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A genetic risk score based on direct associations with coronary heart disease improves coronary heart disease risk prediction in the Atherosclerosis Risk in Communities (ARIC), but not in the Rotterdam and Framingham Offspring, Studies

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Abstract

Objective—Multiple studies have identified single-nucleotide polymorphisms (SNPs) that are associated with coronary heart disease (CHD). We examined whether SNPs selected based on predefined criteria will improve CHD risk prediction when added to traditional risk factors (TRFs).

Methods—SNPs were selected from the literature based on association with CHD, lack of association with a known CHD risk factor, and successful replication. A genetic risk score (GRS) was constructed based on these SNPs. Cox proportional hazards model was used to calculate CHD risk based on the Atherosclerosis Risk in Communities (ARIC) and Framingham CHD risk scores with and without the GRS.

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None.

Results—The GRS was associated with risk for CHD (hazard ratio [HR] = 1.10; 95% confidence interval [CI]: 1.07–1.13). Addition of the GRS to the ARIC risk score significantly improved discrimination, reclassification, and calibration beyond that afforded by TRFs alone in non-Hispanic whites in the ARIC study. The area under the receiver operating characteristic curve (AUC) increased from 0.742 to 0.749 (Δ = 0.007; 95% CI, 0.004–0.013), and the net reclassification index (NRI) was 6.3%. Although the risk estimates for CHD in the Framingham Offspring (HR = 1.12; 95% CI: 1.10–1.14) and Rotterdam (HR = 1.08; 95% CI: 1.02–1.14) Studies were significantly improved by adding the GRS to TRFs, improvements in AUC and NRI were modest.

Conclusion—Addition of a GRS based on direct associations with CHD to TRFs significantly improved discrimination and reclassification in white participants of the ARIC Study, with no significant improvement in the Rotterdam and Framingham Offspring Studies.

Keywords

Genetics; Risk factors; Coronary disease

1. Introduction

Risk assessment plays a pivotal clinical role in prevention strategies and in therapy for coronary heart disease (CHD) at the individual level, and therefore is fundamental to future efforts in personalized medicine in this area. Current risk prediction models are based on traditional risk factors (TRFs) such as age, sex, smoking, lipid levels, and blood pressure, and although extensively validated, these models have limitations [1]. In recent years, multiple studies have assessed the ability of “emerging risk factors,” including biomarkers, imaging, and genetic variants, to improve CHD risk assessment beyond the use of TRFs [2]. Using genetic markers to improve CHD risk prediction has been greatly facilitated by successful genome-wide association studies (GWAS) and candidate gene studies that have led to the discovery of multiple new single-nucleotide polymorphisms (SNPs) associated either with an intermediate phenotype of CHD, such as plasma cholesterol levels, or directly with CHD.

The purpose of this study was to construct a genetic risk score (GRS) by using a novel approach of selecting SNPs that are directly associated with CHD, not associated with known TRF intermediate phenotypes, and improve CHD risk prediction. SNPs were identified based on systematic literature and database review using pre-defined criteria. Both GWAS results and candidate gene approaches were considered. We hypothesized that adding the GRS to TRF-based models would improve the ability of these models to predict CHD events in three independent white population cohorts in the Atherosclerosis Risk in Communities (ARIC), Rotterdam, and Framingham Offspring Studies.

2. Methods

2.1. Setting and participants

The ARIC Study served as our principal cohort for this analysis. We extended our analysis to the Rotterdam Study [3–5] and the Framingham Offspring Study [6,7] as two independent cohort studies to replicate and confirm our findings. Since most of the SNPs in our GRS were identified in Caucasians, we included only non-Hispanic whites in our analysis. See Online Supplementary Material for more details regarding the individual studies.

A detailed description of the design and methods for the ARIC Study has been published elsewhere [8,9]. ARIC participants included in this study were 45–64 years of age when enrolled in 1987–89 and were followed for the development of clinical CHD. A CHD event

was defined as definite or probable myocardial infarction, silent myocardial infarction (indicated by electrocardiogram) between 4 examinations in 1987–1998, definite CHD death, or coronary revascularization). Incident CHD events were determined by annually contacting each ARIC participant and reviewing hospital discharge lists and death certificates.

2.2. Selection of SNPs

Two parallel approaches were employed to identify SNPs for the GRS (Supplementary Table 1). In the first approach, we searched the National Human Genome Research Institute database (updated as of December 2009), which included SNPs identified by means of GWAS and catalogued based on phenotype and/or trait [10]. We considered the key words: “coronary artery disease,” “coronary disease,” “myocardial infarction,” and “early myocardial infarction.” The second approach included SNPs that were identified through candidate gene approaches and were also included in a published GRS for CHD.

Chosen SNPs had to meet the following inclusion criteria: (1) significant association with CHD in addition to no known association with an intermediate cardiovascular phenotype in the literature; (2) for SNPs identified through a GWAS approach, $P < 1 \times 10^{-7}$; (3) replication of the SNPs’ associations with CHD in at least one additional independent sample.

Of the 28 SNPs identified from GWAS, 12 were excluded because of known association with an intermediate phenotype of CHD, 4 were excluded for lack of statistically significant association (by our criterion) in the discovery GWAS, and 3 were excluded because of strong linkage disequilibrium with a selected SNP, rs10757274, which resides in the 9p21 region. A total of 9 SNPs were eligible for the current analysis, including rs9818870, rs2259816, rs9982601, rs12526453, rs1746048, rs6725887, rs6922269, rs501120, and rs10757274 (Supplementary Table 1).

For the second approach, a literature search was conducted using PubMed to identify studies published between January 1985 and December 2009 that examined SNPs as part of a GRS for CHD. The search term “genetic risk score and coronary heart disease” yielded 171 publications. Of those, 107 were excluded because the SNPs were found to be related to an intermediate phenotype associated with CHD, 9 were excluded because the study population was not Caucasian, 3 were excluded for lack of replication in other studies, and 49 were excluded either for negative study findings or because the study outcome focused on phenotypes other than clinical CHD. Two publications revealed four candidate SNPs (rs3900940, rs1010, rs7439293, and rs2298566), which were replicated in additional studies as described in Supplementary Table 1 [11–14].

In total, 13 SNPs met the study inclusion criteria for these analyses: rs9818870, rs2259816, rs9982601, rs12526453, rs1746048, rs6725887, rs6922269, rs501120, rs3900940, rs1010, rs7439293, rs2298566, and rs10757274 (Supplementary Table 2).

2.3. Construction of the unweighted and weighted GRS

For construction of the GRS, each of the 13 SNPs was initially examined for independent association with incident CHD in a Cox proportional hazards model that included TRFs (as defined below). The direction of the SNP’s β coefficient in this model was used to code each SNP so that the risk allele homozygote = 1, heterozygote = 0, and nonrisk homozygote = -1 (e.g., rs10757274: CC = 1, AG = 0, AA = -1). The 13 recoded SNPs were then summed to generate a GRS for each study participant ranging theoretically from -13 to 13. Because risk prediction varied by individual SNP when included in the TRF-based model, we chose to construct a weighted GRS that accounted for the varying degrees of prediction. The ARIC

study weighted the GRS by multiplying each participant's allele score (1, 0, -1) by the SNP's β coefficient from the predictive model (e.g., rs10757274: 1×0.19378 , 0×0.19378 , -1×0.19378). The Rotterdam and Framingham Studies followed this procedure by scoring the alleles for each SNP as they were identified in the ARIC population. The 13 products were then summed to create a weighted GRS for each study participant. Cox proportional hazards models were constructed to estimate the risk of CHD in 10 years by the ARIC risk score [15], the TRFs + unweighted GRS, and TRFs + weighted GRS.

The Framingham Risk Score (FRS), a well-established CHD risk assessment tool, was used for 10-year CHD risk assessment and was calculated by summing points assigned for each risk factor including age, total cholesterol, high-density lipoprotein cholesterol (HDL-C), blood pressure, diabetes, and smoking [1]. Points were assigned separately for men and women because of differences in how Framingham weights the TRF by sex. These data were then combined and the 13 SNPs were individually assessed in the FRS basic model, and their β coefficients were used to calculate the weighted GRS using the methods described above.

2.4. Statistical analysis

Multivariate analyses were conducted using Cox proportional hazards modeling to examine the GRS and weighted GRS as independent predictors of CHD when modeled with TRFs. The ARIC CHD Risk Score (ACRS) was developed in the ARIC Study as a tool to assess coronary risk based on TRFs for CHD. We used the ACRS to examine the addition of the GRS and weighted GRS to TRFs in the ARIC and Rotterdam Studies, while the FRS was used to for the analysis of the Framingham Offspring Study. Several statistical metrics were used to test our expanded models that included the GRS variables. The area under the receiver operator curve (AUC) was used to examine the model's discrimination ability.

The net reclassification index (NRI) and the clinical NRI were calculated to assess improvement between the basic and extended models based on the 10-year CHD risk for low (0–5% 10-year CHD risk), intermediate-low (5–10% 10-year CHD risk), intermediate-high (10–20% 10-year CHD risk), and high (>20% 10-year CHD) risk categories [16]. The integrated discrimination index (IDI), which examines model performance independent of risk categories choice, was also calculated [16]. Calibration of these models was tested using the Grønnesby–Borgan goodness-of-fit statistic. The 10-year CHD event rate was determined based on the Kaplan–Meier survival curve for 10-year incident CHD per 100 individuals for a follow-up time of 18 years. All confidence intervals were established using bootstrapping method.

3. Results

Of the 8542 ARIC participants followed for a maximum of 18 years, 1110 (13.0%) had an incident CHD event. In the Rotterdam and Framingham Offspring Studies, 2068 and 2339 participants had event rates of 13.1% and 9.2%, respectively. Baseline characteristics of each cohort are presented in Supplementary Table 3. Individuals in the Rotterdam Study were older and had higher systolic blood pressure and total cholesterol compared with individuals in the other studies, whereas individuals in the Framingham Offspring Study were younger, more likely to be smokers, and less likely to be diabetic compared with the other cohorts. In all cohorts, most individuals had a GRS of between -2 and 1, as presented in Supplementary Fig. 1.

In the ARIC Study, the unweighted and weighted GRSs ranged from -13 to 13 (mean: -0.971, SD: 2.27); and -1.03 to 1.12 (mean: 0.162, SD: 0.296), respectively. A higher GRS was associated with a higher unadjusted rate of CHD. Those with scores in the highest GRS

tertile (scores 2–13) had higher rates of CHD (rate: 10.34/1000 person-years; 95% confidence interval [CI]: 9.47–11.20) compared to those in the second tertile (scores 0–1) (rate: 7.69/1000 person-years; 95% CI: 6.85–8.52), and first tertile (scores –13 to –1) (rate: 7.36/1000 person-years; 95% CI: 6.42–8.30).

After taking into account the TRFs in the ACRS, the GRS was significantly associated with incident CHD in both the unweighted and weighted analyses in the ARIC Study, with GRS of 1.10 (95% CI: 1.07–1.13) per unit unweighted score increase, and 2.30 (95% CI: 1.9–2.8) per unit weighted score increase. Similar associations with CHD were observed in the Rotterdam Study using the ACRS and in the Framingham Offspring Study using the FRS (Table 1).

When the discrimination ability of the GRS was examined in ARIC, the addition of the unweighted GRS to the ACRS significantly improved the AUC from 0.742 to 0.749 ($\Delta = 0.007$; 95% CI: 0.004–0.013), and the addition of the weighted GRS significantly increased the AUC from 0.742 to 0.751 ($\Delta = 0.009$; 95% CI: 0.006–0.014) (Table 2). The AUC did not significantly improve in the Rotterdam Study or the Framingham Offspring Study (Table 2).

In the ARIC Study, the Grønnesby–Borgan goodness-of-fit test did not show a good fit between the observed and expected number of incident CHD cases based on ACRS alone ($P = 0.003$). However, the addition of either unweighted or weighted GRS improved the goodness-of-fit ($P > 0.05$). In the Rotterdam Study, both the basic ACRS model and the extended GRS model showed a good fit using the Grønnesby–Borgan test ($P > 0.4$).

In the ARIC Study, the largest effect of adding the GRS to the ACRS was observed in the intermediate-CHD risk group. Addition of the unweighted GRS to the ACRS resulted in significant reclassification in the intermediate-risk categories, in which ~22% of participants were reclassified (Tables 3a and 3b). The NRI, which examines correct movement between risk categories, was 6.3% (95% CI: 0.004–0.013) after adding the GRS to the ACRS (Table 2). There was no improvement in total reclassification based on the NRI results for the Rotterdam Study (0.2%) and the Framingham Offspring Study (–0.6%). The clinical NRI, which assesses the intermediate-risk categories only, was 6.5%, 9.4%, and 3.2% in the ARIC, Rotterdam, and Framingham Offspring Studies, respectively (Table 2); however, although recommended when performing reclassification analysis, the clinical NRI has limited significance and should be interpreted carefully as recently published by Pepe et al. [17]. As expected, reclassification after the addition of the weighted GRS to the TRFs was superior to that achieved after the addition of the unweighted GRS across the three studies. In the ARIC Study, the NRI was 7.3% (95% CI: 1.9–12) and clinical NRI was 8.8% (95% CI: 5.3–14.9), after adding the weighted GRS (Table 2); in the Framingham Offspring Study, the NRI was 4.5% and clinical NRI was 12.4%; and in the Rotterdam Study, the NRI was 3.6% and clinical NRI was 15.1%. A modest positive IDI was observed in all three studies (Table 2).

4. Discussion

In this prospective study of middle-aged, white individuals, a GRS comprising 13 SNPs selected by predefined criteria modestly improved CHD risk prediction in the ARIC Study but had no significant effect on risk prediction in the Rotterdam and Framingham Offspring Studies. We followed the criteria for evaluation of novel markers recommended by the American Heart Association Expert Panel on Subclinical Atherosclerotic Diseases and Emerging Risk Factors [18] and determined that gene variation improved CHD risk prediction. In the ARIC Study, we demonstrated improvements in discrimination, calibration, and reclassification after addition of the GRS to traditional CHD risk factors (Table 2). The AUC improvement in the ARIC Study after the addition of the GRS to the

ACRS was similar to that observed when diabetes was added to the ARIC model when it was first created [15]. Although the hazard ratios after addition of the GRS to TRFs were significantly improved in all studies, there was no significant improvement in discrimination or reclassification beyond TRFs in the Rotterdam and Framingham Offspring Studies. The largest improvement in reclassification in the three studies was in the intermediate-risk categories, although it has been suggested that the clinical NRI may be misleading and should be interpreted with caution.

A possible reason for the lack of improvement in reclassification in the Framingham Offspring and Rotterdam Studies may be related to the differences in TRFs among the cohorts and the HRs for each of these TRFs (Supplementary Tables 3–6). Although the SNPs selected for this analysis had no known association with TRFs, their predictive ability may have been influenced by them.

The predefined criteria for SNP selection had a pivotal role in the current study design, with an approach used to select SNPs known to be the best predictors of CHD. One of the SNPs used in our GRS was in the 9p21 region (9p21 allele), and its association with CHD has been extensively validated [19–22]. Studies examining its utility in improving CHD risk prediction showed modest or no improvement in metrics of risk assessment and classification [21–23]. However, a recent study showed a modest improvement in risk prediction using a 12-SNP GRS that was added to the 9p21 allele, which was replicated in a case–control study [24].

Other studies concentrated on SNPs with known intermediate phenotypes associated with CHD. Kathiresan et al selected SNPs based on their previous association with HDL-C and low-density lipoprotein cholesterol (LDL-C); their analysis showed no improvement in discrimination and a modest improvement in reclassification expressed by the NRI [25]. Paynter et al examined a GRS comprised of 101 SNPs associated with intermediate cardiovascular phenotypes, which failed to improve metrics of risk prediction [26]. The reason we chose to exclude all SNPs that are associated with intermediate CHD phenotypes rather than with TRFs alone was related to the possible clinical utility of a GRS. Our major purpose was to add new risk prediction information, the GRS, for which no clinical measurement is available, as exists for biomarkers and imaging modalities, to improve risk prediction.

To increase further the utility of the GRS to improve risk prediction, we applied unweighted and weighted approaches in our analysis. Although the additive unweighted approach is the most frequently used in published studies of genetic variants and CHD risk prediction, it fails to consider the actual effect of each SNP on the trait. SNPs with large and small effect sizes are treated as having the same effect on CHD risk in the unweighted GRS. Therefore, we used a weighted analysis based on the SNP's β coefficient as determined in each study for each SNP. Although this type of analysis is expected to increase the accuracy of the GRS in ARIC, it is limited by the possible exaggeration of predictive ability because the coefficients were calculated and tested in the same cohort. As expected, the weighted GRS improved the AUC and NRI across all studies.

The study had several limitations. The GRS was based on SNPs that were chosen based on predefined criteria. This process will always result in the inclusion of SNPs that do not improve prediction and the exclusion of others that do. In particular, the process of choosing SNPs that were initially identified by the candidate gene approach is challenging. Multiple SNPs have been examined in past years with the candidate gene approach, and no single uniform criterion has been established to validate associations with CHD by this approach. Therefore, among our inclusion criteria, we chose to limit this study to SNPs that were

previously included in CHD risk scores in the published literature. By doing so, we verified that these SNPs had already been tested and proven by a previous study and were also useful for risk prediction. However, multiple SNPs that may have improved the GRS were excluded in this way. Another limitation of our study is that the GRS is applicable to only the white population. The fact that the 9p21 allele is not associated with CHD risk in black individuals [20] limits the use of the GRS in this population. Similarly, SNPs included in the GRS that were tested only in whites also limits the generalizability of these findings to other nonwhite populations. Finally, we have not examined the effect of adding the GRS to imaging measures, such as carotid intima-media thickness (CIMT) and coronary artery calcium, and to biomarkers such as C-reactive protein.

CIMT was recently shown to improve CHD risk prediction in older adults, with an AUC increase of 0.017 (from 0.728 to 0.745, $P = 0.04$) and NRI of 13.7% ($P < 0.001$) [27], which were better than the AUC improvement and NRI in our study. Interestingly, the combination of CIMT and a single SNP in the 9p21 region was shown to improve risk prediction compared with TRFs and CIMT in the ARIC Study [28], suggesting that certain genetic variants and CIMT have an additive effect on CHD risk prediction. The possibility that adding imaging and biomarker variables to the GRS might improve risk prediction is an important question to be addressed in future studies.

4.1. Conclusions

In summary, we constructed unweighted and weighted GRSs based on SNPs chosen according to distinct criteria. Although hazard ratios for CHD were improved with the addition of the GRS to TRFs in the three cohorts examined, CHD risk prediction was significantly improved only in the ARIC study and not in the Rotterdam and Framingham Offspring Studies; however, we believe that the novel method of SNP selection based on direct association with CHD without an association with an intermediate phenotype may be beneficial in the future when additional SNPs that are highly and directly associated with CHD are discovered.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.atherosclerosis.2012.05.035>.

Table 1

Hazards ratios (HR) per unit score increase as calculated for the unweighted and weighted genetic risk score (GRS) in the ARIC, Rotterdam, and Framingham Offspring Studies.

	Study	HR	Lower CI	Upper CI
Unweighted GRS	ARIC	1.10	1.07	1.13
	Rotterdam	1.08	1.03	1.14
	Framingham	1.12	1.10	1.14
Weighted GRS	ARIC	2.30	1.87	2.83
	Rotterdam	2.05	1.50	2.70
	Framingham	1.12	1.10	1.15

HRs were adjusted for age, sex, smoking, diabetes, systolic blood pressure, antihypertensive medication use, total cholesterol, and high-density lipoprotein cholesterol (HDL-C). In the Rotterdam Study, HRs were calculated for participants younger than 65 years. CI indicates 95% confidence interval.

Table 2

AUC, NRI, clinical NRI, and IDI calculations for the unweighted and weighted GRS in the ARIC, Rotterdam, and Framingham Offspring Studies.

		ARIC	Rotterdam	Framingham
Unweighted GRS	AUC baseline model	0.742	0.729	0.773
	AUC extended model	0.749	0.734	0.775
	AUC improvement	0.007	0.005	0.002
	NRI, %	6.3	0.2	-0.6
	Clinical NRI, %	6.5	9.4	3.2
	IDI	0.006	0.004	0.005
Weighted GRS	AUC baseline model	0.742	0.729	0.773
	AUC extended model	0.751	0.735	0.784
	AUC improvement	0.009	0.006	0.011
	NRI, %	7.3	3.6	4.5
	Clinical NRI, %	8.8	15.1	12.4
	IDI	0.006	0.010	0.017

Area under the receiver operating characteristic curve (AUC) calculations were based on a coronary heart disease proportional hazards model including age, sex, smoking, diabetes, systolic blood pressure, antihypertensive medication use, total cholesterol, and HDL-C with and without the addition of the GRS or weighted GRS; IDI indicates integrated discrimination index; NRI, net reclassification index.

Table 3a

Reclassification of participants among ARIC CHD risk score (ACRS) categories after the addition of the unweighted GRS to traditional risk factors (TRFs) in the ARIC study.

10-year CHD risk category	Classification by ACRS alone, n	Classification by ACRS + unweighted GRS, n (%)				Total reclassified for category
		0-5% [low]	>5- 10% [Intermediate-low]	>10- 20% [Intermediate-high]	>20% [high]	
0-5% [Low]	4249	4035 (95.0)	214 (5.0)	0	0	214
Observed event rate		1.4	7.1			
>5- 10% [Intermediate-low]	2373	316 (13.3)	1793 (75.6)	264 (11.1)	0	580
Observed event rate		5.1	7.6	17.5		
>10- 20% [Intermediate-high]	1578	0	253 (16.0)	1229 (77.9)	96 (6.1)	349
Observed event rate			8.9	14.9	18.1	
>20% [High]	342	0	0	58 (17.0)	284 (83.0)	58
Observed event rate				16.3	29.1	
Total	8542	4353	2258	1551	380	1201

Observed event rate is expressed as number of events per 100 people per 10 years of observation.

Reclassification of participants among ACRS categories after the addition of the weighted GRS to TRFs in the ARIC Study.

Table 3b

10-year CHD risk category	Classification by ACRS alone, n	Classification using ACRS + weighted GRS, n (%)				Total reclassified for category	
		0-5% [low]	>5- 10% [Intermediate-low]	>10- 20% [Intermediate-high]	>20% [high]		
0-5% [Low]	4249	3985 (93.8)	264 (6.2)	0	0	264	
Observed event rate		1.4	5.8				
>5- 10% [Intermediate-low]	2373	373 (15.7)	1713 (72.2)	287 (12.1)	0	660	
Observed event rate		4.6	7.8	17.9			
>10- 20% [Intermediate-high]	1578	3 (0.2)	293 (18.6)	1173 (74.3)	109 (6.9)	405	
Observed event rate		0	8.7	15.0	20.1		
>20% [High]	342	0	0	51 (14.9)	291 (85.1)	51	
Observed event rate				20.7	28		
TOTAL	8542	4361	2270	1511	400	1380	

Observed event rate is expressed as number of events per 100 people per 10 years of observation.