Contribution of progenitor cells sharing some serotonergic proteins in the pathophysiology of human aortic and mitral valves degeneration

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Manypeptides (pergolide, fenfluramine, ecstasy) were described as inducers of fibrotic valvular lesions. All these compounds share in common to activate the 5HT2B serotonergic receptor. This observation leads to the hypothesis that cardiac valves express a serotonergic system that could be activated by serotonin (5-HT) or 5-HT receptor (5-HTR) agonists. In this work, we characterized the expression pattern of 5-HT2A, 2B, 4 R and the 5-HT transporter (5ERT) in whole and cell subpopulations of 30 human mitral and aortic valves collected at the time of surgical valve replacement (Aortic: 11 calcified, 5 sclerotic, 4 bicuspid; Mitral: 12 dystrophic). All samples express 5HT2A, 2B, 4 R and SERT, the amount of 5HT2B R mRNA being higher than the 5HT2A R whatever the valve and etiology. 5HT2BR expression is found in endothelial cells (CD31+) at the valve surface, but also inside valve lesions, expressed by interstitial cells (smooth muscle α-actin and vimentin positive cells) located in an abundant glycosaminoglycan matrix. In fact, fibromyxoid lesions and calcified aortic valves express a high amount of CD34+ cells. These cells are endothelial progenitors because they express VEGFR2 and eNOS together with 5HT2R. After collagenase treatment, valve samples were labeled with CD31 and CD34 antibodies: 60.1 ± 11 % of all mitral valvular cells are CD34+ compared to 36.1 ± 8 % of aortic valvular cells (FACS analysis and sorting). To summarize, 5HT2A, 2B, 4 receptors and SERT are expressed in aortic and mitral diseased valves. The amounts of 5HT2A, 2B R mRNA are equivalent between mitral and aortic valves. High amount of CD34+ endothelial progenitors, expressing 5HT2AR and 5HT2BR, are found in degenerated valves. The contribution of the two 5HT2 receptors and endothelial progenitors in valve degeneration is now under investigation.

Functional explorations of genes near genetic risk loci for mitral valve prolapse involve TNS1 and LMCD1 in valve development and integrity

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Nonsyndromic mitral valve prolapse (MVP) is a common degenerative valvulopathy of unknown aetiology that predisposes to heart failure and sudden death. Here we investigate functional evidence for candidate genes near genome-wide association study (GWAS) loci to understand the mechanisms underlying the genetic susceptibility to MVP. To prioritize genes, we used literature and databases (e.g. ENCODE, eQTLs). We analysed the expression pattern of 3 candidate genes during valve development in mouse embryos by immunohistochemistry (IHC) i) completion of endothelial-to-mesenchymal transformation (E13.5), ii) valve sculpting and elongation (E17.5) and iii) adult form (9 months). Morpholino knockdown (KD) was performed for 8 genes in zebrafish and assayed the function of developing atrioventricular (AV) canal by scoring the embryos according to the presence of ephydroid regurgitation. On chr3, the association is in an intron of LMCD1, a repressor of GATA6 previously implicated in cardiac hypertrophy. KD of Lmcd1 in zebrafish resulted in a significant atrioventricular valve defect with regurgitation. On Chr2, the associated variants were upstream to Tns1 encoding a focal adhesion and actin-interacting protein. Tensin1 is expressed during valve morphogenesis in mice and maintained in the adult endothelial and valvular interstitial cells. Hematoxylin and eosin histological staining in 9-month Tns1−/− mice show enlarged posterior mitral leaflet. In addition, zebrafish KD of Tns1 induced AV regurgitation. The functional validation of genes located in or near MVP susceptibility loci identifies new MVP mechanisms and stresses the role of cytoskeleton integrity in valve development. This study reveals LMCD1 and TNS1 at play as early as during valve development and can potentially be targeted during adulthood to improve the natural history of MVP.

Involvement of LRRFip1 gene and canonical Wnt pathway in Mitral Valve Prolapse (MVP)

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Heart valves affect 3% of world population, and surgery is often the only therapeutic mean. A genetic study performed on a family in which several members exhibited a MVP identified a R94G mutation on LRRFip1 gene. LRRFip1 alternative transcription splicing gives rise to five isoforms in humans, three of which are targeted by the mutation (Iso1, 3 and 4). Previous studies only focused on LRRFip1 iso5 that was first described as a transcription factor interacting with positive (Dishevelled) and negative (Flightless-1) regulators of the canonical Wnt β-catenin dependant pathway. As it may participate and regulate crucial events of cardiac valve development and homeostasis involving Wnt pathway, we hypothesised that LRRFip1 could be involved in MVP pathology. We first analysed the expression of LRRFip1 in valves by RNA sequencing and quantitative PCR and showed that LRRFip1 iso5 is expressed in human valves. In mouse, it prevail during embryonic development and then levels down to that other isoforms expression. We thus focused on LRRFip1 iso1. Using cell fractionation, we showed a nuclear localisation of LRRFip1 iso1 while other isoforms are strictly cytoplasmatic. Using luciferase-based Wnt reporter assays and co-IP, we further demonstrated that out of the five isoforms, LRRFip1 iso5 is the strongest interactor of Dvl-1 and Fli-1, and the strongest activator of the canonical Wnt pathway. Although activation requires beta-catenin, it does not involve beta-catenin stabilization nor activation. Using site directed mutagenesis, we mapped the domain responsible for Wnt pathway activation to the 25 amino-acids region surrounding arginine 94 and showed that R94G mutation also decreases Wnt activation. This work demonstrates the involvement of LRRFip1 iso5 in canonical Wnt pathway activation. Taken together, our results suggest a potential role for LRRFip1 in valvulogenesis and/or valve homeostasis regulation that may be impacted by the R94G mutation.