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# Recent doctoral theses (biochemistry, biology and biophysics) in Lithuania

Prepared by Indrė LIPATOVA

## STEM CELL CHARACTERIZATION AND APPLICATION FOR RABBIT'S CARDIAC MUSCLE REGENERATION

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**The dissertation defended:**
23 March 2012

The diverse investigations of stem cells derived from rabbit's skeletal muscle were performed to design stem cell-based technology for improvement of the heart muscle functions after MI. We developed a new method for the rapid and direct estimation of metabolic and viability status of cells based on the fluorescence measurement of natural fluorophore NAD(P)H. We propose that the increase of NAD(P)H fluorescence intensity could be the indicator of myoblast differentiation. NAD(P)H fluorescence measurements also can be used for continuous noninvasive monitoring of myoblast viability. The impact of inflammation caused by transplantation procedures and MI itself on Cx43 expression and permeability properties were investigated by immunoblotting and new fluorescence method in myoblasts and HeLa cells. Stress-activated c-Jun N-terminal kinase signaling pathway has been shown to be responsible for changes in Cx43 expression. The effect of stem cell therapy was determined by echocardiography, optical mapping techniques and by histological examination. Transplantation of SkMs after MI did not improve left ventricle ejection fraction but significantly slowed down the impairment of mechanical function of the heart comparing to control MI. Moreover, we observed the substantial recovery of the electrical activity in MI zone after transplantation of Cx43-EGFP expressing SkMs. Different stem cell types used for tissue engineering were found to be compatible with collagen scaffolds which degraded in 2 months after implantation onto the pericardium. Our results could be valuable for the improvement of stem cell therapy-based treatment of MI.

## DNA CYTOSINE METHYLTRANSFERASE-DIRECTED REACTIONS INVOLVING NON-COFACTOR-LIKE COMPOUNDS

**Zita Liutkevičiūtė**

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**The dissertation defended:**
29 March 2012

For the first time we have shown that DNA cytosine-5 methyltransferases can perform two atypical reactions in the absence of cofactor in vitro: i) reversible covalent addition of short aliphatic aldehydes to the target cytosine producing 5-(1-hydroxyalkyl) cytosine, and ii) coupling thiols and selenols to 5-hydroxymethylated cytosine residues producing corresponding 5-chalcogenomethylcytosines. These are the first demonstrations of reactions involving non-cofactor-like substrates catalyzed by S-adenosyl-L-methionine dependent enzymes, which reveal atypical versatility of DNA cytosine-5 methyltransferases. Mechanistic insights into these reactions indicate that the key step in both cases is the covalent activation of the flipped out target residue by the enzyme. Here it is demonstrated that the methyltransferase-directed condensation of thiols with 5-hydroxymethylcytosine in DNA can be exploited for covalent labelling of the 5-hydroxymethylcytosine residues permitting subsequent enrichment of corresponding fragments from genomic pools. 5-hydroxymethylcytosine is a recently discovered cytosine modification in mammals whose biological function and distribution have not yet been established.

## INVESTIGATION OF *ARTHROBACTER* SP. PLASMIDS

**Rūta Stanislauskienė**

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**The dissertation defended:**
22 June 2012

During this work large molecular weight plasmids from *Arthrobacter* sp. 68b, *A. rhombi* PRH1 and VP3 bacteria were investigated as well as small plasmids from both *A. rhombi* strains. It was determined that genes encoding degradation proteins of phthalic acid, 2 methylpyridine and pyridine are located on plasmid p2MP (113 kb) in *Arthrobacter* sp. 68b. The degradation of phthalate, pyridine and its derivative is an inducible process. It was proved that cells pre-grown with phthalic acid are able to utilise quinolinic acid. Monooxygenase, that cleaves pyridine ring between C2, C3 atoms, is induced by pyridine and 2 methylpyridine. The degradation pathway of the mentioned compounds was proposed. Succinate semialdehyde and succinic acid are formed during utilisation. Genes, encoding 2-hydroxypyridine degradation proteins, are located on large molecular weight plasmid. The phenotype of large *A. rhombi* VP3 plasmid was not determined. Small plasmids from both *A. rhombi* strains were sequenced, open reading frames were determined and identified. Using the minimal replicon of small *A. rhombi* PRH1 plasmid, hybrid vectors pRMU824, pRMU824-Km and pRMU824Tc were constructed for functional gene screening in *Arthrobacter* sp. and *Rhodococcus* spp. bacteria.

# RESTRICTION ENDONUCLEASE-TRIPLEX FORMING OLIGONUCLEOTIDE CONJUGATES WITH CONTROLLABLE CATALYTIC ACTIVITY

**Arūnas Šilanskas**

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**The dissertation defended:**
26 June 2012

Simple mutations within the coding region of critical human genes can lead to the formation of abnormal proteins, resulting in various diseases (e. g. cancer), in failure of an embryo to develop, or premature death. Genetic diseases can only be truly cured via restoration of defective gene function and one of the most promising strategies is based on homologous recombination. Naturally, a homologous recombination occurs with a low frequency (1 in 106 transfected cells), however, it is known that DNA double-strand breaks enhance the efficiency of homologous recombination by several orders of magnitude (up to 10,000-fold). Therefore, gene therapy via homologous recombination requires new molecular tools that should be highly specific and rigorously controllable. In this work we have focused on the development of restriction enzyme-triple helix forming oligonucleotide (TFO) conjugates, where TFO provides specificity for the extended recognition site through the triple helix formation and addresses restriction enzyme to a particular target site where it introduces a double stranded break. We provide proof-of-concept demonstrations of two alternative strategies to control the DNA cleavage activity of restriction endonuclease-TFO conjugates that allows to adopt them in in vivo experiments. To this end, we used restriction endonucleases MunI and Bse634I, which were before structurally and biochemically characterized in our laboratory. We successfully combined the restriction endonuclease photocaging and TFO-coupling to generate a photoswitchable MunI-TFO conjugate and provide the first demonstration that DNA cleavage activity of the caged MunI-TFO conjugate can be spatially and temporally regulated. We also generated Bse634I-TFO conjugate and demonstrated that two monomers assemble into the active dimer on the DNA providing a possible alternative for the catalytic module of the zinc finger nuclease. Moreover, in contrast to the FokI non-specific catalytic domain in zinc finger nucleases, Bse634I retains specificity for the cognate site and therefore is less prone to the off-site cleavage.

## FUNCTION OF MULTICOPPER OXIDASES ADSORBED ON GOLD NANOPARTICLES

**Marius Dagys**

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**The dissertation defended:**
27 September 2012

In this work bioelectrocatalytic functions of multicopper oxidases adsorbed on gold nanoparticles are studied. Laccases and human ceruloplasmin are used as multicopper oxidases. The enzymes have been employed in electrochemical systems consisting of gold electrodes covered by gold nanoparticles and enzymes. For the first time direct electron transfer between gold nanoparticle and active centre of laccase has been reported. Laccases from Trametes hirsuta and Trichaptum abietinum, also human ceruloplasmin, exhibited a direct electron transfer between active centres of the enzyme and gold nanoparticles, meanwhile the laccases exhibited an efficient direct electron transfer based oxygen bioreduction on electrode surface. Heterogeneous electron transfer speed in gold nanoparticle – laccase bioelectrocatalytic systems depended on nanoparticle size. In general, larger diameter (60–90 nm) gold nanoparticle systems exhibited slower oxygen bioreduction than medium sized (40–50 nm) gold nanoparticle systems. Heterogeneous electron transfer constants reached as high as 45 s-1 and 11 s-1 in Trichaptum abietinum and Trametes hirsuta laccase systems, respectively. Direct electron transfer between laccase and gold nanoparticles mechanism has also been studied. It was discussed that T1 site is not detectable at gold nanoparticle surface. Several changes of Trichaptum abietinum laccase catalytic properties upon adsorption on gold nanoparticles have been revealed. It was discussed that probably the optimum curvature of gold nanoparticles is responsible for formation of most efficient direct electron transfer based bioelectrocatalytic systems.

# INVESTIGATION OF THE DEGRADATION OF CARBOXYPYRIDINES IN BACTERIA

**Laimonas Karvelis**

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**The dissertation defended:**
28 September 2012

The main aim of this work was the study of bacteria capable to degrade the pyridine monocarboxylic acids. *Achromobacter* sp. strain JS18 capable to utilize 3-hydroxypyridine-2-carboxylic acid was selected by screening of microorganisms hydroxylating the pyridine ring at unusual positions or transforming pyridine derivatives. The strain 5HP consuming 5-hydroxypyridine-2-carboxylic acid as a sole carbon and energy source was isolated from soil. The 16S rRNA-based phylogenetic analysis showed that the isolate belongs to Pusillimonas genus. It was found that picolinic, nicotinic and dipicolinic acids were metabolized via three distinct inducible pathways in *Achromobacer* sp. JS18. The appropriate biodegradation routes of these acids as well as 3-hydroxypyridine-2-carboxylic acid were proposed. Nicotinic acid, 5-hydroxypicolinic acid and 3-hydroxypyridine induced three distinct metabolic pathways in *Pusillimonas* sp. 5HP cells. All pathways had the same intermediate – 2,5-dihydroxypyridine. For the first time 5-hydroxypicolinate 2-monooxygenase, which catalyzed oxidative decarboxylation of 5-hydroxypicolinic acid, was discovered, partially purified and characterized. The analysis of *Sinorhizobium* sp. L1 cells showed that 3-hydroxypyridine and nicotinic acid were degraded via different metabolic pathways. The *Sinorhizobium* sp. L1 cells converted 3-hydroxymethylpyridine to nicotinic acid. 3-hydroxypyridine and nicotinic acid induced biosynthesis of distinct isoforms of 2,5-dihydroxypyridine dioxygenase in *Sinorhizobium* sp. L1. The gene cluster encoding a nicotinic acid degradation pathway in *Sinorhizobium* sp. L1 was cloned and characterized.

# ROLE OF PERICYTES AND AUTOTAXIN / LYSOPHOSPHATIDIC ACID SIGNALING IN VASCULAR REGRESSION

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**The dissertation defended:**
28 September 2012

Angiogenesis and vascular regression are important for the pathogenesis of a number of conditions such as tumors, ischemic diseases and certain ocular diseases. While molecular mechanisms of blood vessel growth have been studied extensively, less is known about blood vessel stability and regression. New blood vessels are stabilized by pericytes – cells of mesenchymal origin that are found in the vessel wall. In the first part of this study we investigated the molecular mechanism of vessel stabilization by pericytes. We developed a simple in vitro model of pericyte mediated vessel stabilization. In this model, pericytes inhibited regression of vessel-like tubes organized out of endothelial cells. We found that tube association with pericytes in vitro accelerated the metabolism of lysophosphatidic acid (LPA), a vascular regression factor. A drop in the concentration of LPA caused tube stability. Lipid phosphate phosphatase (LPP)-like enzymes were at least in part responsible for the metabolism of LPA. In the second part of this study we investigated whether autotaxin (ATX) – an enzyme that catalyzes LPA production – is important for vascular regression *in vivo*. We generated mice with an endothelial cell specific inactivation of Enpp2, the gene that codes for ATX. We determined that ATX produced by endothelial cells was not required for regression of hyaloid vessels or formation of mature retinal vasculature.

# ARTIFICIAL PHOSPHOLIPIDS SYSTEMS FOR INVESTIGATIONS OF PROTEIN AND PEPTIDE INTERACTIONS WITH BIOLOGICAL MEMBRANES

**Rima Budvytytė**

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**The dissertation defended:**
19 October 2012

The main object of this thesis is tethered bilayer membranes (tBLM) on gold substrates, which are increasingly being used to mimic biomembranes in studies of protein reconstitution and function. We have developed a tBLM as a stable model in which thiolated anchor lipids span a hydrated layer that separates the membrane from its solid support. The dynamic properties of phospholipids in tBLM were studied by fluorescence correlation spectroscopy comparing different amounts of anchor molecules. Electrochemical parameters of these tBLM were studied with electrochemical impedance spectroscopy (EIS). To change the membrane composition, we suggest applying a method of direct lipid exchange between the vesicles and membranes. In this work, EIS was used to determine electrical characteristics of tBLM and also the interaction of tBLM between pores forming toxins: α-hemolysin, antrax toxin and vaginolysin had been studied. We have demonstrated that the pores formed by the toxins significantly alter the membrane conductance. The activation energy of ion transport through the α-HL and PA63 pore barrier was determined. These results show that both toxins form functionl water-filled pores, in which the ions are travelling in an environment similar to the bulk of the solution. A key event in pathogenesis of Alzheimer's disease is thought to be intracellular and extracellular accumulation of low molecular mass peptides – β-amyloids (Aβ1-42). In this work different sizes of soluble synthetic Aβ1-42 oligomers were used to determine their affinity to artificial phospholipid membranes. Membrane composition was found to be one of the important factors affecting the binding of the Aβ oligomers to phospholipid vesicles. The link between the size of soluble Aβ1-42 oligomers and their neurotoxicity was observed.

# STRUCTURAL AND FUNCTIONAL STUDIES OF RESTRICTION ENDONUCLEASES ECORII, BFII AND BSE634I

**Dmitrij Golovenko**

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**The dissertation defended:**
6 November 2012

Due to their unique specificity, restriction endonucleases (REases) have gained widespread application as indispensable tools for *in vitro* manipulation and cloning of DNA. Delineation of the repertoire of the protein folds, providing three-dimensional portraits of the REases by crystallographic methods, should reveal how different folds are tailored to function as restriction enzymes. The major goal of this work was to explore the specificity-structure relationships within PD-(D/E)XK and phospholipase D superfamily enzymes using a combination of crystallographic and biochemical methods. More specifically, we have focused on the Bse634I and EcoRII restriction enzymes belonging to the Cfr10I/NgoMIV/Bse634I branch of PD-(D/E)XK superfamily, and BfiI REase of PLD superfamily. The dissertation presents the crystal structure of the Bse634I REase mutant (R226A) complexed with two alternative target sites 5'-ACCGGT and 5'-GCCGGC, respectively. The analysis of the crystal structures for the first time revealed that the degenerate base pairs recognition by Bse634I is achieved through the combination of direct and indirect readout mechanisms. The crystal structures of the N- and C-terminal domains of EcoRII solved in the DNA bound form revealed different structural mechanisms used for the recognition of the same target sequence 5'-CCWGG. The first structural evidence has been provided showing that the C-terminal domain of EcoRII (EcoRII-C) flips out the central nucleotides A/T while interacting with its target site, enabling the EcoRII-C to use symmetric conserved structural elements for the recognition of the CCGG core. Furthermore, the crystal structure of the EcoRII N-terminal domain (EcoRII-N) provided the first glimpse into the B3 family domain in the DNA-bound form. Finally, the crystal structure of the C-terminal DNA binding domain of BfiI (BfiI-C) bound to the target site 5'-ACTGGG enabled the first structural comparison of two B3-family domains (EcoRII-N and BfiI-C) in the DNA-bound form.

## MECHANISM OF DNA INTERFERENCE BY TYPE II CRISPR / CAS SYSTEMS

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**The dissertation defended:**
7 December 2012

Recently, an adaptive prokaryotic immune system, CRISPR / Cas, was identified that provides acquired immunity against viruses and plasmids. Despite of active research in this field, the molecular mechanism of CRISPR / Cas systems remains unclear. The aims of this study were to perform genetic and biochemical analyses of the *S. thermophilus* Type II CRISPR3 / Cas system cloned and expressed in the heterologous *E. coli* host and to determine the mechanism of DNA interference in Type II CRISPR / Cas systems. In this work it was demonstrated that CRISPR / Cas system can be transferred across distant genera and provides heterologous interference against invasive nucleic acids, hence in this way bacteria might be "vaccinated" against viruses and plasmids. It was established that Cas9 is the sole cas gene necessary for CRISPR-encoded interference. Furthermore, in this work the Cas9–crRNA complex of the *S. thermophilus* CRISPR3 / Cas system has been isolated and it was demonstrated that it cleaves DNA bearing a nucleotide sequence complementary to the crRNA in a proto-spacer adjacent motif dependent manner. Sequence specificity of the Cas9–crRNA complex is dictated by the 42-nt crRNA. We showed that DNA cleavage is executed by two distinct active sites (RuvC and HNH) within Cas9 to generate site-specific nicks on opposite DNA strands. By altering the RNA sequence within the Cas-crRNA complex, programmable endonucleases can be designed both for in vitro and *in vivo* applications, and this thesis provides proof of concept for this novel and promising application.

# CYTOTOXICITY OF ELECTRIC PULSE, BLEOMYCIN, ASCORBATE, AND MENADIONE AND SYNERGISM OF THEIR COMBINATIONS *IN VITRO*

**Rita Saulė**

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**The dissertation defended:**
21 December 2012

Cytotoxicities of an electric field pulse, bleomycin, ascorbate, and menadione *in vitro* and possibilities to increase anticancer efficiency by using their combinations have been studied. The method of the determination of the fraction of electroporated cells based on measuring the release of intracellular potassium ions was successfully adapted for various types of cells in small volume samples (30–50 μl) *in vitro*. The relationships between the parameters of the electric field pulse, which is necessary to electroporate the cells, as well as the dependences of the pore sizes on the pulse duration are almost the same for various cancerous and noncancerous cells, which indicates that the mechanism of electroporation is the same for all these cells. Rat glioma C6 cells were the most sensitive to the action of bleomycin and ascorbate *in vitro*, while Chinese hamster ovary (CHO) cells were most resistant to these cytotoxic agents. Cytotoxicities of menadione and electric field pulse were almost the same for cancerous MH-22A and C6 cells and noncancerous CHO cells. Cytotoxic action of some of the combinations of the cytotoxic agents studied, such as electric pulse and bleomycin, as well as the mixture of vitamins C and $K_3$ at the ratio of 100:1 is synergistic towards cancerous MH-22A and C6 cells and noncancerous CHO cells *in vitro*. Electric pulse decreased cytotoxic dose $LD_{50}$ for bleomycin from 450 to $5 \cdot 10^4$ times, while $LD_{50}$ for menadione was decreased 2.5–5-fold by ascorbate.

## PECULIARITIES OF CHANGES IN CENTRAL AND PERIPHERAL FUNCTIONAL INDICES OF THE CARDIOVASCULAR SYSTEM PERFORMING GLOBAL, REGIONAL AND LOCAL PHYSICAL LOADS

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**The dissertation defended:**
13 April 2012

The aim of the study was to determine the peculiarities of central and peripheral indices of the cardiovascular system while performing global, regional or local type of exercising. In the evaluation of the central and peripheral peculiarities of the cardiovascular system while performing global, regional or local type of exercises in a repetitive manner, this work has revealed that cardiovascular mobilization features depend not only on the active muscle mass: during regional or local type of multiple loads the properties of cardiovascular mobilization depend more on the functional specificity of the muscle groups. It is commonly assumed that muscle blood flow is activated during every exercise. This study shows that when health strengthening exercises are performed in a repetitive manner (local or regional type) on the trainer, the intensity of muscular arterial blood flow decreases and immediately after the exercises, after work hyperaemia begins. Therefore, the selection of optimal rest intervals between exercise repetitions is an important factor for fast adaptation.

## DEPENDENCE OF MOTOR SYSTEM FATIGUE ON FEMALE MENSTRUAL CYCLE PHASE

**Laura Daniusevičiūtė**

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**The dissertation defended:**
21 June 2012

The main aim of the study was to estimate the effect of increased female sex hormone concentration in blood on motor system fatigue, recovery after physical exercise, effectiveness of motion control, cognitive functions and dependence on sex. The study showed that the indices of muscular damage (creatine kinase, low-frequency fatigue) after physical exercise were reduced by the change in female sex hormone concentration, whereas regeneration processes (indices of quadriceps femoris muscle torque, low-frequency fatigue and rectal temperature) as well as memory and attention processes were activated by female sex hormones after physical exercise. After eccentric and concentric loading of the same intensity and duration had been applied, female squat angle was smaller than that of males due to anatomic differences in female and male bone structure.

## DNA METHYLATION MARKERS OF LUNG AND HEAD–NECK TUMOURS

**Asta Ščėsnaitė-Jerdiakova**

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**The dissertation defended:**
22 June 2012

Lung and head-neck tumours are malignancies with high rates of incidences and deaths in Lithuania and world-wide. The most important risk factor for these malignancies is tobacco smoke. Tobacco smoke contains a mixture of well known carcinogens and procarcinogens, which are shown to induce genetic and epigenetic alterations. In this study we aimed to determine the frequency and profile of epigenetic (DNA methylation) changes in pathogenesis of tobacco smoke-related lung and head-neck tumours. Tumour supressor genes (TSGs) p16, p14, RARB, RASSF1, MGMT and DAPK were studied by methylation specific PCR and pyrosequencing in lung, head-neck and salivary gland carcinomas (n = 212, n = 31 and n = 36, respectively). Also, scrapes of oral mucosa from subjects with no cancer indication (n = 11) and histologically normal salivary gland tissues (n = 19) were analysed. MGMT protein expression was analysed by immunohistochemistry in 287 of salivary gland specimens. In lung tumours, promoter hypermethylation was detected in 20.8% of p16, 31.1% of RARB, 28.9% of RASSF1, 15.1% of MGMT and 19.8% of DAPK1 gene. The prevalence of p16 hypermethylation was significantly higher in males than females ($p = 0.018$), squamous cell carcinomas than adenocarcinomas ($p = 0.025$). Hypermethylation of ≥1 gene promoter is associated with TP53 mutations ($p = 0.027$). Methylation index and the frequency of p16 promoter methylation in neversmokers exposed to second-hand tobacco smoke is similar to indices observed in tumours from smokers. In head-neck tumour scrapings, promoter hypermethylation was detected in 23% of p16, 20% of p14, 19% of RASSF1, 20% of RARB, and 50% of MGMT gene. Hypermethylation in ≥1 gene promoter is higher in specimens obtained from smokers than neversmokers ($p = 0.047$). In salivary gland carcinomas and histologically normal tissues promoter hypermethylation was detected in 27.8% and 15.8%, respectively. Epigenetic changes in gene MGMT promoter are associated with loss of MGMT protein expression ($p = 0.021$). The loss of MGMT protein expression in salivary gland tumours is characteristic for patients with poor prognosis (high grade, certain histologic types, lymph node involved tumours). Our study contributes to understanding of tobacco smoke induced molecular alterations in lung and head-neck tumours. Hypermethylation of TSG promoters reflect the harmful effect of tobacco smoke or second-hand tobacco smoke exposure. Analysis of promoter hypermethylation of TSGs in oral scrapings from high risk subjects (smokers) may be a valuable tool for an early prediction of head-neck cancer risk. In salivary gland carcinomas, hypermethylation of gene MGMT promoter causes a loss of MGMT protein expression. This alteration is significant in pathogenesis of salivary gland tumours.

## INVESTIGATION OF MOLECULAR MARKERS OF HUMAN PROSTATE CANCER

**Rasa Sabaliauskaitė**

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**The dissertation defended:**
27 June 2012

In this study, TMPRSS2:ERG fusion transcripts were detected in 58.86% of prostate cancer (PCa) tumour tissues, T1/E4 (46.62%) was the most predominant fusion type. Occurrence of the TMPRSS2:ERG rearrangement was accompanied by an increased production of ERG, revealed increased production of the SPINK1 transcript in the subgroup of TMPRSS2:ERG fusion-negative cases. Our results demonstrated that the expression of TMPRSS2:ERG or TERT (catalytic subunit of telomerase) as a single biomarker was not highly informative for the prediction of biochemical recurrence in PCa. However, the combination of both biomarkers showed significant association with the outcome of the disease. We successfully identified the TMPRSS2:ERG and TERT transcripts in catheterized urine of PCa patients. The detection rate of TMPRSS2:ERG reached 16.39% and was slightly lower than that found by other investigators. In this study we analyzed multifocal PCa samples. We identified different gene expression and TMPRSS2:ERG fusion transcripts isoforms were in multifocal PCa samples. This study identified reduced expression of tumour suppressor genes (GSTP1, RARB, RASSF1 and ZAC) in PCa non-cancerous prostate tissues and TMPRSS2:ERG fusion-positive cases, but we did not get any significant association. Based on microarray data we selected five miRNAs (hsa-miR-33b, hsa-miR-370, hsa-miR-149, hsa-miR-886-3p and hsa-miR-206) which were down-regulated in TMPRSS2:ERG fusion-positive samples. QPCR data from PCa samples and cell lines transfection data show that hsa-miR-149 expression depends on the TMPRSS2:ERG transcript. Our results of XMRV virus analysis are well in line with other XMRV RT-PCR or nested PCR based studies. XMRV virus arose in laboratory and is not related to prostate carcinogenesis.

# GENETIC FACTORS IN PERSONALISED TREATMENT WITH ANTICOAGULANTS AND ANTIPLATELETS: PHARMACOGENETICS OF WARFARIN AND CLOPIDOGREL

**Vacis Tatarūnas**

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**The dissertation defended:**
3 July 2012

Clinical and genetic factors which determine therapeutic activity of warfarin after heart valve surgery during initiation and long-time treatment, as well as factors determining therapeutic activity of clopidogrel in patients treated due to acute coronary syndromes are analyzed in the dissertation. An algorithm for personalised treatment with warfarin has been designed. We have demonstrated that impact of gene polymorphisms on warfarin dosage differs during initiation and long-time treatment. CYP4F2 (G1347A) has the greatest impact on the warfarin dosage during treatment initiation after heart valve surgery, while VKORC1 (G3730A) has the greatest impact on the warfarin dosage during long-time treatment. We have also found that such drugs as ambroxol as well as β2 adrenomimetics reduced the dosage of warfarin. The frequency of clinically important CYP2C9*1,*2,*3 and VKORC1 (G-1639A) genotypes was set in a random sample of Lithuanian population. We also have demonstrated that CYP2C19*1/*1 genotype carriers, clopidogrel users, had a significantly lower platelet aggregation induced by adenosine diphosphate in comparison to CYP2C19 *1/*2 carriers. Totally, 23.2% of study subjects from a random sample of Lithuanian population had *1/*2 genotype leading to a lower antiplatelet activity of clopidogrel, 0.4% of study subjects from a random sample had *2/*2 genotype which causes resistance to clopidogrel.

# INTERACTION OF BETA AMYLOID WITH RAT NEURONAL AND MICROGLIAL CELLS: EXPERIMENTAL INVESTIGATIONS *IN VITRO*

**Paulius Čižas**

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**The dissertation defended:**
28 August 2012

The aim of this study is to investigate the effects of Aβ1-40 and Aβ1-42 peptide aggregates at various degrees of oligomerization on cultivated neurons of brain cells and their toxic mechanisms. 1) To investigate the effect of various Aβ1–40 and Aβ1-42 aggregates on the viability of neuronal-glial cell cultures. 2) To determine the relationship between the toxicity of Aβ1-42 oligomers and their size. 3) To analyze the effect of Aβ1-42 oligomers on membrane potential of neuronal and glial cells. To assess the effect of extracellular Ca2+ concentration, NMDA receptors and activity of endocytosis on neuronal death caused by Aβ1-42 oligomers. 4) To determine the effect of Aβ1-42 oligomers (4–6 nm in size) on neurons in CGC cultures with added macrophages J774 cells. 5) To determine the effect of Aβ1-42 oligomers on respiration of isolated brain mitochondria. Scientific novelty: This study determined the relationship between the size of amyloid and its toxicity to neurons. For the first time it has been shown that small Aβ1-42 oligomers of the size 1–3 nm were toxic to neurons in cell cultures at the concentrations which occur in brain under pathological conditions. However, they did not affect the viability of other cells (microglia and astrocytes) present in the cell cultures. For the first time it has been demonstated that small and large Aβ1-42 oligo-mers can cause neuronal death by different mechanisms: small oligomers by affecting neurons directly, and large oligomers – through glial cells. Our research revealed a phenomenon, which had not been described before, namely, small Aβ1-42 oligomers causing not only neuronal death but also a decline in neuronal cell density. According to other authors (Neniskyte et al., 2011), such a decline might be related to the loss of Aβ affected but still viable neurons due to phagocytosis. It was demonstrated that small Aβ1-42 oligomers provoke changes in the membrane of microglial cells as well as in neuronal membrane. There are NMDA receptors involved in the changes of microglial cell membrane potential. It has been determined that reduced extracellular calcium concentration, cell endocytosis inhibition, which reduces amyloid beta access to the interior of cells, as well as estradiol, which possibly prevents the formation of non-specific mitochondrial pairs in mitochondria, protect neurons from death at selected concentrations of small Aβ1-42 oligomers.

## ASSOCIATION BETWEEN CEREBROVASCULAR AUTOREGULATION AT REST AND DURING EXERCISE

**Aurija Kalasauskienė**

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**The dissertation defended:**
20 September 2012

Aim of the study: to assess the associations between CA at rest and during the exercise. Objectives of the study: to evaluate cerebrovascular autoregulation response at rest in amateur and elite power athletes; to evaluate cerebrovascular autoregulation response to constant physical exercise in amateur and elite power athletes; to assess the differences of cerebrovascular autoregulation response during static and dynamic resistance exercises in amateur and elite power athletes; to assess cerebrovascular autoregulation response to different exercise load in elite power athletes. Our study has shown that cerebral autoregulation responses display high stability – do not change at rest. Autoregulation responses are influenced by the type of physical exercise, however, autoregulation response prominence is independent of the load of physical exercise. Cerebral autoregulation responses display a stronger influence by static than dynamic exercise. In the presence of long-term adaptation to physical exercises, the properties of cerebral blood flow autoregulation response during different physical exercises are dependent on the specificity of training loads. Measurements performed for evaluation of the quasistantic effectiveness of CA (dynamic response time and response velocity) are important not only from physiological point of view but might also be applicable in sport medicine.

## YEAST IN ATOPIC DERMATITIS ETIOLOGY

**Auksė Zinkevičienė**

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Isolation and identification of all yeast species found on skin affected by atopic dermatitis, evaluation of their impact on the synthesis of IgE antibodies, and assessment of the possible cross-reactivity between different yeast species were performed. It was shown that in 36.9% of the cases of atopic dermatitis the affected skin was colonized with yeast belonging to three genera: Candida, Malassezia and Rhodotorula. Systematic and phylogenetic analysis of sequences from atypical Malassezia restricta strain M8 indicated that this isolate could be a member of a new yeast species. Three atypical Malassezia isolates M47, M54 and M235 were identified as non-lipid-dependent variants of Malassezia furfur. It was shown that in atopic dermatitis cutaneous colonization with yeast is two-fold higher in adults than in children. The sera of atopic dermatitis patients have specific IgE antibodies to cross-reactive intracellular yeast antigens. Candida pelliculosa and house dust mites Dermatophagoides pteronyssinus and Dermatophagoides farinae might share some allergenic epitopes. The results of this study suggest that attention should be given to a cutaneous colonization by saprophytic yeast since the immune response to the allergens could further exacerbate allergic inflammation due to cross-reactive epitopes.

# EPIDEMIOLOGY, DIAGNOSTICS AND IMMUNOPROPHYLAXIS OF RABIES IN WILD AND DOMESTIC ANIMALS IN LITHUANIA

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Rabies is one of the oldest and most dangerous human and animal diseases. This is a disease that is transmitted directly from animal to animal and from an animal to a human being. People usually contract this disease from stray domestic animals, therefore, a realistic threat is posed to everyone to become infected with the rabies virus (RV). The only and the most effective way of protecting oneself from rabies after being bitten by an infected or unknown animal is immunoprophylaxis. Between 2003 and 2011, the epidemiological situation of rabies in Lithuania was assessed. The methods used to diagnose rabies are noted for specificity and sensitivity. It has been proved that applying simultaneously direct fluorescent antibody test (FAT) and rabies tissue culture infections test (RTCIT) methods it was possible to ensure a fast and effective determination of RV in the samples of wild and domestic animals under investigation thereby ensuring confirmation of the diagnosis of being infected with RV. During the wild fauna oral rabies vaccination (ORV) period between 2007 and 2011, the philogenetic analysis of RV isolates of domestic and wild animals in the sphere of N gene by means of the reverse transcription-polymerase chain reaction (RT-PCR) was carried out for the first time. In 2006–2011, the assessment of the efficacy of ORV in raccoon dogs and red foxes by means of the quantitative enzyme-linked immunoassay test (ELISA) research method determining antibodies specific to the vaccine rabies virus in blood samples was performed for the first time. We recommend this method to be used to assess the efficacy of ORV of wild fauna. It has been established that vaccine against rabies used in Lithuania is an effective and safe measure of immunoprophylaxis which precludes the spread of rabies.

# CANDIDA BERKHOUT YEASTS: DISTRIBUTION, BIOLOGICAL PECULIARITIES AND SEARCH FOR PREVENTIVE MEASURES AGAINST THEM

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The aim of the work was to determine the distribution of Candida yeasts in various substrates and the surrounding environment, to define their biological characteristics and preventive measures to reduce the pollution. During the study 498 yeast isolates from soil, water, phyllosphere, food products, human body and the surrounding residential and occupational environment were isolated and identified. It was revealed that they belong to 21 genera and 63 species. Candida yeasts comprised 31% of all isolated yeasts; new to Lithuanian mycobiota species were recorded: *C. magnoliae*, *C. saitoana*, *C. oleophila* and *C. sorboxylosa*. Morphological, physiological and biochemical characteristics of Candida yeast were assessed. Acute toxicity / pathogenicity of single doses of *C. albicans* (C.A.4) and *C. parapsilosis* (C.P.1) administered per os or intraperitoneally to warm-blooded animals was ascertained. The assessment of the impact of 11 disinfectants and 8 antifungal preparations on pathogenic Candida yeasts was conducted. The effects of 12 essential oils, Pantoea citrea (T1x, T2x, T3x), *Streptomyces* sp. (Ux, Ux308) on Candida yeasts were determined.

# SLEEP AND THE SENSE OF REST: RELATION BETWEEN SLEEP FRAGMENTATION AND SUBJECTIVE SLEEP QUALITY

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Sleep disorders are one of the most common medical complaints today. There is a growing interest in sleep medicine, attitude of doctors and society has been changing and knowledge about sleep and its disorders is increasing. One of the most tedious and understudied sleep problems is non-restorative sleep. Researchers are still debating about what determines person's sense of rest after the sleep. Recently, much attention has been paid to sleep integrity and a role of sleep fragmentation for the sense of rest. It is thought that sleep fragmentation with short arousals could have effect on the restorative function of sleep. The aim of our study was to analyse sleep structure and sleep quality through sleep cycles, phases and stages and to evaluate structure's relationship with subjective sense of rest after the sleep, irrespective of the type of insomnia. We have analysed three types of arousal (behavioural, vegetative and microarousals) and their dynamics during the night, in different sleep cycles and stages. Subjective sleep quality was evaluated using the Pittsburgh sleep quality index. The results of the work showed that for the subjective sense of rest after the sleep the stability of sleep in the initial than in the final sleep cycles is more important. Regardless of sleep cycle, the sleep stage and arousal type are significant factors for the arousal index values and the increase of all arousal indices in NREM 2 stage (especially an increase of microarousal index) has the strongest impact on the sense of rest after the sleep.

## THE GAIN OF SPINAL CORD MOTONEURONS AND THEIR MODIFICATION

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Motoneurons are the spinal neurons that directly control the muscle contraction. The gain characterizes how the synaptic input to motoneuron is converted into action potential firing and subsequent muscle contraction. The high gain allows a high force and fast contraction, while the low gain is essential for a fine control of movements. The gain of motoneurons is mainly determined by a set of ion channels in membrane and therefore is a subject for modification. It is known that the gain decreases during adaptation of action potential firing. Moreover, the neurotransmitters released during spinal network activity may modify the ion channel activity and therefore adjust the gain to the functional needs. The aim of this study was to evaluate the gain of spinal cord motoneurons and investigate mechanisms of their modification. Spinal motoneurons from adult turtle were used. We found that the gain of motoneurons estimated from triangular current ramps is the same as the steady one obtained from square current steps. Pharmacologically increased conductance of motoneuron membrane does not change the gain. Finally, we demonstrated that persistent inward Na+ current increases excitability and reduces the transient and early gain of spinal motoneurons.