

# The effects of genotype and age on fractional anisotropy and learning in an Alzheimer's mouse model

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## Introduction

Apolipoprotein E (apoE) is a 34 kDa glycoprotein and occurs in three major isoforms (E2, E3 and E4) in humans. ApoE4 is the primary genetic risk factor for Alzheimer's Disease (AD). In addition to its role in lipid transport, apoE is expressed in the brain and the different isoforms exhibit different affinities to beta amyloid, with the lowest affinity associated with apoE4.

The present experiments characterized human apoE targeted replacement/ knock-in mice (apoE-TR) behaviorally at 4, 7 12 or 18 months of age. ApoE-TR mice have yet to be fully characterized and are likely more representative of the human condition than alternative transgenic apoE mouse strains produced via a heterologous promoter. Hippocampi of apoE-TR mice were evaluated neuroanatomically at 4 or 12 months of age via magnetic resonance diffusion tensor imaging (DTI)-measured fractional anisotropy (FA). DTI is an imaging protocol sensitive to the directionality of water flow and recent findings indicate that the DTI-derived measure FA is correlated with dendritic density in the hippocampus [REF ISMRM 2008 poster]. Immunohistochemistry staining of pre- and post-synaptic markers was also conducted in 4 and 12 month apoE-TR mice. We hypothesized that targeted replacement with the apoE4 isoform would impair spatial learning and reduce markers of structural and synaptic plasticity. Further, we hypothesized that a reduction in markers of synaptic and structural plasticity would precede impairments in spatial learning.

## Methods

**Subjects:** Homozygous human apoE2, apoE3, and apoE4 targeted-replacement (apoE-TR) mice were developed by Maeda et al. (Sullivan et. al, 1997; 1998). Briefly, exons 2-4 of the human APOE2, APOE3 and APOE4 gene were used to replace the corresponding genomic DNA at the mouse APOE locus. ApoE-TR mice were acquired from Taconic, Inc. (Hudson, NY) and housed in the IACUC-approved ENH Research Institute mouse colony. Prior to imaging, mice were transcardially perfused with PBS followed by a 4% paraformaldehyde. DTI: DTI was conducted at 26°C on a Bruker vertical bore MR imager operating at a proton frequency of 600 MHz. Diffusion-weighted spin-echo images were acquired using TR = 3000 ms, TE = 27 ms, time between diffusion gradient pulses,  $\Delta$ , = 14 ms, duration of diffusion gradient,  $\delta$ , = 7 ms, field of view = 1.5 cm, and matrix size = 256 x 256. Diffusion sensitizing gradients were applied along six directions:  $[G_x, G_y, G_z] = [1, 1, 0], [1, 0, 1], [0, 1, 1], [-1, 1, 0], [1, 0, -1], [0, -1, 1]$ . Six  $b$  values, [200, 500, 1000, 1500, 2500 and 3500 s/mm<sup>2</sup>], were used along each diffusion gradient direction. A total of ten 500  $\mu$ m thick slices in the coronal orientation were imaged with the five to six slices containing the hippocampus situated in the middle of the slice package. Average FA was calculated separately for hippocampal areas CA1, CA3 and dentate gyrus; average FA was also calculated for primary somatosensory cortex and corpus callosum. All FA calculations were computed using software developed in-house. The scalar metric fractional anisotropy (FA) was calculated from the eigenvalues on a pixel-by-pixel basis. **Immunofluorescence Staining:** Expression of postsynaptic protein PSD-95 and presynaptic protein synaptophysin were evaluated by double staining with microtubule-associated protein (MAP-2). Briefly, coronal brain sections (35 $\mu$ m) were incubated with the primary antibodies rabbit anti PSD-95 (Abcam), rabbit anti-synaptophysin (Chemicon) and mouse anti-MAP-2 (Chemicon). The bound primary antibodies were visualized by incubating with Alexa 488- or 594-conjugated secondary antibodies. For PSD-95 staining, the brain sections were pretreated with 100 mg/ml of pepsin (DAKO) at 37°C in a water bath for 5 min prior to blocking. Fluorescence images were obtained with a confocal scanning laser microscope (LSM 510; Zeiss). All images of MAP-2, synaptophysin, and PSD-95 were taken in the CA3 region of the hippocampus through a 63x oil-immersion objective. **Spatial Water Maze:** In the hidden platform water maze task, mice learned the location of a hidden escape platform across a series of trials. Mice with intact hippocampal functioning learn to find the platform faster than those with impaired spatial functioning. Mice were given 8 trials/day for 3 consecutive days and spatial learning was evaluated by latency to reach the hidden platform.

## Results and Discussion

**Diffusion Tensor Imaging:** The effects of age and genotype on fractional Anisotropy (FA) were examined in a subset of 4 and 12 month old apoE mice ( $n=2-4/\text{cell}$ ) via 2-way ANOVAs (Age by Genotype); separate ANOVAs were computed for each anatomical region. Genotype was significant in dentate gyrus, ( $F(3,15)=3.375$ ,  $p<0.05$ ) as was the Genotype by Age interaction ( $F(3,15)=13.13$ ,  $p<0.001$ ). Age was significant in corpus callosum, ( $F(1,15)=14.48$ ,  $p<0.001$ ); Age trended toward significance in dentate gyrus ( $F(1,15)=3.61$ ,  $p=0.077$ ); see Figure 1. There were no significant main effects or interactions in CA1 or CA3. Post-hoc t-tests were conducted when main effects were present. **Spatial Learning:** Latency to reach the hidden platform was analyzed in a 3-way ANOVA (Test Day by Age by Genotype). The main effect of Age was significant ( $F(3,101)=12.34$ ,  $p<0.001$ ). Post-hoc multiple comparisons revealed the following relationship between age and latency: 4 mo = 7 mo > 12 mo = 18 mo. **Immunofluorescence Staining :** **Synaptophysin:** Immunofluorescence staining revealed an apparent reduction in synaptophysin immunopositive puncta at 12 months for all genotypes. There was also an apparent reduction in synaptophysin staining in 12 month apoE4 mice relative to 12 month apoE2 and apoE3 mice (Figure 3, left panel). **PSD-95:** Immunofluorescence staining also revealed an apparent reduction in PSD-95 immunopositive puncta at 12 months for all genotypes. In addition, immunopositive PSD-95 puncta appeared reduced in apoE4 mice relative to apoE2 and apoE3 mice in both age groups (Figure 3).

## Summary and Future Directions

An age-dependent impairment in spatial learning is present in apoE-TR mice beginning at 12 months of age, coincident with a trend toward an increase in dentate gyrus fractional anisotropy across genotype. Interestingly, post-hoc t-tests revealed that FA decreased in apoE3 while remaining constant (apoE4; apoEKO) or increasing (apoE2) in the other three genotypes. These findings suggest that neuroanatomical evaluation may be insufficient; future experiments will examine other areas interconnected with the hippocampus and known to be important for learning an memory, such as entorhinal cortex and medial septum, should be examined.

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1 Venkatasubramanian PN, Faulkner J, Tom B & Wyrwicz AM. Proc Intl Soc Mag Reson Med. 13 (2005).

