pull down experiments and structural homology modeling, SN binding was mapped to the substrate binding site in the catalytic region of CaMKIIδ. SN attenuated isoproterenol (ISO)-induced autophosphorylation of Thr287-CaMKIIδ in Langendorff hearts, and inhibited CaMKIIδ-dependent ryanodine receptor 2 (Ser2814-RyR2) phosphorylation. SN was also observed to decrease RyR2 open probability in lipid bilayer experiments. In line with CaMKIIδ and RyR2 inhibition, SN treatment decreased Ca^{2+} spark frequency and dimensions in cardiomyocytes during ISO challenge. Ca^{2+} wave frequency was reduced, which corresponded with lower incidence of delayed after-depolarizations and fewer spontaneous action potentials. SN treatment also reduced the incidence of early after-depolarizations during ISO; an effect paralleled by reduced magnitude of L-type Ca^{2+} current. Based on these protective actions of SN, we investigated SN levels in patients with catecholaminergic polymorphic ventricular tachycardia (CPVT) who are prone to Ca^{2+}-dependent arrhythmia. Circulating SN levels were increased in CPVT patients, while levels of established biomarkers were unchanged. In conclusion, SN interacts with the substrate binding site of CaMKIIδ, thereby inhibiting its activity. A consequent reduction in RyR2 and L-type Ca^{2+} channel opening reduces incidence of early and late after-depolarizations. Production of SN may be an endogenous protective mechanism in patients with pathological cardiomyocyte Ca^{2+} handling, supporting its role as an emerging biomarker.

P.1.5-082
The mutational profiles of PIK3CA and TP53 genes and some peculiar ultrastructural aspects in breast cancer

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The *PIK3CA* gene is involved in the phosphatidylinositol 3-kinase/AKT cellular signaling pathway mediating proliferation and cell survival processes, thereby regulating tumor cell growth. The *TP53* is one of the most investigated prognostic or predictive markers in many human cancers. The purpose of the current study was to investigate the mutational status of the *TP53* and *PIK3CA* genes in breast cancer; the molecular results were completed by some ultrastructural details of the tumors – stroma interface during invasive growth of mammary carcinoma. In order to perform molecular analysis and transmission electron microscopic investigations, 22 samples of fresh breast tumor tissue, have been analyzed by Sanger method, respectively the routine TEM protocol. In *PIK3CA* gene, mutational frequency was 36.36% (8/22). Three mutations (37.5%) were identified in exon 9, helical domain and five mutations (62.5%) in exon 20, kinase domain. Regarding the frequency of somatic mutations in *TP53* gene, the mutation rate was 27.3% (6/22) with a predominance of deletion mutations (66.67%). Concerning the fine analysis of the tumor – stroma interface, we refer only to the ability of tumor cells themselves and telocytes (a recently described cell phenotype) present also inside of the peritumoral stroma to produce small lipoprotein sacks termed *extracellular vesicles*. Our results regarding the mutational status of *PIK3CA* and *TP53* genes are in line with those in international database. As regards the ultrastructural details, we have to consider the existence of extracellular vesicles containing an appreciable diversity of (macro)molecules (proteins, segments of genomic DNA, multiple forms of RNA, including miRNA, lipids, metabolites) that act as mediators involved in cell-cell and cell-extracellular matrix interactions, therefore having a high potential to be involved in mammary tumor progression and control.

P.1.5-083
Investigation of serum sclerostin levels in children and adolescents with type-1 diabetes mellitus

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Introduction: Interaction between diabetes mellitus (DM) and osteoporosis is a complex issue and needs careful evaluation. This interaction is especially important in bone metabolism of children with type-1 DM. Sclerostin is one of the factors modulating the Wnt/b-catenin pathway which is essential for normal osteogenesis. Sclerostin supresses mineralization of osteoblastic cells, inhibits osteoblast proliferation and promotes osteoblast apoptosis. The aim of the present study was to measure serum sclerostin levels in children and adolescents with type-1 DM and compare with age- and gender- matched control subjects.
Materials and Methods: The study included 40 children and adolescents with type-1 DM (19 males and 21 females) aged from 7 to 17 years and 40 healthy controls (18 males and 22 females) aged from 6 to 17 years. Serum sclerostin levels were measured by ELISA method using commercially available kit.
Results: Glucose and hemoglobin A1c (HbA1c) levels of children and adolescents with type-1 DM were significantly higher than that of the controls (P = 0.000). However, there was no significant difference between sclerostin levels of the groups.
Discussion and Conclusion: Our result showed that serum sclerotin levels were not changed in children and adolescents with type-1 DM.

P.1.5-084
Immune responses in patients with chronic renal failure before and after transplantation

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Chronic renal failure (CRF) is the end result of chronic renal diseases that characterized progressive loss in the number and function of nephrons. Worldwide, there are up to 400-500 million patients with chronic renal failure, and number of patients is increasing. Thus, the search markers at a very early stage of the kidney disease, as well as rejection, being a challenge for the medical community. The goal of this investigation was to analyze the serum level of 27 cytokines in Chronic kidney insufficiency (CKI) patients.

We examined serum from 240 patients and conditionally healthy people using cytokine protein array kit Bio-Plex Pro™ Human Cytokine 27-plex Assay. Patients were divided into next groups: 1) conditionally healthy donors, 2) patients at pre-dialysis stage, 3) patients received hemodialysis, 4) patients before transplantation, 5) patients after 1 year from transplantation, 6) patients more than 1 year after transplantation.

The cytokine levels of IL-1b and IL-2, and receptor inhibitor IL-1Ra were below level of detection. The level of IFN-g was not significantly different in all groups. The levels of the remaining

measured cytokines IL-4, IL-5, IL-6, IL7, IL-8, IL-9, IL-10, IL-12 b, IL-13, IL-15, IL-17, chemokines IP-10, RANTES, MIP1a, MIP1b, MCP1, eotaxin, growth factors VEGF, FGFb, PDGFbb, G-CSF and GM-CSF, as well as TNF-a were significantly higher (p value < 0.05) in the dialysis patient group and before treatment by compared with other groups. The data suggested that both cellular and humoral immunity in all patients with CRF was activated.

To identify early rejection markers among the cytokines, a data from patients after rejection (8 patients) and other patients after transplantation was separately analyzed in R environment (www.r-project.org). However, no significant differences in the level of cytokines from these groups of patients were observed.

P.1.5-085
The interaction of UDP-N-acetylglucosamine 2-epimerase/N-acetylmannosamine kinase (GNE) and alpha-actinin 2 is altered in GNE myopathy M743T mutant

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GNE Myopathy is a rare neuromuscular recessive disorder caused by missense mutations in GNE, the key enzyme of sialic acid biosynthesis. In an attempt to elucidate GNE functions that could account for the muscle pathophysiology of this disorder, the interaction of GNE with α-actinins has been investigated. Surface plasmon resonance and microscale thermophoresis analysis revealed, that *in vitro*, GNE interacts with α-actinin2, and that this interaction has a 10-fold higher affinity compared to the GNE-α-actinin1 interaction we previously showed. Further, GNE carrying the M743T mutation, the most frequent mutation in GNE myopathy, has a 10-fold lower binding affinity to α-actinin2 than intact GNE. This decrease could eventually affect the interaction, thus causing functional imbalance of this complex in skeletal muscle, that could contribute to the myopathy phenotype.

In vivo, using bi-molecular fluorescent complementation, we show the specific binding of the two proteins inside the intact cell, in a unique interaction pattern between the two partners. This interaction is disrupted in the absence of the C-terminal calmodulin-like domain of α-actinin2, which is altered in α-actinin1. Moreover, the binding of GNE to α-actinin2 prevents additional binding of α-actinin1 but not vice versa. These results suggest that the interaction between GNE and α-actinin1 and α-actinin2 occur at different sites in the α-actinin molecules and that for α-actinin2 the interaction site is located at the C-terminus of the protein.

P.1.5-086
Tau toxicity on plasma membrane Ca2+-ATPase (PMCA) is prevented and reversed by calmodulin

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The maintenance of intracellular free Ca^{2+} at the properly low level in eukaryotic cells involves a system of high-affinity Ca^{2+} membrane pumps. These transporters use the energy of ATP hydrolysis to pump cytosolic Ca^{2+} out of the cell (Plasma Membrane Ca^{2+}-ATPase, PMCA) or into internal stores (Sarco Endoplasmic Reticulum Ca^{2+}-ATPase, SERCA, and Secretory Pathway Ca^{2+}-ATPase, SPCA). Increasing evidence point out a link between Ca^{2+} homeostasis dysregulation, aging and aging-related diseases such as Alzheimer's disease (AD). AD is characterized by the accumulation of β-amyloid peptide in senile plaques and neurofibrillary tangles of tau protein. In this work, we show that calmodulin, the major endogenous activator of PMCA prevents the inhibition of the Ca^{2+}-ATPase activity of PMCA by tau and also reverses the inhibition of PMCA by tau. Furthermore, functional studies with native and truncated variants of human PMCA4b pointed out that tau binds to the C-terminal tail of PMCA, in a site located between the transmembrane domain 10 and the calmodulin binding domain. These results point toward a relationship between PMCA and neurodegeneration by tau protein, and the role of calmodulin as a neuroprotective agent against one of the major molecular hallmarks of Alzheimer's disease.

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P.1.5-087
Activity of the reproductive axis in the adult female is modified by pre-pubertal stress

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Environmental conditions in early life can have a substantial influence on female reproductive function. Earlier studies suggested that the challenging environment encountered by young pre-pubertal women may affect their subsequent fertility: menarche was delayed, they had lower salivary progesterone levels and early menopause. Variation in reproductive function might be determined by epigenetic regulation and, we hypothesized, this might be due to changes in expression of key genes in the reproductive axis. We established a mouse model for early life immunological stress in order to investigate its effects on reproductive function and elucidate the mechanisms involved. After weaning, female pups were divided into two groups: one treated for 7 d with 1.5–2.5% DSS in the drinking water to induce mild colitis, while the other served as untreated controls. Vaginal opening was assessed as a measure of puberty, and was significantly delayed by 3.4 days compared to untreated littermates. Furthermore, ovarian *Pgr* mRNA levels were significantly lower in DSS-treated mice compared to controls, at 2–3 and 8–12 month. Similarly, ovarian *Lhr* mRNA levels were lower in the treated mice at both time points, while mRNA levels of *Amh*, which is produced in pre-antral follicles, appeared higher in ovaries of the treated mice. These results suggest that ovarian gene expression is altered long-term by the pre-pubertal treatment, with possible implications for reproductive function and fertility. Our preliminary breeding experiments revealed that the DSS-treated mice are fertile, and the litter sizes significantly larger than those of untreated controls. Taken together, our results suggest that reproductive strategy in adult life is altered by pre-pubertal treatment, and indicate that this might involve an increase in numbers of follicles that start to develop in each cycle, perhaps to increase the likelihood of reproductive success which, in women, would also lead to an earlier menopause.