Longitudinal characterization of Apolipoprotein E targeted replacement mice at 7 T

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Introduction: In the brain, a major role for ApoE is to maintain a constant supply of neuronal lipids that are necessary for normal brain function. The three common ApoE isoforms (ε2, ε3 or ε4) differ by only a single amino acid which has significant effects on the tertiary structure and function of the protein. Even in populations with a low ε4 allele frequency, gene dosage of ε4 increases the age-adjusted relative risk for developing AD (1). By utilizing the targeted replacement (TR) ApoE mice model simultaneous measurement of morphology, cerebral blood flow and metabolite concentration were carried out using MRI.

Methods: All animal procedures were conducted under protocols that were approved by our institutional IACUC. Female TR homozygous ε2, ε3 and ε4 mice were studied. MRI was conducted on a BioSpec 7T system with an actively-decoupled cross-coil setup (Bruker BioSpin, Billerica, MA). Animals were anesthetized with isoflurane and the core body temperature was monitored and maintained at 37°C. Structural imaging was accomplished with a T2-weighted 3D RARE sequence covering a field of 16 × 16 × 19.2 mm^3 with 128 x 128 x 64 matrix points (TR = 2600 ms, TE(eff) = 74 ms, RARE factor = 16). A PRESS sequence was used to obtain spectroscopy on a 9 mm^3 voxel placed on the hippocampus with VAPOR water suppression and the following parameters: TE = 16 ms, TR = 3 s, 32 averages and 16 repetitions. Single-slice CASL measurement were performed using a 2.0 s continuous RF labeling pulse (60 mG), followed by a RARE image acquisition (TR=2.5 s, TE=8.0 ms, ETL=16). Whole brain and sub-region segmentation was performed by applying an atlas-based segmentation followed by manual correction. LCModel (2) was used for spectral analysis, and CASL images were processed in Matlab. Due to attrition, animal numbers were reduced from their original size of 12 per group to 8 ε2, 10 ε3, and 12 ε4 at 14 months, and 5 ε2, 5 ε3, and 8 ε4 at 20 months. One ε3 and one ε4 mouse at the 14 month time point, and one ε3 mouse at the 20 month time point died in the scanner and did not provide MRS or CBF data at the respective time point.

Results: *volumetry:* At the 14 month time point, there was a reduction of hippocampal volume in both ε3 and ε4 compared to ε2. No other significant differences between the 3 lines were observed in whole brain volume or any of the 3 segmented regions (hippocampus, ventricles, and cortex) that were analyzed. At the 14 month and 20 month, ε4 mice consistently showed reduced cortical, hippocampal, and whole-brain volumes. However, the reduction is not statistically significant except the cortex measured at 14 month (p=0.012, uncorrected).

CBF: The only significant difference observed was a reduction in thalamic perfusion of ε4 from ε2 at the 14-month point. There were no other significant differences in cerebral blood flow measurements amongst the three genotypes in the thalamus, cortex or hippocampus. However, the ε4 group shows a trend of lower perfusion compared to the ε2 and ε3 groups in the every region analyzed at both time points (fig 1).

MRS: Spectroscopy measurements in the hippocampus showed a significant decrease in the levels of creatine (total) in ε2 compared to ε3 and ε4 at 14 and 20 months, as well as a significant decrease in the level of myo-inositol in ε2 compared to ε4 at 20 months. There were no significant changes observed in any of the other metabolites measured, though, for animals scanned at both time point, the rate of change *over time* of myo-inositol and total creatine were significantly greater for ε3 (0.18 +/- 0.09 mM/6 months and 0.09 +/- 0.03 mM/6 months, respectively) than for ε4 (-0.11 +/- 0.06 mM/6 months and -0.01 +/- 0.02 mM/6 months, respectively).

Discussion: Creatine, along with phosphocreatine (PCr), acts as an energy buffer through the creatine kinase reaction to maintain a constant supply of ATP. It has been shown by *in vivo* ^{31}P studies in humans that PCr levels decrease initially with mild dementia and then increase as dementia worsens (3). Decreased cognitive performance in healthy individuals has also been correlated to increased creatine levels (4). Thus increasing creatine levels may be an early indication of declining cognition. Concerning the change in total creatine (creatine + PCr) over time in these relatively old animals, ε4 animals remained unchanged at high levels, ε3 animals increased significantly, and ε2 remained unchanged at lower levels (though there was a small, non-significant increase in ε2 over time). This trend seems to reflect what is known about the neuroprotective nature of the ε2 allele (5). The trend of hypoperfusion in the ε4 mice compared to the other isotypes is consistent with the morphometry results, which show trends of atrophy in ε4 cortex, hippocampus, and whole brain, compared to other isotypes, but the small sample size and cohort attrition over time make a rigorous assessment of these biomarkers of state very challenging. Beginning the assessment of these endpoints when the animals are very young will avoid the complication of attrition in these fragile animals. From this work in these relatively old animals, it appears that the longitudinal change in total creatine may give valuable information on the phenotypic impact of ApoE isotype.

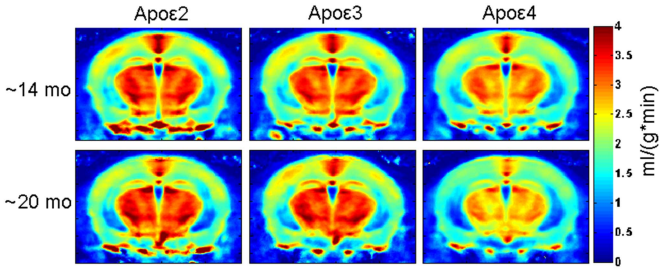

Figure 1. Shown above are the mean perfusion maps for each genotype at each time point. Clearly, there is a deficit in perfusion in Apoε4 mice that increases with time.

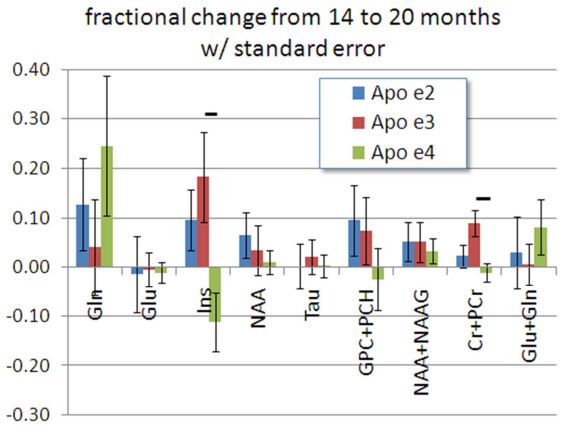

Figure 2. Relative change over time (14 months to 20 months) in the observed metabolites. ε3 and ε4 isotypes differed significantly (p<0.05, uncorrected) in change in myo-inositol and total creatine

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