Regional Transverse Relaxation Alterations in the APP/PS1/Tau Alzheimer's Disease Transgenic Mouse Model Following Diet Induced Iron Loading

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Introduction: There is converging evidence that iron overload is involved in both amyloid-beta (Aβ) plaque and neurofibrillary tangle (NFT) formation. The incorporation of the Aβ fibrils into plaques is accelerated in an iron-rich environment and iron has been shown to accumulate in neurons with NFTs and enhance the ability of phosphorylated Tau (pTau) to aggregate into NFTs *in vitro*. Our previous results have demonstrated that hypo-intensities on T_2- and T_2*-weighted datasets coincide with Aβ plaques in AD and transgenic neural tissue[1]. In addition, our data also revealed that plaque morphology and inflammatory response differ between AD and APP/PS1 mouse tissue. However, there are crucial unanswered questions in the current literature on how iron and amyloid fibrils are involved in plaque and tangle genesis in the living brain and the neurotoxic impact of amyloidogenesis. We hypothesize that iron is a cofactor in the genesis of Aβ plaques and NFTs; furthermore, the lower iron concentration found in the transgenic neural tissue (in addition to inherent human and animal model distinctions) accounts for the dissimilarity between AD and transgenic plaque morphology, pTau pathology, and inflammatory response. We also hypothesize that iron plays a synergistic function in relation to Aβ plaque neurotoxicity. To test our hypothesis we are recapitulating the homeostatic iron imbalance present within the AD brain in the transgenic mouse model using a novel lipophilic iron compound (TMH-Ferrocene) that passively crosses the intestinal lumen[2]. The goal of this research is to 1) determine the *in vivo* relationship between iron and AD pathology, 2) generate an improved AD mouse model, and 3) establish the cyto-architectural basis of AD pathology in relation to MR metrics.

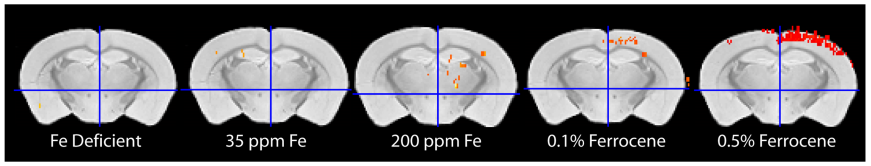

Figure 1: Group based T_2* parametric differences of significant voxels (0m > 3m) for each diet

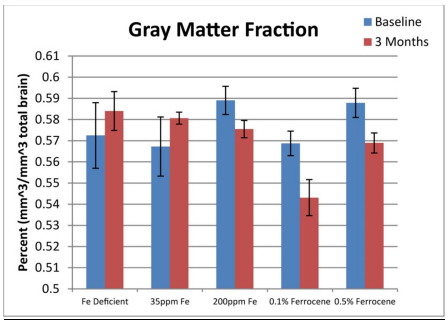

Figure 2: Segmented gray matter fraction (volume region/total brain volume) comparison of mice at baseline and 3 months of diet.

Methods: Five groups of six APP/PS1/Tau transgenic mice (N=30, total), acquired from Jackson Labs, were randomized into five diet groups consisting of Fe deficient (trace, 2-6 mg/kg), 35 mg/kg Fe, 200 mg/kg Fe, 0.1% TMH-Ferrocene (171 mg/kg Fe) and 0.5% TMH-Ferrocene (885 mg/kg Fe) at 10 weeks of age. Mice were scanned prior to starting their randomly assigned diets (0 month, baseline) and after three months of *ad libitum* diet administration. All mice were treated using standard IACUC protocols and 1.5% inhaled isoflurane anesthetic was used during scans. All MRIs were acquired using a 7.0T Bruker MedSpec system with a 23mm birdcage volume RF coil (Bruker Biospin). A $3DT_1$ was taken for spatial registration at a resolution of 100 x 100 x 250µm, and eight-echo MGE T_2* (5-50ms) and eight-echo MSME T_2 (11-88ms) datasets were acquired at a resolution of 100 x 100 x 500 µm. Parametric relaxation T_2, T_2*, R_2 and R_2* maps were generated using a linear model. All datasets were coregistered and resliced to match the $3DT_1$ dataset using SPM 5 with the SPMmouse toolkit[3]. Datasets were then skullstripped, realigned to a template mouse brain, and segmented into white matter (WM), gray matter (GM) and cerebral spinal fluid (CSF) with a probability map for volumetric analysis. For parametric map analysis, the relaxation maps and $3DT_1$ datasets were normalized to the template brain and voxel based analysis was performed using a group based method in SPMmouse (cluster size ≥ 5 and *p*Value ≤ 0.005). Region of interest (ROI) based transverse relaxation metrics were acquired by overlaying a 20-region atlas[4] over the normalized parametric maps and significance was determined using a T-test.

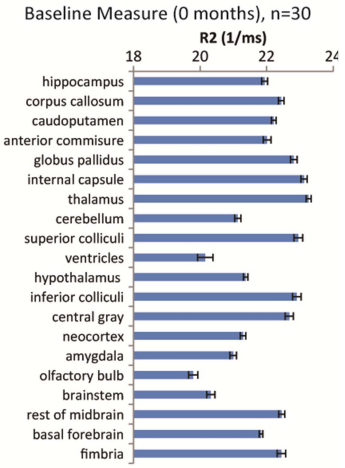

Figure 3: Baseline measures of R_2 for all twenty regions of interest (n=30).

Results: Group based T_2* statistical parameter maps of mice fed the iron diets for three months are seen in Fig. 1. Regions in the R_2 ROI analysis have shorter transverse relaxation, hypothesized to be indicative of regions with higher iron concentration and increased Aβ plaque load. A step-wise decrease in cortical T_2* is found with increasing iron diet in the same cortical region. Gray matter volume as a fraction of total brain volume for each group is seen in Fig 2. A trend in cortical volume reduction is found in the high iron diets, with 0.1% group showing a significant decrease in GM fraction. Baseline ROI R_2 relaxation measures are presented in Fig 3. The baseline ROI analysis of regions known to harbor high iron[5] show increased R_2 rates with minimal error within the measures. Comparison of the parametric map ROI analysis between groups at three months of diet administration is seen in Table 1. Several regions in the high iron diet 0.5% ferrocene have increased R_2 rates, indicative of iron loading. The other diet groups also showed an increased R_2 rate which is hypothesized to be due to plaque progression.

Discussion: The parametric map group analysis and segmented changes confirm that high iron diets are significantly altered in the APP/PS1/Tau mouse brain. Transverse relaxation is known to be a measure of plaque formation and iron loading. The trending significant changes in Figure 1 are hypothesized to reflect an accumulation of iron and Aβ plaques genesis in the cortex. The data here represent the first time point (month 3) of a twelve month iron loading study, which will also incorporate quantitative histological analysis. The synergistic role of iron in amyloid plaque and pTau tangle formation has not been fully elucidated *in vivo*. The lack of high focal iron in the Aβ plaques, decrease in overall brain iron, and dissimilarity in $A\beta_{42}$ and $A\beta_{40}$ composition, morphology, and inflammatory response within the transgenic model compared to AD raises several fundamental questions regarding toxicity, plaque, and tangle genesis. Our research will generate new information for understanding the role of homeostatic iron overload in Aβ plaque and NFT formation within the AD brain to determine how iron levels affect plaque morphology, pTau formation, iron management, inflammatory response, and cognition. This will lead to a more accurate AD transgenic murine model to better mirror the processes occurring in AD. This has implications for future investigation of pharmacological intervention therapies associated with amyloid, pTau, and related hypotheses.

Subregion	0 Fe	35ppm	200ppm	0.1%	0.5%
hippocampus	--	--	--	--	↑**
corpus callosum	--	--	--	--	--
caudoputamen	↑**	--	--	--	↑**
anterior commisure	↑*	--	--	--	↑***
globus pallidus	↑*	--	--	--	--
internal capsule	--	--	--	--	--
thalamus	--	--	--	--	↑*
cerebellum	--	--	--	--	--
superior colliculi	--	--	--	--	↑*
ventricles	--	--	--	--	--
hypothalamus	--	--	--	--	↑*
inferior colliculi	↑*	--	--	--	--
central gray	--	--	--	--	↑**
neocortex	↑***	--	↑*	--	↑***
amygdala	--	--	--	--	--
olfactory bulb	--	--	--	--	--
brainstem	--	--	--	--	--
rest of midbrain	--	--	--	--	--
basal forebrain	↑**	--	--	--	↑***
fimbria	--	--	--	--	--

Table 1: ROI based R_2 changes after 3 months on diet. (* = $p < 0.05$; * = $p < 0.01$; *** = $p < 0.001$)

References: **1** – Meadowcroft *et al*. JMRI 2009; 29(5): 997-1007, **2** – Nielsen *et al.*, Biochem Pharm 1993;45(2):385-391, **3** – Sawiak *et* al., Neurobiol Dis. 2009 Jan;33(1):20-7 **4** – Ma *et al.*, Front Neuroanat 2008; 2(1): 1-10. **5** – Connor *et al.*, Neuroscience 1997; 91(1): 255-261.