Association between seven single nucleotide polymorphisms involved in inflammation and proteolysis and abdominal aortic aneurysm

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Background: **Abdominal aortic aneurysm (AAA) formation involves an inflammatory and proteolytic process. Previous studies suggest that AAA is a multifactorial disease with a strong genetic background. This study evaluated the role of seven important functional single nucleotide polymorphisms (SNPs) in AAA.**

Methods: **This was a case-control study of two independent populations: 397 AAA patients (mean aortic diameter, 6.2 ± 1.4 cm) and 393 controls (mean diameter, 2.4 ± .2 cm) recruited from Greece (the main cohort), and 400 patients (mean diameter: 5.4 ± 1 cm) and 400 controls (mean diameter, 2.4 ± .6 cm) recruited from the United Kingdom (replication cohort). The functional SNPs analyzed were rs3025058, rs3918242, rs2276109, rs1801133, rs1799752, rs1799983, and rs16874954. These regulate the following enzymes: matrix metalloproteinases (MMPs), angiotensin-converting enzyme, endothelial nitric oxide synthase, methylenetetrahydrofolate reductase (MTHFR), and platelet-activating factor acetylhydrolase or lipoprotein-associated phospholipase A2.**

Results: **Genotype distributions (univariate analyses) did not differ significantly between cases and controls in the main or the replication cohort, with the exception of the MMP-3 rs3025058 SNP, where differences were borderline significant (odds ratio [OR], 1.42; 95% confidence interval [CI], 1.02-1.97; $P = .04$) in the replication cohort. Adjusted analyses for age, sex, smoking, hypertension, and hypercholesterolemia disclosed no differences in either cohort. For SNPs that had previously been associated with AAA presence, meta-analysis of currently available data together with the two study cohorts disclosed positive associations for the MMP-3 rs3025058 (OR, 1.15; 95% CI, 1.06-1.25; $P = .0009$) and MTHFR rs1801133 (OR, 1.07; 95% CI, 1.02-1.12; $P = .0088$).**

Conclusions: **The SNPs included in this analysis were not associated with AAA presence in either study population. However, meta-analysis of the currently available data disclosed a positive association for MMP-3 rs3025058 and MTHFR rs1801133. (J Vasc Surg 2015;61:1120-8.)**

Abdominal aortic aneurysm (AAA) has a reported prevalence rate for men aged >65 years of 1.7% to 7.2% in various populations.[1-3] Even though several environmental factors are implicated in the development of the disease, there is a strong genetic diathesis, with family history being one of the strongest predictors of AAA presence.[4,5] In fact, individuals with a first-degree relative with AAA have up to a 12-fold increased risk of developing the pathology themselves.[6]

Previous reports have investigated single nucleotide polymorphisms (SNPs) in patients with AAA using a candidate-gene or a genome-wide approach, and various positive as well as negative associations have been reported in separate populations and meta-analyses.[4,7-18] However, most candidate-gene analyses have largely included patients with small AAAs from nongeographically uniform populations. In addition, the reported odds ratios (ORs) for the identified risk alleles are not in agreement with the strong heritability pattern observed in epidemiologic studies, suggesting that multiple risk loci are associated with the disease, with many yet to be identified.[12,13]

Inflammation and proteolysis, together with loss of smooth muscle cells, increased oxidative stress, and fragmentation of elastin constitute the hallmarks in the pathophysiology of AAA formation.[19-22] The key histopathologic findings in AAA tissue include a widespread inflammatory infiltrate characterized by the presence of proteolytic molecules such as matrix metalloproteinases (MMPs).[21,23] Various functional inflammatory and proteolytic SNPs have been reported so far, mostly in cohorts

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with cardiovascular disease.[4] This study investigated the prevalence of important functional inflammatory, proteolytic, and atherosclerotic SNPs (rs3025058,[9] rs3918242,[9] rs2276109,[9] rs1801133,[24] rs1799752,[25] rs1799983,[26] and rs16874954[27]), some of which have previously been associated with AAA presence, in patients with AAA compared with controls (no AAA), using two separate populations and also providing a meta-analysis of the currently available data.

METHODS

This study was approved by the Aristotle University Institutional Ethics Committee. All participants provided informed consent.

Study populations. This is a case-control study of two independent populations from discrete geographic areas. The main population was white Caucasians undergoing elective endovascular or open repair of an infrarenal AAA between January 2008 and April 2011 (consecutive elective patients who provided consent) in a tertiary referral center in Northern Greece (cases) and a group of geographically matched controls, without an AAA, recruited within the same interval. Demographics, medication, medical history, and cross-sectional abdominal imaging were prospectively recorded in an electronic registry. Cases and controls were of Mediterranean origin.

Patients with an AAA were eligible for repair if they had a maximal aortic diameter >5 cm, a maximal aortic diameter <5 cm with a rapidly increasing sac (>1 cm/y), or a symptomatic AAA. Excluded were patients with inflammatory aneurysms or soft-tissue pathologies related with aneurysmosis. All controls were inpatients who had already undergone computed tomography or ultrasound imaging of their abdominal aorta within the last 2 weeks of recruitment, documenting a maximal diameter <3.0 cm. Controls were recruited from the general surgery department affiliated with the specific institution and had already been admitted for nonvascular intra-abdominal pathologies.

A second group of patients and controls (replication cohort) from the United Kingdom was used to validate the initial findings. Samples were recruited from the Leicestershire regional screening program and the inpatient and outpatient populations of the Leicester regional Vascular Surgical Unit. The control group was screened for AAA by ultrasound imaging and were all aged >65 years but not selected in any other way. Recruitment took place from 2002 to 2008. Clinical information was gathered by participant interview.

Before performing a meta-analysis of our findings, we undertook a systematic review of the literature was using the electronic PubMed and EMBASE databases in May 2013 to identify all previous studies that had investigated the SNPs described in this analysis. The following terms were used: "abdominal aortic aneurysm" *and* "gene" *or* "single nucleotide polymorphism." The search yielded 3496 abstracts, which were screened by A.S., and 42 papers were included. Meta-analysis of our two populations was performed for the SNPs that had previously been associated with AAA presence in different groups.

Genetic analyses. Overall, seven functional SNPs were included in this analysis. A review of the literature using the electronic PubMed and EMBASE databases in July 2009 (A.S.) identified SNPs that were functional (affected the product encoded by the specific gene), were associated with inflammatory and proteolytic molecules involved in AAA pathogenesis, or were associated with hypertension and atheromatosis, and had previously been investigated in populations with AAA. This search yielded seven SNPs, which were then included in this study.

DNA extraction. Peripheral venous blood was drawn into 5 mL ethylenediaminetetraacetic acid tubes upon recruitment and stored at −80°C until DNA was extracted using the QuickGene-810 system (Fujifilm, Singapore). Resulting DNA samples were stored at 4°C until analysis. Quality control for each batch of extractions was performed by running a blank tube through the entire process, omitting only the addition of blood. If any DNA was found in the blank tube, the entire DNA batch was rejected.

Subject samples were identified by sample identification numbers. Clinical data were not available during genotyping.

Genotyping. All samples for both cohorts were genotyped in the same laboratory. The following SNPs were identified using LightSNip (TIB MOLBIOL GmbH, Berlin, Germany) on the Roche LightCycler 480 system (Roche Diagnostics Corp, Indianapolis, Ind), which is based on real-time polymerase chain reaction and melting-curve analysis: rs3025058,[9] rs3918242,[9] rs2276109,[9] rs1801133,[24] rs1799752,[25] rs1799983,[26] and rs16874954.[27] The reaction mixture contained polymerase chain reaction-grade water (7.2 μL), reagent mix (primers and probe; 0.5 μL), FastStart DNA Master (1.0 μL) and magnesium chloride (0.8 μL; 25 mM). One microliter of DNA was used. The LightCycler 480 instrument was programmed according to the instructions for use for each LightSNip (Supplementary Table, online only).

Statistical analyses. Statistical analyses were performed using SPSS 17.0 software (SPSS, Chicago, Ill) and R 3.0 software (The R Foundation for Statistical Computing, http://www.r-project.org/foundation/). Hardy-Weinberg equilibrium was evaluated using the χ^2 test. Genotype distributions (univariate analyses) were also compared using the χ^2 test. A recessive model, a dominant model, and an additive model were used for each SNP when univariate analyses were performed. The dominant model compared those with one or two rare alleles (heterozygotes and homozygotes) with the group of homozygous individuals, and the recessive model compared individuals with two rare alleles (homozygotes) with the combined group of heterozygous wild-type homozygous individuals. The additive model assumed that there is a linear gradient in risk.

Categorical variables are presented as frequencies and percentages. Parametric variables are presented as mean ± standard deviation and nonparametric variables as median and interquartile range. Sample size power