Clinical Utility of Multimarker Genetic Risk Scores for Prediction of Incident Coronary Heart Disease: A Cohort Study Among Over 51,000 Individuals of European Ancestry

Carlos Iribarren, MD, MPH, PhD; Meng Lu, MD, MS; Eric Jorgenson, PhD; Manuel Martínez, BSc; Carla Lluis-Ganella, MSc, PhD; Isaac Subirana, MSc, PhD; Eduardo Salas, MD, PhD; Roberto Elosua, MD, PhD

Background—We evaluated whether including multilocus genetic risk scores (GRSs) into the Framingham Risk Equation improves the predictive capacity, discrimination, and reclassification of asymptomatic individuals with respect to coronary heart disease (CHD) risk.

Methods and Results—We performed a cohort study among 51,954 European-ancestry members of a Northern California integrated healthcare system (67% female; mean age 59) free of CHD at baseline (2007–2008). Four GRSs were constructed using between 8 and 51 previously identified genetic variants. After a mean (±SD) follow-up of 5.9 (±1.5) years, 1,864 incident CHD events were documented. All GRSs were linearly associated with CHD in a model adjusted by individual risk factors: hazard ratio (95% confidence interval) per SD unit: 1.21 (1.15–1.26) for GRS_8, 1.20 (1.15–1.26) for GRS_12, 1.23 (1.17–1.28) for GRS_36, and 1.23 (1.17–1.28) for GRS_51. Inclusion of the GRSs improved the C statistic (ΔC statistic = 0.008 for GRS_8 and GRS_36; 0.007 for GRS_12; and 0.009 for GRS_51; all P<0.001). The net reclassification improvement was 5% for GRS_8, GRS_12, and GRS_36 and 4% for GRS_51 in the entire cohort and was (after correcting for bias) 9% for GRS_8 and GRS_12 and 7% for GRS_36 and GRS_51 when analyzing those classified as intermediate Framingham risk (10%–20%). The number required to treat to prevent 1 CHD after selectively treating with statins up-reclassified subjects on the basis of genetic information was 36 for GRS_8 and GRS_12, 41 for GRS_36, and 43 for GRS_51.

Conclusions—Our results demonstrate significant and clinically relevant incremental discriminative/predictive capability of 4 multilocus GRSs for incident CHD among subjects of European ancestry. (Circ Cardiovasc Genet. 2016;9:531-540. DOI: 10.1161/CIRCGENETICS.116.001522.)

Key Words: clinical effectiveness • cohort studies • coronary disease • genetic predisposition to disease • risk factors

Methods

Study Cohort
This study used genome-wide genetic data available on the GERA cohort (Genetic Epidemiology Resource in Adult Health and Aging)
of 110,266 adult male and female Kaiser Permanente of Northern California members. The cohort has been described in detail elsewhere. In brief, the GERA cohort was formed by including all racial and ethnic minority participants in the larger cohort of the RPGEH (Research Program on Genes, Environment and Health) with saliva samples (19% of the total); the remaining participants were drawn randomly from White non-Hispanic participants (81% of the total). All RPGEH participants responded to a self-administered questionnaire in 2007/2008 that included information on medical history, ancestry, health behaviors (smoking, alcohol consumption, diet, physical activity, and reproductive history), and current weight and height. The study was approved by the Kaiser Foundation Research Institute Institutional Review Board, and subjects gave informed consent. Of the 110,266 subjects, 97,973 had complete genetic data for estimating the GRSSs. Among those, the following sequential exclusions were applied: 17,556 non-European ancestry subjects, 15,265 for being <30 years or >74 years, 1,544 for having prior CHD, and 11,198 for missing data on ≥1 Framingham Risk Score Components, resulting in a final analytic cohort of 51,954 people. The outcome of interest was incident CHD events, including hospital primary discharge diagnoses of myocardial infarction, angina (stable or unstable) or coronary revascularization procedures (coronary bypass or percutaneous intervention), or death because of CHD. The International Classification of Diseases–Ninth Revision and International Classification of Diseases–Tenth Revision codes used in event ascertainment are given in Table I in the Data Supplement.

Genotyping
Genotyping was conducted at the Institute for Human Genetics, University of California San Francisco, using custom-designed Affymetrix Axiom arrays. To maximize genome-wide coverage of common and less common variants, 4 specific arrays were designed for individuals of Non-Hispanic White, East Asian, African American, and Latino race/ethnicity. In the analyses presented here, only the Non-Hispanic White assay data (674,513 SNPs) were used. The genome-wide arrays gave high-quality genotypes, with high genotype call rates (average 99.7%) and SNP reproducibility (99.9%), and results from the Non-Hispanic White array have been reported. We originally selected 51 SNPs previously identified to be associated with CHD risk factors included in the classical risk functions (total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, blood pressure, smoking, and diabetes mellitus) in accordance to the data available at GWAS catalog reviewed in August 2010 for GRSS_8 and GRSS_12 and in January 2013 for GRSS_36. We also defined a GRS_51 that included the 47 variants reported to be associated with CAD independently and independently of their association with risk factors included in the classical risk functions. For clarification purposes, GRSS_12 contains 8 variants from GWAS, GRSS_36 contains 32 variants from GWAS, and GRSS_51 contains 47 variants from GWAS, and all of them contain 4 variants to incorporate the ALOX5AP haplotype. These GWAS usually analyze individual genetic variants and do not take into consideration haplotypes. The ALOX5AP presents an haplotype, called haplotype B, that has been reported to be associated with CHD in different populations. This haplotype consisted of rs10507391-A, rs93155050-A, rs17228242-G, and rs17216473-A (these 4 SNPs were imputed). Because the haplotype diversity could capture genetic variability associated risk better than individual genetic variants, and there were consistent data supporting the association between the ALOX5AP Haplotype B and CHD, we included this haplotype variant in 3 of the 4 GRSSs (GRSS_12, GRSS_36, and GRSS_51). No significant deviations from Hardy–Weinberg equilibrium were noted for any of the SNPs. We have verified in 1000 genomes SNAP (SNP Annotation and Proxy Search; https://www.broadinstitute.org/mpg/snap/ldsearch.php) that all 51 SNPs are independent (linkage disequilibrium r 2<0.50). Weighted GRSSs were calculated using the formula where beta is the estimated effect size reported for each variant, SNP is the number of copies of each individual SNP evaluated (with values 0, 1, or 2), and n is number of SNPs. A weight of 0.131 was applied to the presence of the haplotype. Ten-year CHD risk was estimated using the Framingham risk function described by Wilson et al. We did not use the more recently developed Pooled Cohorts Equation because it applies to all cardiovascular disease, including stroke, whereas the CARDIoGRAMplusC4D Consortium focused on coronary artery disease. Age, sex, education level, race/ethnicity, smoking status, alcohol consumption, body mass index, and family history of heart disease were available from the RPGEH survey; systolic and diastolic blood pressures were obtained from primary care outpatient visits closest to the survey date, and lipid panels and serum creatinine (closest to survey date) were obtained from the health plan laboratory database. Diabetes mellitus status was derived by cross-linkage with the Kaiser Permanente of Northern California diabetes mellitus registry. Hypertension and hypercholesterolemia treatment was ascertained using the Pharmacy Information Management System (PIMS), relying on prescription dispensing (at the time of the RPGEH survey or ≤2 years prior) of drugs falling into the corresponding therapeutic class. Estimation of glomerular filtration rate was done using the Modification of Diet in Renal Disease Study (MDRD) formula. We used standard parametric and nonparametric methods to compare the characteristics of different groups of individuals according to quintiles of the GRSSs. We then tested the association of each GRS (both as a continuous variable in SD units and as quintiles with the lowest quintile as the referent group) with incident CHD using Cox proportional hazards models, with sequential adjustment for classical CHD risk factors. Subjects were right-censored at different times depending on incident events, vital status, or health plan membership status. Model a included only the GRS (ie, no covariates); model b included the individual FRS variables (age, sex, total cholesterol, high-density lipoprotein cholesterol, systolic and diastolic blood pressure, smoking status, and diabetes mellitus); model c included model b covariates plus family history of heart disease, and model d included additional covariates that are not part of the FRS, namely education level, body mass index, antihypertensives, lipid-lowering drugs, and alcohol consumption. We expected 5% of the cohort to develop CHD, in which case we had 80% power to detect a hazard ratio of 1.04 per standard deviation of the GRS in the full sample. To test the proportionality of hazards assumption, we plotted Schoenfeld residuals against time and tested the interaction of each GRS with follow-up time. There was no visual evidence of departure from zero slope, and none of the interactions were statistically significant (P=0.10 for GRSS_8, P=0.08 for GRSS_12, P=0.87 for GRSS_36, and P=0.52 for GRSS_51). Therefore, there was no evidence that the proportionality of hazards assumption was violated. In addition, we used 3 different statistical approaches to assess the potential value of including the GRSSs in risk prediction: (1) the goodness-of-fit of the models using the Hosmer–Lemeshow test.
Iribarren et al  Genetic Risk Scores for CHD

(Continued)

Results

The mean (SD) age of the cohort was 59 (9) years, and 67% of the subjects were female (Table 1). Eighty-two percent of the patients were college educated; 5% reported current smoking; and 38% were former smokers. Although 57% of the patients reported low or medium alcohol intake, 4% reported high alcohol intake. The prevalence of diabetes mellitus was 12%, and 23% of the patients had a body mass index in the obesity range. Forty-six percent of the patients were on antihypertensive medication, and about a third was taking cholesterol-lowering agents. Moderately to severe kidney dysfunction (glomerular filtration rate <60) was present in 16% of the individuals. Overall, 72% of the patients had a low (<10%) FRS, 16% had an intermediate–low (10%–15%) FRS, 7% had an intermediate–high (15%–20%) FRS, and 6% had a high (≥20%) FRS. Thirty-one percent of the patients reported family history of angina/heart attack.

The Pearson correlation coefficients between the 4 GRSs ranged from 0.64 (between GRS_8 and GRS_51) to 0.94 (between GRS_8 and GRS_12). GRSs means were significantly higher in subjects with events compared with subjects without events (all P<0.001; Figure 1).

As expected by design (ie, selection of SNP components), GRS_8, GRS_12, and GRS_36 were not associated with the classical risk factors, with the exception of slightly higher use of cholesterol-lowering drugs by subjects in quintile 5 versus quintile 1 (Table IIA–IID in the Data Supplement).

Table 1. Characteristics of the GERA Cohort, European Descent Subjects (n=51 954)

| Baseline Characteristics | Mean±SD or n (%) |
|-------------------------|-----------------|
| Age at survey, y (mean±SD) and n (%) | 59.4±9.1 |
| 30–54 | 14 785 (28.3%) |
| 55–64 | 19 995 (38.5%) |
| 65–74 | 17 174 (33.1%) |
| Sex, n (%) | |
| Male | 17 270 (33.2%) |
| Female | 34 684 (66.8%) |
| Education level, n (%) | |
| Less than college | 6638 (12.8%) |
| College or higher | 42 422 (81.7%) |
| Missing | 2894 (5.6%) |
| Smoking status, n (%) | |
| Never | 29 586 (56.9%) |
| Former | 19 818 (38.1%) |
| Current | 2550 (4.9%) |
| Alcohol consumption, n (%) | 4.6±6.7 |
| Abstinence | 18 509 (35.6%) |
| Low (<8 in men, <4 in women) | 14 379 (27.7%) |
| Medium (8–21 in men, 4–14 in women) | 14 860 (28.6%) |
| High (≥21 in men; >14 in women) | 18 76 (3.6%) |
| Missing | 2330 (4.5%) |
| Diabetes mellitus, n (%) | 6429 (12.4%) |
| Body mass index, kg/m² (mean±SD) and n (%) | 27.2±5.7 |
| <18 | 304 (0.6%) |
| 18–24.9 | 19 846 (38.2%) |
| 25–29.9 | 18 022 (34.7%) |
| ≥30 | 12 160 (23.4%) |
| Missing | 1622 (3.1%) |
| Systolic blood pressure, mm Hg (mean±SD) | 125.8±15.4 |
| Diastolic blood pressure, mm Hg (mean±SD) | 74.3±9.7 |
| Antihypertensives, % | 23 682 (45.6%) |
| HDL-C, mg/dL (mean±SD) | 56.6±15.8 |
| Total cholesterol, mg/dL (mean±SD) | 198±36.4 |
| Cholesterol-lowering drugs, n (%) | 17 398 (33.5%) |
| GFR, ml/min per 1.73 m², (mean±SD) and n (%) | 73.5±15.3 |
| <60 | 81 18 (15.6%) |
| 60–90 | 36 079 (69.4%) |
| ≥90 | 60 47 (11.6%) |
| Missing | 17 10 (3.3%) |
| Framingham Risk Score (median [IQR]) and n (%) | 8.4±6.3 |
| Low (0–10)% | 37 199 (71.6%) |
| Intermediate-Low (10–15)% | 8 216 (15.8%) |
| Intermediate–High (15–20)% | 3 504 (6.8%) |
| High (≥20%) | 2 985 (5.7%) |
| Family history of angina/heart attack, n (%) | |
| No | 34 421 (66.3%) |
| Yes | 15 918 (30.6%) |
| Missing | 1 615 (3.1%) |

GERA indicates Genetic Epidemiology Resource in Adult Health and Aging; GFR, glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; and IQR, interquartile range.
A greater prevalence of family history of angina/heart attack in quintile 5 versus quintile 1 was found in all GRSs. On the contrary, and also consistent with expectation, GRS_51 was significantly associated with high-density lipoprotein cholesterol, total cholesterol, and the FRS in addition to use of cholesterol-lowering drugs and family history of angina/heart attack.

After a mean (SD) follow-up time of 5.9 (1.5) years, 1864 incident CHD events were documented (1077 in men and 787 in women). For all the GRSs, there was a positive linear association among the quintiles of GRS and age-adjusted rates of incident CHD, and the trend lines for the 4 GRSs were overlapping (Figure 2). In unadjusted models, each SD increment in GRS was associated with 1.21, 1.20, 1.23, and 1.23 increased hazard of incident CHD for GRS_8, GRS_12, GRS_36, and GRS_51, respectively (Table 2). Adjustment for FRS individual components and additional available covariates had no appreciable effect on the risk estimates. No evidence of gender interaction was found in either minimally or fully adjusted models (all \( P > 0.60 \)); thus, no results are presented separately for men and women. When the GRSs were modeled as quintiles, quintile 5 (versus quintile 1) was associated with hazard ratios (95% confidence interval [CI]) of 1.75 (95% CI, 1.51–2.03), 1.74 (95% CI, 1.50–2.02), 1.77 (95% CI, 1.53–2.05), and 1.95 (95% CI, 1.68–2.27) for GRS_8, GRS_12, GRS_36, and GRS_51, respectively, in the unadjusted models. Again, there was little or no attenuation of the risk estimates after multivariable adjustment. The strength of independent association of all the individual risk factors with incident CHD is shown in Table IV in the Data Supplement.

The results of all the Hosmer–Lemeshow tests showed a good fit of the models, including the GRS in the equation (all \( P \) values ≥0.20; Table 3). The area under the curve after the addition of the GRSs to models already containing the FRS changed only marginally, although the results were statistically significant for all GRSs. For all the GRSs, both the integrated discrimination improvement and the NRI presented a better performance when analyzing only the subset of individuals classified in the intermediate-risk category. Notably, the contribution to reclassification was mainly from subjects who went on to develop CHD. Both reclassification as a continuous value (integrated discrimination improvement) and as a categorical value (NRI) was better for GRS_8 and GRS_12 than for GRS_36 and GRS_51. The reclassification tables are provided in Table V in the Data Supplement. After correcting for bias, adding GRSs to the risk function in the intermediate-risk population up-reclassified 9% of subjects.

Figure 1. Distributional properties of the 4 genetic risk scores (GRS).
using GRS_8 or GRS_12 and 7% of subjects using GRS_36 or GRS_51 to high-risk category (Table 4). We estimated that systematic treatment with statins to all the subjects in the intermediate group would have prevented 168 CHD events (699x0.24), avoiding 1 CHD event for every 70 individuals treated (11 770/168) based on the theoretical efficacy of statins (24% reduction in CHD incidence). The number of subjects reclassified to the high-risk group required to be treated to prevent 1 CHD with statins were 36 for GRS_8 and GRS_12, 41 for GRS_36, and 43 for GRS_51 (Table 4). Thus, the inclusion of GRS_8 or GRS_12 in a 2-stage screening scenario would be ≈1.9x more effective in preventing events than the systematic use of statins in the intermediate-risk group (the corresponding figures for GRS_36 and GRS_51 were 1.7 and 1.6, respectively).

**Discussion**

In our cohort, 4 GRSs, including 8 to 51 genetic variants previously shown to predict CHD, were independently associated with CHD incidence. The 4 GRSs improved the discrimination capacity of the Framingham risk function and reclassification, particularly in the intermediate-risk group. Of note, the extent of reclassification was slightly better using GRS_8 and GRS_12 compared with that using GRS_36 and GRS_51 and was mostly driven by incident CHD cases. We have also assessed the GRS clinical utility and its effect on clinical outcomes. Using the GRSs in a 2-stage screening scenario, 7% to 9% of the intermediate-risk individuals are reclassified into a high-risk group. We estimated that this approach would prevent 1.6x to 1.9x more events than would systematic use of statins in the intermediate-risk group.

We replicate here the association between GRS_8 and risk of CHD previously reported in the REGICOR (Registre Gironi del COR) and Framingham cohorts. Further, we tested the comparative utility of 3 additional GRSs enriched with additional SNPs previously known to be associated with CHD. For any of the 4 GRSs, and after multivariable adjustment, there was more than a 1.75-fold increase in the age-adjusted rate of incident of CHD comparing quintile 5 to quintile 1. The strength of the unadjusted association was similar across enrichment, with increasing number of SNPs included in the GRS. As expected (because some of the SNPs selected for GRS_51 were related to risk factors), we observed some attenuation of the risk relation in models including GRS_51. Therefore, there was no clear advantage of GRS_12, GRS_36, or GRS_51 over GRS_8. It remains to be seen if incorporation of a much higher number of SNPs associated with CHD using a more liberal threshold for tested SNPs in GWAS could improve the GRSs analyzed here.
These results are in agreement with previous studies that have analyzed the association between different GRSs and CHD incidence. Interestingly and similar to prior studies, this association was independent of other cardiovascular risk factors and of family history of CHD, indicating the value of genomic information above and beyond self-reported family history.

From the clinical standpoint, the GRSs have to demonstrate their value at different levels in accordance to guidelines. The first level is the assessment of the predictive added value: do the GRSs add predictive information to established, standard risk markers? After the addition of the GRSs to models already containing the FRS, we observed a small but statistically significant increment in the discrimination capability (C statistic). The improvement in the discrimination capacity has been reported in some studies but not in others. Second, does the GRS changed predicted risk sufficiently to change clinical attitude/recommended therapy as determined by where the subject is allocated? New statistical metrics, such as reclassification, have been developed to characterize that change. In our study, we report a modest (4%–5% for all GRSs) but significant reclassification when including the GRSs in the predictive risk function. The extent of reclassification was more evident (between 7% and 9%) when considering only the intermediate-risk group, which is the most interesting group from the clinical point of view. Our results in this group are, therefore, in the low range of prior reports, where NRI ranged from 6% to 27%. However, it should be noted that these previous estimates were not corrected for bias as described by Paynter and Cook.

From the clinical standpoint, the GRSs have to demonstrate their value at different levels in accordance to guidelines. The first level is the assessment of the predictive added value: do the GRSs add predictive information to established, standard risk markers? After the addition of the GRSs to models already containing the FRS, we observed a small but statistically significant increment in the discrimination capability (C statistic). The improvement in the discrimination capacity has been reported in some studies but not in others. Second, does the GRS changed predicted risk sufficiently to change clinical attitude/recommended therapy as determined by where the subject is allocated? New statistical metrics, such as reclassification, have been developed to characterize that change. In our study, we report a modest (4%–5% for all GRSs) but significant reclassification when including the GRSs in the predictive risk function. The extent of reclassification was more evident (between 7% and 9%) when considering only the intermediate-risk group, which is the most interesting group from the clinical point of view. Our results in this group are, therefore, in the low range of prior reports, where NRI ranged from 6% to 27%. However, it should be noted that these previous estimates were not corrected for bias as described by Paynter and Cook. Third, does use of the GRSs improve clinical outcomes, especially when tested in a randomized clinical trial? This level is much more difficult to evaluate mainly because of the high cost of clinical trials and the availability of potentially useful markers. Alternatively, we have performed a

| Table 2. Association Between 4 Genetic Risk Scores and Incident CHD Among GERA Subjects of European Descent (n=51 294) |
|---------------------------------------------------------------|
| **Hazard Ratio (95% CI)** | **GRS_8** | **GRS_12** | **GRS_36** | **GRS_51** |
| **Model 1a** | 1.21 (1.15–1.26) | 1.20 (1.15–1.25) | 1.23 (1.18–1.29) | 1.25 (1.20–1.31) |
| **Model 1b** | 1.22 (1.17–1.28) | 1.22 (1.16–1.27) | 1.25 (1.19–1.31) | 1.26 (1.21–1.32) |
| **Model 1c** | 1.22 (1.17–1.27) | 1.21 (1.16–1.27) | 1.24 (1.19–1.30) | 1.26 (1.20–1.32) |
| **Model 1d** | 1.21 (1.15–1.26) | 1.20 (1.15–1.26) | 1.23 (1.17–1.28) | 1.23 (1.17–1.28) |
| **Model 2a** | | | | |
| **Quintile 2** | 1.12 (0.96–1.32) | 1.14 (0.97–1.34) | 1.24 (1.06–1.45) | 1.31 (1.12–1.54) |
| **Quintile 3** | 1.32 (1.13–1.54) | 1.38 (1.18–1.61) | 1.32 (1.13–1.55) | 1.40 (1.20–1.64) |
| **Quintile 4** | 1.40 (1.20–1.63) | 1.43 (1.22–1.66) | 1.50 (1.28–1.74) | 1.44 (1.23–1.68) |
| **Quintile 5** | 1.75 (1.51–2.03) | 1.74 (1.50–2.02) | 1.77 (1.53–2.05) | 1.95 (1.68–2.27) |
| **Model 2b** | | | | |
| **Quintile 2** | 1.16 (0.99–1.36) | 1.17 (0.99–1.38) | 1.20 (1.03–1.41) | 1.31 (1.11–1.53) |
| **Quintile 3** | 1.34 (1.14–1.56) | 1.40 (1.20–1.63) | 1.29 (1.10–1.51) | 1.38 (1.18–1.61) |
| **Quintile 4** | 1.45 (1.24–1.69) | 1.46 (1.25–1.71) | 1.49 (1.28–1.73) | 1.45 (1.24–1.69) |
| **Quintile 5** | 1.84 (1.58–2.13) | 1.82 (1.57–2.11) | 1.79 (1.54–2.08) | 1.99 (1.72–2.31) |
| **Model 2c** | | | | |
| **Quintile 2** | 1.16 (0.98–1.36) | 1.17 (0.99–1.37) | 1.21 (1.03–1.41) | 1.30 (1.11–1.53) |
| **Quintile 3** | 1.33 (1.14–1.55) | 1.40 (1.20–1.63) | 1.29 (1.10–1.50) | 1.37 (1.17–1.61) |
| **Quintile 4** | 1.44 (1.24–1.68) | 1.45 (1.24–1.70) | 1.48 (1.27–1.73) | 1.44 (1.23–1.68) |
| **Quintile 5** | 1.82 (1.57–2.11) | 1.81 (1.56–2.10) | 1.78 (1.53–2.06) | 1.97 (1.70–2.28) |
| **Model 2d** | | | | |
| **Quintile 2** | 1.19 (1.01–1.40) | 1.19 (1.01–1.40) | 1.25 (1.06–1.47) | 1.29 (1.09–1.51) |
| **Quintile 3** | 1.34 (1.14–1.57) | 1.38 (1.18–1.62) | 1.32 (1.12–1.55) | 1.33 (1.13–1.56) |
| **Quintile 4** | 1.45 (1.24–1.69) | 1.48 (1.26–1.73) | 1.48 (1.26–1.73) | 1.35 (1.15–1.59) |
| **Quintile 5** | 1.82 (1.57–2.12) | 1.80 (1.55–2.09) | 1.76 (1.52–2.05) | 1.84 (1.58–2.15) |

Model 1a indicates GRS/SD as a continuous variable only; Model 1b, GRS/SD as a continuous variable plus individual Framingham risk score variables (age, sex, total cholesterol, HDL-C, systolic blood pressure, diastolic blood pressure, diabetes mellitus, smoking status). Model 1c: Model 1b covariates plus family history of heart disease. Model 1d: Model 1c covariates plus education level, body mass index, anti-hypertensives, lipid lowering, alcohol consumption. Model 2a: GRS as quintiles only. Model 2b: GRS as quintiles plus individual Framingham risk score variables (age, sex, total cholesterol, HDL-C, systolic blood pressure, diastolic blood pressure, diabetes mellitus, smoking status). Model 2c: Model 2b covariates plus family history of heart disease. Model 2d: Model 2c covariates plus education level, body mass index, anti-hypertensives, lipid lowering, alcohol consumption. CHD indicates coronary heart disease; CI, confidence interval; GERA, Genetic Epidemiology Resource in Adult Health and Aging; GRS, genetic risk scores; and HDL-C, high-density lipoprotein cholesterol.
Our results indicate that use of genetic testing in identifying the subjects at high risk could prevent between 1.6× and 1.9× more events than systematic allocation of statins to all individuals of the intermediate group. These results are more conservative to those reported by Tikkanen et al., who reported that the genetic testing would prevent 2.5× more events than would random allocation of statins. Additionally, we have analyzed whether the up-reclassified subjects would have a higher benefit from statins, similarly to the work by Mega et al.39 They noted that, in primary prevention, their GRS would identify subjects who could benefit more from statin therapy and that the mean individuals needed to treat with statin to prevent 1 CHD in a 10-year period would move from 61 to 33. Our results are similar to those reported by Mega et al because 36 to 43 individuals would need to be treated to avoid 1 CHD event in the genetically screened population. We think that these results are conservative because we have only used the statin effect associated with the decrease in 1 mmol/L of total cholesterol when for patients at high risk a higher decrement is recommended. 2,40 Moderate-intensity statin therapy is expected to lower low-density lipoprotein

| Table 3. Model Calibration, Discriminative Capacity, Reclassification, and Clinical Utility Parameters for Incident CHD for Each of the 4 GRS Among GERA Subjects of European Descent (n=51,294) |
|-----------------------------------------------|--------------|--------------|--------------|--------------|
| Harrell C statistic                          | GRS_8        | GRS_12       | GRS_36       | GRS_51       |
| Model with FRS                              | 0.692        | 0.692        | 0.692        | 0.692        |
| Model with FRS+GRS                          | 0.700        | 0.699        | 0.700        | 0.701        |
| P value for difference                      | <0.001       | <0.001       | <0.001       | <0.001       |
| Hosmer–Lemeshow chi-square*                 |              |              |              |              |
| Model with FRS                              | 23.9 (9); 0.005 | 23.9 (9); 0.005 | 23.9 (9); 0.005 | 23.9 (9); 0.005 |
| Model with FRS+GRS                          | 9.9 (9); 0.36  | 10.9 (9); 0.28 | 12.3 (9); 0.20 | 8.9 (9); 0.45 |
| Integrated discrimination improvement (IDI)† |              |              |              |              |
| All cohort                                  | 0.20 (0.13–0.32) | 0.19 (0.11–0.31) | 0.25 (0.14–0.39) | 0.26 (0.15–0.39) |
| Intermediate risk subset                    | 0.31 (0.12–0.57) | 0.30 (0.10–0.57) | 0.28 (0.11–0.53) | 0.29 (0.11–0.56) |
| Category-based net reclassification index in the full cohort |              |              |              |              |
| Subjects with CHD events                    | 0.06 (0.03–0.08) | 0.06 (0.03–0.08) | 0.05 (0.03–0.08) | 0.05 (0.03–0.08) |
| Subjects without CHD events                 | 0.00 (–0.01 to −0.00) | 0.00 (–0.01 to −0.00) | −0.01 (–0.01 to −0.00) | −0.01 (–0.01 to −0.00) |
| All subjects                                | 0.05 (0.03–0.08) | 0.05 (0.03–0.08) | 0.05 (0.02–0.07) | 0.04 (0.02–0.07) |
| Bias-corrected category-based net reclassification index in the intermediate risk subset |              |              |              |              |
| Subjects with CHD events                    | 0.10 (0.05–0.14) | 0.10 (0.06–0.15) | 0.09 (0.04–0.13) | 0.10 (0.05–0.14) |
| Subjects without CHD events                 | −0.01 (−0.02 to 0.00) | −0.01 (−0.02 to 0.00) | −0.02 (−0.03 to −0.01) | −0.03 (−0.04 to −0.02) |
| All subjects                                | 0.09 (0.04–0.13) | 0.09 (0.05–0.14) | 0.07 (0.03–0.11) | 0.07 (0.02–0.11) |

CHD indicates coronary heart disease; CI, confidence interval; FRS, Framingham risk score; GERA, Genetic Epidemiology Resource in Adult Health and Aging; and GRS, genetic risk scores.

*Estimate (df); P value.
†Estimate (95% CI), rescaled by multiplying by 100.

| Table 4. Clinical Utility Parameters for Incident CHD for Each of the 4 GRS Among GERA Subjects of European Descent Classified as Intermediate Risk (n=11,498) |
|-----------------------------------------------|--------------|--------------|--------------|--------------|
| One-stage screening                          | Individuals to be Treated (A) | Events Predicted at 10 y (B) | Events Potentially Prevented (C=B×0.24) | Individuals Needed to Treat to Prevent 1 CHD Event (D=A/C) | Efficiency of the 2-Stage vs 1-Stage Approach |
| GRS_8                                        | 828          | 94.7         | 23           | 36           | 1.9 (70/36) |
| GRS_12                                       | 801          | 92.3         | 22           | 36           | 1.9 (70/36) |
| GRS_36                                       | 921          | 94.3         | 23           | 41           | 1.7 (70/41) |
| GRS_51                                       | 931          | 89.3         | 21           | 43           | 1.6 (70/43) |

CHD indicates coronary heart disease; GERA, Genetic Epidemiology Resource in Adult Health and Aging; and GRS, genetic risk scores.
cholesterol by ≥30% to <50% and high-intensity statin therapy by ≥50%. Moreover, we have not taken into consideration that there may be an increment in the compliance of patients after receiving genetic testing. Statins are considered effective in reducing cardiovascular morbidity and mortality in high-risk patients. However, although adherence to statins improves morbidity and mortality, it remains suboptimal. For some researchers, one of the most important causes of nonadherence is the so-called statin intolerance, mainly because of muscle-related symptoms. In any case, nonadherence is a general problem to any drug and is not necessarily associated to adverse reactions. In the case of statins, the knowledge that patients with high genetic risk obtain a great benefit from statin therapy may motivate patient to adhere to statin therapy. A higher adherence to statins and a higher reduction of cardiovascular risk has already been observed in patients by the knowledge of a relevant genetic contribution to their disease. Fourth, does use of the GRS improved clinical outcomes sufficiently to justify the additional cost of testing and treatment? GRS_8 has already proved to be cost-effective in a theoretical model based on data from 2 cohorts. Future work needs to be done to establish the cost-effectiveness of GRS_12, GRS_36, and GRS_51.

We have identified some limitations in our study. Because of population admixture and the fact that the genetic variants were ascertained in a European population, these results may not be generalizable to non-European populations. Future work by our group and others will address the need for ethnic-specific validation of these 4 multilocus GRS, as well as development of ethnic-specific multilocus GRSs. Moreover, the mean follow-up of the study was 5.9 years when common risk functions estimate 10-year risk. Although routine lipid panels in clinical care require 8 hours of fasting, we did not have information on compliance with the fasting requirement or actual hours of fasting. The GERA cohort has a high representation of the upper end of the educational spectrum, which could limit its generalizability to populations with lower educational attainment. On the contrary, the study has several strengths, including an unprecedented large sample size and availability of high-quality genotypic data and of traditional cardiovascular risk factors and other variables (such as body mass index, alcohol intake, renal function, and family history of CAD) not included in the Framingham equation. Rather than a Health Maintenance Organization (claims data), Kaiser Permanente of Northern California is an integrated healthcare system where utilization comes from their own hospitals and outpatient clinics. As long as members remain in the plan, ascertainment of inpatient services is essentially complete. Among people in the GERA cohort, >97% have at least 5 years of continuous membership and >83% have at least 10 years of continuous membership; their average duration of membership is 23.5 years. We have focused on incident rather than on prevalent events. We have also used state-of-the-art methodology to assess the performance of novel biomarkers. Finally, the Kaiser population has a higher proportion of individuals with ideal cardiovascular metrics in comparison to those published for adults in United States, which places more individuals at the low-risk category and makes it more difficult for GRSs to reclassify subjects to high-risk categories. Despite this, we have observed similar results for GRS_8 than previously reported.

This study goes beyond a simple replication of prior results and fills gaps in knowledge, such as the relative contribution of GRSs with increasing number of loci and different known relatedness to risk factors and the link to clinical utility. In addition, we think that this work is a significant and important contribution to the field of cardiovascular genetics for the following reasons: (1) this is large, well-characterized cohort with individual data and not a meta-analysis; (2) it demonstrates the potential of data generated by an integrated healthcare system to conduct robust, cost-effective, large-scale genetic epidemiological studies; (3) our analysis is noteworthy because it indicates the usefulness of genetic information in a population with good control for risk factors, which places more individuals at the low-risk category and makes it more difficult for GRSs to reclassify subjects to high-risk categories.

In summary, the analyzed GRSs improved the predictive capacity of a classical risk function, especially in the intermediate-risk group. The clinical utility results presented here build the evidence and the case for incorporation of parsimonious multilocus GRSs in predictive algorithms for primary prevention of CHD and may help advance the realization of precision medicine.

Disclosures

Dr Iribarren received a research grant from GenDiag/Ferrer in Code, Inc for this study. M. Martínez is a member of the board of GenDiag and has a services contract relationship with GenDiag. Dr Lluis-Ganella is an employee of Ferrer in Code, company that commercializes a product based on GRS_12. Dr Salas is an employee of Gendiag.exe (company participated by Gendiag) and inventor in a patent application based on the GRS_12, whose applicant is Gendiag.exe. Dr Elouua is a member of the scientific advisory board of GenDiag and inventor in a patent application based on the GRS_12, whose applicant is Gendiag.exe. The other authors report no conflicts.

References

1. Goff DC Jr, Lloyd-Jones DM, Bennett G, Coady S, D’Agostino RB, Gibbons R, et al; American College of Cardiology/American Heart Association Task Force on Practice Guidelines. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;129(25 suppl 2):S49–S73. doi: 10.1161/01. Cir.0000437741.48606.98.
2. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, et al; European Guidelines on Cardiovascular Prevention & Rehabilitation (EACPR); ESC Committee for Practice Guidelines (CPG). European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Eur Heart J. 2012;33:1635–1701. doi: 10.1093/ eurheartj/ehs092.
3. Greenland P, Smith SC Jr, Grundy SM. Improving coronary heart disease risk assessment in asymptomatic people: role of traditional risk factors and noninvasive cardiovascular tests. Circulation. 2001;104:1863–1867.
4. Naghavi M, Libby P, Falk E, Casscells SW, Litovskys S, Rumberger J, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part II. Circulation. 2003;108:1772–1778. doi: 10.1161/01.CIR.0000087481.55887.C9.

Disclosures

Dr Iribarren received a research grant from GenDiag/Ferrer in Code, Inc for this study. M. Martínez is a member of the board of GenDiag and has a services contract relationship with GenDiag. Dr Lluis-Ganella is an employee of Ferrer in Code, company that commercializes a product based on GRS_12. Dr Salas is an employee of Gendiag.exe (company participated by Gendiag) and inventor in a patent application based on the GRS_12, whose applicant is Gendiag.exe. Dr Elouua is a member of the scientific advisory board of GenDiag and inventor in a patent application based on the GRS_12, whose applicant is Gendiag.exe. The other authors report no conflicts.

References

1. Goff DC Jr, Lloyd-Jones DM, Bennett G, Coady S, D’Agostino RB, Gibbons R, et al; American College of Cardiology/American Heart Association Task Force on Practice Guidelines. 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;129(25 suppl 2):S49–S73. doi: 10.1161/01. Cir.0000437741.48606.98.
2. Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, et al; European Guidelines on Cardiovascular Prevention & Rehabilitation (EACPR); ESC Committee for Practice Guidelines (CPG). European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). Eur Heart J. 2012;33:1635–1701. doi: 10.1093/ eurheartj/ehs092.
3. Greenland P, Smith SC Jr, Grundy SM. Improving coronary heart disease risk assessment in asymptomatic people: role of traditional risk factors and noninvasive cardiovascular tests. Circulation. 2001;104:1863–1867.
4. Naghavi M, Libby P, Falk E, Casscells SW, Litovskys S, Rumberger J, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: Part II. Circulation. 2003;108:1772–1778. doi: 10.1161/01.CIR.0000087481.55887.C9.
40. Stone NJ, Robinson JG, Lichtenstein AH, Bairey Merz CN, Blum CB, Eckel RH, et al; American College of Cardiology/American Heart Association Task Force on Practice Guidelines. 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. Circulation. 2014;129(25 Suppl 2):S1–45. doi: 10.1161/01.cir.0000437738.63853.7a.

41. Schneider KI, Schmidtke J. Patient compliance based on genetic medicine: a literature review. J Community Genet. 2014;5:31–48. doi: 10.1007/s12687-013-0160-2.

42. Kullo IJ, Jouni H, Austin EE, Brown SA, Kruisselbrink TM, Isseh IN, et al. Incorporating a genetic risk score into coronary heart disease risk estimates: effect on low-density lipoprotein cholesterol levels (the MiGENES Clinical Trial). Circulation. 2016;133:1181–1188. doi: 10.1161/CIRCULATIONAHA.115.020109.

43. Umans-Eckenhausen MA, Defesche JC, Sijbrands EJ, Scheerder RL, Kastelein JJ. Review of first 5 years of screening for familial hypercholesterolaemia in the Netherlands. Lancet. 2001;357:165–168. doi: 10.1016/S0140-6736(00)03587-X.

44. Umans-Eckenhausen MA, Defesche JC, van Dam MJ, Kastelein JJ. Long-term compliance with lipid-lowering medication after genetic screening for familial hypercholesterolemia. Arch Intern Med. 2003;163:65–68.

45. Ramirez de Arellano A, Coca A, de la Figuera M, Rubio-Terres C, Rubio-Rodriguez D, Gracia A, et al. Economic evaluation of Cardio inCode(R), a clinical-genetic function for coronary heart disease risk assessment. Appl Health Econ Health Policy. 2013;11:531–542.

46. Dong C, Rundek T, Wright CB, Anwar Z, Elkind MS, Sacco RL. Ideal cardiovascular health predicts lower risks of myocardial infarction, stroke, and vascular death across whites, blacks, and Hispanics: the northern Manhattan study. Circulation. 2012;125:2975–2984. doi: 10.1161/CIRCULATIONAHA.111.081083.

**CLINICAL PERSPECTIVE**

According to the Framingham Risk Score, a sizable proportion of patients are at intermediate risk (10-year risk 10% to 20%) and, thus, are not candidates for aggressive clinical management. In our cohort of 51 954 European-descent patients, 23% were at intermediate risk, yet 40% of coronary heart disease (CHD) events (720/1813) occurred among them. Therefore, a way to discern who would benefit from stepped-up care has important clinical implications. We contrasted 4 versions of weighted multilocus genetic risk scores (GRSs) as a way to improve the selection of patients requiring aggressive clinical management. We have shown that (1) there was more than a 1.75-fold increase in the age-adjusted rate of incident CHD in the subjects with high genetic load compared with those with low genetic load; (2) the GRSs with 8 and 12 variants performed just as well or better than the ones with 36 or 51 insofar as risk prediction, reclassification, and clinical utility; (3) the inclusion of GRS_8 and GRS_12 in the risk prediction model up-reclassified 9% of subjects in the intermediate group, and the contribution to reclassification came exclusively from subjects who went on to develop CHD; (4) the number of subjects needed to treat to prevent 1 CHD event was lower for GRS_8 and GRS_12 (36) than for GRS_36 (41) and GRS_51 (43), indicating better clinical utility and prevention of 1.6 to 1.9 times more events than systematic use of statins. Our results build the evidence for incorporating a parsimonious set of multilocus GRSs in predictive algorithms for CHD primary prevention.