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A 45-SNP genetic risk score is increased in early-onset coronary artery disease but independent of familial disease clustering

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ABSTRACT

Background and aims: Common genetic risk variants may contribute to the heritability of early-onset coronary artery disease (CAD). We aimed to investigate the association of a genetic risk score (GRS) with age upon CAD-onset and to test the association between the GRS, familial clustering, and CAD severity in early-onset CAD.

Methods: 134 early-onset CAD patients (<40 years), 446 late-onset CAD patients (male >55 years/female >65 years), and 89 healthy controls were genotyped for 45 CAD-associated SNPs and a GRS was created. In early-onset CAD patients, family pedigrees with information on 1585 1st and 2nd degree relatives were used to calculate a stratified log-rank family score (SLFS) as a measure of familial clustering.

Results: Early-onset patients had a higher mean GRS than late-onset CAD patients ($p = 0.02$) and healthy controls ($p < 0.0001$). In the adjusted model, a GRS increase of one SD was associated with 1.2 years (95% CI 0.1–2.2) earlier onset. The GRS was not associated with the SLFS in the regression model ($p = 0.41$) and did not differ between SLFS tertiles ($p = 0.98$). The SLFS predicted the number of affected coronary vessels (OR [95% CI] per SD increase in SLFS: 2.0 [1.4–3.0]), whereas the association between the GRS and CAD severity was not statistically significant (OR [95% CI] per SD increase in GRS: 1.3 [0.9–1.9]).

Conclusions: The GRS was increased in early-onset CAD patients, but not associated with the SLFS, suggesting that these common genetic variants are of minor importance in familial clustering of early-onset CAD. Furthermore, family pedigree analysis may predict CAD severity more precisely than common variants.

1. Introduction

Coronary artery disease (CAD) is a multifactorial disease with a substantial genetic contribution to disease risk [1]. In recent years, large genome-wide association studies (GWAS) have identified a number of common genetic risk variants (single-nucleotide polymorphisms, SNPs) associated with CAD and myocardial infarction (MI) [2–9]. Though each variant has a low effect-size individually, it has been demonstrated that pooling risk variants in a genetic risk score (GRS) predicts future cardiovascular events and may identify patients that could benefit from preventive treatment [10–14].

Young cases of CAD tend to cluster in families, and age at clinical presentation of CAD is inversely associated with the degree of heritability [1,15,16]. In one of the pioneer GWAS, it was demonstrated that a common variant at the 9p21 locus predicted age of first MI [2]. Furthermore, early-onset MI has been associated with an increased GRS [17,18]. Therefore, familial clustering in patients with early-onset CAD may arise from inheritance of several genetic variants (i.e. polygenic burden) with low effect sizes. A recent study found family history to be independent of a 30-SNP GRS in patients referred for coronary angiography [19]. This study, however, was based on self-administered questionnaires dichotomizing familial history instead of using gold standard pedigree interviews. Besides the risk of misclassification it does not capture the number of affected family members and age upon CAD onset, which may

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influence heritability and hence reduce the ability to predict future disease among relatives [16,20,21].

Therefore, we aimed to quantify the polygenic burden (measured as a 45-SNP GRS) in early-onset CAD patients and to investigate whether early-onset individuals with a strong familial clustering of CAD had a larger proportion of common risk alleles. Furthermore, we examined whether these measures of heritability were associated with CAD severity.

2. Patients and methods

2.1. Design and study population

The present study was a cross-sectional study in patients with early- and late-onset CAD. The recruitment of patients has previously been described in detail [22–24]. Briefly, all patients were recruited from the Western Denmark Heart Registry (WDHR) and interviewed between 2007 and 2015. All patients were ≥18 years of age at time of inclusion and had angiographically verified CAD with a prior coronary revascularization procedure. Data on medical history, cardiovascular risk factors, and medication was systematically collected, and blood samples were drawn. Age at CAD onset was the age at the time of the first coronary revascularization procedure. Early-onset CAD patients (<40 years) were recruited as part of studies on heritability [22]. As a measure of CAD severity, the cumulative number of affected coronary vessels was recorded from medical files upon inclusion and categorized as one-, two- or three-vessel disease based on coronary angiographies. A vessel was considered affected in case of a revascularization procedure or visualization of a >50% stenosis. For the present study, patients with familial hypercholesterolemia or familial relations to other study participants were excluded. Late-onset CAD patients (male ≥55 years, female ≥65 years) were recruited from studies exploring the antiplatelet effect of aspirin [23]. In addition, volunteers with no sign of CAD, which had previously been recruited through local advertisement [24], served as a healthy control group for the present study.

The study was approved by the National Committee on Health Research Ethics (record number: 1304078).

2.2. Family history

Patients with early-onset CAD were requested to obtain a cardiovascular disease history from 1st and 2nd degree relatives ≥18 years. Upon attendance, a family pedigree was drawn by a physician (M.K.C.). A family history of CAD was considered present if a patient reported a history of MI or any coronary revascularization procedure in a 1st or 2nd degree relative.

For all patients with early-onset CAD, the family pedigrees were used to calculate a stratified log-rank family risk score (SLFS), which is a single measure of family history severity. The SLFS was chosen for the present study since it simultaneously considers the age at time of CAD onset in relatives, taking into account the relationship and differences in CAD risk among men and women [21]. The calculation and validation of the SLFS has previously been described [21]. Briefly, for a given family member type (in the following we consider fathers) the age of CAD onset (i.e. observed events) of all affected fathers is used to construct time intervals. Each time interval is assigned a log-rank score based on the number of observed events and censored events in the period. The score of the father is the cumulated log-rank scores of the time intervals up to the time of his event (CAD onset) or censoring (current age or age at death). A value of one is added to the score of the father in case he is affected by CAD. The SLFS for a patient with early-onset CAD is calculated as the mean of the scores of all relatives in the family, where a higher score reflects a higher degree of familial clustering.

2.3. Genotyping

Genomic DNA was extracted from whole blood and diluted in 10 mmol/l Tris-HCl 0.1 mmol/l EDTA suspension buffer to a DNA concentration of 10–20 pmol/μl. Genotyping was performed on a Fluidigm platform (Fluidigm Corp., South San Francisco, CA, USA) according to the manufacturer's description. Assays for SNPs from 46 CAD risk loci, identified in populations with European ancestry [25], were designed using the online Fluidigm D3 Assay Design tool. Specific target amplification (STA) was performed prior to genotyping according to the manufacturer's description. The Fluidigm SNP Genotyping Analysis software was used to analyse the cluster plots. All cluster plots were manually inspected for each chip separately. Four samples with 23 SNPs or less successfully genotyped were excluded, hence the final data consisted of 669 samples. One SNP (rs17114036) was excluded due to poor clustering on all chips. The remaining 45 SNPs all had a call rate above 0.98, except rs2252641 and rs964184 with call rates above 0.85, due to our conservative calling. All genotypes were successfully called in 560/669 = 83.7% of samples, whereas 43 SNPs or more were successfully called in 656/669 = 98.1% of samples. No significant difference was found in the distribution of missing genotypes between early-onset CAD, late-onset CAD or control individuals.

For all SNPs, the genotype distribution did not deviate significantly from Hardy-Weinberg equilibrium (Bonferroni-corrected threshold of $p = 0.0011$ [0.05/45 SNPs]). All allele frequencies were found to be consistent with the HapMap CEU population (Northern and Western European ancestry).

Fifty-three individuals (8% of our sample) were run as independent duplicates for quality control. There was 100% consistency between the 2367 genotypes successfully called in both samples (theoretically 2385 genotypes).

2.4. Genetic risk score

A weighted multi-locus GRS was calculated based on the 45 SNPs successfully genotyped. The score was calculated for each patient as the sum of the number of risk alleles (0–2) weighted by the log of the odds ratio (OR) for each SNP. The ORs were retrieved from the respective original discovery papers (Supplementary Table 1). To avoid a value of zero in case of a missing genotype, the value (score) for that particular SNP was set to the group-specific average.

2.5. Statistical analysis

Data are presented as mean ± standard deviation (SD), median (interquartile range [IQR]) or numbers (percentages). Patient characteristics were assessed using the χ^2 test, the Wilcoxon rank-sum test, and the Student's t-test.

Associations between continuous outcome variables (age at onset, SLFS) and explanatory variables were assessed using one-way analysis of variance (when divided into groups) and multivariable linear regression. The relationship between the SLFS and the GRS, respectively, and CAD severity was evaluated by ordinal logistic regression. For regression analyses the GRS and SLFS were standardized. In the adjusted models, age, sex, body mass index (BMI), current smoking, antihypertensive treatment, statin treatment, and diabetes were incorporated simultaneously. However, in ordinal regression analyses only the GRS and SLFS were added to the model due to the low number of observations with more than one vessel affected. A p-value of ≤0.05 was considered significant.

Linear regression models were validated by checking QQ-plots