of the diseases in these cases. In the present study, IgG antibodies against egg-albumin, bovine casein and Neu5Gc were measured in the cerebrospinal fluid of 13 healthy individuals and in patients with mild(17), moderate(15) and severe(13) Alzheimer's Disease(AD). **Methods:** For the detection of antibodies, the antigens were attached at the bottom of ELISA plates, pre-treated with highly hydrophilic microlon resin. **Results:** If a concentration equal to mean+2SD of healthy individuals is considered as the cut-off for the characterization of positive samples, no positive samples against egg-albumin and casein were observed at the groups of healthy, mild and moderate AD patients(0 out of 45), while 5 antibody positive samples out of 13(38.5%) were detected at the group of severe AD patients(Fisher exact test value 0.01), with extremely high antibody concentration in these samples, reaching 23-42 folds the cut-off value of anti-egg albumin and 5-10 folds the cut-off value of anti-casein antibodies(Fig.1). Low concentrations of anti-Neu5Gc antibodies were detected in all AD patients. Structural alignment analysis revealed tree different areas of egg-albumin with structural similarity with beta amyloid peptide and one area with similarity with tau protein, both of which are involved in AD development. High sequence homology between bovine κ-casein and reelin a protein involved in neuronal function was also found. **Conclusions:** The results indicate a probable correlation of anti-egg albumin and anti-casein antibodies with severe Alzheimer's disease, in some cases, which may worth further investigation. References 1. Vered Padler-Karavani etal. Cancer Res. 71(9), 3352, 2011. 2.K. Zarogoulidis, P. Eleftheriou, etal. European Respiratory Journal, 2015. **3.**P. Eleftheriou etal. IJGC, 27(S4), 108, 2017. 4.P. Eleftheriou, etal. Biomed. Res. Int., 2014. 5. EG. Severance, etal. Bipolar Disord. 12(8), 834, 2010.

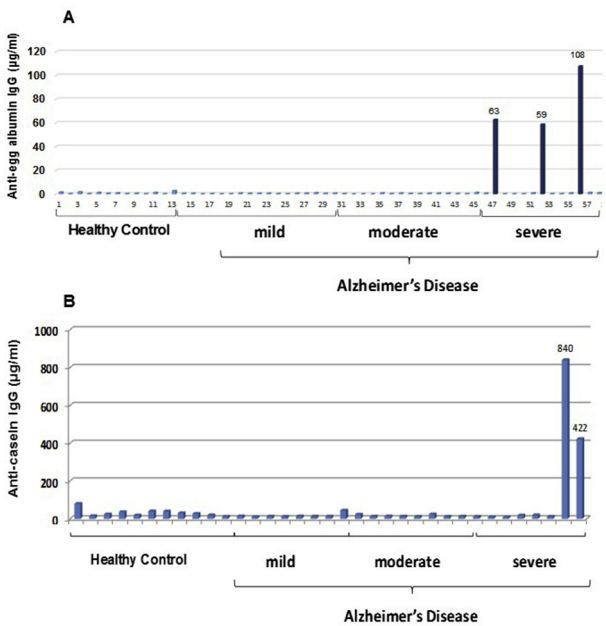

Figure 1. Antibodies against egg-albumin (A) and bovine casein (B) in the cerebrospinal fluid of healthy individuals and in patients with mild, moderate and severe Alzheimer's Disease. The patients or healthy individuals did not participate in any active or passive immunization therapies. The concentration is expressed in arbitrary units. For the quantification of the results, five cells of the ELISA plate were coated with different concentrations of purified human IgG and used for the calibration curve.

P2-210 **WITHDRAWN**

P2-211 **VARIABILITY IN THE SMALL-NUCLEOLAR RNAS COMMONLY USED FOR MICRORNA NORMALISATION IN POSTMORTEM BRAIN TISSUE OF ALZHEIMER'S AND VASCULAR DEMENTIA**

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Background: Measurement of MicroRNAs with specificity is challenging, given their small size and shared sequence similarities. qRT-PCR has been proposed as the gold standard for microRNA profiling where it is even used to validate the results derived from other platforms. However, qRT-PCR requires normalization to a reference gene and there remains no consensus nor evaluation on the most commonly used reference genes for microRNA profiling in post-mortem brain tissue. We tested the variability of the 3 most commonly used small RNAs on microRNA screenings in dementia. **Methods:** Post-mortem brain tissue was dissected from the posterior cingulate cortex of 66 brains, including Alzheimer's disease, vascular dementia and controls. miRvana isolation kit and Quant-iT RiboGreen assay were used for isolation and quantification. qRT-PCR was performed with TaqMan assays for RNU6B, RNU44 and RNU48. Analysis was performed according to the CT-difference to the median of individual calibrators, the relative quantity to the geometric mean, the relative quantity using absolute values and the software BestKeeper. **Results:** RNU48 showed the lowest coefficient of variability between samples with a range from 49-69%. The most commonly used calibrator, RNU6B, showed a coefficient of variability ranging from 118-134% and RNU44 a range from 121-133%. These results were also observed in plots of the data. No significant differences were found when analysed according to diagnosis. **Conclusions:** Although no significant differences were observed between diagnostic groups, using reference genes with high variability can introduce bias when investigating microRNA expression. We have previously observed that RNU6B was relatively stable in samples with Braak stage 0-V, however, a significant 3-fold decrease was present in Braak stage VI. Thus, significant differences in microRNA levels might be an artefact of unstable calibrators. Absolute quantification is recommended and further stringency in qRT-PCR can be performed with more stable calibrators using the relative standard curve method.

P2-212 **HIGH FAT DIET EXACERBATES CAPILLARY STALLING AND ALZHEIMER'S DISEASE-RELATED PATHOLOGY IN THE APP/PS1 MOUSE MODEL**

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Background: Obesity has long been associated with increased risk for developing Alzheimer's disease (AD) as well as with increased

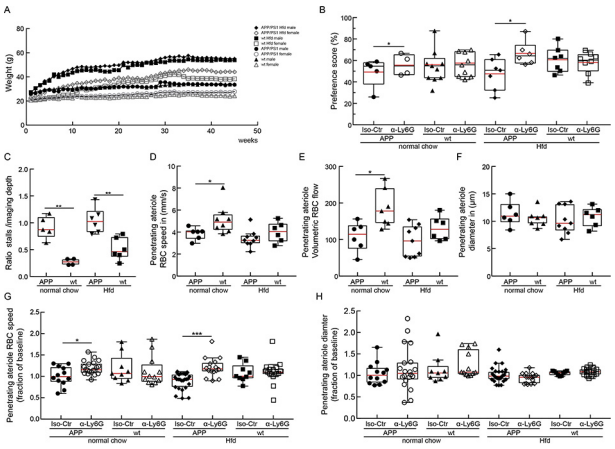

Figure 1. Stalled capillaries causing reduced CBF in AD and wt mice on a western high fat diet. (A) Graph shows the body weights of wild-type (wt) and AD mice on a HFD or normal chow. (B) Preference score in object replacement task for AD-HFD, AD, wt-HFD and wt mice analyzed 3-6 hr after a single administration of α-Ly6G or Iso-Ctr antibodies compared to baseline measurements. (C) Number of capillaries with stalled blood flow in AD-HFD AD, wt-HFD and wt. (D) RBC speed (E) Volumetric RBC flow and (F) diameters at 10 month of age (5-month on HFD) AD-HFD, AD, wt-HFD and wt. (G) RBC speed and (H) capillary diameter of AD-HFD, AD, wt-HFD and wt mice, measured 60-90 min after α-Ly6G or Iso-Ctr antibody administration in 13-month old mice (9 month on HFD).

severity of AD symptoms. We have recently shown that ∼1.5% of cortical capillaries have transiently stalled blood flow due to adhered neutrophils in transgenic mouse models of AD, leading to an overall reduction in cerebral blood flow (CBF) that can be restored by administering antibodies against the neutrophil-specific cell surface protein Ly6G. Wildtype (wt) mice showed a much lower rate of capillary stalling of ∼0.4%. This study aims to explore whether a high fat diet (HFD) exacerbates this capillary stalling phenomena in AD mice. **Methods:** 3-4 month old APP/PS1 (AD) and wt mice were fed a western HFD or a control diet for 12 months. *In vivo* multiphoton microscopy was used to quantify the number of flowing and stalled cortical capillaries and quantify blood flow speed in penetrating arterioles after 6 and 12 months of HFD. Behavioral tests were performed monthly from the beginning of cognitive decline in the AD models to assess short-term memory. At each time point for cognitive testing and in vivo imaging, measurements were made before and after animals received anti-Ly6G antibody to reduce the incidence of capillary stalling. **Results:** The incidence of stalled blood flow in capillaries was elevated in 18-month old AD mice (0.9% on control diet; 1.1% on HFD) as compared to wt (0.3% on control diet; 0.6% on HFD) (Fig 1). In turn, the HFD caused a 20% increase in capillary stalling and a 27% decrease in CBF in both AD and wt mice relative to control diets. AD-HFD animals displayed enhanced motor-sensory and cognitive deficits compared to AD control animals after 6 months of HFD whereas wt-HFD animals display cognitive deficits relative to wt control animals after 9 months of HFD. Anti-Ly6G treatment reduced capillary stalls, increased brain blood flow and improved short-term memory performance in APP-HFD animals. **Conclusions:** We demonstrated that capillary stalling contributes to enhanced AD progression in obese APP/PS1 mice, suggesting that this mechanism is one potential link between obesity and AD risk and severity.

P2-213 EARLY STRUCTURAL AND BEHAVIOURAL DIFFERENCES IN APPSWE/PS1ΔE9 MOUSE MODEL OF ALZHEIMER'S DISEASE

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Background: Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by decline in cognitive functions, including memory impairment. We examined structural changes in neuronal morphology in CA1 region of the hippocampus in APPSwe/PS1ΔE9 (APP/PS1) mice. We further evaluated these structural changes with hippocampal dependent learning tasks. **Methods:** Golgi staining - Brains from 1 month old APP/PS1 mice (wild-type and transgenic) were fixed for Golgi staining and 100 µm serial sections were cut for spine and morphometric analyses. Novel-object recognition (NOR), Object-place recognition (OPR), Object-in-context recognition (OCR) were used to study different aspects of episodic memory. For analysis, the test phase in all the three tasks were converted into a discrimination ratio. The Morris water maze (MWM) - The mouse is trained to navigate a pool with opaque water and find the hidden platform. The reversal phase started on day 6, at which point the platform was moved to the opposite quadrant of the tank. Results at the probe trial represented spatial memory. Contextual fear conditioning (cFC) – It involves pairing of a conditioned stimulus with an aversive unconditioned stimulus (footshock). As a result of these pairings, animals freeze to the neutral stimulus such as the training context (CS) upon subsequent presentations. **Results:** Apical dendritic spine loss, including reduction in mushroom spine density is seen as early as 1 month of age in CA1 region of the hippocampus of APP/PS1 mice. Significant deficits were observed upon hippocampal dependent episodic-like memory task requiring the binding of an object memory into a spatial context such as OCR and OPR. No difference was seen in NOR, which unlike the other components of episodic-like memory is considered not to critically rely on the hippocampus. In MWM, mice showed no difference in acquisition but had less preference for the target quadrant compared to the wildtype upon reversal learning. APP/PS1 mice also showed contextual fear learning deficits. **Conclusions:** Our study demonstrates that AD-relevant pathological changes affecting structural and behavioral features start early and progress with increasing age in APP/PS1 mice.

P2-214 THE IMPACT OF AGE ON CELL PROLIFERATION IN HIPPOCAMPAL SUBGRANULAR ZONE IN ADULT MOUSE BRAIN

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Background: The hippocampus is one of the brain areas damaged earlier in the development of Alzheimer's disease. This damage is thought to be linked to memory loss in Alzheimer's patients. The adult brain retains an ability to produce new hippocampal granule neurons. New neurons are thought to be involved in new memory formation, learning, stress response, and emotion. Therefore, the new hippocampal neuron production potentially can be