Integrating Polygenic and Classical Risk Factors for Cardiovascular Risk Stratification in a Polish Primary-Care Cohort

Running title: *Polygenic and classical cardiovascular risk in Poland*

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Abstract:

Background: Polygenic risk scores (PRS) promise improved cardiovascular risk stratification, but most validation derives from UK or Nordic biobanks. The transferability of these scores to Central European populations and their incremental value over classical risk factors in routine care remain unestablished.

Objectives: To determine whether a coronary PRS derived from external European data transfers to a Polish primary-care population and adds discrimination for prevalent cardiovascular disease beyond classical risk factors.

Methods: In the nationwide Polish LIPIDOGEN2015 primary-care cohort, we derived PRS for coronary heart disease, myocardial infarction (MI) and stroke from FinnGen data with continuous shrinkage priors and applied them to imputed genotypes. We compared allele frequencies of 1,397 unrelated participants with a non-Finnish European (NFE) reference and, in 1,328 participants with clinical data, related the PRS to coronary artery disease (CAD), MI and stroke recorded at baseline and to a composite including death during follow-up, with and without classical risk factors or SCORE2.

Results: Allele frequencies correlated with the NFE reference (r=0.97). Per standard deviation (SD), the coronary PRS was associated with prevalent CAD (odds ratio 1.36, 95% CI 1.11 to 1.67) and the composite endpoint (1.26, 95% CI 1.04 to 1.51), as were two validated genome-wide scores. Adding it to classical risk factors changed the area under the curve (AUC) for the composite endpoint by 0.0017 (95% CI −0.0005 to 0.0076), from 0.860 to 0.861; results with SCORE2 were similar. The study had 80% power for odds ratios per SD of 1.31 (CAD) and 1.27 (composite).

Conclusions: A coronary PRS derived from external European data retains its association with prevalent CAD without re-derivation in this Polish primary-care cohort but, in this cross-sectional, event-limited sample, adds little discrimination to classical risk factors. Its value for predicting future events in this setting remains to be tested against incident outcomes.

Key words: polygenic risk score; cardiovascular disease; primary care; SCORE2; genotype imputation; Poland; risk stratification

Highlights:

- We tested a coronary polygenic risk score (PRS), built with continuous shrinkage from a FinnGen GWAS, on imputed genotypes in a nationwide Polish primary-care cohort.
- Allele frequencies of 41,037 directly genotyped variants matched the non-Finnish European reference (Pearson r=0.97); each SD of the PRS, computed from 1,062,486 imputed variants, was associated with 1.36-fold higher odds of prevalent coronary artery disease (95% CI 1.11 to 1.67; P=0.003).
- Adding the PRS to a model of traditional risk factors, or to SCORE2, did not materially improve discrimination in this cross-sectional, event-limited sample (change in AUC 0.0017, 95% CI −0.0005 to 0.0076).
- The study had 80% power only for odds ratios of 1.31 or more per SD for coronary artery disease, so modest incremental effects of the PRS cannot be excluded.

Introduction

Polygenic risk scores (PRS) aggregate the small effects of many common variants into a single measure of inherited susceptibility, and for coronary artery disease (CAD) they identify individuals whose genetic risk approaches that conferred by monogenic disease.[1,2] However, the evidence supporting them has been generated almost entirely in a small number of large biobanks, predominantly in the United Kingdom and the Nordic countries. Programs that pool data across many cohorts, such as the National Heart, Lung, and Blood Institute Trans-Omics for Precision Medicine (TOPMed) program, are broadening the populations available for genetic research,[3] but Central and Eastern European populations remain sparsely represented among those used to derive and test polygenic scores. Whether a score derived in one setting retains its properties in another population is an empirical question,[4] and one that matters for any health system considering genomic risk stratification in routine care.

Central Europe is an informative test case. The region carries one of the highest burdens of cardiovascular mortality worldwide;[5] lipid disorders affect most adults in Poland;[6] familial hypercholesterolemia (FH) remains underdiagnosed and undertreated;[7,8] and most cardiovascular risk assessment takes place in primary care rather than in specialist cardiology. Whether a polygenic score transfers into this setting is therefore a relevant question. Transfer, however, is necessary rather than sufficient for clinical use: a score that transfers must also add information to the risk assessment already performed.

Two questions therefore need to be separated, although the polygenic literature often conflates them. The first is whether a score transfers, that is, whether the variants it uses are present at similar frequencies and act in the same direction in the new population. The second is whether it adds information once classical risk factors are measured. A score can transfer well and still be clinically redundant, because age, sex, lipids and blood pressure already capture most of the discriminable variation in risk.

We addressed both questions in the nationwide Polish LIPIDOGEN2015 primary-care cohort. We derived PRS for coronary heart disease, myocardial infarction and stroke from FinnGen summary statistics using continuous shrinkage priors. We applied them to imputed genotypes of 1,328 participants with linked clinical data. We compared allele frequencies in the cohort with a non-Finnish European reference. We related the scores to cardiovascular disease recorded at baseline, alone and in combination with classical risk factors and the SCORE2 risk equations. Because disease was recorded at a single baseline visit, this is a cross-sectional analysis of prevalent disease rather than a test of predicting future events.

Methods

Study Population

LIPIDOGEN2015 is the genetic sub-study of LIPIDOGRAM2015, a nationwide survey of adults attending primary care in Poland. We previously described its design, recruitment and baseline characteristics,[9] and the protocol of the present polygenic risk project.[10] Between December 2015 and June 2016, 438 primary-care physicians in 398 practices across all 16 Polish regions, selected at random with the number per region proportional to the regional population, enrolled 13,724 adults aged 18 years or older, with no upper age limit, who attended the practice for any reason. Individuals with dementia or mental illness that precluded informed consent, and those who declined consent, were not enrolled.[9] The study follows the Transparent Reporting of a Multivariable Prediction Model for Individual Prognosis or Diagnosis (TRIPOD) guideline.[11]

At a single baseline visit, the physician recorded medical history, including physician-diagnosed CAD, myocardial infarction and stroke; current treatment; and family history of cardiovascular disease, using a standardized questionnaire, and measured office blood pressure following European guidelines. A central laboratory analyzed fasting venous blood (after at

least 12 hours of fasting) within 12 hours of collection and measured low-density lipoprotein cholesterol (LDL-C) directly; for 137 participants in the analytic sample, the recorded value was the Friedewald estimate, and excluding them did not change the results. We therefore measured all risk factors once, at baseline, and after the diagnosis of any prevalent disease. We ascertained deaths until December 2021 by linking the national personal identification number (PESEL) to the death register of Statistics Poland, where registration of every death is mandatory; causes of death were not available. Vital status was known for 1,309 participants in the analytic sample; the 19 without it were counted as alive in the composite endpoint and excluded from the death analysis.

The LIPIDOGEN2015 clinical dataset available to this project comprised 1,549 participants, of whom 1,507 could be linked to a genotyped DNA sample through the identifier bridge between the genotyping laboratory and the clinical database. After excluding 46 participants whose sample was on a BeadChip that failed genotyping, 60 whose sample failed quality control and 73 removed in relatedness filtering (see below), the analytic sample comprised 1,328 participants (Figure 1). No participant was excluded from the analytic sample for missing data.

The Bioethical Commission of the Chamber of Physicians approved the study protocol on December 2, 2015 (Resolution No. K.B.Cz.-0018/2015).[9,10] All individuals signed an informed consent form to participate in the study and gave their permission to use anonymous questionnaire data, laboratory test results, and secure biological material for statistical and scientific studies. The study was conducted in accordance with the Declaration of Helsinki.

Genotyping and Quality Control

Samples were genotyped on the Illumina Global Screening Array (GSA-24v3-0_A2.bpm manifest with the GSA-24v3-0_A1_ClusterFile.egt cluster file) across 67 BeadChips. We converted intensity data to genotype calls and then to variant call format against the GRCh38

reference genome in a Nextflow pipeline[12] using bcftools,[13] and set individual genotype calls with a GenCall score below 0.15 to missing. We excluded 2 BeadChips for degraded intensity data, leaving 1,560 samples and 610,818 variants. The mean sample call rate was 98.4%, and 1,502 of 1,560 samples met the 0.97 threshold.

Using PLINK 2,[14] we removed variants with missingness above 2%, minor allele frequency below 1% or departure from Hardy-Weinberg equilibrium at $P<1\times10^{-6}$, and samples with missingness above 2%, leaving 1,474 samples and 450,336 variants. We removed one from each pair of relatives with a KING kinship coefficient above 0.0884,[15] corresponding to second-degree relatedness, leaving 1,397 unrelated participants. Allele frequency comparisons used these directly assayed variants; we computed polygenic scores from imputed genotypes.

Genotype Imputation

We imputed the 1,474 quality-controlled samples with Beagle 5.5[16] against the phased high-coverage 1000 Genomes Project panel of 3,202 genomes,[17] restricted to biallelic single-nucleotide variants with a minor allele frequency of at least 0.5% in the panel, using GRCh38 genetic maps. We ran imputation locally against this public panel, so no individual-level data were sent to an external imputation server. We retained 8,379,553 variants with an estimated dosage R^2 (DR2) of at least 0.8.

Ancestry

We computed principal components by projecting the cohort onto the 1000 Genomes Project reference panel[17] after linkage-disequilibrium pruning. On the first two components, all 1,397 unrelated participants lay closest to the European reference centroid; 3 lay more than six standard deviations from the European reference distribution, between the European and admixed American clusters, and were retained. We retained the first five principal components as covariates for genome-wide analyses. In genome-wide association analyses of the outcomes, adjusted for age, sex and the first five principal components, the genomic control inflation factor

was 1.01 to 1.03, indicating no appreciable residual population stratification, and the same approach recovered known lipid loci (APOE for LDL-C, $P=9.2\times10^{-17}$; CETP for high-density lipoprotein cholesterol, $P=5.0\times10^{-10}$).

Polygenic Score Derivation

We derived weights from FinnGen release 11 summary statistics[18] for coronary heart disease, myocardial infarction and stroke using PRS-CS, a Bayesian method with continuous shrinkage priors,[19] with the global shrinkage parameter learned from the data and the European 1000 Genomes linkage-disequilibrium reference panel of 1,120,696 HapMap 3 variants. We used summary statistics rather than previously published weights so that all three scores came from one large, recent European resource and one method. PRS-CS re-estimates the effect of each variant jointly with those of its correlated neighbors, so its posterior weights differ from the marginal effects of the genome-wide association study (Pearson r=0.42 across 1,111,426 variants); for the 14 variants of a previously published 27-variant coronary score[20] present among the weights, the direction of effect agreed in all. We applied the weights to the imputed dosages, which covered 1,062,486 of the 1,111,426 weighted variants (96%), by summing dosage-weighted effects; standardized each score to zero mean and unit variance across all genotyped samples; and averaged replicate samples of the same participant. We reconciled variant identifiers to reference single-nucleotide polymorphism cluster identifiers (rsIDs) before scoring because mixing identifier conventions can silently drop variants.

To test whether the results depended on the score or on imputation, we also computed the same PRS-CS score restricted to the 161,020 HapMap 3 variants directly assayed on the array, which requires no imputation; the 27-variant coronary score pre-specified in our study protocol,[10] which corresponds to the score of Mega et al[20] (PGS Catalog[21] PGS000010; 25 of 27 variants available, 96% of the total weight), and the 50-variant score of Tada et al[22] (PGS000011; 39 of 50 variants, 77% of the weight), both from directly assayed variants; and

two validated genome-wide coronary scores, metaGRS[2] (PGS000018) and GPS-CAD[1] (PGS000013), from imputed dosages (61% and 86% of their total absolute weight available, respectively).

Outcomes and Classical Risk Model

CAD, myocardial infarction (MI) and ischemic stroke were physician-recorded diagnoses at the baseline visit and are therefore prevalent rather than incident. We defined the composite endpoint as the presence of any of these conditions at baseline or death from any cause during follow-up, with each participant counted once, regardless of how many components applied. Of the 152 participants with the composite endpoint, 131 had prevalent CAD, MI or stroke, 21 qualified by death alone, and 58 met two or more components (Supplementary Table S1). Because the composite endpoint is dominated by prevalent disease, we analyzed it, the three prevalent conditions and their union (prevalent CAD, MI or stroke, without deaths) as binary outcomes with logistic regression, and analyzed all-cause death separately with Cox regression (31 deaths among the 1,309 participants with known vital status during a median follow-up of 5.7 years; 14 of the deaths occurred in participants free of cardiovascular disease and diabetes at baseline).

The classical model comprised age, sex, LDL-C, high-density lipoprotein cholesterol (HDL-C), systolic blood pressure, current smoking, diabetes and body mass index, a set of established risk factors that overlaps with those of contemporary European risk algorithms such as SCORE2.[23] We compared three nested models: classical risk factors alone; the PRS with age and sex; and the classical model with the PRS added. Because treatment started after diagnosis lowers measured LDL-C and blood pressure, we repeated the comparisons with additional adjustment for statin or any lipid-lowering therapy, antihypertensive treatment and family history of cardiovascular disease, and in participants with directly measured LDL-C.

A classical model derived and evaluated in the same data may be optimistic, so we also used externally derived risk equations as base models. We computed SCORE2 (ages 40 to 69 years) and SCORE2-OP (70 years and older), recalibrated for high-risk countries, which include Poland,[23,24] and, as a secondary benchmark, the US pooled cohort equations (PCE),[25] in the 1,059 participants aged 40 years or older. SCORE2 does not include diabetes, so we also fitted it with diabetes as an additional term. Here, the equations serve as externally validated summaries of risk factors, not predictions of future events, and we applied them to all participants in the age range, including those with prevalent disease.

Statistical Analysis

We summarized discrimination by the area under the receiver operating characteristic curve (AUC) with 95% confidence intervals (CI), and compared nested models with the DeLong test for correlated curves,[26] the likelihood ratio test and a bootstrap 95% CI for the change in AUC (1,000 resamples). The categorical net reclassification improvement (NRI) at predicted probabilities of 5%, 10% and 20% and the integrated discrimination improvement (IDI),[27] with bootstrap CIs, are reported in the Supplement. We estimated optimism by bootstrap and by repeated (5 × 5-fold) cross-validation and, for MI and stroke, which had few events per variable, also fitted L2-penalized models within the cross-validation. We assessed calibration of out-of-fold predictions using calibration intercept and slope, Brier score and the Hosmer-Lemeshow test. We assigned participants to low, intermediate and high tiers of predicted probability (below 5%, 5% to below 10%, and 10% or above) under the classical and the combined models, and cross-tabulated the two assignments separately for participants with and without the endpoint; because these probabilities describe prevalent disease, the tiers are descriptive. To test whether the PRS association depended on baseline risk, we used the SCORE2 risk categories from the 2021 European prevention guideline (low-to-moderate, high and very high, with age-specific thresholds)[28] and included a PRS-by-category interaction term. We report associations as odds

ratios (OR) per standard deviation (SD) of the score and as top-versus-bottom quintile contrasts, both adjusted for age, sex as recorded in the clinical dataset, and the first five principal components (PC1-5). We estimated the smallest OR per SD detectable with 80% power at a two-sided α of 0.05 for each endpoint from the number of cases and the variance of the score after adjustment for covariates, and checked the estimate by simulation.

We compared cohort allele frequencies with the non-Finnish European (NFE) reference of the Genome Aggregation Database (gnomAD),[29] obtained through the Ensembl Variant Effect Predictor,[30] by Pearson correlation across all directly genotyped array variants that passed quality control and carried a reference annotation (array genotypes only, not imputed dosages), and, for individual variants, by a two-sided binomial test against the reference frequency treated as a known proportion. We considered a variant divergent at $P<1\times10^{-6}$, which is more stringent than Bonferroni correction for the 38,647 gene-annotated variants tested (1.3×10^{-6}). Because a fixed external reference also captures platform and population differences, we calibrated per-variant results against the genome-wide background by comparing the proportion of divergent variants among the 68 array variants in nine low-density lipoprotein (LDL) pathway genes with that among the other 38,579 gene-annotated variants using Fisher's exact test. P values are exact for the variants reported individually and use the normal approximation to the binomial for this genome-wide comparison. Analyses used PLINK v2.0.0-a.7.1 M1, bcftools 1.20, Beagle 5.5, PRS-CS and Python with pandas, statsmodels, scikit-learn and lifelines. We considered two-sided P values below 0.05 significant, without adjustment for multiplicity across endpoints; the analysis is framed as estimation rather than hypothesis screening, and confidence intervals should be read accordingly.

Results

Participants

Of 1,549 participants with clinical data, 1,507 were linked to a genotyped sample, and 1,328 were included in the analytic sample (Figure 1). Table 1 shows their characteristics. The mean age was 50.5 years (269 participants were younger than 40 years and 35 were 70 or older); 531 (40.0%) were men; mean LDL-C was 3.35 mmol/L; and 360 (27.1%) were taking a statin and 529 (39.8%) were taking antihypertensive treatment. Prevalent CAD was recorded in 119 (9.0%) participants, MI in 48 (3.6%) and ischemic stroke in 19 (1.4%); 31 of the 1,309 with known vital status (2.4%) died during follow-up, and 152 (11.4%) met the composite endpoint. The 221 participants with clinical data who were not analyzed were younger (mean 47.6 versus 50.5 years) and had a slightly lower body mass index; the standardized differences were 0.22 or less for all characteristics (Supplementary Table S2).

Transferability of the Score

Across 41,037 directly genotyped array variants that passed quality control and carried an NFE reference annotation, cohort allele frequencies tracked the reference closely (Pearson r=0.975, Spearman r=0.952, mean absolute difference in allele frequency 0.028). These variants serve only to compare allele frequencies and do not constitute the PRS, which was computed from 1,062,486 imputed variants (see Polygenic Score Derivation). The close agreement indicates that the cohort's genetic background resembles that of the European population in which the weights were estimated, a necessary condition for transfer.

Association With Prevalent Cardiovascular Outcomes

Table 2 and Figure 2A show associations of the coronary score with each endpoint. Each SD of the score was associated with higher odds of prevalent CAD (OR 1.36, 95% CI 1.11 to 1.67; P=0.003), of MI (OR 1.49, 95% CI 1.08 to 2.07; P=0.016) and of the composite endpoint

(OR 1.26, 95% CI 1.04 to 1.51; P=0.017). Supplementary Table S3 reports the ORs for the score and classical risk factors in the combined model.

The prevalence of CAD was 5.3% in the bottom quintile and 12.0% in the top quintile of the score (Figure 2A), and the odds of CAD in the top quintile were 3.41 (95% CI 1.64 to 7.10) times those in the bottom quintile (P=0.001). Quintile contrasts are shown for comparison with earlier studies; they use only two-fifths of the sample and overstate the gradient relative to the per-SD estimates.

The trait-matched scores were less informative. The MI score was associated with MI less strongly than the coronary score (OR per SD 1.31, 95% CI 0.94 to 1.83). Neither the stroke score (1.00, 95% CI 0.61 to 1.62) nor the coronary score (1.17, 95% CI 0.73 to 1.86) was associated with ischemic stroke; with 19 cases, the stroke estimates cannot distinguish a failed transfer from an absence of power. All-cause death, analyzed separately, was not associated with the coronary score (hazard ratio per SD 1.04, 95% CI 0.73 to 1.48; 31 deaths), including in participants free of cardiovascular disease and diabetes at baseline (0.74, 95% CI 0.44 to 1.25; 14 deaths); both estimates are compatible with a wide range of effects (Supplementary Table S4).

The association with CAD was similar to previously validated scores (Supplementary Table S5). The validated genome-wide scores computed from imputed dosages gave ORs per SD for CAD of 1.44 (95% CI 1.17 to 1.77) for metaGRS and 1.28 (95% CI 1.04 to 1.57) for GPS-CAD, and the 27-variant protocol score gave 1.33 (95% CI 1.09 to 1.62). Restricted to directly assayed variants, the PRS-CS score, which correlated with the imputed score (r=0.77), gave a weaker estimate of 1.21 (95% CI 0.99 to 1.49), consistent with the information lost without imputation. Adjusting for lipid-lowering or antihypertensive treatment or for family history of cardiovascular disease, or restricting to participants with directly measured LDL-C, kept the

OR per SD of the PRS for CAD in the classical model between 1.30 and 1.33 (Supplementary Table S6).

Incremental Value Over Classical Risk Factors

Table 3 and Figure 2B show the nested model comparisons. For the composite endpoint, the classical model reached an AUC of 0.860 (95% CI 0.831 to 0.888). Adding the PRS changed this to 0.861 (95% CI 0.833 to 0.890), an increase of 0.0017 (bootstrap 95% CI −0.0005 to 0.0076; DeLong P=0.44). For CAD, the increase was 0.0035 (bootstrap 95% CI −0.0007 to 0.0116; P=0.38). The likelihood ratio test indicated that the score carried independent information for CAD (P=0.009), but not enough to meaningfully improve discrimination. In repeated cross-validation, the composite-endpoint AUC was 0.846 for the classical model and 0.847 with the PRS added. The NRI confidence intervals included zero for every endpoint, and the IDI was small, the largest being 0.0101 for CAD (95% CI 0.0004 to 0.0295) (Supplementary Table S7).

The interpretation of the intermediate model deserves care. A model containing the PRS with age and sex reached an AUC of 0.824 for the composite endpoint, which invites the reading that the score is strongly predictive. It is not. The PRS alone gave an AUC of 0.554 (95% CI 0.507 to 0.602), barely above chance. Age alone gave an AUC of 0.799 and age with sex 0.822, so almost all the discrimination of that intermediate model comes from age. Figure 2B shows the four curves together.

The classical model was not materially overfitted for the larger endpoints: its optimism-corrected AUC was 0.851 for the composite endpoint, 0.838 for CAD and 0.895 for MI, and penalized estimation gave a similar cross-validated AUC for MI (0.889). For ischemic stroke, with 2.4 events per variable, the cross-validated AUC fell to 0.738, and to 0.637 with penalization, so the stroke models should not be interpreted. Out-of-fold predictions were well calibrated for the composite endpoint, CAD and MI (calibration slopes 0.88 to 0.94; Hosmer-

Lemeshow P≥0.45) but not for stroke (slopes 0.60 and 0.63) (Supplementary Table S8 and Figure S1).

With SCORE2 as the base model, in the 1,059 participants aged 40 years or older, the PRS remained associated with CAD (OR per SD 1.31, 95% CI 1.07 to 1.61; likelihood ratio P=0.008) but changed the AUC only from 0.733 to 0.740 (DeLong P=0.38); with the PCE, the AUC changed from 0.773 to 0.777 (Supplementary Table S9). The association of the PRS did not differ across SCORE2 risk categories (interaction P=0.77 for CAD and 0.89 for the composite endpoint; Supplementary Table S10), and restricting the classical-model comparison to participants aged 40 to 69 years gave similar results (change in AUC for CAD 0.0041, DeLong P=0.41).

Risk Tiers Under the Classical and Combined Models

Under the classical model, the prevalence of the composite endpoint rose from 1.6% in the low tier (677 participants) through 6.4% in the intermediate tier (218) to 29.3% in the high tier (433), which held 84% of all participants with the endpoint (Table 4 and Figure 3A). The combined model produced a similar gradient (1.5%, 5.2% and 30.4%). Adding the PRS moved 7 of the 152 participants with the composite endpoint to a different tier (6 to a higher and 1 to a lower tier) and 90 of the 1,176 without it (38 higher, 52 lower) (Table 4). The gradient across tiers is therefore a property of the classical risk factors.

Lipid-Pathway Allele Frequencies

Three variants in genes of the low-density lipoprotein pathway, shown in Figure 3B and Table 5, differed markedly in frequency from the NFE reference. In 1,397 unrelated participants, APOB rs1801703 was carried at 1.79% versus 0.81% in the reference (2.21-fold); LDLR rs10423288 at 2.62% versus 1.30% (2.01-fold); and APOE rs445925 at 9.81% versus 6.67% (1.47-fold), each at $P<1\times10^{-6}$ on exact binomial testing. None is a pathogenic FH variant: LDLR rs10423288 is benign in ClinVar, APOB rs1801703 is benign to likely benign

in ClinVar, with conflicting submissions, and APOE rs445925 has no ClinVar entry. This analysis uses the unrelated set rather than the analytic sample, because allele frequency estimation gains from the larger denominator and does not require the clinical covariates.

Calibration against the rest of the array changes the interpretation of these differences. Against the fixed external reference, 25.6% of the 38,579 gene-annotated array variants outside the LDL pathway departed at $P<1\times10^{-6}$, reflecting genotyping-platform and population differences. Across all 68 array variants in nine LDL-pathway genes (Supplementary Table S11 gives the count per gene), the proportion was 32.4% (22 variants), not significantly higher (Fisher's exact test P=0.21), and the departures ran in both directions (15 variants more common and 7 less common than the reference). The three variants above were among those more common in the cohort. They therefore differ in frequency, but they do not show that the LDL pathway as a whole is enriched in this population.

Discussion

This study asked two questions about a coronary PRS in a Polish primary-care population and answered each differently. The score transfers and is associated with prevalent CAD, but it adds little measurable discrimination once classical risk factors are in the model.

Two lines of evidence support transfer. Allele frequencies of 41,037 directly genotyped array variants, used only for this comparison, track the NFE reference at r=0.97; and the PRS itself, computed from 1,062,486 imputed variants, is associated with prevalent CAD with a direction and size that agree with published estimates. These findings suggest that the score retains its association with prevalent CAD without re-derivation in this Polish cohort. They do not establish its prospective clinical applicability, which requires incident outcomes, nor that it transfers to other Central European populations. Transfer is expected to degrade with genetic distance rather than at national borders,[4] but allele frequencies, linkage disequilibrium and the

baseline risk against which a score is judged differ between countries, and both steps, transfer and incremental value, would need to be confirmed in each population before clinical use.

The size of the association falls within the range reported for coronary scores in European-ancestry populations, although comparisons are approximate because our estimates refer to prevalent disease. The OR per SD of 1.36 for CAD is similar to the hazard ratios per SD of 1.24 and 1.38 reported for a genome-wide score in the ARIC and MESA cohorts[31] and smaller than the 1.71 reported for metaGRS in UK Biobank.[2] For the 27- and 50-variant scores, top-versus-bottom quintile hazard ratios of 1.7 to 1.9 have been reported,[20,22] against an OR of 2.31 for the 27-variant score here. The small incremental value also matches prospective studies: adding a genome-wide score to the PCE did not improve the C statistic in ARIC or MESA,[31] and in UK Biobank it raised the C statistic from 0.76 to 0.78.[32] The 2026 ACC/AHA multisociety dyslipidemia guideline lists high polygenic risk, when measured, among the risk-enhancing factors that can inform lipid-lowering decisions,[33] a role that rests on relative risk in the tail of the distribution rather than on population-wide discrimination.

The small incremental value is equally clear and, we would argue, more important. Adding the score to a model containing age, sex, lipids, blood pressure, smoking, diabetes and body mass index moved the AUC by 0.0038 or less for the composite endpoint, CAD and MI, with bootstrap confidence intervals that include no change; with SCORE2 or the PCE as the base model, the change was 0.0065 or less and not significant. Several explanations apply simultaneously and cannot be fully separated in these data.

First, the outcomes are prevalent rather than incident. A PRS measures lifetime susceptibility, and its natural comparator is future risk in people who are currently well. Asking it to discriminate disease that has already occurred, in a sample where age is recorded contemporaneously, sets it against a competitor that has already absorbed the consequences of that susceptibility. Risk factors were also measured after diagnosis, when treatment had already

modified them: LDL-C was inversely associated with the composite endpoint (OR per SD 0.60, 95% CI 0.48 to 0.74), the signature of lipid-lowering treatment after diagnosis, and adjustment for treatment did not change the results. Age, lipids and blood pressure at baseline are also partly downstream of the very genetics the score measures.

Second, the sample is event-limited. With 152 participants meeting the composite endpoint, 119 cases of CAD and 48 of MI, the study had 80% power to detect ORs per SD of 1.27, 1.31 and 1.51, respectively, and 1.91 for stroke (Supplementary Table S12 and Figure S2). Published per-SD effects of coronary scores range from about 1.2 to 1.7, so the study was adequately powered only for CAD effects of 1.31 or more, and it was underpowered for MI and stroke. For incremental discrimination, which depends on a far smaller signal, the margin is narrower still. The correct statement is that we did not detect a meaningful incremental value, not that we excluded it.

Third, without imputation, array coverage would have limited the score. The array carried 161,020 of the 1,120,696 HapMap 3 variants used by PRS-CS; after imputation, the score covered 1,062,486 weighted variants, and its association with CAD was stronger than that of the array-only score (OR per SD 1.36 versus 1.21). Two validated genome-wide scores gave similar estimates. Yet the change in AUC remained small for every score, which places the limit on incremental value in the design of this study (prevalent outcomes and few events) rather than in the genotyping.

Fourth, overfitting of the classical model does not explain the result. Its optimism-corrected and cross-validated AUCs were within 0.02 of the apparent values for the composite endpoint, CAD and MI, and the externally derived SCORE2 and PCE base models led to the same conclusion.

Interpretation of the Lipid-Pathway Findings

The lipid-pathway allele frequencies should not be over-read. The three variants that differ most visibly from the reference are not FH mutations: none is classified as pathogenic in ClinVar, and carrying one neither identifies an affected individual nor qualifies a family for cascade screening. Once calibrated against the genome-wide background, the LDL pathway as a whole was no more divergent from the reference than the rest of the array. These data therefore document transfer at the allele-frequency level and bear on neither the prevalence of FH in Poland nor the case for FH screening.

Limitations

The disease outcomes are prevalent and were recorded at baseline, so these data cannot support causal or predictive claims about future events. The predicted probabilities and risk tiers describe prevalence rather than future risk. Non-fatal events during follow-up were not recorded; only deaths were ascertained, without causes, and the 14 deaths among participants free of cardiovascular disease and diabetes at baseline are too few for an incident-outcome analysis, which would require linkage to hospitalization records. The composite endpoint combines prevalent disease with deaths accrued during follow-up, so we also report its components separately. We measured risk factors once, after diagnosis and treatment of prevalent disease. We did not measure creatinine, so we could not compute the PREVENT equations and SCORE2-Diabetes, which require estimated glomerular filtration rate. Imputation used the 1000 Genomes panel rather than a larger or population-specific reference, which limits accuracy for rarer variants and the coverage of some published scores. The analytic sample depends on an identifier bridge between the genotyping laboratory and the clinical dataset, which linked 1,507 of 1,549 participants; analyzed and non-analyzed participants differed little. The allele frequency comparison uses an external reference derived from sequencing rather than a matched Polish series genotyped on the same array; per-variant

differences therefore mix population and platform effects, so we calibrated them against the genome-wide background. Finally, we did not adjust for multiple endpoints, so nominally significant results should be read as estimates rather than independent confirmations.

Conclusions

A coronary PRS derived from external European summary statistics transfers to a Polish primary-care cohort in allele frequency and effect direction, and is associated with prevalent CAD. In this cross-sectional, event-limited sample, it added little discrimination to classical risk factors or to SCORE2. Whether it improves the prediction of future cardiovascular events in this population remains to be tested in a cohort with incident outcomes.

Article Information

The results will be presented during the American Heart Association (AHA) Scientific Sessions 2026 in the oral moderated session "*Building Better Models: Machine Learning and Novel Frameworks for Risk Prediction*" on Saturday, Nov. 7.

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Data Availability

Individual-level genotype and phenotype data are subject to the consent under which LIPIDOGEN2015 was collected. Analysis code, quality-control thresholds and the derived summary tables underlying every figure are held in the project repository and can be made available on reasonable request.

References

1. Khera AV, Chaffin M, Aragam KG, et al. Genome-wide polygenic scores for common diseases identify individuals with risk equivalent to monogenic mutations. Nat Genet. 2018;50(9):1219-1224. doi: 10.1038/s41588-018-0183-z PMID: 30104762.
2. Inouye M, Abraham G, Nelson CP, et al. Genomic Risk Prediction of Coronary Artery Disease in 480,000 Adults: Implications for Primary Prevention. J Am Coll Cardiol. 2018;72(16):1883-1893. doi: 10.1016/j.jacc.2018.07.079 PMID: 30309464.
3. Taliun D, Harris DN, Kessler MD, et al. Sequencing of 53,831 diverse genomes from the NHLBI TOPMed Program. Nature. 2021;590(7845):290-299. doi: 10.1038/s41586-021-03205-y PMID: 33568819.
4. Martin AR, Kanai M, Kamatani Y, Okada Y, Neale BM, Daly MJ. Clinical use of current polygenic risk scores may exacerbate health disparities. Nat Genet. 2019;51(4):584-591. doi: 10.1038/s41588-019-0379-x PMID: 30926966.
5. Roth GA, Mensah GA, Johnson CO, et al. Global Burden of Cardiovascular Diseases and Risk Factors, 1990-2019: Update From the GBD 2019 Study. J Am Coll Cardiol. 2020;76(25):2982-3021. doi: 10.1016/j.jacc.2020.11.010 PMID: 33309175.
6. Banach M, Mastalerz-Migas A, Wita K, Myśliwiec M. Poland takes a lead in effective lipid disorders management healthcare programmes in Europe. Am J Prev Cardiol. 2025;24:101346. doi: 10.1016/j.ajpc.2025.101346 PMID: 41321469.
7. Myśliwiec M, Bandura M, Wołoszyn-Durkiewicz A, et al. 2024 Polish recommendations for the management of familial hypercholesterolemia in children and adolescents. Arch Med Sci. 2024;20(6):1741-1753. doi: 10.5114/aoms/196329 PMID: 39967943.
8. Lewek J, Sosnowska B, Starostecka E, et al. Clinical reality and challenges with familial hypercholesterolemia patients' management. 2024 results from the Regional Center for Rare Diseases (RCRD) Registry in Poland. Int J Cardiol. 2025;419:132667. doi: 10.1016/j.ijcard.2024.132667 PMID: 39442759.
9. Jóźwiak JJ, Kasperczyk S, Tomasik T, et al. Design and rationale of a nationwide screening analysis from the LIPIDOGRAM2015 and LIPIDOGEN2015 studies. Arch Med Sci. 2022;18(3):604-616. doi: 10.5114/aoms.2020.96052 PMID: 35591817.
10. Banach M, Fronczek M, Osadnik T, et al. Risk of adverse cardiovascular events based on common genetic variants in 8-year follow-up of the LIPIDOGEN2015 population using the polygenic risk score (PRS): study design and methodology. Arch Med Sci. 2024;20(5):1452-1460. doi: 10.5114/aoms/192147 PMID: 39649254.

11. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent Reporting of a multivariable prediction model for Individual Prognosis or Diagnosis (TRIPOD): the TRIPOD statement. Ann Intern Med. 2015;162(1):55-63. doi: 10.7326/M14-0697 PMID: 25560714.

12. Di Tommaso P, Chatzou M, Floden EW, Barja PP, Palumbo E, Notredame C. Nextflow enables reproducible computational workflows. Nat Biotechnol. 2017;35(4):316-319. doi: 10.1038/nbt.3820 PMID: 28398311.

13. Danecek P, Bonfield JK, Liddle J, et al. Twelve years of SAMtools and BCFtools. Gigascience. 2021;10(2):giab008. doi: 10.1093/gigascience/giab008 PMID: 33590861.

14. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience. 2015;4:7. doi: 10.1186/s13742-015-0047-8 PMID: 25722852.

15. Manichaikul A, Mychaleckyj JC, Rich SS, Daly K, Sale M, Chen WM. Robust relationship inference in genome-wide association studies. Bioinformatics. 2010;26(22):2867-2873. doi: 10.1093/bioinformatics/btq559 PMID: 20926424.

16. Browning BL, Zhou Y, Browning SR. A One-Penny Imputed Genome from Next-Generation Reference Panels. Am J Hum Genet. 2018;103(3):338-348. doi: 10.1016/j.ajhg.2018.07.015 PMID: 30100085.

17. Byrska-Bishop M, Evani US, Zhao X, et al. High-coverage whole-genome sequencing of the expanded 1000 Genomes Project cohort including 602 trios. Cell. 2022;185(18):3426-3440.e19. doi: 10.1016/j.cell.2022.08.004 PMID: 36055201.

18. Kurki MI, Karjalainen J, Palta P, et al. FinnGen provides genetic insights from a well-phenotyped isolated population. Nature. 2023;613(7944):508-518. doi: 10.1038/s41586-022-05473-8 PMID: 36653562.

19. Ge T, Chen CY, Ni Y, Feng YA, Smoller JW. Polygenic prediction via Bayesian regression and continuous shrinkage priors. Nat Commun. 2019;10(1):1776. doi: 10.1038/s41467-019-09718-5 PMID: 30992449.

20. Mega JL, Stitziel NO, Smith JG, et al. Genetic risk, coronary heart disease events, and the clinical benefit of statin therapy: an analysis of primary and secondary prevention trials. Lancet. 2015;385(9984):2264-2271. doi: 10.1016/S0140-6736(14)61730-X PMID: 25748612.

21. Lambert SA, Gil L, Jupp S, et al. The Polygenic Score Catalog as an open database for reproducibility and systematic evaluation. Nat Genet. 2021;53(4):420-425. doi: 10.1038/s41588-021-00783-5 PMID: 33692568.

22. Tada H, Melander O, Louie JZ, et al. Risk prediction by genetic risk scores for coronary heart disease is independent of self-reported family history. Eur Heart J. 2016;37(6):561-567. doi: 10.1093/eurheartj/ehv462 PMID: 26392438.

23. SCORE2 working group and ESC Cardiovascular risk collaboration. SCORE2 risk prediction algorithms: new models to estimate 10-year risk of cardiovascular disease in Europe. Eur Heart J. 2021;42(25):2439-2454. doi: 10.1093/eurheartj/ehab309 PMID: 34120177.

24. SCORE2-OP working group and ESC Cardiovascular risk collaboration. SCORE2-OP risk prediction algorithms: estimating incident cardiovascular event risk in older persons in four geographical risk regions. Eur Heart J. 2021;42(25):2455-2467. doi: 10.1093/eurheartj/ehab312 PMID: 34120185.

25. Goff DC Jr, Lloyd-Jones DM, Bennett G, et al. 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 PMID: 24222018.

26. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics. 1988;44(3):837-845. doi: 10.2307/2531595 PMID: 3203132.

27. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. Stat Med. 2008;27(2):157-172. doi: 10.1002/sim.2929 PMID: 17569110.

28. Visseren FLJ, Mach F, Smulders YM, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J. 2021;42(34):3227-3337. doi: 10.1093/eurheartj/ehab484 PMID: 34458905.

29. Chen S, Francioli LC, Goodrich JK, et al. A genomic mutational constraint map using variation in 76,156 human genomes. Nature. 2024;625(7993):92-100. doi: 10.1038/s41586-023-06045-0 PMID: 38057664.

30. McLaren W, Gil L, Hunt SE, et al. The Ensembl Variant Effect Predictor. Genome Biol. 2016;17(1):122. doi: 10.1186/s13059-016-0974-4 PMID: 27268795.

31. Mosley JD, Gupta DK, Tan J, et al. Predictive Accuracy of a Polygenic Risk Score Compared With a Clinical Risk Score for Incident Coronary Heart Disease. JAMA. 2020;323(7):627-635. doi: 10.1001/jama.2019.21782 PMID: 32068817.

32. Elliott J, Bodinier B, Bond TA, et al. Predictive Accuracy of a Polygenic Risk Score-Enhanced Prediction Model vs a Clinical Risk Score for Coronary Artery Disease. JAMA. 2020;323(7):636-645. doi: 10.1001/jama.2019.22241 PMID: 32068818.

33. Writing Committee Members, Blumenthal RS, Morris PB, et al. 2026 ACC/AHA/AACVPR/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA Guideline on the Management of Dyslipidemia: A Report of the American College of Cardiology/American Heart

Association Joint Committee on Clinical Practice Guidelines. Circulation. 2026;153(17):e1154-e1276. doi: 10.1161/CIR.0000000000001423 PMID: 41824552.

Figure Legends

Figure 1. Participant flow. Left, genotyping: of 1,608 samples on 67 BeadChips, 1,560 samples yielded genotype calls at 610,818 variants; quality control on variant missingness, minor allele frequency, Hardy-Weinberg equilibrium and sample missingness left 1,474 samples and 450,336 variants, and removal of one of each related pair at a kinship coefficient above 0.0884 left 1,397 unrelated samples, used for the allele frequency analyses. Right, participants: of 1,549 participants with clinical data, 1,507 were linked to a genotyped sample; after excluding participants whose sample failed genotyping (46), quality control (60) or relatedness filtering (73), the analytic sample comprised 1,328 participants. We excluded no participants for missing data.

Figure 2. Polygenic risk score performance. A, Prevalence by quintile (Q1 to Q5) of the coronary PRS, computed from imputed genotypes, for the composite endpoint, CAD and MI, with the OR per SD adjusted for age, sex and the first five principal components. B, Receiver operating characteristic curves for the composite endpoint under four models: classical risk factors alone, classical risk factors with the PRS, the PRS with age and sex, and the PRS alone. The separation between the last two curves shows that the age-and-sex model's discrimination is attributable to age rather than the PRS. AUC indicates area under the receiver operating characteristic curve; OR, odds ratio; and PRS, polygenic risk score.

Figure 3. Risk tiers and lipid-pathway allele frequencies. A, Prevalence of the composite endpoint across tiers of predicted probability (below 5%, 5% to below 10%, 10% or above) under the classical model and under the combined model with the polygenic risk score, in the 1,328 participants of the analytic sample. B, Alternate allele frequencies for three variants in genes of the low-density lipoprotein (LDL) pathway in the 1,397 unrelated participants, compared with the gnomAD non-Finnish European (NFE) reference, with fold difference on the right. None of the three is a pathogenic familial hypercholesterolemia (FH) variant. After calibration against the genome-wide background, the LDL pathway as a whole was not more divergent from the reference than the rest of the array (see Results). The two panels use different denominators. gnomAD indicates Genome Aggregation Database; LDL, low-density lipoprotein; and NFE, non-Finnish European.

Tables

Table 1. Baseline characteristics of the analytic sample.

Characteristic	Analytic sample (n=1,328)
Age, years, mean (SD)	50.5 (12.0)
Age below 40 years, n (%)	269 (20.3%)
Age 40 to 69 years, n (%)	1,024 (77.1%)
Age 70 years or older, n (%)	35 (2.6%)
Men, n (%)	531 (40.0%)
Body mass index, kg/m2, mean (SD)	28.2 (5.0)
Systolic blood pressure, mmHg, mean (SD)	128.6 (17.0)
Total cholesterol, mmol/L, mean (SD)	5.36 (1.17)
LDL cholesterol, mmol/L, mean (SD)	3.35 (1.05)
HDL cholesterol, mmol/L, mean (SD)	1.43 (0.41)
Triglycerides, mmol/L, mean (SD)	1.77 (1.43)
Current smoking, n (%)	219 (16.5%)
Diabetes, n (%)	196 (14.8%)
Hypertension, n (%)	551 (41.5%)
Antihypertensive treatment, n (%)	529 (39.8%)
Dyslipidemia, n (%)	996 (75.0%)
Family history of cardiovascular disease, n (%)	596 (44.9%)
Statin therapy, n (%)	360 (27.1%)
Any lipid-lowering therapy, n (%)	429 (32.3%)
Antiplatelet therapy, n (%)	126 (9.5%)
Coronary artery disease, n (%)	119 (9.0%)
Myocardial infarction, n (%)	48 (3.6%)
Ischemic stroke, n (%)	19 (1.4%)
Death during follow-up, n (%)	31 (2.4%)
Composite endpoint, n (%)	152 (11.4%)

HDL indicates high-density lipoprotein; LDL, low-density lipoprotein; and SD, standard deviation. Disease outcomes are prevalent at baseline; deaths are those during follow-up, among the 1,309 participants with known vital status. The composite endpoint is the presence of coronary artery disease, myocardial infarction or ischemic stroke at baseline, or death during follow-up, with each participant counted once.

Table 2. Coronary polygenic risk score and prevalent cardiovascular outcomes.

Endpoint	Cases	AUC (95% CI)	OR per SD (95% CI)	P	Top vs bottom quintile OR (95% CI)	P
Composite endpoint	152	0.554 (0.507 to 0.602)	1.26 (1.04 to 1.51)	0.017	2.22 (1.16 to 4.24)	0.016
Coronary artery disease	119	0.583 (0.531 to 0.636)	1.36 (1.11 to 1.67)	0.003	3.41 (1.64 to 7.10)	0.001
Myocardial infarction	48	0.605 (0.529 to 0.682)	1.49 (1.08 to 2.07)	0.016	3.57 (1.14 to 11.23)	0.029
Myocardial infarction, MI score	48	0.579 (0.507 to 0.651)	1.31 (0.94 to 1.83)	0.11	1.94 (0.60 to 6.24)	0.27
Ischemic stroke	19	0.530 (0.393 to 0.666)	1.17 (0.73 to 1.86)	0.52	1.09 (0.26 to 4.61)	0.90
Ischemic stroke, stroke score	19	0.493 (0.366 to 0.621)	1.00 (0.61 to 1.62)	0.99	1.46 (0.30 to 7.09)	0.64

Scores computed from imputed genotypes, n=1,328. Rows show the coronary heart disease score unless a trait-matched score is named. ORs per SD and quintile contrasts adjusted for age, sex and the first five principal components; AUCs are for the score alone, with DeLong 95% CIs. AUC indicates area under the receiver operating characteristic curve; CI, confidence interval; MI, myocardial infarction; OR, odds ratio; and SD, standard deviation.

Table 3. Nested model comparison: does the polygenic score add to classical risk factors?

Endpoint	Cases	Classical AUC (95% CI)	Combined AUC (95% CI)	Change in AUC (bootstrap 95% CI)	DeLong P	LRT P	Cross-validated AUC, classical / combined
Composite endpoint	152	0.860 (0.831 to 0.888)	0.861 (0.833 to 0.890)	+0.0017 (−0.0005 to 0.0076)	0.44	0.052	0.846 / 0.847
Coronary artery disease	119	0.849 (0.815 to 0.883)	0.853 (0.819 to 0.886)	+0.0035 (−0.0007 to 0.0116)	0.38	0.009	0.838 / 0.839
Myocardial infarction	48	0.910 (0.882 to 0.937)	0.913 (0.887 to 0.940)	+0.0038 (−0.0005 to 0.0126)	0.082	0.15	0.891 / 0.895
Ischemic stroke	19	0.825 (0.754 to 0.895)	0.824 (0.752 to 0.895)	−0.0007 (−0.0103 to 0.0189)	0.47	0.92	0.738 / 0.727

Classical model: age, sex, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, systolic blood pressure, smoking, diabetes, body mass index. Combined model: classical model with the trait-matched polygenic score added (coronary score for the composite endpoint and coronary artery disease). Change in AUC with bootstrap 95% CI (1,000 resamples); cross-validated AUCs are means over five repeats of five-fold cross-validation. Supplementary Table S7 reports the PRS with age and sex, the PRS alone, NRI and IDI. AUC indicates area under the receiver operating characteristic curve; LRT, likelihood ratio test; and PRS, polygenic risk score.

Table 4. Risk tiers under the classical and combined models, by composite endpoint status.

Tier	Low	Intermediate	High	Total
Participants with the composite endpoint (n=152): classical-model tier (rows) by combined-model tier (columns)				
Low	9	2	0	11
Intermediate	1	9	4	14
High	0	0	127	127
Participants without the composite endpoint (n=1,176): classical-model tier (rows) by combined-model tier (columns)				
Low	644	22	0	666
Intermediate	30	158	16	204
High	0	22	284	306
Prevalence of the composite endpoint in each tier, % (participants)				
Classical model	1.6 (677)	6.4 (218)	29.3 (433)	11.4 (1,328)
Combined model	1.5 (684)	5.2 (213)	30.4 (431)	11.4 (1,328)
Share of all participants with the composite endpoint in each tier, %				
Classical model	7.2	9.2	83.6	100.0
Combined model	6.6	7.2	86.2	100.0

Tiers of predicted probability of the composite endpoint: low, below 5%; intermediate, 5% to below 10%; high, 10% or above. In the upper two panels, rows show the tier under the classical model and columns show the tier after adding the polygenic risk score. Probabilities describe prevalence, not future risk.

Table 5. Low-density lipoprotein pathway alleles compared with a non-Finnish European reference.

Variant	Consequence	ClinVar	Cohort frequency, %	Reference frequency, %	Fold enrichment	P
APOB rs1801703	missense variant	benign to likely benign, with conflicting submissions	1.79	0.81	2.21	4.3×10^{-7}
LDLR rs10423288	intron variant	benign	2.62	1.30	2.01	5.6×10^{-8}
APOE rs445925	downstream gene variant	no entry	9.81	6.67	1.47	4.3×10^{-10}

Computed in 1,397 unrelated participants (2,794 alleles). P values from the exact binomial test against the reference frequency treated as a known proportion; each is below the Bonferroni threshold for the 38,647 gene-annotated variants tested. Against the same reference, 25.6% of the other gene-annotated array variants depart at $P<1\times10^{-6}$, and the pathway as a whole is not more divergent (Fisher's exact test P=0.21); see Results. APOB indicates apolipoprotein B; APOE, apolipoprotein E; gnomAD, Genome Aggregation Database; LDLR, low-density lipoprotein receptor; and NFE, non-Finnish European.

Supplementary Material

Integrating Polygenic and Classical Risk Factors for Cardiovascular Risk Stratification in a Polish Primary-Care Cohort

Banach M, Stempor P, Osadnik T, et al.

Supplementary Methods

Genotype imputation and polygenic scoring

The 1,474 quality-controlled samples were imputed chromosome by chromosome with Beagle 5.5 (build 27Feb25.75f), with the reference converted to the bref3 format, against the phased high-coverage 1000 Genomes Project panel (3,202 genomes, GRCh38) restricted to biallelic single-nucleotide variants with minor allele frequency of at least 0.5% in the panel, and with the PLINK-format GRCh38 genetic maps distributed with Beagle. Target variants were restricted to sites present in the reference with identical alleles. Dosages of variants with DR2 of at least 0.8 (8,379,553 variants) were imported into PLINK 2. PRS-CS was rerun on the full set of 1,120,696 HapMap 3 variants of its European 1000 Genomes linkage-disequilibrium reference, with the same settings as the array-based score (global shrinkage parameter learned from the data, FinnGen release 11 summary statistics, effective sample size 401,611), giving 1,111,426 weights per trait, of which 1,062,486 were scored. Scores were computed with PLINK 2 --score from dosages, standardized across all genotyped samples and averaged over replicate samples of the same participant. Published scores were taken from the harmonized GRCh38 scoring files of the PGS Catalog and matched on chromosome, position and effect allele (with the other allele checked at multi-allelic sites); coverage is reported as the share of the total absolute weight that could be scored (Table S5).

External risk equations

SCORE2 (ages 40 to 69) and SCORE2-OP (70 and older) were computed from the published coefficients and baseline survival, with the recalibration scales for the high-risk region, which includes Poland; the coefficients were checked against two independent public implementations. SCORE2 was computed without a diabetes term, as published for people without diabetes, and diabetes was added as a separate covariate in a second model. The pooled cohort equations (white equations) reproduced the guideline's worked examples (2.05% for the example woman and 5.38% for the example man, against 2.1% and 5.3% published with rounded intermediate sums). Both equations were applied to all 1,059 participants aged 40 years or older, including those with prevalent disease, and entered the logistic models as the logit of the predicted 10-year risk. SCORE2 risk categories followed the 2021 ESC prevention guideline: below 50 years, low-to-moderate below 2.5%, high 2.5% to below 7.5%, very high 7.5% or above; 50 to 69 years, below 5%, 5% to below 10%, 10% or above; 70 years or older, below 7.5%, 7.5% to below 15%, 15% or above. Effect modification was tested with a likelihood ratio test of PRS-by-category interaction terms (2 degrees of freedom).

Statistical methods

Confidence intervals for the change in AUC, NRI and IDI are bootstrap percentile intervals over 1,000 resamples of participants, with both models refitted in each resample; optimism-corrected AUCs follow Harrell's bootstrap procedure. Cross-validated AUCs are the mean over five repeats of stratified five-fold cross-validation of the AUC of pooled out-of-fold predictions. Penalized models used L2-penalized logistic regression on standardized predictors, with the penalty chosen by inner five-fold cross-validation over ten values. Calibration was assessed on out-of-fold predictions averaged over the repeats, by logistic recalibration

(intercept and slope) and the Hosmer-Lemeshow test over deciles of predicted probability. Power was computed for a Wald test of the PRS coefficient in a logistic model adjusted for age, sex and PC1-5, with standard error $1/\sqrt{(n\, p(1-p)\, \mathrm{Var}(PRS)\, (1-R^2))}$, where p is the endpoint prevalence and R^2 is that of the PRS on the covariates; the smallest detectable OR per SD at 80% power and two-sided α of 0.05 is $\exp((1.96 + 0.84) \times SE)$. For CAD at an OR of 1.3 per SD, simulation (300 replicates, outcomes simulated from the observed covariate effects) gave a power of 75% against 78% analytically. All-cause death was analyzed with Cox models adjusted for age and sex, with time from the baseline visit to death or to the end of follow-up (December 2021).

Table S1. Composite endpoint: components and overlap.

Component combination	Participants
CAD	62
CAD + MI	43
death	21
ischemic stroke	11
CAD + death	5
CAD + ischemic stroke	4
CAD + MI + ischemic stroke + death	2
CAD + MI + death	2
CAD + MI + ischemic stroke	1
ischemic stroke + death	1
Total with the composite endpoint	152
of whom with prevalent CAD, MI or stroke	131
of whom by death alone	21
of whom with two or more components	58

Analytic sample, n=1,328. Each participant is counted once, in the combination of components that applies. Component totals: CAD 119, MI 48, ischemic stroke 19, death during follow-up 31. CAD indicates coronary artery disease; MI, myocardial infarction.

Table S2. Participants with clinical data: analyzed versus not analyzed.

Characteristic	Analyzed (n=1,328)	Not analyzed (n=221)	Standardized difference	P
Age, years	50.45 (11.96)	47.65 (13.81)	+0.22	0.005
Body mass index, kg/m2	28.22 (4.99)	27.39 (4.96)	+0.17	0.021
LDL cholesterol, mmol/L	3.35 (1.05)	3.39 (1.05)	−0.04	0.54
HDL cholesterol, mmol/L	1.43 (0.41)	1.44 (0.39)	−0.01	0.85
Total cholesterol, mmol/L	5.36 (1.17)	5.44 (1.21)	−0.07	0.35
Triglycerides, mmol/L	1.77 (1.43)	1.88 (2.50)	−0.06	0.52
Systolic blood pressure, mmHg	128.64 (17.01)	127.60 (16.46)	+0.06	0.39
Diastolic blood pressure, mmHg	79.54 (10.62)	78.76 (9.77)	+0.08	0.28
Men	531 (40.0%)	94 (42.5%)	−0.05	0.51
Current smoking	219 (16.5%)	33 (14.9%)	+0.04	0.62
Diabetes	196 (14.8%)	23 (10.4%)	+0.13	0.095
Hypertension	551 (41.5%)	83 (37.6%)	+0.08	0.30
Antihypertensive treatment	529 (39.8%)	81 (36.8%)	+0.06	0.41
Statin therapy	360 (27.1%)	50 (22.6%)	+0.10	0.19
Family history of cardiovascular disease	596 (44.9%)	112 (50.7%)	−0.12	0.13
Coronary artery disease	119 (9.0%)	17 (7.7%)	+0.05	0.61
Myocardial infarction	48 (3.6%)	7 (3.2%)	+0.02	0.85
Ischemic stroke	19 (1.4%)	3 (1.4%)	+0.01	1.00
CAD, MI or stroke	131 (9.9%)	19 (8.6%)	+0.04	0.62
Death during follow-up	31 (2.4%)	4 (1.8%)	+0.04	0.81

Mean (SD) or n (%). Not analyzed: no linked genotyped sample (42), sample on an excluded BeadChip (46), sample removed in quality control (60), removed in relatedness filtering (73). P values from Welch's t test or Fisher's exact test.

Table S3. Odds ratios of the classical risk factors and the polygenic score in the combined model.

Variable	Composite endpoint, OR (95% CI)	Coronary artery disease, OR (95% CI)	Myocardial infarction, OR (95% CI)
Age (per SD)	3.74 (2.80 to 5.01), P<0.001	3.43 (2.50 to 4.71), P<0.001	3.54 (2.17 to 5.79), P<0.001
Male sex	2.13 (1.39 to 3.27), P<0.001	1.75 (1.10 to 2.78), P=0.017	7.43 (3.28 to 16.85), P<0.001
LDL cholesterol (per SD)	0.60 (0.48 to 0.74), P<0.001	0.66 (0.53 to 0.83), P<0.001	0.43 (0.30 to 0.63), P<0.001
HDL cholesterol (per SD)	0.87 (0.69 to 1.11), P=0.27	0.83 (0.63 to 1.08), P=0.17	1.06 (0.74 to 1.53), P=0.74
Systolic blood pressure (per SD)	1.13 (0.93 to 1.36), P=0.23	1.07 (0.87 to 1.31), P=0.54	1.12 (0.83 to 1.51), P=0.47
Current smoking	1.08 (0.64 to 1.82), P=0.78	1.06 (0.60 to 1.88), P=0.84	1.44 (0.65 to 3.19), P=0.36
Diabetes	1.70 (1.08 to 2.68), P=0.023	1.99 (1.22 to 3.23), P=0.006	1.24 (0.60 to 2.58), P=0.57
Body mass index (per SD)	1.30 (1.05 to 1.61), P=0.017	1.30 (1.04 to 1.64), P=0.024	1.08 (0.76 to 1.54), P=0.66
Coronary PRS (per SD)	1.22 (1.00 to 1.48), P=0.053	1.33 (1.07 to 1.66), P=0.010	
MI PRS (per SD)			1.28 (0.91 to 1.81), P=0.16

Mutually adjusted logistic models (classical risk factors plus the trait-matched polygenic score, computed from imputed genotypes). Continuous variables are standardized, so their ORs are per SD and comparable with the OR per SD of the score. The inverse association of LDL cholesterol reflects treatment after diagnosis of prevalent disease (see Discussion).

Table S4. Prevalent endpoints and all-cause death analyzed separately.

Endpoint	Score	Model	Cases / n	OR or HR per SD (95% CI)	P
Composite endpoint	coronary score	logistic, age, sex, PC1-5	152 / 1,328	1.26 (1.04 to 1.51)	0.017
Prevalent CAD, MI or stroke	coronary score	logistic, age, sex, PC1-5	131 / 1,328	1.33 (1.09 to 1.62)	0.004
Coronary artery disease	coronary score	logistic, age, sex, PC1-5	119 / 1,328	1.36 (1.11 to 1.67)	0.003
Myocardial infarction	MI score	logistic, age, sex, PC1-5	48 / 1,328	1.31 (0.94 to 1.83)	0.11
Ischemic stroke	stroke score	logistic, age, sex, PC1-5	19 / 1,328	1.00 (0.61 to 1.62)	0.99
Myocardial infarction	coronary score	logistic, age, sex, PC1-5	48 / 1,328	1.49 (1.08 to 2.07)	0.016
All-cause death	coronary score	Cox, age, sex	31 / 1,309	1.04 (0.73 to 1.48)	0.84
All-cause death, free of CVD and diabetes at baseline	coronary score	Cox, age, sex	14 / 1,046	0.74 (0.44 to 1.25)	0.26

Logistic models adjusted for age, sex and the first five principal components; Cox models for death adjusted for age and sex, in participants with follow-up data. Prevalent CAD, MI or stroke excludes deaths. CVD indicates cardiovascular disease; HR, hazard ratio.

Table S5. Association with prevalent disease and incremental value across polygenic scores and genotype data.

Score	Genotypes	Coverage	CAD, OR per SD	MI, OR per SD	Composite, OR per SD	CAD, change in AUC	CAD, LRT P
PRS-CS coronary score (primary)	imputed	1,062,486 variants (96% of weights)	1.36 (1.11 to 1.67)	1.49 (1.08 to 2.07)	1.26 (1.04 to 1.51)	+0.0035	0.009
PRS-CS MI score	imputed	1,062,486 variants	1.24 (1.00 to 1.52)	1.31 (0.94 to 1.83)	1.15 (0.95 to 1.39)	+0.0027	0.069
PRS-CS coronary score, array variants only	array	161,020 variants	1.21 (0.99 to 1.49)	1.35 (0.97 to 1.87)	1.15 (0.96 to 1.39)	+0.0007	0.15
27-variant protocol score (PGS000010)	array	25/27 variants (96% of weight)	1.33 (1.09 to 1.62)	1.54 (1.14 to 2.08)	1.19 (0.99 to 1.43)	+0.0060	0.001
50-variant score (PGS000011)	array	39/50 variants (77% of weight)	1.26 (1.03 to 1.54)	1.60 (1.18 to 2.17)	1.14 (0.95 to 1.37)	+0.0031	0.037
metaGRS (PGS000018)	imputed	1,272,220 variants (61% of weight)	1.44 (1.17 to 1.77)	1.84 (1.31 to 2.58)	1.30 (1.08 to 1.57)	+0.0066	0.001
metaGRS (PGS000018)	array	194,744 variants (12% of weight)	1.22 (0.98 to 1.50)	1.60 (1.14 to 2.25)	1.16 (0.96 to 1.41)	+0.0038	0.043
GPS-CAD (PGS000013)	imputed	5,958,982 variants (86% of weight)	1.28 (1.04 to 1.57)	1.84 (1.32 to 2.57)	1.27 (1.05 to 1.53)	+0.0037	0.024
GPS-CAD (PGS000013)	array	423,926 variants (8% of weight)	1.14 (0.93 to 1.40)	1.53 (1.10 to 2.14)	1.13 (0.94 to 1.36)	+0.0004	0.045

n=1,328. ORs per SD adjusted for age, sex and PC1-5. Change in AUC: classical model with versus without the score, for CAD. Coverage of published scores is the share of the total absolute weight that could be scored; the array-only versions of metaGRS and GPS-CAD cover a small fraction of their weight and are shown only to illustrate why imputation was needed. Correlation of each score with the primary score: GRS27 0.50 (array score), metaGRS 0.52, GPS-CAD 0.53, array-only PRS-CS 0.77.

Table S6. Sensitivity analyses: treatment, family history and directly measured LDL cholesterol.

Endpoint	Model	Subset	n / cases	PRS OR per SD (95% CI)	Change in AUC	LRT P
Composite endpoint	classical model	all	1,328 / 152	1.22 (1.00 to 1.48)	+0.0017	0.052
Composite endpoint	classical model	direct LDL-C only	1,191 / 142	1.19 (0.97 to 1.46)	+0.0015	0.090
Composite endpoint	+ statin	all	1,328 / 152	1.20 (0.98 to 1.46)	+0.0014	0.076
Composite endpoint	+ any lipid-lowering	all	1,328 / 152	1.19 (0.98 to 1.46)	+0.0012	0.080
Composite endpoint	+ antihypertensive treatment	all	1,328 / 152	1.21 (0.98 to 1.48)	+0.0009	0.070
Composite endpoint	+ family history of CVD	all	1,328 / 152	1.21 (1.00 to 1.48)	+0.0012	0.055
Composite endpoint	+ all three	all	1,328 / 152	1.19 (0.97 to 1.46)	+0.0004	0.091
Prevalent CAD, MI or stroke	classical model	all	1,328 / 131	1.30 (1.06 to 1.60)	+0.0035	0.012
Prevalent CAD, MI or stroke	classical model	direct LDL-C only	1,191 / 121	1.28 (1.03 to 1.59)	+0.0023	0.023
Prevalent CAD, MI or stroke	+ statin	all	1,328 / 131	1.28 (1.03 to 1.58)	+0.0028	0.023
Prevalent CAD, MI or stroke	+ any lipid-lowering	all	1,328 / 131	1.27 (1.03 to 1.57)	+0.0025	0.024
Prevalent CAD, MI or stroke	+ antihypertensive treatment	all	1,328 / 131	1.30 (1.04 to 1.61)	+0.0025	0.018
Prevalent CAD, MI or stroke	+ family history of CVD	all	1,328 / 131	1.30 (1.06 to 1.61)	+0.0031	0.013
Prevalent CAD, MI or stroke	+ all three	all	1,328 / 131	1.28 (1.03 to 1.59)	+0.0022	0.025
Coronary artery disease	classical model	all	1,328 / 119	1.33 (1.07 to 1.65)	+0.0035	0.009
Coronary artery disease	classical model	direct LDL-C only	1,191 / 111	1.31 (1.05 to 1.64)	+0.0027	0.017
Coronary artery disease	+ statin	all	1,328 / 119	1.30 (1.04 to 1.62)	+0.0029	0.020
Coronary artery disease	+ any lipid-lowering	all	1,328 / 119	1.30 (1.04 to 1.62)	+0.0027	0.021
Coronary artery disease	+ antihypertensive treatment	all	1,328 / 119	1.33 (1.06 to 1.66)	+0.0029	0.012
Coronary artery disease	+ family history of CVD	all	1,328 / 119	1.33 (1.07 to 1.65)	+0.0027	0.009
Coronary artery disease	+ all three	all	1,328 / 119	1.31 (1.04 to 1.64)	+0.0027	0.020
Myocardial infarction	classical model	all	1,328 / 48	1.28 (0.91 to 1.80)	+0.0038	0.15
Myocardial infarction	classical model	direct LDL-C only	1,191 / 44	1.25 (0.87 to 1.78)	+0.0041	0.23
Myocardial infarction	+ statin	all	1,328 / 48	1.25 (0.88 to 1.78)	+0.0041	0.21
Myocardial infarction	+ any lipid-lowering	all	1,328 / 48	1.24 (0.88 to 1.76)	+0.0038	0.21
Myocardial infarction	+ antihypertensive treatment	all	1,328 / 48	1.29 (0.90 to 1.83)	+0.0031	0.16
Myocardial infarction	+ family history of CVD	all	1,328 / 48	1.28 (0.91 to 1.82)	+0.0048	0.16
Myocardial infarction	+ all three	all	1,328 / 48	1.27 (0.89 to 1.81)	+0.0027	0.19

Each model adds the named variable(s) to the classical model, with and without the polygenic score (imputed genotypes). All three: any lipid-lowering therapy, antihypertensive treatment and family history of cardiovascular disease. Direct LDL-C only: participants whose LDL cholesterol was measured directly rather than estimated by the Friedewald equation.

Table S7. Discrimination, optimism, reclassification and integrated discrimination.

Endpoint	Cases (events per variable)	Apparent AUC, classical / combined	Optimism-corrected AUC	Cross-validated AUC	Penalized CV AUC	PRS with age and sex AUC	PRS alone AUC (95% CI)	NRI 5/10/20% (95% CI)	IDI (95% CI)

Composite endpoint	152 (19.0)	0.860 / 0.861	0.851 / 0.852	0.846 / 0.847		0.824	0.554 (0.507 to 0.602)	+0.031 (−0.048 to 0.084)	+0.0045 (0.0000 to 0.0184)
Coronary artery disease	119 (14.9)	0.849 / 0.853	0.838 / 0.840	0.838 / 0.839		0.818	0.583 (0.531 to 0.636)	+0.032 (−0.059 to 0.118)	+0.0101 (0.0004 to 0.0295)
Myocardial infarction	48 (6.0)	0.910 / 0.913	0.895 / 0.899	0.891 / 0.895	0.889 / 0.892	0.878	0.579 (0.507 to 0.651)	+0.068 (−0.063 to 0.206)	+0.0003 (−0.0029 to 0.0196)
Ischemic stroke	19 (2.4)	0.825 / 0.824	0.767 / 0.761	0.738 / 0.727	0.637 / 0.621	0.816	0.493 (0.366 to 0.621)	0.000 (−0.137 to 0.309)	+0.0003 (−0.0019 to 0.0313)

Classical model versus classical model with the trait-matched polygenic score (imputed genotypes). Optimism correction by bootstrap (1,000 resamples); cross-validation 5 × 5-fold; penalized: L2 logistic regression within the cross-validation, fitted for the two endpoints with fewest events. NRI and IDI with bootstrap 95% CIs.

Table S8. Calibration of out-of-fold predictions.

Endpoint	Model	Calibration intercept	Calibration slope	Brier score	Hosmer-Lemeshow P
Composite endpoint	classical	+0.005	0.93	0.0810	0.63
Composite endpoint	combined	+0.006	0.93	0.0805	0.60
Coronary artery disease	classical	0.000	0.94	0.0685	0.69
Coronary artery disease	combined	0.000	0.94	0.0676	0.46
Myocardial infarction	classical	−0.015	0.89	0.0317	0.54
Myocardial infarction	combined	−0.014	0.88	0.0320	0.59
Ischemic stroke	classical	−0.031	0.63	0.0142	0.004
Ischemic stroke	combined	−0.033	0.60	0.0143	0.006

Out-of-fold predicted probabilities from 5 × 5-fold cross-validation, averaged over repeats. Intercept and slope from logistic recalibration; the Hosmer-Lemeshow test uses deciles of predicted probability. Figure S1 shows the calibration plots.

Table S9. Externally derived risk equations as base models, and the classical model at ages 40 to 69.

Base model	Endpoint	Cases	AUC, base	AUC, base + PRS	DeLong P	LRT P	PRS OR per SD (95% CI)
SCORE2/SCORE2-OP	Composite endpoint	149	0.742	0.746	0.40	0.045	1.21 (1.00 to 1.45)
SCORE2/SCORE2-OP + diabetes	Composite endpoint	149	0.771	0.774	0.57	0.058	1.20 (0.99 to 1.45)
SCORE2/SCORE2-OP	Coronary artery disease	117	0.733	0.740	0.38	0.008	1.31 (1.07 to 1.61)
SCORE2/SCORE2-OP + diabetes	Coronary artery disease	117	0.771	0.775	0.65	0.010	1.32 (1.07 to 1.62)
SCORE2/SCORE2-OP	Myocardial infarction	47	0.742	0.745	0.62	0.17	1.24 (0.91 to 1.69)
SCORE2/SCORE2-OP + diabetes	Myocardial infarction	47	0.765	0.768	0.59	0.19	1.23 (0.90 to 1.68)
Pooled cohort equations	Composite endpoint	149	0.779	0.782	0.50	0.054	1.21 (1.00 to 1.46)
Pooled cohort equations + diabetes	Composite endpoint	149	0.783	0.786	0.44	0.058	1.20 (0.99 to 1.46)
Pooled cohort equations	Coronary artery disease	117	0.773	0.777	0.53	0.009	1.32 (1.07 to 1.63)
Pooled cohort equations + diabetes	Coronary artery disease	117	0.782	0.785	0.58	0.010	1.32 (1.07 to 1.63)
Pooled cohort equations	Myocardial infarction	47	0.796	0.799	0.66	0.20	1.23 (0.89 to 1.69)
Pooled cohort equations + diabetes	Myocardial infarction	47	0.796	0.799	0.63	0.20	1.23 (0.89 to 1.69)
Classical model, ages 40 to 69 (n=1,024)	Composite endpoint	134	0.826	0.828	0.51		1.21 (0.99 to 1.47)
Classical model, ages 40 to 69 (n=1,024)	Coronary artery disease	105	0.815	0.819	0.41		1.31 (1.06 to 1.63)
Classical model, ages 40 to 69 (n=1,024)	Myocardial infarction	41	0.896	0.901	0.060		1.23 (0.86 to 1.77)

SCORE2/SCORE2-OP and pooled cohort equations: n=1,059 participants aged 40 years or older; the equations enter as the logit of predicted 10-year risk. PRS OR per SD from the model with the base equation; in the age 40 to 69 rows, from the model with age, sex and PC1-5.

Table S10. Association of the polygenic score by SCORE2 risk category.

Endpoint	SCORE2 category	n	Cases	PRS OR per SD (95% CI)	P	Interaction P
Composite endpoint	low-to-moderate	511	36	1.28 (0.90 to 1.81)	0.16	0.89
	high	373	58	1.25 (0.93 to 1.67)	0.14	
	very high	175	55	1.14 (0.83 to 1.58)	0.42	
Coronary artery disease	low-to-moderate	511	29	1.34 (0.92 to 1.96)	0.13	0.77
	high	373	46	1.23 (0.89 to 1.71)	0.21	
	very high	175	42	1.48 (1.02 to 2.13)	0.037	
Myocardial infarction	low-to-moderate	511	11	1.09 (0.59 to 2.02)	0.78	0.67
	high	373	17	1.18 (0.71 to 1.96)	0.53	
	very high	175	19	1.52 (0.89 to 2.60)	0.12	

Categories of the 2021 ESC prevention guideline with age-specific thresholds (Supplementary Methods). Models within each category adjusted for the SCORE2 linear predictor; interaction P from a likelihood ratio test of PRS-by-category terms (2 degrees of freedom).

Table S11. Low-density lipoprotein pathway variants per gene and significance criteria.

Gene	Array variants	$P<1\times10^{-6}$ (more common in cohort)	Bonferroni, pathway	Bonferroni, genome-wide
LDLR	13	6 (2)	10	6
APOB	20	5 (4)	6	5
PCSK9	4	1 (1)	1	1
LPA	5	1 (1)	2	1
APOE	6	4 (3)	5	4
LDLRAP1	4	2 (2)	2	2
ABCG5	2	0 (0)	1	0
ABCG8	9	2 (1)	2	2
LIPA	5	1 (1)	3	1
total	68	22 (15)	32	22

Variants on the quality-controlled array in nine LDL-pathway genes, 1,397 unrelated participants, against the gnomAD non-Finnish European reference. Bonferroni thresholds: pathway $0.05/68 = 7.4\times10^{-4}$; genome-wide $0.05/38{,}647$ gene-annotated variants $= 1.3\times10^{-6}$. $P<1\times10^{-6}$ in 32.4% of pathway and 25.6% of background variants (Fisher's exact test P=0.21). Per-variant P values for this comparison use the normal approximation to the binomial; Table 5 of the main text gives exact binomial P values.

Table S12. Statistical power of the analysis.

Endpoint	Cases	Smallest detectable OR per SD (80% power)	Power at OR 1.2	Power at OR 1.3	Power at OR 1.5
Composite endpoint	152	1.27	57%	86%	100%
Coronary artery disease	119	1.31	48%	78%	99%
Myocardial infarction	48	1.51	24%	43%	79%
Ischemic stroke	19	1.91	12%	20%	42%
Prevalent CAD, MI or stroke	131	1.29	51%	82%	99%
All-cause death (Cox)	31	1.65 (HR)			

n=1,328; two-sided α 0.05. Published per-SD estimates for coronary scores range from about 1.2 to 1.7 (Discussion). Figure S2 shows power across effect sizes.

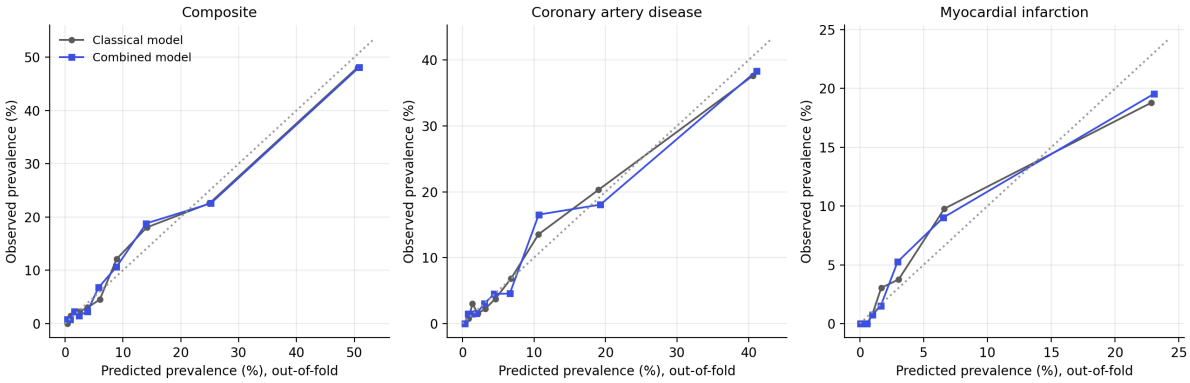


Figure S1. *Calibration of out-of-fold predicted probabilities, classical and combined models (imputed polygenic score), for the composite endpoint, coronary artery disease and myocardial infarction. Points are deciles of predicted probability; the dotted line is perfect calibration.*

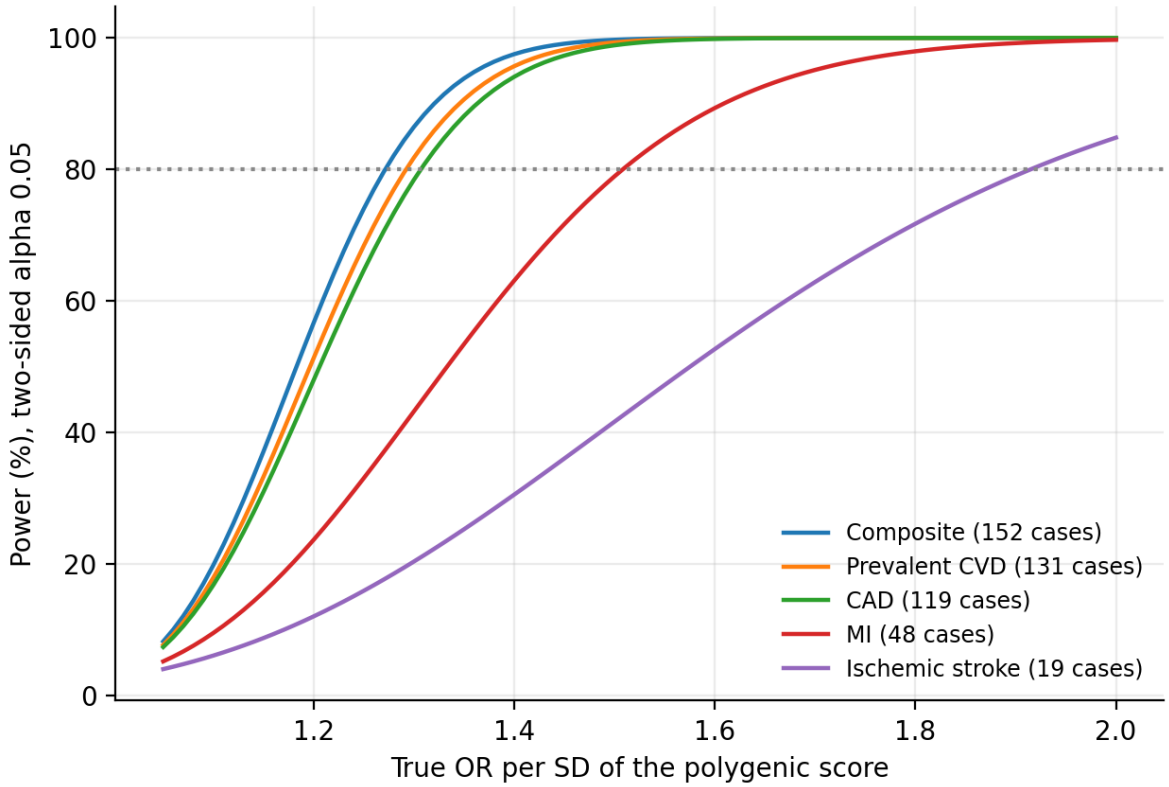


Figure S2. *Power to detect an association of the polygenic score per SD, by endpoint, for the case counts of the analytic sample (two-sided α 0.05).*