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A genetic risk score of 45 coronary artery disease risk variants associates with increased risk of myocardial infarction in 6041 Danish individuals

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ABSTRACT

Background: In Europeans, 45 genetic risk variants for coronary artery disease (CAD) have been identified in genome-wide association studies. We constructed a genetic risk score (GRS) of these variants to estimate the effect on incidence and clinical predictability of myocardial infarction (MI) and CAD.
Methods: Genotype was available from 6041 Danes. An unweighted GRS was constructed by making a summated score of the 45 known genetic CAD risk variants. Registries provided information (mean follow-up = 11.6 years) on CAD (n = 374) and MI (n = 124) events. Cox proportional hazard estimates with age as time scale was adjusted for sex, BMI, type 2 diabetes mellitus and smoking status. Analyses were also stratified either by sex or median age (below or above 45 years of age). We estimated GRS contribution to MI prediction by assessing net reclassification index (NRI) and integrated discrimination improvement (IDI) added to the European SCORE for 10-year MI risk prediction.
Results: The GRS associated significantly with risk of incident MI (allele-dependent hazard ratio (95%CI): 1.06 (1.02–1.11), $p = 0.01$) but not with CAD ($p = 0.39$). Stratification revealed association of GRS with MI in men (1.06 (1.01–1.12), $p = 0.02$) and in individuals above the median of 45.11 years of age (1.06 (1.00–1.12), $p = 0.03$). There was no interaction between GRS and gender ($p = 0.90$) or age ($p = 0.83$). The GRS improved neither NRI nor IDI.
Conclusion: The GRS of 45 GWAS identified risk variants increase the risk of MI in a Danish cohort. The GRS did not improve NRI or IDI beyond the performance of conventional European SCORE risk factors.

1. Background

Cardiovascular disease is the leading cause of death due to non-communicable disease and is projected to cause 23.3 million deaths globally in 2030 [1]. Cardiovascular disease includes coronary artery disease (CAD) caused by atherosclerosis of the coronary arteries, ultimately leading to myocardial infarction (MI). Several modifiable lifestyle-related risk factors such as smoking, dyslipidemia, type 2 diabetes mellitus and elevated blood pressure have been firmly established to increase the risk of CAD [2]. Clinical risk scores based on readily measurable traits have been constructed to address and ascertain risk tendency in the general European population [2]. However, apart from lifestyle-related risk factors, a genetic predisposition for CAD also exists. A third of all patients experiencing CAD have a family-history of CAD and individuals with a family-history of premature MI are at 1.5–2-fold increased risk of MI [3]. Furthermore, it has been estimated that the

Abbreviations: GRS, genetic risk score; CAD, coronary artery disease; MI, myocardial infarction; SNP, single-nucleotide polymorphism; SBP, systolic blood-pressure; DBP, diastolic blood-pressure.

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heritability of MI is around 50% [4,5]. Consequently, there is a hypothetical possibility to expand personalized preventive strategies from conventional risk factors by identifying genetic at-risk individuals and prevent future disease. Until now, 45 CAD risk variants have been identified in genome-wide association studies (GWAS) of Europeans to increase risk of MI, ischemic heart disease or clinically "silent" coronary atherosclerosis [6–16]. The identification of genetic variants has been performed in cross-sectional epidemiological studies – whereas the predictive ability is estimated in prospective studies with several years of time to follow-up. Prospective studies have shown that a genetic risk score (GRS) based on up to 46 CAD risk variants improves the clinical prediction of CAD or MI besides that of conventional CAD risk-factors [17–19].

In a Danish prospective cohort, we aimed to replicate the association of a GRS based on 45 CAD risk variants with increased risk of MI and CAD. We also aimed to reproduce the improved clinical risk prediction of incident CAD and MI of a GRS on 45 risk variants compared to the European SCORE CAD risk factors.

2. Methods

2.1. Study samples

The Inter99 study is a randomized, non-pharmacological intervention study for the prevention of ischemic heart disease on 13,016 participants between 30 and 60 years randomly selected from the Civil Registration System. These individuals were randomized to high-intensity (90%) or low-intensity (10%) lifestyle intervention. The study was conducted at the Research Centre for Prevention and Health in Glostrup, Denmark (ClinicalTrials.gov: NCT00289237). 6784 (52%) showed up at the baseline-examination. Written informed consent was obtained from all participants. The study was approved by the Regional Ethical Committee of Copenhagen and is in accordance with the scientific principles of the Helsinki Declaration II. All participants received individual lifestyle counseling, with focus on lifestyle behavior (smoking, physical activity, diet and alcohol). Detailed characteristics of Inter99 have been published previously [20,21]. Genotype information was available on 6127 individuals. However, we removed 86 individuals having missing values of systolic blood-pressure, total cholesterol levels, smoking status, BMI, genotype or information on MI/CAD before baseline examination. The complete data set was therefore 6041 individuals for model 1 and 5736 individuals for model 2 as 305 individuals had missing values for glucose-tolerance and smoking (266 and 39 individuals, respectively). See information on models below. Baseline characteristics of the study participants are shown in Table 1.

2.2. Information on myocardial infarction and coronary artery disease

Information on incident MI, CAD and death from MI and CAD was obtained from the Danish National Patient Registry and the National Cause of Death Registry using ICD-10 codes [22,23]. Vital status was obtained from The Central Person Registry, which keeps updated records on vital status, emigration and registers all deaths. Having a cardiovascular event was defined as the first non-fatal or fatal diagnosis with MI (ICD-10: I21–I21.9) and CAD (ICD-10: I20–I25) as an A- or B-diagnosis or cause of death. We pooled data on morbidity and mortality to attain maximal statistical power. Data were available in the registries up until July 11th 2011. The mean follow-up time (SD) was 11.6 years (1.3).

2.3. Quantitative traits

Height and body weight were measured in light indoor clothing without shoes. BMI was calculated as the weight divided by height in meters squared (kg/m^2). Levels of serum triglyceride, serum total cholesterol and HDL-cholesterol were measured in the fasting state. LDL-cholesterol was calculated by the Friedewald equation [24]. Remnant cholesterol (cholesterol content in VLDL and IDL) was calculated as [remnant cholesterol] = [total cholesterol] – [HDL cholesterol] – [LDL cholesterol] [25]. Individuals had blood pressure measured twice in the supine position after a five minute rest and the mean was subsequently calculated.

2.4. Binary traits

Glucose tolerance was estimated either by self-reported status as type 2 diabetic or according to WHO 1999 criteria after an oral glucose tolerance test (OGTT) at the baseline examination. We created a binary variable from glucose tolerance status: 1) normal glucose tolerance or impaired glucose tolerance and 2) type 2 diabetes mellitus. Information on smoking status was obtained by self-reported questionnaires. Smoking habits were divided into four categories of 1) habitual smoker, 2) occasional smoker, 3) former smoker and 4) never smoker.

2.5. Genotyping

Genotyping of 45 single nucleotide polymorphisms (SNPs) was

Table 1
Baseline characteristics of Inter99 participants.

		Non-cases	MI cases	CAD cases	$p_{non\text{-}cases\ vs.\ MI}$	$p_{non\text{-}cases\ vs.\ CAD}$
N		5667	124	374		
Sex	Women (%)	2941 (51.9%)	28 (22.6%)	139 (37.2%)	5×10^{-10}	0.0001
Age	Years (±SD)	45.9 (±7.9)	49.8 (±7)	50.3 (±7)	1×10^{-8}	$<2 \times 10^{-16}$
BMI	kg/m^2 (±SD)	26.2 (±4.6)	27.6 (±4.3)	27.5 (±4.6)	0.0004	0.0004
Fasting serum total cholesterol	mmol/l (±SD)	5.5 (±1.1)	6.0 (±1.2)	6.0 (±1.2)	1.3×10^{-5}	1×10^{-12}
Fasting serum HDL-cholesterol	mmol/l (±SD)	1.4 (±0.4)	1.2 (±0.3)	1.3 (±0.4)	4.6×10^{-11}	5.3×10^{-6}
Fasting serum LDL-cholesterol	mmol/l (±SD)	3.9 (±1.0)	3.9 (±1.2)	3.9 (±1.2)	0.008	2.3×10^{-5}
Fasting serum triglycerides	mmol/l (IQR)	1.1 (0.8–1.5)	1.5 (1.0–2.1)	1.3 (0.9–2.0)	$1.3 \times 10^{-8*}$	$1.8 \times 10^{-13*}$
Fasting non-HDL cholesterol	mmol/l (±SD)	4.1 (±1.1)	4.7 (±1.2)	4.6 (±1.2)	8.6×10^{-9}	2.4×10^{-16}
Fasting serum remnant cholesterol	mmol/l (IQR)	0.5 (0.4–0.7)	0.7 (0.5–1.0)	0.6 (0.4–0.9)	$4.7 \times 10^{-5*}$	$4.5 \times 10^{-7*}$
Systolic blood pressure	mmHg (±SD)	130 (±17)	139 (±18)	138 (±19)	6.5×10^{-8}	3×10^{-15}
Smoking status	% smokers	35.4	45.5	41.8	0.02	0.01
Fasting plasma glucose	mmol/l (±SD)	5.6 (±1)	6.1 (±1.7)	6.0 (±1.7)	0.0003	3.9×10^{-6}

The table describes the characteristics of Inter99 according to status as non-case or MI/CAD case. MI cases are included in CAD cases. Values are mean ± SD or median and interquartile range. P-values are Student's t-test. *: non-parametric t-tests. BMI: body mass index; HDL: high-density lipoprotein cholesterol; LDL: low-density lipoprotein cholesterol; MI: myocardial infarction; CAD: coronary artery disease; IQR: interquartile range.