Study of the contribution of copy number variation to the pathogenesis of bicuspid aortic valve associated aortopathy

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Introduction

Affecting 1-2% of the population, bicuspid aortic valve (BAV) is the most common congenital heart malformation. Although it frequently remains asymptomatic, 10-20% of individuals with BAV develop life-threatening thoracic aortic aneurysms (TAA). Up to 10% of patients with left-sided heart defects have been reported to carry a deleterious copy number variation (CNV). Hence, we hypothesize that **deleterious CNVs may also contribute to the pathogenesis of BAV/TAA**.

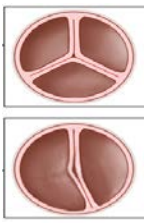


Figure 1: The aortic valve.
The aortic valve usually consists of three semilunar shaped leaflets. A bicuspid aortic valve consists of two unequally sized leaflets, resulting from a fusion of two out of the three valve leaflets.

Methods

95 unrelated BAV/TAA patients

↓ HumanCytoSNP-12 BeadChip

1,390 CNVs

↓ Filtering
1. Low frequency CNVs (<1%)
   - 35 cohorts of DGV
   - HapMap database
2. Potential role in cardiovascular system
   - Gene function
   - Gene expression
   - Animal models

16 prioritised CNVs

↓ Experimental confirmation

7 candidate CNVs

↓ Extra genetic evidence of CNV involvement
1. Overlapping CNVs in patients with CHD
   - DECIPHER
   - In-house WES data of 67 BAV/TAA patients
2. Disruption of highly interactive chromatin domains (Online Hi-C data)

2 candidate genes: *DGCR6* and *TBX20*

↓ In search for extra genetic evidence for gene involvement
Rare variant burden analysis between 637 BAV/TAA patients and gnomAD database

TBX20

Conclusion

I. No major contribution of deleterious CNVs to the aetiology of BAV/TAA

II. Causal CNVs or risk-modifying CNVs may exist though

III. Evidence for involvement of *TBX20* in the aetiology of BAV/TAA

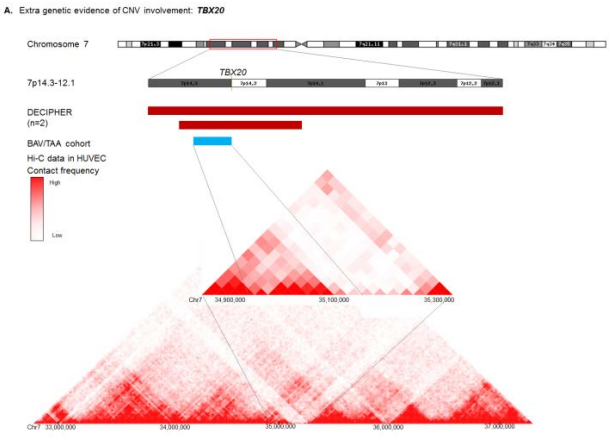
Results

In our BAV/TAA cohort, a total of 7 candidate CNVs were identified, of which the characteristics are summarised in Table 1. Figure 2 demonstrates all the genetic evidence for its involvement in BAV/TAA disease.

Table 1: Candidate CNVs.

Cytogen. band	CN	Validation technique	Freq. (%)	Protein-coding genes	CN of gene of interest	Additional evidence	
						CNV cases	TAD boun.
1q42.13	3	MLPA	0.26	*GJC2*, *GUK1*, *HIST3H2A*, *HIST3H2BB*, *HIST3H3*, *IBA57*, *OBSCN*, *RNF187*, *TRIM11*, *TRIM17*, *ARF1*, *c1orf35*, *MRPL55*, ***RHOU***, ***WNT3A***	2	No	Only for *RHOU*
7p14.2*	3	qPCR	0.02	*DPY19L1*, *NPSR1*, ***TBX20***	2	Yes	Yes
13q22.1	3	MAQ	0.01	***KLF12***	3	No	No
16p13.11	3	MLPA	0.56	*ABCC6*, ***NOMO3***, *NPIPA7*, *NPIPA8*	2	No	Yes
19p12	3	MAQ	0.19	***ZNF626***	3	Yes	No
19p13.2	3	MLPA	0.03	***FBN3***	3	No	No
22q11.21*	1	MAQ	0.9	*PRODH*, ***DGCR6***, *USP18*	1	Yes	Yes

Gene of interest in bold; underlined genes are not affecting the cardiovascular system (autosomal recessive/dominant, susceptibility genes) (*GJC2*, OMIM608804, 613480, 613206; *IBA57*, OMIM615330, 616451; *PRODH*, OMIM239500, 600850). Dup: duplication. Del: Deletion. MLPA: Multiplex Ligation-dependent Probe Amplification. qPCR: quantitative Polymerase Chain Reaction. MAQ: Multiplex Amplicon Quantification. Bp: base pairs. CN: Copy number. Freq.: frequency; Cytogen: cytogenetic; TAD boun.: Topological Associated Domain boundary. *Identified within the same BAV/TAA patient.



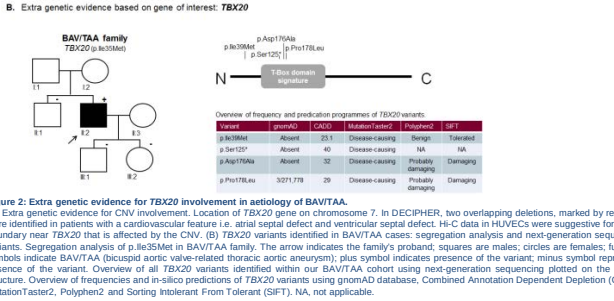


Figure 2: Extra genetic evidence for *TBX20* involvement in aetiology of BAV/TAA.
(A) Extra genetic evidence for CNV involvement. Location of *TBX20* gene on chromosome 7. In DECIPHER, two overlapping deletions, marked by red bars, were identified in patients with a cardiovascular feature i.e. atrial septal defect and ventricular septal defect. Hi-C data in HUVECs were suggestive for a TAD boundary near *TBX20* that is affected by the CNV. (B) *TBX20* variants identified in BAV/TAA cases: segregation analysis and next-generation sequencing variants. Segregation analysis of p.Ile35Met in BAV/TAA family. The arrow indicates the family's proband; squares are males; circles are females; full filled symbols indicate BAV/TAA (bicuspid aortic valve-related thoracic aortic aneurysm); plus symbol indicates presence of the variant; minus symbol represents absence of the variant. Overview of all *TBX20* variants identified within our BAV/TAA cohort using next-generation sequencing plotted on the protein structure. Overview of frequencies and in-silico predictions of *TBX20* variants using gnomAD database, Combined Annotation Dependent Depletion (CADD), MutationTaster2, Polyphen2 and Sorting Intolerant From Tolerant (SIFT). NA, not applicable.

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