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.

P322

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

A. Kostina[1]; A. Kiselev[1]; H. Bjorck[2]; O. Irtyuga[1]; A. Sergushichev[3]; Y. Baranov[3]; P. Eriksson[2]; A. Kostareva[1]; A. Malashicheva[1]
[1]Almazov National Medical Research Centre, Saint Petersburg, Russian Federation; [2]Karolinska Institute, Stockholm, Sweden; [3]ITMO University, Saint Petersburg, Russian Federation

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.

P323

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

I. Parra-Izquierdo[1]; I. Castanos-Mollor[1]; J. Lopez[2]; C. Gomez[1]; A. San Roman[2]; M. Sanchez Crespo[1]; C. Garcia-Rodriguez[1]
[1]Instituto de Biología y Genética Molecular (CSIC-Universidad de Valladolid), Valladolid, Spain; [2]ICICOR, CIBER de Enfermedades Cardiovasculares (CIBERCV), Hospital Clinico Universitario, Valladolid, Spain

Funding Acknowledgements: Grants SAF2013-44521-R, BIO/VA47/14 and BIO/VA36/15; Fundación Domingo Martínez; PI14/00022 and CIBERCV. Fellowships from the UVA and CyL government.
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.

P324

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

L. Mourino-Alvarez[1]; F. De La Cuesta[2]; T. Sastre-Oliva[1]; M. Baldan-Martin[1]; N. Corbacho-Alonso[1]; T. Martin-Rojas[1]; LF. Lopez-Almodovar[3]; G. Alvarez-Llamas[4]; LR. Padial[5]; MG. Barderas[1]
[1]National Hospital of Paraplegics, Vascular Physiopathology, Toledo, Spain; [2]Centre for Cardiovascular Science, Queen's Medical Research Institute, University of Edinburgh, Edinburgh, United Kingdom; [3]Hospital Virgen de la Salud, Cardiac surgery, Toledo, Spain; [4]Foundation Jimenez Diaz, Department of Immunology, Madrid, Spain; [5]Hospital Virgen de la Salud, Cardiology, Toledo, Spain

Funding Acknowledgements: Instituto de Salud Carlos III [PI11-02239, PI14-01917], FONDOS FEDER [RD06/0014/1015, RD12/0042/0071] and the Spanish Society of Cardiology
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.

P325

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

Y. Herry
Diponegoro University, Department of Cardiology and Vascular Medicine, Semarang, Indonesia

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.

P326

New signaling pathways potentially involved in human mitral valve prolapse

P. Songia; V. Myasoedova; P. Gripari; V. Valerio; L. Fusini; L. Cavallotti; G. Tamborini; M. Pepi; P. Poggio
Cardiology Center Monzino IRCCS, Milan, Italy

Funding Acknowledgements: Fondazione Gigi e Pupa Ferrari ONLUS
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-

ical treatments have been identified yet, leaving the surgical intervention the only effective treatment.
Hypothesis. Our aim was to evaluate circulating microRNA (miRNA) profile in human myxomatous MVP to identify the pathological processes and thus new potential therapeutic targets.
Methods: We analyzed plasma obtained from 30 patients that underwent mitral valve repair due to MVP and 30 controls. TaqMan Array Human MicroRNA Card A (v2.0) was used to assess the expression levels of 384 miRNA. Validation were performed using real-time PCR and expressed as log fold change (logFC). Functional analysis were carried out with Cytoscape (v3.4.0) and ClueGO (v2.3.3). In vitro studies were performed on valve endothelial cells isolated from MVP specimens.
Results: MiRNA profiling revealed that in MVP patients 6 miRNAs were up-regulated, while 22 were down-regulated when compared to controls. Validation analyses confirmed that miR-150-5p (logFC=+0.46±0.06; p<0.0001), miR-210-3p (logFC=+0.23±0.06; p=0.01), miR-451a (logFC=+0.50±0.09; p<0.0001), and miR-487a-3p (logFC=+0.54±0.16; p=0.003) were significantly up-regulated in MVP. MiR-27a-3p (logFC=-0.32±0.09; p=0.004), miR-323a-3p (logFC=-0.36±0.10; p=0.004), miR-361-5p (logFC=-0.35±0.09; p=0.0002), and miR-376c-3p (logFC=-1.37±0.36; p=0.003) were significantly down-regulated in MVP. Functional analysis identified several biological processes: 1) cellular response to oxidative stress and mechanical stimulus; 2) regulation of stress fiber assembly; 3) apoptosis; 4) transforming growth factor beta signaling pathway; 5) adherens junction and focal adhesion regulation; 6) response to hypoxia-inducible factor 1 signaling pathway; 7) endothelial and smooth muscle cell proliferation; 8) ErbB and apelin signaling pathways. Finally, endothelial cells, under oxidative stress stimuli, showed a positive regulation of myxomatous degeneration with a concomitant release of miR-150-5p (logFC=+3.73±0.2; p<0.0001). Conclusions. To the best of our knowledge, this is the first study performed on human plasma and isolated valve endothelial cells from MVP patients, showing a strong association of miRNA and MVP pathology. The new identified pathways could represent new pharmacological targets to slow-down or even halt MVP progression.

P328

Gender features of myocardial infarction and stroke risk in general population with vital exhaustion in russia / siberia: who program monica-psychosocial

VV. Gafarov; EA. Gromova; DO. Panov; AV. Gafarova; IV. Gagulin; EA. Krymov
Institute of Internal Medicine, Novosibirsk, Russian Federation

Objective: To determine the gender differences influence of vital exhaustion (VE) in the risk of myocardial infarction and stroke in the general population aged 25–64 years old in Russia/Siberia.
Materials and Methods: In the III screening WHO program "MONICA-psychosocial" surveyed a random representative sample of the population aged 25-64 in 1994 (men = 657, women n = 870).
Results: VE level were: men 66.8%, in women 75.7%. The risk of MI among men with VE was HR =2. RR of MI in persons with VE were higher among divorced women HR = 5.4, than men HR = 4.7. Risk MI was higher in men with VE: primary education HR = 2.2; have never married HR = 3.7, widowed male HR = 7. Risk of stroke in patients with VE were higher in women HR = 3.34, than men HR=3.1. Risk stroke was higher only in men with VE: with incomplete secondary - primary education HR = 4.8; men, divorced HR = 3.8, widowed men at HR = 3.6.
Conclusion: Prevalence of VE was higher in women than in men. VE is a predictor of MI in men and stroke in both genders.

P329

Regulation of LTBP expression as a modulator of TGFb availability in patients with BAV

F-A Poujade; L. Du; V. Paloschi; P. Eriksson
Karolinska Institute, Department of Medicine, Stockholm, Sweden

Funding Acknowledgements: Swedish Research Council (Vetenskapsrådet), Swedish Heart and Lung Foundation
Introduction: Bicuspid aortic valve (BAV) is the most common cardiac defect in human, estimated to affect 1-2% of the general population. However, surprisingly, more than 50% of patients undergoing aortic valve and/or ascending aortic surgery display a BAV, rather than a normal tricuspid aortic valve (TAV). People with BAV are consequently 50-70% more likely to develop ascending aortic aneurysm later in life, with no forewarning symptoms.
The association between BAV and ascending aortic aneurysm is believed to be two-fold. Firstly, the valve malformation disturbs the normal blood flow within the system, generating stress to the endothelial cells lining the interior of the aortic tissue, which might in turn modify signalling pathways. Secondly, it is likely that the genetic changes responsible for the development of a BAV also interfere with the structure of the ascending aorta, which has a common embryologic origin with the aortic valve, rendering it less resilient and more susceptible to dilatation and rupture.
Purpose: The aim of this project is to understand the extent to which cells issued from patients with BAV and TAV differ, and how these differences explain the aetiology of BAV-associated aortopathy. Specifically, the present work focuses on latent TGF-β-binding proteins (LTBPs) as regulators of TGF-β activity, which is of crucial importance in ascending aortic aneurysm development.
Methods: Although the exact role of TGF-β in aneurysm initiation and development is still unclear, it seems to differ between BAV-related and otherwise occurring aneurysms. Previous work demonstrated a difference in TGF-β availability between BAV and TAV patients, possibly due to differential sequestering of TGF-β in the extracellular matrix by latent TGF-β-binding proteins (LTBPs). Using electrophoretic mobility shift assay (EMSA) and luciferase reporter assays, the regulation of LTBP expression is studied in BAV and TAV systems, and compared.
Results: We have revealed the presence of protein binding regions in the promoter sequence of LTBP1. Further work is required to confirm whether these interactions are responsible for the regulation of LTBP1 transcription, which could explain the difference in LTBP level observed during aneurysm between BAV and TAV patients.
Conclusion: Differential regulation of LTBP expression in BAV and TAV, through the variation of transcription factors activity or other regulatory elements could explain, at least in part, the differences observed during aneurysm development between BAV and TAV patients.

P330

Modifications of short-term heart rate varibility and intrinsic pacemaker variability in an experimental model of metabolic syndrome

CJ. Calvo[1]; OJ. Arias-Mutis[2]; A. Diaz[1]; E. Blanch[1]; L. Such-Miquel[1]; L. Such[1]; A. Alberola[1]; FJ. Chorro[1]; M. Zarzoso[1]
[1]*University of Valencia, Valencia, Spain;* [2]*CIBERCV, Valencia, Spain*

Funding Acknowledgements: GV2015-062, UV-INV-PRECOMP14-206372, PROMETEOII/2014/037, CIBERCV CB16/11/0486
Introduction: Metabolic syndrome (MetS) describes a cluster of cardiovascular and metabolic alterations such as abdominal obesity, reduced HDL and elevated LDL cholesterol, elevated triglycerides, glucose intolerance and hypertension. Diagnosis requires that any three out of these five criteria are present. MetS has been linked with a higher prevalence of cardiovascular mortality, including sudden cardiac death, but the mechanisms are not well understood. One possible mechanism underlying may be an abnormal modulation of autonomic activity, which can be quantified analyzing heart rate variability (HRV).
Purpose: To investigate the modifications that MetS produces in short-term HRV and the intrinsic modulation of pacemaker variability in isolated heart.
Methods: Male NZW rabbits were randomly assigned to a control (n=12) or a MetS group (n=13), fed during 28 weeks with high-fat (10% hydrogenated coconut oil and 5% lard), high-sucrose (15% dissolved in water) diet. After anesthesia (2% isoflurane), a 15 min ECG recording was performed (lead 1) at week 28. Then, their hearts were isolated and, after 15 min of stabilization, 15 min volume-conducted ECG was recorded. We analyzed short RR time series in vivo and in isolated heart using the following parameters: 1) Time domain: RR, HR, SDNN, triangular index (Ti), RMSSD and TINN; 2) Frequency domain: very low frequency (VLF), low frequency (LF), high frequency (HF), and LF-HF ratio; 3) Non-linear analysis: Poincaré (SD1, SD2) and sample entropy; 4) Time-frequency analysis (wavelet-based). Multivariate analysis of variance (MANOVA) was used for statistical analysis (p<0.05).
Results: Poincaré analysis of HRV showed a decreased SD2 at week 28 in MetS animals, indicative of a reduced parasympathetic activity and non-linear variability. We did not find changes in the rest of time-domain, frequency domain, non-linear and time-frequency parameters of HRV between groups. When comparisons were made within groups, we found a decrease in HR and Ti, and an increase in HF components of HRV (total power and normalized) when comparing week 28 and isolated heart measurements in both control and MetS groups (Table), suggesting a predominance of sympathetic activity in vivo. SD1 and SD2 of Poincaré plot increased in the isolated heart of MetS animals but remained unchanged in controls. No differences were found in the measured HRV parameters in isolated heart between control and MetS groups.
Conclusion: MetS produced changes in non-linear indices of short-term HRV indicative of a decreased parasympathetic activity at week 28. In isolated heart, and thus not submitted to extrinsic nervous or humoral influences, intrinsic pacemaker variability does not seem to be modified by the administration of a high-fat, high-sucrose diet during 28 weeks.

HRV parameter	Control		MetS	
	In vivo	*Ex vivo*	*In vivo*	*Ex vivo*
RR (ms)	238±29	297±63†	257±27	300±44†
HR (bpm)	254±32	210±39†	241±26	203±27†
Ti (ms)	8.9±4.4	2.8±1.5†	7.7±2.9	2.9±1.9†
HF power (%)	39.5±39.4	86.4±7.4†	21.9±28.3	79.7±23.3†
HF normalized (%)	41.8±4.1	86.3±7.3†	21.8±3.6	88.1±4.8†
Time-freq. HF power (%)	44.7±37.4	87.1±7.1†	35.9±25.7	87.2±5.5†
SD1 (ms)	5.8±5.6	14.2±13.9	4.8±3.8	11.3±17.6†
SD2 (ms)	20.1±11.5	16.6±14.0	13.4±6.0*	16.2±17.1†

HRV parameters. *p<0.05 vs. control. †p<0.05 *vs.* in vivo week 28.

Abstract P330 Figure.

P331

Are there gender specific differences in elderly regarding exercise treatment of cardiovasular diseases?

A. Lelbach[1]; B. Albert[2]; A. Koller[2]
[1]*Dr. Rose Private Hospital, V.I.P. Department, Budapest, Hungary;* [2]*Semmelweis University, Budapest, Hungary*

Funding Acknowledgements: Supported: Hungarian National Science Research Fund (OTKA, K108444)
Introduction: Hypertension (HT) affects 25% of the world's population and a major risk factor of cardiovascular diseases (CVD) (Carpio-Rivera, 2016). According to the Hungarian Hypertonia Register's data (Kekes, 2009) the prevalence of HT under 55 years is lower in women than in men but in the population above 75 years it is the opposite. Based on the research of Kekes et al. to reach the optimal blood pressure in overweight elderly patients is especially difficult.
Applied methods: We have reviewed and critically analyzed the available literature regarding the exercise treatment and gender specific differences in hypertensive elderly in connection with the lowering of CVD risks.