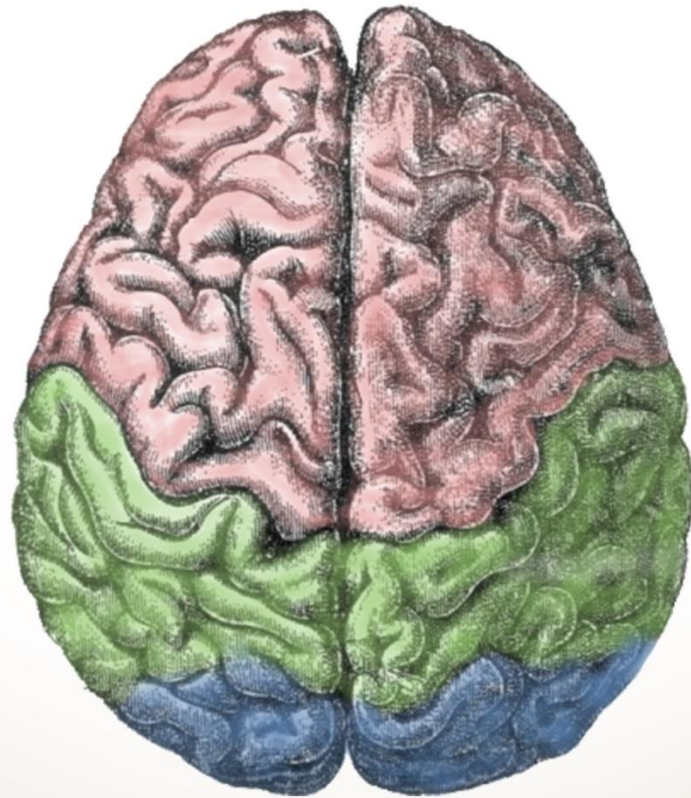


THE 2016 ALZHEIMER'S DISEASE CONGRESS



ABSTRACTS

7TH – 9TH JUNE 2016
LONDON, UK

EuroSciCon 

This three day international academic congress will bring to light the current research and treatments being developed for Alzheimer's Disease.

Alzheimer's Disease has been recognised as the most prevalent form of dementia; an ever-growing issue in an ageing society. Over three days the Alzheimer's Disease summit will discuss many aspects of disease progression, development and treatment in an informal academic setting. Topics for discussion include prediction and prevention strategies, vaccine development, drug discovery and care. With plenty of opportunity for networking and debate, this informal international meeting will bring you up to date with current research and thinking regarding Alzheimer's Disease.

This event has [CPD accreditation](#)

This abstract book will be finalised two weeks before the event

www.lifescienceevents.com/alz2016

#Alz2016

Table of Contents

Invited Speakers Abstracts.....	7
Alzheimer's Disease: a clinical viewpoint.....	7
Inflammation in Dementia with Lewy bodies and Alzheimer's Disease	7
Associations of sorLA/SORL1 with Alzheimers disease.....	7
Calcium, Memory and Alzheimer's disease	7
Cholesterol's contribution to autophagy deficits in Alzheimer's Disease.....	7
Computational studies on the toxicity mechanisms of amyloid beta peptides aggregation in relation to Alzheimer's disease	8
Where did we go wrong in the study of APP processing	8
Exploring the Glutamatergic Hypothesis for the Pathogenesis of Down Syndrome: Potential Lessons for our Understanding of Alzheimer's Disease.....	8
The involvement of type-1 interferon signalling and resultant neuroinflammation Alzheimer's Disease.....	8
Loss of neuroprotective lipids and myelin as a key sensitizing factor in Alzheimer's pathogenesis.	8
The role of Pb in AD pathogenesis	9
Good things in small packages: The molecular chaperone action of the small heat shock chaperone proteins.....	9
Functional imaging markers of cognitive decline in preclinical Alzheimer disease	9
PET imaging biomarkers in Alzheimer's Disease	9
The role of heparan sulfates in the amyloid pathology of Alzheimer disease.....	10
Link between the modifiable risk factors of Alzheimer's disease and neuroinflammation.....	10
Granulovacuolar degeneration, a neurodegenerative change that accompanies tau pathology	10
Sigma-1 receptor ligand and SSRI Fluvoxamine modulates production of amyloid beta peptides and is protective in J20 Alzheimer disease mice.....	10
Early molecular and electrophysiological alterations during the asymptomatic stage of Alzheimer's disease: hippocampus versus retina	10
The regulation of metastable proteins in neurodegenerative Diseases.....	11
Characterizing Prodromal Alzheimer's Disease Using Multimodality Imaging.....	11
Psychological treatments for depression and anxiety in dementia and mild cognitive impairment: systematic review and meta-analysis.....	11
EEG biomarkers and profiling of AD mouse lines	11
Neurophysiological vulnerability to aging associated with the Alzheimer's risk variant in CLU gene.....	12
Multiple roles of cholinergic neurons in the modulation of amyloid production	12
Chaperoning Tau aggregation	12
Predicting Progression to Mild Cognitive Impairment in Cognitively Unimpaired Individuals Using Neuroimaging Biomarkers	12
Targeting Nucleophilic Attack During the Lag-phase of Beta-Amyloid Oligomerization	12
The drug therapy to increase stem cells for treatment of Alzheimer/s disease	13
What makes an amyloidogenic protein toxic? Dissecting the sequence - self-assembly -toxicity relationship for amyloid peptides.	13
The role of proliferating astrocytes in Alzheimer's disease	13
Potential therapeutic strategies of Cerebrolysin in Alzheimer's Disease	13

Drugs used in multiple pathologies, with stresses common to Alzheimers disease, offer candidate drugs and vaccine potential for Alzheimers disease	14
Paradigm Shift: Semantic memory decline as a biomarker of preclinical Alzheimer’s disease.....	14
Smell identification function in Alzheimer's disease	14
Dystrophic neurites are sites of microtubule disruption, BACE1 elevation, and increased A β generation: the potential role of A β oligomers.....	14
Biomedical nanotechnology and Alzheimer's. Can the disease be reversed?.....	15
Alpha2 adrenergic receptor as a novel target for Alzheimer's disease	15
Type 2 diabetes mellitus accelerate tau pathology in nonhuman primate	15
Day 1:	16
Oral Presentation Abstracts	16
UBIQUITOME PROFILE IN DOWN SYNDROME BRAIN PRIOR TO AND AFTER DEVELOPMENT OF ALZHEIMER NEUROPATHOLOGY	16
WHAT IF DEMENTIA WAS DUE TO CALCAREOUS?.....	16
GLIAL REGULATION OF NEOCORTICAL SYNAPTIC TRANSMISSION: IMPLICATION FOR ALZHEIMER’S DISEASE	17
THE IMPAIRMENT OF BILIVERDIN REDUCTASE-A PROMOTES BRAIN INSULIN RESISTANCE IN ALZHEIMER DISEASE: A NOVEL MECHANISM	18
Day 2:	19
Oral Presentation Abstracts	19
MINI SKQ (SEMANTIC KNOWLEDGE QUESTIONNAIRE): 12 QUESTIONS TO HIGHLIGHT SEMANTIC DETERIORATION IN ALZHEIMER’S DISEASE.....	19
RETINAL BIOMARKERS IN AD	20
OXIDATIVE SIGNATURE OF CSF FROM MILD COGNITIVE IMPAIRMENT AND ALZHEIMER DISEASE PATIENTS	20
Day 3:	21
Oral Presentation Abstracts	21
THE USE OF NEUROPEPTIDES OF “ADEMENT” FOR THE DEVELOPMENT DELAY OF THE NEURODEGENERATIVE PROCESSES: THE STUDY ON THE MODEL OF DROSOPHILA MELANOGASTER.....	21
MICRORNA CONTRIBUTION IN A NEW THERAPEUTIC STRATEGY FOR ALZHEIMER’S DISEASE	22
Poster Presentation Abstracts	24
AN ORIGINAL MEDICINE FROM TROPHINOTROPINE GROUP FOR THE TREATMENT OF CEREBROVASCULAR AND NEURODEGENERATIVE DISEASES.....	24
EYE MOVEMENT BEHAVIOR AND ALZHEIMER’S DISEASE: OCULOMOTOR PARAMETERS PREDICTIVE OF COGNITIVE DECLINE.....	25
THE IMPACT OF TIME AND COGNITIVE EFFORT IN EYE MOVEMENT BEHAVIOR: CONTRIBUTIONS OF OCULOMOTOR PARAMETERS IN THE EARLY DETECTION OF ALZHEIMER’S DISEASE.	26
TARGETING OF PERK KINASE VIA SMALL MOLECULE INHIBITORS AS A NEW TREATMENT THERAPY FOR ALZHEIMER’S DISEASE	26
THE ROLE OF PERK KINASE AND UNFOLDED PROTEIN RESPONSE IN THE PATHOGENESIS OF ALZHEIMER’S DISEASE	27
COMPONENTS OF UNFOLDED PROTEIN RESPONSE SIGNALING PATHWAY AS A THERAPEUTIC TARGET FOR ALZHEIMER’S DISEASE	28
MOLECULAR GENETIC INSIGHTS IN ALZHEIMER’S DISEASE	29
GOLD NANOPARTICLES-BASED RAPID ANTI-AGGREGATION DRUG SCREENING METHOD FOR REALIZING PERSONALIZED MEDICINE OF ALZHEIMER’S DISEASE.....	30

REAL-TIME MONITORING OF REACTIVE OXYGEN SPECIES GENERATED IN NEURONAL CELLS BY AMYLOID β AGGREGATES VIA A NOVEL PLASMONIC METHOD	30
MEMORY PALACES TO IMPROVE QUALITY OF LIFE IN ALZHEIMER'S DISEASE	31
THE ROLE OF NUCLEAR-MITOCHONDRIAL INTERACTIONS THAT INFLUENCE NEUROCOGNITIVE IMPAIRMENT IN ALZHEIMER'S DISEASE	31
FUNCTIONAL BRAIN NETWORKS IN EARLY DIFFERENTIAL DIAGNOSIS OF DEMENTIA.....	32
THE IMPAIRMENT OF BILIVERDIN REDUCTASE-A PROMOTES BRAIN INSULIN RESISTANCE IN ALZHEIMER DISEASE: A NOVEL MECHANISM.	32
ALZHEIMER'S DISEASE RISK ALLELES AND CUMULATIVE GENETIC RISK ARE LINKED TO HETEROGENEITY IN MICROGLIA AND PATHOPHYSIOLOGY	33
NITRO-PROTEOMICS TO DISCOVER SYNAPTOSOMAL BIOMARKERS OF NEUROINFLAMMATION IN ALZHEIMER'S DISEASE..	34
A SYSTEMATIC REVIEW OF POSITIVE PSYCHOLOGY MEASURES FOR FAMILY CARERS OF PEOPLE LIVING WITH DEMENTIA..	34
USE OF AUTOMATED TECHNOLOGY-BASED ADHERENCE AIDS TO IMPROVE MEDICATION ADHERENCE IN PATIENTS WITH DEMENTIA: A SYSTEMATIC LITERATURE REVIEW	35
LONG-TERM EFFECTS OF IMMUNOSTIMULATION ON SYNAPTIC PLASTICITY AND NEURODEGENERATION	36
CHEMICAL MECHANISMS OF HDL DYSFUNCTIONAL IN ALZHEIMER'S DISEASE	36
ROLE OF ELECTRONEGATIVE LDL IN DYSREGULATED HEPATIC GLYCOSYLATION ASSOCIATED OBSTRUCTIVE SLEEP APNEA .	37
APOLIPOPROTEIN E -491 GENE POLYMORPHISM AND ALZHEIMER'S DISEASE: A SYSTEMATIC REVIEW AND META-ANALYSIS	37
ATP-BINDING CASSETTE TRANSPORTER G1 AND APOLIPOPROTEIN M ARE "NOVEL PLAYERS" IN CHOLESTEROL TRANSPORT AT THE BLOOD-BRAIN BARRIER	38
WHITE MATTER CHANGES IN ALZHEIMER'S DISEASE: A FOCUS ON OLIGODENDROCYTES	39
PROBING AMYLOID BETA-INDUCED CELL DEATH USING A FLUORESCENCE-PEPTIDE CONJUGATEN IN ALZHEIMER'S DISEASE MOUSE MODEL	40
BEXAROTENE MODULATES CHOLESTEROL AND AMYLOID-BETA METABOLISM IN AN IN VITRO MODEL OF THE BLOOD-BRAIN BARRIER.....	40
IN VITRO AND IN VIVO INVESTIGATIONS OF TYROSINE OXIDATION IN AMYLOID-BETA ($A\beta$)	41
ABERRANT CELLULAR MORPHOLOGY IN INDUCED HUMAN ASTROCYTE MODELS OF ALZHEIMER'S DISEASE.	41
EEG MEASURES OF NEURAL PLASTICITY IN MILD COGNITIVE IMPAIRMENT AND ALZHEIMER'S DISEASE.....	42
RELATIONSHIP BETWEEN CORTEX AND AUTONOMIC NERVOUS SYSTEM BY MENTAL ACTIVITY IN PATIENTS WITH MILD COGNITIVE IMPAIEMENT	42
EFFECT OF THE KETOGENIC DIET ON BLOOD-BRAIN BARRIER.....	43
EFFECT OF BEXAROTENE ON $A\beta$ PEPTIDE EFFLUX ACROSS THE HUMAN BLOOD-BRAIN BARRIER IN VITRO	44
RELATIONSHIP BETWEEN CORTEX AND AUTONOMIC NERVOUS SYSTEM BY MENTAL ACTIVITY IN PATIENTS WITH MILD COGNITIVE IMPAIEMENT	45
TAU PATHOLOGY IN AGED CYNOMOLGUS MONKEYS WITH LONG-TERM TYPE 2 DIABETES MELLITUS.	45
THE ROLE OF EXTRACELLULAR VESICLES IN THE PROCOAGULANT PROFILE OF ALZHEIMER'S DISEASE PATIENTS.....	46
INTERACTIONS OF SIMVASTATIN AND APOJ WITH APP PROCESSING AND AMYLOID-B CLEARANCE IN BLOOD-BRAIN BARRIER ENDOTHELIAL CELLS.....	47
A DEMENTIA SCREENING INVESTIGATION FOR INHABITANTS OF RURAL AREAS IN JAPAN.....	48
ANTIBODIES TO SIGNALING MOLECULES AND RECEPTORS ARE ASSOCIATED WITH LONGITUDINAL RISK OF PSYCHOSIS AND DEATH IN ALZHEIMER'S DISEASE	48
A HAPLOTYPE OF THE PURINERGIC <i>P2Y12</i> GENE IS ASSOCIATED WITH INCREASED RISK OF ALZHEIMER'S DISEASE.....	49

Invited Speakers Abstracts

Alzheimer's Disease: a clinical viewpoint

Dr Amit Arora, University Hospital of North Staffordshire, British Geriatrics Society, Stoke On Trent, UK

Inflammation in Dementia with Lewy bodies and Alzheimer's Disease

Dr Jay Amin, University of Southampton, Clinical Neurosciences Division, Memory Assessment and Research Centre, Moorgreen Hospital, Southampton, Hampshire, United Kingdom

There is growing evidence supporting a role for inflammation in the aetiology of Alzheimer's Disease (AD) but currently there is little evidence in Dementia with Lewy bodies (DLB).

We are conducting a clinical observational study and post-mortem neuropathology study to investigate whether central and systemic inflammatory processes are altered in DLB compared to AD and controls, and whether these changes are associated with the neuropathological and clinical features of the disease.

A greater understanding of the role of inflammation in DLB will determine if pharmacological therapies based on established inflammatory changes in AD are likely to be viable in DLB.

Associations of sorLA/SORL1 with Alzheimers disease

Dr. Olav Michael Andersen, Aarhus University, Aarhus, Denmark

The gene encoding the sorLA protein (SORL1) is associated with early- and late-onset Alzheimer's disease (AD), and sorLA protein level is decreased in brains from many AD patients.

We have initially identified sorLA as a sorting receptor for APP, where neuronal sorLA activity protects against the amyloidogenic cleavage of APP.

Our recent studies have identified novel ways to regulate intraneuronal levels of functional sorLA available to act in APP transport and processing, including control mechanisms for sorLA shedding and identification of cis- and trans-regulatory elements that control receptor expression.

In conclusion, we describe novel mechanisms that influence sorLA activity that may be involved in AD onset.

Calcium, Memory and Alzheimer's disease

Sir Michael Berridge, The Babraham Institute, Babraham Research Campus, Cambridge, United Kingdom

Alzheimer's disease (AD) begins with a decline in memory. Accumulation of the amyloid β (Ab) protein in the brain alters the calcium signalling mechanism responsible for memory formation. A progressive increase in the resting level of Ca^{2+} mimics the elevation in calcium that occurs during sleep that is responsible for memory erasure. In AD, therefore, such a rapid erasure of memories soon after they are acquired during the wake period means that they are not retained long enough to be stored during sleep. Vitamin D may reduce the risk of AD by reducing the resting level of calcium

Cholesterol's contribution to autophagy deficits in Alzheimer's Disease

Dr Anna Colell, Institute of Biomedical Research of Barcelona, Spain

Aberrant autophagy in Alzheimer's Disease (AD) is evidenced by a massive build-up of autophagy intermediates. The exact mechanisms that lead to this dysfunction are largely unknown; however, as similar defects are observed in Niemann-Pick type C1 knockout mice, which accumulate cholesterol in endolysosomes, we hypothesize that the impaired cholesterol homeostasis described in AD may play a role. Using APP/PS1 mice that overexpress the active truncated form of sterol regulatory element-binding protein-2 (SREBP-2) we demonstrate that cholesterol can regulate beta-amyloid-induced autophagy by blocking the autophagolysosome maturation and impeding the correct engulfment of damaged mitochondria.

Computational studies on the toxicity mechanisms of amyloid beta peptides aggregation in relation to Alzheimer's disease

Lock Chew, Nanyang Technological University, Singapore

In this talk, I will present our computational studies on various potential toxicity mechanisms resulting from the aggregation of amyloid beta peptides mediated by the cell membrane, or induced through the protein S100A9. In addition, the effect of amyloid beta peptide on DNA damage in association with neuronal dysfunction will be discussed. Through our simulation studies, we have uncovered approaches that can circumvent the oligomerization of the amyloid beta peptides using molecules such as the curcumin and heme. The underlying mechanisms in which this occur will be highlighted in this talk. By elucidating these diverse mechanisms obtained through our extensive molecular dynamics simulations, I hope to throw light on the pathogenesis of Alzheimer's disease as well as the possible means for its prevention.

Where did we go wrong in the study of APP processing

Dr. Ming Chen, University of South Florida, Tampa, United States

Why and how are plaques and tangles formed in sporadic Alzheimer's disease (sAD)? To this central question, I believe that plaques and tangles are resulting from natural aging, not from any "aberrant pathways". From this starting point, I revisit current concepts in APP processing and emphasize that A β levels are determined by α -secretase alone, thus boosting this enzyme's activity is the only rational approach for intervention. Indeed, perception of sAD as a "discrete disease" and overly focusing on the presumptive " β - and γ -secretases" are the reasons why sAD has remained as an enigma today (see my paper: <http://www.ncbi.nlm.nih.gov/pubmed/26052267>).

Exploring the Glutamatergic Hypothesis for the Pathogenesis of Down Syndrome: Potential Lessons for our Understanding of Alzheimer's Disease.

Dr Alberto Costa, Case Western Reserve School of Medicine, Cleveland, OH, United States

In this talk, I will provide a brief account of the relationship between Down syndrome and Alzheimer's disease. This will be followed by a short description of the rational basis of current translational work on potential pharmacological therapies for Down syndrome, with an emphasis on the "glutamatergic hypothesis". Finally, I will describe the rationale behind the design of a new, Phase II clinical trial of the drug memantine on improving cognition of adolescents and young adults with Down syndrome (NCT02304302 at <http://www.clinicaltrials.gov>), which is being conducted at University Hospitals Case Medical Center and the Cleveland Clinic.

The involvement of type-1 interferon signalling and resultant neuroinflammation Alzheimer's Disease

Dr. Peter Crack, The University of Melbourne, Melbourne, Australia

The CNS can exhibit features of inflammation in response to injury, infection or disease. This presentation will focus on the involvement of the type-1 interferon (IFN) signalling system in regulating the neuroinflammation that is resultant in Alzheimers disease (AD) neuropathology. The data presented in this talk highlights that type-1 IFNs are a previously unrecognised protein class involved the development and progression of the neural injury/degeneration seen in AD. We propose that type-1 IFN signalling plays a key role in the regulation of the cellular environment that elicits neuroinflammation AD.

Loss of neuroprotective lipids and myelin as a key sensitizing factor in Alzheimer's pathogenesis.

Dr Anthony Simon Don, University of New South Wales, Sydney, NSW, Australia

The greatest genetic risk factor for Alzheimer's Disease (AD) is inheritance of the E4 allele of the major brain lipoprotein, Apolipoprotein E. ApoE-containing lipoprotein particles carry abundant myelin lipids and potent signalling lipids such as neuroprotective sphingosine 1-phosphate (S1P). We have observed a pronounced depletion of these lipids in pre-clinical and clinical AD, and hypothesize that loss of neuroprotective lipids sensitizes vulnerable brain regions to synapse loss and neuronal atrophy in AD. In particular, loss of S1P tracks very closely with early disease pathology. S1P receptor agonists that are currently used in multiple sclerosis therapy may be applicable for AD.

The role of Pb in AD pathogenesis

Dr. Yansheng Du, Indiana University School of Medicine, Indianapolis, United States

Accumulation of beta-amyloid (Abeta) in brain extracellular space is believed to be an initial feature of Alzheimer's disease (AD) pathogenesis. Recent human epidemiological evidences suggest an association between human lead (Pb) exposure and altered gene expressions relevant to the Abeta production. Animal data also show a reduced Abeta clearance from the Pb-exposed brain. In the current study, we used human Tg-SWDI APP transgenic mice, which genetically over-express amyloid plaques at age of 2–3 months, to investigate roles of Pb in amyloid plaque formation. Data showed that subchronic oral Pb exposure (50 mg/kg Pb acetate) increased the Abeta levels in the CSF, brain cortex and hippocampus; there was also a significant increase of amyloid plaques in brains of these animals. Interestingly, these mice showed an impaired spatial learning ability. Mechanistic studies revealed that Pb damaged the blood-brain barrier and directly participated in physiochemical reaction and facilitated the Abeta fibril formation. Our effort to use the dynamic contrast-enhanced computed tomography (DCE-CT) to study effects of Pb on real-time brain regional blood flow, blood volume, and BBB permeability is currently in progress.

Good things in small packages: The molecular chaperone action of the small heat shock chaperone proteins

Associate Professor Heath Ecroyd, ARC Future Fellow, School of Biological Sciences, Illawarra Health and Medical Research Institute, University of Wollongong, Australia

The small heat-shock molecular chaperone proteins (sHsps) are one of the cell's first-lines of defence against protein aggregation. They therefore represent attractive therapeutic targets for the treatment of diseases associated with protein aggregation such as Alzheimer's disease. Recently our work has focused on defining the key region(s) in sHsps responsible for their chaperone activity. We have demonstrated that the core alpha-crystallin domain is a potent inhibitor of amyloid- β peptide fibril formation and its associated cytotoxicity. Our experiments therefore identify a novel, small and highly structured 'functional unit' of sHsps which potentially enables more rational design of sHsp activators and inhibitors.

Functional imaging markers of cognitive decline in preclinical Alzheimer disease

Dr. Panteleimon Giannakopoulos, University Hospitals of Geneva, Genève, Switzerland

We investigated whether subtle cognitive deterioration in healthy elderly individuals could be predicted by ASL imaging and EEG markers. A longitudinal study included 75 stable controls (sCON) and 73 deteriorated controls (dCON) at 18-month clinical follow-up and 65 patients with mild cognitive impairment (MCI). Continuous EEG was recorded during a n-back working memory task and two-dimensional pulsed ASL was performed at the baseline visit. Reduced ASL in the posterior cingulate cortex was associated with the development of subtle neuropsychological deficits. Three EEG indices distinguished the two control groups: alpha and beta even related desynchronization (dCON > sCON) and beta inter-trial coherence (dCON < sCON). These results will be discussed as a paradigm of predictive biomarkers in preclinical forms of dementia.

PET imaging biomarkers in Alzheimer's Disease

Professor Karl Herholz, University of Manchester, Manchester, United Kingdom

Positron emission tomography (PET) is providing functional and molecular imaging for differential diagnosis and monitoring of progression in dementia. Regional changes of synaptic activity, which show characteristic patterns in many neurodegenerative diseases, can be imaged with FDG PET, typically 1-2 years before the onset of dementia. Several ligands are now available to demonstrate fibrillary cortical amyloid beta deposits, including 11C-PIB (used in clinical research since more than 10 years) and 18F-ligands florbetapir, florbetaben, and flutemetamol, which have received licenses for diagnostic use by FDA and EMA. They offer very high sensitivity to detect Alzheimer's disease (AD), and their accuracy has been demonstrated by post-mortem pathological verification. Positive findings with these tracers can probably precede the clinical manifestation of dementia by up to 20 years. They are commonly being used as imaging biomarkers in clinical trials of anti-amyloid agents, and their ability to increase diagnostic certainty has been demonstrated in clinical settings. Recently, PET tracers have also been developed for imaging of pathological tau deposits, which are common in AD and other neurodegenerative conditions.

The role of heparan sulfates in the amyloid pathology of Alzheimer disease

Ms Charlotte Jendresen, Department of Pharmacology, University of Oslo and Oslo University Hospital, Oslo, Norway

Heparan sulfate proteoglycans are found in extracellular matrices and cell surfaces in all vertebrate tissues. They co-localize with amyloid-beta (A β) in Alzheimer disease (AD) amyloid plaques. In vitro studies show that heparan sulfates (HS) augment A β -aggregation, but their role in vivo has remained uncertain. HS is fragmented by heparanase. By overexpressing heparanase in an A β precursor protein (A β PP) transgenic mouse model, we found that fragmented cerebral HS lowered the amyloid burden in A β PP-transgenic mice. We suggest that HS plays an important role in amyloid deposition in AD by increasing aggregation and possibly spreading of A β in the brain.

Link between the modifiable risk factors of Alzheimer's disease and neuroinflammation

Dr Andis Klegeris, University of British Columbia Okanagan Campus, Kelowna, British Columbia, Canada

Studies have identified diets high in polyunsaturated fats, sedentary lifestyle, as well as lifestyle-induced diseases, such as type 2 diabetes mellitus, as risk factors for developing Alzheimer's disease. The same risk factors can also contribute to gliosis and sustained neuroinflammatory environment, which may drive progression of the specific mechanisms of this neurodegenerative disease. Several cellular and molecular mechanism linking select modifiable risk factors of Alzheimer's disease and neuroinflammation will be described. This line of research could identify novel targets for treating or managing Alzheimer's diseases since adverse glial activation and neuroimmune mechanisms are thought to contribute to the disease pathogenesis.

Granulovacuolar degeneration, a neurodegenerative change that accompanies tau pathology

Dr Christoph Köhler, Institute of Anatomy, University of Cologne, Cologne, Germany

Severe granulovacuolar degeneration (GVD) of neurons in the hippocampus is a neuropathological feature of Alzheimer's disease, in addition to senile plaques and neurofibrillary tangles. GVD inclusions, possibly a special form of autophagic vacuole, usually occur together with pathological changes of the microtubule-associated protein tau, but to date, the relationship between both remains elusive. We studied human tau transgenic mice and mice with A β -plaque pathology. Those neurons in human tau transgenic mice that developed an advanced stage of tau hyperphosphorylation and early tau fibrillary pathology displayed GVD inclusions. The findings support a link between GVD and tau pathology.

Sigma-1 receptor ligand and SSRI Fluvoxamine modulates production of amyloid beta peptides and is protective in J20 Alzheimer disease mice

Associate Professor John BJ Kwok, Neuroscience Research Australia, Sydney, Australia

Alzheimer's disease (AD) is a neurodegenerative disease with abnormal Amyloid beta (A β) peptide accumulation beginning decades before symptom onset. A prophylactic treatment aimed at arresting the amyloidogenic pathway will have to be tolerated for at least 20 years. Fluvoxamine, an SSRI and a potent sigma-1 receptor ligand, decreased gamma-secretase activity and A β secretion in two cellular models. Finally, a subset analyses of J20 mice with low transgene human APP (Swedish and Indiana double mutation) expression, long term chronic treatment with fluvoxamine significantly protected against A β 1-42 neuropathology and cognitive deficits in the treated mice.

Early molecular and electrophysiological alterations during the asymptomatic stage of Alzheimer's disease: hippocampus versus retina

Dr Slavica Krantic, UPMC, CNRS, Paris, France

Accumulation of amyloid-beta (A β) plays a major role in Alzheimer's disease (AD) by initiating a series of events including synaptic dysfunction, glia activation and hyperphosphorylation of tau, that culminates in widespread neuronal death. AD is diagnosed 10-20 years after the pathology has begun. Such long incubation period could offer a window of therapeutic intervention if the disease could be diagnosed earlier. The first molecular (TNF α induction) and electrophysiological (neuronal excitability, networks coupling) alterations occur in the hippocampus and retina while A β is still virtually undetectable. The potential of these alterations to become the earliest AD biomarkers will be discussed.

The regulation of metastable proteins in neurodegenerative Diseases

Rishika Kundra, University of Cambridge, St. Johns College, Cambridge, United Kingdom

Protein misfolding disorders like Alzheimer's (AD) are associated with the formation of aberrant aggregates. The cell has evolved a highly complex proteostasis system to preserve the structural and functional integrity of proteins and maintain protein solubility. We reported the presence of large number of proteins that are inherently metastable and at risk of aggregation. Biochemical pathways associated with neurodegenerative conditions are enriched in these metastable proteins and they were downregulated in AD, accompanied by a transcriptional downregulation of protein homeostasis components rendering the proteome more susceptible to aggregation. We are now looking at the proteostasis complement of these metastable proteins.

Characterizing Prodromal Alzheimer's Disease Using Multimodality Imaging

Dr. Val Lowe, Mayo Clinic Rochester, Rochester, United States

Characterizing Prodromal and Early Alzheimer's Disease Using Multimodality Imaging

Neuroimaging with positron emission tomography (PET) and magnetic resonance imaging (MRI) provides important biomarker information that aids in understanding Alzheimer's disease (AD) and the AD clinicopathologic spectrum. Amyloid imaging with ¹¹C Pittsburgh compound B (PiB-PET) and other PET ligands is used to infer the presence of amyloid- β (A β) in the brain. Structural MRI, ¹⁸F-fluorodeoxy-glucose (FDG) and tau PET are used to infer neurodegenerative pathology. Given the importance of early diagnosis with respect to prognosis and treatment, the National Institute on Aging-Alzheimer's Association (NIA-AA) clinical diagnostic guidelines for AD and preclinical AD now include biomarkers, both cerebrospinal and neuroimaging, as integral components of the diagnostic algorithm. It is therefore of particular importance to understand the utility of imaging biomarkers for the evaluation of AD-related pathology in the spectrum of prodromal to early AD dementia.

While progress in the field of neuroimaging is substantial, the relationship between pathologic findings and antemortem multimodality neuroimaging is newly being investigated. We will therefore discuss the data which evaluates the association multimodality imaging and pathology. Data with regard to multimodality imaging and correlation with clinical cohorts will also be discussed as it pertains to prodromal and early AD dementia.

Psychological treatments for depression and anxiety in dementia and mild cognitive impairment: systematic review and meta-analysis

Dr Vasiliki Orgeta, University College London, London, United Kingdom

Anxiety and depression are common in people with dementia and mild cognitive impairment (MCI), but there is uncertainty about the effectiveness of both pharmacological and psychological therapies. This talk considers current evidence base on emotional well-being in people with dementia by presenting the results of a systematic review and meta-analysis of randomised controlled trials (RCTs) of psychological treatment versus usual care in people with dementia and MCI. The talk will conclude with recommendations for current and future research in the field.

EEG biomarkers and profiling of AD mouse lines

Professor Bettina Platt, University of Aberdeen, Institute of Medical Sciences, Foresterhill, Aberdeen, Scotland, United Kingdom

Changes in sleep/wake pattern and electroencephalogram (EEG) profiles are common in Alzheimer disease (AD) and now assumed to contribute to disease onset and progression. Corresponding traits have been studied in animal models but often do not recapitulate human symptomatology. We have developed a wireless recording device (NAT) that allowed us to longitudinally monitor EEG and vigilance profiles of low expression models of AD (PLB lines, expressing tau, APP and/or PS1). Using novel computational tools, we were able to identify progressive, vigilance stage-, brain region- and age-specific changes. It will be discussed whether such endophenotypes are suitable as translational biomarkers of AD.

Neurophysiological vulnerability to aging associated with the Alzheimer's risk variant in CLU gene

Dr Natalya Ponomareva, Research Center of Neurology, Russian Federation

CLU CC (rs11136000) genotype is associated with risk of Alzheimer's disease (AD) (Harold et al., 2009; Lambert et al., 2009; Golenkina et al., 2010). The mechanisms by which this variant confers AD susceptibility remain largely unknown. Herein we describe how CLU CC genotype impacts brain neurophysiology during normal aging. The results imply that the genotype is related to the preclinical dysregulation of hippocampal networks and to the decreased cognitive networks efficiency during cognitive tasks. These factors may contribute causally to the pathogenesis of AD.

Multiple roles of cholinergic neurons in the modulation of amyloid production

Dr Jane Rylett, Schulich Medicine & Dentistry, London, Ontario, Canada

Alzheimer disease is associated with increased amyloidogenic processing of amyloid precursor protein (APP) to β -amyloid ($A\beta$), cholinergic neuron loss with decreased choline acetyltransferase (ChAT) activity, and cognitive dysfunction. Both 69- and 82-kDa ChAT are expressed in cholinergic neurons in human brain with 82-kDa ChAT localized to neuronal nuclei, suggesting alternative functions. 82-kDa ChAT in nuclei of cholinergic neurons decreases with increasing age and lost in mild cognitive impairment and AD. Genes for proteins regulating APP processing are differentially expressed in 82-kD ChAT-expressing cells, and predicted effect is decreased amyloidogenic APP processing; BACE1 levels and activity and of $A\beta$ 1-42 from neurons cultured from AD-model mice are reduced. These studies indicate a novel relationship between cholinergic neurons and APP processing, with 82-kDa ChAT acting as a negative regulator of $A\beta$ production. Decreasing levels of 82-kDa ChAT due to increasing age or neurodegeneration could alter the balance towards increasing $A\beta$ production, with this potentiating decline in cholinergic neuron function.

Chaperoning Tau aggregation

Dr. Stefan Rüdiger, Utrecht University, Utrecht, Netherlands

This cellular control of protein homeostasis is essential for life, derailing of this control is fatal. In case of Alzheimer, aggregation of the protein Tau is an obligate step in the development of the disease. Molecular chaperones such as the Hsp90 chaperone are the first line of the cellular defense system against protein damage and aggregation. Here, we present a structural model of an Hsp90-Tau complex. We found Hsp90 to bind to the Tau's microtubule-binding repeat region, which forms the toxic aggregates. Together this suggests a direct role for Hsp90 in dealing with Tau aggregation.

Predicting Progression to Mild Cognitive Impairment in Cognitively Unimpaired Individuals Using Neuroimaging Biomarkers

Dr. Cynthia M. Stonnington, Mayo Clinic College of Medicine, Scottsdale, United States

Neuroimaging biomarkers can detect disease pre-clinically but their prognostic value, alone or in combination, is uncertain. In this talk, I describe new data from the Arizona APOE Cohort in which we used regional volumetric MRI and FDG PET measurements to distinguish between cognitive unimpaired older adults who did or did not subsequently progress to the clinical diagnosis of amnesic Mild Cognitive Impairment (MCI) due to AD within the next 2 years. Participants followed in the Arizona APOE Cohort have a reported first degree family history of possible AD dementia and were enriched for APOE4 gene dose.

Targeting Nucleophilic Attack During the Lag-phase of Beta-Amyloid Oligomerization

Adrien W. Schmid, Ecole Polytechnique Fédérale de Lausanne (EPFL), Proteomics Core Facility, Life Sciences, Lausanne, Switzerland

Beta-amyloid (Ab) oligomers account for the decline in loss of memory associated with dementia and therefore represents an early diagnostic and therapeutic target for the treatment of Alzheimer's disease (AD). In order to elucidate the mechanisms of peptide misfolding in the early stages of AD pathogenesis, it is important to develop sensitive and specific assays to identify key molecular triggers before the development of clinical symptoms. We have elucidated key molecular changes associated with Ab oligomerization using mass spectrometry, which has enabled us to design novel epitope targets for anti-ab antibodies that specifically target Ab at the early stages of oligomerization.

The drug therapy to increase stem cells for treatment of Alzheimer/s disease

Dr. Kiminobu Sugaya, University of Central Florida, Orlando, United States

We reported that human neural stem cells (NSCs) transplantation significantly improved the cognitive function of aged rat. However, ethical and technical issues put off the clinical use of stem cells for Alzheimer's disease (AD) therapy. We have developed technologies to overcome these issues. One is to dedifferentiate adult stem cells into pluripotent cells using an embryonic stem cell gene. Another is to increase endogenous stem cell by systemic administration of a small molecule compound. We also found that amyloid precursor protein (APP) cause glial differentiation of NSCs. Here, I will discuss how to increase neurogenesis under the AD pathological conditions.

What makes an amyloidogenic protein toxic? Dissecting the sequence - self-assembly -toxicity relationship for amyloid peptides.

Professor Louise Serpell, University of Sussex, Falmer, United Kingdom

Many proteins and peptides with different amino acid sequences share the ability to self-assemble to form amyloid fibrils. A large number of these have been implicated in protein misfolding diseases but some are also known to perform functional roles in living organisms. Amyloid fibrils are also being utilised to form functional materials, highlighting the need to better understand the structure-function relationship.

We have been exploring how the sequence of amyloidogenic peptides determines the ability to self-assemble and in turn, how this relates to the formation of toxic oligomers and mature amyloid fibrils. A variant of the Alzheimer's A β peptide has been designed in order to examine the specific structural variations that lead to A β toxicity. Key differences have been identified in the aggregation propensity, which are closely linked to cellular uptake and functional effects. We have also identified a dityrosine crosslinked A β that is related to the neurodegeneration observed in Alzheimer's disease. In very recent work, we have reported that A β can directly lead to memory loss in a model organism.

Here we will describe how these striking observations have led to insights into the mechanism of A β toxicity, providing a platform to better understand deleterious effects of oligomeric proteins in disease and how amyloid fibrils may be controlled for functional and non-toxic roles.

References

1. K. E. Marshall, R. Marchante, W. F. Xue, and L. C. Serpell, Prion The relationship between amyloid structure and cytotoxicity. 2014 8, 28860

The role of proliferating astrocytes in Alzheimer's disease

Dr Magdalena Sastre, Division of Brain Sciences, Hammersmith Hospital, Imperial College London, London, UK

Dr. Magdalena Sastre graduated in Sciences and did her PhD in Biology and Health Sciences at the University of the Balearic Islands, Spain. She trained in Neuroscience in the USA (Cornell University and New York University) and in Germany (Universities of Munich, Bonn and Frankfurt).

She is interested in the molecular mechanism by which inflammation affects neurodegenerative diseases, in particular Alzheimer's disease.

Her scientific contributions include the study of the intracellular signalling cascade of the amyloid precursor protein and how it affects its cleavage and the formation of amyloid- β peptide. In addition, she has focused her research in the use of anti-inflammatory drugs as potential therapy for neurodegenerative diseases, and in the cofactors of the peroxisome proliferator-activated receptor- γ (PPAR- γ).

Potential therapeutic strategies of Cerebrolysin in Alzheimer's Disease

Dr Hari Shanker Sharma, Uppsala University, Uppsala, Sweden

Nanodelivery of drugs induce better therapeutic effects in preventing neurological diseases and their effects are also prolonged than the parent compounds. Thus, the need of the hour to examine whether drugs tagged with different kinds of nanoparticles may have different effects following their nanodelivery in treating neurological diseases e.g., Alzheimer's Disease (AD). AD is mainly characterized by deposition of amyloid -peptide (ABP) in various brain regions leading to cell and tissue destruction. It is widely believed that breakdown of the blood-brain barrier (BBB) to serum constituents activates a series of abnormal reactions leading to immunological, biochemical, and pathological changes culminating in AD. Thus, to

reduce the BBB breakdown and induce neuroregeneration or neurorepair using several neurotrophic factors in combination could alleviate AD symptoms. Our laboratory is engaged to find out whether Cerebrolysin a multimodal drug (Ever NeuroPharma, Austria) comprising a well-balanced composition of several neurotrophic factors and active peptide fragments could induce neuroprotection in animal models of AD. The new developments in this strategies will be discussed to reduce AD induced brain pathology.

Drugs used in multiple pathologies, with stresses common to Alzheimers disease, offer candidate drugs and vaccine potential for Alzheimers disease

Professor Joan Smith Sonneborn, Emeritus Zoology & Physiology, University of Wyoming, Laramie, United States

Alzheimer's disease common denominators include: oxidative stress, toxic abeta 42, aggregated amyloid, tau pathology, and insulin resistance. Mitochondrial location of the plaques prompted use of successful-targeted mitochondrial antioxidants. Other disease interventions possibilities include: antisense microRNAs that inhibit the age-related reduction in stress resistance; TERT target upregulation used as a dose dependent hormetic agent; Neuregulin, NrF2 stability, identified as longevity promotion agents in disease-free mole rat, and cold shock protein RMB3 with synapse restoration; targeted agents, and diabetic drugs. Vaccines against Alzheimer's disease remain an option, with targets that include not only tau, but toxic forms of amyloid.

Paradigm Shift: Semantic memory decline as a biomarker of preclinical Alzheimer's disease

Professor Annalena Venneri, Department of Neuroscience, University of Sheffield, Royal Hallamshire Hospital, Sheffield, UK

Detection of Alzheimer's disease (AD) in routine neurological management of middle-aged/elderly adults is challenging. Standard assessments of episodic memory and brain atrophy are useful to detect the prodromal mild cognitive impairment stage of AD but fail to identify the earliest preclinical cases. Longitudinal research has indicated that at this earliest stage of AD there is a subtle decline of semantic memory. A focus on latent and more qualitative aspects of semantic performance (which transcend the simple raw quantitative score) might be a better biomarker of preclinical AD. Sticking to episodic memory may be effective to diagnose prodromal disease, but does not offer many opportunities for clinical research to progress towards cognitive biomarkers of earlier application.

Smell identification function in Alzheimer's disease

Dr Latha Velayudhan, Oxleas NHS Foundation Trust and University of Leicester, United Kingdom

Olfactory dysfunction in general and impaired odour identification in particular, have been reported in Alzheimer's disease (AD) and are found to occur at early stages of the disease. Olfactory identification deficits are also known in other neurodegenerative disorders. Olfactory identification testing can be a useful diagnostic aid for AD and the talk will demonstrate smell dysfunction in AD compared to other dementias (OD), people with mild cognitive impairment (MCI) and non-demented controls (NDC). The association of smell identification dysfunction with activities of daily living, non-cognitive symptoms and structural MRI imaging will also be discussed.

Dystrophic neurites are sites of microtubule disruption, BACE1 elevation, and increased A β generation: the potential role of A β oligomers

Professor Robert Vassar, Feinberg School of Medicine, Northwestern University Interdepartmental Neuroscience, Chicago, IL, USA

Alzheimer's dystrophic neurites accumulate the β -site amyloid precursor protein (APP) cleaving enzyme (BACE1). Live-cell imaging of primary neurons treated with A β 42 oligomers showed beaded neurites with disrupted microtubules. Brain sections from AD patients and 5XFAD transgenic mice exhibited peri-plaque dystrophic neurite halos with BACE1 elevation and aberrant tubulin localization. At the EM level, axonal dystrophies were devoid of microtubules and replete with autophagic intermediates. Microtubule motors were aberrantly localized in peri-plaque dystrophies. BACE1 accumulation in dystrophies caused increased BACE1 cleavage of APP and A β generation. Our study suggests that A β disrupts microtubules to cause dystrophic neurites and exacerbate amyloid pathology.

Biomedical nanotechnology and Alzheimer's. Can the disease be reversed?

Professor Christopher Whiteley, National Taiwan University of Science and Technology, Taipei, Taiwan
Deposits of aggregated A β -peptide senile plaques and the accumulation of arginine within neuroglial cells in the brain are classic observations in the neuropathology of Alzheimer's disease. Neuronal nitric oxide synthase (nNOS) induces fibrillogenesis when incubated with amyloid peptide fragments. Addition of gold/silver nanoparticles to these induced fibrils decreases their concentration by > 90 % within 10 sec. Three mechanisms for this reversal are: (1) depletion of free A β monomer in solution and blocking potential aggregation sites on the nNOS molecule (2) hydrophobic interaction between the A β peptide and nanoparticle (3) disruption of binary adducts between A β -peptides and nNOS by nanoparticles.

Alpha2 adrenergic receptor as a novel target for Alzheimer's disease

Dr. Qin Wang, University of Alabama at Birmingham, Birmingham, United States
Alzheimer's disease (AD) involves multiple genetic and environmental risk factors that lead to disruption of Abeta homeostasis. We discover that a G protein-coupled receptor, namely the alpha2A adrenergic receptor (AR), is involved in AD pathogenesis through modulating SorLA-dependent endocytic sorting of amyloid precursor protein. Significantly, blockade of alpha2A-AR in mice with profound AD-related pathology reduces neuropathology and ameliorates cognitive deficits. Our study suggests alpha2A-AR as a novel target for AD, and clinical alpha2AR antagonists may be repurposed for AD treatment.

Type 2 diabetes mellitus accelerate tau pathology in nonhuman primate

Dr. Zhiming Zhang, University of Kentucky College of Medicine, Lexington, United States
Type 2 diabetes mellitus (T2DM) has long been considered as a risk factor for Alzheimer's disease (AD). Since both T2DM and AD are closely related to aging, aged macaques with T2DM could be natural candidates for studying AD. Here, we provide the evidence that T2DM accelerate tau pathology in the brain of aged cynomolgus monkeys with long-term T2DM. In details, significantly higher number of AT180- and AT8-positive pre-NFT, iNFT cells and dystrophic neuritis were found in the hippocampus in T2DM aged comparing with normal aged monkeys, which suggests the acceleration of p-tau pathology duo to T2DM.

Day 1:

Oral Presentation Abstracts

Oral presentations will be added after the submission deadline

UBIQUITOME PROFILE IN DOWN SYNDROME BRAIN PRIOR TO AND AFTER DEVELOPMENT OF ALZHEIMER NEUROPATHOLOGY

A. Tramutola*, F. Di Domenico, D. A. Butterfield, M. Perluigi

*presenting author: Department of Biochemical Sciences "A. Rossi-Fanelli", Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, ITALY.

Aims. Among the putative mechanisms proposed to be common factors in Down Syndrome (DS) and Alzheimer disease (AD) neuropathology, defects in protein quality control has emerged as a unifying mechanism of neurodegeneration. Considering that disturbance of protein degradative systems have been demonstrated in DS and that oxidized/misfolded proteins require poly-ubiquitinylation for degradation via the ubiquitin proteasome system (UPS), this study aimed to investigate the disturbance of protein poly-ubiquitinylation contributes to accelerated aging and neurodegeneration in DS.

Results. Post-mortem brains from DS subjects, prior to and after development of AD neuropathology were analyzed. By selectively isolating poly-ubiquitinylated proteins, we were able to identify the specific proteins with an altered pattern of poly-ubiquitinylation as a function of age. Interestingly, we found that oxidation is coupled with poly-ubiquitinylation for the majority of proteins, which are mainly involved in protein quality control and energy metabolism.

Innovation. This is the first study showing alteration of the poly-ubiquitinylation profile as a function of aging in DS brain compared with healthy controls. Understanding the onset of the altered ubiquitome profile in DS brain may contribute to identification of key molecular regulators of age-associated cognitive decline.

Conclusions. Disturbance of poly-ubiquitinylation machinery is considered to be a key feature of aging and neurodegeneration. But in the case of DS, the age-associated deficit of proteolytic systems further exacerbates the accumulation of oxidized/misfolded/poly-ubiquitinylated proteins, which are not correctly degraded and may become harmful to neurons and contribute to AD-related neuropathology.

WHAT IF DEMENTIA WAS DUE TO CALCAREOUS?

Dr. A. Scibetta

Corso Italia 39, Ronchis (Udine), Italy

1. Definite data: Calcium (Ca⁺⁺) in neuronal membrane, presence of carbonic acid (HCO₃⁻), pH 7,4 , temperature 36,5°, presence of water in the interstitial fluid.
2. In nature $H_2O + CO_2 > H_2CO_3 > H^+ - HCO_3^- > 2H^+ + CO_3^{2-} + Ca^{2+} > CaCO_3 + H^+$
3. Transitive Theory, similar conditions are present also in the human brain.
4. As this is salt (Ca⁺⁺⁺ HCO₃⁻ > CaCO₃), it will precipitate directly in its site and block the activation of its canal. Subsequently, the resistance properties or the state of tension changes in that point of the membrane. The fluidity of the cell membrane of the neuron does not change but its pulsatility.
5. There are two inductions. In the second one, I observed from my clinical experience, what appears to be highly evidenced and transcribed in a cerebral RMN of a patient suffering from dementia and ischemic attacks is: the presence of an increase of the liquor spaces both above and under the

cerebellar tentoria, a reduction in the thickness of the cortex with an increase in the ridges, the presence of hypo-dense areas, maybe due to ischemic episodes. Therefore, the ischemic areas differentiate themselves from the hypotrophic areas.

6. So as to activate the areas where there are neurons with micro-calcifications, it is necessary to have spikes that are able to overcome the mechanical resistances of the calcareous.
7. Hebbian's theory states that memory is developed when new proteins are formed in the neurons and by modification of the dendrites. We can deduce from this that the neuron together with its dendrites gets to a certain-molecular weight and then it stabilizes; it may form other dendrites but the space however tends to be reduced. If we multiply this process thousands of times in a small area as the hippocampus, where short term memory is located, it will get compressed and therefore explain why this kind of memory gets damaged in old age and makes it impossible for it to produce new long-term memory. The aging of the hippocampus would be a process of saturation.
8. Neurology and psychiatry: The ability to know what one wants depends on the character and social aspects of the individual. Senile BIOLOGICAL dementia is the loss of the ability to survive and impossibility to look after one's body; this may be another way in which human biology prepares itself to die. In ascertained biological senile dementia, there are no choices but only acceptance.
9. Consideration: if Calcium, carbonic acid and water form the calcium carbonate; Why we don't think that this new hypothesis could be the etiopathogenesis of dementia?
10. Other definite data are going to be presented during the oral presentation.

GLIAL REGULATION OF NEOCORTICAL SYNAPTIC TRANSMISSION: IMPLICATION FOR ALZHEIMER'S DISEASE

Dr Yuriy Pankratov, University of Warwick, School of Life Sciences, Coventry, United Kingdom

Brain function depends on the interaction between two cellular circuits: neurons transmitting electrical signals and glial cells which maintain the wellbeing and function of neurons. Neuroglia, in particular astrocytes, are central elements of homeostasis and defense mechanisms of the brain. Astrocytes are endowed with multiple receptor and signalling cascades that allow them to monitor the brain microenvironment and signal back through the secretion of numerous factors and neurotransmitters. Astrocytes therefore are strategically positioned to act as integrating command centres that can orchestrate neuroprotection and convey a positive influence of active lifestyle on memory and cognition.

An impairment of glial function has been implicated to the pathogenesis of many neurological disorders, including Alzheimer's Disease. Recent data, including our work, show that astroglia can modulate synaptic transmission and plasticity via release of gliotransmitters, such as ATP, glutamate and D-Serine. In particular, we discovered that astrocyte-derived ATP can cause significant attenuation of phasic and tonic GABAergic currents. Furthermore, we showed that synergistic action of astrocyte-derived ATP and glutamate is important for the induction of long-term synaptic plasticity in the neocortex. We also found out that glutamatergic antagonist memantine, clinically used for treatment of AD, has much higher affinity to astroglial NMDAR receptors than to NMDA receptor subtypes, predominant in the brain neurons. However, the age- and AD-related changes in the mechanisms of astrocyte-neuron interaction remained almost unexplored.

In our recent work, we studied the impact of physiological and pathological ageing on astroglial Ca²⁺-signalling and glial-neuron communications in the brain cortex of Alzheimer disease model mice. We obtained unique data on quantal synaptic currents in the neocortex of aged wild-type and AD model APP/PS1 mice. We observed the considerable decline in the astrocytic Ca²⁺-signaling towards the old age (12-24 months) and significant decrease in the astrocytic signaling in the APP/PS1 mice. Consistent with

changes in the Ca²⁺-signaling, the release of ATP, glutamate and D-Serine from astrocytes declined in the old age and in the AD model mice.

Rather surprisingly, majority of neocortical pyramidal neurons in Alzheimer's disease model retained large number of functional synapses. However, impairment of glia-derived regulation significantly altered the balance between excitation and inhibition. The significant decline in the quantal size of excitatory AMPA and NMDA receptor-mediated synaptic currents contrasted with significant up-regulation of phasic and tonic GABAergic currents. This was followed by the significant age- and AD-related deficit in the long-term synaptic plasticity. Importantly, the neocortical synaptic plasticity could be rescued by additional activation of astrocytic Ca²⁺-signalling. Exposure of neocortical tissue to low dose of memantine, efficient only for glial NMDA receptors, also had beneficial effect on synaptic plasticity. Moreover, the negative impact of ageing and AD-related pathologies on the glial signalling and synaptic currents was remedied by environmental enrichment.

Combined, our data show that efficient astrocyte-neuron interaction is important for maintaining the balance between excitation and inhibition in cortical networks. This interaction can significantly decline with ageing and thus contribute to the age- and pathology-related cognitive impairment. Conversely, identification of environmental and pharmacological factors that can facilitate glia-neuron communication may help to ameliorate the negative impact of ageing and disease on cognition.

THE IMPAIRMENT OF BILIVERDIN REDUCTASE-A PROMOTES BRAIN INSULIN RESISTANCE IN ALZHEIMER DISEASE: A NOVEL MECHANISM

*E. Barone**, F. Di Domenico, D. A. Butterfield, M. Perluigi

*presenting author: Department of Biochemical Sciences "A. Rossi-Fanelli", Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, ITALY.

Clinical studies suggest a link between peripheral insulin resistance and cognitive dysfunction. Interestingly, post-mortem analyses of Alzheimer disease (AD) subjects demonstrated insulin resistance in the brain proposing a role for cognitive deficits observed in AD. However, the mechanisms responsible for the onset of brain insulin resistance (BIR) need further elucidations. Biliverdin reductase-A (BVR-A) emerged as a unique Ser/Thr/Tyr kinase directly involved in the regulation of insulin signaling. Indeed, once activated via Tyr phosphorylation by insulin receptor (IR), BVR-A is able to phosphorylate the insulin receptor substrate (IRS)-1 on inhibitory domains, representing an upstream regulator in the insulin signaling cascade. Because we previously demonstrated the oxidative/nitrosative stress (OS/NS)-induced impairment of BVR-A in human Alzheimer disease (AD) brain, here we hypothesize that BVR-A dysregulation could be associated with the onset of BIR in AD.

To this aim, we performed a longitudinal analysis to evaluate BVR-A protein levels and activation in the hippocampus of 3xTg-AD and WT mice at 3, 6, 12 and 18 months of age (n=6/group). Changes of BVR-A have been then correlated with changes about (i) IR/IRS1 protein levels and activation state (ii) total OS/NS markers levels (PC, HNE, 3-NT), (iii) changes of A β and tau pathology, (iv) TNF- α levels and (v) mTOR activation. Subsequently, ad hoc experiments have been performed in SH-SY5Y cells treated with (i) insulin, (ii) hydrogen peroxide/peroxynitrite or (iii) a specific silencing RNA (siRNA) for BVR-A, to clarify the molecular mechanism(s) underlying changes observed in mice.

Our results highlighted two distinct phases in the hippocampus of 3xTg-AD mice: a first insulin signaling hyper-activation (3-6 months) followed by a persistent IRS1 inhibition and thus insulin resistance at both 12 and 18 months. In this picture, we found that BVR-A levels and activation start to decline early, at 6 months of age, prior the accumulation of A β and tau pathology, and remain persistently reduced until 18 months, possibly because the increased OS/NS levels in the same time-frame. In addition, because TNF- α is known to inhibit BVR-A promoter activity and TNF- α has been also demonstrated to be a conceivable mediator of BIR in AD, we wondered to check whether reduced BVR-A levels were associated with changes of TNF- α . Interestingly, an increase of TNF- α levels is evident only at 18 months, thus highlighting the impairment of BVR-A as an early event in the onset of BIR. Similar changes have been found during the

normal ageing process in WT mice, but later in life. Experiments on SH-SY5Y cells further confirmed that either lack of BVR-A or OS/NS-induced impairment of BVR-A promotes BIR. Finally, we identified the sustained activation of mTOR, as one of the feedback mechanisms leading to insulin resistance following BVR-A impairment both in mice and cells.

In conclusion, we propose a novel mechanism for which: OS/NS-induced impairment of BVR-A is firstly responsible for a sustained activation of IRS1, which then causes the stimulation of negative feedback mechanisms (i.e. mTOR) aimed to turn-off IRS1 hyper-activity and thus insulin resistance. Similar alterations characterize the normal ageing process in mice, positing BVR-A impairment as a possible bridge in the transition from normal ageing to AD.

Day 2:

Oral Presentation Abstracts

MINI SKQ (SEMANTIC KNOWLEDGE QUESTIONNAIRE): 12 QUESTIONS TO HIGHLIGHT SEMANTIC DETERIORATION IN ALZHEIMER'S DISEASE

I. Simoes Loureiro and L. Lefebvre

Cognitive psychology and neuropsychology department, University of Mons (UMONS), Place du Parc, 18, 7000, Mons. Email: isabelle.simoesseloureiro@umons.ac.be

Objective

Semantic memory disturbance is, with episodic memory deficit, one of the first symptom in Alzheimer's disease (AD). However, semantic knowledge is not always investigated in the neuropsychological assessment and is rather reflected through non-specific semantic measurements as through naming tasks. The Semantic Knowledge Questionnaire (SKQ) explores semantic impairments in AD patients. Initially proposed by Laiacona et al. (1933) and revised in a French and more concise form by Simoes Loureiro and Lefebvre (2015), SKQ assesses different levels of hierarchy and attributes in semantic memory by the mean of 120 questions about 30 objects (Q1: questions about general superordinate aspects; Q2: questions about intracategorical aspects; Q3: questions about perceptual attributes and Q4 : questions about thematical/functional attributes).

The objective of this work is to create a shorter version of SKQ, with the most discriminant questions between AD and controls, in order to highlight semantic impairments during neuropsychological assessment.

Method and results

We administered SKQ in its full version (120 items) to 39 healthy senior (MMSE \geq 28) and 35 mild AD (MMSE $>$ 20). An item by item analysis was conducted to compare control group and AD group in order to pick up the most differentiated items between both groups with chi-square. 12 items discriminating both group at a level of significance of $p=.001$ were selected (three Q2; four Q3 and four Q4). We also performed correlational analyses for non-parametric data (Kendall's Tau correlation) to ensure that the failure to these 12 items are well correlated with AD ($p=.001$). Finally, a Bravais-Pearson correlation analysis confirms the correlation between the score at mini-SKQ and the full version of SKQ ($r=.992$; $p=.001$).

Conclusion

The Mini-SKQ is a fast and easily administered questionnaire. Results indicated favorable indicators to screen semantic knowledge. The failure to the items of the mini-SKQ is highly correlated to AD. These first observations underline that mini-SKQ could potentially be attractive for screening semantic memory deterioration.

RETINAL BIOMARKERS IN AD

U.A.Kayabasi¹, R.C.Sergott²

1- Lifemed Health Center Istanbul 34778 Turkey

2- Wills Eye Hospital, Philadelphia, USA

Background and aims:

Optical coherence tomography (OCT) and fundus autofluorescence (FAF) examinations provide us with important information about neurodegenerative diseases. Since the optic nerve and retina share similar structures with the brain, any defect detected by OCT and/or FAF may be related to a disease in the nervous system.

Methods:

We examined 50 patients with dementia due to Alzheimer's disease (AD). The FAF examinations were performed after which OCT was done through the abnormal (hyperfluorescent or hypofluorescent) spots or regions. We gave curcumin capsules to all the patients and 20 age-matched healthy controls before the tests. OCT and FAF images were examined in a masked fashion by two neuro-ophthalmologists

Results:

In 45 patients with AD, shining dots and spots were mostly seen in the outer plexiform, ganglion and nerve fiber layers on OCT. Patients who were given curcumin demonstrated patchy hypofluorescent areas with hyperfluorescent dot like lesions on FAF and OCT revealed brighter spots in different retina layers. In the control group, no change was detected after curcumin use.

Conclusion:

We believe that detection of lesions before the onset of dementia or very early in the course of the disease is extremely important. Since curcumin binds to beta-amyloid, the differences in images before and after curcumin use can be explained by beta-amyloid accumulation in the retina. OCT and FAF can be trustable biomarkers in the course of AD.

OXIDATIVE SIGNATURE OF CSF FROM MILD COGNITIVE IMPAIRMENT AND ALZHEIMER DISEASE PATIENTS

F Di Domenico*, E Barone, A Lloret, J Vina and M Perluigi

*presenting author: Department of Biochemical Sciences "A. Rossi-Fanelli", Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, ITALY.

Several studies suggest that pathological changes in Alzheimer's disease (AD) brain begin around 10-20 years before the onset of cognitive impairment. Therefore, the identification of biomarkers that can support early diagnosis and predict development of dementia would, could be crucial for patient care and evaluation of drug efficacy. Although, the levels of A β 42, tau, and p-tau, in the cerebrospinal fluid, are well-established diagnostic biomarkers of AD, there is still a need for additional brain-related biomarker that can increase diagnostic accuracy and specificity and that can be evaluated, eventually, at the systemic level. Our study was focused on the analysis of oxidative stress-related modifications of the CSF proteome, from subjects with AD and amnesic mild cognitive impairment (aMCI). A targeted proteomics approach has been employed to discover novel CSF biomarkers. CSF samples from aMCI, AD and CTR patients were collected and analyzed using a combined redox proteomics approach to identify the specific oxidatively modified proteins. The majority of carbonylated proteins identified by redox proteomics are found early in the progression of AD, indeed oxidatively modified CSF proteins were already present in aMCI compared with controls and remain oxidized in AD. Our data suggests that oxidative stress is a primary event in the development of AD and that the oxidative modification of selected proteins initiate many years before severe dementia is diagnosed, reflecting the alteration occurring at brain level.

Day 3:

Oral Presentation Abstracts

Oral presentations will be added after the submission deadline

THE USE OF NEUROPEPTIDES OF “ADEMENT” FOR THE DEVELOPMENT DELAY OF THE NEURODEGENERATIVE PROCESSES: THE STUDY ON THE MODEL OF DROSOPHILA MELANOGASTER

Makarenko O.M., Wolfe I., Petrov P.I.

Predslavinskaya street 14, apt.27, 03150, Kyiv, Ukraine

For the treatment of neurodegenerative diseases of a human being the limited quantity of therapeutic agents is used, which mechanism of action have been studied not enough. One of such pharmacological preparations is Adement– a peptide agent, having its neurotrophic action on animal models and intensive neurospecific activity, and also the ability to metabolic regulation, neuroprotection and functional modulation.

Studying of the anti-neurodegenerative action of Adement on the model of *Drosophila melanogaster*.

The study regarding the influence of therapeutic agents on the processes, which occur in the nervous system and lead to neuron death, significantly depend on the use of a suitable model. In our experiments the mutant lines of *drosophila* with the induced changes in the brain structure acted as a test-system. The mutant lines were characterized by the appearance of degenerative changes through the whole brain structure and by a shortened life time. The line of the wild type Oregon acted as control. There has been studied the influence of different concentrations of Adement on the dynamics of the occurrence of neurodegenerative changes. Adement was included in the growth medium for larva feeding, recounting the concentration of a high daily dose for a person of the medium weight. In our studies Adement was administered in one- and thirty times concentrations (0,12 and 3,6 ml/100 ml of medium accordingly). The dynamics of the changes in the brain tissue structure has been investigated by means of the preparation of histologic specimen of brain cuts in the individuals of different age.

Adement in low concentrations slowed down the occurrence of dead zones in the brain structure for 5-7 days. The administration of 30-times dose proved the more intensive effect in mutant lines with early neurodegeneration. After the action of such dose, the dead zones in the brain tissue appeared since the 15th day of life. At present there is a search of the most effective combinations of the medicines with different mechanisms of action, as the individual influence of the pharmacological agent is nondurable and unilateral. In this connection, we worked out the scheme of studying of the combined stepwise action of preparations: Adement (neuroprotection) and Nimotope (neuroretardation). The restoration of the mutant phenotype to the normal one was observed depending on the line genotype during the period of 22-28 days of life. The stability of the neurodegeneration delay was accompanied by the increase of the life duration in different mutant lines by 10-33%.

The administration of Adement as a neuroprotector in combination with a neuroactivator and neuroretardator is the most effective.

MICRORNA CONTRIBUTION IN A NEW THERAPEUTIC STRATEGY FOR ALZHEIMER'S DISEASE

T. Viegas^{1,2}, V. Carmona^{2,3}, J. Guedes², A. R. Oliveira^{2,4}, L. Pereira de Almeida^{2,3}, C. Resende Oliveira^{2,4}, J. P. de Magalhães⁵, J. Peça², A. L. Cardoso²

1 Faculty of Medicine, University of Coimbra, Coimbra, Portugal

2 CNC - Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

3 Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal

4 Faculty of Science and Technology, University of Coimbra, Coimbra, Portugal

5 Institute of Integrative Biology - University of Liverpool, Liverpool, United Kingdom

Corresponding Author: A. T. Viegas

Postal address: Centro de Neurociências e Biologia Celular

Rua Larga, Faculdade de Medicina, 2^o piso

Universidade de Coimbra, Pólo I

3004-504 Coimbra, Portugal

Ageing is considered a major health concern in modern society, making the understanding of senescence and senescence-associated diseases a challenge for biomedical research. Brain ageing frequently underlies cognitive decline, being a major risk factor for neurodegenerative conditions such as Alzheimer's disease (AD), which is the most common form of dementia worldwide. However, the specific mechanisms underlying the relationship between brain ageing and neurodegeneration remain unclear. Recent studies have proposed a pivotal role for microRNAs (miRNAs) in ageing and, hence, in the development of neurodegenerative disorders. These short regulatory RNAs bind to the 3' UTR region of target mRNAs, inhibiting translation or degrading the mRNA, therefore altering the expression of their target genes. Researches using high throughput techniques have demonstrated that dynamic changes occur in the transcriptome with age, providing non-coding RNA gene candidates for further studies. Accordingly, miRNAs have been reported to have significant impact on mammalian brain ageing and neurodegeneration; making this area of research a fundamental area of interest. More specifically, in neurodegenerative disorders, deregulation of specific miRNAs targeting key proteins of the disorder, such as APP and BACE1 in AD, has been reported.

Although recent outcomes suggest a relationship between miRNAs regulation and ageing, deciphering the underlying molecular mechanisms in order to provide new therapeutic strategies remains a crucial challenge and this constitutes an extremely promising and timely research area. We and others have hypothesize that miRNAs are pivotal in ageing and neurodegeneration and that the identification of miRNA profiles as "molecular signatures" may contribute to the development of new therapies for neurodegenerative disorders associated with ageing. In this context, this study aims to modulate the levels of selected miRNAs predicted to target key proteins involved in AD in order to clarify their contribution to this disease and evaluate the potential therapeutic benefit of this kind of strategy.

Through bioinformatic tools, we have identified miRNAs with high affinity to the 3'UTR of both APP and BACE1 mRNAs and we have performed a biochemical validation of these binding sites, through the luciferase assay, upon co-transfection of HT-22 cells with miRNA mimics. Our results demonstrate that the selected miRNAs target APP and BACE1, since there was a decrease in luciferase activity in the presence of mimics of these miRNAs. Additionally, a decrease in APP and BACE1 mRNA levels was observed upon transfection of HT-22 and HEK-293 cells with lentiviral constructions containing the selected miRNAs precursor sequences.

MiR-B was selected for in vivo studies, since its overexpression was able to decrease the expression of both APP and BACE1 proteins in human and mouse neuronal cell lines. miR-B modulation was achieved in the brain of the 3xTg AD animal model, following stereotactic injection of lentivirus encoding the miR-B precursor sequence.

Behavioral tests designed to specifically analyze the long-term, short-term and working memory, showed a significant improvement in the cognitive function of the animals treated with miR-B, with respect to the untreated group and to the negative control group. Moreover, histological, stereological and biochemical analysis showed that animals injected with miR-B present a strong reduction in the number of A β plaques in the subiculum and hippocampus, as well as a significant reduction in human APP and mouse BACE1 mRNA and protein levels, compared to control groups.

Overall, our study demonstrates that miRNA modulation is a promising strategy to decrease APP and BACE1 expression, leading to a reduction of A β deposition and to the amelioration of cognitive function in 3xTg-AD animals. Given the high conservation of the selected miRNAs across species, new significant insights into the ageing process may arise from this study, supporting new diagnostic and therapeutic avenues for Alzheimer's disease and other ageing-related disorders.

Poster Presentation Abstracts

Poster abstracts will be finalised weeks before the event

AN ORIGINAL MEDICINE FROM TROPHINOTROPINE GROUP FOR THE TREATMENT OF CEREBROVASCULAR AND NEURODEGENERATIVE DISEASES

Makarenko O.M., Wolfe I.

Predslavinskaya street 14, apt.27, 03150, Kyiv, Ukraine

On the basis of fundamental and biomedical disciplines neuroscience really appeared giving new possibilities of medicines therapy.

“Cerebral” is an original medicine and a founder of the new group of medicines – trophinotropins, that open a new era in the treatment of acute cerebrovascular and chronic organic neurodegenerative diseases.

The founder of the new group of the trophinotropin’s row is a neurotropic medicine “Cerebral”, that change the content of neuropeptides and some aminoacids (GLU, ASP, GLI, ALA) in the neocortex of animals with experimental model of bihemispheric stroke. Administration of this medicine was accompanied with 70% decrease of animals’ mortality. Obtained from female pigs after hemorrhagic stroke of the brain the recovery medicine “Cerebral” is a peptide substance of trophinotropins in stroke brain tissues.

The high medical action of “Cerebral” and a real lack of sight effects in hemorrhagic stroke treatment provides with entering molecules of medicine substances into the terminations of the olfactory nerves after intranasal administration of “Cerebral” solution. After that the medicine molecules transported by axonal in different areas of the CNS (limbic system) with the mechanism of retrograde axonal substances transport.

“Cerebral” accelerated the progress of structural and functional restoration of alterative neurons (reparative effect), have neurocytoprotective and different pharmacological properties – trigger and neuroactivative actions in post-stroke periods, like the medicines of a few pharmacological groups.

“Cerebral” is suggested for the treatment in the acute period of stroke and at decompensation phases, it essentially simplifies the treatment of the patients and it is safer for treatment. These medicine was used for treatment of neurological diseases, the conditions of which were accompanied with blood introduction into the brain and cerebrospinal fluid of the child, sick with infantile cerebral palsy), some other organic brain disorders.

Polypharmacological actions of “Cerebral” are directed at immunodeficiency. Cerebral is based on a new endopharmacological principle of the patient’s therapy and is a scientific basis for getting of the Therapeutic Individual Medicine for the concrete patient’s treatment in the nearest future at the concrete stage of disease development.

EYE MOVEMENT BEHAVIOR AND ALZHEIMER'S DISEASE: OCULOMOTOR PARAMETERS PREDICTIVE OF COGNITIVE DECLINE.

MLGF Pereira, MVZACamargo, AF Belan, B Santos, OV Forlenza.

Laboratory of Neuroscience
Department of Psychiatry
Faculty of Medicine, University of Sao Paulo
Rua Doutor Ovidio de Campos 785
05403-010 Sao Paulo - S.P. - Brazil

Background: Eye movement analysis has proved to be a valuable method to explore higher-order cognitive processes, namely detecting memory and visuospatial processing deficits (Rizzo et al., 2004). Different oculomotor parameters have been studied and they have shown some capacity to distinguish cognitive decline in clinical populations, namely AD, however, little is known about the oculomotor profile in MCI subjects and its relation to cognitive decline in this group. (Crutcher et al., 2009; Hanulla et al., 2010; Nakashima et al., 2010; Lagun et al. 2011).

Methods: 90 age-matched participants were divided into 3 groups, 22 subjects diagnosed with AD (M= 72,73 / SD= 5,22), 41 subjects with MCI (M= 69,45 / SD= 8,59) and 27 normal age-matched control subjects (NC) (M= 69,26 / SD= 7,68). They performed a set of computerized cognitive tasks (visuospatial perception tasks involving abstract and common figures and also scenes), while their eye movement behavior was measured. The following oculomotor parameters were analysed within predefined regions of interest (ROI): time to first fixation (TFF), fixation duration (FD), fixation count (FC), visit duration (VD) and visit count (VC). Eye movement behaviors were compared in order to determine if they can effectively discriminate between groups.

Results: (1) Selected ROIs has shown to be sensitive in distinguishing the different groups throughout the oculomotor parameters: MCIxNC groups (FC>TFF>FB>VD), ADxMCI groups (TFF>VD>FC>FB) and with a significantly higher discrimination between ADxNC (TFF>VD>FB>FC); (2) when comparing 'familiar' (previously seen) stimuli to the other ('new') stimuli, FC significantly discriminated NC and AD subjects ($p<.01$) and, although it did not discriminate between NC and MCI, data suggested a strong tendency in that direction ($p=.06$). Results also presented no significant difference between AD and MCI group for the FC parameter.

Conclusion: Eye movement behavior can accurately distinguish between NC, MCI and AD groups. When looking to FC, one of the most discriminating parameter according to the literature, MCI and AD groups presented a similar oculomotor behavior, showing less fixations in the familiar stimuli. It can be assumed that MCI subjects could not remember recently presented images, which suggests that this group may present a similar oculomotor profile to the one associated with Alzheimer's disease. Further researches controlling MCI subgroups may reinforce the usefulness of oculomotor parameters in the early detection of memory decline prior to AD, as suggested by the present study.

THE IMPACT OF TIME AND COGNITIVE EFFORT IN EYE MOVEMENT BEHAVIOR: CONTRIBUTIONS OF OCULOMOTOR PARAMETERS IN THE EARLY DETECTION OF ALZHEIMER'S DISEASE.

MVZACamargo, MLGF Pereira, AF Belan, B Santos, OV Forlenza.

Laboratory of Neuroscience
Department of Psychiatry
Faculty of Medicine, University of Sao Paulo
Rua Doutor Ovidio de Campos 785
05403-010 Sao Paulo - S.P. - Brazil

Background: Research has been showing that oculomotor measurements can accurately detect higher-order cognitive deficits, namely in memory and visuospatial processing deficits (Rizzo et al., 2004; Garbutt et al., 2008; Crutcher et al., 2009). Literature has still failed to analyze the impact of the cognitive effort on the oculomotor parameters used to distinguish healthy subjects from those with cognitive decline due to Alzheimer's disease.

Methods: 26 participants were divided into 3 groups, 6 subjects diagnosed with AD, 10 subjects with MCI and 10 normal control subjects (NC). Subjects were instructed to look at two initial images - familiarization phase. After a time delay (5 seconds; 2 minutes) – test phase - two other images were presented, one of them identical to one of the previously seen (familiar stimulus). The subject was instructed to select the novel image compared to the familiar one. This novel image was either similar or non similar to the familiar stimulus. The experiment consisted of four blocks of five trials (2 minutes/similar; 2 minutes/non-similar; 5 seconds/similar; 5 seconds/non-similar). The following oculomotor parameters were analysed within predefined regions of interest (ROI) for each group: fixation count (FC) and visit duration (VD).

Results: Eye movement behaviors were compared in order to determine if they can effectively discriminate between groups. Both time and stimuli feature discriminated between groups when analyzing FC and VD. The 5 second trials discriminated between groups, although the 5 seconds/similar trials showed a higher efficiency in this discrimination. The same effect was obtained with the time delay of 2 minutes, which seemed to be more discriminative when associated with cognitive effort (2 minutes/similar).

Conclusion: Eye movement behavior can accurately distinguish between NC, MCI and AD groups. Time delay (2 minutes) presented a more accurate discrimination between groups. The same effect was observed for the cognitive effort (similar features). The combination of both similarity and longer time delay seemed to be even more accurate in this discrimination. These findings suggest that both cognitive effort and time delay represent a difficulty level increment on cognitive tasks that seemed to be useful when detecting cognitive decline prior to AD.

TARGETING OF PERK KINASE VIA SMALL MOLECULE INHIBITORS AS A NEW TREATMENT THERAPY FOR ALZHEIMER'S DISEASE

Rozpędek W.^a, Nowak A.^a, Pytel D.^b, Diehl J.A.^b, Majsterek I.^a.

^a Department of Clinical Chemistry and Biochemistry, Military-Medical Faculty, Medical University of Lodz, Lodz, Poland;

^b Department of Biochemistry and Molecular Biology, Hollings Cancer Center, Medical University of South Carolina, Charleston, USA

The main hallmark of Alzheimer's disease (AD) is the deposition of A β ₄₂ plaques among the neurons in tissue brain. Although the etiology of AD is not completely clear, abundant experimental evidence suggests that mutation in the Amyloid Precursor Protein (APP) gene initiates the amyloidogenic pathway, where the general role is played by enzyme secretase β . As a result, the level of the non-physiological form of A β is increased. Subsequent accumulation of misfolded and unfolded proteins in the Endoplasmic Reticulum (ER) lumen triggers ER stress and activation of the Unfolded Protein Response (UPR) signaling branches. Activated Protein kinase R-like endoplasmic reticulum kinase (PERK) causes phosphorylation of eIF2 α that

leads to the attenuation of global translation and, conversely, contributes to increased synthesis of proteins such as secretase β and Activating Transcription Factor 4 (ATF4). Prolonged ER stress conditions promotes translation of CCAAT-enhancer-binding *protein* homologous *protein* (CHOP), which results in apoptotic nerve cells death, memory loss and aggravation of cognitive function in AD patients.

The purpose of the present study was to explore the ability of selected small-molecule compounds to the inhibition of PERK-mediated signalling pathway, that may contribute to preventing the excessive accumulation of senile plaques among the neurons, neuronal loss and significant decline in cognitive abilities in AD.

We selected set of potential PERK inhibitors included 80 000 compounds by utilizing docking software. To evaluate their biological activity the Time Resolved Fluorescence test was utilized. We obtained 209 compounds for further analysis and their specific ability to inhibition only PERK kinase was measured by evaluating PERK phosphorylation at a concentration range of 250nM to 5000nM (250nM, 500nM, 1000nM, 5000nM) of each inhibitor using the Radioactive Kinase Assay. This analysis showed that inhibitor marked no. 3 significantly inhibited PERK kinase at 1000 nM and higher concentrations. We utilized cell line SH-SY5Y, with overexpression of APP gene. SH-SY5Y cells were treated with previously selected inhibitor at a concentrations: 50 μ M, 25 μ M, 12.5 μ M, 6.25 μ M, 3.12 μ M and with thapsigargin to evoke ER stress conditions. Moreover, we had two positive controls: SH-SY5Y without inhibitor compound and without thapsigargin, and the second one without inhibitor compound, but treated with thapsigargin. Then, we extracted total proteins from cultured lines and we measured PERK activity by evaluating the level of PERK phosphorylation using the Western Blot technique.

As a result, significant inhibition of PERK action was noted at 25 μ M and higher concentrations. In conclusion, our results suggest that targeting of components of UPR pathways on the molecular level presents a new point of view on the therapeutic strategy for AD. Deactivation of PERK kinase via small-molecule inhibitors is identified as a novel, potential therapeutic target for neurodegenerative diseases. It may be postulated that inhibition of PERK activity may contribute to preventing the excessive accumulation of A β ₄₂ among the neurons and, as a result, neuronal loss and memory impairment in AD.

This work was supported by grant HARMONIA no. 2013/10/M/NZ1/00280 from the Polish National Science Centre, grant PRELUDIUM no. 2015/19/N/NZ3/00055 from the Polish National Science Centre and by grant of Medical University of Lodz, Poland no. 502-03/5-108-05/502-54-170.

THE ROLE OF PERK KINASE AND UNFOLDED PROTEIN RESPONSE IN THE PATHOGENESIS OF ALZHEIMER'S DISEASE

Rozpędek W.^a, Nowak A.^a, Pytel D.^b, Diehl J.A.^b, Majsterek I.^a.

^a Department of Clinical Chemistry and Biochemistry, Military-Medical Faculty, Medical University of Lodz, 1 Hallera Sq., 90-647 Lodz, Poland;

^b Department of Biochemistry and Molecular Biology, Hollings Cancer Center, Medical University of South Carolina, HCC-709, 86 Jonathan Lucas Street, Charleston, SC 29425, USA

Alzheimer's disease (AD) is a progressive disease entity, which is closely connected with memory and other important mental function impairment. AD is one of the most common type of dementia, that is associated with gradual degeneration of neurons in tissue brain. Recent estimates suggest that the prevalence of AD may quadruple by 2050. Due to that fact AD may become one of the principal public health issues globally, that emphasizing the need for effective therapies, since current treatment may only temporally improve symptoms of AD.

There is abundant evidence that characteristic hallmarks of AD is deposition of toxic, non-physiological form of Amyloid β consisting of 42 amino acids (A β ₄₂) in tissue brain as well as synaptic neurodegeneration, but the etiology of AD still remains unclear. Recent data suggest that pathogenesis of AD is linked to the pathology of molecular mechanisms termed Unfolded Protein response (UPR). One of the transmembrane receptors of Endoplasmic Reticulum (ER) is protein kinase RNA - like endoplasmic

reticulum kinase (PERK), that is strongly associated with the pathological mechanisms activated under stress conditions, that are evoked by the accumulation of unfolded and misfolded proteins within ER lumen of nerve cells. Above-mentioned un-physiological conditions triggers ER stress and subsequently activation of UPR branches. Stress-dependent activation of PERK kinase contributes to its trans-oligomerisation and trans-autophosphorylation. Activated PERK kinase phosphorylates serine 51 at α subunit of the Eukaryotic Initiation Factor 2 (eIF2) that leads to the global translation attenuation apart from selective mRNAs such as Activating Transcriptor Factor 4 (ATF4) and secretase β . Protein secretase β is an enzyme that plays the vital role in the processing of Amyloid Precursor Protein (APP), since it initiates amyloidogenic pathway that results in generation of A β 42 with ability to aggregation in tissue brain as a toxic senile plaques. Surprisingly, excessive, prolonged ER stress conditions, which the initial aim is to support cell survival, may switch into the pro-apoptotic signalling network. Generally, ATF4 is a transcription factor that regulates a wide range of genes, which play a crucial role in cell adaptation to stress conditions, but paradoxically, during long-termed ER stress, ATF4 may also stimulate gene of CCAAT-enhancer-binding protein homologous protein (CHOP), which is responsible for initiation the apoptotic cascade of AD neurons.

Due to that the fact dysregulated translation, increased expression of genes secretase β , ATF4 and CHOP, as a result of PERK-mediated eIF2 α phosphorylation, may be a major contributor to structural and functional neuronal loss resulting in memory impairment in AD patients. Thus, blocking PERK-dependent eIF2 α phosphorylation through specific, small-molecule PERK branch inhibitors seems to be a potential treatment strategy for AD individuals. That may contribute to the restoration of global translation rates and reduction of expression of ATF4, CHOP and secretase β . Importantly, that treatment strategy can block accelerated β -amyloidogenesis by reduction in APP cleaving via the secretase β -dependent amyloidogenic pathway.

This work was supported by grant HARMONIA no. 2013/10/M/NZ1/00280 from the Polish National Science Centre, grant PRELUDIUM no. 2015/19/N/NZ3/00055 from the Polish National Science Centre and by grant of Medical University of Lodz, Poland no. 502-03/5-108-05/502-54-170.

COMPONENTS OF UNFOLDED PROTEIN RESPONSE SIGNALING PATHWAY AS A THERAPEUTIC TARGET FOR ALZHEIMER'S DISEASE

Rozpędek W.^a, Nowak A.^a, Pytel D.^b, Diehl J.A.^b, Majsterek I.^a.

^a Department of Clinical Chemistry and Biochemistry, Military-Medical Faculty, Medical University of Lodz, 1 Hallera Sq., 90-647 Lodz, Poland;

^b Department of Biochemistry and Molecular Biology, Hollings Cancer Center, Medical University of South Carolina, HCC-709, 86 Jonathan Lucas Street, Charleston, SC 29425, USA

Alzheimer's disease (AD) is a progressive disorder, which the main hallmark is irreversible loss of neurons in brain tissue. Stimuli that contribute to neuronal cell death include misfolded and unfolded protein aggregation in the Endoplasmic Reticulum (ER) lumen. Due to that fact abundant evidence have suggested that cellular stress conditions trigger disruptions of ER homeostasis, which evoke ER stress. Rapid response of neurons to that un-physiological conditions is closely associated with the activation of pro-adaptive signalling branches named Unfolded Protein Response (UPR). That results in subsequent phosphorylation of Eukaryotic Initiation Factor 2 alpha (eIF2 α) by activated protein kinase RNA-like endoplasmic reticulum kinase (PERK), rapid downregulation of global protein synthesis and preferential expression of genes such as Activating Transcriptor Factor 4 (ATF4) and secretase β . Interestingly, prolonged activation of UPR axis may evoke a paradoxical response through the initiation of apoptotic neurons death. Pharmacologically decreasing, by inhibition PERK-dependent molecular pathway, the levels of eIF2 α phosphorylation and other proteins involved in AD pathogenesis may be a potential treatment strategy for AD.

Latest evidence suggest that utilizing a specific inhibitor of PERK kinase termed GSK2606414 in mouse model of AD eliminates the attenuation of global protein translation. Furthermore, phosphorylation of eIF2 α was inhibited and due to the fact that the level of ATF4 and CHOP was significantly decreased neurons of tissue brain were protected against apoptotic cell death.

Moreover, small molecule inhibitors ISBIR (Integrated Stress Response Inhibitor) possess the ability to reverse effects of PERK kinase activation under sustained ER stress conditions. It has been reported that ISBIR may selectively attenuate only PERK kinase. ISBIR contributes to significant decrease in the levels of ATF4 and CHOP, but without any alterations in the level of phosphorylated eIF2 α . It allows to conclude that ISBIR may arrest effects of PERK kinase activation and reverse protein translation under ER stress conditions. Importantly, it has been reported that ISBIR plays a key role in memory and learning processes, since markedly enhances memory consolidation in wild type mice.

Utilizing inhibitor termed Sephin1 contributes to the suppression of creation the protein complex DNA damage-inducible protein-Protein Phosphatase 1 (GADD34-PP1). CHOP, a transcription factor of GADD34, directly leads to translational recovery, increases of ER nascent protein loads, thus promotes ER stress and apoptotic cell death of AD neurons. Sephin1 inhibits above-mentioned negative feedback loop, that promotes dephosphorylation of eIF2 α and subsequently triggers protein translation recovery in stressed cells. It allows to conclude that small-molecule inhibitor like Sephin1 protects AD neuros against apoptosis and memory impairment in AD patients.

This work was supported by grant HARMONIA no. 2013/10/M/NZ1/00280 from the Polish National Science Centre, grant PRELUDIUM no. 2015/19/N/NZ3/00055 from the Polish National Science Centre and by grant of Medical University of Lodz, Poland no. 502-03/5-108-05/502-54-170.

MOLECULAR GENETIC INSIGHTS IN ALZHEIMER'S DISEASE

Rozpedek W.^a, Nowak A.^a, Pytel D.^b, Diehl J.A.^b, Majsterek I.^a.

^a Department of Clinical Chemistry and Biochemistry, Military-Medical Faculty, Medical University of Lodz, 1 Hallera Sq., 90-647 Lodz, Poland;

^b Department of Biochemistry and Molecular Biology, Hollings Cancer Center, Medical University of South Carolina, HCC-709, 86 Jonathan Lucas Street, Charleston, SC 29425, USA

Alzheimer's disease (AD) is characterized by aggregation of toxic senile plaques among the neurons in the brain and synaptic neurodegeneration. Detailed genetic and biochemical investigations suggest that abnormal deposition of A β leads to significant loss of neurons in brain tissue. Amyloid β composed of 42 amino acids (A β 42), that play a central role in initiating AD, was identified as a major component of extracellular senile plaques. It is worthy to note that increased level of A β 42 is strictly correlated with significant disturbances in the processing of type I transmembrane protein termed Amyloid Precursor Protein (APP). Development of AD symptoms is associated with occurrence of approximately 30 mutations in a gene encoding APP, that is located on chromosome 21. Processing of APP protein is regulated by three proteolytic enzymes such as: secretase α , β and γ . Especially secretase β , also termed BACE1, is involved in amyloidogenic pathway. Adversely, processing of APP by secretase α ensues by the non-amyloidogenic, physiological molecular pathway, since it cleavages APP in the middle of A β sequence, that prevents the creation of full-length A β 42. APP cleaving in specific site K687/L688 release two products such as: soluble APPs α degraded in extracellular environment, and fragment C83, subsequently cleaved by secretase γ on non-amyloidogenic parts called p7 and p3. Firstly, secretase β cleaves APP at M671/D672 into APPs β and C99, that as a membrane-bound protein is cleaved by secretase γ at G708/G709, G709/V710, V711/I712, that generates A β 37-40 or A713/T714, which creates A β 42. The second fragment produced during APP processing by secretase γ is the carboxyl terminus of A β named AICD (APP intracellular domain). Mutations on chromosome 21. located near the cleavage site by secretase β or γ contribute to APP processing by amyloidogenic pathway. Also, mutations in the middle of A β are associated with a significant decrease in APP processing by secretase α , thus promote the amyloidogenic pathway. Altogether pathogenesis of AD is linked to disturbances on the molecular level. Generation of non-physiological forms of protein like A β 42 may evoke ER stress conditions within Endoplasmic Reticulum (ER) lumen, that leads to the activation of the Unfolded Protein Response (UPR) signaling pathway. Under long-termed stress conditions UPR may switch signal form pro-adaptive, which the main aim is to recover cell homeostasis, into apoptotic cell death, that is closely connected with significant decrease in brain mass resulting in cognitive impairment in AD patients.

This work was supported by grant HARMONIA no. 2013/10/M/NZ1/00280 from the Polish National Science Centre, grant PRELUDIUM no. 2015/19/N/NZ3/00055 from the Polish National Science Centre and by grant of Medical University of Lodz, Poland no. 502-03/5-108-05/502-54-170.

GOLD NANOPARTICLES-BASED RAPID ANTI-AGGREGATION DRUG SCREENING METHOD FOR REALIZING PERSONALIZED MEDICINE OF ALZHEIMER'S DISEASE

Kim HY, Kwon JA and Choi I*

Department of Life Science, University of Seoul, 163 Siripdae-ro, Dongdaemun-gu, Seoul 130-743, Republic of Korea.

**Corresponding author: inheechoi1@uos.ac.kr*

The extracellular accumulation of amyloid- β aggregates is currently considered as one of the most indisputable factor implicated in Alzheimer's disease. However, the conventional methods to screen anti-aggregation drugs for the Alzheimer's disease are limited for rapid screening effective drugs because of long incubation times, labor-intensive, complicated sample pretreatment steps, and expensive instrumentation. Here, we present a nanoparticle-assisted rapid colorimetric drug screening method for finding out effective anti-aggregation drugs. In our method, nanoparticles act as catalytic activators, which accelerate the kinetics of the protein aggregation. Simultaneously, these nanoparticles exhibit colorimetric responses according to their embedded shapes on the structure of protein aggregates. By using this method, we successfully achieved rapid screening of anti-aggregation drugs for Alzheimer's disease since effective drugs can attenuate protein aggregation process with depending on their binding affinity to target protein. As a result, we observed colorimetric responses for anti-aggregation effects of potential drug candidates with the naked eye (without any instrumentation) within a few minutes. Moreover, we quantitatively evaluated the drug effect under various destabilizing conditions through corresponding spectral shifts collected by microplate reader. We believe that these findings will accelerate developing not only drug screening method for conquering protein conformational diseases such as aging-related neurodegeneration including Alzheimer's disease, Lou Gehrig's disease and Parkinson's disease but also therapeutic methods for curing protein conformational diseases.

REAL-TIME MONITORING OF REACTIVE OXYGEN SPECIES GENERATED IN NEURONAL CELLS BY AMYLOID β AGGREGATES VIA A NOVEL PLASMONIC METHOD

Kim Y, Kim HY, Kwon JA and Choi I*

Department of Life Science, University of Seoul, 163 Siripdae-ro, Dongdaemun-gu, Seoul 130-743, Republic of Korea.

**Corresponding author: inheechoi1@uos.ac.kr*

The accumulation of amyloid β ($A\beta$) peptides-induced cellular oxidative stress is widely known as a main cause of Alzheimer's disease (AD). The generation of reactive oxygen species (ROS) by $A\beta$ aggregates of brain tissues leads to apoptotic neuronal cell death implicated in AD. However, the current methods are difficult to be utilized for detecting ROS of living cell because of their poor sensitivity, reproducibility, selectivity, stability and *in vivo* applicability. Here, we present a novel method for real-time monitoring of ROS based on plasmon resonance energy transfer (PRET) phenomena between plasmonic nanoparticles and redox-active cytochrome *c* (Cyt *c*). Based on this principle, we observed the dynamic spectral changes in the fingerprint quenching dip of individual plasmonic nanoparticles following the redox state of the Cyt *c* induced by ROS. By using this method, ROS levels were determined from millimolar concentration range to physiologically relevant micromolar and nanomolar concentration range *in vitro*. Furthermore, we successfully monitored the generation of ROS in Neuro-2a cells incubated with $A\beta$ aggregates in real-time. We believe that our approach could provide a powerful method for achieving dynamic, high spatial monitoring of ROS affecting diverse neurological disorders as well as AD.

MEMORY PALACES TO IMPROVE QUALITY OF LIFE IN ALZHEIMER'S DISEASE

K. Bormans (i), K. Roe, D. De Wachter

(i) Faculty of Social Sciences, KULeuven, Leuven School for Mass Communication Research, KULeuven, Mechelsestraat 92, 3000 Leuven, Belgium

Among elderly adults, retrieval of proper names is a source of unease and distress. This naming deficit is an early symptom of patients coping with neurodegenerative disorders such as Alzheimer's disease (AD). Individuals with Mild Cognitive Impairment (MCI) are at great risk of being in a prodromal phase of developing Alzheimer's disease. Memory enhancement training is an upcoming intervention in the field of Neurology and Geriatrics. These interventions have already proven to increase cognition in individuals with Mild Cognitive Impairment. Yet no studies have attempted to combine two powerful communication strategies (i.e. Method of Loci and Face-Name mnemonic) with virtual reality to improve the memory of significant others. This study has the ambition to optimize these communication strategies by externalizing and customizing memory palaces for subjects in early AD. Professional architects will construct virtual scale models of the house these subjects presently live in. They function as memory palaces in which photos of significant others are connected to the architectural, spatial environment. In addition we decorate the actual houses in accordance with the scale models. Thus (virtual) reality supports and facilitates participants during their familiar walk along the loci route. We hypothesize this to generate a convenient instrument allowing participants to independently improve recall and recognition of familiar faces. By extension we propose to reinforce the dialogue that seemed to evaporate between patient and environment. At follow up we expect an increase in memory and quality of life for early AD subjects and their significant others.

Keywords. Alzheimer's disease, cognitive rehabilitation, communication strategies, method of loci, quality of life, virtual environments, augmented reality

THE ROLE OF NUCLEAR-MITOCHONDRIAL INTERACTIONS THAT INFLUENCE NEUROCOGNITIVE IMPAIRMENT IN ALZHEIMER'S DISEASE

Sandra Paulina Smieszek, Case Western Reserve University Cleveland Clinic

Numerous studies have linked the pathogenesis of Alzheimer's Disease (AD) to mitochondrial dysfunction and genetic variation within mitochondrial genomes. There are also clear connections between mitochondrial function and various processes governed by nuclear genes, in particular iron metabolism and transport. In a different phenotype known as HAND HIV-Associated Neurocognitive Disorder individuals do show neurocognitive decline resembling that of AD. Due to the influence of combined antiretroviral therapy (CART), earlier severe forms of NCI such as the AIDS dementia complex have given way to HAND, a more subtle, progressive cognitive dysfunction similar to Alzheimer's Disease. Some evidence of pathological consistencies between AD and HAND have been reported, including beta-amyloid deposition and accumulation. It is hypothesized that HIV accelerates aging processes within the brain leading to an early-onset of mild AD-like symptoms, however evidence supporting this connection is minimal, in part due to our lack of knowledge on the etiology of both of these conditions. We have observed multiple interactions between mitochondrial haplogroups and specific iron-related nuclear genotypes, suggesting that genetic variation within the iron/mitochondrial functional axis influences NCI. We are able to replicate these findings within the Alzheimer's Disease group of patients using sequencing data. We have identified highly significant common variants and pathways. That sheds light into the common pathways being activated in both HAND related decline and AD and may be of profound importance for the understanding of AD pathogenesis.

FUNCTIONAL BRAIN NETWORKS IN EARLY DIFFERENTIAL DIAGNOSIS OF DEMENTIA

Nazem A, Tang CC, Ma Y, Peng S, Spetsieris P, Dhawan V, Eidelberg D

The Elmezzi Graduate School of Molecular Medicine, The Feinstein Institute for Medical Research, Northwell Health, New York 11030

Clinical differential diagnosis between Alzheimer's disease (AD) and behavioral variant frontotemporal dementia (bvFTD) can be challenging especially early in the course of the disease. Biomarkers that improve the accuracy of early diagnosis of dementia syndromes can play an important role in patient selection and stratification in clinical trials designed to test disease-modifying agents to intervene with the specific underlying neurodegenerative mechanisms.

In this study, we hypothesized that metabolic disruption in bvFTD is specific, and can be identified as a disease-related spatial covariance pattern with significantly different expression between bvFTD and AD. To this end, FDG PET data from 10 clinically confirmed bvFTD patients and 10 age and gender matched healthy controls underwent spatial covariance mapping with principal component analysis to identify an abnormal network topography associated with bvFTD (generic bvFTD-related metabolic pattern, bvFTDRP). Subject scores for the first principal component (PC1, VAF=21.12%) significantly discriminated bvFTD patients from healthy controls (validation $p < 0.0001$). This bvFTDRP was characterized by covarying metabolic reductions in the anterior cingulate cortex, medial frontal lobe, inferior frontal gyrus, orbitofrontal cortex and insula. This topography demonstrated a close voxel-wise correlation ($r = 0.71$; $p < 0.001$; Figure 2A) with an analogous disease-related pattern identified by analysis of FDG PET data from a combined group comprised of 7 bvFTD subjects with confirmed FTLD pathology and 7 healthy subjects scanned at Universität München. The bvFTDRP exhibited a significant voxel-wise correlation ($r = -0.64$, $p < 0.001$; Figure 2B) with the previously characterized normal default mode network (DMN) topography. Nonetheless, the bvFTDRP topography was unrelated ($r = -0.02$; Figure 2C) to the recently characterized Alzheimer's disease-related covariance pattern (ADRP) topography. Indeed, bvFTDRP expression levels differed for bvFTD and AD patients ($p < 0.001$).

In conclusion, bvFTDRP was characterized by disease-related topographical features, involving key regions in the normal DMN. bvFTDRP expression levels (subject scores) discriminated between bvFTD subjects and healthy controls as well as AD patients.

THE IMPAIRMENT OF BILIVERDIN REDUCTASE-A PROMOTES BRAIN INSULIN RESISTANCE IN ALZHEIMER DISEASE: A NOVEL MECHANISM.

E. Barone*, F. Di Domenico, D. A. Butterfield, M. Perluigi

*presenting author: Department of Biochemical Sciences "A. Rossi-Fanelli", Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, ITALY.

Clinical studies suggest a link between peripheral insulin resistance and cognitive dysfunction. Interestingly, post-mortem analyses of Alzheimer disease (AD) subjects demonstrated insulin resistance in the brain proposing a role for cognitive deficits observed in AD. However, the mechanisms responsible for the onset of brain insulin resistance (BIR) need further elucidations. Biliverdin reductase-A (BVR-A) emerged as a unique Ser/Thr/Tyr kinase directly involved in the regulation of insulin signaling. Indeed, once activated via Tyr phosphorylation by insulin receptor (IR), BVR-A is able to phosphorylate the insulin receptor substrate (IRS)-1 on inhibitory domains, representing an upstream regulator in the insulin signaling cascade. Because we previously demonstrated the oxidative/nitrosative stress (OS/NS)-induced impairment of BVR-A in human Alzheimer disease (AD) brain, here we hypothesize that BVR-A dysregulation could be associated with the onset of BIR in AD.

To this aim, we performed a longitudinal analysis to evaluate BVR-A protein levels and activation in the hippocampus of 3xTg-AD and WT mice at 3, 6, 12 and 18 months of age ($n = 6$ /group). Changes of BVR-A have been then correlated with changes about (i) IR/IRS1 protein levels and activation state (ii) total OS/NS markers levels (PC, HNE, 3-NT), (iii) changes of A β and tau pathology, (iv) TNF- α levels and (v) mTOR

activation. Subsequently, ad hoc experiments have been performed in SH-SY5Y cells treated with (i) insulin, (ii) hydrogen peroxide/peroxynitrite or (iii) a specific silencing RNA (siRNA) for BVR-A, to clarify the molecular mechanism(s) underlying changes observed in mice.

Our results highlighted two distinct phases in the hippocampus of 3xTg-AD mice: a first insulin signaling hyper-activation (3-6 months) followed by a persistent IRS1 inhibition and thus insulin resistance at both 12 and 18 months. In this picture, we found that BVR-A levels and activation start to decline early, at 6 months of age, prior the accumulation of A β and tau pathology, and remain persistently reduced until 18 months, possibly because the increased OS/NS levels in the same time-frame. In addition, because TNF- α is known to inhibit BVR-A promoter activity and TNF- α has been also demonstrated to be a conceivable mediator of BIR in AD, we wondered to check whether reduced BVR-A levels were associated with changes of TNF- α . Interestingly, an increase of TNF- α levels is evident only at 18 months, thus highlighting the impairment of BVR-A as an early event in the onset of BIR. Similar changes have been found during the normal ageing process in WT mice, but later in life. Experiments on SH-SY5Y cells further confirmed that either lack of BVR-A or OS/NS-induced impairment of BVR-A promotes BIR. Finally, we identified the sustained activation of mTOR, as one of the feedback mechanisms leading to insulin resistance following BVR-A impairment both in mice and cells.

In conclusion, we propose a novel mechanism for which: OS/NS-induced impairment of BVR-A is firstly responsible for a sustained activation of IRS1, which then causes the stimulation of negative feedback mechanisms (i.e. mTOR) aimed to turn-off IRS1 hyper-activity and thus insulin resistance. Similar alterations characterize the normal ageing process in mice, positing BVR-A impairment as a possible bridge in the transition from normal ageing to AD.

ALZHEIMER'S DISEASE RISK ALLELES AND CUMULATIVE GENETIC RISK ARE LINKED TO HETEROGENEITY IN MICROGLIA AND PATHOPHYSIOLOGY

Yi Cao, Patrick Haddick, Karen Cuenco, Melanie Huntley, Jennifer Tom, Tushar Bhangale, Shyamali Mandal, William Forrest, Robert Graham, *Marcel van der Brug**
Genentech, Inc., 1 DNA Way, South San Francisco, CA, 94080, USA

A major challenge in developing therapeutics for Alzheimer's disease (AD) is the wide range of heterogeneity in the clinical and pathophysiological phenotypes.

To better understand molecular heterogeneity within AD and identify disease pathways and candidate diagnostic biomarkers, we characterized a cohort of 100 cases and 35 age matched controls. We serially sectioned temporal lobe brain tissue from AD cases at different disease stages (Braak III to VI) and performed whole genome sequencing, RNAseq, and IHC for fibrils, plaques, microglia, and astrocytes, sequentially on adjacent sections. With this large spatially proximal, cross-platform dataset we were able to study genotype, pathology and cellular interactions. We observed enrichment of GWAS risk alleles in genes that are expressed in microglia, and that the cumulative genetic risk was also associated with microglia number. We also found nominal associations between individual GWAS hits CD2AP, PTK2B (rs10948363, rs28834970) and plaque density and microglia phenotypes.

NITRO-PROTEOMICS TO DISCOVER SYNAPTOSOMAL BIOMARKERS OF NEUROINFLAMMATION IN ALZHEIMER'S DISEASE

*TS. Wijasa*¹, F. Brosseron¹, MT. Heneka^{1,2}.

1German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

2University Hospital Bonn, Dept. of Neurology, Clinical Neuroscience Unit, Bonn, Germany

Background: Alzheimer's disease (AD) is pathologically characterized by the presence of senile plaques, neurofibrillary tangles, and early synapse loss. Another important characteristic of AD is neuroinflammation, which can result in increased levels of nitrated proteins in the brain, due to glial NOS2 expression. This study aims to characterize the nitrosylation fingerprint of synaptic proteins in a mouse model of AD to discover new potential biomarkers, followed up by analyzing human brain tissue.

Methods: Male APP/PS1 mice age 3 and 12 months old are used as AD model with wild type as controls. NOS2-deficient mice with the same age are used to see the role of nitric oxide in neuroinflammation. Synaptosomes are prepared from whole brain excluding cerebellum and brain stem. Protein S-nitrosylation modification is detected using modified mass tag labeling strategy. All samples are subjected to liquid chromatography and mass spectrometry for quantitative proteome analysis.

Result: Synaptosome samples of 12 animals per group have been collected. The MS analysis using IodoTMT sixplex detected 469 nitrosylated proteins, of which 38 proteins were localized in the synaptosomes.

Conclusion: A modified mass tag labeling workflow for multiplex quantitative proteomics of nitrosylated proteins has been established. Identification of the modified proteins will establish a link between nitrosylation of synaptic proteins and Alzheimer Disease and potentially provide new biomarker candidates.

A SYSTEMATIC REVIEW OF POSITIVE PSYCHOLOGY MEASURES FOR FAMILY CARERS OF PEOPLE LIVING WITH DEMENTIA

J. Stansfeld, C. Stoner, J. Wenborn, M. Vernooij-Dassen & M. Orrell

Jacki Stansfeld,

Research & Development Department

North East London Foundation Trust

1st Floor Maggie Lilley Suite

Goodmayes Hospital

Barley Lane

Ilford

Essex

IG3 8XJ

Introduction: The importance of positive psychology in understanding the wellbeing and experiences of family carers of people with dementia is increasingly being recognised. Despite this, outcome measures used in research with family carers of those with dementia are often centered on concepts such as burden and depression. There is a scarcity of positive psychology measures developed for or validated in this population (Tarlow et al., 2015).

Aim: By employing standardised criteria, this review aimed to assess the quality of positive psychology measures developed for or already in use with family carers of people with dementia and to determine their potential utility in future interventional studies.

Methods: We performed a systematic review of positive psychology measures for family carers of people with dementia. The databases searched were PsychINFO, CINHALL, MEDLINE, EMBASE and PubMed. Two reviewers independently assessed full-texts for inclusion and performed a quality assessment of each of the scale development studies identified to examine the psychometric properties reported.

Results: This review identified 10 positive psychology outcome measures (and 6 validation papers of these scales) within the constructs of self-efficacy, spirituality, resilience, gain, and meaning.

Conclusion: Several outcome measures were identified that may have potential utility for future interventional studies, but it is clear that there is still work to be done to develop and refine more positive psychology measures for this population. A lack of reporting of the psychometric properties by development authors limited the conclusions that could be drawn. It is recommended that authors aim to report this in the future.

USE OF AUTOMATED TECHNOLOGY-BASED ADHERENCE AIDS TO IMPROVE MEDICATION ADHERENCE IN PATIENTS WITH DEMENTIA: A SYSTEMATIC LITERATURE REVIEW

S. Curran, M. Dodd, M. Stead and R. Bougoffa

Protomed House, 6 John Bradshaw Court, Congleton Business Park, Congleton, Cheshire, CW12 1LB
Corresponding author: Rosie Bougoffa, Mednet Consult Ltd., 40 Otley Road, Leeds, LS6 2AL

Scope:

Patients with dementia are particularly susceptible to medication non-adherence and may be vulnerable to medication-related adverse outcomes, which are associated with significant morbidity and admission to secondary care (Maidment, I. et al. *International Journal of Geriatric Psychiatry*, 2012, 27, 439-442).

It is suggested that automated technology-based reminders may be beneficial in improving medication adherence. A qualitative study has demonstrated that patients find compliance aids, particularly those that are monitored, simplify their medication regimens and increase their sense of control (Lecouturier, J. et al. *British Journal of General Practice*, 2011, 61, 93-100).

Search methods:

Systematic electronic and manual searches were performed. Two reviewers screened titles and abstracts, before selecting studies to include in the review using pre-determined criteria.

Results:

176 titles and abstracts were screened, with 35 full text articles reviewed. 3 studies were included in the literature review, with a total of 36 patients with mild dementia or cognitive impairment. Interventions included telephone or video calls before each dosage time, pre-recorded video reminders, and a medication reminder device with audible and visual stimuli.

Medication monitoring through telephone or video calls was suggested to improve medication adherence in patients with dementia. Caregiver burden was additionally reduced. Video reminders were not shown to increase medication adherence any more than caregiver reminders.

A medication reminder device was shown to increase medication adherence in 83% of patients with mild cognitive impairment after one month, with continued stability or improvement after 3 months.

Conclusion:

Although minimal studies are available, current literature appears to favour the use of medication reminder devices and technological advances, compared with caregiver help alone. Further investigation is warranted. To this end, a study investigating the use of Biodose Connect®, a unique medicines delivery system enabling live, remote monitoring, tailored interventions and adherence management in patients with dementia will be undertaken.

LONG-TERM EFFECTS OF IMMUNOSTIMULATION ON SYNAPTIC PLASTICITY AND NEURODEGENERATION

Authors: *Saqib Faiz*¹, Martin Korte², Michael T. Heneka^{1, 3}

¹German Center for Neurodegenerative Diseases (DZNE), Bonn, Germany

²Helmholtz Centre for Infection Research, Neuroinflammation and Neurodegeneration - NIND, Braunschweig, Germany

³University Hospital Bonn, Dept. of Neurology, Clinical Neuroscience Unit, Bonn, Germany

Introduction: Recurrent systemic infection is a driving force for neurodegeneration and, in particular, Alzheimer's disease. It has been reported that about 71% of septic patients develop septic encephalopathy (SE), an acute condition leading to potentially irreversible cerebral dysfunction. SE is caused by systemic inflammatory processes evoked as an immune response to bacterial lipopolysaccharide (LPS) or other endotoxic bacterial cell wall components. Acute peripheral inflammation can negatively impact neuronal plasticity and learning behavior in rodents; however, how these are affected in time upon immune stimulation remains largely unexplored.

Methods: Young (4 months old) and old (16 months old) wild type C57Bl6/J and APP/PS1 transgenic mice received a single dose of 0.4 µg/g body weight of LPS administered peripherally, after which learning behavior was tested through Morris water maze (MWM) and open field tests. Right brain hemispheres (excluding cerebellum and brain stem) were removed to obtain synaptosomal preparations, while the left hemispheres were dissected to extract proteins from hippocampus and cortex for cytokine measurements.

Results: Impairments in learning and increased anxious behavior were observed in old animals upon exposure to LPS from *Salmonella enterica* serogroup typhimurium, which also led to a reduction of pre- and post-density synaptic proteins, such as PSD95, synaptophysin, synaptotagmin and MUNC-18. Cytokine levels of IL-1β and TNF-α were largely increased in hippocampus as well as cortex of LPS-treated old mice. These results were further validated in an in vitro model of sepsis.

Conclusion: Taken together, our results suggest that peripheral stimulation of the immune system may impair the structure and function of synapses long after the immune challenge; however, the effect is dependent on the stimulatory component.

CHEMICAL MECHANISMS OF HDL DYSFUNCTIONAL IN ALZHEIMER'S DISEASE

Wei-Hsiang Chen, Shi-Hui Law, Hsiao-Ting Lu, *Liang-Yin Ke*, Mei-Chuan Chou, Chu-Huang Chen, Hua-Chen Chan

Background:

HDL plays an important role in preventing neurodegenerative disorders and anti-inflammatory activity. In contrast, dysfunctional HDL impairs ATP-binding cassette, sub-family A member 1 (ABCA1) and scavenger receptor class B member 1 (SRB1)-mediated cholesterol efflux pathways in Alzheimer's disease (AD). To delineate the mechanisms, we examined the chemical properties of their high-density lipoprotein (HDL).

Methods and results:

HDL isolated from AD patients (HDL-C, 51±11 mg/dL; n=41) exhibited greater mobility in agarose gel electrophoresis than LDL of healthy control subjects (HDL-C, 52±12 mg/dL; n=24), secondary to an increased representation of H5 (23.48±17.83% vs. 4.24±3.22%; P<0.001), the most electronegative subfraction of HDL identified by anion-exchange chromatography. RAW 264.7 cells incubated with 22-NBD-cholesterol were used to test the cholesterol efflux by 25, 50, 75, 100 µg/mL HDL from AD or healthy control subjects. Result showed that the AD-HDL was significantly defected in the function of reverse cholesterol transport. By using LC/MSE (ACQUITY UPLC M-Class System, Xevo G2 QTof), the lipid components were quantified to test the differences of HDL from AD and in healthy control subjects. Lyso-phosphatidylcholines (LPC 16:0 and 18:3 (6Z, 9Z, 12Z) significantly decrease in AD patients (8.06 and 14.3 fold respectively, P<0.001). By LC/MSE and ProteinLynx Global SERVER (PLGS), we confirmed that apoCIII

was increased and albumin was decreased in AD-HDL, which enhanced lipid raft formation and inflammation.

Conclusions: The AD-HDL is attributed in part to elevated percentage of electronegative subfraction (H5), impaired function of reverse cholesterol transport and decreased content of LPC. ApoCIII-rich H5 from AD enhanced lipid raft formation, which is associated with augmented inflammatory reactions.

ROLE OF ELECTRONEGATIVE LDL IN DYSREGULATED HEPATIC GLYCOSYLATION ASSOCIATED OBSTRUCTIVE SLEEP APNEA

Yu-Ching Tan, Shi-Hui Law, Ying-Hui Lee, Hua-Chen Chan, Mei-Chuan Chou, Chu-Huang Chen, *Liang-Yin Ke*

Background:

Obstructive Sleep Apnea (OSA) is a common sleep disorder accompanied by frequent episodes of apnea and hypopnea, often correlates with metabolic syndrome (MetS) and hypoxia-mediated Alzheimer's disease. However, detailed mechanisms are still not clear. Because electronegative low-density lipoprotein (LDL), L5, is atherogenic in vitro and in vivo, we therefore hypothesized that hypoxia will enhance the elevation of plasma L5 in OSA patients, in turn to increase the risk of cardiovascular and cerebrovascular diseases.

Methods and results:

LDL isolated from OSA patients exhibited a higher percentage of L5 than healthy control subjects ($2.4 \pm 0.7\%$ vs. $9.6 \pm 8.2\%$; $P < 0.01$). By using LC/MSE (ACQUITY UPLC M-Class System, Xevo G2 QTof), the lipid components were quantified. Lyso-phosphatidylcholines has increased significantly in LDL which isolated from OSA patients (5.45 folds, $P < 0.001$). In cell studies, hypoxia (1% O₂, 5% CO₂) induced glial fibrillary acidic protein (GFAP) and O-Linked N-Acetylglucosamine Transferase over-expression in a time-dependent manner. Pharmaceutical inhibitor such as 0.2 μ M Azaserine attenuated the hypoxia-induced GFAP and OGT expression. In contrast, polypeptide N-acetylgalactosaminyl transferase 2 (GALNT2) expression was decreased under hypoxic conditions. Through disruption of OGT/ O-GlcNAc hydrolase (OGA), proteins produced in hepatocytes were extensively glycosylated.

Conclusions: Hypoxia disrupts the balance of OGT/OGA expression and leads to the extensive protein glycosylation in liver cells, which can be an important mechanism for the over production of L5 in OSA patients.

APOLIPOPROTEIN E -491 GENE POLYMORPHISM AND ALZHEIMER'S DISEASE: A SYSTEMATIC REVIEW AND META-ANALYSIS

Wen-Li Hsu^{1,4}, Liang-Yin Ke^{1,2,3}, Shi-Hui Law², Chu-Huang Chen^{1,3,5,6}, Ming-Hsien Tsai³

¹Lipid Science and Aging Research Center, Kaohsiung Medical University (KMU), Kaohsiung, Taiwan;

²Department of Medical Laboratory Science and Biotechnology, KMU, Kaohsiung, Taiwan.

³Center for Lipid Biosciences, KMU Hospital, KMU, Kaohsiung, Taiwan;

⁴The Institute of Basic Medical Sciences, College of Medicine, NCKU, Tainan, Taiwan;

⁵Vascular and Medicinal Research, Texas Heart Institute, Houston, TX, USA;

⁶New York Heart Research Foundation, Mineola, NY, USA;

Background:

Alzheimer's Disease (AD) is a complex disease associated with the genetic polymorphisms as well as environmental factors. In particular, polymorphisms of apolipoprotein E (apoE), which locate on the promoter or the coding region for the 299 amino acids (or a ~34 KDa protein), have been recognized as a factor correlated with the onset of AD. The three major common types of apoE gene polymorphism in the coding region were well studied and systematic reviewed. However, the -491 A/T promoter polymorphism is still controversial in different populations and in different case-control studies.

Methods and results:

We performed a comprehensive meta-analysis by electronic databases which are available on site of Pubmed, LISTA (EBSOC) or Medline. Through relevant literature search from 1998 to 2016, we aim to define

the association of apoE -491 A/T polymorphism and AD. The MeSH terms including “apolipoprotein E”, “apoE”, “-491”, “-491A/T” “promoter”, “polymorphism”, “Alzheimer’s disease”, “Alzheimer”, and “AD” were utilized as search keywords. 835 references were identified by keywords; 45 references were screened by abstract. Following with detailed review, 24 studies were included into this meta-analysis. The genotypes were grouped as AA or non-AA (including AT or TT) and allele type was grouped as A allele and T allele. The fixed or random effect model was used to evaluate the pooled odds ratio (OR) by STATA 14 software Metan and Metafunnel command. Overall, a total of 6130 AD cases and 4690 controls were reevaluated. Results show that genotype AA is significantly associated with AD risk (OR=1.116, 95% CI=1.051~1.185, p<0.001). In addition, allele type A is also significantly associated with AD risk (OR=1.052, 95%CI=1.021~1.083, p=0.001).

Conclusions: Although apoE promoter -491 polymorphism is not obviously increased the risk of AD in comparison to apoE e4 polymorphism, it is still show significant effect on the AD risk in our systematic review and meta-analysis study.

ATP-BINDING CASSETTE TRANSPORTER G1 AND APOLIPOPROTEIN M ARE "NOVEL PLAYERS" IN CHOLESTEROL TRANSPORT AT THE BLOOD-BRAIN BARRIER

Alexandra Kober¹, Anil Paul Chirackal Manavalan¹, Elham Fanaee Danesh¹, Martina Zandl¹, Carmen Tam-Amersdorfer¹, Nicole Albrecher¹ Gunther Marsche², Michael Holzer², Gerhard Kostner³, Andreas Holmer⁴, Björn Dahlbäck⁴, and Ute Panzenboeck¹

¹Institute of Pathophysiology and Immunology; ²Institute of Experimental and Clinical Pharmacology; ³Institute for Molecular Biology and Biochemistry, Medical University of Graz, Austria; ⁴Department of Laboratory Medicine, Division of Clinical Chemistry, Lund University, Sweden

Background: Impaired cholesterol and lipoprotein metabolism in the brain has been linked to AD. The BBB restricts exchange with plasma lipoprotein cholesterol and maintains cerebral cholesterol homeostasis via a mechanism of compensatory elimination. Unlike cholesterol, the oxysterols 24(S)-hydroxycholesterol (24(S)OH-C) and 27-hydroxycholesterol (27OH-C) readily cross the BBB bidirectionally and promote expression of several targets including ATP binding cassette transporter (ABC)A1 and apolipoprotein (apo)A-I. The presence of HDL apolipoproteins is required for cholesterol and lipid transfer within the brain and loss of ABC G1 and G4 result in the accumulation of oxysterols in the brain. ApoM, a mainly HDL-associated apolipoprotein, has been shown to be positively associated with pre-β HDL concentrations and pre-β HDL formation in plasma, suggesting an important role in HDL metabolism and potentially in cellular cholesterol homeostasis.

Aims: In light of our recent findings that modulation of cellular cholesterol metabolism alters APP synthesis and processing in porcine brain capillary endothelial cells (pBCEC), this study aimed to identifying ‘novel’ transporters/proteins involved in cholesterol and oxysterol transport at the BBB using the established in vitro model.

Methods and Results: RTQ-PCR analyses and immunoblotting revealed that in addition to ABCA1 and SR-BI, pBCEC also express ABCG1 and ABCG4. ABCG1 but not ABCG4 expression was up-regulated (up to 10-fold) by LXR activation. Immunofluorescent staining showed that both proteins are localized intracellular and ABCG1 co-localizes to early and late endosomes. Site-specific biotinylation and immunoprecipitation identified ABCG1 also on the plasma membrane and verified LXR induced ABCG1 gene expression in pBCEC. Functional studies on ABCG1 using siRNA interference and sterol efflux assays were performed. ABCG1 silencing (by ~50%) reduced HDL mediated [³H]-cholesterol efflux (by ~50%) but did not influence [³H]-24(S)-hydroxycholesterol efflux from pBCEC. Moreover, we show for the first time that, in addition to apoA-I, pBCEC express and secrete apoM. The presence of HDL enhanced mRNA expression of apoM and HDL enriched with apoM promoted cholesterol efflux from pBCEC more efficiently as compared to control HDL.

Conclusion: LXR activation of ABCG1 and modulation of apoM levels may influence cholesterol turnover and HDL formation and maturation at the BBB and adds two more targets that support regulation of cholesterol metabolism at the interphase between the brain and the circulation.

Contact:

e-mail: alexandra.kober-macher@medunigraz.at

Telephone number: 0316 380 7671

Address: Heinrichstrasse 31a

8010 Graz

WHITE MATTER CHANGES IN ALZHEIMER'S DISEASE: A FOCUS ON OLIGODENDROCYTES

S. E. Nasrabady^{1,3}, E. P. Cortes^{3,4}, JP. Vonsattel^{3,4}, J. E. Goldman^{2,3}, A. M. Brickman³

622 West 168th Street, PH18-324

New York, NY, 10032

¹⁾ Division of Late Life Disorders, Department of Psychiatry, Columbia University ²⁾ Department of Pathology and Cell Biology, Columbia University ³⁾ Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Department of Neurology, Columbia University ⁴⁾ New York Brain Bank, Columbia University

Introduction: Pathologically Alzheimer's disease (AD) is characterized by two hallmark changes in grey matter: extracellular neuritic plaques, made of amyloid-beta (A β), and neurofibrillary tangles, which are interneuronal aggregates of phosphorylated tau protein. However, recent neuroimaging studies implicate white matter abnormalities in the pathogenesis and course of AD. Despite these fairly consistent observations, the underlying pathophysiology of radiologically-defined white matter abnormalities is poorly understood. Since the myelin that comprises the white matter is produced by oligodendrocytes changes in the number and/or function of these cells may be one source of white matter abnormalities in AD. In addition to production, oligodendrocytes maintain and repair myelin and are also responsible for synthesizing brain cholesterol. The purpose of this study is to investigate the changes in white matter in AD with a focus on oligodendrocyte lineage cells and to explore the mechanistic basis of the imaging abnormalities.

Methods: Using Olig2 immunohistochemistry we are quantifying the changes in the distribution of oligodendrocytes in two white matter brain areas: frontal and parietal areas, which correspond to areas that show the greatest amount of radiological white matter abnormalities in aging and AD. Within each area we are studying the oligodendrocyte changes in periventricular, deep, and subcortical regions. For analysis, formalin (10%) fixed tissue is processed and paraffin embedded and 7 μ m sections are mounted on slides and will be stained with H&E and Olig2/hematoxylin.

Results: To date we have collected the brain tissue of 30 AD patients (19 female, 11 male; average age: 82.5 years) and 12 controls (8 females, 4 males; average age: 72 years). Standardized pathological assessment showed that 36% of the AD cases had severe neuronal loss in grey matter and 17% had preserved normal neuronal density and 33% of the cases showed mild neuronal loss or neuronal loss was limited to distinct areas (14% remained unspecified). Atrophy degree was scored from 0 to 4. In general, in both grey and white matter the majority of cases (46%) were graded for 2nd degree atrophy, however white matter frontal area shows the highest 3rd degree atrophy amongst white and gray matter areas. White matter vessels showed thickened fibrosis walls and enlarged perivascular spaces in 66% and 60% of the cases, respectively. The proportion of Olig2 positive nuclei to the total nuclei number was significantly increased in deep white matter of AD cases (N=7) in comparison with controls (N=6). In addition, there was an upward trend in Olig2 positive nuclei in subcortical white matter, while periventricular white matter showed no change in the number of Olig2 positive nuclei.

The assessment of white matter oligodendrocytes number and nuclei size distribution is ongoing. In addition, because of the abundant vessel abnormalities in white matter, as the future plan we will also focus on the oligodendrocyte changes around these vessels. Vascular and myelin markers (trichrome and LFB, respectively) will be used to measure the extent of the anatomical deficits in white matter of Alzheimer's disease patients.

PROBING AMYLOID BETA-INDUCED CELL DEATH USING A FLUORESCENCE-PEPTIDE CONJUGATE IN ALZHEIMER'S DISEASE MOUSE MODEL

H K JIN ^{1,2} AND JAE-SUNG BAE ^{1,3}

¹Stem Cell Neuroplasticity Research Group, Kyungpook National University, Daegu, Korea;

²College of Veterinary Medicine, Kyungpook National University, Daegu, Korea;

³Department of Physiology, School of Medicine, Cell & Matrix Research Institute, Kyungpook National University, Daegu

With the increasing worldwide incidence of Alzheimer's disease (AD), there is a critical need for the discovery of more effective diagnostic methods. However, development of diagnostic tools in AD has been hindered by obstacles such as the absence of exact biomarkers. Apoptosis caused by amyloid-beta (A β) plays an important role in AD pathology; therefore, provides an attractive biological target for the diagnosis of AD. The present study aimed to evaluate the potential of small peptide, named ApoPep-1 (Apoptosis-targeting peptide-1) as a new apoptosis imaging agent in AD. The fluorescein-conjugated ApoPep-1, but not the control peptide, targeted apoptotic cells in the brain of amyloid precursor protein (APP)/presenilin 1 (PS1) mice. We also observed fluorescence signals during in vivo imaging of apoptotic cells using ApoPep-1, and fluorescence levels increased in an age-dependent manner in APP/PS1 mice. Ex vivo imaging of isolated brains in APP/PS1 mice further confirmed the targeting of ApoPep-1 to apoptotic cells. The fluorescein-labeled ApoPep-1 co-localized with brain cells such as neurons, astrocytes, and microglia, all of which undergo apoptosis in the APP/PS1 mice brain. These findings demonstrate that ApoPep-1 can target apoptotic brain cells, and be used for experimental investigations relevant to apoptosis in AD.

BEXAROTENE MODULATES CHOLESTEROL AND AMYLOID-BETA METABOLISM IN AN IN VITRO MODEL OF THE BLOOD-BRAIN BARRIER

Elham Fanaee-Danesh¹, Martina Zandl¹, Marielies Reiter¹, Anil Paul Chirackal Manavalan¹, Chaitanya Chakravarthi Gali¹, Nicole Maria Albrecher¹, Alexandra Kober-Macher¹, Ute Panzenboeck¹

¹Department of Pathophysiology and Immunology, Medical University Graz, Austria

Using an in vitro model of the blood-brain barrier (BBB) we have shown that primary porcine brain capillary endothelial cells (pBCEC) express and release apoA-I that may assemble with cellular cholesterol to form HDL, a pathway that is enhanced by treatment with nuclear receptor agonists. These apo/lipoproteins may also interact with A β or its precursor protein (APP). Pharmacological modulation of cellular cholesterol metabolism could contribute to redirect APP synthesis and processing by cerebrovascular endothelial cells towards the beneficial, non-amyloidogenic pathway. This study aims to investigate the effects of a pharmacologic retinoid-X receptor (RXR) agonist, bexarotene on pathways of APP processing, A β production and transfer across the BBB. So far, our results demonstrate that cellular cholesterol efflux, the obligatory first step in reverse cholesterol transport, to both apoA-I or HDL3 was enhanced upon administration of 100 nM bexarotene to pBCEC for 24 h. Treatment of pBCEC with bexarotene increased mRNA expression levels of APP, ADAM10, ApoA-I, SREBP2, LRP1, MDR1, MRP1, and BCRP, whereas BACE mRNA expression as well as activity was decreased. ROS levels in pBCEC were significantly decreased in response to 100 nM bexarotene. Transport studies revealed that bexarotene treatment enhanced A β clearance across pBCEC Transwells to the apical/serum compartment. Bexarotene suppressed cholesterol biosynthesis and esterification.

Our results strongly suggest that this nuclear receptor agonist exerts beneficial effects on cholesterol and A β metabolism at the BBB.

IN VITRO AND IN VIVO INVESTIGATIONS OF TYROSINE OXIDATION IN AMYLOID-BETA (A β)

Yousra Al-Hilaly, Thomas Williams, Julian Thorpe, Alaa Abdul Al-Sada, Louise Serpell
School of Life Sciences, University of Sussex, Brighton BN1 9QJ, UK

Dityrosine is a tyrosine dimer that results from the ortho-ortho coupling of two tyrosine residues. The formation of dityrosine cross-links is one of the irreversible oxidative modifications that may mediate the toxicity of amyloid-beta (A β) in Alzheimer's disease and it has also been implicated in many diseases, including Parkinson's disease, cystic fibrosis, atherosclerosis and cataracts in the eye lens. In this regard, dityrosine has been used as an important biomarker for identification of oxidative protein damage, as well as aging diseases. Many studies have shown that A β oligomers, rather than amyloid fibrils, are the principle pathogenic species in Alzheimer's disease. The monomeric species of A β can assemble and couple through dityrosine to give A β oligomers (eg. dimer and trimer). We have investigated the formation of dityrosine cross-links in A β 42 formed by covalent ortho-ortho coupling of two tyrosine residues under conditions of oxidative stress with elevated copper and have shown that dityrosine can be formed in vitro in A β oligomers and fibrils and that these links further stabilize the fibrils. Using a specific antibody for dityrosine and immunogold labeling transmission electron microscopy, we have revealed the prevalence of dityrosine cross-links in amyloid plaques in brain tissue and in cerebrospinal fluid from AD patients. A β dimers may be stabilized by dityrosine crosslinking. These results indicate that dityrosine cross-links may play an important role in the pathogenesis of Alzheimer's disease and can be generated by reactive oxygen species catalyzed by Cu²⁺ ions. The observation of increased A β and dityrosine in CSF from AD patients suggests that this could be used as a potential biomarker of oxidative stress in AD.

ABERRANT CELLULAR MORPHOLOGY IN INDUCED HUMAN ASTROCYTE MODELS OF ALZHEIMER'S DISEASE.

VC JONES, A VERKHRATSKY, L MOHAMET

School of Pharmacy and Biomedical Sciences, University of Central Lancashire, MB024 Maudland Building, Fylde Road, Preston, PR1 2HE

Astrocyte dysfunction is a defining feature of many neurological disorders, including Alzheimer's disease (AD). Astroglial changes in animal models of AD are represented by both atrophy and hypertrophy, the latter representing defensive functional remodelling, known as astrocyte reactivity. While these results are interesting, their interpretation is complicated by limitations of the model, which does not fully recreate the progression of AD seen in humans. Here, we utilise induced pluripotent stem cell (iPSC) technology to derive enriched populations of induced astrocytes from patients with familial AD (PSEN M146L) and sporadic AD (ApoE4+/+), as well as from healthy individuals. Astrocytes derived from iPSCs from both familial and sporadic AD patients demonstrated acutely aberrant morphological deficits (including an overall cell size reduction and loss of processes) and abnormal expression of astroglial markers (including dramatic mislocalisation of S100B) compared to healthy controls. Taken together, these data suggest that human astrocytes harbouring these AD-associated mutations are inherently abnormal and that this pathology is independent of the influence of neurones.

EEG MEASURES OF NEURAL PLASTICITY IN MILD COGNITIVE IMPAIRMENT AND ALZHEIMER'S DISEASE

M. J. Spriggs, L. J. Tippett, & I. J. Kirk

Author affiliations: Brain Research New Zealand,

Centre for Brain Research, New Zealand

Cognitive Neuroscience Research Group, The University of Auckland

Corresponding author: M. J. Spriggs. mspr827@aucklanduni.ac.nz

The School of Psychology, The University of Auckland

Private Bag 92019

Auckland 1142, New Zealand

It is currently widely agreed that disruptions in synaptic plasticity are an early neuropathological change in Alzheimer's disease (AD; Klein, 2006; Knoblock & Mansuy, 2008; Liao et al., 2014), and that synapse loss is the most reliable correlate of cognitive symptoms (Overk & Masliah, 2014; Spires-Jones & Hyman, 2014; Su et al., 2010). However, to date, a lack of non-invasive measures of neural plasticity in humans have limited its application in clinical settings. In association with Brain Research New Zealand and the Dementia Prevention Research Clinic (DPRC) at the University of Auckland, the aim of the current research is to develop electrophysiological measures of neural plasticity in humans that may be used as indicators of disease progression. Specifically, we hope to test whether simple perceptual tasks presented during EEG recording can be used to identify disrupted neural plasticity at the synaptic and network level in MCI and early AD. This is in line with the overall goals of the DPRC; to understand the underlying causes, treatment, management and prevention of AD, and to improve the wellbeing of all ageing New Zealanders.

RELATIONSHIP BETWEEN CORTEX AND AUTONOMIC NERVOUS SYSTEM BY MENTAL ACTIVITY IN PATIENTS WITH MILD COGNITIVE IMPAIRMENT

Fokin V., Ponomareva N., Medvedev R., Lagoda O., Tanashian M.,

Research Center of Neurology, Volokolamskoe sh.80, Moscow, Russia 125367, fvf@mail.ru

Mental activity is accompanied by the activation of the autonomic nervous system (ANS), and may cause mental stress and pronounced changes in brain energy metabolism in patients with mild cognitive impairment (MCI).

132 MCI patients, men and women, were examined during the performance of cognitive tests: proofreading, verbal fluency and verbal memory Luria test. Blood pressure, heart rate, Doppler measure of linear blood flow velocity (LBV) in cerebral arteries, DC potentials of the brain were recorded. Glucose in blood, cortisol and insulin in saliva were examined before and after the performance of cognitive tests.

During the performance of cognitive tests significant increase in blood pressure and heart rate, as well as the DC potentials in different area of the head was observed. After the tests the level of glucose showed a decrease (-0.68±0.2 mmol/l), and the concentration of cortisol and insulin showed a significant increase (11.6±3.3 nmol/l; 4.7±1.2 pmol/l). Lowering of blood glucose level was associated with worse performance of the proofreading test ($r=0.44$, $p=0.001$) and better performance of verbal fluency test ($r=0.28$, $p=0.04$).

Better performance of verbal fluency test correlated with the increasing of heart rate during the test ($r=0.41$; $p=0.001$). The increase of DC potential in left temporal area was associated with better verbal memory in Luria's test ($F=6.9$, $p=0.01$). LVB in the main cerebral arteries of the head was not significantly changed during cognitive tasks. However the increase of LVB during the test in right middle cerebral artery was associated with worse performance of proofreading test ($F=6.6$; $p=0.02$).

The results suggest that the performance of different cognitive tests is accompanied by similar pattern of ANS reactivity. However successful performance of cognitive tests correlated with different characteristics of ANS reactivity, this may be due to the activation of different cortical and ANS structures during cognitive processing.

The study was supported by grant RFBR N15-04-05066-a

EFFECT OF THE KETOGENIC DIET ON BLOOD-BRAIN BARRIER

M. Corsi^{1,2}, P. Candela¹, A. Fuso², L. Fenart¹, R. Businaro^{2*} and F. Gosselet^{1*}

¹Artois University, Blood-Brain Barrier Laboratory (Laboratoire de la barrière hémato-encéphalique, LBHE) EA 2465-IMPRT 114, Faculté Jean Perrin, rue Jean Souvraz S.P.18, F-62300 Lens, France

²Department of Medico-Surgical Sciences and Biotechnologies, Sapienza University of Rome

* Corresponding author: R. Businaro (rita.businaro@uniroma1.it) and F. Gosselet (fabien.gosselet@univ-artois.fr).

Introduction: Alzheimer's disease (AD) is an age related progressive neurodegenerative disorder characterized by an abnormal biosynthesis and accumulation of the neurotoxic β -amyloid ($A\beta$) peptide in the brain. AD remains asymptomatic for a considerable time before cognitive decline becomes clinically evident. For this reason, the establishment of appropriate nutritional protocols, in the early stage, may be more effective than any drug treatments in the fight against this disease. Among them, Ketogenic Diet (KD) is very interesting. This is a high in fat, adequate-proteins and low carbohydrates diet which produces ketone bodies (KBs) (alternative energy source to glucose for the brain) in the body. Nowadays, relations between KBs and cerebral $A\beta$ accumulation are not clear. Most studies focus on the neuronal component and forget the vascular component of AD represented by the blood-brain barrier (BBB), located at the cerebral microvessels level. Consequently, it seems essential to focus on the BBB physiology, and in particular on the BBB's functions of the receptors/transporters and enzymes involved in $A\beta$ peptide transport and metabolism, to better understand the influence of a KD on the onset and the evolution of this disease.

Methods: For 4 weeks, Wild type mice (129SV) were maintained on KD and Control Diet (CD). Body weight was measured. Glucose and Beta-hydroxybutyrate (BHB) levels were assessed in blood sample. Microvessel fractions were isolated from total brain (Coisne et al., 2005) and qPCR analyses were performed to study expression of transporters, receptors and enzymes involved in amyloid transport and metabolism at the BBB level.

Results: KD fed animals showed increased levels of BHB which was accompanied by an increased expression of Monocarboxylate Transporters 1 (MCT1) and Glucose transporter 1 (GLUT1) at the BBB level. There were not changes in the level of Glucose and body weight in these mice. In addition we observed modifications in the expression of some transporters involved in $A\beta$ exchanges such as Low density lipoprotein receptor-related protein 1 (LRP1) and Multidrug resistance-associated protein 1 (MRP1). The expression of $A\beta$ Synthesis enzymes remains unchanged, instead an increase for the $A\beta$ degradation enzyme Endothelin Converting Enzyme 1 (ECE1) was observed.

Discussion: These preliminary results show that dietary factors and in particular KD can modulate the expression of some actors implicated in brain $A\beta$ metabolism at the BBB level. Further investigations are necessary, in particular using transgenic mice, to confirm our results.

Coisne C, Dehouck L, Faveeuw C, Delplace Y, Miller F, Landry C, Morissette C, Fenart L, Cecchelli R, Tremblay P, Dehouck B. (2005) Mouse syngenic in vitro blood-brain barrier model: a new tool to examine inflammatory events in cerebral endothelium. *Lab Invest.* Jun;85(6):734-46

EFFECT OF BEXAROTENE ON A β PEPTIDE EFFLUX ACROSS THE HUMAN BLOOD-BRAIN BARRIER IN VITRO

P. Candela*, C. Coisne*, E. Sevin, M-C. Boucau, L. Fenart and F. Gosselet

Artois University, Blood-Brain Barrier Laboratory (Laboratoire de la barrière hémato-encéphalique, LBHE) EA 2465-IMPRT 114, Faculté Jean Perrin, rue Jean Souvraz S.P.18, F-62300 Lens, France. * Equal contributors and corresponding authors: Dr. P. Candela (pietra.candela@univ-artois.fr) and Dr. C. Coisne (caroline.coisne@univ-artois.fr)

Alzheimer's disease (AD) is mainly associated with excessive β -amyloid peptide (A β) accumulation in the brain. This phenomenon would result from (i) dysregulation of brain cholesterol homeostasis and (ii) impaired bidirectional A β exchanges between blood and brain compartments. Both mechanisms appear to be closely related and controlled by the blood-brain barrier (BBB) which is localized at the brain capillary endothelium level. Thus in search of therapeutics to treat AD, nuclear retinoid X receptors (RXR) have emerged as promising targets. By regulating brain cholesterol homeostasis, the anticancer drug bexarotene, a RXR agonist, has been shown to restore cognitive functions and decrease brain amyloid burden in animal models of AD (Cramer et al., 2012). When several investigations tried to reproduce and explain the therapeutic potential of bexarotene, focuses were mainly given to the neuro-glial component of the disease while omitting the key role played by the BBB in this process. Therefore our work aimed at investigating the effect of bexarotene on A β transport across the endothelial cells forming the BBB using a human in vitro system (Cecchelli et al., 2014). Our previous results demonstrated that bexarotene treatment provoked ABCB1 transporter upregulation in BBB cells along with a decrease in β -amyloid peptide influx (i.e A β transport from blood to brain compartment across the BBB) (Kuntz et al., 2015). However the effect of bexarotene on A β peptide efflux (i.e A β transport from brain to blood compartment across the BBB) still needs to be fully characterized. Preliminary results showed a two-fold increase in A β peptide efflux across the human in vitro BBB upon bexarotene treatment. Real time RT-PCR data showed modulation of the expression of various transporters expressed in endothelial cells upon drug treatment. Blocking transporters such as ABCB1 and, to a lesser extent, LRP family members together with drug treatment partially reversed bexarotene effect on A β transport, indicating their involvement in this process. Therefore, complementary investigations are still compulsory to fully understand which effectors are involved in the drug action on the A β peptide transport across the blood-brain barrier with the goal to develop novel targeted therapeutic approaches.

Cecchelli R, Aday S, Sevin E, Almeida C, Culot M, Dehouck L, Coisne C, Engelhardt B, Dehouck MP, Ferreira L. (2014). A stable and reproducible human blood-brain barrier model derived from hematopoietic stem cells. *PLoS One*, 9(6):e99733.

Cramer PE, Cirrito JR, Wesson DW, Lee CY, Karlo JC, Zinn AE, Casali BT, Restivo JL, Goebel WD, James MJ, Brunden KR, Wilson DA, Landreth GE. (2012). ApoE-directed therapeutics rapidly clear β -amyloid and reverse deficits in AD mouse models. *Science*, 335(6075):1503-6.

Kuntz M, Candela P, Saint-Pol J, Lamartinière Y, Boucau MC, Sevin E, Fenart L, Gosselet F. (2015). Bexarotene Promotes Cholesterol Efflux and Restricts Apical-to-Basolateral Transport of Amyloid- β Peptides in an In Vitro Model of the Human Blood-Brain Barrier. *J Alzheimers Dis*, 48(3):849-62.

RELATIONSHIP BETWEEN CORTEX AND AUTONOMIC NERVOUS SYSTEM BY MENTAL ACTIVITY IN PATIENTS WITH MILD COGNITIVE IMPAIRMENT

Fokin V., Ponomareva N., Medvedev R., Lagoda O., Tanashian M.,

Research Center of Neurology, Volokalamskoe sh.80, Moscow, Russia 125367, fvf@mail.ru

Mental activity is accompanied by the activation of the autonomic nervous system (ANS), and may cause mental stress and pronounced changes in brain energy metabolism in patients with mild cognitive impairment (MCI).

132 MCI patients, men and women, were examined during the performance of cognitive tests: proofreading, verbal fluency and verbal memory Luria test. Blood pressure, heart rate, Doppler measure of linear blood flow velocity (LBV) in cerebral arteries, DC potentials of the brain were recorded. Glucose in blood, cortisol and insulin in saliva were examined before and after the performance of cognitive tests.

During the performance of cognitive tests significant increase in blood pressure and heart rate, as well as the DC potentials in different area of the head was observed. After the tests the level of glucose showed a decrease (-0.68 ± 0.2 mmol/l), and the concentration of cortisol and insulin showed a significant increase (11.6 ± 3.3 nmol/l; 4.7 ± 1.2 pmol/l). Lowering of blood glucose level was associated with worse performance of the proofreading test ($r=0.44$, $p=0.001$) and better performance of verbal fluency test ($r=0.28$, $p=0.04$).

Better performance of verbal fluency test correlated with the increasing of heart rate during the test ($r=0.41$; $p=0.001$). The increase of DC potential in left temporal area was associated with better verbal memory in Luria's test ($F=6.9$, $p=0.01$). LVB in the main cerebral arteries of the head was not significantly changed during cognitive tasks. However the increase of LVB during the test in right middle cerebral artery was associated with worse performance of proofreading test ($F=6.6$; $p=0.02$).

The results suggest that the performance of different cognitive tests is accompanied by similar pattern of ANS reactivity. However successful performance of cognitive tests correlated with different characteristics of ANS reactivity, this may be due to the activation of different cortical and ANS structures during cognitive processing.

The study was supported by grant RFBR N15-04-05066-a

TAU PATHOLOGY IN AGED CYNOMOLGUS MONKEYS WITH LONG-TERM TYPE 2 DIABETES MELLITUS.

ZM Zhang^{1,2*}, F Yue³, Y Ai¹, CL Lu³, RC Grondin^{1,2}, GA Gerhardt^{1,2}, DM Gash^{1,2}

¹. Department of Anatomy and Neurobiology, University of Kentucky College of Medicine, Lexington, KY, USA

². GLP Neuroscience Service Center, University of Kentucky College of Medicine, Lexington, KY, USA

³. Wincon TheraCells Biotechnologies Co., Ltd, 3 Gaoxindong 3rd Road, Nanning, Guangxi, China

Several epidemiological and clinical studies show that diabetes increases the risk to develop Alzheimer's disease (AD) (Baglietto-Vargas, Shi et al. 2016) with type 2 diabetes mellitus (T2DM) patients being more likely to exhibit increased susceptibility to AD (Kimura 2016). These clinical studies strongly support the notion that aging patients with diabetes are at higher risk of developing AD. In testing these theories, we organized a preclinical study to invest if T2DM can accelerate tau pathology in particular in aged cynomolgus monkeys with long-term T2DM. The monkey criterion were: age (>22 years old), fasting plasma glucose (FPG) >6.9 mmol/L, and glycated hemoglobin (Hb-A1c) >6.5% for aged monkeys with T2DM. In addition, T2DM animals had significantly higher levels of plasma insulin ($P<0.0001$) and abnormal lipid profiles. Five additional aged animals with normal range of FPG, and Hb-A1c were used as normal controls. Antibodies (AT 180, AT8, AT100) were used for detecting tau pathogenesis and A β 1-16 was used for detecting senile plaques in some key brain regions associated with AD including hippocampus, parts of

entorhinal, frontal and temporal cortex. The result of histological analysis demonstrated that significantly higher numbers of AT180 and AT8 positive neurons were found in the CA1, CA2 and CA3, subiculum and entorhinal cortex in the brains of T2DM animals compared to normal controls. AT100-positive neurons with collapsed basal dendrites were also more often observed in T2DM hippocampus and its surrounding fields. Additionally to increased NFTs in T2DM animals, A β positive senile plaques were also significantly increased in T2DM compared to normal controls. These data strongly support the notion that T2DM can accelerate tau pathology in aged cynomolgus monkeys with long-term T2DM, and aging diabetic nonhuman primates could be used as a highly relevant AD-model for testing therapeutic interventions in AD.

Key Words: Diabetes, Alzheimer's disease, Tau, Cynomolgus monkeys, beta-amyloid

THE ROLE OF EXTRACELLULAR VESICLES IN THE PROCOAGULANT PROFILE OF ALZHEIMER'S DISEASE PATIENTS

J. E. Nielsen¹, S. Papaioannou², G. Christiansen³, S. Andersen⁴, S. Li⁴, S. R. Kristensen^{1, 5}, S. Pedersen^{1, 5}

¹)Department of Clinical Biochemistry, Aalborg University Hospital, Aalborg, Denmark

²)Aalborg University Hospital, Aalborg, Denmark

³)Department of Biomedicine, Aarhus University, Aarhus, Denmark

⁴)Department of Geriatric Medicine, Aalborg University Hospital, Aalborg, Denmark

⁵)Department of Clinical Medicine, Aalborg University Hospital, Aalborg Denmark

Introduction: The most common form of dementia is Alzheimer's Disease (AD), with an estimated global prevalence to 46.8 million people. AD has been linked to vascular pathologies and a prothrombotic state has been observed in this patient group. Extracellular vesicles (EVs) harbour procoagulant properties, as they can express tissue factor (TF) and phosphatidylserine on their surface, and has been shown to mediate a procoagulant response in pathological conditions. Isolation of EVs is still up for investigation and consensus between researchers for the most suitable isolation method has not yet been reached.

Aim: The aim of this study was to (1) compare EV isolation proficiency using isolation methods size exclusion chromatography (SEC) and ultracentrifugation (UC). (2) Determine a procoagulant profile of plasma and in vitro spiked EVs in AD patients compared to healthy controls. (3) Correlate particle concentration and age with coagulation parameters.

Materials and methods: Peripheral blood from 13 healthy controls (69.77 ± 2.13 SEM) and 7 AD patients (82.25 ± 1.28 SEM) was centrifuged at $3,220 \times g$ for 15 min at room temperature and plasma was filtered through a $0.22 \mu\text{m}$ filter. EV isolation was performed using SEC and UC. Sample purity was determined by particle- and protein-concentrations investigated using nanoparticle tracking analysis and bicinchoninic acid assay. Confirmation of EVs was validated by western blot (WB) and transmission electron microscopy with immunogold labeling (TEM/IEM). Coagulation profiles of plasma and in vitro spiked EVs were determined by the calibrated automated thrombography, procoagulant phospholipid activity (PPL), and rotational thromboelastometry.

Results: Both isolation methods showed good isolation proficiency. UC samples showed positive signals for EV markers in WB, while TEM/IEM for SEC and UC samples showed CD9+ EVs. Increased thrombin generation was observed in AD patients compared to healthy controls, with significant difference in time to peak (ttPeak), although PPL activity were indifferent. In vitro spiked EVs from AD patients and healthy controls did not affect thrombin generation, but increased PPL activity. A correlation between age and ttPeak was found (p value = 0.0199).

Conclusion: UC is recommended as the most suitable method for EV isolation from plasma. AD patients exhibited a TF dependant prothrombotic state compared to healthy controls. In vitro spiked EVs from AD patients and healthy controls increased PPL activity, but thrombin generation was unaffected. Thrombin generation (ttPeak) was shown to correlate with age.

Disclosure of Interest: None Declared

INTERACTIONS OF SIMVASTATIN AND APOJ WITH APP PROCESSING AND AMYLOID-B CLEARANCE IN BLOOD-BRAIN BARRIER ENDOTHELIAL CELLS

Martina Zandl¹, Elham Fanaee-Danesh¹, Nicole M Albrecher¹, Chaitanya Gali¹, *Marielies Reiter*¹, Steffen Storck², Ahmed Saeed³, Alexandra Kober¹, Claus Pietrzik², Ingemar Björhem³, Mark R Wilson⁴, Ute Panzenboeck¹

¹ Department of Pathophysiology and Immunology, Medical University of Graz, Austria

² Institute for Pathobiochemistry, University Medical Center of Johannes Gutenberg University Mainz, Mainz, Germany

³ Division of Clinical Chemistry, Department of Laboratory Medicine, Karolinska University Hospital, Karolinska Institute Huddinge, Sweden

⁴ School of Biological Sciences, University of Wollongong, Australia

Amyloid- β peptides ($A\beta$) accumulate in cerebral capillaries indicating a central role of the blood-brain barrier (BBB) in the pathogenesis of Alzheimer's disease (AD). Although a close connection between apolipoprotein-, cholesterol- and $A\beta$ metabolism is evident, the interconnecting mechanisms operating in brain capillary endothelial cells (BCEC) are poorly understood. Apolipoprotein (apo)J, also known as clusterin, is present in lipoprotein particles and regulates cholesterol and lipid metabolism in the CNS, which are disturbed in AD. ApoJ expression is increased in AD brains and ApoJ binds, prevents fibrillization, and enhances endocytosis and clearance of $A\beta$. Our central study aim is to define the involvement of apoJ and cellular cholesterol homeostasis in amyloid precursor protein (APP) processing/ $A\beta$ metabolism at the BBB.

Primary porcine (p)BCEC were incubated in the presence or absence of plasma-derived apoJ and modulators of cholesterol metabolism prior to analyses of APP/ $A\beta$ and apoJ mRNA and protein expression levels. $A\beta$ transport studies were aimed to elucidate the role of apoJ and the HMGCoA reductase inhibitor simvastatin in $A\beta$ clearance across the BBB.

Simvastatin treatment [5 μ M] increased both apoJ and full-length APP in primary, porcine (p)BCEC. In pBCEC simvastatin also reduced $A\beta$ uptake and cell-associated $A\beta$ oligomer levels, leaving extracellular $A\beta$ peptides increased. The addition of purified apoJ increased APP, had no effect on $A\beta$ oligomer levels, but enhanced $A\beta$ clearance across the in vitro model of the BBB. ApoJ silencing decreased APP and $A\beta$ oligomer levels in pBCEC. In addition, treatment with $A\beta$ 1-40 revealed increased expression of both apoJ and lipoprotein receptor-related protein 1 (Lrp1), which were primarily secreted from pBCEC and promoted $A\beta$ clearance.

We suggest an important role of cellular cholesterol homeostasis and apoJ in modulating APP/ $A\beta$ metabolism at the posttranslational level and $A\beta$ clearance in cerebrovascular endothelial cells.

Contact:

e-mail: martina.zandl@medunigraz.at

Telephone number: +43 316 380 7671

Address: Heinrichstrasse 31a

A-8010 Graz

A DEMENTIA SCREENING INVESTIGATION FOR INHABITANTS OF RURAL AREAS IN JAPAN

Yukihisa Matsuda, Reiko Hashimoto, Sachiko Takemoto, Masao Ishizaki, Yasuhiro Kawasaki

Kanazawa Medical University, Uchinada 920-0293, Japan

In Japan, the number of dementia patients is increasing at present, and societies in rural areas are tending towards becoming super-aging societies. It is hard for inhabitants who live in these rural areas to access hospitals. Thus, there are very few opportunities to diagnose and treat for dementia in its early stage. From 2013, we conducted a dementia screening project based on a dementia awareness training program known in Japan as the Dementia Supporter Caravan. We chose three areas where the percentages of inhabitants over 65 years old were 50% to 73%. The screening tests consisted of an Action Observation Sheet which had an assessment scale for symptoms of dementia (AOS; 52 items) and a Brain Function Battery (BFB; 12 tests). The BFB consisted of a series of questions and tests, and a BFB score was correlated with a Mini Mental State Examination and Clinical Dementia Rating ($r = 0.96, -0.89$, respectively). Over 30 trained testers conducted door-to-door canvassing for two days. Close to half the number of inhabitants participated in this study. The results of screening tests indicated that over half of the participants showed low BFB scores which were below the cut-off point. In the results of the AOS, 11% of participants had low-level healthy scores, but the remaining participants had scores showing borderline, mild, and severe symptoms of dementia. These screening results indicate that a significant number of inhabitants of rural areas are highly likely to be diagnosed with dementia if they were tested in a hospital.

ANTIBODIES TO SIGNALING MOLECULES AND RECEPTORS ARE ASSOCIATED WITH LONGITUDINAL RISK OF PSYCHOSIS AND DEATH IN ALZHEIMER'S DISEASE

Lasse M. Gill, MD1*, Anders Lund, Medical Student2, Christian A. Vedeler, MD, PhD4,5, Einar K. Kristoffersen, MD, PhD2,3, Harald Heidecke, MScPharm, PhD9, Ralf Dechend, MD, PhD10,11, Kai Schulze-Forster, MScPharm, PhD9, Dominik N. Muller, MScPharm, PhD10,12, Victoria S. von Goetze, Scientific Student9, Gabriela Riemekasten, MD, PhD13, Petra Vogelsang, MSc, PhD1,3, Dag Aarsland, MD, PhD6,7, Jan Erik Nordrehaug, MD, PhD2,8

1 Department of Internal Medicine, Haraldsplass Deaconess Hospital, Bergen, Norway

2 Department of Clinical Science, University of Bergen, Bergen, Norway

3 Department of Immunology and Transfusion Medicine, Haukeland University Hospital, Bergen, Norway

4 Institute of Clinical Medicine, University of Bergen, Bergen, Norway

5 Department of Neurology, Haukeland University Hospital, Bergen, Norway

6 Department of Old Age Psychiatry, Institute of Psychiatry, Psychology and Neuroscience, Kings College, United Kingdom

7 Centre for Age-Related Diseases (SESAM), Stavanger University Hospital, Norway

8 Department of Cardiology, Stavanger University Hospital, Stavanger, Norway

9 CellTrend GmbH, Luckenwalde, Berlin, Germany

10 Experimental and Clinical Research Center, Charité Medical Faculty and the Max-Delbrück Center for Molecular Medicine, Berlin, Germany

11 HELIOS-Klinikum Berlin-Buch, Berlin, Germany

12 Max-Delbrück Center for Molecular Medicine, Berlin, Germany

13 Department of Rheumatology, University Hospital Schleswig-Holstein, Lübeck, Germany

*Lasse Melvaer Gill

Haraldsplass Deaconess Hospital

Ulriksdal 8

5009 Bergen, Norway

lassegill@gmail.com

Background

Antibodies to signaling molecules and receptors are increased in Alzheimer's disease (AD) and may act on the brain's vasculature, immune system or interact with signal transmission. We investigated the effect of 33 antibodies to signaling molecules and receptors, including dopaminergic, serotonergic, muscarinic and adrenergic receptor antibodies on cognitive decline, neuropsychiatric symptoms and mortality.

Methods

Ninety one patients with mild AD were followed with annual measurement of the Mini Mental State Examination (MMSE) and the Neuropsychiatric Inventory (NPI) for 5 years. Antibodies were measured in sera with a full-receptor ELISA in u/mL. Because of the statistical distributions of antibody levels and multicollinearity, antibody levels were pre-transformed and stored as their principal components (PC) for use in multivariate analyses. MMSE decline (n = 81 with 2 measurements) was analyzed with a linear mixed effect model, NPI (n = 79 with 3 measurements) by generalized estimating equations (GEE). The different domain scores approximated a gamma distribution. Covariates for NPI analysis were age, sex, co-morbidity (Cumulative illness rating scale, CIRS. > 4), executive function (Trail Making A) and MMSE < 24. Survival was estimated with Cox Proportional Hazard Analysis (CPHA) (n = 91) with the last censoring date 05.11.2015. Covariates for survival was age, sex, body mass index and CIRS > 4.

Results

Antibodies loaded on four PCs explaining 85% of the variance. The first PC was loaded with factor loadings >0.9 by dopaminergic receptor antibodies, 5-HT1AR-ab, 5-HT2BR-ab and 5-HT6R-ab. In GEE PC1 had a significant effect on the domain score for delusions (t(t = time); ($\beta = 1.176$, $p < 0.001$), t2; ($\beta = -0.359$, $p < 0.001$), t3; ($\beta = 0.032$, $p < 0.001$)), hallucinations (t; ($\beta = 0.474$, $p = 0.084$), t2; ($\beta = -0.195$, $p = 0.039$), t3; ($\beta = 0.021$, $p = 0.025$)) and aberrant motor behavior (t; ($\beta = -0.977$, $p = 0.008$), t2; ($\beta = 0.321$, $p = 0.002$), t3; ($\beta = -0.029$, $p = 0.003$)). The first PC (HR 1.232, $p = 0.013$) and the fourth (HR 1.430, $p = 0.005$) PC had a negative impact on long term survival. The fourth PC had its factor loadings from NGF-ab and RAGE-ab. Post-hoc step-by-step analysis suggest that VEGFA-ab, D1R-ab, D42R-ab and RAGE-ab impact on mortality. There were no effects on cognitive decline.

Conclusion

Antibodies to dopaminergic and serotonergic receptors are associated with mortality and psychiatric symptoms in AD. Possible mechanisms include interplay between peripheral antibodies and central receptors for signal transmission in the brain or immune-regulation and psychiatric symptoms.

A HAPLOTYPE OF THE PURINERGIC P2Y12 GENE IS ASSOCIATED WITH INCREASED RISK OF ALZHEIMER'S DISEASE

CH Andersson, O Hansson, L Minthon, N Andreasen, K Blennow, H Zetterberg, I Skoog, A Wallin, S Nilsson and P Kettunen

Institute of Neuroscience and Physiology, Department of Psychiatry and Neurochemistry, The Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; Department of Neuropathology, Nuffield Department of Clinical Neurosciences, University of Oxford, Oxford, OX3 9DU, United Kingdom

Alzheimer's disease (AD) has been characterized by a chronic low-grade inflammatory response in the brain, primarily driven by microglia, the innate immune cells of the brain. The purinergic ADP-binding receptor P2Y12 is expressed by microglia and animal studies have suggested that P2Y12 and the other purinergic receptors may have an impact on the development of neurodegenerative diseases. However, to our knowledge no genetic studies have yet shown associations of P2Y receptors and risks of neurodegenerative diseases. Therefore, we investigated the possible association between genetic variation in P2Y12 and the risk of AD in a Swedish cohort consisting of AD patients and controls. We found a P2Y12 haplotype associated with increased risk of AD; an association that remained significant after correction for multiple testing. The genetic variation in P2Y12 was also investigated in the AD patients in regards to cognitive function and levels of total tau, phosphorylated tau and A β ₁₋₄₂, all characteristic cerebrospinal fluid biomarkers for AD. We found a number of SNPs nominally associated with A β ₁₋₄₂ levels, but these associations did not survive correction for multiple testing.