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A genetic risk score predicts cardiovascular events in patients with stable coronary artery disease

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ABSTRACT

Background: Genetic risk scores (GRSs) may predict cardiovascular risk in community-based populations. However, studies investigating the association with recurrent cardiovascular events in patients with established coronary artery disease (CAD) are conflicting.

Methods: We genotyped 879 patients with high-risk stable CAD and created a GRS based on 45 single nucleotide polymorphisms previously reported to be associated with CAD in genome-wide association studies. Patients were categorised into high or low GRS according to the median GRS and followed for recurrent cardiovascular events using national Danish registries. The primary endpoint was a composite of myocardial infarction, coronary revascularisation, and cardiovascular death.

Results: Median (interquartile range) follow-up time was 2.8 (2.4–3.8) years. The cumulative incidence proportions of the primary endpoint at 1 and 3 years were 6.4% and 11.5% in high-GRS patients vs. 2.5% and 7.3% in low-GRS patients. The corresponding relative risks were 2.56 (95% confidence interval (CI) 1.29–5.07), and 1.57 (95% CI 1.02–2.44).

The adjusted hazard ratio (HR) of the primary endpoint was 1.50 (95% CI 1.00–2.25). The most pronounced effect of a high GRS was observed on coronary revascularisations (adjusted HR 2.10 [95% CI 1.08–4.07]). Risks of cardiovascular death (adjusted HR 1.07 [95% CI 0.46–2.48]) and all-cause death (adjusted HR 1.15 [95% CI 0.65–2.03]) were unaffected.

Conclusions: A GRS predicts recurrent cardiovascular events in high-risk stable CAD patients. The observed effect was mainly driven by coronary revascularisations.

1. Introduction

Genetic predisposition is a major risk factor for the development of coronary artery disease (CAD), including myocardial infarction (MI). A family history of premature MI is associated with a doubled risk of a first MI, and heritability is estimated to be 50% [1].

In recent years, large-scale genome-wide association studies (GWASs) have identified a number of common genetic variants (single-nucleotide polymorphisms [SNPs]) associated with CAD and/or MI [2–9]. Although most variants are in non-coding regions of the genome, where the causal variants are difficult to pinpoint, they represent genetic loci playing a role in CAD development [10]. Each of the identified SNPs only confers a small increase in susceptibility to CAD and MI. However, adding up the effect of multiple SNPs in genetic risk scores (GRSs) has been shown to predict incident cardiovascular events in various community-based populations free of CAD [11–14]. Moreover, adding GRSs may improve discrimination and reclassification on top of current models based on traditional risk factors [11–15].

In contrast to the primary prevention setting, evidence on the association between GRSs and recurrent cardiovascular events in patients with established CAD is limited and contradictory [16–22]. A genetic susceptibility may lead to adverse events by modifying known and unknown pathophysiological pathways leading to progression of CAD and MI. However, the importance of GRSs in patients already at high risk of a new event is uncertain. Therefore, we aimed to investigate whether a GRS predicts recurrent cardiovascular events in patients with established CAD.

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2. Methods

2.1. Setting

We conducted an observational cohort study including 879 high-risk patients with stable CAD. All patients were genotyped for 45 genetic risk variants reported to be significantly associated with CAD and/or MI in GWASs [10]. A GRS was calculated, and patients were followed from inclusion until the first event of interest, death, or December 31, 2012, whichever came first. All patients provided informed written consent. The project was approved by The Central Denmark Region Committees on Health Research Ethics (record number: 1-10-72-210-15) and by the Danish Data Protection Agency (record number: 1-16-02-400-15).

2.2. Study cohort

Patients with documented stable CAD were recruited from the Western Denmark Heart Registry from November 2007 to January 2011. The study cohort and the inclusion criteria have previously been described in detail [23]. Briefly, patients were above 18 years of age and had angiographically verified CAD with at least one visually documented stenosis ≥50%. The cohort was considered at high risk of developing cardiovascular events, since all had a history of prior MI, type 2 diabetes, or both. None of the patients had suffered cardiovascular events or had been undergoing revascularisation during the last year before enrolment, and they were all on mono-antiplatelet therapy with aspirin 75 mg daily.

2.3. Genotyping

Blood samples from 883 patients were available for genotyping. DNA was extracted from whole blood and diluted in 10 mM TRIS-HCL 0.1 mM EDTA suspension buffer to a DNA concentration of 10–20 pmol/μl. Genotyping was performed on a Fluidigm BioMark HD (Fluidigm Corp., South San Francisco, CA, USA). The genotyping facility was blinded to the clinical data. The online Fluidigm D3 Assay Design tool was used to design the genotyping assays for SNPs from 46 loci genome-wide significantly associated with CAD and/or MI in populations of European ancestry (Supplementary, Table S1) [10]. Specific target amplification was performed prior to genotyping and cluster plot outputs were analysed using the Fluidigm SNP Genotyping Analysis software. All cluster plots were manually inspected for each chip separately. All plots showed a clear separation of all three genotypes. One SNP (rs17114036 at the 1p32.2 locus) failed on all chips and four samples with <50% of SNPs successfully genotyped were excluded. Hence, the final dataset contained information on 45 SNPs and 879 patients.

All 45 SNPs had successfully been genotyped in 729 (82.9%) of samples, whereas ≥44 SNPs where successfully genotyped in 863 (98.2%) of patients. The genotype distribution did not deviate from Hardy-Weinberg equilibrium for any of the SNPs (Bonferroni-corrected threshold of $p = 0.0011$ [0.05/45 SNPs]). The reference allele and allele frequencies for all SNPs were consistent with the HapMap CEU population. For quality control, DNA from 77 randomly chosen patients (around 9% of our sample) were included a second time on the last DNA plate and genotyped for all SNPs. After QC filtering, 3438 pairs of genotypes were available for comparison, revealing 3437 consistencies (99.97% consistency rate).

2.4. Construction of the GRS

The GRS was calculated as the sum of the number of risk alleles (0, 1 or 2) at each locus weighted by the log of the odds ratio for each allele reported in the original discovery GWAS. In the few instances of a missing genotype, to avoid a value of zero, the average of the cohort (a number between 0 and 2) for that SNP was used to calculate the GRS.

Table 1
Baseline characteristics.

	Low GRS ($n = 440$)	High GRS ($n = 439$)	Total ($n = 879$)	p-Value
Age	65 ± 10	65 ± 9	65 ± 9	0.54
Male sex	339 (77)	350 (80)	689 (78)	0.33
Prior MI	388 (88)	389 (89)	777 (88)	0.84
Prior PCI/CABG	413 (94)	419 (95)	832 (95)	0.30
Prior stroke	22 (5)	27 (6)	49 (6)	0.44
Diabetes	119 (27)	121 (28)	240 (27)	0.86
Renal failure[a]	90 (20)	79 (18)	169 (19)	0.36
Antihypertensive treatment	405 (92)	404 (92)	809 (92)	0.81
Statin treatment	398 (91)	397 (90)	795 (91)	0.74
Current smoking	105 (24)	87 (20)	192 (22)	0.15
Systolic BP (mmHg)	142 ± 21	141 ± 20	142 ± 20	0.58
Diastolic BP (mmHg)	83 ± 12	83 ± 11	83 ± 11	0.94
Body mass index	28 ± 4	28 ± 4	28 ± 4	0.96
Creatinine (mM)	82 (71–97)	82 (71–95)	82 (71–96)	0.48

Data are presented as mean ± SD, n (%) or median (IQR).[a] Estimated glomerular filtration rate ≤60 ml/min. BP, blood pressure; CABG, coronary artery bypass graft surgery; GRS, genetic risk score; MI, myocardial infarction; PCI, percutaneous coronary intervention.

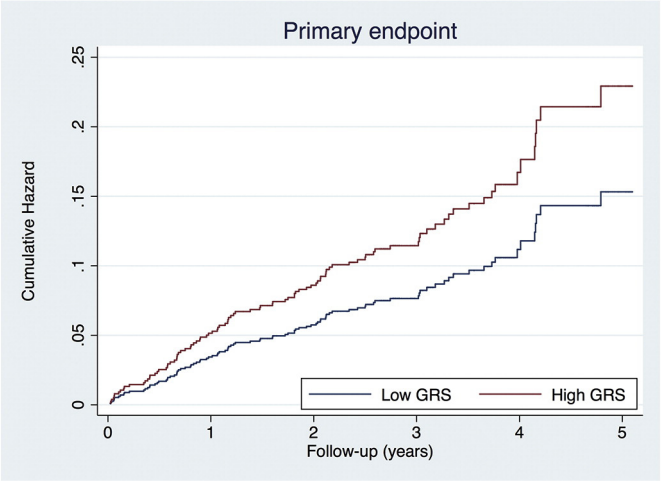

Fig. 1. Title Cumulative hazard of the primary endpoint (composite of cardiovascular death, myocardial infarction, and coronary revascularisation). Legend Adjusted for age, sex, prior myocardial infarction, diabetes, body mass index, smoking,and renal failure (estimated glomerular filtration rate ≤60 ml/min). GRS, genetic risk score.

2.5. Follow-up and endpoint definitions

Follow-up was performed using national medical registries and the Danish Civil Registration System [24]. In Denmark, the tax-supported health care system provides free and unlimited access to public hospitals, which ensures coding of all deaths, diagnoses, and procedures in national registries. Linking individual medical records to national registries is possible due to the civil registration number; a unique number assigned to all citizens at birth or upon immigration.

The civil registration number was used to extract endpoint data from the Western Denmark Heart Registry [25], the Danish Stroke Registry [26], and the Danish Register of Causes of Death [27]. All coronary events were validated by physicians (SDK, ELG, and SBL) blinded to the genotype data. MI was defined according to the universal definition [28], and coronary revascularisation was any percutaneous coronary intervention or coronary artery bypass graft surgery performed in the absence of an MI. Cardiovascular death was defined according to the International Classification of Diseases 10th Revision (ICD-10) codes I00–I25, I27, I30–I52, and I60–I72. Ischaemic stroke was defined as ICD–10 codes I63 and I64.

The primary endpoint was a composite of MI, coronary revascularisation, and cardiovascular death. For secondary endpoints, we considered all-cause death, cardiovascular death, MI, coronary revascularisation, and ischaemic stroke, separately, and a composite of all cardiovascular events including cardiovascular death, MI, ischaemic stroke, and coronary revascularisation.

2.6. Statistical analysis

Data are presented as numbers (proportion), mean ± standard deviation (SD), or median (interquartile range [IQR]). Patients were categorised as having a "low GRS" or a "high GRS", according to the median GRS (the distribution of the GRS is displayed in Supplementary, Fig. S1). Differences in baseline characteristics between the two groups were calculated using the Chi-square test, Student's t-test, or the Wilcoxon rank-sum test, as appropriate.

The 1-year and 3-year cumulative incidences of the endpoints were calculated for each group considering death a competing risk in the non-fatal endpoints [29]. Relative risk estimates and confidence intervals (CI) of the primary endpoint were generated using the pseudo-observations method [30], however, they were not calculated for the secondary endpoints, due to the low number of events in some categories. Cox proportional hazards-regression was used to compute unadjusted and adjusted hazard ratios (HR) of the primary and secondary endpoints, and cumulative hazard curves were used to graphically illustrate the results. The primary endpoint was adjusted for age, sex, prior MI, diabetes, body mass index (BMI), smoking, and renal failure (estimated glomerular filtration rate ≤60 ml/min), whereas secondary endpoints were only adjusted for age due to few events. All co-variables were simultaneously added to the model. The proportional hazards assumption was tested using log-log plots and found valid in all cases.

To investigate the association between the GRS and the time to the primary endpoint, we used a multivariable linear regression model. For these analyses, the GRS was standardised. Hence, the reported beta coefficient corresponds to the change per SD increase in the GRS.

Given the arbitrary cut-off point of the GRS, we performed post-hoc sensitivity analyses adding the standardised GRS to the models as a continuous variable and HRs were calculated per SD. Additionally, we performed an analysis categorising patients into three groups (quartile 1, quartile 2 + 3, and quartile 4) in which HRs were calculated using quartile 1 as the reference group.

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