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.

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

| Chromosome | CN | Variants | Probing technique | Protein-coding genes | CNV frequency | Additional evidence | TAD length \%
|------------|----|----------|------------------|----------------------|---------------|---------------------|---------
| 1p26.13    | 3  | MLPA     | 0.26             | B3G3, G3G1, HSPA1B, INTB1P18, FMR1, PAK1, TNN1, TNNIT1, APRIL, VDR, SEMA4D, SMGR1 | Only for RMHD |                     |
| 1p14.2*    | 3  | qPCR     | 0.02             | DPF4, SPG5B, NPY3R1 | 2            | Yes                 |                     |
| 13q22.1    | 3  | MAQ      | 0.01             | CEP216 | 3            | No                  | No                  |
| 16p13.11   | 3  | MLPA     | 0.06             | ARSE, AMN95S, UVC | 2            | No                  |                     |
| 19p12      | 3  | MAQ      | 0.19             | 3              | No                  | Yes |                     |
| 19p13.2    | 3  | MLPA     | 0.03             | TBX20 | 3            | No                  | No                  |
| 22q11.21*  | 1  | MAQ      | 0.9              | ARCE, TDP52, SFN | 1            | Yes                 | 9                   |

Gene of interest in bold; underlined genes are not affecting the cardiovascular system (autosomal recessive/dominant, susceptibility genes) (GUCY2C, CDH19, DMD, EVC, PTPN11, USH1C, SMAD4, B3G3, CEAS2, PSEN1, PSEN2, PDGFRB, NDRG1, MYH6, ELN, LMNA, TNNT2); Duplication (DGV); HapMap database; MutationTaster2, Polyphen2 and Sorting Intolerant From Tolerant (SIFT). *Identified within the same BAV/TAA patient.

In search for extra genetic evidence for CNV involvement

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.

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. an inherited notch and ventricular septal defect. Hi-C data in gnomAD 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 identified variants in patients with CHD and in a CHD proband. The arrow indicates the family’s proband; squares are males; circles are females; 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.