genotype-phenotype correlation in large families. Our data highlights the importance of AC cascade genetic screening to determine the clinical significance of rare genetic variants.

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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 anti-calcific 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.

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In vitro studies to understand gender differences in calcific aortic valve disease: crosstalk between JAK-STAT and TLR pathways

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Introduction: Calcific aortic valve disease (CAVD) has become an important social and economic burden. Inflammation has been pointed out as a key event in CAVD pathogenesis, in which the role of the immune modulator type I interferon (IFN) remains unknown. On the other hand, as male sex is a risk factor for CAVD, possible gender differences may have gone unnoticed in studies performed mostly with cells explanted from male patients.
Purpose: To elucidate the role of type I IFN on inflammation and calcification of human aortic valve interstitial cells (AVIC) isolated from male and female patients.
Methods: Control and stenotic AVICs were exposed to IFN-α and lipopolysaccharide (LPS) alone or combined. Western Blot and ELISA were used to analyze pro-inflammatory/pro-osteogenic molecules and signaling pathways. To test whether stimuli promotes osteogenic differentiation of AVIC, osteoblast markers and α-smooth-muscle actin were analyzed by qPCR and immunofluorescence, respectively. Alizarin red staining and calcium deposits quantification were performed to evaluate in vitro calcification.
Results: Our data showed that IFN-α and LPS cooperated to activate signal transducer and activator of transcription (STAT)-1 and nuclear factor (NF)-κB. The combination of stimuli also triggered the secretion of pro-inflammatory and pro-osteogenic molecules with a reported role in AVIC calcification. Consistent with this, IFN-α alone and combined with LPS promoted α-smooth muscle actin downregulation, thus suggesting cell differentiation. Further analysis indicated an IFN-α mediated early upregulation of osteoblast markers in male but not in female AVIC, pointing to potential differences in calcification. In vitro calcification assays revealed that male AVIC were more prone to calcification, as suggested by higher calcium deposition. Strikingly, IFN-induced calcification was totally abrogated by using the JAK inhibitor tofacitinib. qPCR revealed the tendency to lower levels of IFN-α receptor subunit-1transcripts in female AVIC. In addition, signaling pathways analysis showed a synergistic phosphorylation of protein-kinase-B (Akt) only in female AVIC, a kinase that may be involved in calcification differences.
Conclusions: IFN-α and LPS crosstalk promotes higher inflammatory and osteogenic responses in male AVIC, which correlates with the higher rate of metaplasia reported in male patients. Our data point to JAK-STAT pathways as potential therapeutic targets for CAVD.

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New molecular panel with high sensitivity and specificity for early diagnosis of degenerative aortic stenosis

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Background: Using animal models for the study of degenerative aortic stenosis (DAS) is crucial to avoid the differences between the analyzed subjects, something common in these patients due to its age and the associated pathologies. Specifically, rabbit models are ideal since this animal has many similarities to humans, including valve histology and lipid metabolism.
Purpose: Our objective was to characterize proteins involved in the first stages of DAS, searching for candidates for early diagnostic suitable to be transferred to the clinic.
Methods: In this work, we have analyzed aortic valves (AV) from healthy and mild stenotic rabbits. Male New Zealand White rabbits were divided in control and pathological group (cholesterol-enriched diet plus vitamin D2). After the sacrifice, AV were harvested and their proteins were analyzed using 2D-DIGE. Differentially expressed proteins were measured in plasma from the same rabbits to corroborate their potential as diagnostic indicators and in plasma from human subjects to confirm their feasible translation to the clinical field.
Results: Fifteen spots were found differentially expressed corresponding to 8 unique proteins. Of them, 5 proteins were measured in plasma samples from rabbit and 3 were also altered in human plasmas: transitional endoplasmic reticulum ATPase, tropomyosin alpha-1 chain and L-lactate dehydrogenase B chain. ROC curves were performed for these proteins, separately and as a panel, in order to establish its sensibility and specificity. In all cases, the area under the curve was higher than 0.73 and the p-value below 0.037. The diagnostic power of the three proteins as a panel was much better than the proteins alone.
Conclusion: Here, we have defined a new panel with potential for the early diagnostic of DAS, something that should not be undervalued. Starting the treatment during the asymptomatic period of the disease may reduce mortality and improve the outcome of these patients.

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Statin inhibits synthesis of type I collagen in patients with rheumatic heart disease

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Background: Rheumatic heart disease (RHD) remains a health burden in developing countries with lack of pharmacological agents to slow this disease process. Increasing of collagen synthesis plays an important role in the progression of fibrosis, thickening and calcification of cardiac valves in patients with RHD. Statins have been known to have anti-fibrotic and anti-inflammatory effect; however their effect on on the synthesis of type I collagen, primary collagen component of human cardiac valves, has not been elucidated.
Purpose: To evaluate the effect of simvastatin on the synthesis of type I collagen in patients with RHD
Methods: This experimental randomized pretest-posttest control group study was performed in 31 RHD patients confirmed by echocardiographic finding. Patients with age $>$ 75 years old, unstable hemodynamic, previous percutaneus or valvular heart surgery, renal failure, and previous statins treatment were excluded. Treatment and control groups received a standard medical therapy with (16 patients) and without (15 patients) simvastatin 40 mg/day for 4 weeks, respectively. Carboxy-terminal propeptide of type I procollagen (PICP) is used as a marker of type I collagen synthesis. PICP blood serum was taken from peripheral vein blood and measured by ELISA method prior and after 4 weeks treatment.
Results: There were no significant differences in clinical and echocardiographic baseline paramaters between treatment and control groups. Most of cardiac valve abnormality was mitral stenosis concomitant with atrial fibrillation. Prior serum level of PICP were similar between treatment and control groups (709$\pm$269 versus 671$\pm$242 ng/mL, p=0.699). A significantly lower of PICP serum level was found in statin treated group than untreated patients (583$\pm$236 versus 879$\pm$316 ng/mL, p=0.008). There were minor side effect reported, including mild myalgia and nausea in two (2) patients treated with statin.
Conclusion: Simvastatin may reduce synthesis of type I collagen in patients with RHD which could represent potential pharmacological agents to slow the disease process.

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New signaling pathways potentially involved in human mitral valve prolapse

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Introduction: Mitral valve prolapse (MVP) with severe regurgitation is one of the most common pathology of the mitral valve, afflicting more than 175 million people worldwide. No pharmacolog-