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Novel *NOTCH1* mutations in patients with bicuspid aortic valve disease and thoracic aortic aneurysms

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Objectives: Bicuspid aortic valve is a common condition and is associated with a significantly increased risk of developing thoracic aortic aneurysms and acute aortic dissection. Patient-specific prediction of the risk of developing thoracic aortic aneurysm, however, is imprecise. We hypothesize that genotypic variations in patients with bicuspid aortic valves contribute to this observed variability in aortic phenotype. We, therefore, investigated the potential relationship between mutations in regions of *NOTCH1* recently reported to be associated with bicuspid aortic valve and the phenotype of bicuspid aortic valve and thoracic aortic aneurysms in unrelated patients undergoing surgical repair.

Methods: We performed a targeted mutational analysis of *NOTCH1* using genomic DNA from 48 unrelated subjects with concomitant bicuspid aortic valve and thoracic aortic aneurysm using denaturing high-performance liquid chromatography and DNA sequencing. We focused on exons in which mutations associated with bicuspid aortic valve have been reported previously. Results were compared with control subjects with trileaflet aortic valves (n = 94), bicuspid aortic valves, and normal aortas (n = 22) and in subjects with tricuspid aortic valves and thoracic aortic aneurysms (n = 28).

Results: Four unique, nonsynonymous (3 novel) variants were identified in 5 (10.4%) of 48 patients with concomitant bicuspid aortic valves and thoracic aortic aneurysms compared with only 3 (2.1%) of 144 control subjects ($P = .02$). Of these, 2 novel missense mutations, A1343V and P1390T, were observed only in patients with bicuspid aortic valves and tricuspid aortic aneurysms.

Conclusions: This targeted analysis involving *NOTCH1* exons previously implicated in familial and sporadic bicuspid aortic valve demonstrates overrepresentation of *NOTCH1* missense variants among patients with bicuspid aortic valves and thoracic aortic aneurysms. Identification of aneurysm-predisposing susceptibility genes may lead to gene-directed surgical therapy of the ascending aorta for patients with bicuspid aortic valves.

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Bicuspid aortic valve (BAV) disease is the most common congenital cardiovascular malformation and is responsible for a large proportion of patients coming to aortic valve replacement (AVR).[1] Individuals with BAV are at increased risk of developing thoracic aortic aneurysms (TAA), acute aortic dissection, and premature death relative to the normal population[2] and have a demonstrably higher incidence of postoperative aortic dissection after AVR.[3] Accordingly, some surgeons advocate prophylactic replacement of an "even seemingly normal" ascending aorta at the time of AVR for BAV.[3] Aneurysmal disease does not develop in all patients with BAV, however, and such an approach would needlessly subject a large number of patients to a procedure with a predictably higher operative risk.[4] Clinical risk stratification, however, is imprecise.

Abbreviations and Acronyms

AVR = aortic valve replacement
BAV = bicuspid aortic valve
DHPLC = denaturing high-performance liquid chromatography
PCR = polymerase chain reaction
TAA = thoracic aortic aneurysm
TAV = trileaflet aortic valve
TGF = transforming growth factor

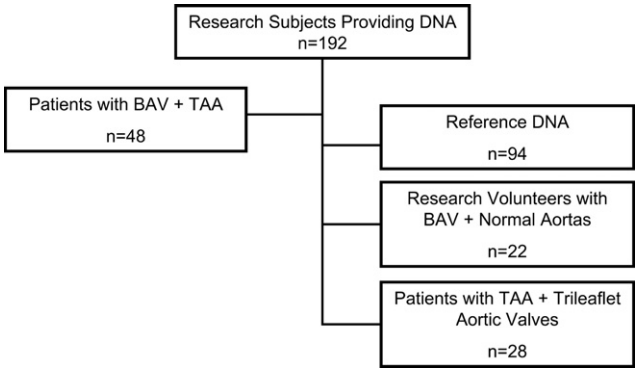

Figure 1. Flow chart of research subjects. DNA was analyzed from 192 subjects, BAV/TAA (n = 48), BAV with normal aortas (n = 22), and TAV/TAA (n = 28). *BAV,* Bicuspid aortic valve; *TAV,* trileaflet aortic valve; *TAA,* thoracic aortic aneurysm.

Both TAA disease and BAV disease are inheritable disorders in at least some cases. Familial clustering of BAV disease shown by Clementi,[5] Cripe,[6] and their colleagues has demonstrated heritability (h^2) of 89% with autosomal dominant transmission and incomplete penetrance of the condition. In addition to Marfan syndrome and the more recently identified Loeys–Dietz syndrome,[7] a genetic basis for some cases of familial TAA and dissection has been mapped by Milewicz, Basson, and their colleagues to 3 genetic loci: 5q13-14,[8] 11q23,[9] and 3p24-25,[10,11] the last of which has proven to be the transforming growth factor (TGF) β-2 receptor.

Recently, an association between mutations in *NOTCH1* and aortic valve disease has been described. *NOTCH1* encodes for a transmembrane protein that activates a signaling pathway with an active role in cardiac embryogenesis, including aortic and pulmonary valve development as well as the development and maintenance of the aorta and other great vessels.[12-15] Garg and colleagues[16] reported *NOTCH1* mutations in 2 pedigrees with an assortment of cardiovascular disease phenotypes, including BAV. Subsequently, Mohamed and coworkers[17] reported 2 mutations in different exons of the same gene in patients with nonfamilial BAV; however, the status of the aorta was not described in the former and only briefly mentioned in the latter study. To date, no studies have identified genetic mutations common to both BAV and TAA phenotypes.

Recent advances in the field of genomics introduce the possibility of genetic profiling as is currently used in pharmacogenomics. Identification of genes predictive of TAA in patients with BAV may foster "gene-directed" surgical therapy of the ascending aorta. We hypothesize that genetic variability among patients with BAV may explain the phenotypic variability in the subset of patients with BAV in whom TAA develops. We, therefore, performed a targeted mutational analysis of *NOTCH1* from genomic DNA obtained from patients with BAV and TAA using denaturing high-performance liquid chromatography (DHPLC) and direct DNA sequencing. These results were compared with DNA from 188 reference alleles obtained from control subjects (n = 94) with normal, trileaflet aortic valves (TAV), as well as a limited number of subjects with BAV and normal aortas (n = 22) or TAV and TAA (n = 28).

Materials and Methods

After institutional review board approval, written informed consent was obtained for all 192 unrelated subjects. We performed a targeted mutational analysis of the 4 exons (11, 20, 25 and 29) previously implicated in aortic valve disease[16,17] in 48 unrelated patients with combined BAV and TAA. Comparisons were made among subjects with BAV and normal aortas (n = 22), TAV and TAA (n = 28), and reference alleles from control subjects with TAV (n = 94) as seen in Figure 1.

We defined an abnormal aorta as an ascending aorta that measured more than 4.0 cm at its greatest diameter. DNA for all BAV and TAA subjects was extracted either from peripheral lymphocytes using the Puregene DNA extraction kit (Gentra, Inc, Minneapolis, Minn) or from aortic tissue obtained at the time of surgery (Wizard SV genomic DNA purification system; Promega Corp, Madison, Wis). Genomic DNA for 94 subjects with TAV was acquired from patients residing in Olmsted County, Minnesota, participating in an epidemiologic heart disease study.[18] Echocardiograms were available for control subjects to confirm valve phenotype.

Using polymerase chain reaction (PCR), DHPLC, and direct DNA sequencing, we performed a targeted mutational analysis of the 4 exons (11, 20, 25, and 29) of *NOTCH1* previously implicated in BAV,[16,17] as previously described.[19] PCR amplification primers were designed with Oligo software (Molecular Biology Insights, Inc, Cascade, Colo). PCR primers are shown in Table 1. In brief, DHPLC is a sensitive method used to elucidate unknown gene mutations. It is based on thermal energy found in the formation and separation of double-stranded DNA fragments containing a mismatch in the base pairing between the "wild type" and "mutant," or heteroduplex, DNA strands. PCR-amplified DNA is injected onto a solid phase column that is heated to a specific temperature (individually optimized for each unique PCR product), which allows for partial denaturing of the DNA sequence of interest. A linear acetonitrile gradient based on the size of the