

to obtain membrane-specific loss of function (iii) mice lacking the AF2 ligand-dependant-transcriptional function domain of ER $\alpha$  (AF2 $^{\Delta}$ ) and (iv) wild type (WT) mice. Hypertension was induced using Angiotensin II (Alzet minipumps) and blood pressure measured using tail cuff. After 28 days vascular structure and function were measured in vitro. Angiotensin II-induced hypertension was greater in ER $\alpha$ -/- and in AF2 $^{\Delta}$  mice than in WT and C451A-ER $\alpha$  mice. These data suggest that the protective effect of ER $\alpha$  against elevated blood pressure involved nuclear AF2-mediated transcriptional functions rather than MISS-mediated function. Changes in pressure were associated with proportional changes in endothelium-dependent dilation and wall thickness. Thanks to this work, we hope to better understand how ER $\alpha$  is able to mediate the protective effects of estrogen against hypertension.

## 0343

### Essential role of P2Y6 UDP receptor in Angiotensin-II dependent arterial hypertension

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Extracellular nucleotides are responsible for pleiotropic effects in the vasculature. Uracyl nucleotides are vasoactive and trophic agents and promote inflammation. The participation of specific P2 receptors in these effects remains undefined and their potential contribution in arterial hypertension is unknown. Objective: To evaluate the contribution of the UDP receptor P2Y6 in hypertension in mouse. Methods: Arterial contraction was evaluated using a wire myograph. Blood pressure was measured following nucleotides iv infusion and experimental hypertension was induced either by Angiotensin-II (Ang-II 1mg/kg/j) or DOCA-salt (1%) in uni-nephrectomized mice. Histological approaches, immunofluorescence and RTqPCR were used to evaluate the nature of vascular remodeling. Results: P2Y6 displayed the highest arterial expression level among other P2Y receptors. Contraction of conductance (thoracic aorta) and resistance (mesenteric) arteries was abrogated in P2ry6-/- mice in response to UDP and UTP while other vasoconstrictor induced normal responses. P2Y6 receptor triggered a moderated intracellular calcium increase while RhoA (calcium facilitating pathway) activation was abrogated in P2ry6-/- mice. Both genetic deletion and pharmacological blockade of P2Y6 receptor abolished Ang-II-induced blood pressure increase (40 mmHg in wild type mice). By contrast, hypertensive response in DOCA-salt was equivalent in both genotypes. Following Ang-II treatment, P2ry6-/- mice developed a reduced arterial hypertrophic remodeling and fibrosis but equivalent immune cell recruitment/infiltration compared to wild type. These changes were corroborated to reduced mRNA expressions of TGF $\beta$  and NADPH oxidase subunits. Conclusions: Vascular P2Y6 receptor contributes to exaggerated vascular tone, hypertrophy and fibrosis in the context of Ang-II-dependent hypertension. Its absence or pharmacological blockade limits vascular damages and prevents blood pressure increase associated to hypertension.

## 0356

### Disseminated arterial calcification and enhanced myogenic response are associated with Abcc6 deficiency in a mouse model of pseudoxanthoma elasticum

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Beside calcification, the impact of ABCC6 deficiency on the vasculature remains unclear. We investigated arterial structure and function in Abcc6-/-

mice, a model of the human Pseudoxanthoma Elasticum (PXE). Arterial calcium accumulation determined by atomic absorption spectrometry was 1.5- to 2-fold higher in Abcc6-/- than in wild-type mice. Calcium also accumulated locally leading to a specific punctuated pattern. Abcc6-/- mesenteric arteries mounted on a wire myograph displayed slight increase in arterial vasoconstrictor tone in response to phenylephrine and thromboxane A2. Interestingly, myogenic tone (Bayliss effect) determined using a pressure myograph was significantly elevated in Abcc6-/- compared to wild type arteries. Arterial blood pressure was not significantly modified in Abcc6-/- animals, despite higher variability. These changes were accompanied with deregulated gene expression (RTqPCR) in both liver and resistance arteries. Old Abcc6-/- mouse mesenteric arteries expressed markers of both osteogenic (Runx2, opn) and chondrogenic lineage (Sox9, col2a1). Surprisingly Enpp1 and Alpl genes encoding ectonucleotide pyrophosphatase/phosphodiesterase 1 and alkaline phosphatase were deregulated within Abcc6-/- liver and this was corroborated with reduced alkaline phosphatase circulating levels in PXE patients. As a conclusion, scattered calcium depositions result from osteochondrogenic trans-differentiation of vascular cells. The lower elasticity and increased myogenic tone evidenced in aged Abcc6-/- mice suggest a reduced control of local blood flow, which in turn may alter vascular homeostasis. Our findings argue in favor of a deregulated arterial function and may help to decipher consequences of ABCC6 deficiency since PXE is a significant risk factor for small vessel disease and particularly ischemic stroke.

## 0416

### Vascular smooth muscle cells are responsible for a prothrombotic phenotype of spontaneously hypertensive rat arteries

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**Objective:** The hypothesis that hypertension confers a hypercoagulable state arises from the complications associated with hypertension, stroke and myocardial infarction. Our objective was to determine whether spontaneous hypertension causes changes in the thrombin generating capacity of the vascular wall.

**Approach and Results:** We used spontaneously hypertensive rats (SHR) compared with Wistar rats. The addition of thoracic aorta rings of SHR to a Wistar or SHR plasma pool resulted in a greater increase in thrombin generation compared to the addition of equivalent rings from Wistar. Comparison of 5 week-old and 12 week-old rats indicate that established hypertension is required to induce increased thrombin generation within the vessel wall. Whereas no difference was observed for endothelial cells, thrombin formation was higher at the surface of cultured aortic smooth muscle cells (SMCs) from SHR than from Wistar. Exposure of negatively-charged phospholipids was higher on SHR than on Wistar aortic rings as well as on SMCs. Tissue factor activity was higher in SHR SMCs. Twelve week-old SHR exhibited accelerated FeCl $_3$ -induced thrombus formation in carotid arteries and the resulting occlusive thrombi are disaggregated by blockade of glycoprotein Ib $\alpha$ -von Willebrand factor interactions. SHR SMCs were more sensitive to thrombin-induced proliferation than Wistar SMCs. This cellular effect was totally abolished by a protease-activated receptor 1 inhibitor.

**Conclusions:** The prothrombotic phenotype of the SHR vessel wall was due to the ability of SMCs to support greater thrombin generation and resulted in accelerated occlusive thrombus formation after arterial injury, which is sensitive to glycoprotein Ib $\alpha$ -von Willebrand factor inhibitors.

## 0194

### Angiotensin II type 2 receptor reduces metabolic and vascular effects of type 1 diabetes in the mouse

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The renin-angiotensin system has a key role in cardiovascular homeostasis, mainly through activation of angiotensin II type 1 (AT1R) and type 2 (AT2R)