Abstract: **P322**

**Notch signaling pathway is attenuated in aortic endothelial cells of patients with aortic pathologies associated with bicuspid aortic valve**

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Background: Bicuspid aortic valve (BAV) is the most common congenital heart malformation occurring in 1-2% of the population. The higher velocity and eccentric blood flow jets caused by BAV leads to increased shear stress on the ascending aortic wall, thereby increasing the risk of ascending aortic aneurysm. Notch signaling pathway is indispensable for heart development and maintenance of vascular system during postnatal life. NOTCH1 mutations have been associated with BAV, yet variants are present in only a minority of individuals with BAV. Notch signaling in the endothelium has been shown to be uniquely positioned to mediate the anticalcific response to shear stress within the valve.

**Purpose:**
To identify novel mutations in NOTCH1 gene in cohort of unrelated patients with BAV-associated aortic pathology; to elucidate whether shear stress response is impaired in endothelial cells of patients with BAV-associated aortic pathology.

**Methods:**
All regions of NOTCH1 gene including the coding exons, introns and 5’-3’ UTR were sequenced in 27 patients with BAV-associated aortic pathology using target NGS approach. Obtained data were processed with GATK3.5, annotated with ANNOVAR and analysed including population databases ExAC and gnomAD. Human aortic endothelial cells (HAEC) were isolated from tissue fragments of BAV-associated thoracic aortic aneurysm patients and from healthy donors used as controls. HAEC were subjected to oscillatory flow imitating disturbed flow in the aorta with BAV. Expression of corresponding responsive genes was estimated by qPCR. Activity of Wnt/β-catenin pathway was studied using TCF luciferase reporter.

**Results:**
Three novel mutations, two missense mutations (Exon 15, N816D; Exon 18, R955C) and a nonsense mutation (Exon 20, Q1108X) were found in the NOTCH1 gene. Using CADD tool for scoring the deleteriousness of single nucleotide variants identified mutations were determined as like-pathogenic with scaled C-score equal 23.1, 29 and 36 correspondingly.

Expression of genes related to antioxidant, antiatherogenic and proinflammatory pathways was significantly changed in HAEC of patients at baseline level as well as after oscillatory flow. Relative level of TCF activity reflecting Wnt activation was significantly elevated in the HAEC of patients in response to Wnt activation while the fold activation of Wnt activity was decreased in the diseased cells. AXIN2 expression level reflected the same tendency showing failure of activation in response to Wnt activation.

**Conclusions:**
Three novel like-pathogenic mutations were identified in NOTCH1 in 27 patients with BAV-associated aortic pathology, highlighted the role of Notch signaling in development of aortic pathology. Aortic endothelial cells of patients with BAV have impaired response to shear stress due to defective NOTCH/WNT/BMP cross-talk.