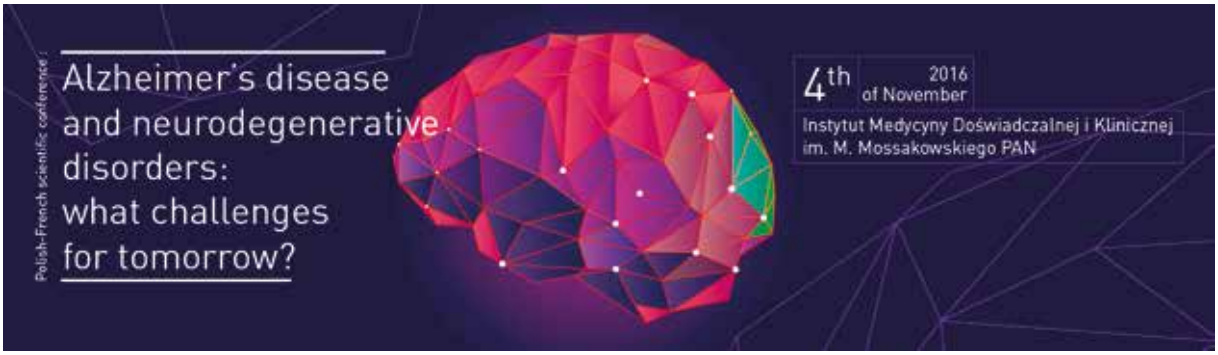

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Polish-French scientific conference:

Alzheimer's disease and neurodegenerative disorders: what challenges for tomorrow?

Mossakowski Medical Research Centre
of Polish Academy of Sciences

4th of November 2016

PREFACE

Polish-French conference "Alzheimer's disease and neurodegenerative disorders: what challenges for tomorrow?" was held at Mossakowski Medical Research Center of Polish Academy of Sciences on the 4th of November 2016. The event aimed to present the latest scientific achievements in the field of Alzheimer's disease and other neurodegenerative diseases. The conference was organized by **French Embassy in Warsaw, French Institute in Warsaw, Polish Academy of Sciences and Mossakowski Medical Research Center.** The conference was opened by Stanislas Pierret, Director of French Institute, Prof. Maria Barcikowska-Kotowicz, Director of Mossakowski Medical Research Center, and by Prof. Stanislaw Czuczwar, Vice-President of Polish Academy of Sciences. The invited guests were eminent specialists in the field of neuroscience and molecular biology. The professors lectures were given by French experts **Prof. Luc Zimmer** and **Prof. Philippe Corcia,** and Polish experts, **Prof. Tomasz Gabryelewicz** and **Prof. Konrad Reydak.** The professors lectures were complemented by oral presentations of young, talented and promising scientists: **Dr. Raphaëlle Pardossi-Piquard** and **Dr. Olivier Nicole** from France and **Dr. Michalina Wężyk** and **Dr. Anna Barczak** from Poland. Conference was attended by nearly 130 participants (#130 registrations, but a bit more than 90 people were there), including 23 poster presentations assembling various approaches and sectors in the study of Alzheimer's disease and neurodegenerative disorders. The conference was strengthening Polish-French cooperation in the field with an emphasis on young scientists networking. It is our hope that this conference will help in future scientific exchange and sharing valuable patient's derived material for the research. This is particularly important in the era of large-scale high throughput methods where single factors or single genes are no longer in the center of the study. There is an urgent need to join the forces and to conduct the research on thousands patients targeting entire gene conglomerates, the influence of multiple environmental factors and, consequently, whole genome, epigenome, and proteome that are the challenges for today and tomorrow's neurodegenerative research.

Dr. Michalina Wężyk, Mossakowski Medical Research Center
Dr. Sebastien Reymond, French Embassy in Warsaw
Dr. Antonin Borgnon, French Embassy in Warsaw
Organizing Committee

ORAL PRESENTATIONS

[1]

Toward PET molecular imaging of functional serotonin receptors during Alzheimer's disease

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Evidence accumulates suggesting that drugs targeting the serotoninergic system could play an important therapeutic role in Alzheimer's disease (AD), particularly in terms of cognitive enhancement. Among the serotonergic targets, the 5-HTT1A and the 5-HT6 receptor subtypes are of particular interest to treat cognitive or non-cognitive symptoms in AD and several pharmaceutical and biotech companies are currently developing new drugs specifically targeting these receptors.

It is crucial that discovery and development of clinical candidate compounds are accompanied by parallel development of suitable positron emission tomography (PET) tracers to allow for a rational and robust testing of pharmacological hypotheses with neuroimaging. If PET is a powerful imaging modality mainly used to visualize and asses the distribution/density of targeted receptors in a living subject (animal, human), we propose that comparing PET imaging obtained using an agonist radiotracer, which binds selectively to functional receptors, with the PET imaging obtained using an antagonist radiotracer would provide original information on 5-HT receptor impairment during AD. This exploration of functional and active receptors at pre-dementia stages can open up the possibility of better pathophysiological understanding, differential diagnosis or assessment of the impact of pro-cognitive therapy.

[2]

Genetics of amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is the most frequent motor neuron disorder in adulthood. This always fatal condition is characterized by upper and lower motor neurons degeneration in bulbar and spinal territories leading to death by respiratory failure after a median duration of 36 months. Although a huge literature clearly supports a major role of genetics in ALS, pathophysiology of ALS remains unknown. 10% of cases are familial and over the last 20 years, more than 25 genes have been linked to the disease, among which four (SOD1, TARDBP, FUS and mainly C9orf72 genes) explained more than 60% of familial (FALS) cases and around 10% of sporadic (SALS) cases. After an overview of recent findings of genetics in ALS, we will bring evidence that strongly support that ALS is a oligogenic affection and finally discuss whether genotype-phenotype correlations could be drawn in ALS. This clearly must be taken into account in clinical practice and more specifically in case of genetic counselling. In conclusion, genetics plays a key role in pathophysiology of ALS and probably will be the matter of promising clinical trials in the next years.

[3]

C99 as an early contributor to AD pathology

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Endosomal-autophagic-lysosomal (EAL) dysfunction is an early and prominent neuropathological feature of Alzheimers's disease, yet the exact molecular mechanisms contributing to this pathology remain undefined. By combined biochemical, immunohistochemical and ultrastructural approaches, we demonstrate a link between EAL pathology and the intraneuronal accumulation of the β-secretase-derived βAPP fragment (C99) in two *in vivo* models, 3xTgAD mice and adeno-associated viral-mediated C99-infected mice. We present a pathological loop in which the accumulation of C99 is both the effect and causality of impaired lysosomal-autophagic function. The deleterious effect of C99 was found to be linked to its aggregation within EAL-vesicle membranes leading to disrupted lysosomal proteolysis and autophagic impairment. This effect was Aβ independent and was even exacerbated when γ-secretase was pharmacologically inhibited. No effect was observed in inhibitor-treated wild-type animals suggesting that lysosomal dysfunction was indeed directly linked to C99 accumulation. In some brain areas, strong C99 expression also led to inflammatory responses and synaptic dysfunction. Taken together, this work demonstrates a toxic effect of C99 which could underlie some of the early-stage anatomical hallmarks of Alzheimer's disease pathology. Our work also proposes molecular mechanisms likely explaining some of the unfavorable side-effects associated with γ-secretase inhibitor-directed therapies.

[4]

Behavioral and electrophysiological characterization of the learning and memory deficits induced in mouse model of Alzheimer's disease

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Post-learning hippocampal sharp wave-ripples (SWRs) generated during slow wave sleep are thought to play a crucial role in memory formation. While in Alzheimer's disease, abnormal hippocampal oscillations have been reported, the functional contribution of SWRs to the typically observed spatial memory impairments remains unclear. These impairments have been related to degenerative synaptic changes produced by soluble amyloid beta oligomers (Aβos) which, surprisingly, seem to spare the SWR dynamics during routine behavior. To unravel a potential effect of Aβos on SWRs in cognitively-challenged animals, we submitted vehicle- and Aβo-injected mice to spatial recognition memory testing. While capable of forming short-term recognition memory, Aβ mice exhibited faster forgetting, suggesting successful encoding but an inability to adequately stabilize and/or retrieve previously acquired information. Without prior cognitive requirements, similar properties of SWRs were observed in both groups. In contrast, when cognitively challenged, the post-encoding and -recognition peaks in SWR occurrence observed in controls were abolished in Aβ mice, indicating impaired hippocampal processing of spatial information. These results point to a crucial involvement of SWRs in spatial memory formation and identify the Aβ-induced impairment in SWRs dynamics as a disruptive mechanism responsible for the spatial memory deficits associated with Alzheimer's disease.

[5]

Apraxic variant of Alzheimer's disease – case presentation

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Corticobasal syndrome (CBS) is increasingly reported in subjects with confirmed Alzheimer's disease underlying pathology. With the exception of memory problems, investigations point to severe apraxia, slowly progressive left hemi-Parkinsonism, myoclonus, visuospatial disturbances, resembling Corticobasal degeneration (CBD). 55 yo female with problems in occupational and everyday activities, reporting impairment in memory, visuospatial domains as well as in writing, speech and calculation was hospitalised with initial diagnosis of CBD. Severe dementia (MMSE 14) was diagnosed, with parkinsonism and dysfunctions in learning, episodic memory, executive functions along with dispropor-

tionally pronounced apraxia, spatial disorientation, writing, speech, mathematical skills dysfunctions. Neuroimaging examination revealed the presence of multiple small vascular changes in frontal white matter and ventricular areas together with the cortical atrophy in the frontal and parietal lobes, but hippocampal regions were intact. CSF-AD biomarkers profile with decreased level of beta-amyloid and increased levels of total and phosphorylated tau proteins was characteristic for Alzheimer's disease. Final diagnosis was AD-CBS plus syndrome and despite donepezil treatment, her condition was rapidly worsening, with psychotic features and complete loss of the independency. After 3 years of observation the MMSE = 0, subject is mute, presents motor problems and requires full care.

[6]

DNA damage stress response in Alzheimer's disease

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Whole-transcriptome profiling of primary cell lines of fibroblasts derived from fEOAD patients with mutations in PSEN1 revealed disturbances in signaling pathways linked with regulation of cell cycle and DNA damage response (DDR). The transcriptomic data were further subjected to functional validation in terms of DDR. We found disturbed activity of ATR and ATM kinases, expressed by their activation status and abnormally increased phosphorylation of their downstream effectors, Chk1 and Chk2 kinases. These effects were accompanied by an increased phosphorylation of BRCA1 at Ser1524 in fEOAD cells. Simultaneously, we have observed a drop in nucleic BRCA1 (Ser1524) level in fEOAD, suggesting disturbances in translocation of BRCA1 to nucleus. Abnormal subcellular localization of phosphorylated BRCA1 could interfere with DNA repair and redirect the cells to apoptosis that was found to occur at a greater extent in fEOAD patients than in control cell line, what was demonstrated by an increase of percentage of apoptotic cells using flow cytometry and by the content of nuclear enzyme PARP cleaved by caspase 3 to fragments of 89 and 24 kDa. We concluded that BRCA1 may influence presenilin 1 reprocessing and this opens very interesting novel research area linking Aβ42-based pathology in familial AD with PSEN1 mutations and possibly pathology in sporadic AD, suggesting that BRCA1-targeted treatment could work for both types of AD.

This work is supported by Polish National Science Centre grant "SONATA6" no. G11192013/09/D/NZ3/01348 and Statutory Grant at MMRC from Ministry of Science and Higher Education.

[7]

A project within the EU Joint Programme for Neurodegeneration Biomarkers for Alzheimer's disease and Parkinson's disease

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The main goal for the BIOMARKAPD project was to standardize the sampling and measurement for the already known biomarkers, as well as to develop new ones for AD and PD, and standardize clinical use biomarkers. This has been done by developing and validating protocols for these

processes and to give training courses for the staff. As a result, most of the centers in Europe are performing this procedure in a common, standardized way. The protocols for analysis of CSF Aβ, P- and T-Tau (AD markers) has been done by developing both for clinical practice and for clinical trials. Some scientific highlights of this project consists; (1) The largest subject-level meta-analysis on the prevalence of amyloid abnormality in non-demented subjects was performed; (2) A novel ELISA for neurogranin, a dendritic marker, was validated clinically; (3) A novel fully automated method for CSF Aβ42 measurement was validated; (4) A capillary isoelectric focusing immunoassay for Aβ fragments was developed and validated. Some structural highlights consists: (1) Connecting researchers who work on biomarkers for Alzheimer's and Parkinson's diseases around Europe; (2) The partners set-up a central and virtual biobanks and 5 litres of CSF has been collected; (3) The reference method developed in BIOMARKAPD will now be used for CSF Aβ42 T-tau and P-tau.

POSTERS

[1]

Basal forebrain cholinergic neurons morphology in mouse models of Alzheimer-type and frontotemporal dementia-type tauopathy

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Loss of basal forebrain cholinergic neurons (BFCN) is a hallmark of the Alzheimer's disease (AD) thought to contribute to the cognitive dysfunctions. To this date, the mechanisms underlying cholinergic neurons degeneration remain uncertain. The present study aimed to investigate the relationship between tau neurofibrillary degeneration and cholinergic defects of two tauopathic mouse models: Line 1 (L1), with mild Alzheimer's disease-like tauopathy and Line 66 (L66) with severe frontotemporal lobar degeneration-like tauopathy (FTLD). Experiments were carried out on 3- and 9-month-old animals. Immunohistochemical stainings were performed for cholinergic markers ChAT and p75NTR. BFCNs were less numerous and showed lower expression of ChAT and p75NTR in L1-AD mice, but not in L66-FTLD mice as compared to wild type NMRI mice. The impairments in L1 were observed in interneurons of striatum as well as in projection neurons in medial septum, vertical and horizontal limb of diagonal bands and magnocellular basal nucleus of both age-groups. In summary, obtained results may suggest a loss of cholinergic phenotype or even neuronal deaths in L1-AD animals as early as in 3 month of age with no change in function in L66-FTLD animals at the same age.

This work was founded by NCN grant 2014/15/B/NZ4/05041.

[2]

The point mutations of APP gene (Amyloid beta precursor protein) as a target for SNP-selective RNA degradation by ribonuclease H, using modified antisense oligonucleotides

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Amyloid β precursor protein is an integral membrane protein and occurs especially abundant in the synapses of neurons. It undergoes regulated intramembrane proteolysis that generates beta amyloid polypeptide (Aβ) whose fibrillar form plays a major role in the pathogenesis of Alzheimer's disease. Exons 16 and 17 of the APP gene encode the Aβ peptide. Point mutations of these exons affect metabolism and stability of the Aβ, being the cause of some percentage of familial, dominant AD. In the presented study, three cases of familial, dominant AD-causing mutations: London (V717I), Flemish (A692G) and Arctic (E693G) were investigated as a targets for allele-selective RNA degradation by cellular ribonuclease H. Two different and new approaches using modified antisense oligonucleotides were applied. In total, about 90 antisense oligonucleotides, carrying different nucleotide and non-nucleotide modifications, were tested to activate RNase H to selectively cut the mutant RNA without affecting the wild type form. The highest selectivity of degradation was observed for Flemish RNA variants, that include C-to-G nucleotide substitution. Among 44 designed oligonucleotides to this particular case, 20 caused selective degradation of mutant RNA variant in in vitro assay, but only 8 differentiated the alleles ratio in HeLa cells.

[3]

Purinergic P2X7 receptor is involved in alpha-synuclein-mediated neuronal cell death via dysregulation of PI3K/AKT and AMPK-mTOR signaling pathways

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In parallel to the alpha-synuclein (ASN) hypothesis of Parkinson's disease (PD) aetiology, purinergic receptors have been recently identified as mediators of Lewy bodies accumulation and dysregulation of dopaminergic neurotransmission. However, the mechanisms underlying the disturbances in purinergic signalling in PD are not well established. The aim of our study was to investigate the role of purinergic receptors in mechanisms of ASN-evoked cell death. As a research model we used neuroblastoma (SH-SY5Y) cell line as well as rat synaptoneurosomes treated with exogenous soluble ASN. We observed that exogenous ASN activates P2X7 receptor (P2X7R) resulting in calcium influx and pannexin channel-dependent ATP release. Treatment with either non-selective (PPADS) or selective (AZ11645373) P2X7R antagonist prevented the elevation of cytosolic calcium as well as cytotoxicty evoked by extracellular ASN. We identified that downstream intracellular signalling of ASN induced cytotoxicity implicate two signalling pathways: P2X7R-PI3K/AKT and P2X7R-AMPK-mTOR axis. When exposed to exogenous ASN, overactivation of P2X7R perturbs the balance between those pathways leading to concurrent blockade of the mTOR signalling and neuronal cell death. Our study provide new insight into our knowledge of the relationship between purinergic signalling and ASN and provides a further rationale for anti-purinergic therapy of synucleinopathies.

Supported by NSC grant 2013/09/D/NZ3/01359.

[4]

Cholinesterases inhibition of new tacrine-melatonin heterodimers

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Alzheimer's disease (AD) is the most common form of neurodegenerative dementia affecting elderly and middle-aged people. Cholinesterases (ChE) – acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are acetylcholine (ACh) hydrolyzing enzymes and their inhibition still represents the currently employed approach for the treatment of AD.

As a continuation of our studies, we designed compounds with inhibitory activity against cholinesterases. In this study we report biological activity of several series of hybrid molecules – heterodimeric compounds combining substituted tacrine with a melatonin or cyclic melatonin derivative.

The cyclic derivative – structurally similar to physostigmine – highly improve the neurotransmission of acetylcholine.

The activity of the synthesized compounds was evaluated with spectrophotometric Ellman's method. This method of evaluation of the activity of cholinesterase inhibitors is based on the fact that AChE/BuChE interacts with the inhibitor, thus preventing the acetyl/butyrylcholine hydrolysis. The inhibitor diminishes the enzyme activity which is dependent on the inhibitor concentration.

The new compounds of these novel hybrids exhibit inhibitory activity against cholinesterases, especially BuChE, and can be used as an important starting point for further investigation of the possible use of these compounds in the therapy of neurodegenerative diseases.

This work was supported by the National Science Center Grant DEC-2011/03/B/ST5/01593.

[5]

Discrimination between Alzheimer patients and controls by means of EEG connectivity measures

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Alzheimer disease (AD) instrumental assessment includes biomarkers based on amyloid β and tau proteins in cerebrospinal fluid (CSF), MRI and PET. These procedures are expensive and not widely available (PET, MRI) or invasive (PET, CSF). A promising cheap, largely available, repeatable and non-invasive technique is electroencephalography (EEG). AD deteriorates neuronal networks, so functional brain connectivity as estimated from EEG was hypothesized to discriminate between AD and normal elderly (Nold) individuals.

Resting state eyes-closed EEG data were recorded (19 electrodes of 10-20 system) in 42 Nold and 42 AD subjects with dementia. The connectivity measures: coherences and Directed Transfer Functions (DTF – a measure of directed signal propagation in brain) were analyzed in delta, theta, alpha, beta and gamma bands.

Compared to Nold group, AD showed decrease of EEG coherence and posterior-to-anterior decrease of propagation as revealed by DTF, especially at theta (4-8 Hz) and alpha (8-13 Hz) bands. The features best discriminating between both groups were determined by means of statistical tests and principal components analysis. The classification yielded sensitivity = 86%, specificity = 70%. Including alpha frequency peak into the classification parameters resulted in sensitivity = 90% and specificity = 0.67%. These results indicate promising perspectives of AD assessment with EEG.

Supported by FP7 project DECIDE, www.eu-decide.eu

[6]

Periodontitis and periopathogens as a risk factor for Alzheimer's disease and stroke

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Studies have shown that systemic, peripheral infections affect AD patients. Chronic infection can cause slow progressive dementia and cortical atrophy.

Emerging evidence suggests that poor oral health influences the initiation and/or progression of diseases such as atherosclerosis (including myocardial infarction and stroke), diabetes mellitus and neurodegenerative diseases. Periodontal disease (PD) is a common chronic infectious disease often resulting in tooth loss. An inflammatory response in the periodontal tissues is caused by microorganisms present in dental plaque. Specific bacterial ligands increase the expression of proinflammatory molecules, which activates the innate and adaptive immune systems. Evasion of pathogens from destruction by the host immune reactions leads to persistent infection, chronic inflammation, neuronal destruction and Aβ deposition. Aβ has been shown to be a pore-forming antimicrobial peptide, indicating that Aβ accumulation might be a response to infection.

We started epidemiological studies on a prevalence of PD among Polish AD and post-stroke patients. The first step was focused on a periodontal status analysis in a cohort of 120 patients after (within 72 hours) hemorrhagic and non-hemorrhagic stroke. We found significant differences in the BOP and API values between patients and control subjects which confirm that PD can be predisposing factor to stroke.

[7]

Plasma levels of hsa-miR-107-5p and hsa-miR-650-5p in relation to APOE genotypes in Alzheimer's disease patients

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Background and aims: Alzheimer's disease (AD) is characterized by brain deposition of amyloid-β (Aβ). Apolipoprotein E (APOE) may be involved in Aβ clearance. There are 3 common APOE variants: E2, E3 and E4. The miR-650 probably regulate the expression of APOE. The miR-107 was shown to influence amyloid cascade and is downregulated in AD. The aim of the study was the analysis of relative levels of miR-107 and miR-650 in plasma of AD patients and in control group in relation to APOE genotype.

Material and methods: We investigated 35 AD patients, 42 control subjects and 37 persons in comparative group. The plasma levels of miR-107 and miR-650 were measured by qPCR and normalized per external standard – cel-miR-39-3p.

Results: Preliminary study has shown that the miR-107 was insignificantly decreased in plasma of AD patients and the level of miR-650 was increased in AD patients as compared to controls. In comparative group the normalized relative level of miR-107 was higher than miR-650, in AD patients the relation was reversed. Moreover, the APOE E4 genotype was correlated with decreased levels of both miR-107 and miR-650.

Conclusions: It seems that miR-107 and miR-650 may be associated with pathogenesis of AD, mediated by APOE gene.

[8]

The organization of health care for patients in daily care centers for people with dementia and quality of life of caregivers

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The research was devoted to the issues of organization of patient carewith dementia in Poland in the context of assessing quality of life for those who care.

Both in Poland and the world is still growing number of older people, so we should be interested in problems of people experiencing their own age and to move away from stereotypes defining seniors peripheral social position. In Poland, in the absence of sufficient professional, institutional forms of support for this group of patients, most commonly the entire burden of care falls on family carers. Long-term care for a person with dementia creates multiple load, among which the most important are: psychological stress, physical, economic and social. The main purpose of the research study was to assess whether there is a link between the organization of health assistance (understood as home care vs. institutional care, when the patients spend time in daily care centers) and the quality of life of carers. The data obtained allowed to determine the direction and intensity of the relationship between psychosocial variables and quality of life in both groups were similar and differed among themselves.

[9]

Assessment of sensitivity biomarkers for the determination of Amyotrophic Lateral Sclerosis (ALS) progress

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Amyotrophic lateral sclerosis (ALS) is one of the progressive neurodegenerative diseases of upper and lower motoneurons, leading to death within 3-5 years (with a median survival of 3 years from the symptom onset), mainly because of respiratory inefficiency and hypoxemia. ALS occurs either in familial (fALS) or, more frequently, in sporadic forms (sALS). Approximately 2 per 100,000 people worldwide are affected every year. The aetiology of ALS remains still unclear [Folia Neuropathol 2011, 49(1): 1-13]. Currently, effective treatments for ALS are not available, hence the discovery of sensitive biomarkers for the disease activity can offer tools for the rapid diagnosis and provide some insights into the pathophysiology of ALS, as well as for new therapeutic strategies [Transl Neurodegener 2015, 4: 17]. The present study demonstrates evaluation of the sensitivity, the specificity and relations of erythropoietin (EPO), some metalloproteinases (MMPs), as well as their tissue inhibitors (TIMPs) levels, in sALS patients with mild and severe symptoms. The results of this analysis and our previous clinical studies [J Neural Transm 2010, 117(3): 343-7; Eur J Neurol 2010, 17(2): 226-31] suggest the discriminative and the prognostic potential of CSF EPO and MMP-2 as biomarkers for the recognition/monitoring of ALS progress.

Authors thank Dr Piotr Janik for his neurological consultation in ALS patients and valuable remarks. This study was supported by the statutory budget of the MMRC PAS (Z-526, topic 23). Computations and analyses were carried out using the computational infrastructure of the Biocentrum – Ochota project (POIG.02.03.00-00-0030/09).

[10]

The combination of mass spectrometry and fluorescent methods to study amyloid-beta peptide aggregation inhibition

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Amyloid-β peptide (AB) varies in length from 39 to 43 amino acids and is generated during degradation of amyloid precursor protein. It is believed this peptide is involved in the development of Alzheimer's disease (AD) but its pathological role is not fully understood. To understand the amyloid-β peptide role in Alzheimer's disease a lot of

analytical tools have been proposed, among others mass spectrometry and fluorescent methods.

In our lab we decided to use the combination of these two methods to study AB (1-43) aggregation inhibition in the presence of protein hydrolysates derived from different sources. For this purpose, the AB was incubated in Tris buffer and the progress of aggregation was monitored by triple quadrupole mass spectrometer in multi reaction monitoring mode (MRM) as well as by spectrofluorometer.

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[11]

Oligomeric states of Human Cystatin C variants – atomic force microscopy and spectroscopic studies

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Human Cystatin C (hCC) is a small protein consisting of 120 amino acids that belongs to the type 2 cystatin family, which function is the inhibition of papain- and legumain-like proteases [1]. What is interesting, this protein is fully active in monomeric form, but was observed in dimeric and oligomeric states as well as fibrils.

Hereby we present a study conducted to obtain and characterize high molecular weight oligomers from wild type hCC as well as oligomers of hCC variants with single-point mutations, particularly concerning the residues 68^{th} and 57^{th}. The first is the location of naturally occurring mutation (L68Q) leading to hereditary cystatin C amyloid angiopathy (HCCAA-I) and the second mutation is responsible for the conformational instability leading the dimer formation [2]. Both, oligomers and fibers, were visualized by AFM and TEM techniques. Additionally, we assessed the secondary structure content for all studied proteins using infrared spectroscopy.

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[12]

Studies on budding yeast Hsp31p protein orthologous to Parkinson's disease-associated DJ-1 and to *Candida albicans* Glx3

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Saccharomyces cerevisiae Hsp31p belongs to a DJ-1/ThiJ/PfpI family together with human DJ-1 protein, whose mutations are implicated in hereditary early onset Parkinson's disease and with Glx3 protein that may be involved in *Candida albicans* pathogenicity. Hsp31p was previously shown to be important for survival in the stationary phase of growth and under oxidative stress. Recently, it was identified as a chaperone or as glutathione-independent glyoxalase. To unveil the role played by this protein in budding yeast cells, we investigated its involvement in the protection against diverse environmental stresses. Here, we show that HSP31 gene is controlled by multiple transcription factors, including Yap1p, Cad1p, Msn2p, Msn4p, Haa1p and Hsf1p. They mediate the HSP31 responses to oxidative, osmotic and thermal stresses, to toxic products of glycolysis: methylglyoxal and acetic acid, and to the diauxic shift. We also demonstrate that the absence of the HSP31 gene sensitizes cells to these stressors.

Overproduction of Hsp31p rescues the sensitivity of glo1Δ cells to methylglyoxal and the increased sensitivity of the ald6Δ strain to acetic acid. We postulate that *S. cerevisiae* Hsp31p may have broader substrate specificity than previously proposed and is able to eliminate various toxic products of glycolysis. Elucidating the role of this protein will bring us closer to unraveling the molecular function of its medically important orthologs, human DJ-1 and *C. albicans* Glx3 proteins.

[13]

VDAC and cell viability of the inducible PC12 model of Huntington's disease

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Huntington disease (HD) is a fatal neurodegenerative disorder characterized by a selective loss of neurons, especially from the striatum and deep layers of cerebral cortex. The disease belongs to polyglutamine expansion diseases because is caused by CAG trinucleotide repeat expansion in exon 1 of HTT gene encoding huntingtin (Htt). The repeats number higher than 35 results in its mutant form (mHtt) regarded as HD triggering factor. It is now obvious that mitochondria play a vital role in HD pathogenesis while Voltage-Dependent Anion selective Channel (VDAC) is described as crucial for the organelle functioning. Therefore we decided to estimate the effect of Htt and mHtt expression on VDAC functioning. For that purpose we applied HD model based on PC12 cells derived from a pheochromocytoma of the rat adrenal medulla. The model consists of PC-12HD-Q23 and PC-12HD-Q74 cells with induced (doxycycline) and monitored (GFP labeling) expression of Htt and mHtt, respectively. The obtained results including functional properties of VDAC isolated from PC-12HD-Q23 and PC-12HD-Q74 cells indicate that VDAC may constitute an important element of cytotoxic effect caused by mHtt.

The PC12 cell lines were obtained from David Rubinsztein and Andreas Wyttenbach, UK.

[14]

ATM gene alterations in Polish patients with ataxia-telangiectasia

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Ataxia-telangiectasia (AT, MIM#208900) is a complex genetic neurodegenerative and immunodeficiency disorder, is inherited in an autosomal recessive manner. AT is characterized by cerebellar degeneration, immunodeficiency, premature aging, cancer predisposition, and radiation sensitivity. AT results from mutations in the ataxia telangiectasia mutated gene (ATM).

We screened 105 samples from 49 AT families using cDNA sequencing and multiplex ligation-dependent probe amplification.

58 ATM mutations were identified in 40 patients (72.5%), among which 7 mutations have not previously been reported. New detected variants are: c.8441delC, c.6145T>G, c.434T>G, c.6754_6754delAfsX5, c.4007_4008insA, c.7606G>A and simultaneous deletion of 62 and 63 exons of gene. The mutation types are diverse, including nonsense (47.5% all detected mutations), splicing (38.9%), missense alterations (15.3%) and 1 large genomic deletion. Only 2 mutations have been found in homozygous state ([c.4007_4008insA];[c.4007_4008insA]), ([c.9021_9022insA]; [c.9021_9022insA]). Most frequent mutations among our AT patients are: c.5932G>T (7), c.6095G>A (10), c.7630-2A>C (11).

In this study, we confirmed status of recurrent mutations, but also detected new changes in ATM sequence. Most of the Polish patients are compound heterozygotes and none of them having the same combination of mutations, what makes molecular diagnostic more difficult.

[15]

Gender differences in cell death in cells harboring Leber's hereditary optic neuropathy mutations

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Leber's hereditary optic neuropathy (LHON) is a maternally inherited form of central vision loss due to optic nerve degeneration caused by point mutations in mitochondrial DNA (mtDNA). In most cases, the mutated mtDNA is present at about 100% in every cell, but only retinal ganglion cells (RGC) are affected. LHON in general has an early onset between the age of 20 and 30 years and male preponderance (men are four to five times more likely to develop the disease). The aim of this study is to determine gender differences in cell death under the influence of sex hormones.

Primary results have shown no difference in the level of apoptosis in LHON affected males and healthy controls after application of both testosterone and estradiol. The obtained results confirmed findings of other authors that cell death in cells with LHON mutations takes place via a caspase-independent pathway. In the female control cell line the level of apoptosis was much higher after induction of oxidative stress and application of testosterone even in low concentrations, but in regular conditions the same levels of testosterone did not trigger apoptosis.

[16]

AMPA receptors modulate Store-Operated Calcium Entry and interact with STIMs in rat cortical neurons

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The process of store-operated calcium entry (SOCE) leads to refilling the endoplasmic reticulum (ER) with calcium ions (Ca^{2+}) after their release into the cytoplasm. The interaction between (ER)-located proteins (STIM1, STIM2) and plasma membrane-located Ca^{2+} channel protein (ORAI1) mediates the formation of complexes and underlies SOCE in non-excitable cells. Our previous data indicated that STIMs are involved in Ca^{2+} homeostasis in neurons, form complexes with endogenous ORAI1, but play a distinct role in SOCE. In contrast to non-excitable cells, Ca^{2+} influx in neurons is modulated mainly by voltage-gated Ca^{2+} channels and ionotropic receptor-operated Ca^{2+} channels. Here we report that endogenous STIM1 and STIM2 interact with endogenous GluA1 and GluA2, AMPA receptors (AMPARs) subunits, using co-immunoprecipitation assays. To assess the role of AMPARs in SOCE, they were inactivated by their specific inhibitors. Single-cell Ca^{2+} measurements showed that in the presence of NBQX or CNQX SOCE was ~3.7 or ~2.2 times decreased, respectively. In addition, AMPA-induced calcium signal was reduced by 80% or by 53% by SOCE inhibitors, ML-9 or SKF96365, respectively. Altogether, our data suggest that STIMs in neurons can control AMPA-induced Ca^{2+} entry as a part of the mechanism of SOCE.

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[17]

Electrochemical Biosensors for Detection of Alzheimer's Disease Markers

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Alzheimer disease is the most common form of dementia. Therefore the development of biosensors destined for screening of naturally occurring compounds, which might be used as the preventing agents, as well as for early diagnostics of Alzheimer disease is very demanding. We proposed the several biosensors destined for the above purposes.

The immobilization of Aβ1-40 was performed on Au-colloid modified gold electrodes as well as on the HS-aliphatic acid monolayer through EDC/HNS activation. Theses types of biosensors were used for determination of interaction between Aβ1-40 and selected alkaloids.

In the next type of biosensors, RAGE domains were covalently immobilized on the redoxactive monolayer

through interactions with polyhistidine tag. These biosensor were applied for determination Aβ peptides, S100B protein and glycated albumin, the potential markers of Alzheimer disease.

Cyclic voltammetry and electrochemical impedance spectroscopy and surface plasmon resonance were used as the measuring systems. The presented biosensors might be very useful tools for early diagnosis of Alzheimer disease.

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[18]

Dissociation of amyloid aggregates with photo-switchable molecular levers

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As a consequence of the society aging in Europe, the occurrence of cognitive impairment and dementia is rapidly becoming a significant challenge. Up to 70% of dementia cases in EU is due to the Alzheimer's disease (AD) – a neurodegenerative disease with no cure. With the increasing proportion of the elderly among Europeans, this problem is dramatically growing, especially in Western Europe, where the population suffering from dementia is reaching now ~7.5 million people. Moreover, AD is becoming a severe economic issue. The cost of dementia for the 2015 in Europe has been estimated for 200 billion Euros, and increased by 25% in the last five years. Despite huge and long term research efforts there is still no cure for AD. Considering that, a question rises – where else shall we look for new and effective treatments? We believe that the answer for this big question may lie in a newly designed derivatives of a small but very portentous photoresponsive molecule called azobenzene.

[19]

Two faces of one disease – difference in cell cycle regulation and apoptotic response between lymphocytes from familial and sporadic Alzheimer's disease patients

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Alzheimer's disease (AD) was first described over 100 years ago. It is the most common cause of dementia with an estimated prevalence of 30 million people worldwide. A growing body of data has shown that AD is characterized by complex alterations in cellular processes that occur not only in neurons, but also in peripheral cells such as lymphocytes. Recently we have demonstrated that lymphocytes from the sporadic form of AD (SAD) show G1 phase arrest and increased levels of protein p21, the key regulator of apoptosis and the G1/S cell cycle checkpoint. Since it is known that p21, besides controlling the G1/S checkpoint, can regulate apoptosis, we conducted studies to determine if p21 levels play a role in the cellular response to an oxidative stress challenge like 2d-ribose (2dRib) treatment.We report here that cells from familial AD (FAD) aremore resistant to 2dRib-induced cell death than control or SAD cells. p21 mRNA and protein levels significantly increased in FAD cells in response to 2dRib. In addition, we found a higher cytosolic accumulation of p21 in FAD cells. Transcriptional activation of p21 was shown to be dependent on p53, as it can be blocked by PFT-a and was correlated with phosphorylation of p53. Thus in human B-lymphocytes under oxidative stress evoked by 2dRib, 7 PS1 mutants seem to strongly exacerbate phosphorylation of p53 exhibiting a gain of function effect over wtPS1. Altogether, our results showed that the mechanism of apoptotic response to acute oxidative stress distinguishes cells from SAD and FAD patients.

[20]

Potent 5-HT6 receptor antagonists for the treatment of Alzheimer's disease

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Alzheimer's disease, an irreversible neurodegenerative disorder, constitutes one of the most frequent forms of dementia. AD is characterized by progressive deterioration of cognitive functions. In recent years 5-HT6 receptor has emerged as a promising molecular target for the treatment of cognitive deficits associated with AD [1].

Herein we present the development of novel class of 5-HT6R antagonists based on 1H-pyrrolo[3,2-c]quinoline core. The study allowed for identification of compound 14 (S)-1-[(3-chlorophenyl)sulfonyl]-4-(pyrrolidine-3-yl-amino)-1H-pyrrolo[3,2-c]quinoline, more selective and potent 5-HT6R antagonist than the reference compound SB-742457. Further evaluation of 5-HT6Rs constitutive activity at Gs signaling revealed that 14 behaved as a neutral antagonist, while SB-742457 was classified as an inverse agonist [2,3].

Compounds 14 and SB-742457 reversed phencyclidine memory deficits and displayed procognitive properties in cognitively unimpaired animals in NOR tasks. Additionally, compound 14 demonstrated higher anxiolytic effect than SB-742457 in Vogel test and showed similar antidepressant-like properties in FST.

These results support therapeutic potential of 5-HT6R antagonists and inverse agonists in the treatment of cognitive decline associated with neuropsychiatric disorders like autism, Alzheimer's or Parkinson's disease.

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[21]

New rare variants of TREM 2 gene involved in neurodegeneration

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Introduction: The genetic basis of late-onset Alzheimer disease is complex. Variation in multiple genes have been suspected to lead to the disease. Several candidate genes are involved in immune response and inflammation pathways. TREM2 (triggering receptor expressed on myeloid cell2) is one of them. It is known to be expressed on the cell membrane of a subset of myeloid cells, including microglia. TREM2 increases phagocytic pathways and suppresses inflammatory reactivity. Reduce function of TREM2 may be connected with Alzheimer's conditions and other neurodegenerative disorders. Homozygous loss-of-function mutations in this gene cause Nasu-Hakola disease. Several studies have reported the TREM2 R47H rare variant to be a risk factor for AD.

Methods: TREM2 exon2 in 208 neurologically normal controls, 274 AD, 194 ALS and 135 FTD patients were sequenced.

Results: Nine rare variants located in exon 2 of TREM2 were identified. Seven of them were reported previously. Novel synonymous variant (G29G) and single nucleotide insertion in 3' intron splice site (c.41-2_3insA) were identified for the first time, only in ALS patients.

Conclusion: Little is known about the biochemical mechanisms that underline the connection between neurodegeneration and TREM2 however the results indicate that rare variants are associated with an increase in neurodegenerations susceptibility.

[22]

Combined metabolomics and transcriptomics approaches to assess the IL-6 blockade as a therapeutic of ALS: deleterious alteration of lipid metabolism

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Playing an important role in the regulation of systemic metabolic regulation and neuroinflammation, interleukin-6, a major cytokine of the inflammatory response, has been proposed as a target for management of amyotrophic lateral sclerosis. Although one pilot clinical trial provided promising results in humans [1], another one recent preclinical study showed that knocking-out interleukin-6 gene in mice carrying amyotrophic lateral sclerosis did not improve clinical outcome [2]. We aimed to determine the relevance of the IL-6 pathway blockade in a mouse model of ALS, by using a pharmacological antagonist of interleukin 6, a murine surrogate of tocilizumab, namely MR16-1. We first characterized immunological and metabolic status of untreated SOD1*G93A (mSOD1) mice comparatively to wild-type mice, and then we compared treated versus untreated mSOD1 mice.

Metabolomics and transcriptomics analyses revealed that metabolic effects of IL-6 blockade were mild compared to metabolic disturbances observed in ALS condition, which include especially tryptophan, arginine and proline metabolism pathways (including polyamines). MR16-1treatment mainly affected lipid metabolism. Immunological analysis showed a significant increase of regulatory T cells count (p = 0.0268) and a decrease of CXCL1 (mKC) concentrations in plasma (p = 0.0479). Finally, a deleterious clinical effect of MR16-1 was revealed, with a speeding up onset of weight loss (p = 0.0041) and decreasing body weight (p < 0.05).

As metabolic pathways involved in MR16-1 therapy have been previously clearly described in ALS, we may suspect that IL-6 blockade had negative effect through a multiparametric effect, despite a significant anti-inflammatory effect. Together, these results indicate that IL-6 blockade did not improve clinical outcome of mSOD1 mouse model of ALS.

Key words: interleukin-6, metabolomics, transcriptomics.

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