P3-420 CRITICAL "OMES" IN AGING AND NEURODEGENERATIVE PATHOLOGY. FROM THEORY TO PRACTICE

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Background: The presentation is centered on the notions issued in the recently growing field of OMICS. **Methods:** Taking into account that the 'omics approach' is not at all a sophisticated "translation" of the usual language in esoteric terms, but a newly proposed methodological tool striving to put a new conceptual order in the scientific thinking, and that the core of 'omics' is to perform an integrative attempt able to reveal the intimate core of that field and to drive it to practice improvement, the definition and pursuit of critical 'omes' in aging and neurodegenerative pathology could be a useful methodological attempt. **Results:** We present some partial results obtained by us in two nationally founded research projects, in which we are trying to investigate, on cohorts of helthy elderly by comparison with age matched patients, the relationships of some methylome and genome/epigenome particularities with the nutriomic and sociomic characteristics of that cohorts. **Conclusions:** We agree and comment the idea that the integrative pursuit of different neuromes and of their interplay with certain environmental (ecomes, nutriomes, chronomes) and inner organism omes such as metabolome,

P3-421 THE ROLE OF THE GGA PROTEIN FAMILY UPON BACE TRANSPORT AND APP PROCESSING IN ALZHEIMER'S DISEASE

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Background: Abeta generation is caused by sequential cleavage of the amyloid precursor protein (APP) by two proteases, first beta-site of APP-cleaving enzyme (BACE) followed by Gamma-secretase. Abeta accumulates in senile plaques in Alzheimer's disease (AD). APP and BACE traffic together. GGA1, a member of the Golgi-localized gamma-ear-containing ARF binding (GGA) protein family interacts with BACE phosphorylation dependent via its VHS domain in the Golgi and influences its subcellular distribution.Therefore we were interested in the question whether GGA2 or GGA3 or both also colocalize and interact with BACE and alter its subcellular occurrence and distribution. **Methods:** We applied confocal imaging and a novel technique to show close protein-protein vicinity in living cells with fluorescence protein tags: spectral fluorescence lifetime imaging microscopy (FLIM). Spectral FLIM is a novel technique, which combines spectral resolved and time resolved detection. Additionally an electrochemoluminescence-based assay was established to measure the influence of GGA1, 2 and 3 upon APP processing. **Results:** We found colocalization of GGA1, GGA2 and GGA3 with BACE1 at perinuclear compartments. The colocalization of GGA2 and BACE1 was enhanced in endosomal as well as Golgi structures whereas the colocalizations of GGA3 and BACE1 were smaller and could only be shown at the ER or Golgi. Regarding the GGAs and BACE FLIM we found a clear decrease in donor lifetime indicating interaction between all three GGAs and BACE. By performing control experiments with deletion mutants of GGAs and BACE we were able to show that the VHS domain of the GGA proteins and the DXXLL-motive in the BACE protein are responsible for the interaction. We observed reduced sAPP secretion upon GGA overexpression. This effect seems not to be reversible with VHS-deletion mutants of the GGAs. Therefore we suggest a BACE-independent influence of GGA1, 2 and 3 upon the APP transport and processing. **Conclusions:** These results indicate that all GGAs may have differential impact upon the transport of BACE1 and APP and may therefore play an essential role in APP cleavage and the subsequent Abeta generation.

P3-422 IMPAIRED BALANCE OF MITOCHONDRIAL FISSION AND FUSION IN SUSCEPTIBLE NEURONS OF ALZHEIMER DISEASE

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Background: Mitochondrial dysfunction is a prominent and early feature of Alzheimer disease (AD), although the underlying mechanisms remain elusive. Nonetheless, emerging evidence suggest that mitochondrial function is dependent on the dynamic balance of fission and fusion events which are regulated by a machinery involving large dynamin-related GTPases that exert opposing effects. While an impaired balance of mitochondria fission and fusion is being increasingly implicated in various neurodegenerative diseases, few studies have examined this aspect in AD. **Methods:** To address this issue, we investigated mitochondria morphology and distribution in biopsy brains from normal subjects and those from AD patients. The levels of fission and fusion in human brain were analyzed using immunoblot analysis. Additionally, these proteins were overexpressed or knocked-down in rat primary neurons. **Results:** We found that mitochondria were redistributed away from axons and became more heterogeneous in AD neurons as evidenced by both confocal and electron microscopy studies. Immunoblot analysis revealed that levels of DLP1, OPA1, and Mfn1/2 were significantly decreased while levels of Fis1 were significantly increased in AD. Interestingly, all these proteins appeared to accumulate in the soma but not in the processes of pyramidal neurons in AD hippocampus in contrast to the even distribution in the cytoplasm and processes of pyramidal neurons in age-matched control hippocampus. We then overexpressed or knocked down functional mitochondrial fission/fusion proteins in rat primary neurons. Remarkably, in situations where functional protein changes mimicking that in AD, we found similar changes in mitochondrial distribution to that observed *in vivo* in AD neurons. We further demonstrated that elevated oxidative stress and increased amyloid-beta production are likely the potential pathogenic factors that cause this impaired balance of mitochondrial fission/fusion. **Conclusions:** AD brains demonstrate mitochondrial distribution and morphological abnormalities compared to age-matched controls. Levels of mitochondria fission/fusion proteins are changed in AD autopsy brains. Manipulation of mitochondria fission/fusion proteins mimicking that in AD causes similar mitochondrial abnormalities in rat primary neurons. ADDLs causes DLP1 downregulation and mitochondria abnomalities in mitochondria in primary neurons. Work in the authors' laboratories is supported by the National Institutes of Health (AG024028) and Alzheimer's Association (IIRG-07-60196).

P3-423 LRRK2 REGULATES THE STRESS RESPONSE IN C. ELEGANS AND MAMMALIAN CELLS

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Background: Mutations in leucine-rich repeat kinase 2 (LRRK2) have been identified to cause late-onset Parkinsons disease (PD), and lead to inclusions containing either alpha-synuclein or tau protein. The LRRK2 kinase domain is highly homologous to that of the mixed-lineage kinases (MLKs), which are MAPKKKs that act upstream of MAPKKs in the mitogen-activated protein kinase (MAPK) pathway. We generated lines of C. elegans expressing neuronally directed human LRRK2 and observed striking differences in responses to stresses associated

with PD. To analyze potential mechanisms of action, we used co-immunoprecipitation and luciferase reporter assays and investigated the regulation of MAPK signaling by LRRK2. **Methods:** LRRK2 constructs (WT, G2019S, R1441C, kinase dead) were expressed in C. elegans driven by the neuronal specific synaptobrevin promoter. The WT and G2019S lines were integrated, while the other lines were expressed as arrays. C. elegans lines were also crossed to lines carrying GFP driven by the dopamine transporter promoter. For mammalian cell work, epitope tagged LRRK2 constructs were transiently expressed in 293 cells. **Results:** Expressing LRRK2 strongly potentiated toxicity caused by tunicamycin, heat shock or proteasomal inhibition, which are stresses associated with increased protein misfolding. LRRK2 expression protected C. elegans against agents causing mitochondrial dysfunction, such as rotenone or paraquat, but protection by G2019S LRRK2 was less than protection by wild type LRRK2. In each case, knockdown of lrk-1, the C. elegans homologue of LRRK2 produced the opposite effect. Disease related mutations in LRRK2 specifically increased susceptibility of dopaminergic neurons. Binding studies in mammalian cells indicate that LRRK2 binds to MKK3, 6 and 7, which are upstream kinases regulating the p38 and JNK signaling cascades. LRRK2 appears to act by regulating the subcellular localization of the MKKs. Knockdown of the C. elegans equivalent of p38 or JNK abrogated the actions of LRRK2 in C. elegans. **Conclusions:** Our data indicate that LRRK2 modulates the p38 and JNK stress kinase cascades through interaction with MKK3, 6 and 7, and this regulation allows LRRK2 to modulate the response of neurons to stresses associated with neurodegenerative disease.

P3-424 DIET-INDUCED HYPERHOMOCYSTEINEMIA INCREASES AMYLOID-BETA LEVELS IN A MOUSE MODEL OF ALZHEIMER'S DISEASE BY MODULATING THE ENDOPLASMATIC RETICULUM STRESSOR RESPONSE

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Background: High plasma homocysteine levels have been recognized as a risk factor for Alzheimer's disease (AD). However, the underlying mechanism is still elusive. Previous *in vitro* studies have demonstrated that homocysteine, by increasing the expression levels of Herp--an endoplasmatic reticulum stressor protein, elevates amyloid-beta formation. In the current study, we tested the hypothesis that in vivo high homocysteine levels may increase brain amyloid-beta levels and deposition whereby modulating Herp expression. **Methods:** To this end hyperhomocysteinemia was induced by feeding Tg2576 mice, a transgenic mouse model over-expressing the human APP Swedish mutation, with a methionine-enriched diet. After 7 months on the diet, Tg2576 and their control mice were sacrificed at the age of 15 months and their brains were harvested. One hemisphere of the brain was used for amyloid-beta immunostaining; the other one went through two-step extraction (RIPA and formic acid) for ELISA and western blot analyses. **Results:** Compared to control group, diet-treated Tg2576 mice have higher level of homocysteine, Herp protein, and amyloid-beta40/42 in the formic acid extraction of both cortex and hippocampus. Immunostaining studies also showed that diet-treated Tg2576 mice had more amyloid-beta plaques deposits than control mice on regular chow diet. By contrast, no significant difference in total APP, beta- or alpha-secretase levels were observed between the diet-treated mice and control group. **Conclusions:** We conclude that diet-induced hyperhomocysteinemia, significantly increases amyloid-beta levels and deposition in the Tg2576 mice whereby modulating the expression levels of Herp protein. Given the unchanged total APP level and the unaltered alpha- and beta secretase pathways, we conclude that Herp modulates in vivo the pro-amyloidotic effect of hyperhomocysteinemia by interfering with APP proteolytic pathways after its beta cleavage (e.g. gamma-secretase).

P3-425 ORAL HEALTH STATUS IN ALZHEIMER TYPE DEMENTIA PATIENTS

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Background: To evaluate the oral health status in patients with Alzheimer Dementia (AD), and the association of the disease severity with the oral findings. **Methods:** The study was conducted on the study group (31 AD patients), and the control group (47) from neurology department of the institute. Cognitive status was evaluated with the Mini Mental Status Examination scoring (MMSE) system. Oral parameters, such as decaying, missing, filled teeth (DMFT) index, and present teeth (PT) were evaluated in the patients. Oral hygiene habits (OHH), plaque index (PI), denture status and mucosal lesions including denture related stomatitis were also investigated. **Results:** The mean age of the patients in the study and control group were 67.68 $\pm$ 7.16, and 65.11 $\pm$5.93 respectively. The mean MMSE scores were 18.48$\pm$4.74 vs 28.29$\pm$0.83 in the study and control group respectively. The ratio of the patients with removable dentures (upper and/or lower arch) was higher in AD group than the control group [$p < 0.05$; 22 of 31 (%71) patients vs 26 of 47 (%55), respectively]. Stomatitis, DMFT were significantly higher and PT were significantly lower in AD patients than in control subjects ($p < 0.05$).Additionally epulis fissuratum, polyps and ulcerative lesions were observed in oral mucosa in 2, 3 and 2 of the patients with AD. There was a negative correlation with MMSE scores with DMFT and PI ($p < 0.05$) in AD patients, where as PT was positively correlated with MMSE scores (Pearson correlation test, $p < 0.001$). Tooth brushing and denture cleaning were irregular in 22 of 31 (70%) patients with AD. The ratio of the subjects who forgets to remove the denture during night was significantly higher in study group than in control group ($p < 0.001$). **Conclusions:** In this study, decreased cognitive functions in AD patients have been demonstrated to result with worsening of OHH and increased denture related mucosal lesions. These findings were considered due to decreased denture care including the removal of the denture in the night in the patients with decreased cognitive functions. The caregivers should be instructed for the importance of this issue to prevent devasting dental problems.

P3-426 QUALITY OF LIFE IN MILD COGNITIVE IMPAIRMENT THAT DOES NOT PROGRESS TO ALZHEIMER DISEASE: LONGITUDINAL ANALYSES OF THE INVESTIGATION TO DELAY THE DIAGNOSIS OF ALZHEIMER'S DISEASE WITH EXELON (INDDEX) STUDY

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Background: Quality of life (QoL) in subjects with Mild Cognitive Impairment who do not progress to Alzheimer Disease (Stable MCI) has not been fully investigated. Information on the measurable changes in QoL ratings and their longitudinal course has potentially important clinical, social and economic implications. Our objective was to characterize the four-year changes on QoL and concurrent neuropsychiatric symptoms (NPS) in subjects with Stable MCI. **Methods:** The InDDex Study was a 48-month double-blind, randomized controlled trial of rivastigmine to delay Alzheimer Disease (AD) in MCI, with assessments at 6-month intervals. MCI criteria included having cognitive symptoms, a CDR global score=0.5 and a score<9 on the NYU delayed paragraph recall. We defined Stable MCI as including all subjects who did not progress to AD. We evaluated placebo arm data on those who completed the 48-month visit (n=209). Subject QoL was assessed with the QOL-AD scale, which includes informant (QOL-I) and subject (QOL-S) ratings. NPS were assessed with Neuropsychiatric Inventory (NPI) and the Beck Depression Inventory (BDI). Baseline and 48-month change descriptive data were computed, and mixed-effects longitudinal regression analyses performed with all