Homocysteine Mini-Conference
Poznań 2020

Biocentrum, Poznań University of Life Sciences

September 25, 2020

Dear Conference Participants,

We are delighted to welcome you to the hybrid Homocysteine Mini-Conference Poznań 2020 held at the Poznań University of Life Sciences. This is the second meeting of researchers interested in homocysteine biology and pathophysiology organized in Poznań by Professor Hieronim Jakubowski group, Department of Biochemistry and Biotechnology, Poznań University of Life Sciences. The main goal of this conference is to bring together people working at academic and research institutions who share common scientific interests, facilitate exchange of their latest research findings, to discuss emerging ideas and potential collaborations.

We are pleased to host 23 participants, including 9 speakers, and 3 poster presenters, from Lublin, Łódź, Gdańsk and Poznań. The participants are affiliated with the Poznań University of Life Sciences, the Poznań University of Medical Sciences, the Institute of Human Genetics PAS, the University of Lódź, the Medical University of Gdańsk and the Medical University of Lublin.

The Homocysteine Mini-Conference Poznań 2020 received the honorary patronage of the Rector of the Poznań University of Life Science, Professor Krzysztof Szoszkiewicz.

The conference is generously sponsored by Qiagen, IKA, CellService, A.P. Instruments, Promega, EPRO and Animalab.

Due to the pandemic, please follow the sanitary regime, e.g. use masks to cover your faces, use hand disinfectants, and obey the social distancing rules.

On behalf of the Scientific and Organizing Committee

Hieronim Jakubowski

Joanna Perła-Kaján

Scientific committee

Hieronim Jakubowski

Joanna Perła-Kaján

Organizing committee

Joanna Perła-Kaján

Joanna Suszyńska-Zajczyk

Ewa Bretes

Łukasz Witucki

Olga Włoczkowska

Hieronim Jakubowski

Conference website:

https://hcy2020.syskonf.pl/

Administered by Olga Utyro and Joanna Perła-Kaján

Scientific Program

11:00 – 12:00	Registration and setting up posters
12:00 – 12:10	Welcome by Joanna Perła-Kaján and Hieronim Jakubowski
12:00 – 13:45	**Session 1** Chair Agata Chmurzyńska
12:10 – 12:50	**Hieronim Jakubowski**, Rutgers University-New Jersey Medical School Genetic attenuation of paraoxonase 1 activity induces pro-atherogenic changes in plasma proteomes of mice and humans
12:50 – 13:10	**Jerzy Bełtowski**, Medical University of Lublin Effect of lipophilic and hydrophilic statins on paraoxonase 1 expression in the kidney and renal handling of Hcy thiolactone
13:10 – 13:30	**Joanna Mikołajczyk-Stecyna**, Poznań University of Life Sciences Effect of maternal nonalcoholic fatty liver disease and dietary choline intake on body mass and body composition in rat offspring
13:30 – 13:45	**QIAGEN** presentation
13:45 – 14:05	Coffee break
14:05 – 15:20	**Session 2** Chair Hieronim Jakubowski
14:05 – 14:25	**Łukasz Witucki**, Poznań University of Life Sciences Silencing *Cbs* or *Blmh* gene promotes accumulation of amyloid beta via the Phf8/H4K20me1/mTOR/autophagy pathway in mouse neuroblastoma N2A-APPswe cells
14:25 – 14:45	**Andżelika Borkowska**, Medical University of Gdańsk Homocysteine - Akt kinases dependent modulator of endothelial iron metabolism
14:45 – 15:05	**Joanna Perła-Kaján**, Poznań University of Life Sciences Proteom-wide analysis of protein lysine *N*-homocysteinylation in *Saccharomyces cerevisiae*
15:05 – 15:20	**IKA** presentation
15:20 – 16:00	Lunch and Poster session
16:00 – 17:00	**Session 3** Chair Joanna Perła-Kaján
16:00 – 16:20	**Olga Włoczkowska**, Poznań University of Life Sciences Autoantibodies against *N*-homocysteinylated proteins impair cognition: the VITACOG trial
16:20 – 16:40	**Ewa Bretes**, Poznań University of Life Sciences Opposite associations of cysteine and cysteinylglycine with stroke
16:40-17:00	**Kamila Borowczyk**, University of Lódź Determination of *N*-acetylhomocysteine by high performance liquid chromatography with ultraviolet detection in the form of S-quinolinium derivative
17:00– 17:20	Closing ceremony

Genetic attenuation of paraoxonase 1 activity induces pro-atherogenic changes in plasma proteomes of mice and humans

Marta Sikora[1], Ewa Bretes[2], Joanna Perła-Kaján[2], Łukasz Marczak[1], Hieronim Jakubowski[2,3]

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Background: High-density lipoprotein (HDL) promotes reverse cholesterol transport and also possesses anti-inflammatory, anti-oxidative, and antithrombotic activities. A calcium-dependent hydrolytic enzyme, paraoxonase 1 (PON1), carried on HDL in the blood, may contribute to these anti-atherogenic activities. The *PON1-Q192R* polymorphism involves a change from glutamine (Q variant) to arginine (R variant) at position 192 of the PON1 protein, which affects its activity and determines its atheroprotective function. However, molecular basis of the protective function of PON1 against cardiovascular disease (CVD) are not fully understood.

Aim: To get insight into the function of PON1 in human disease, we examined how genetic attenuation of PON1 levels/activity affects plasma proteomes of mice and humans.

Methods: Healthy participants (n = 100; 49 ± 17-years-old, 50% men) were randomly recruited from the Poznań population. Three month-old *Pon1*$^{-/-}$ (n=17) and *Pon1*$^{+/+}$ (n=8) mice were used in these experiments. Blood was collected on EDTA, and plasma separated by centrifugation. PON1 genotypes were established by PCR. Plasma proteomes were analyzed using label-free mass spectrometry. Bioinformatic analysis was carried out using the Ingenuity Pathway Analysis resources to identify proteins/molecular pathways interacting with PON1 in humans and mice.

Results: *PON1-Q192R* polymorphism and *Pon1*$^{-/-}$ genotype induced similar changes in plasma proteomes of humans and mice, respectively. Top molecular network affected by these changes involved proteins participating in lipoprotein metabolism both in humans and mice. *Pon1*$^{-/-}$ genotype induced other changes in the mouse plasma proteome, not found in *PON1-192QQ* humans, which affected a biological network containing proteins involved in the acute phase/immune response. Overall, these changes in plasma proteomes are associated with a pro-atherogenic phenotype.

Conclusion: Our findings suggest that PON1 interacts with molecular pathways involved in lipoprotein metabolism, acute/inflammatory response, and complement/blood coagulation that are essential for normal plasma homeostasis. Dysregulation of those interactions can account for the involvement of PON1 in CVD.

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Effect of lipophilic and hydrophilic statins on paraoxonase 1 expression in the kidney and renal handling of Hcy thiolactone

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Background: Statins (3-hydroxy-3-methylglutarylcoenzyme A reductase inhibitors) reduce LDL-cholesterol and are commonly used in primary and secondary prevention of cardiovascular diseases. Although statins excellently improve survival rates, in a subset of patients they induce significant and sometimes dangerous adverse effects such as hepatotoxicity, myopathy, neuropathy, decrease in myocardial contractility, cognitive decline, etc. Atorvastatin and rosuvastatin are two most commonly used statins nowadays. Atorvastatin is lipophilic and easily permeates plasma membranes being active not only in the liver (the primary target organ responsible for LDL reduction) but also in other tissues. In contrast, rosuvastatin is hydrophilic and membrane-impermeable. Consequently, rosuvastatin has less adverse effects in extrahepatic organs. However, rosuvastatin is in large part excreted by the kidney and recent studies indicate that it induces renal tubular injury and proteinuria in some patients. Previous studies have demonstrated that statins may inhibit paraoxonase 1 (PON1) expression in the liver by inhibiting synthesis of oxysterols, the endogenous agonists of liver X receptor (LXR). Apart from the liver, PON1 is substantially expressed in the kidney where it may protect against protein N-homocysteinylation by hydrolyzing Hcy thiolactone which is in high amounts excreted by the kidneys.

Aim: We examined the effect of atorva- and rosuvastatin on PON1 expression and activity in the liver and kidney as well as on protein N-homocysteinylation.

Methods: Male Wistar rats were treated with atorvastatin (20 mg/kg/day) or rosuvastatin (5 mg/kg/day) for 3 weeks. PON1 expression and activity was measured at the mRNA and protein levels by qRT-PCR and ELISA, respectively. Total plasma Hcy as well as protein-bound Hcy thiolactone were measured. In addition, urinary excretion of Hcy thiolactone was measured as well.

Results: Both statins have similar lipid-lowering effects as well as reduced PON1 expression in the liver and plasma PON1 activity by about 20%. Statins had no effect on plasma Hcy or protein-bound Hcy thiolactone. Only rosuvastatin but not atorvastatin decreased PON1 mRNA (-42%) and protein (-35%) in the renal cortex. Statins had no effect on glomerular filtration rate. Rosuvastatin increased urinary concentration and fractional excretion of Hcy thiolactone as well as increased protein N-homocysteinylation in the renal cortex and medulla. The effects of rosuvastatin on renal PON1 and Hcy thiolactone but not on plasma lipids were attenuated by co-treatment with synthetic LXR agonist, T0901317. In addition, rosuvastatin but not pravastatin increased sterol responsive element-binding protein)SREBP) and reduced LXR activity in the kidney.

Conclusions: Rosuvastatin but not pravastatin decreases PON1 synthesis in the kidney presumably by inhibiting LXR signaling. As a consequence, rosuvastatin increases local Hcy thiolactone concentration and protein N-homocysteinylation which may contribute to tubular injury observed in some rosuvastatin-treated patients. LXR agonists may be useful in prevention/treatment of rosuvastatin-induced kidney injury.

Effect of maternal nonalcoholic fatty liver disease and dietary choline intake on insulin, leptin, and adiponectin levels in male rat offspring.

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Background: Both maternal metabolic status and nutrition during pregnancy and lactation may have programming effects on offspring's metabolism.

Aim: To examine the role of dietary choline supply during pregnancy and lactation in rat dams suffering from nonalcoholic fatty liver disease (NAFLD) on insulin, leptin, and adiponectin serum levels in their progeny.

Methods: The research protocol was approved by the local ethics committee. The study groups included the offspring of: 1. healthy dams receiving choline during pregnancy and lactation (the control group); 2. NAFLD dams receiving choline during pregnancy and lactation (NN); 3. NAFLD dams receiving choline during pregnancy and a choline-deficient diet during lactation (ND); 4. NAFLD dams receiving a choline-deficient diet during pregnancy and a supply of choline during lactation (DN); and 5. NAFLD dams receiving a choline-deficient diet during both pregnancy and lactation (DD). Insulin, leptin, and adiponectin serum levels were assessed in male rats from each group on day 24 (24d), day 90 (90d), and month 12 (12m) The differences were analyzed by using one-way ANOVA test with Fisher (NIR) post-hoc test. *P* values < 0.05 were taken as significant.

Results: The mean adiponectin concentration in serum was significantly higher in offspring of the maternal choline deficient groups: DD, DN and ND than in the NN and the control group at 24d ($p<0.05$). At 90 d the difference was observed only between the DD and DN groups ($p=0.048$), and at 12m the adiponectin concertation did not differ between the groups. We observed no differences of leptin level in serum between the studied groups at 24d and 12m, but at 90d the leptin concentration was the highest in DN group and significantly higher in comparison to the K ($p=0.034$), NN ($p=0.008$), ND ($p=0.042$), and DD ($p=0.009$). Insulin concentration was significantly higher only at 12m in the DN and ND groups as compared to the K ($p=0.027$ and $p=0.023$, respectively), but at 24d the serum insulin level was the highest in the DN group and at 90d the lowest in the ND group.

Conclusions: Maternal NAFLD and dietary choline status during pregnancy and lactation can affect insulin, leptin, and adiponectin serum concentration in rat male offspring.

The project was financed by the National Science Centre, Poland (2016/21/D/NZ9/00360).

Silencing *Cbs* or *Blmh* gene promotes accumulation of amyloid beta *via* the Phf8/H4K20me1/mTOR/autophagy pathway in mouse neuroblastoma N2A-APPswe cells

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Background: Dysfunction of Cystathionine-β-synthase (CBS) or Bleomycin Hydrolase (BLMH) genes, which causes accumulation of homocysteine (Hcy) and/or homocysteine thiolactone (HTL), is linked to neurodegenerative disease, including Alzheimer's (AD). Dysregulation of the mechanistic target of rapamycin (mTOR) signaling/autophagy pathways and amyloid beta (Aβ) accumulation are hallmarks of AD. Mechanistic role of CBS and BLMH in the development and progression of AD is not fully understood.

Aim: We tested a hypothesis that silencing of *Cbs* or *Blmh* gene expression promotes Aβ accumulation via a pathway involving epigenetic effects of Hcy and HTL on mTOR signaling/autophagy in mouse neuroblastoma N2A-APPswe cells.

Methods: Neuroblastoma N2A-APPswe cells (N2A-APPswe) harboring a human transgene with mutation in the amyloid precursor protein (APP) gene were grown on the complete DMEM/F12 medium. Cbs and Blmh genes were silenced using transfection with siRNAs. The non-transfected cells were treated with 20-200 μM Hcy or HTL. Aβ accumulation, proteins involved in the mTOR/autophagy pathway, including the Phf8 histone demethylase and Lys20 methylation in histone 4 (H4K20me1), were quantified by Western blotting and Immunofluorescence.

Results: Levels of Aβ immunofluorescent signal were significantly elevated in cells after silencing Cbs- or Blmh gene relative to non-transfected control. At the same time Western blots showed upregulation of APP expression, increased levels of the epigenetic mark on H4K20me1, increased mTOR and its phosphorylated form, and decreased autophagy markers Beclin1, Atg5, and Atg7, compared to untreated cells. Treatments of non-transfected N2A-APPswe cells with Hcy or HTL caused similar changes in these markers.

Conclusion: Silencing of Cbs or Blmh gene, or treatment with Hcy or HTL (Cbs or Blmh substrate, respectively) results in epigenetic up-regulation of mTOR signaling, mediated by increased H4K20 methylation. Up-regulation of the mTOR pathway leads to down-regulation of autophagy, which in turn causes Aβ accumulation in mouse neuroblastoma N2A-APPswe cells.

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Proteom-wide analysis of protein lysine *N*-homocysteinylation in *Saccharomyces cerevisiae*

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Background: Protein *N*-homocysteinylation by a homocysteine (Hcy) metabolite, Hcy-thiolactone, is an emerging post-translational modification (PTM) that has been linked to human diseases. The yeast *Saccharomyces cerevisiae* is widely used as a model eukaryotic organism in biomedical research, including studies of PTMs. However, patterns of global protein *N*-homocysteinylation in yeast are not known.

Hypothesis: *N*-Homocysteinylation sites overlap with other PTMs and affect yeast cellular proteostasis.

Methods: Cultures of the yeast *S. cerevisiae* (strain BY4742, a lysine auxotroph derived from S288C) in SD medium containing required supplements were incubated with Hcy (1 - 10 mM, 3 h, 24°C). *N*-Hcy-Lys peptides were identified in tryptic digests of yeast proteins by an increase in mass of 174 Da (iodoacetamide-modified *N*-Hcy) or 163 Da (*S*-methyl methanethiosulfonate-modified *N*-Hcy) and by peptide fragmentation spectra. *N*-Hcy-Lys sites were also identified in yeast proteins modified with L-Hcy-thiolactone *in vitro*. The sequence motifs around *N*-Hcy-sites were analyzed using Two Sample Logo and pLogo tools. Cellular proteomes were analyzed by using SILAC and iTRAQ on a Q Exactive mass spectrometer. DAVID and STRING resources were used for bioinformatic analyses.

Results: We identified 68 *in vivo* and 197 *in vitro N*-homocysteinylation sites at protein lysine residues (*N*-Hcy-Lys). The position-wise enrichment of amino acids in the *in vivo N*-Hcy-Lys sites showed that *N*-homocysteinylated lysines in closest proximity were surrounded mostly by neutral, hydrophobic and buried amino acid residues (A, Q, V, S). pLogo analysis of *in vitro N*-Hcy-Lys sites have shown that the only statistically significant enrichment occurred at position -1, with Ala residue being more frequent in *N*-Hcy-peptides (frequency 17.22%, *p* value 0.002). Some of the *N*-homocysteinylation sites overlap with previously identified other PTM sites. Protein *N*-homocysteinylation induced in yeast cells by elevated Hcy, which increases Hcy-thiolactone biosynthesis *in vivo*, was associated with significant dysregulation of protein expression. We identified 70 Hcy-responsive yeast proteins: 38 up-regulated (1.06-3.58-fold) and 32 down-regulated (0.21-0.88-fold). Upregulated proteins are involved in amino acid biosynthesis, vitamin B6 and red-ox metabolism. Down-regulated proteins are involved in ribosome biogenesis and translation, protein folding, apoptotic signaling, oxidative stress response, S-adenosylmethionine biosynthesis, and sulfate assimilation.

Conclusions: Our study provides the first global survey of *N*-homocysteinylation in the yeast *S. cerevisiae* and the accompanying dysregulation of cellular proteostasis caused by elevated Hcy level. Homologous proteins and *N*-homocysteinylation sites are likely to be involved in Hcy-linked pathophysiology in humans and experimental animals.

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Homocysteine - Akt kinases dependent modulator of endothelial iron metabolism

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Background: Hyperhomocysteinemia is an independent risk factor for cardiovascular diseases such as ischemic heart diseases, stroke, peripheral vascular disease, atherosclerosis as well as dementia, brain atrophy and others. There is evidence that iron can mediate homocysteine (Hcy) toxicity. However, molecular mechanism of iron dependent Hcy toxicity has not been well understood.

Aim: The aim of this study was to investigate the effect of Hcy on iron metabolism in HUVEC and SH-SY5Y cells.

Methods: HUVEC and SH-SY5Y cells were treated with 3mM Hcy for defined time.

Results: We demonstrate that Hcy induced upregulation of ferritins L and H in HUVEC cells in a time dependent manner and had no effect on ferritins in SH-SY5Y cells. The change in ferritin expression was preceded by a significant decrease in the cellular level of the active form of Akt kinase (p-Akt) in HUVEC but not in SH-SY5Y cells. In order to confirm the involvement of Akt kinases and FOXO3a in ferritins upregulation, the HUVEC cells were transfected with siRNA against Akt1, Akt2, Akt3 and FOXO3a respectively. A significant increase in ferritin L and H protein levels was observed in Akt siRNA transfected cells, while in cells transfected with FOXO3a siRNA decrease in ferritins level was noticed. Moreover, in the HUVEC cells treated with Hcy for 6 days active form of kinase Akt return to control value and it was accompanied by drop in ferritin L and H protein levels. Cytotoxicity of hydrogen peroxide (H_2O_2) significantly decreased in HUVEC cells pre-treated with Hcy for 24 h.

Conclusions: These data indicate that Hcy induces an increase in cellular ferritin level, and the process is mediated by alterations in Akt-FOXO3a signaling pathway.

Autoantibodies against *N*-homocysteinylated proteins impair cognition: the VITACOG trial

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Background: Hyperhomocysteinemia (HHcy) is a risk factor for dementia and Alzheimer disease (AD). Hcy-lowering B-vitamins treatment slows age-related brain atrophy and cognitive decline in patients with mild cognitive impairment (MCI). A reactive homocysteine (Hcy) metabolite, Hcy-thiolactone modifies protein lysine residues to generate auto-immunogenic *N*-Hcy-protein. Anti-*N*-Hcy-protein auto-antibodies are associated with stroke. However, it is not known whether anti-*N*-Hcy-protein auto-antibodies are associated with cognition.

Hypothesis: We hypothesize that anti-*N*-Hcy-protein antibodies predict cognitive performance in humans and that this effect is modified by B vitamins supplementation.

Methods: A single-center, randomized, double-blind controlled trial of high dose folic acid, vitamins B_6 and B_{12}. Patients with MCI participating in the VITACOG trial (n=196, 76.8-years-old, 60% women) were randomly assigned to receive a daily dose of folic acid (0.8 mg), vitamin B_{12} (0.5 mg) and B_6 (20 mg) (n=133) or placebo (n=133) for 2 years. Cognition was analyzed by neuropsychological tests. Brain atrophy was quantified in a subset of patients (n=187) by MRI. Anti *N*-Hcy-protein auto-antibodies were quantified by ELISA.

Results: Multiple regression analysis showed that baseline anti-*N*-Hcy-protein antibodies were significantly associated with hemoglobin (β=0.27, *P*=0.001), stroke (β=-0.15, *P*=0.047), and baseline measures of performance in several cognitive domains: global cognition (Minimental State Examination - MMSE: β=0.15, *P*=0.039), verbal episodic memory (Hopkins Verbal Memory Test-revised, Total Recall - HTLV TR: β=0.18, *P*=0.028), and complex attention/processing speed (Map Search: β=-0.18, *P*=0.031). In a placebo group at two years, measures of cognition in two domains: global memory (Telephone Inventory for Cognitive Status modified - TICSm: β=-0.19, *P*=0.040) and attention/speed (Trail Making A: β=0.18, *P*=0.033) were significantly associated with baseline anti *N*-Hcy-protein auto-antibodies. Global cognition tended to be associated with anti *N*-Hcy-protein auto-antibodies (MMSE: β=-0.18, *P*=0.077). Attention/speed was significantly associated with tHcy (Trail Making A: β=0.26, *P*=0.022) but global memory (TICSm: β=-0.11, *P*=0.382) and global cognition were not (MMSE: β=-0.06, *P*=0.670). MMSE, Trail Making A, and TICSm at two years were also significantly associated with brain atrophy and a corresponding neuropsychological test at baseline. In the B vitamins group at two years, these measures of cognition were not associated with baseline anti-*N*-Hcy-protein autoantibodies or tHcy.

Conclusions: Anti-*N*-Hcy-protein autoantibodies impair cognition in the global memory, global cognition, and attention/speed domains in MCI patients. B vitamins treatment abrogates these effects. These findings identify a novel positive aspect of B vitamin supplementation.

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Opposite associations of cysteine and cysteinylglycine with stroke

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Background: Stroke is a second, after coronary heart disease, leading cause of morbidity and mortality worldwide. According to WHO in 2018 stroke mortality accounted for 9,76 % of total mortality in Poland. Although elevated plasma total homocysteine (tHcy) is an emerging risk factor for stroke, a role of other thiols as possible stroke risk factors has not been examined.

Aim: We investigated associations between plasma thiols (Cys, CysGly, GSH) and stroke. We also examined how these associations are modified by tHcy, traditional risk factors, and genetic factors such as MTHFR_677C>T, MTHFR_1298A>C, CBS_1224-2A>C, CBS_833T>C, CBS_844_45ins68, CBS_9276G>A (associated with Hcy levels), PON1_192Q>R, PON1_55L>M, BLMH_443I>V, and BPHL_259L>S (associated with Hcy-thiolactone levels).

Participants and methods: Stroke patients (n = 198, 68.4±11.7-year-old, 44.9% women) were from the Department of Neurology and Cerebrovascular Disorders, Poznań University of Medical Sciences, L. Bierkowski Hospital. Healthy volunteers were used as a control group (n = 299, 49.9±16.7-year-old, 59.9% women). Gender, age and previous history of all subjects were recorded. Blood samples were collected from July 2017 to November 2019. Fasting glucose, total cholesterol, HDL-C, LDL-C, triglycerides (TG), creatinine and eGFR (MDRD) were measured in the hospital's diagnostic laboratory. Plasma thiols (tHcy, Cys, GSH and CysGly) were quantified by standard procedures. Genotyping was done using polymerase chain reaction - restriction fragment length polymorphism (PCR-RFLP) method. Statistical analysis was performed using the Statistica data analysis software system v.13. An unpaired *t*-test was used for comparisons between two groups of variables with normal distributions. Associations between stroke and other variables were studied by Pearson's correlations. Stroke determinants were analyzed by multiple logistic regression. Genotype distributions were compared between stroke patients and control group using the Pearson Chi-test. The Bioethics Committee of the Karol Marcinkowski Poznan University of Medical Sciences approved the study protocol.

Results: In multivariate regression analysis, male sex, age, and plasma Cys were positively associated with stroke, whereas HDL-C, LDL-C and CysGly were negatively associated. No significant association with stroke was observed for Hcy, GSH, glucose, total cholesterol, TG, creatinine, and the *MTHFR*, *CBS*, *PON1*, *BLMH*, *BPHL* genotypes.

Conclusions: Elevated Cys and decreased CysGly levels are novel risk factors for stroke, independent of Hcy and traditional risk factors.

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Determination of *N*-acetylhomocysteine by high performance liquid chromatography with ultraviolet detection in the form of *S*-quinolinium derivative

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Background: *N*-acetylhomocysteine (AcHcy) is an acetylated homocysteine derivative that might have potential anticoagulant properties. Literature data regarding AcHcy are incomplete and refer only to some physico-chemical properties of the compound. Due to the limited amount of information about this analyte, it was necessary to conduct research that broaden the knowledge.
Aim: The aim of the study was to develop a new analytical protocol for the quantitative analysis of AcHcy based on high performance liquid chromatography with UV detection. Due to the limited commercial availability of this analyte, it was also necessary to improve a procedure of AcHcy synthesis and synthetize the analyte in our laboratory.
Methods: The AcHcy was obtained by acetylation of homocysteine or homocystine with acetic anhydride. An analytical method based on high performance liquid chromatography with UV detection was developed to detect AcHcy. For analysis a ZORBAX SB – C18 chromatography column (155 × 4.6 mm, 5 Å) was used. The mobile phase consisted of acetonitrile and 0.15% acetic acid (pH .2).
Results: We have proven that homocystine is preferred to be the starting substrate of the synthesis. The experiments indicated that 10 – 40- fold molar excess of acetic anhydride, added in portions at equal time intervals was required. The total time of acetylation at 4 °C was 30 min.
Conclusions: For the determination of AcHcy with the use of high performance liquid chromatography with UV detection, the procedure of sample preparation must be based derivatization with 2-chloro-1-methylquinolinium tetrafluoroborate.

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The c.293G>A polymorphism of *DJ-1* gene and the level of oxidative stress markers in patients with Parkinson's disease

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Background: Parkinson's disease (PD) is the second most common neurodegenerative disorder in the world, affecting about 2% of population above 60 years of age. Although the etiology of this age-related disease remains unknown, the involvement of genetic factors in its pathomechanism is substantial. *LRKK2*, *SNCA*, *PRKN*, *PINK1* and *DJ-1* are one of the many genes associated with familial PD. The last one encodes DJ-1 protein which plays key role in cell's defense against oxidative stress.

Aim of the study: The aim of this study was to analyze c.293G>A polymorphism of *DJ-1* gene, homocysteine (Hcy) and glutathione (GSH) plasma concentration, in correlation with clinical symptoms of PD.

Materials and methods: The study included 40 PD patients and 40 controls. Mean age of participants was 52±8 years. The presence of polymorphism was detected by high resolution melting (HRM) analysis followed by Sanger sequencing. Plasma Hcy and GSH concentrations were determined by high performance liquid chromatography with electrochemical detection (HPLC/EC).

Results: The GA genotype of c.293G>A *DJ-1* polymorphism was identified only in 5 patients with PD and in none of the control volunteers. There was no significant difference between Hcy levels in neither study nor control group. The carriers of the studied polymorphism were characterized by higher concentration of Hcy ($p=0.0138$). Plasma GSH levels were significantly higher in PD patients as compared to the control group. Moreover, a positive correlation was found between the levels of Hcy and GSH in PD patients ($p=0.0116$, $R=0.4220$). PD patients with the c.293G>A *DJ-1* polymorphism had slightly more severe symptoms of the disease in comparison to patients without this polymorphism. However, their response to L-dopa treatment remained good.

Conclusion: Although, the c.293G>A polymorphism of *DJ-1* gene is likely a risk factor for PD, it does not affect concentration of oxidative stress markers (Hcy, GSH), in significantly harmful manner.

P 02

The GC-MS assay for homocysteine thiolactone quantification in human saliva

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Background: Investigation of the role of homocysteine thiolactone (HTL) in living organisms began in the 70's. Despite many years of research work, its physiological and pathological role in humans still remains unclear. Recently, it has been recognized that urinary HTL can act as a risk predictor of acute myocardial infarction, independent from other established risk factors and plasma homocysteine [1]. Up till now, few methods enabling determination of HTL in biofluids have been developed. So far, its presence has been documented in human and mouse plasma and urine. Here, we show that HTL is also present in human saliva [2].

Aim: The main aim of our studies has been focused on providing new powerful tool for HTL identification and quantification in human saliva. Since gas chromatography – mass spectrometry (GC-MS) is one of increasingly popular technique, but shows a negligible extent of utility of HTL analytics, we have decided to extend its application.

Methods: The assay for the determination of salivary HTL in the form of its volatile trimethylsilyl derivative by GC-MS have been elaborated for the first time. Sample preparation procedure involves three critical steps, including isolation (liquid-liquid extraction), lyophilization and derivatization followed by gas chromatographic separation.

Results and conclusions: An attractive and high-throughput GC-MS assay was proven to be an excellent tool for the determination of HTL in human saliva. Using this method, we show that in apparently healthy individuals (n = 18), salivary HTL varied from 0.07 to 0.19 μmol L^{-1}, while average salivary HTL was 0.10 ± 0.02 μmol L^{-1} in men and 0.11 ± 0.03 μmol L^{-1} for women. Despite some limitations, the assay could be considered as a valuable analytical tool useful for thoroughly studies of the physiological and pathological role of HTL in living systems.

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P 03

Folate, but not homocysteine concentration, is improved after three-month iron and folic acid supplementation in young women

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Background: The effect of simultaneous iron and folic acid (IFA) supplementation and gene polymorphism on biochemical parameters and blood morphology has been not fully characterized.

Aim: The aim of this study was to characterize the effects of three-month IFA supplementation in young women with suboptimal folate and iron levels. We also wanted to determine associations between polymorphism of genes related to folate and iron status on biochemical parameters and blood morphology.

Methods: This study was conducted with the approval of the local ethical committee (approval no. 917/16). We enrolled 244 women, aged 18-35 years old to a case-control study in 2016-2018 in Poznań, Poland. The cases (the S group) had suboptimal folate and/or iron levels, while the controls (the C group) had adequate iron and folate concentrations. Women in the group S received supplements that contained iron (14 mg) and folic acid (200 µg) and were taking them for three months. Folate, homocysteine, iron, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC) and blood morphology were analyzed before and after the supplementation. Polymorphic variants of *MTHFR* (rs1801133), *DHFR* (rs70991108), *RFC* (rs1051266), DMT (rs224589), and *TFR2* (rs7385804) genes were determined.

Results: Differences between the C and S groups were noticed in Δfolate (-0.45 ± 2.93 ng/ml vs. 3.12 ± 5.38 ng/ml, $P < 0.001$), ΔUIBC (67.34 ± 123.46 µg/dl vs. -12.18 ± 122.33 µg/dl, $P < 0.01$) and ΔTIBC (82.75 ± 133.48 µg/dl vs. 6.53 ± 126.81 µg/dl, $P < 0.01$), but were not observed in homocysteine concentration after three months of IFA supplementation. Individuals with TT and GT genotypes of rs224589 in the S group had an increase in WBC concentration, unlike GG homozygotes ($P < 0.001$).

Conclusions: To conclude, three-month IFA supplementation can increase folate concentration, but not homocysteine concentration in women with suboptimal level of folate and iron. Three-month IFA supplementation can also improve iron status. However, the investigated polymorphisms are not associated with the effects of the intervention.

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