Our genetic risk score (FDR267) was associated with a 1.45 increase in odds ratio and a 1.32 increase in hazard ratio per standard deviation of the score. The score modestly improved the area under the curve for predicting cardiovascular disease (CVD) in the European and African American groups.

Results:
African American groups.

regression and Cox proportional hazards analyses in the European and in the Atherosclerosis Risk in Communities cohort using logistic

with CAD in the UK Biobank cohort meta-analysis. FDR267 was tested (95% confidence interval, 1.39-1.51) increase in odds ratio and a 1.32 (95% confidence interval, 1.26-1.38) increase in hazard ratio per standard deviation of the score. The score modestly improved the area

Our study investigated the performance of a false discovery rate 267-marker genetic risk score (FDR267) in the
under the curve (AUC) statistic when added to a clinical model (ΔAUC = 0.0112, P = 0.0002), FDR267 predicted incident CAD (C-index = 0.60), although it did not improve on clinical risk factors (ΔAUC = 0.0159, P = 0.0965). Individuals in the top quintile of FDR267 genetic risk were at approximately 2-fold increased risk compared with the bottom quintile, which is comparable to risk associated with self-reported family history. The performance of FDR267 was less robust in the African American sample.

Conclusions: FDR267 is significantly associated with CAD in the European sample, with an effect size comparable to self-reported family history. FDR267 discriminated between individuals with and without CAD, but did not improve CAD risk prediction over clinical variables. FDR267 was less predictive of CAD risk in African Americans.

Atherosclerosis Risk in Communities (ARIC) cohort. We stratified the ARIC cohort by ethnicity (European Americans and African Americans) and tested the association between genetic risk and CAD in each sample. Next, we asked whether FDR267 could add predictive value to a model that considers only traditional Framingham risk factors. Finally, we compared the predictive value of FDR267 against a positive self-reported family history.

Materials and Methods

Study population

ARIC is a prospective cohort population of 15,792 European and African Americans aged 45 to 64 years recruited at 4 separate field centres in the United States between 1987 and 1989. These individuals were extensively examined at recruitment; baseline sociodemographic and medical data were gathered. A total of 3 follow-up examinations occurred every 3 years; the time ranges were 1990-1992, 1993-1995, and 1996-1998. One final visit occurred between 2011 and 2013. Yearly follow-up assessments by telephone were also conducted to maintain contact with patients. The event definition for CAD was fatal or nonfatal myocardial infarction (MI), revascularization procedure, and silent MI detected by electrocardiogram. Detailed methodology has been previously published.14 Of these 15,792 individuals, 12,771 were genotyped with the Affymetrix 6.0 chip after quality control or that could be reliably imputed on the basis of an INFO score of >0.3. For the remaining SNPs that did not meet these criteria, we sought reliable proxies by linkage disequilibrium (r² > 0.8) using PLINK 1.9 and the 1000 Genome phase 3 version 5 dataset.

The weighted GRS was calculated with PLINK 1.9 using regression coefficients from the UK Biobank study obtained from the CARDIoGRAMplusC4D website (http://www.cardiogramplusc4d.org/) using the following equation:

\[
\text{Genetic risk score} = \sum_{i=1}^{x} w_i D_i
\]

\[x = \text{total set of SNPs, } w_i = \text{beta, } D_i = \text{dosage of risk allele}\]

Statistical analyses

The computed GRSs were standardised by subtracting the mean and dividing by the standard deviation to obtain odds ratio or hazard ratio (HR) changes per standard deviation of the score. Correlations between continuous variables were tested using the Pearson product-moment correlation. Association with binary outcome variables was tested by fitting data to a logistic regression model and testing for model significance using the analysis of variance chi-square test. Density

1.29-1.51) du rapport des cotes et d’une augmentation de 1,32 (IC à 95 %, 1,26-1,38) du risque relatif par l’écart-type des scores. Le score a modestement améliorer l’aire sous la courbe (ASC) lorsqu’il a été ajouté à un modèle clinique (ΔASC = 0.0112, P = 0.0002). Le FDR267 a prédit les nouveaux cas de MC (C-index [Indice de concordance] = 0.60), mais il n’a pas amélioré les facteurs de risque cliniques (ΔASC = 0.0159, P = 0.0965). Les individus dans le quintile supérieur du risque génétique du FDR267 ont montré un risque accru d’environ 2 fois par rapport au quintile inférieur, soit un risque comparable au risque associé aux antécédents familiaux auto-rapportés. La performance du FDR267 s’est révélée moins robuste chez les Afro-Américains.

Conclusions : Le FDR267 est associé de manière significative à la MC dans l’échantillon d’Européens et a une taille de l’effet comparable aux antécédents familiaux auto-rapportés. Le FDR267 a fait la discrimination entre les individus atteints ou non atteints de MC, mais n’a pas amélioré la prédiction du risque de MC par rapport aux variables cliniques. Le FDR267 a moins bien prédit le risque de MC chez les Afro-Américains.
distribution differences were tested for difference using the Kolmogorov-Smirnov test. A Cox proportional hazards model was fitted for incidence analysis and tested for goodness-of-fit using the likelihood ratio test. Uno’s concordance index was estimated for the Cox proportional hazard models.16 Receiver-operator curves (ROCs) were constructed, and the area under the curve (AUC) was calculated to assess the discrimination ability of different models. Differences in AUC between models were evaluated using DeLong’s test.17 All statistical analyses were performed on Rv3.5.1 and SAS v9.4 (SAS Institute, Inc, Cary, NC). R packages obtained from the CRAN mirror and used include “survival,” “survminer,” “ggplot2,” “PredictABEL,” “ggpubr,” “pROC,” “rms,” and “qwraps2.”

Results

Baseline demographic and modifiable risk factors

After quality-control analysis of the ARIC population, 10,578 individuals had all data available for analysis. The baseline demographic and Framingham risk factor characteristics by ethnic group are found in Table 1. A total of 836 events occurred during the follow-up period, with 1138 cases of CAD when considering both individuals with CAD at baseline and those who had an event during follow-up.

Associations among GRS, clinical risk factors, and CAD

After quality control, 265 of the 301 SNPs identified in the UK Biobank study were found to have been directly genotyped or reliably imputed with an imputation quality score > 0.3 in the ARIC population. Two additional SNPs were included in the GRS build as proxies with an $r^2 > 0.8$ to the reported SNPs. The list of SNPs included in the final GRS build (FDR267) can be found in Supplementary Table S1.

When tested against traditional Framingham risk factors, FDR267 was significantly associated with total and high-density lipoprotein (HDL) cholesterol, systolic blood pressure, anti-hypertensive treatment, and type 2 diabetes in the European sample and systolic blood pressure in the African American sample (Supplementary Table S2). FDR267 was normally distributed in both samples and was significantly right shifted in cases compared with controls (Fig. 1). The odds ratio of CAD per standard deviation of FDR267 was 1.45 (95% confidence interval [CI], 1.39-1.51; $P < 0.001$) and 1.05 (95% CI, 0.99-1.11; $P = 0.411$), respectively, for the European and African American samples when corrected for age and sex.

Prediction of incident CAD by FDR267

The 431 individuals with CAD at baseline were excluded from survival-to-event analyses. The HR per standard deviation of FDR267 when corrected for age and sex was 1.45 (95% confidence interval [CI], 1.39-1.51; $P < 0.001$) and 1.05 (95% CI, 0.99-1.11; $P = 0.411$), respectively, for the European and African American samples when corrected for age and sex.
Individuals in the fifth quintile of FDR267 had an HR of 1.89 (95% CI, 1.42-2.44) for CAD when compared with the lowest quintile. Survival curves built for individuals at increasing degrees of genetic risk (low = first quintile, moderate = 2-4 quintiles, and high = fifth quintile) are shown in Supplementary Figure S1.

**FDR267 and clinical risk**

Incremental discriminatory value of FDR267 was assessed by ROC analysis (Fig. 2). In the European sample, the AUC for a model based on clinical factors (systolic blood pressure, total and HDL cholesterol, smoking status, type 2 diabetes, age, and sex) was 0.74 (95% CI, 0.72-0.76). Including FDR267 to the model resulted in a modest and significant increase in the AUC (ΔAUC = 0.0112, P = 0.0002) (Table 3 and Supplementary Fig. S2). Likewise, when we investigated CAD incidence, adding genetic risk to a model with Framingham risk factors alone did not significantly change the C-index (Table 4 and Supplementary Fig. S3), giving similar results to those seen with the AUC analysis in Table 3.

**Comparison of FDR267 with self-reported family history**

The ROCs for the European and African American samples are shown in Figure 3. When tested against self-reported family history of CAD in at least 1 first-degree relative, the ORs for FDR267 were 2.00 (95% CI, 1.70-2.35; P < 0.001) and 1.61 (95% CI, 1.17-2.22, P = 0.00379) for the European and African American samples, correcting for sex and age, respectively. The respective HRs associated with a positive self-reported family history were 1.72 (95% CI, 1.56-1.90; P < 0.001) and 1.51 (95% CI, 1.25-1.83; P = 0.0319), when correcting for age and sex. The corresponding C-indices for FDR267 were 0.53 (95% CI, 0.51-0.56) and 0.55 (95% CI, 0.49-0.61), respectively. There was no significant difference in the C-index when comparing the FDR267 and family history models in the European sample (ΔC = -0.0292, P = 0.2325) or the African sample, after adjusting for age and sex.

**Discussion**

In European individuals, FDR267 was associated with a 1.45 (95% CI, 1.39-1.51; P < 0.001) and a 1.32 (95% CI, 1.26-1.38; P < 0.001) increase in CAD odds ratio and HR, respectively, per standard deviation of the score. The score modestly improved the AUC statistic when added to a clinical model. It predicted incident CAD (C-index = 0.60), although it did not improve on clinical risk factors (ΔC = 0.0159, P = 0.0965). Individuals in the top quintile of FDR267 genetic risk are at an approximately 2-fold increased risk compared with the bottom quintile, which is comparable to risk associated with self-reported family history of CAD. The performance of FDR267 in the African American sample was notably weaker.

FDR267 was built from variants evaluated by the 2017 UK Biobank GWAS meta-analysis for CAD. There are multiple strategies to filter out nonrelevant SNPs when building a GRS. These include, but are not limited to thresholding by significance, linkage disequilibrium, effect size, and biological significance. Traditionally, only independent SNPs that have reached a significance threshold of P < 5 × 10⁻⁷ are considered to be statistically significant for the model after Bonferroni correction. However, the stringency of this threshold risks
excluding SNPs with smaller, but real, effects on disease risk. Indeed, when GRSs are built solely from SNPs that reach this level of significance, they are only able to explain 10% of CAD heritability. Therefore, we sought to expand the SNP selection by filtering SNPs by an FDR (<5%) approach. The 304 independent SNPs that reach an FDR < 5% have been shown in the UK Biobank cohort to explain 21.2% of CAD heritability.

When tested in the ARIC European sample, a higher burden of genetic risk measured by FDR267 was indeed associated with CAD and could predict incident CAD. Individuals in the top quintile of genetic risk had an approximately 2-fold increase in CAD risk compared with those in the lowest quintile (HR, 1.89), an effect comparable to that conferred by a positive family history (HR, 1.72). Of note, there was no significant difference in risk discrimination between genetic risk assessed by FDR267 and that by family history. Although FDR267 is associated with self-reported family history, its effect on CAD risk was not significantly attenuated when adjusting for family history (HR, 1.29; 95% CI, 1.20-1.38). Therefore, FDR267 may capture components of an individual’s genetic risk for CAD that cannot be explained through family history.

Compared with a model with Framingham risk factors alone, inclusion of FDR267 improved discrimination of CAD cases from controls in Europeans (ΔAUC = 0.0112, P = 0.0002). Yet it did not add predictive value to the model. The modest increase in the C-index (ΔC = 0.0159) did not reach statistical significance. This is possibly because a large proportion of the genetic risk measured by FDR267 is already, indirectly, being captured by baseline clinical variables. Indeed, FDR267 is significantly associated with multiple Framingham risk factors (ie, total and HDL cholesterol, systolic blood pressure, and type 2 diabetes). Moreover, many of the SNPs in FDR267 are also implicated in GRSs for the clinical risk factors. Nonetheless, the HR associated with FDR267 is not significantly attenuated when correcting for clinical risk factors: HR, 1.27 (95% CI, 1.18-1.36) vs 1.32 (95% CI, 1.26-1.38). It is also likely that our sample size limits further clarification of this issue.

These results suggest modest utility of GRS testing in the 45- to 64-year-old population, for whom there are already well-validated clinical risk models. There is overlap between information provided by assessing pertinent risk phenotypes prevalent in this age group (ie, type 2 diabetes, dyslipidemia, and hypertension) and that measured by FDR267. However, there may be greater value of GRS testing in early screening for high-risk individuals. The low prevalence of risk phenotypes in a young population makes CAD risk assessment difficult. However, this is not to say that certain individuals do not possess high baseline genetic risk for CAD. In fact, our score was able to identify individuals at 2-fold increased risk

Table 3. Areas under the curve of ROCs for 3 models in the European and African American samples

| Model                     | European (AUC (95% CI)) | African (AUC (95% CI)) |
|---------------------------|-------------------------|------------------------|
| FDR267 genetic risk only  | 0.60 (0.58-0.62)         | 0.53 (0.49-0.56)       |
| Clinical risk only        | 0.74 (0.72-0.76)         | 0.72 (0.69-0.75)       |
| Clinical risk plus FDR267 | 0.75 (0.74-0.77)         | 0.72 (0.69-0.75)       |

AUC, area under the curve; CI, confidence interval; FDR267, false discovery rate 267-marker genetic risk score; ROC, receiver-operator curve.

Table 4. C-indices for 3 models in the European and African American participants

| Model                     | European (C-index (95% CI)) | African (C-index (95% CI)) |
|---------------------------|-----------------------------|---------------------------|
| FDR267 genetic risk only  | 0.60 (0.56-0.64)             | 0.54 (0.48-0.60)          |
| Clinical risk only        | 0.72 (0.70-0.75)             | 0.75 (0.70-0.80)          |
| Clinical risk plus FDR267 | 0.74 (0.72-0.77)             | 0.75 (0.70-0.80)          |

CI, confidence interval; FDR267, false discovery rate 267 marker genetic risk score.
without considering any clinical risk factors. Early identification of such individuals and targeting lifestyle or medical interventions might prove useful in mitigating CAD risk.7,8

Our results also show that compared with European subjects, FDR267 is weakly associated with CAD and has less predictive value for incident CAD in African Americans. Although the lack of statistical significance could be explained by an underpowered sample, there is nonetheless a consistent pattern of less robust evaluation metrics for CAD risk in the African American sample. Among Framingham risk factors, FDR267 was significantly associated only with systolic blood pressure in the African American sample. The strong association of FDR267 with lipid phenotypes in the European sample was not observed in the African American sample.

Haplotype variability, Eurocentric content of SNP genotyping arrays, and SNP ascertainment bias have all been suggested as reasons why GRSs are misestimated for individuals of African descent.25-27 Indeed, effect allele frequencies and thus effect estimates for lipid phenotypes were shown to be imperfectly correlated between white and African groups in the Million Veteran Program study.22 The UK Biobank population consists predominantly of Europeans (94.6%), with only 1.6% of the cohort being black or black British.28,29 With such a discrepancy between the recruitment of the 2 ethnicities, it would follow that our GRS is biased toward the most represented ethnicity. Our results highlight the importance in considering ethnic diversity when developing risk modelling tools.

Study limitations

The main limitations of this study include our inability to assess FDR267 in other ethnic groups and the likelihood that polygenic risk scores comprising a larger number of variants would show better clinical performance. A previous 25-SNP GRS was shown in a multi-ethnic sample of 8556 participants to have a consistent association with CAD across Europeans, South Asians, Southeast Asians, and Arabs, but similar to our study, it was not significant in Africans.18 As with FDR267, addition of the 25-SNP GRS to clinical risk factors did not markedly improve CAD risk prediction.20 Very recently, extremely large GRSs have been constructed using millions of SNPs, almost all of which are not individually significantly associated with CAD risk.18 These enormous and complex polygenic scores seem to better predict CAD status, with significant ORs between high- and low-risk scores in the range of 3 to 4.18 These “mega” or “meta” GRSs also appear to substantially improve discrimination over family history and clinical variables.18 If an inexpensive laboratory test of such mega-GRSs can be developed, they might have potential clinical utility.

Conclusions

Our GRS was significantly associated with CAD and provided modest predictive utility for incident CAD. Despite comparable predictive power with family history and improved ability to discriminate prevalent cases of CAD when added to a model with traditional risk factors, FDR267 does not improve on risk assessment. The clinical use of FDR267 is further limited by its inconsistent assessment of risk in a non-European population.

Funding Sources

R.A.H. is supported by the Jacob J. Wolfe Distinguished Medical Research Chair, the Edith Schulich Vinet Research Chair in Human Genetics, and the Martha G. Blackburn Chair in Cardiovascular Research. R.A.H. has received operating grants from the Canadian Institutes of Health Research (Foundation Grant) and the Heart and Stroke Foundation of Ontario (G-18-0022147).

Disclosures

R.A.H. has received honoraria for membership on advisory boards and speakers’ bureaus for Aegerion, Akcea, Amgen, Boston Heart, Gemphire, Regeneron, and Sanoﬁ, all unrelated to the topic of this manuscript. The other authors have no conflicts of interest to disclose.

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Supplementary Material
To access the supplementary material accompanying this article, visit the online version of the Canadian Journal of Cardiology at www.onlinecjc.ca and at https://doi.org/10.1016/j.cjco.2019.01.003.