Human leukocyte antigen class II immune response genes, female gender, and cigarette smoking as risk and modulating factors in abdominal aortic aneurysms

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Objective: Aortic inflammation and the genes that regulate the immune response play an important role in abdominal aortic aneurysm pathogenesis. However, the modulating effects of such genetic and other environmental factors on the severity on aneurysm inflammation is not known. The objective of this study was to determine the influence of the human leukocyte antigen (HLA) class II genes, gender, and environmental factors on degree of abdominal aortic aneurysm tissue inflammation.

Methods: Aneurysm specimens were obtained at the time of operation from 96 consecutive patients who underwent abdominal aortic aneurysm repair and were graded for degree of histologic inflammation. Multivariate analysis was used to determine the association of genetic and environmental factors with degree of inflammation and to determine the HLA-associated disease risk for aneurysm.

Results: Active cigarette smoking and female gender were independently associated with high-grade tissue inflammation identified histologically (odds ratio [OR], confidence interval [CI]: 5.6, 1.6 to 19.3; and 6.0, 1.4 to 26.2, respectively), and a specific HLA allele (DR B1*01) was inversely associated with inflammation (OR, CI: 0.2, 0.04 to 0.7). Overall, the HLA-DR B1*02 and B1*04 alleles were significantly associated with disease risk, more than doubling risk for abdominal aortic aneurysm (OR, CI: 2.5, 1.4 to 4.3; and 2.1, 1.2 to 3.7, respectively).

Conclusion: Active cigarette smoking and female gender are significant disease-modulating factors associated with increased abdominal aortic aneurysm inflammation. In addition, the HLA class II immune response genes possess both disease modulating and disease risk properties, which may be useful in early aneurysm detection and surveillance. (J Vasc Surg 2002;35:988-93.)
ment. However, the disease risk for aneurysm associated with the HLA class II genes is not well quantified, and the modulating effects of the immune response genes on aneurysm inflammation are not known.26-28 The purpose of this study was to determine the association of gender, cigarette smoking, and HLA type with the degree of local abdominal aortic aneurysm in inflammation and to establish the disease risk for abdominal aortic aneurysms associated with HLA class II genes.

METHODS

HLA class II genotyping was performed in 142 consecutive patients who underwent elective abdominal aortic aneurysm repair at the Mayo Clinic. All patients in the cohort were white, which reflects both the racial distribution of the disease and the geographic location of the study. Full thickness sections from the proximal anterolateral portion of the aneurysm were available for histologic study in 96 patients from the cohort. Procurement of tissue at the time of aneurysm repair was at the discretion of the surgeon and did not interfere with conduct of the operation. Tissue was not obtained in 20 patients because of surgeon discretion, and 26 specimens were excluded because they were not full-thickness sections through the aneurysm wall.

Cross sections of aneurysm wall were stained with hematoxylin and eosin and graded for degree of inflammation by one pathologist without knowledge of HLA or clinical data. All elements of the aortic wall (intima, media, and adventitia) were examined for the presence and severity of inflammation and graded as either low-grade or high-grade inflammation. Low-grade inflammation included those specimens with no inflammatory cells (lymphocytes, macrophages, or plasma cells) or with only scattered, adventitial inflammatory cells without aortic wall thickening (<2.0 mm; Fig 1A). High-grade inflammation consisted of specimens with confluent, adventitial inflammatory cells (lymphocytes, macrophages, or plasma cells) or germinal centers, with fibrous aortic wall thickening (≥2.0 mm; Fig 1B).

Genetic comparison. Blood samples from 118 ethnically matched individuals with no known aortic, cardiovascular, or autoimmune disease were obtained for HLA class II genotyping and comparison with aneurysm patients (n = 142). DNA was extracted from whole blood of patients and control subjects with the DNA Isolation Kit for Mammalian Blood (Roche Molecular Biochemicals, Boehringer Mannheim, Indianapolis, Ind) and the QIAamp Blood Kit (Qiagen, Chatsworth, Calif) according to manufacturer procedures. HLA-DRB1 genotyping was performed with a Micro SSP HLA DRB kit (One Lambda, Canoga Park, Calif). Trays provided sequence-specific oligonucleotide primers for the 12 major allelic variants at the HLA-DR B1 locus. Matched primer pairs resulted in the amplification of target sequences. Polymerase chain reaction was performed in a 9600-thermal cycler (PE Biosystems, Foster City, Calif) with the following conditions: denaturation at 96°C for 130 seconds, annealing at 63°C for 60 seconds; followed by 9 cycles of 96°C for 10 seconds and 63°C for 60 seconds; then 20 cycles of 96°C for 10 seconds, 59°C for 50 seconds, and 72°C for 30 seconds. Amplified DNA was separated with agarose gel electrophoresis and visualized with ethidium bromide and ultraviolet light.

Clinical factors. Data were abstracted from the medical records of all 142 patients. Smoking status was catego-
 allelés in the study cohort (n = 96). Smoking status was estimated in a pack-years calculation. The median age of the 12 major alleles in the study cohort (n = 142) was compared with the frequency in controls (n = 118), initially with univariate logistic regression analysis. Alleles that were significantly enhanced (P < .05) or that showed a trend towards enhancement (P = .05 to .09) in univariate analysis results were identified as possible risk alleles. Alleles were determined to be randomly distributed if their P values were more than .10. The risk alleles identified with univariate analysis were placed into a stepwise logistic regression analysis to develop a multivariate disease-risk model reported as odds ratios (ORs) and 95% confidence intervals (CIs).

Univariate logistic regression was performed to determine the association of clinical factors (gender, smoking status, pack-years smoked, aneurysm size, age, and family history of aortic aneurysm) with either low-grade or high-grade aneurysm inflammation (n = 96). Patient HLA types then were added with the clinical data, and a multivariate logistic regression model was established to determine any HLA associations with degree of inflammation. The possibility of two-way interactions in the multivariate models reported was investigated. Institutional review board approval was granted and patient consent was obtained for the genotyping, tissue procurement, and clinical data abstraction necessary for this study.

RESULTS

Tissue study. Among the 96 patients who had histologic grading of abdominal aortic aneurysm inflammation, the median age was 71 years (range, 42 to 90 years) and males predominated (n = 119; 84%; Table I). One hundred twenty-four patients (87%) were current or former smokers, and 20 patients (20%) reported a family history of aortic aneurysms. The median aneurysm diameter was 6.0 cm (range, 3.5 to 10 cm).

Univariate logistic regression results determined female gender and current smoking to be independently associated with high-grade aneurysm inflammation (Table II). No other clinical factors, including pack-years smoked, aneurysm size, patient age, or family history of aortic aneurysm, were associated with severity of tissue inflammation. Only two of the 16 women (12%) who had aortic aneurysm tissue grading were undergoing estrogen replacement therapy. One was a current smoker found to have high-grade aortic aneurysm inflammation, and the other was a former smoker found to have low-grade inflammation.

A multivariate logistic regression model that included the HLA genotypes and clinical data showed that a specific HLA class II allele, B1*01, was associated inversely with aneurysm inflammation (OR, 0.2; CI, 0.04 to 0.7; Fig 2). In this model of genetic and environmental factors, female gender (OR, 6.0; CI, 1.4 to 26.2) and current smoking (OR, 5.6; CI, 1.6 to 19.3) were again shown to be independently associated with high-grade aortic inflammation. These factors had no two-way interaction, and the overall model P value was less than .001.

Genetic study. The median age of the 142 patients who underwent HLA genotyping was 71 years (range, 42 to 90 years), and males predominated (n = 119; 84%; Table I). One hundred twenty-four patients (87%) were current or former smokers. Twenty-eight patients (20%) reported a family history of aortic aneurysms. The median aneurysm diameter was 6.0 cm (range, 3.5 to 10 cm).

The HLA-DR B1*02 (42% versus 25%; P < .01) and B1*04 (35% versus 25%; P = .08) alleles were more common in the aneurysm cohort compared with controls and were identified as risk alleles (Fig 3). In contrast, the B1*01 (18% versus 27%; P = .09), B1*08 (6% versus 14%; P = .04), and B1*14 (1% versus 10%; P < .01) alleles were diminished in the aneurysm cohort compared with controls. The seven remaining major alleles at the HLA-DR B1 locus were distributed evenly among the study cohort and controls (P ≥ .10). A multivariate, disease risk model showed the B1*02 (OR, 2.5; CI, 1.4 to 4.3) and B1*04 (OR, 2.1; CI, 1.2 to 3.7) alleles as independently associated with abdominal aortic aneurysm (Fig 4; overall model P value = .007). There were no two-way interactions between these alleles, suggesting the OR in a B1*02/04 heterozygous case to be additive (near 4.5 fold).
DISCUSSION

This study shows for the first time that active cigarette smoking and female gender are independently associated with severity of local aortic aneurysm inflammation. In addition, these findings show the B1*01 allele, a specific genetic factor, to be negatively associated with local aortic aneurysm inflammation. Finally, this study quantifies significant disease risk for abdominal aortic aneurysm associated with genes within the HLA class II region of chromosome 6.

Genetic studies. These findings support results from earlier studies that identified the B1*02 (which includes the B1*15 and B1*16 alleles) and B1*04 alleles as important in white, Japanese, and black patients with abdominal aortic aneurysms.26-28 This study extends previous observations by providing a quantitative assessment of the contribution of HLA alleles to disease risk for abdominal aortic aneurysm in a large cohort of patients.

Cigarette smoking, female gender, and aortic inflammation. This study provides new data that active cigarette smoking is associated with increased inflammation within the wall of abdominal aortic aneurysms. This finding is consistent with the premise that smoking affects the aorta through immune-mediated pathways and provides insight into the increased expansion and rupture rates observed in patients with aneurysm who smoke.11,12,29-32 Importantly, neither former smoking nor pack-years smoked were associated with increased aortic inflammation, suggesting an acute and not a cumulative effect. This observation underscores the importance of smoking cessation and supports recent studies that have found longer periods of cessation to correlate with decreasing risk of aneurysm.15,16

The association of female gender with high-grade aneurysm tissue inflammation, independent of smoking or HLA status, has not been reported. These findings may reflect women’s overall susceptibility to immune-related arterial diseases, such as giant cell arteritis and Takayasu’s disease.33,34 However, these data also allow a possible explanation for the aggressive course of aneurysms in women, specifically pertaining to the greater risk of aneurysm rupture in women compared with men.8-11 Furthermore, these findings raise important questions regarding the influence of estrogen replacement therapy on the magnitude of aneurysm inflammation and the influence of estrogen on the aggressive course of aneurysms in postmenopausal women. The current findings suggest that women should be included in aneurysm early detection efforts, especially older women or women with other genetic or environmental risk factors.

Human leukocyte antigen DR B1*01 as a protective allele. An important new finding from this study is that a specific HLA allele protects against local aortic inflammation. This observation suggests that antigen bound to HLA molecules in B1*01+ individuals may dilute or divert proinflammatory reactions within the aorta. The overall antiinflammatory effect of this allele may be significant enough to reduce the risk for aneurysm because B1*01+ individuals were relatively underrepresented in the study cohort (Fig 3). Interestingly, underexpression of the B1*01 allele is also observed in patients with giant cell

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**Fig 2.** Multivariate logistic regression analysis results show association of genetic and environmental factors with high-grade aortic inflammation. DR, CI, confidence interval.
arteritis, another inflammatory arterial disease. This observation in separate analyses of different diseases suggests a genuine role of this allele in modulating arterial wall inflammation.

**Disease risk versus disease modulation.** Surprisingly, the primary HLA risk alleles for abdominal aortic aneurysm (B1*02 and B1*04) did not influence tissue inflammation as measured in this study. These findings suggest that polymorphic HLA molecules influence aneurysm pathogenesis through different mechanisms or at different phases of pathogenesis (ie, disease initiation versus disease modulation). HLA molecules selectively bind and present antigenic peptides to elicit and amplify adaptive immune responses. The distinct association of the B1*02 and B1*04 alleles with disease risk and the B1*01 allele with disease modulation suggests an interesting model in which antigen-dependent mechanisms are relevant at several points in aneurysm pathogenesis.

**Study limitations.** HLA risk was examined only in patients who underwent elective abdominal aortic aneurysm repair. The role of these genes as factors in the more commonly encountered small abdominal aortic aneurysms and their potential role in predicting aneurysm expansion or rupture remains to be determined. In addition, the methods used to grade inflammation in this study were strictly observational. However, this common histologic approach revealed strong clinicopathologic associations without the need for advanced molecular techniques or a larger sample size. Finally, the abdominal aorta was not specifically imaged in the control group, which makes it possible that persons in this group had abdominal aortic aneurysms. In this unlikely case, the HLA risk of such persons would be the same as the actual study cohort and their removal from the control group would only increase or strengthen the reported ORs.

**Clinical implications.** In spite of these limitations, this study has valuable implications for the management of abdominal aortic aneurysms relating to early detection and risk factor modification. Specifically, more than doubling of the disease risk for aneurysms in B1*02 and B1*04 individuals is significant enough to consider these genetic markers for clinical screening purposes. Families of B1*02 and B1*04 donors may also be considered kindreds at higher disease risk for abdominal aortic aneurysm.

The association of female gender with high-grade aneurysm inflammation combined with the known clinical course of aneurysms in women suggests that women should be included in early aneurysm detection programs. The results of this study also emphasize the importance of smoking cessation in patients at genetic risk for, or with known, abdominal aortic aneurysms. An aggressive smoking cessation program in patients with aneurysms is likely to be cost-effective because smoking increases aneurysm expansion and therefore the likelihood of complications or the need for operation.

**CONCLUSION**

This study provides quantitative data on specific genetic risk factors for abdominal aortic aneurysms within the HLA class II immune response genes. These genetic factors represent biomarkers that may advance aortic screening and early aneurysm detection programs. In addition, this study shows that female gender is associated with increased aneurysm inflammation, providing histologic evidence for the aggressive clinical behavior of aneurysms in women. Finally, this study emphasizes the need for assertive smoking cessation efforts to reduce immune-mediated destruction of the aorta in patients at genetic risk for, or with known, abdominal aortic aneurysms.
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