Genotype–phenotype correlation in patients with bicuspid aortic valve and aneurysm

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Objectives: Bicuspid aortic valve is the most common congenital cardiac abnormality, occurring in 1% to 2% of the population, and often associates with ascending aortic aneurysm. Based on familial studies, bicuspid aortic valve with aneurysm segregates in an autosomal dominant manner with incomplete penetrance. *NOTCH1* mutations have been reported in 6 families with prominent valve calcification and dysfunction and low penetrance of aneurysm. We sought to determine the contribution of *NOTCH1* mutations to the more common phenotype of highly penetrant aneurysms with low penetrance of bicuspid aortic valve and with rare valve calcification or dysfunction.

Methods: All exons and splice junctions of *NOTCH1* were sequenced in probands from 13 affected families presenting with bicuspid aortic valve with ascending aortic aneurysm in the absence of valve calcification. In addition, mutation analysis was performed on a single individual with aneurysm and calcified tricuspid aortic valve. Sequences were aligned and compared with the reference genomic sequence.

Results: Corroborating previous studies, analysis of the single sporadic patient with calcified aortic valve in the presence of ascending aortic aneurysm revealed a novel heterozygous missense mutation in *NOTCH1* resulting in a nonsynonymous amino acid substitution (p.T1090S, c.C3269G) of an evolutionarily conserved residue. This change was not observed in controls. In contrast, we did not identify any pathologic *NOTCH1* mutations in the 13 families segregating noncalcified bicuspid aortic valve with highly penetrant aortic aneurysm.

Conclusions: These data suggest that there are phenotypic differences that distinguish families with and without *NOTCH1* mutations, indicating a genotype–phenotype correlation with potential implications for patient diagnosis, counseling, and management. (J Thorac Cardiovasc Surg 2013;146:158-65)

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Supplemental material is available online.

Bicuspid aortic valve (BAV) is the most common congenital cardiac abnormality[1,2] affecting about 1% to 2% of individuals in the general population. It is characterized by fusion or incomplete formation of valve commissures during valvulogenesis.

Patients with BAV have varying degrees of valvular dysfunction, ranging from severe (including stenosis and regurgitation) to absent.[2] Patients with BAV may also have several additional cardiovascular phenotypes, including valve calcification and ascending aortic aneurysm (AscAA).[2-5] However, there is poor correlation between the extent of valve dysfunction and the incidence or severity of aortic aneurysm.[6-9] Furthermore, patients with BAV and AscAA have been noted to have dilation of the pulmonary trunk with histologic findings of cystic medial necrosis and elastic fiber fragmentation.[4,10] Taken together, these data suggest that the underlying gene defect(s) causing BAV can directly alter vessel wall homeostasis. However, recent studies continue to suggest that eccentric blood flow patterns and/or aortic wall stress, attributed at least in part to perturbation of valve morphology and function, contribute to the increased propensity to aortic dilatation and dissection in patients with BAV.[11,12]

It has been shown that BAV, either in the presence or absence of these additional clinical features, is highly heritable,[13,14] and appears to segregate in an autosomal dominant manner with reduced penetrance.[4,15,16] However, the molecular basis of the disorder is not yet well understood.

In 2005, Garg and colleagues[17] reported an association between inactivating mutations in *NOTCH1* and autosomal dominant aortic valve disease with prominent calcification. Twelve affected individuals in 2 unrelated families were

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Abbreviations and Acronyms
AscAA = ascending aortic aneurysm
BAV = bicuspid aortic valve
TAV = tricuspid aortic valve

identified to have *NOTCH1* mutations. Nine had BAV, with 4 of the 9 also affected by aortic aneurysm. Two other groups have described 4 additional missense *NOTCH1* mutations associated with isolated BAV cases, most of whom had valve calcification and aortic aneurysm.[18,19] Table E1 presents the clinical information for individuals with previously reported *NOTCH1* mutations. Thus, it appears that in some but not all patients with BAV, *NOTCH1* mutations are responsible for early valve calcification, significant valvular dysfunction, and aortic aneurysm.

Many patients with BAV have a distinct phenotype, in which the BAV is associated with dilation of the ascending aorta in the absence of early or aggressive valve calcification.[3,20] The aneurysms can be observed at the aortic root or, more commonly, just distal to the sinotubular junction.[8,21] In such families, the disease is inherited in an autosomal dominant pattern with reduced penetrance. Family members of affected probands can show AscAA in the absence of aortic valve abnormality and vice versa, suggesting that these manifestations share a common etiology but are not interdependent.[4] In fact, in these families, AscAA appears to be more highly penetrant when compared with the penetrance of BAV.

NOTCH1, encoded on 9q34.3, is a member of the type 1 transmembrane receptor protein family. Notch signaling is an evolutionarily conserved intercellular pathway that plays a key role in multiple developmental processes by regulating cell fate decisions.[22] Given the reported connection between calcific aortic valve disease, aortic aneurysm, and *NOTCH1* mutations, we sought to determine the contribution of *NOTCH1* mutations to noncalcific BAV with highly penetrant AscAA.

MATERIALS AND METHODS

Study Subjects

This study was approved by the Institutional Review Board of the Johns Hopkins University School of Medicine. Individuals were recruited, enrolled, and evaluated at the Medical Genetics Clinic of the Johns Hopkins Hospital as previously described.[4] Comprehensive clinical analysis for the proband and the extended family was completed before genotyping. History, physical examination, and echocardiograms were obtained for all individuals who gave informed consent. Figure 1 presents the pedigrees of families analyzed.

Criteria for Inclusion

Each person was evaluated based on clinical history, examination, and echocardiography.[4] Individuals were considered affected with BAV if cardiovascular imaging showed complete or partial fusion of any aortic valve commissure. Individuals were assigned affected status for aneurysm if either the aortic root or more distal ascending aorta showed a dimension with a z score >2 when standardized to age and body size. The z scores were calculated using the Wave Form Echo Program from Boston Children's Hospital for pediatric patients or standard normograms for patients aged 19 years and older.[23] In addition, family members with a history of aortic dissection, rupture, or surgery were coded as affected. The individual from each family selected for sequencing is indicated by an arrow in Figure 1.

Criteria for Exclusion

Families demonstrating dysmorphic features or manifestations of a connective tissue disorder were excluded from this study.

NOTCH1 Mutation Analysis

Genomic DNA was isolated from ethylenediaminetetraacetic acid-treated peripheral blood using the DNeasy Blood Kit according the manufacturer's protocol (Qiagen, Valencia, Calif). Mutation analysis of the entire *NOTCH1* coding sequence was performed using direct DNA sequencing of polymerase chain reaction-derived amplicons. Amplification of all 34 exons and splice junctions of *NOTCH1* was achieved using 42 primer pairs derived from previously published sequencing studies (sequences available upon request)[17,18] and the REDExtract-N-Amp PCR Ready Mix (Sigma-Aldric, St Louis, Mo) per manufacturer's instructions. An ABI capillary sequencer (AB, Foster City, Calif) was used for bidirectional sequencing of each amplicon. The resulting sequences were analyzed for variants using MacVector software (MacVector, Inc, Cary, NC) by alignment to the *NOTCH1* human reference genome sequence (NG_007458.1).

If a putative mutation was identified, all available family members in that pedigree were genotyped. Sequence conservation was assessed by protein alignment in ClustlW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The accession numbers of each aligned protein are as follows: *Homo sapiens* NP_060087, *Pan troglodytes* XP_001171581, *Equus caballus* XP_001498632, *Bos taurus* DAA24217, *Canis familiaris* XP_537795, *Rattus norvegicus* NP_001099191, *Mus musculus* NP_032740, *Gallus gallus* XP_415420, *Xenopus laevis* NP_001081074, *Oncorhynchus mykiss* NP_00112916, *Danio rerio* NP_571516, and *Drosophila melanogaster* NP_476859.

RESULTS

Clinical Findings

Pedigrees for the 13 families and the sporadic case included in this study are shown in Figure 1. Ten of the families described in our study (families A, D, G, I, J, K, L, M, Q, and R) were previously reported[4]; 3 new families (families F, H, and S) were also studied, as well as the individual with sporadic calcified tricuspid aortic valve (TAV) and aneurysm of the ascending aorta (pedigree T). Three previously described families (families N, O, and P) were excluded due to the lack of sufficient DNA.

All the selected probands had BAV with root or ascending aortic aneurysm, with the exception of the proband in family A. This proband (AII:1) presented with BAV, and was considered affected because she had 2 children with AscAA and another with BAV.

Detailed clinical descriptions for families A, D, G, I, J, K, L, M, Q, and R were previously reported.[4] These families include multiple generations of individuals segregating AscAA, BAV, or the combination, in addition to other left

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