single-nucleotide polymorphisms reached significance in individual or combined analyses. A genetic risk score (GRS) was created by combining the risk alleles of the 5 significantly associated loci. The OR of CHD/GRS unit was 1.19 (95% confidence interval: 1.13 to 1.26; p < 0.0001).

Individuals with GRS ≥8 (19% of diabetic subjects) had almost a 2-fold increase in CHD risk (OR: 1.94, 95% confidence interval: 1.60 to 2.35) as compared with individuals with GRS ≤5 (30% of diabetic subjects). Prediction of CHD was significantly improved (p < 0.001) when the GRS was added to a model including clinical predictors in the combined samples.

**Conclusions**

Our results illustrate the consistency and differences in the determinants of genetic susceptibility to CHD in diabetic patients and the general populations. (J Am Coll Cardiol 2011;58:2675–82) © 2011 by the American College of Cardiology Foundation

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Mortality due to coronary heart disease (CHD) has been declining overall during the past few decades in most industrialized countries (1). However, during the same time period, the number of CHD deaths attributable to diabetes has been increasing (2). Two factors account for these contrasting trends. First, although the prevalence of other risk factors—such as smoking, hypertension, and hypercholesterolemia—has been reduced by prevention programs, the incidence of diabetes has been steadily rising (3). Second, the excess cardiovascular risk experienced by diabetic subjects (a 2- to 4-fold increase as compared with the nondiabetic population) has not significantly declined during the same period of time (2). Clearly, there is an urgent need for more effective approaches to curb the current diabetes epidemic and to prevent CHD in those subjects who have developed diabetes. However, little is known about the factors underlying the excess cardiovascular risk in diabetic patients.

Studies in diabetic and nondiabetic subjects suggest that the risk of CHD is influenced by genetic factors (4), and a number of predisposing loci have been recently identified in...
the general population through genome-wide association studies (GWAS) (5–9). However, whether these genetic markers predispose to increased cardiovascular complications in diabetes remain uncertain.

In this study, we genotyped 12 CHD-susceptibility loci identified by GWAS of the general populations and examined their associations with CHD risk in 3 independent cohorts of patients with type 2 diabetes. We also assessed the joint genetic effects of these loci by creating a genetic risk score (GRS) and evaluated its prediction value for CHD among diabetic patients.

Methods

Study subjects. DIABETIC COHORTS IN NURSES’ HEALTH STUDY AND HEALTH PROFESSIONAL FOLLOW-UP STUDY. The study samples for the present analysis were selected from 2 diabetic cohorts nested in the NHS (Nurses’ Health Study) (10) and HPFS (Health Professional Follow-Up Study) (11) studies (Online Appendix), including 1,188 women and 999 men. Diabetes cases were defined as self-reported diabetes confirmed by a validated supplementary questionnaire. The National Diabetes Data Group criteria were used to define diabetes, because all study subjects were diagnosed with diabetes before the release of the American Diabetes Association criteria in 1997 (12). The validity of this method has been confirmed (13,14). These patients met the following selection criteria: 1) they were incident cases of type 2 diabetes diagnosed between the cohort baseline (1976 for NHS, and 1986 for HPFS) and the first collection of blood sample (1990 for NHS, and 1994 for HPFS); 2) they had blood samples available; and 3) they were free of other chronic diseases such as cardiovascular disease and cancer at blood collection (15–17). The study was approved by the Human Research Committee at the Brigham and Women’s Hospital, Boston, and all participants provided written informed consent.

For the purpose of the present study, CHD was defined as the occurrence of a fatal or nonfatal myocardial infarction or coronary artery bypass grafting during follow-up through 2006. Nonfatal myocardial infarction was confirmed by reviewing medical records with the criteria of the World Health Organization of symptoms plus either typical electrocardiographic changes or elevated levels of cardiac enzymes. Physicians who reviewed the records had no knowledge of the self-reported risk factors. Cardiovascular deaths were confirmed by review of medical records or autopsy reports with the permission of the next of kin. Sudden deaths were included in the fatal CHD category. We excluded those subjects who were diagnosed with CHD before the diagnosis of diabetes, who were diagnosed with stroke and/or angina, who were non-Caucasian minorities, and who were missing all genotypes. After these exclusions, 335 women and 203 men were removed, leaving 853 women (309 CHD case subjects and 544 control subjects) and 796 men (345 CHD case subjects and 451 control subjects) who were analyzed in this study.

JOSLIN HEART STUDY. The JHS study (Joslin Heart Study) consists of a series of non–Hispanic White CHD cases and control subjects, all with type 2 diabetes, who lived in the greater Boston and attended the Joslin Clinic and/or the Beth Israel Deaconess Medical Center at the time of their recruitment. The study protocol and informed consent procedures were approved by the Joslin Committee on Human Studies and the Beth Israel Deaconess Medical Center Committee on Clinical Investigations. All subjects gave written informed consent. The recruitment and clinical characteristics of the subjects recruited up to 2006 were previously described (18). Type 2 diabetes was defined as diabetes that was diagnosed at age 30 years or older according to American Diabetes Association criteria (19) and did not require insulin treatment for at least 2 years after its diagnosis. The CHD case participants (n = 422) were a random sample of patients with type 2 diabetes who had a stenosis >50% in a major coronary artery or a main branch thereof that was documented by cardiac catheterization at the Beth Israel Deaconess Medical Center between 2001 and 2008. All eligible participants were enrolled in the study at the time of catheterization and examined within 1 month after the procedure. Sixty percent of the case patients received diabetes management care at the Joslin Clinic. Control subjects (n = 435) were randomly selected from among Joslin patients who were identified between 2001 and 2008 as fulfilling the following criteria: 1) current age between 55 and 74 years; 2) type 2 diabetes for 5 years or more; 3) negative cardiovascular history (i.e., normal resting electrocardiogram, absence of cardiac symptoms, and no hospital stay for cardiovascular events); and 4) normal response to an exercise treadmill test performed for screening purposes. All control participants were recruited within 6 months after the exercise treadmill test. History of myocardial infarction, smoking, hypertension, and hypercholesterolemia and treatment with glucose-lowering drugs were determined by a questionnaire administered at the time of examination.

Measurement of hemoglobin A1c and high-density lipoprotein. Hemoglobin A1c (HbA1c) and high-density lipoprotein (HDL) were measured in 1990 to 1991 in the NHS study, in 1993 to 1999 in the HPFS study and at examination in the JHS study. The HbA1c was measured by immunoassay (Hitachi 911 Analyzer, Roche Diagnostics, Indianapolis, Indiana) in the NHS and HPFS studies and
by high-performance (pressure) liquid chromatography (Tosoh Bioscience, South San Francisco, California) in the JHS study. The coefficients of variation were 3.8% in the NHS and HPFS studies and 2.1% in the JHS study. The HDL was measured on a Hitachi 911 analyzer (Roche Diagnostics) in the NHS and HPFS studies and on an Ortho Vitros 5.1 Chemistry Analyzer (Ortho-Clinical Diagnostics, Rochester, New York) in the JHS study. The coefficients of variation were <3.0% with both methods.

**Single-nucleotide polymorphism genotyping.** Deoxyribonucleic acid was extracted from the buffy coat fraction of centrifuged blood with the QIAmp Blood Kit (Qiagen, Chatsworth, California) in the NHS and HPFS studies and the chloroform/phenol method in the JHS study. All subjects were typed for 15 single-nucleotide polymorphisms (SNPs) tagging 12 loci that were previously found to be associated with coronary heart disease at the genome-wide significance level of 5 · 10⁻⁸ in GWAS of the general population (Online Table 1). In the NHS and HPFS participants, the genotyping was carried out with the OpenArray SNP Genotyping System (BioTrove, Woburn, Massachusetts). Replicate quality control samples (10%) were included and genotyped with >99% concordance. In the JHS study, genotyping was carried out by the Joslin DERC Genetics Core by means of TaqMan assays implemented on an ABI PRISM 7700 HT Sequence Detection System (Applied Biosystems, Foster City, California). Genotyping quality was tested by including 6 blinded duplicate samples in each 96-well assay. The average agreement rate was >99%. The call rate was >95% in all 3 studies.

**Statistical analysis.** The SAS statistical package was used for all analyses (version 8.2 for UNIX, SAS Institute, Cary, North Carolina). Chi-square tests were used to assess whether genotypes were in Hardy-Weinberg equilibrium (HWE) and to compare genotype frequencies between control subjects. All p values are 2-sided.

**Indivdual locus analyses.** Crude odds ratios (ORs) of CHD and their 95% confidence intervals (CIs) were estimated for each SNP and in each study by means of logistic regression models in which CHD was considered as the dependent variable and the SNP genotypes as the independent variables according to an additive model. Associations were then summarized across the 3 studies by meta-analyses with STATA (version 7.0, StataCorp, College Station, Texas). The presence of heterogeneity among the 3 studies was tested by means of chi-square statistics. Because this test was not significant for any of the SNPs, we calculated summary ORs according to a fixed-effect model (i.e., by averaging the natural logarithms of the ORs from individual studies), weighted by the inverses of their variances (20). The 3 studies combined had 90% power (alpha = 0.05) to detect ORs in the 1.13- to 1.18 range at the disease allele frequencies considered in this study. Haplotype analysis was conducted with the THESIAS program, which is based on the Stochastic-EM algorithm (21). We selected the haplotypes on the basis of a frequency >1% in control subjects.

**Calculation of a GRS.** A GRS was calculated from 5 SNPs that were significantly associated with CHD in the 3 studies combined. For the GRS calculation, we assumed that each SNP was independently associated with risk according to an additive genetic model, which performs well even when the true genetic model is unknown or wrongly specified (22). In the main analysis, we assumed that each SNP in the panel contributed equally to the risk of CHD and calculated the GRS by summing the number of risk alleles at each polymorphic locus. This score ranged from 0 (no risk allele at any of the 5 loci) to 10 (2 risk alleles at each locus). However, in sensitivity analyses, we also calculated a weighted GRS by multiplying the number of risk alleles at each locus (0, 1, or 2) for the corresponding beta coefficient from the meta-analysis and then summing the products.

**Evaluation of GRS performance.** We analyzed the associations between GRS and CHD by means of logistic regression. Predictor coefficients were estimated by regression models including: 1) only the GRS; 2) only clinical predictors (age, sex, HbA1c, HDL, and history of smoking, hypertension, and hypercholesterolemia); and 3) both the GRS and clinical predictors. The area under the receiver-operator characteristic curve (AUC) was used as an overall measure of prediction accuracy with a sensitivity cutoff of 0.90. Because it is difficult to empirically estimate the variance of the accuracy measure estimates, standard errors for model coefficients and accuracy measures were estimated by a perturbation-resampling method (23). Because apparent accuracy measure estimates can be overly optimistic when the same set of data is used to estimate both the model parameters and the accuracy of the resulting risk score, we considered a general 3-fold cross-validation procedure in which the data were randomly split into a training set (2/3*n) and a validation set (1/3*n). For each of 200 random splits, we estimated the model parameters with the training set and calculated the accuracy measure on the basis of the validation set. The resulting cross-validated AUC was an average over all random splits. Confidence intervals were centered around the cross-validation estimate with width determined by the perturbed variance estimate. To evaluate the incremental value provided by the GRS, we compared the predictive accuracy of the model with GRS with the model without GRS, both including all clinical predictors. A CI for the difference in AUC when the GSR was added was constructed with the estimated difference in AUC with variance on the basis of the perturbation-resampling method, which accounts for the correlation between the 2 accuracy measure estimates.

To quantify the improvement in the proportion of explained variation due to the addition of GRS to the clinical predictors (24), we used the sum of squares for logistic regression as a basis for calculating the proportion of explained variation.
**Results**

Clinical characteristics of cases and control subjects. Clinical characteristics of participants at baseline (NHS and HPFS) or examination (JHS) are summarized in Table 1 according to study and CHD status. Within each study, age at examination and body weight were similar in subjects with and without CHD. In the HPFS study, CHD cases were younger than control subjects at diabetes diagnosis, whereas no significant case-control differences in this variable were observed in the NHS and JHS studies. In the JHS study, the proportion of men was significantly higher in CHD cases than in CHD control subjects. In all 3 populations, CHD cases had higher HbA1c (a measure of poor glycemic control), lower HDL values, and a more frequent history of hypertension and hypercholesterolemia than subjects without evidence of CHD. A history of smoking was almost twice as common in cases as in control subjects in the JHS study; a more modest, nonsignificant association between smoking and CHD was observed in the NSH and HPFS studies.

**Association between individual loci and CHD.** All SNPs were common in the study samples, with risk allele frequency comparable to the Hapmap reference (CEU) (Online Table 2). All SNPs but 1 (rs6725887) were in HWE. The SNP rs6725887 showed a significant HWE deviation ($p < 0.05$) in both the NHS and HPFS cohorts (in non-CHD control subjects as well as in cases and control subjects combined), possibly due to diabetic patients not being representative of the general population or to genotyping errors. Therefore, we excluded this SNP from further analysis.

No significant evidence of heterogeneity was observed among the 3 studies in the effect of the SNPs on CHD risk (all $p$ values for heterogeneity >0.05), and no significant differences were observed in the association between SNPs and CHD between sexes. Five SNPs (rs4977574 [CDKN2A/2B], rs12526453 [PHACTR1], rs646776 [CELSR2-PSRC1-SORT7], rs2259816 [HNF1A], and rs11206510 [PCSK9]) showed an association with CHD that went in the same direction across studies and was in agreement with the association pattern described in the previous GWAS reports (Table 2). When the 3 studies were analyzed together, the ORs did not change after adjustment for age, sex, glycemic control, HDL, and history of smoking, hypertension, and hypercholesterolemia, indicating that the effect of these SNPs was not mediated by an effect on other cardiovascular risk factors. Two other SNPs (rs9818870 at MRAS and rs998260 at MRPS6–SCL5A3–KCNE2) approached

**Table 1.** Characteristics of the Participants

<table>
<thead>
<tr>
<th></th>
<th>NHS</th>
<th>HPFS</th>
<th>JHS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CHD Absent (n = 544)</td>
<td>CHD Present (n = 309)</td>
<td>p Value</td>
</tr>
<tr>
<td>Age at baseline/examination, yrs</td>
<td>60 ± 6</td>
<td>60 ± 6</td>
<td>0.9</td>
</tr>
<tr>
<td>Age at diabetes diagnosis, yrs</td>
<td>52 ± 11</td>
<td>52 ± 9</td>
<td>0.3</td>
</tr>
<tr>
<td>Duration of diabetes, yrs</td>
<td>6 ± 8</td>
<td>8 ± 8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.8 ± 6.2</td>
<td>30.0 ± 6.4</td>
<td>0.88</td>
</tr>
<tr>
<td>HbA1c</td>
<td>6.7 ± 1.7</td>
<td>7.2 ± 1.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of hypertension</td>
<td>35.5</td>
<td>45.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of hypercholesterolemia</td>
<td>25.5</td>
<td>35.0</td>
<td>0.002</td>
</tr>
<tr>
<td>HDL, mg/dl</td>
<td>52 ± 15</td>
<td>49 ± 14</td>
<td>0.002</td>
</tr>
<tr>
<td>Smoking</td>
<td>Never</td>
<td>47.7</td>
<td>39.9</td>
</tr>
<tr>
<td>Past</td>
<td>40.3</td>
<td>45.8</td>
<td>54.6</td>
</tr>
<tr>
<td>Current</td>
<td>12.0</td>
<td>14.3</td>
<td>5.7</td>
</tr>
</tbody>
</table>

*Values are n, %, or mean ± SD. SDs. Characteristics at baseline for the NHS Nurses’ Health Study and HPFS (Health Professional Follow-up Study) studies and at examination for the JHS study (Joslin Heart Study).*

**BMI** = body mass index; **CHD** = coronary heart disease; **HbA1c** = hemoglobin A1c; **HDL** = high-density lipoprotein.
nominal significance in the combined analysis, but their association with CHD went in the opposite direction of that observed in the GWAS, the risk allele being associated with protection (Table 2).

No significant association with coronary artery disease was observed for the 2 haplotypes defined by SNPs rs2048327, rs3127599, rs7767084, and rs10755578 in the SLC22A3 gene cluster on chromosome 6q26–q27 that was observed for the 2 haplotypes defined by SNPs rs2048327, rs3127599, rs7767084, and rs10755578 in the SLC22A3 gene cluster on chromosome 6q26–q27 that was observed for the 2 haplotypes defined by SNPs rs2048327, rs3127599, rs7767084, and rs10755578 in the SLC22A3 gene cluster on chromosome 6q26–q27. Relative to the most frequent haplotype (TCTC), the ORs of CHD for the predisposing haplotype CCTC were not significantly different from 1 with this sample size (1.21 [95% CI: 0.91 to 1.56], 0.93 [95% CI: 0.73 to 1.20], 0.98 [95% CI: 0.79 to 1.23], and 0.98 [95% CI: 0.79 to 1.23] in the NHS, HPFS, and JHS studies and combined analysis, respectively. The ORs were larger for the other predisposing haplotype CTTG but were not significantly different from 1 in the HPFS study (1.10 [95% CI: 0.93–1.28], 1.15 [0.91–1.45], 0.96 [0.78–1.19], and 1.04 [0.91–1.18], respectively for the HPFS, NHS, JHS, and combined analyses, respectively).

### Table 2: Associations of Reported CHD SNPs With CHD Risk in NHS, HPFS, and JHS

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Genes (chr)</th>
<th>Risk Allele</th>
<th>Odds Ratios, 95% CI</th>
<th>p Value Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4977574</td>
<td>CDKN2A/CDKN2B (9p21)</td>
<td>G</td>
<td>1.13 (0.93–1.37)</td>
<td>1.07 (0.88–1.30)</td>
</tr>
<tr>
<td>rs17465637</td>
<td>MiA3 (1q41)</td>
<td>C</td>
<td>1.03 (0.83–1.29)</td>
<td>1.15 (0.91–1.45)</td>
</tr>
<tr>
<td>rs9818870</td>
<td>MRAS (3q22)</td>
<td>T</td>
<td>0.85 (0.65–1.13)</td>
<td>0.97 (0.73–1.32)</td>
</tr>
<tr>
<td>rs12526453</td>
<td>PHACTR1 (6p24)</td>
<td>C</td>
<td>1.12 (0.91–1.38)</td>
<td>1.23 (0.99–1.52)</td>
</tr>
<tr>
<td>rs9982601</td>
<td>MRPS6-SLCSA3-KCNE2 (21q22)</td>
<td>T</td>
<td>0.93 (0.73–1.18)</td>
<td>0.86 (0.66–1.10)</td>
</tr>
<tr>
<td>rs646776</td>
<td>CELSR2-P8RBC1-SORT1 (1p21)</td>
<td>T</td>
<td>1.10 (0.86–1.41)</td>
<td>1.31 (1.03–1.67)</td>
</tr>
<tr>
<td>rs2259816</td>
<td>HNF1A (12q11)</td>
<td>T</td>
<td>1.06 (0.86–1.31)</td>
<td>1.25 (1.01–1.55)</td>
</tr>
<tr>
<td>rs1746048</td>
<td>CXCL12 (10q11)</td>
<td>C</td>
<td>1.08 (0.81–1.44)</td>
<td>0.89 (0.69–1.17)</td>
</tr>
<tr>
<td>rs1122608</td>
<td>LDLR (19p13)</td>
<td>G</td>
<td>1.12 (0.88–1.42)</td>
<td>1.07 (0.85–1.35)</td>
</tr>
<tr>
<td>rs11206510</td>
<td>PCSK9 (1p32)</td>
<td>T</td>
<td>1.05 (0.80–1.37)</td>
<td>1.44 (1.10–1.88)</td>
</tr>
</tbody>
</table>

| chr | chromosome; CI = confidence interval; SNP = single-nucleotide polymorphism; other abbreviations as in Table 1.

### Table 3: Joint Effect of the Loci Significantly Associated With CHD

<table>
<thead>
<tr>
<th>Score</th>
<th>Odds Ratios (95% CI)</th>
<th>p Value Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5</td>
<td>1.00 (1.00–1.00)</td>
<td>1.00 (1.00–1.00)</td>
</tr>
<tr>
<td>6–7</td>
<td>1.15 (0.79–1.67)</td>
<td>1.18 (0.81–1.71)</td>
</tr>
<tr>
<td>≥8</td>
<td>1.53 (1.10–2.15)</td>
<td>1.87 (1.34–2.60)</td>
</tr>
</tbody>
</table>

### Abbreviations as in Tables 1 and 2.
0.000), consistent with the weaker association between the GRS and CHD observed in this study (Table 3). When the 3 studies were considered together, the apparent AUC was 0.715 (95% CI: 0.693 to 0.736) for the model with both the GRS and the clinical predictors, as compared with 0.699 (95% CI: 0.677 to 0.720) for the model including only clinical variables, and 0.577 (95% CI: 0.554 to 0.600) for the model including only the GRS. The corresponding cross-validated values were 0.695 (95% CI: 0.674 to 0.717), 0.682 (95% CI: 0.660 to 0.704), and 0.574 (95% CI: 0.550 to 0.597). The cross-validated increase in AUC determined by the GRS, although relatively small (0.013, 95% CI: 0.008 to 0.018), was significant at the 0.001 level.

Reclassification analysis. Addition of the GRS to the clinical model produced a significant improvement in net reclassification as measured by the NRI in the HPFS (0.2857; 95% CI: 0.197 to 0.237). The cross-validated NRI across the 3 studies was significant (0.2776, 95% CI: 0.185 to 0.370).

Genetic markers versus family history. We finally assessed whether the genetic markers could account for part of the variance of CHD family history and explain its predisposing effect on CHD. We limited this analysis to the HPFS and NHS studies, for which complete data on the occurrence of CHD in parents were available. A family history of CHD—defined as the report of at least 1 affected parent—was significantly associated with an increased risk of CHD in both the NHS (OR: 1.38, 95% CI: 1.00 to 1.91) and HPFS studies (OR: 1.56, 95% CI: 1.04 to 2.32). After adjusting for other risk factors and case-control status, we did not observe any association between family history and the genetic score in either cohort (p = 0.68 and p = 0.99, respectively). In both studies, the change in AUC resulting from adding the genetic markers to a predictive model that
Discussion

In this study of 3 CHD case-control series, we found that 5 of 12 loci previously identified as predictors of CHD in GWAS of the general population also affected CHD risk in the presence of type 2 diabetes. We also showed that the genetic determinants of cardiovascular risk in diabetic patients might be different from the general population.

At all the 5 loci associated with CHD, the association went in the same direction in the 3 series of diabetic subjects and was consistent with that previously reported in the general population (5–9). Some variability was observed in the strength of the associations among the 3 samples, although no significant evidence of heterogeneity was detected at any of the 5 loci, indicating that such differences were compatible with chance. At 2 of the CHD loci (rs4977574 and rs646776), the effect estimates obtained by a meta-analysis of our 3 studies were similar to those previously reported in the general population (8), whereas at the other 3 loci (rs12526453, rs2259816, and rs11206510) effects seemed to be stronger (Table 2, Online Table 1) (7,8).

Our data indicate that these genetic markers, when considered jointly, might exert sizable influence on CHD risk, even though the individual genetic effects seem to be moderate. Individuals with more than 8 risk alleles had almost a 2-fold increase in CHD risk as compared with individuals with 5 risk alleles. Screening the genetic susceptibility might provide important information to discriminate diabetic individuals at high-risk for cardiovascular complications from those at lower risk, considering the relatively high proportion of the 2 extreme groups (19% vs. 30%) in the diabetic population. An added value of the genetic markers is that they can offer information on CHD risk early in life when other cardiovascular risk factors such as hypertension, hypercholesterolemia, or poor glycemic control have yet to emerge. Such a feature is especially attractive if we consider that type 2 diabetes is being diagnosed at an increasingly young age (26).

The genetic markers significantly improved CHD risk prediction when added to conventional risk factors such as age, body mass index, sex, smoking, degree of glycemic control, HDL, and history of hypertension and hypercholesterolemia. This effect, however, was modest. These results are in line with the previous observations that currently identified genetic variants might contribute modestly to the prediction of common disorders such as type 2 diabetes and cancer (27,28). However, our data suggest that adding the genetic information to the model might lead to a 28% net gain with respect to moving the risk estimates toward the correct direction.

Our data suggest that the architecture of genetic susceptibility to CHD might be different in diabetic patients from that in the general population. Across all 3 studies, 2 loci, MRAS and MRPS6–SCL5A3–KCNE2, consistently showed associations with CHD risk that went in the opposite direction from that in the general population, although such effects did not reach statistical significance in the combined analyses. Some other loci, such as the haplotype system at the SLC22A3–LPAL2–LPA locus identified in the general population, were not associated with CHD risk in diabetes. However, the frequencies of the previously reported predisposing haplotypes at the SLC22A3–LPAL2–LPA locus were low in the study samples, approximately 0.02 for CCTC and 0.13 to 0.14 for CTTG, and the failure to replicate the associations might have been partly due to the inadequate power. The mechanisms underlying the different genetic effects in diabetic and nondiabetic populations are not clear. Our previous findings suggest hyperglycemia or other metabolic abnormalities of the diabetic milieu might modulate the genetic effects on cardiovascular risk in diabetes (18). However, we cannot exclude the possibility that the observed differences between the diabetic patients in our study and those in the general population might be due to chance or to differences in study designs. Future adequately powered studies including both diabetic and nondiabetic subjects are warranted to verify our findings.

The differences in genetic effects between diabetic subjects and the general population raise the hypothesis that genetic predictors of CHD might exist that are specific to diabetes. Identification of these genes will require GWAS that are specifically targeted to the diabetic population. The existence of other as yet unidentified genetic predictors of CHD is supported by the fact that the currently identified genetic markers did not explain the predisposing effect of family history on CHD observed in our study. A similar pattern has been observed for other common disorders, such as type 2 diabetes itself (27), prompting an assessment in the published data of the reasons that might account for such "missing heritability" (29). Part of the familial clustering of CHD might be due to the sharing of environmental risk factors among family members, in addition to the existence of as yet unidentified genetic factors. Such putative shared environment, however—if it plays a role—should act through mechanisms other than those of known risk factors, such as smoking, body mass index, or dyslipidemia, because the predictive effect of family history was unaffected by adjustment for these variables.

Study strengths and limitations. Our study has several main strengths, namely the replication design with 3 independent cohorts of diabetic patients, a rigorous definition of CHD, and a sample size that was adequate for the detection of additive genetic effects of the magnitude reported in the published data. Some limitations, however, should be acknowledged. One limitation concerns the generalizability of
our findings. The NHS and HPFS cohorts consist of health professionals, and the JHS study consists of patients receiving their care in an academic environment. Whether our findings can be extended to the general population of diabetic subjects remains to be determined. However, our previous genetic analyses in these cohorts are highly consistent with the observations in other populations (15,30,31). Our study was also restricted to non-Hispanic Whites to avoid the possible confounding effect of race. Other genetic markers might be more effective, on the basis of the known differences in linkage disequilibrium patterns among races, in capturing the predisposing effect of the loci described in this article in other racial groups. Different loci might also be involved in the modulation of CHD risk in other races.

Conclusions

Five loci recently found to be associated with CHD in GWAS of the general population were also associated with CHD among diabetic subjects. Our findings demonstrate similarities in the genetic susceptibility to CHD between the diabetic and nondiabetic populations but also highlight possible peculiarities in the genetic architecture of susceptibility to CHD in diabetes.

Acknowledgments

The authors thank all the participants of the study.

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REFERENCES


Key Words: CHD • diabetes • genetics.

APPENDIX

For supplementary figures, tables, and text, please see the online version of this article.