12. Pharmacogenomics, Pharmacogenetics and Clinical Pharmacology

12.001 Impact of Arginase 1 and Arginase 2 on erectile dysfunction risk and disability. Lacchini R[1], Blanco ALF[2], Muniz JJ[1], Nobre YTDA[3], Cologna AJ[3], Martins ACP[3], Tanus-Santos JE[2] [1]EERP-USP – Enfermagem Psiquiátrica e Ciências Humanas, [2]FMRP-USP – Farmacologia, [3]FMRP-USP – Cirurgia

Introduction: Arginase 1 and Arginase 2 are homologous enzymes that convert L-Arginine to Urea and L-ornithine and compete with nitric oxide synthases for L-arginine. Higher Arginase 1 and 2 activity seem to be responsible for a reduced nitric oxide production by endothelium in disease states, including erectile dysfunction. **Aim**: Here we aim to assess whether Arginase 1 and 2 plasma levels, plasma arginase activity, or genetic factors are associated with erectile dysfunction risk and disability. **Methods**: Blood samples were collected from healthy controls (n=106) and erectile dysfunction patients (n=110) after completion of the 5-IIEF questionnaire (international index of erectile dysfunction). All subjects given their informed consent and this work was approved by Institutional Review Board at the Faculty of Medicine of Ribeirao Preto (Process number 5469/2008). Arginase 1 and 2 plasma levels were assessed by ELISA, while plasma arginase activity was measured by Urea production by spectrophotometry. Genotypes of ARG1 (rs2781659, rs2781667, rs2246012 and rs17599586) and ARG2 (rs3742879 and rs10483801) were assessed by Taqman genotyping assays by real time polymerase chain reaction. **Results**: Arginase 2 is increased in Clinical ED and is associated with increased risk for ED. ARG1 rs2781659 AA and rs2781667 TT genotypes are associated with lower IIEF scores (higher disability) only in Clinical ED. Similarly, ARG1 GTCC haplotype associated with higher IIEF scores only in Clinical ED. **Conclusion**: This study provides evidences that Arginase 2 levels on plasma may serve as risk factor for erectile dysfunction. Besides, Arginase 1 genetic factors are associated with altered disability in erectile dysfunction patients. This study was supported by CAPES, CNPq and FAPESP

12.002 Protein kinase C genotypes and haplotype modify the antihypertensive responses to enalapril. Oliveira-Paula GH[1], Lacchini R[1], Fontana V[1], Silva PS[2], Biagi C[3], Tanus-Santos JE[1] [1]FMRP-USP – Farmacologia, [2]FCM-Unicamp – Farmacologia, [3]Santa Casa de Araçatuba

Introduction: Protein kinase C (PKC) up-regulation plays an important role in hypertension. Angiotensin converting enzyme inhibitors (ACEi) decrease PKC expression and activity and these effects are partially associated with the antihypertensive responses to ACEi. Therefore, it is possible that variations in the *PRKCA* gene may affect the antihypertensive responses to these drugs. **Aims**: To evaluate whether polymorphisms in *PRKCA* gene modify the responses to ACEi enalapril in hypertensive patients. **Methods**: Hypertensive patients (n=104) were prospectively treated only with enalapril 10 mg/day (n=48) or 20 mg/day (n=56) for 60 days. We compared the effect of *PRKCA* polymorphisms on changes in BP after enalapril treatment. Genotypes for rs887797, rs1010544 and rs16960228 *PRKCA* polymorphisms were determined using real-time polymerase chain reaction. The software PHASE 2.1 was used to estimate the haplotype frequencies. **Results**: Patients carrying the variant genotypes (TC+CC) for the rs1010544 polymorphism showed more intense decreases in mean BP in response to enalapril 20 mg/day compared with TT carriers (TT=-12.5±8.5 mmHg *vs.* TC+CC=-18.1±8.3 mmHg; $P<0.05$). In addition, the variant genotypes (GA+AA) for the rs16960228 polymorphism were associated with lower decreases in mean BP in response to enalapril 20 mg/day compared with GG genotype (GG=-16.2±9.2 mmHg *vs.* GA+AA=-9.6±4.4 mmHg; $P<0.05$). Interestingly, the CTA haplotype was associated with lower decreases in diastolic BP after treatment enalapril 20 mg/day compared with other haplotypes (CTA=-5.3±6.5 mmHg *vs.* CTG=-15.1±12.4 mmHg, CCG=-15.6±9.4 mmHg and TTG=-14.7±10.1 mmHg; $P<0.05$). No significant effects of genotypes and haplotypes of *PRKCA* gene were found on BP responses to enalapril 10 mg/day. **Conclusion**: These findings show evidence that *PRKCA* polymorphisms modify the antihypertensive responses to enalapril. While the variant genotypes for the rs1010544 polymorphism are associated with better responses to enalapril, the variant genotypes for the rs16960228 polymorphism and the CTA haplotype predispose to worse responses to this drug. **Financial support**: CNPq and FAPESP This study was approved by the Institutional Review Board at the Faculty of Medicine of Ribeirao Preto, University of Sao Paulo (Process 9237/2009).

12.003 Endothelin-1 production and expression of microrna s in preeclamptic patients responsive and nonresponsive to antihypertensive therapy in an *in vitro* model of preeclampsia. Dias MC, Sandrim VC, Bovolato ALC, Deffune E IBB-Unesp

Introduction: Preeclampsia is characterized by hypertension and proteinuria at ≥ 20 weeks of gestation and it is the worldwide leading cause of fetal-maternal morbidity and mortality [1,2]. Antihypertensive therapy is used to control the blood pressure in preeclampsia, but most of preeclamptic women do not respond to any antihypertensive drug, which increases the severity of the clinical outcome of these patients compared to those who are responsive[3]. Its pathophysiology involves an endothelial dysfunction manifested by unregulated production of vasoactive substances such as endothelin-1 (ET-1), a vasoconstrictor peptide, and the increase of its production modulate blood pressure[4]. MicroRNAs (miRNAs) are small (18–24 nt) endogenous noncoding single-stranded RNAs, which regulate post transcriptionally the expression of some genes, including the endothelin-1 gene (EDN-1)[5]. **Aims**: to compare the miRNAs expression and endothelin-1 production by human umbilical vein endothelial cells (HUVEC) incubated with plasma from preeclamptic patients responsive (PER) and nonresponsive (PENR) to antihypertensive therapy. We choose to investigate the miRNAs 125a, 125b, let-7a, let-7b and let-7c because they are expressed in HUVECs and they are predicted to regulate ET-1, based on bioinformatic analysis (www.microrna.org). **Methods**: HUVEC were incubated with 20% (v/v) plasma from PER (n=4) and PENR (n=4) for 24h. ET-1 production was measured in the cell supernatants by ELISA and the miRNAs expression in the cells was analyzed by qRT-PCR. **Results**: ET-1 concentration was not significantly different between the PER and PENR (2.11±0.48 pg/mL; 0.82±0.22 pg/mL, respectively). The miRNAs let-7a, let-7b and let-7c were significantly down-regulated in the nonresponsive group compared to the responsive (fold change: let-7a=0.4; let-7b=0.4; let-7c=0.4), but the expression of the mir-125a and mir-125b was not significantly different. **Conclusions**: The miRNAs let-7a, b and c were significantly less expressed in the nonresponsive group, showing a genetic difference in the post-transcriptional regulation between patients that respond and do not respond to antihypertensive therapy. **References:** [1] Report of the National High Blood Pressure Education Program Working Group. Am J Obstet Gynecol, v.183, p.1, 2000. [2] World Health Organization. Am J Obstet Gynecol, v.158, p.80, 1988. [3] Sandrim, V.C. Pharmacogenomics J, v.10, n.1, p.40, 2010. [4] LaMarca, B.D. Hypertension, v.51, p.982, 2008. [5] Bartel, D.P. Cell, v.136, p.215, 2009. **Financial Support**: Capes Research Ethical Committee (REC) approval: process number 035/2009 - Hospital Santa Casa de Belo Horizonte