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A multi-locus genetic risk score for abdominal aortic aneurysm

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ABSTRACT

Background: We investigated whether a multi-locus genetic risk scores (GRS) was associated with presence and progression of abdominal aortic aneurysm (AAA) in a case - control study.
Methods and Results: The study comprised of 1124 patients with AAA (74 ± 8 years, 83% men, 52% of them with a maximal AAA size ≤ 5 cm) and 6524 non-cases (67 ± 11 years, 58% men) from the Mayo Vascular Disease Biorepository. AAA was defined as infrarenal abdominal aorta diameter ≥3.0 cm or history of AAA repair. Non-cases were participants without known AAA. A GRS was calculated using 4 SNPs associated with AAA at genome-wide significance ($P \leq 10^{-8}$). The GRS was associated with the presence of AAA after adjustment for age, sex, cardiovascular risk factors, atherosclerotic cardiovascular diseases and family history of aortic aneurysm: odds ratio (OR, 95% confidence interval, CI) 1.06 (1.04 −1.09, $p < 0.001$). Adding GRS to conventional risk factors improved the association of presence of AAA (net reclassification index 14%, $p < 0.001$). In a subset of patients with AAA who had ≥2 imaging studies (n = 651, mean (SE) growth rate 2.47 (0.11) mm/year during a mean time interval of 5.41years), GRS, baseline size, diabetes and family history were each associated with aneurysm growth rate in univariate association (all $p < 0.05$). The estimated mean aneurysm growth rate was 0.50 mm/year higher in those with GRS > median (5.78) than those with GRS ≤ median ($p = 0.01$), after adjustment for baseline size ($p < 0.001$), diabetes ($p = 0.046$) and family history of aortic aneurysm ($p = 0.02$).
Conclusions: A multi-locus GRS was associated with presence of AAA and greater aneurysm expansion.

Abdominal aortic aneurysm (AAA) is conventionally defined as a transverse aortic diameter ≥3.0 cm [1]. The prevalence of AAA increases with age and is about 12.8% and 4.1% in men and women >65 years old, respectively [2]. Acute rupture is a devastating outcome that is associated with a high mortality of nearly 80% [3]. No pharmacological treatment is available to effectively limit disease progression. Early identification through ultrasound screening followed by elective aneurysm repair has been shown to decrease aneurysm-related mortality [4]. Given the significant disease burden and paucity of treatment options, there is a need to identify biomarkers of AAA that may enable individualized screening.

AAA is a multifactorial disease with a heritable component [5]. Genome-wide association studies (GWAS) have found several common single nucleotide polymorphisms (SNPs) to be associated with AAA [6–11]. Whether such variants can improve prediction of presence of AAA beyond conventional risk factors is unknown. The risk of rupture largely depends on aneurysm size and growth rate. Factors that influence aneurysm expansion remain unclear. In particular genetic factors that relate to aneurysm growth are largely unknown. A study of participants in the UK small aneurysm trial found that the 9p21 locus which is associated with atherosclerosis and presence of AAA, was not associated with aneurysm expansion [12]. Whether genetic predisposition to AAA expansion is due to the additive effect of multiple susceptibility alleles is unknown.

We hypothesized that a multi-locus GRS based on SNPs associated with AAA in GWAS may improve disease prediction beyond conventional risk factors and would be associated with aneurysm growth. To test these hypotheses, we performed genotyping using Illumina Human 610 and 660W Quad-v1in participants in the Mayo Clinic Vascular Disease Biorepository [13].

Abbreviations: AAA, abdominal aortic aneurysm; ASCVD, atherosclerotic cardiovascular disease; CHD, coronary heart disease; CI, confidence interval; EHR, electronic health record; GWAS, Genome-wide association studies; OR, odds ratio; SNP, Single nucleotide polymorphism; T2D, Type 2 diabetes.

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1. Methods

1.1. Study participants

The VDB at Mayo Clinic consists of patients referred for noninvasive vascular evaluation in the Gonda Vascular Center and stress electrocardiographic laboratory, and was initiated in 2008. The design and selection criteria have been reported previously [13]. Briefly, the purpose of this registry is to identify novel biomarkers, including genetic susceptibility markers for common and rare vascular diseases. Until August 2013, more than 11,814 adults have been recruited. Blood samples of participants were drawn at the recruitment. High-density genotyping data were available in 8062 (68%) participants. For the purpose of the current study, we included 7648 (9594.7%) patients, including 1124 with AAA as cases and 6524 non-cases who have ASCVD or were referred for cardiovascular risk assessment but without ASCVD. Demographic information, conventional risk factors and comorbidities were ascertained by previously validated algorithms using ICD-9-CM diagnosis codes, procedure codes, medication use and laboratory data from the institutional electronic health records (EHR). A questionnaire on physical activity, lifestyle and family history was given to each participant at the time of consent and scanned into the database after completion. All participants gave informed consent. The study protocol was approved by the Institutional Review Board of the Mayo Clinic.

1.2. Ascertainment of cases and non-cases of AAA

We sampled subjects based on their AAA status. AAA cases were defined as having 1) an infrarenal abdominal aortic diameter $\geq$3 cm, or 2) a history of open or endovascular AAA repair. Patients with AAA often have similar risk profiles as those with atherosclerotic cardiovascular disease (ASCVD) or have ASCVD concomitantly. To test whether a GRS for AAA can differentiate patients with AAA from those who may have ASCVD, participants not known to have AAA (including lack of billing codes for aortic aneurysm) were selected as non-cases. Such non-cases could have ASCVD in different arterial locations. We manually reviewed 100 non-cases with any abdominal imaging study in the EHR. None of them had AAA mentioned in the radiology report.

AAA cases were manually reviewed to confirm the maximal aneurysm size (either anteroposterior or transverse diameter). Radiology reports used to screen included abdominal ultrasound, computerized tomography, magnetic resonance imaging and angiography. To assess AAA progression, the latest or the pre-operation measure of AAA size in the EHRs was collected for all AAA cases. Based on previous reports that >85% of adults with ectasia of abdominal aorta will progress to a size $\geq$3.0 cm [14], and that infrarenal aortic diameter $\geq$ 2.5 cm was associated with significantly increased risk of cardiovascular events and mortality compared to those with a diameter < 2.5 cm [15], we included aortic size $\geq$ 2.5 cm as baseline measure if subsequent measure reaches or exceeds 3 cm. Growth rate was used to assess aneurysm expansion, defined as (latest/pre-operation minus first diameter)/time interval (mm/year). Time interval was calculated in years. We required the shortest follow-up time be at least 3 months for analyses of aneurysm growth.

1.3. Genotyping and calculation of GRS

Genomic DNA was extracted from whole blood samples drawn at the recruitment. Genotyping was performed in Mayo Clinic core lab according to standard protocols using Illumina Infinium Human core Exome Array, and Illumina Human 610 and 660W Quad-v1. Sample call rates were all >95%. Four SNPs had been genotyped for all participants. rs599839 was imputed using the cosmopolitan 1000Genomes Project reference panel using SHAPEIT2 for phasing and IMPUTE2 software for imputation. The IMPUTE 2 information score for this SNP was 0.94. All SNPs followed Hardy–Weinberg equilibrium (all $p > 0.05$). We used logistic regression to estimate the effect in our data set of five SNPs from independent loci (linkage disequilibrium = 0) that were associated with AAA at a *P*-value $\leq 10^{-8}$ (Table 1). To be conservative in the analyses, we used Z-tests to assess whether the risk estimates of SNPs in our dataset were substantially different from that in the published literature. Except for *LDLR* (rs6511720G, $P = 0.008$ for Z test), risk estimates for four SNPs were not significantly different from that in previous studies (all $P > 0.05$). Therefore, we excluded rs651172G in the calculation for GRS. We assumed an additive genetic model to construct GRS for each individual by summing the number of risk alleles for each of four SNPs weighted by estimated effect sizes in the GWAS catalog or from the largest meta-analysis and then rescaled by the number of SNPs divided by summed effect size of each SNP, as reported previously [16].

1.4. Ascertainment of cardiovascular risk factors and ASCVD

Demographic information was abstracted from the EHR as structured data and conventional cardiovascular risk factors (hypertension, diabetes and dyslipidemia) and ASCVD were ascertained by previously validated algorithms using ICD-9 billing codes and natural language processing [17]. Family history of aortic aneurysm in first-degree relatives and smoking status were ascertained from the study questionnaire. Participants were considered smokers if they had smoked more than 100 cigarettes in the past [18,19]. ASCVD was defined as a history of having any of coronary heart disease, stroke, carotid arterial stenosis or peripheral arterial disease.

1.5. Statistical methods

Descriptive statistics were used to compare demographic information and conventional cardiovascular risk factors between cases and non-cases. Continuous variables were presented as mean (standard deviation) and dichotomous variables as numbers (percentages). Comparisons were performed after adjustment for age and sex. To assess the association of GRS with AAA, logistic regression analysis was performed 1) without adjustment; 2) with adjustment for age and sex; and 3) additionally adjusting for body-mass index, hypertension, diabetes, smoking, dyslipidemia, ASCVD and family history. To assess whether GRS can improve disease identification beyond conventional risk factors, the C-statistic, net reclassification index and integrated discrimination improvement were estimated. The association of GRS with aneurysm growth rate in a linear regression model violated homoscedasticity assumption when both were used as continuous variables. Therefore, we dichotomized GRS based on the median of 651 cases with at least two size measures at an interval $\geq$3 months. Logistic regression analysis was performed after adjustment for baseline size and other covariates associated with aneurysm growth rate in the univariate analysis. The association of age, sex and conventional risk factors with aneurysm expansion and interaction with GRS were also assessed. Two sub-analyses were performed to assess: 1) whether GRS can improve disease identification beyond age, sex and smoking history-main factors considered in initiating screening; and 2) whether GRS was associated with clinically high-risk aneurysm expansion defined as either with an aneurysm growth rate $\geq$10 mm/year or with unstable features requiring urgent intervention (rupture or penetrating ulcers). Analyses were