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Incremental predictive value of 152 single nucleotide polymorphisms in the 10-year risk prediction of incident coronary heart disease: the Rotterdam Study

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Abstract

Objective: To examine the incremental predictive value of genetic risk scores of coronary heart disease (CHD) in the 10-year risk prediction of incident CHD.

Methods: In 5899 subjects, we used 152 single nucleotide polymorphisms (SNPs) associated with coronary artery disease by the CARDIoGRAMplusC4D consortium to construct three weighted genetic risk scores: (i) GRS_{gws} based on 49 genome-wide significant SNPs; (ii) GRS_{fdr} based on 103 suggestively associated SNPs; and (iii) GRS_{all} based on all 152 SNPs. We examined the changes in discrimination and reclassification of incident CHD when adding the genetic risk scores to models including traditional risk factors. We repeated the analysis for prevalent CHD.

Results: The genetic risk scores were associated with incident CHD despite adjustment for traditional risk factors and family history: participants had a 13% higher rate of CHD per standard deviation increase in GRS_{all}. GRS_{all} improved the C-statistic by 0.006 [95% confidence interval (CI): 0.000, 0.013] beyond age and sex, 0.003 (95% CI: −0.001, 0.008) beyond traditional risk factors and 0.003 (95% CI: −0.001, 0.007) beyond traditional risk factors and family history. The genetic risk scores did not improve reclassification. GRS_{all} strongly improved both discrimination and reclassification of prevalent CHD, even beyond traditional risk factors and family history, with a C-statistic improvement of 0.009 (0.003, 0.015).

Conclusions: Although the genetic risk scores based on 152 SNPs were associated with incident CHD, they did not improve risk prediction. This discrepancy may be the result of SNP discovery for prevalent rather than incident CHD, since the SNPs do improve prediction for prevalent disease.

Key words: genetic risk scores, coronary heart disease, prediction, prevention

Key Messages

- Genetic risk scores do not improve risk prediction of incident coronary heart disease (CHD) over traditional risk factors.
- The same genetic risk scores do improve the identification of individuals with prevalent CHD.
- Adding suggestively associated genetic variants to the genetic risk score strengthened the association with incident CHD.
- The genetic risk scores provided largely independent information on top of family history.

Introduction

Primary and secondary prevention programmes are widely performed using risk prediction models based on traditional risk factors to identify individuals at high risk for coronary heart disease (CHD). Optimizing these risk prediction models could therefore directly translate into improved prevention and management of CHD-related morbidity and mortality. As CHD has a strong heritable component,[1,2] adding genetic markers to prediction models could improve risk prediction. This assumption has been tested in studies using genetic risk scores based on single nucleotide polymorphisms (SNPs).[3–13] Overall, the studies show that prediction is not meaningfully improved by currently validated CHD SNPs.[3–13] Nevertheless, the set of CHD SNPs is growing through the efforts of international consortia, and a recent genome-wide association study (GWAS) by the CARDIoGRAMplusC4D consortium raised the number of independent CHD SNPs from 31to 153.[14] Collectively these SNPs explain around 10% of the genetic variance,[14] which suggests that we are now in a better position to implement SNPs in risk prediction of CHD.

These SNPs, however, were identified using case-control and cross-sectional designs. In these study designs, SNPs associated with a favourable prognosis after CHD events may be overrepresented in cases. As a consequence, the association of these SNPs may not fully translate to incident CHD, leading to markers that are spuriously associated with CHD.

We hypothesized that adding genetic risk scores based on CHD SNPs would improve 10-year CHD risk prediction when added to traditional risk factors.To evaluate our hypothesis, we constructed three genetic risk scores based on CHD SNPs found by the CARDIoGRAMplusC4D consortium. We then examined whether risk prediction improved when we added the genetic risk scores to three models including: (i) age and sex; (ii) age, sex and traditional risk factors; and (iii) age, sex, traditional risk factors and family history. To examine differences between incident and prevalent CHD, we repeated the analysis for prevalent CHD.

Methods

Study population

This study was conducted within the Rotterdam Study, an ongoing prospective population-based cohort study of inhabitants of Ommoord, a district of Rotterdam in The Netherlands. The Rotterdam Study has been described in detail elsewhere.[15,16] In the year 1990, inhabitants of Ommoord who were 55 years old or over were invited to participate. Baseline examination lasted from 1990 to 1993 and included 7983 participants, of whom 7758 gave their informed consent for follow-up data collection. Follow-up examinations were carried out every 3 to 5 years. The study was approved by the Medical Ethics Committee of Erasmus University, Rotterdam, The Netherlands, and all included participants gave their written informed consent.

Genotyping and imputation

Genotyping was successfully conducted in 5899 participants who agreed to be followed-up, using the Illumina 550 K. Imputation was done with reference to HapMap release 22 CEU using the maximum likelihood method implemented in MACH.[17–19] The imputation quality of the SNPs is presented in Supplementary Table 1 (available as Supplementary data at *IJE* online).

Genetic risk scores

To construct genetic risk scores, we used 153 uncorrelated SNPs associated with CHD by the CARDIoGRAMplusC4D consortium, of which 49 attained genome-wide significance and the remaining 104 had a false discovery rate (FDR) of less than 10% in an FDR analysis.[14] Out of the 153 SNPs, 152 were either genotyped or imputed in the Rotterdam

Study. We calculated weighted dosages by multiplying the risk allele (the allele previously reported to increase the risk of CHD) dosage of each SNP with its previously reported effect size (lnOR)[14]. GRS_{gws} was constructed using the 49 genome-wide significant SNPs, GRS_{fdr} using the 103 additional SNPs that were found in the FDR analysis, and GRS_{all} using all 152 SNPs. Genetic risk scores were computed using the PredictABEL package in R version 2.15.1 (R Foundation for Statistical Computing, Vienna, Austria).[20]

Coronary heart disease

CHD events included myocardial infarctions, all CHD mortality and revascularization. Cardiovascular outcome definitions as well as data collection methods are presented in detail elsewhere.[21] In brief, participants with general practitioners in the district of Ommoord were continuously monitored for fatal and nonfatal cardiovascular events through automated linkage with files from general practitioners and hospitals. Participants with general practitioners outside Ommoord were monitored through annual checks of their medical records. All reported events were independently reviewed and coded by two research physicians. Codes on which the research physicians disagreed were discussed to reach consensus, and a medical expert in cardiovascular disease subsequently reviewed all events.

Traditional risk factors and family history

Serum total and high-density lipoprotein (HDL) cholesterol concentrations were determined at baseline within 2 weeks after sampling by an automated enzymatic procedure in non-fasting blood samples (Kone Specific Analyzer, Kone Instruments). Blood pressure was measured while seated, using a random-zero sphygmomanometer at the right brachial artery. The average of two consecutive measurements was used. Diabetes was defined as fasting plasma glucose levels $\geq$7 mmol/l or non-fasting plasma glucose $\geq$11.1 mmol/l or use of medications indicated for the treatment of diabetes. Current smoking status (yes/no), family history of myocardial infarction in first-degree relatives (yes/no), lipid-lowering medication use (yes/no) and antihypertensive medication use (yes/no) were assessed during a structured interview at baseline by trained research assistants.

Statistical analyses

Statistical analyses were done using SPSS version 20 (IBM, Armonk, NY) and R version 2.15.1. Missing values for all covariates were imputed using expectation maximization in SPSS. Participants with prevalent CHD at baseline were excluded, and hazard rates were computed using Cox proportional hazard models. Three adjustment models were used. Model 1 was adjusted only for age and sex. Model 2 was further adjusted for total and HDL cholesterol, systolic blood pressure, prevalent type 2 diabetes, antihypertensive medication, lipid-lowering medication and current smoking. Model 3 was additionally adjusted for family history of myocardial infarction. In addition to standard *P*-values, we computed Bonferroni corrected *P*-values for the associations of the genetic risk scores with incident CHD, using the p.adjust function in R. We applied a correction for nine statistical tests (the three genetic risk scores were each tested in three models). All models met the assumption of proportional hazards, which was tested for each model using the 'cox.zph' function in R. Absolute 10-year risk was estimated as explained by Wilson *et al.*[22] These predicted risks were used to classify participants into low (<5%), intermediate-low (5–10%), intermediate-high (10–20%) and high (>20%) risk categories. Changes in C-statistic were used to assess improvements in discrimination, and the categorical net reclassification improvement (NRI) was used to assess improvements in reclassification.[23] NRIs were calculated using the prospective form applicable to survival data as introduced by Pencina *et al.*[24] We used 10 000 bootstrap resamples to generate 95% confidence intervals for changes in C-statistic and prospective NRI. We performed several additional analyses. First, improvements in prediction were also calculated in the subgroup of 2082 participants who were under 65 years old at baseline. Second, we examined the association of the genetic risk scores with myocardial infarction and estimated the corresponding improvements in prediction. Furthermore, we used Cox proportional hazard models to examine the association between family history and incident CHD, using different adjustments: age- and sex-adjusted, further adjusted for traditional risk factors and further adjusted for each of the genetic risk scores.

The genetic risk scores used SNPs that were identified for prevalent rather than incident CHD. To examine whether this affects their predictive value, we repeated the analysis separately for prevalent cases. For prevalent CHD, odds ratios were computed using logistic regression, and both the predicted risks and NRIs were calculated using PredictABEL.[20] Nagelkerke's R^2 was used to estimate the variance in incident and prevalent CHD explained by different combinations of risk factors.[25]

Results

Out of 5899 participants, 485 participants had prevalent CHD at baseline. During a mean follow-up period of 12.8 years, 964 CHD events (460 myocardial infarctions)

occurred among the remaining 5414 individuals. Of these events, 571 (270 myocardial infarctions) occurred within 10 years. Baseline characteristics of the study population are shown in Table 1, and baseline characteristics by CHD status are shown in Supplementary Table 2 (available as Supplementary data at *IJE* online).

All three genetic risk scores were associated with incident CHD. The associations were attenuated when adjusting for traditional risk factors, and further attenuated when additionally adjusted for family history. These associations are shown in Table 2. The association between family history and incident CHD largely remained stable when the genetic risk scores were added to the model (Supplementary Table 3, available as Supplementary data at *IJE* online).

Improvements in discrimination and reclassification of incident CHD are shown in Table 3. The largest improvement in risk prediction was achieved by GRS_{all} beyond age and sex ($\Delta C = 0.006$, 95% CI: 0.000, 0.013); however, it did not improve reclassification. Furthermore, improvements in discrimination or reclassification beyond traditional risk factors or traditional risk factors + family history were very modest. In participants under the age of 65, the genetic risk scores led to greater improvements in prediction than in the entire sample, although these were accompanied by larger confidence intervals (Supplementary Table 4, available as Supplementary data at *IJE* online). The associations and improvements in prediction were considerably weaker for incident MI than for prevalent CHD (Supplementary Tables 5 and 6, available as Supplementary data at *IJE* online).

All three genetic risk scores were associated with prevalent CHD (Supplementary Table 7, available as Supplementary data at *IJE* online), and these associations were stronger than the associations with incident CHD. Improvements in the prediction of prevalent CHD were almost always markedly higher than improvements in prediction of incident CHD events (Supplementary Table 8, available as Supplementary data at *IJE* online). All three genetic risk scores improved discrimination beyond the three models. GRS_{all} improved discrimination the most (ΔC 0.009 beyond traditional risk factors and family history, 95% CI: 0.003, 0.015). GRS_{all} also improved reclassification beyond the three models, whereas GRS_{gws} only improved reclassification beyond age + sex and traditional risk factors. GRS_{fdr} did not improve reclassification beyond any of the models.

The percentage of variance in incident and prevalent CHD explained by the genetic risk scores, risk factors and their combinations are shown in Supplementary Table 9 (available as Supplementary data at *IJE* online). Genetic risk scores consistently explained a larger proportion of the variance of prevalent CHD than of incident CHD: GRS_{all} explained 1.5% of the variance of prevalent CHD, but only 0.7% of the variance of incident CHD. In both cases, only 0.1% of the variance was also explained by family history. GRS_{all} explained a larger proportion of the variance of both incident and prevalent CHD than family

Table 1. Baseline characteristics of the 5899 participants included in this study

	Mean (SD) or percentage
Age (years)	69.3 (9.0)
Sex (% males)	40.9
Total cholesterol (mmol/l)	6.6 (1.2)
HDL cholesterol (mmol/l)	1.34 (0.4)
Lipid-lowering medication use	2.5
Antihypertensive medication use	13.3
Systolic blood pressure (mmHg)	139.2 (22.3)
Diastolic blood pressure (mmHg)	73.7 (11.5)
Prevalent type 2 diabetes	10.6
Current smoking	23.1
BMI (kg/m^2)	26.3 (3.7)
Parental history of myocardial infarction	37.2

BMI, body mass index.

Table 2. Hazard ratios (95% confidence intervals) per SD change of genetic risk scores for incident CHD

	Model 1	*P*-value	Bonferroni-corrected *P*-value*	Model 2	*P*-value	Bonferroni-corrected *P*-value*	Model 3	*P*-value	Bonferroni-corrected *P*-value*
GRS_{gws}	1.13 (1.06, 1.20)	0.00014	0.0013	1.12 (1.05, 1.19)	0.00054	0.0049	1.11 (1.05, 1.19)	0.00076	0.0068
GRS_{fdr}	1.09 (1.03, 1.17)	0.0051	0.046	1.08 (1.01, 1.15)	0.02	0.18	1.07 (1.01, 1.14)	0.032	0.29
GRS_{all}	1.15 (1.08, 1.23)	1.1×10^{-5}	9.9×10^{-5}	1.13 (1.06, 1.21)	0.00012	0.0011	1.13 (1.06, 1.20)	0.00022	0.0020

GRS_{gws}, genetic risk score including only CHD SNPs significant according to genome-wide significance; GRS_{fdr}, genetic risk score including only CHD SNPs significant according to false discovery rate analysis; GRS_{all}, genetic risk score including all significant CHD SNPs.

Model 1: age- and sex-adjusted; Model 2: further adjusted for total and HDL cholesterol, systolic blood pressure, prevalent type 2 diabetes, antihypertensive medication, lipid-lowering medication and current smoking; Model 3: further adjusted for family history of myocardial infarction.

*Bonferroni-corrected *P*-values are corrected for nine statistical tests.

Table 3. Improvements in discrimination and reclassification of incident CHD when adding genetic risk scores to 10-year risk prediction models

	C	ΔC	NRI
Model 1	0.684		
GRS_{gws}		0.004 (−0.001, 0.009)	0.023 (−0.021, 0.067)
GRS_{fdr}		0.004 (−0.001, 0.008)	0.003 (−0.04, 0.046)
GRS_{all}		0.006 (0.000, 0.013)	0.034 (−0.014, 0.081)
Model 2	0.716		
GRS_{gws}		0.002 (−0.001, 0.006)	0.014 (−0.019, 0.047)
GRS_{fdr}		0.002 (−0.001, 0.005)	0.01 (−0.024, 0.044)
GRS_{all}		0.003 (−0.001, 0.008)	0.022 (−0.018, 0.061)
Model 3	0.716		
GRS_{gws}		0.002 (−0.001, 0.006)	0.016 (−0.019, 0.051)
GRS_{fdr}		0.002 (−0.001, 0.004)	0.007 (−0.026, 0.04)
GRS_{all}		0.003 (−0.001, 0.007)	0.017 (−0.025, 0.058)

C, C-statistic before adding genetic risk scores to the model; ΔC, improvement in C-statistic when adding the genetic risk score to base models; NRI, net reclassification improvement when adding the genetic risk score to base models; GRS_{gws}, genetic risk score including only CHD SNPs significant according to genome-wide significance; GRS_{fdr}, genetic risk score including only CHD SNPs significant according to false discovery rate analysis; GRS_{all}, genetic risk score including all significant CHD SNPs.

Model 1 includes age and sex; Model 2 further includes total and HDL cholesterol, systolic blood pressure, prevalent type 2 diabetes, antihypertensive medication, lipid-lowering medication and current smoking; Model 3 further includes family history of myocardial infarction.

history, age, total cholesterol, systolic blood pressure, smoking and lipid-lowering medication use.

Discussion

In this study we showed that genetic risk scores, based on up to 152 SNPs so far identified for prevalent CHD, are associated with incident CHD, though they do not lead to clinically relevant improvements in 10-year risk prediction of CHD.

SNPs could be used in CHD risk prediction in two different settings. The first is to use genetic data in adults and elderly subjects to improve risk prediction beyond current CHD risk prediction models. Our results show that currently available SNPs are not sufficient for this application. A second use of SNPs is to estimate the future risk of CHD earlier in life. This could be in the form of lifetime risk, or in the form of 10-year risk at different ages. In this setting, SNPs are already useful if they improve prediction over age and sex. Our study suggests that current GWAS findings may be more useful for this setting.

Several studies have shown that genetic risk scores based on SNPs for prevalent CHD are associated with incident CHD, though improvements in prediction are generally very small.[3–7] Ganna *et al* have previously tested a genetic risk score similar to GRS_{gws},[7] and they found slightly larger improvements in discrimination and reclassification. In contrast to our study, they recalculated the weight of each included SNP in an independent prospective cohort. This step may partly explain the differences between our studies. Another study suggested that SNPs might be especially useful in specific subgroups such as middle-aged men.[4] Our study was not sufficiently powered to examine predictive improvements in this subgroup, but we did find greater improvements in prediction when we limited our analysis to participants under 65 years old.

Our genetic risk scores were based on GWA studies. Given that collecting the large number of cases needed for adequate statistical power is easier in a case-control setting with prevalent cases, a large proportion of studies included in these GWA studies are composed of case-control studies. Such a design, though statistically more powerful, may lead to the identification of SNPs that are related to improved survival after events rather than SNPs that increase the risk of event. This is known as Neyman's bias or incidence-prevalence bias.[26] If so, the identified SNPs for CHD, and hence the genetic risk score herewith evaluated, might represent a mixture of SNPs associated with CHD risk and SNPs associated with an improved survival after a CHD event. Indeed, we found a striking rise in the incremental value of the genetic risk scores when we used prevalent CHD as the outcome instead of incident CHD. Furthermore, a previous study of prevalent CHD also found a large C-statistic improvement beyond traditional risk factors (0.008) in contrast to the small improvements found by studies of incident CHD.[8] This difference suggests that the inability of SNPs to contribute to risk prediction is in part explained by the cross-sectional discovery panel. This is also supported by our findings, as percentage of variance explained. For instance, the variance explained by GRS_{all} in prevalent CHD was twice as large as in incident CHD. This bias may hamper the ability of genetic risk scores to improve prediction of first CHD events in populations free of CHD.[27] We present only preliminary evidence that this is influencing risk prediction: prevalent events occurred earlier in life than incident events, and this may partly explain the observed differences in risk prediction. Individuals experiencing CHD events at a younger age may be genetically enriched for CHD SNPs. In line with this, the percentage of individuals with a family history of myocardial infarction is slightly higher in prevalent cases than in incident cases.

A potential solution may be to recalculate the weight of each included SNP in an independent prospective cohort, as done by Ganna *et al.*[7] Nevertheless, this approach still assumes that important SNPs for prevalent CHD are also important for incident CHD, and did not lead to substantially higher indices of discrimination and reclassification.

Instead, it may be necessary to conduct a GWAS on incident CHD restricted to prospective cohort studies.

Conducting large-scale genetic studies in prospective cohort studies is likely to lead to more clinically relevant SNPs for prediction, but there are further developments that may also achieve this goal. First, increasing the discovery GWAS sample size will continue to lead to more effective genetic risk scores, by identifying new SNPs and by refining the effect estimates of known SNPs. Chatterjee *et al*. projected that the predictive performance of genetic risk scores for CHD may keep improving as GWAS samples increase to as much as 10 times their current size.[28] Our study also supports intensifying the discovery effort: the most effective risk score not only included SNPs robustly associated with CHD, but also 103 further SNPs suggestively associated with CHD. Second, denser genotyping arrays, denser imputation panels, and exome and whole-genome sequencing studies may yield low-frequency and rare variants for CHD that were hidden from GWAS. Whereas common variants usually have small effect sizes due to evolutionary constraints, rarer variants may also have intermediate to large effect sizes. Therefore, although a single rare variant only explains a small proportion of variance in the general population, it can explain a large proportion of variance in families where it is present.

Family history only overlapped slightly with the genetic risk scores in the variance of CHD explained, providing largely independent information. Our results suggest that family history largely tags genetic variants that are not well covered by GWAS, or aspects of the shared environment that are independent of traditional risk factors. These hidden risk factors appear to affect CHD risk by increasing the burden of subclinical atherosclerosis.[29]

This study has certain strengths and limitations. First, we examined the association between the genetic risk scores and both incident and prevalent CHD in the same population, allowing us to compare these associations. Since associated SNPs were identified using the largest available GWAS of CHD, a relatively large set of CHD SNPs with well-estimated weights was used, including multiple independently associated SNPs per locus when known. Previous studies have focused on genome-wide significant SNPs to include only the most robustly associated SNPs. This was also our approach for GRS_{gws}, but by including both genome-wide significant SNPs and suggestively associated SNPs in GRS_{all}, we were able to create a stronger genetic instrument than GRS_{gws}. In addition, this study included individuals of 55 years and older, which corresponds well with the target population for prediction. On the other hand, our population consisted entirely of Caucasians, and our results may not be generalizable to other populations. Furthermore, we used a crude measure of family history. First, family history was only available for myocardial infarction and not for CHD in general. Second, family history was obtained during an interview, and may not always be complete. Third, participants were only asked about first-degree relatives. However, these limitations reflect difficulties in measuring family history that also arise in clinical practice.

Whereas our results do not support a role for currently available common SNPs in CHD risk prediction in the traditional setting, they do suggest that SNPs could already improve prediction of future CHD earlier in life, when other variables used in prediction are not yet available. Our results also suggest that SNPs identified through GWAS of prevalent disease may not be optimally suited for the prediction of incident disease. This mismatch may extend to other diseases with high mortality rates.

Supplementary Data

Supplementary data are available at *IJE* online.

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