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

Kathleen C. Kent, PhD[a], **Melissa L. Crenshaw, MD**[b], **Denise L. M. Goh, MD**[c], and **Harry C. Dietz, MD**[a,d]

[a]McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, Baltimore, Md

[b]Medical Genetics Services, All Children's Hospital, a member of Johns Hopkins Medicine, St Petersburg, Fla

[c]Department of Paediatrics, National University of Singapore, National University Hospital, Singapore, Republic of Singapore

[d]Howard Hughes Medical Institute, Bethesda, Md

Abstract

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.

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 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, *Rat-tus 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 ventricular outflow track abnormalities. Three additional pedigrees (F, G, and S) consisted of families with affected members within a single generation. Two of these (F and G) were trios with the offspring presenting with BAV and AscAA.

Proband demographic and clinical data are provided in Table 1. Of the BAV/AscAA probands included in the familial cohort (n = 13), 10 (77%) were male and the median age was 22 years. Two of the probands (15%) were reported to have unicommissural aortic valves, and 2 (15%) had BAV with unknown morphology. Of the 9 probands with known BAV morphology, 8 (62%) had fusion of the right/left coronary commissure whereas 1 (8%) showed fusion of the right/noncoronary commissure. Twelve (92%) of the probands lacked calcification of the aortic valve, whereas the most senior proband at 64 years old (SII:6), was shown to have moderate asymmetric aortic valve calcification with severe aortic stenosis in addition to mitral annular calcification. However, his age (64 years) is a potential confounding variable because aortic valve calcification is commonly observed in individuals aged 60 years and older.[1,2]

Amongst these probands, 1 individual (JII:3) was reported to have moderate to severe aortic regurgitation but no aortic valve stenosis. Moderate aortic stenosis and regurgitation was observed in the proband of family G (GII:1). Severe aortic stenosis with mild aortic regurgitation was seen in the proband of family S. Of the remaining 10 probands, 4 had no stenosis or regurgitation, 3 had mild stenosis and regurgitation, 2 had mild regurgitation alone, and 1 had mild stenosis alone. Eight of 13 probands (62%) had surgery to repair an AscAA with a majority (n = 5) undergoing a valve-sparing procedure.

In contrast, the sporadic individual (TII:4) with an extensively calcified TAV and a diffuse fusiform AscAA measuring 4.3 cm distal to the STJ was 56 years old at ascertainment. His 3 older siblings had normal echocardiograms. The patient's father (TI:1) lived to 81 years of age and died from pulmonary failure; he was known to have significant arthrosclerosis. The patient's mother (T:I:2) died at age 87 years. Although there was a family history of obesity, there was no known history of aortic aneurysm or BAV.

NOTCH1 Sequencing Results

After sequencing all 34 exons and splice junctions of *NOTCH1* in probands from each of 13 affected families, all known polymorphic variants were cataloged (Table 2). Minor allele frequencies were ascertained from the 1000 Genomes Project European ancestry population because this best represents the demographics of our families.

A single novel intronic variant was identified, exon 16+20G>A in the proband (SII:6) from family S. This variant, although noncoding, could theoretically have an effect on splicing; however, it does not have an intuitive effect on messenger RNA processing or stability. Additionally, this variant was identified in his unaffected brother (SII:3), further supporting its neutrality. The proband from family S (SII:6) was also heterozygous for a nonsynonymous variant in exon 25 p.P1377S (c.C4129T) (Figure 2). This variant was absent in his affected brother (individual SII:5) with BAV and coarctation of the aorta in the absence of AscAA and present in his unaffected brother (SII:3) (Figure 2, B). Protein alignment analysis showed that this amino acid is not highly conserved, because rat and

mouse NOTCH1 show serine at this position whereas humans have proline (Figure 2, B). This variant was previously observed in BAV cases and was rejected as pathogenic in those studies because it was also detected in similar frequencies in control individuals.[18,19] Hence, this variant is also unlikely to be pathogenic.

A coding variant was also identified in the proband from family T (TII:4). This individual had AscAA and early and severe calcification and dysfunction of a TAV. He was heterozygous for a threonine to serine (p.T1090S;c.C3269G) substitution in exon 20 (Figure 2, C). This mutation is located within an epidermal growth factor-like domain and involves a residue that is highly conserved through evolution. It was not observed in 180 human control chromosomes and was also absent from publicly available databases, including the 1000 genomes project. No additional family members were available for segregation analysis.

No other variants were identified in the probands from the other pedigrees.

Discussion

The results of this study indicate that *NOTCH1* mutations do not contribute significantly to the more common phenotype of noncalcific BAV and AscAA. The underlying genetic cause for families with highly penetrant AscAA and low penetrance of BAV in the absence of aggressive valve calcification or dysfunction has yet to be elucidated.

BAV with AscAA

BAV with AscAA is a phenotype that shows autosomal dominant inheritance with reduced penetrance and variable expression; however, the disease mechanism has not been fully illustrated.[4,15,16] Thus we maintain high incentive to identify a molecular marker for disease predisposition. Mutations in *NOTCH1* have been associated with aortic valve disease in familial as well as sporadic cases.[17-19] Garg and colleagues[17] compellingly demonstrated the link between inactivating mutations in *NOTCH1* and autosomal dominant aortic valve disease with pronounced calcification in 2 unrelated families. Nine individuals had BAV and amongst these, dilated ascending aorta was noted in four individuals. One individual with AscAA, TAV, no valve calcification, and normal valve function was negative for the familial *NOTCH1* mutation.[17] Four additional missense mutations were subsequently reported in sporadic BAV cases.[18,19] Each of these cases had a phenotype of dysfunctional BAV with stenosis/insufficiency and coexisting AscAA and all but 1 also showed prominent calcification. Taken together, these patient phenotypes indicate that valve malformation, calcification, and dysfunction are the predominant phenotype caused by altered *NOTCH1* signaling (Table E1).

Because the contribution of *NOTCH1* mutations to BAV with AscAA in the absence of valve calcification has not been established, we chose to approach this question by sequencing families with highly penetrant AscAA and low penetrance of aortic valve dysmorphology and dysfunction.

The absence of *NOTCH1* mutations in our cohort, although identifying a potentially pathogenic *NOTCH1* mutation in a sporadic individual with AscAA and prominent calcification, suggests that there are phenotypic differences that distinguish families with and without *NOTCH1* mutations. We suggest that at least 2 distinct disease mechanisms exist: a *NOTCH1*-dependent mechanism that produces stenotic, insufficient and/or calcified aortic valve with rare aneurysm, and a *NOTCH1*-independent mechanism that produces highly penetrant AscAA in the presence of a noncalcified and often normally functioning BAV. Identification of the causative gene for BAV patients is important not only for understanding the mechanism of disease, but also from the perspective of managing the

disease, especially given that aortic valve calcification, dysfunction, and aortic aneurysm show an age-dependent onset.

Screening for *NOTCH1* Mutations and Follow-up

In general we show that *NOTCH1* mutations are exceedingly rare, with now a total of 7 reported to date (Table E1). Therefore, it would be low yield and cost-ineffective to genotype all families with BAV and AscAA, in particular families with decreased penetrance of BAV and highly penetrant AscAA who have a low incidence of early valve calcification. When screening family members of patients with *NOTCH1* mutations, the presence of BAV, aortic valve calcification, and/or aortic valve dysfunction suggests these individuals are affected, and hence require continual care and monitoring. On the other hand, the absence of BAV does not exclude the presence of a pathologic *NOTCH1* mutation or the predisposition for aneurysm, because data from our lab and others show variable clinical expression of *NOTCH1* mutations in the context of TAV. More study is necessary to determine if valve calcification can be a robust read out for *NOTCH1* mutations. It is also notable that a full phenotypic description of children with *NOTCH1* mutations is lacking.

For patients with the more common presentation of non-calcific BAV with aortic aneurysm, due to the variable penetrance, variable expression and age-dependent onset of AscAA, we recommend that family members of affected individuals be regularly monitored by echocardiogram for AscAA regardless of their aortic valve morphology. First-degree as well as more distantly related individuals should have regular echocardiograms and imaging studies that include the ascending aorta distal to the sinotubular junction.

Based on our observations and other reported studies,[17-19] we suggest that patient management should differ between patients with BAV and families with and without *NOTCH1* mutations.

Surgical Management

Individuals with BAV have an increased risk for aortic aneurysms and dissections. Although aneurysms and dissections can be observed in individuals in the absence of aortic valve abnormalities, the incidence of such abnormalities in patients with BAV is 8- to 10-fold greater than in patients with no aortic valve abnormalities.[2,3,14,24,25] BAV with AscAA carries a significant burden of disease with important surgical implications. We propose that there may be different etiologies of different subclasses of BAV. In terms of implications for management, disruption of NOTCH1 expression primarily affects valve calcification, morphology, and function, and AscAA generally occurs in the setting of significant hemodynamic perturbations. For those patients with BAV with unequivocally pathologic *NOTCH1* mutations, there should be significant concern regarding the long-term durability of the native aortic valve. Such patients will likely require valve replacement within their lifetime, and they may benefit from early valve replacement because this may reduce their risk of secondary aortic aneurysms.

In contrast, in the more common presentation of high penetrance of AscAA with low penetrance of BAV (with minimal calcification or dysfunction), it appears that the underlying gene defect is altering aortic wall homeostasis as a primary manifestation. In this scenario, it seems appropriate to consider sparing the natural aortic valve. In these patients, valve repair or replacement alone would not be predicated to mitigate the risk for aneurysm progression and/or dissection.

Limitations

The limitations of this study must be recognized. There is a possibility of incomplete ascertainment of the phenotype. Whereas BAV is congenital, the onset of both aortic valve calcification and AscAA are age-dependent, leading to the possibility of misclassification. Although our proposed correlation between early valve calcification and the presence of a *NOTCH1* mutation is based on (1) the mutation-negative status of 13 of 13 families without significant valve calcification, (2) our identification of a *NOTCH1* mutation in the sole individual in our study with early valve calcification, and (3) the documentation of aggressive valve calcification in a vast majority of patients previously reported to have *NOTCH1* mutations (Table E1), further prospective work is needed to define the full predictive power of this apparent phenotype–genotype correlation. Furthermore, although we provide significant genetic evidence that the novel missense mutation (T1090S) observed in our case of AscAA in the presence of calcified aortic valve likely contributes to the phenotype, we must be cautious when concluding that this is absolutely the cause of disease in the absence of functional studies. Further analysis was limited by the inability to study additional family members. Another limitation is that we did not analyze the untranslated regions of the *NOTCH1* gene or look for duplications/deletions involving *NOTCH1*.

In conclusion, BAV with the predisposition for aortic aneurysm is not a single disorder. Understanding of disease genes and elucidation phenotype–genotype correlations will inform patient diagnosis, counseling, and management.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations and Acronyms

AscAA	ascending aortic aneurysm
BAV	bicuspid aortic valve
TAV	tricuspid aortic valve
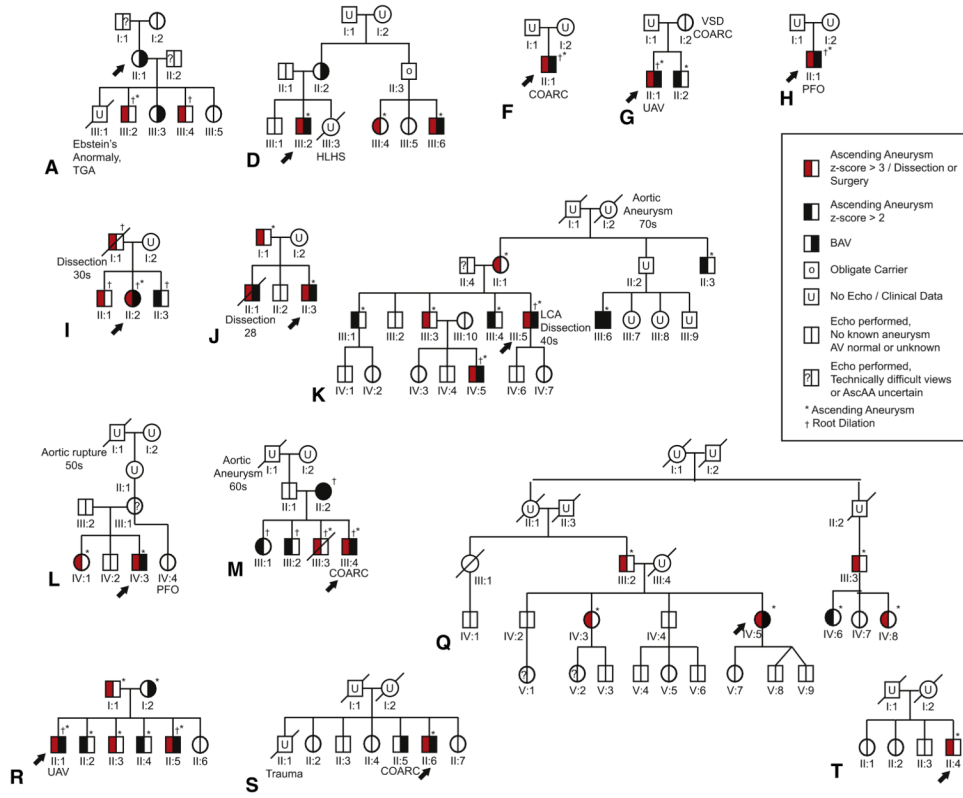
Figure 1.
Pedigrees of 14 families: 13 with bicuspid aortic valve (*BAV*)/ascending aortic aneurysm (*AscAA*), and 1 with calcified tricommissural aortic valve/AscAA. Arrows denote the family member selected for *NOTCH1* sequence analysis. AscAA includes both aortic root and ascending aortic dilation. *TGA*, Transposition of the great arteries; *HLHS*, hypoplastic left heart syndrome; *COARC*, coarctation of the aorta; *VSD*, ventricular septal defect; *UAV*, unicommissural aortic valve; *PFO*, patent foramen ovale; *LCA*, left cerebral artery. Adapted with permission from Loscalzo and colleagues.[4]
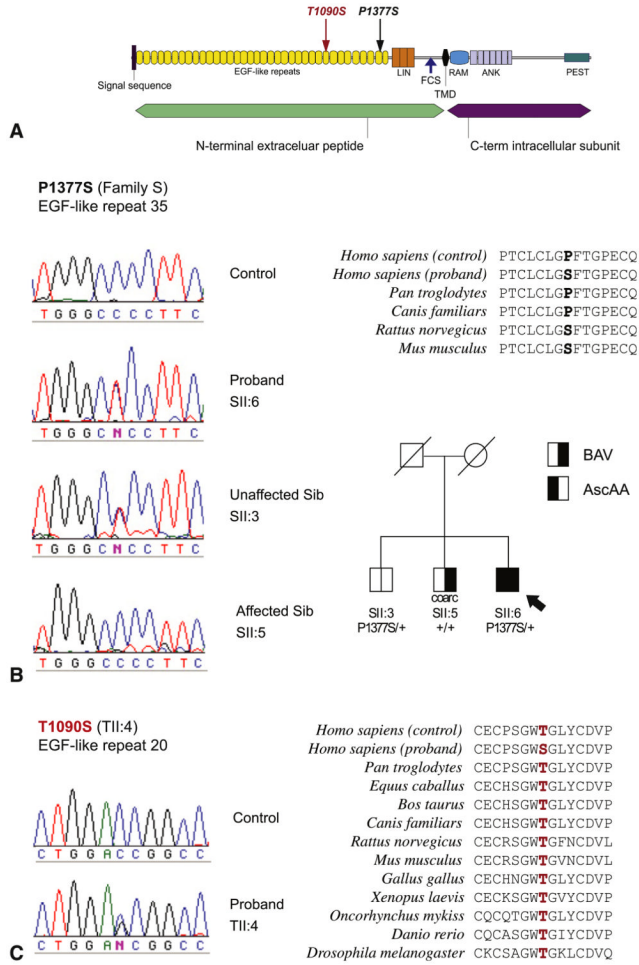
Figure 2.
Characterization of coding variants identified within *NOTCH1*. A, Schematic of NOTCH1 protein domains with the location of each variant indicated. B, Variant P1377S in family S is present in proband SII:6 and unaffected sibling (Sib) SII:3, but absent in a control and affected sibling SII:5. Protein conservation reveals proline at position 1377 is not conserved in rat and mouse. C, Variant T1090S in proband TII:4. This substituted amino acid is conserved down to fruit fly. *EGF*, Epidermal growth factor; *LIN*, LIN-12/Notch repeats; *FCS*, furin cleavage site; *TMD*, transmembrane domain; *RAM*, RAM domain; *ANK*, ankyrin repeats; *PEST*, proline, glutamate, serine, threonine-rich degradation motif; *BAV*, bicuspid aortic valve; *AscAA*, ascending aortic aneurysm.

Table 1

Proband demographic and clinical data

Proband	ID	Gender	Age (y)	Valve status	Cusp fusion	Aortic stenosis	Aortic regurgitation	Calc	AscAo dilation (age of surgery [y])
A	II:1	F	42	BAV	R-L	–	–	–	No AscAA
D	III:2	M	26	BAV	R-L	Mild	Mild	–	AscAo z>3
F	II:1	M	25	BAV	R-L	–	–	–	Valve-sparing AR replacement (25); coarc
G	II:1	M	20	UAV	N/A	Moderate	Moderate	–	Combined AV/AscAo replacement (15)
H	II:1	M	9	BAV	R-L	Very mild	Mild	–	Valve-sparing AR replacement (9)
I	II:2	F	15	BAV	R-L	–	Mild	–	AR z>2; STJ z>3; AscAo z>3
J	II:3	M	22	BAV	R-L	–	Moderate to severe	–	AR z>3; AscAo z>3
K	III:5	M	49	BAV	Unk	Mild	–	–	Valve-sparing AR replacement (49)
L	IV:3	M	7	BAV	R-non	–	–	–	Combined AV/AscAo replacement (5)
M	III:4	M	16	BAV	R-L	–	–	–	AR z>3; STJ z>3; AscAo z>3; coarc
Q	IV:5	F	49	BAV	R-L	–	Mild	–	Valve-sparing AR replacement (44)
R	II:1	M	22	UAV	N/A	Mild	Mild	–	Valve-sparing AR replacement (15)
S	II:6	M	64	BAV	Unk	Severe	Mild	Moderate	Combined AV/AscAo replacement (64)
T	II:4	M	56	TAV		–	–	+	AscAo z>3

Calc, Calcification; *AscAo*, ascending aorta; *F*, female; *BAV*, bicuspid aortic valve; *R-L*, right-left commissural fusion; *AscAA*, ascending aortic aneurysm; *M*, male; *AR*, aortic root; *coarc*, coarctation of the aorta, *N/A*, not applicable; *AV*, aortic valve; *z*, z score; *STJ*, sinotubular junction; *Unk*, unknown; *R-non*, right noncommissural fusion; *UAV*, unicommissural aortic valve; *TAV*, tricuspid aortic valve.

Table 2

Genetic variants within the human *NOTCH1* gene in bicuspid aortic valve (BAV)/ascending aortic aneurysm (AscAA) probands

SNP ID	Genomic position (NG_007458.1)	*NOTCH1* gene position	cDNA position (NM_017617.3)	AA change	Minor Allele	MAF Probands	MAF 1000 gen
rs7028061	g.4849 T>C	5′ UTR - 76 T/C			A	0.38	0.329
rs4489420	g.26979 T>C	Exon 3	c.312T>C	p.N104N	A	0.54	0.487
rs72775789	g.27550 C>A	Exon 4 - 49 C/A			T	0.04	0.01
rs2229975	g.31331 G>A	Exon 5	c.852G>A	p.P284P	T	0.15	0.228
rs9411208	g.33042 C>T	Exon 8 + 7 C/T			G	0.50	0.513
rs9411254	g.33359 C>T	Exon 9 - 43 C/T			G	0.35	0.636
rs11145767	g.33525 A>G	Exon 9 + 10 A/G			T	0.38	0.636
rs10781498	g.33617 C>T	Exon 9 + 102 C/T			G	0.40	0.636
rs11574887	g.33620 C>T	Exon 9 + 105 C/T			A	0.08	0.075
rs4880100	g.34560 A>G	Exon 10 - 133 A/G			T	0.62	0.636
rs4880099	g.34650 T>C	Exon 10 - 43 T/C			A	0.38	0.387
rs3124603	g.35062 A>G	Exon 11 - 9 A/G			T	0.64	0.612
rs62579232	g.35591 C>T	Exon 12 + 94 C/T			A	0.31	0.168
rs3812609	g.36347 G>A	Exon 13 + 70 G/A			T	0.15	0.165
rs2229971	g.37307 T>C	Exon 14	c.2265T>C	p.N755N	A	0.69	0.66
rs3812608	g.37787 G>A	Exon 15 + 21 G/A			C	0.58	0.483
Novel	g.39655 G>A	Exon 16 + 20 G/A			Novel	0.04	No data
rs11574894	g.39709 G>A	Exon 16 + 74 G/A			T	0.08	0.056
rs9411206	g.39738 T>C	Exon 16 + 103 T/C			A	0.38	0.413
rs3125002	g.39878 T>C	Exon 17 - 104 T/C			A	0.54	0.466
rs3125001	g.39978 G>A	Exon 17 - 4 G/A			C	0.67	0.575
rs36119806	g.40146 C>T	Exon 17 + 12 C/T			A	0.08	0.109
rs3124598	g.41685 A>G	Exon 19 - 31 A/G			T	0.27	0.429
rs11145765	g.41959 G>A	Exon 19 42 G/A			T	0.08	0.097
rs3125000	g.41971 A>G	Exon 19 + 54 A/G			T	0.31	0.429
rs35987207	g.41998_41999ins C	c.3171+81_3171+82insC			No data	0.50	No data
rs3812603	g.42331 A>G	Exon 20 - 71 A/G			T	0.33	0.429

SNP ID	Genomic position (NG_007458.1)	NOTCH1 gene position	cDNA position (NM_017617.3)	AA change	Minor Allele	MAF Probands	MAF 1000 gen
rs3124597	g.42576 A>G	Exon 20 + 21 A/G			T	0.38	0.429
rs4880098	g.44335 G>A	Exon 24 + 75 G/A			C	0.50	0.549
rs11145764	g.44833 G>A	Exon 25 - 73 G/A			C	0.52	0.55
rs61751542	g.45020 C>T	Exon 25	c.4129C>T	p.P1377S	A	0.04	0.016
rs13300218	g.45598 C>T	Exon 26 - 85 C/T			A	0.08	0.1
rs11574903	g.46169 C>T	Exon 26 + 55 C/T			A	0.23	0.219
rs10521	g.47532 C>T	Exon 27	c.5094C>T	p.D1698D	G	0.54	0.596
rs12344155	g.48256 T>C	Exon 28 - 43 T/C			G	0.08	0.1
rs3124594	g.48831 T>C	Exon 30 - 43 T/C			A	0.42	0.472
rs11574906	g.49126 A>T	Exon 30 + 87 A/T			A	0.08	0.098
rs11574908	g.49928 C>T	Exon 31 - 12 C/T			A	0.10	0.083
rs9632944	g.51932 T>C	Exon 33 + 44 T/C			G	0.08	0.098
rs2229974	g.53603 C>T	Exon 34	c.6555C>T	p.D2185D	G	0.40	0.423

SNP ID, Single nucleotide polymorphism identification; cDNA, complementary DNA; AA, amino acid; MAF, minor allele frequency.