

## Correlation between Plaque Counts and Metabolite Concentrations in Transgenic Mouse Model of Alzheimer's Disease

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### Introduction

Previously, reduced levels of N-acetylaspartate (NAA) and glutamate (Glu) were observed in various mouse models of Alzheimer's disease (AD) (1-3). Dramatic increase in the concentration of myo-inositol (mIns) with age was observed for APP-PS1 mouse model of AD (3). Additionally, the treatment of transgenic AD mice with anti-A $\beta$  antibody regimes appeared to slow the rate at which mIns normally increases in AD mice (4).

In this project, we looked at the correlation between concentrations of metabolites obtained *in vivo* using  $^1\text{H}$  MRS just prior to sacrifice and the plaque counts obtained from histological Thio-S stained brain sections.

### Methods

Eighty double transgenic APP-PS1 mice were entered into a 4-month treatment study. APP-PS1 mice were randomly assigned to five treatment groups (1 mg/kg ponezumab, 3 mg/kg ponezumab, 10 mg/kg ponezumab, 10 mg/kg 2H6-D, control antibody). Treatment consisted of weekly intraperitoneal injection of the antibodies supplied by Pfizer, Inc.

*In vivo*  $^1\text{H}$  NMR spectra were obtained using a previously described LASER sequence (5). MR experiments were performed with a 9.4-T (31-cm horizontal bore) magnet equipped with Varian INOVA console to localize a 18  $\mu\text{L}$  voxel placed in the hippocampus and the cortex (3).

The obtained spectra were analyzed using LCModel with the spectra of eighteen metabolites and the experimentally measured spectrum of macromolecules included in the basis set. The quantification was obtained using tCr resonance as an internal standard and assuming 8 mM concentration.

Animals were sacrificed at the end of treatment, perfused with PBS and fixed with neutral buffered 10% formalin after an overdose with pentobarbital. The brain was removed and fixed further in formalin overnight and equilibrated in 0.1 M sodium phosphate, pH 7.4, for 24 h. The brains were sectioned and stained with Thio-S. The Thio-S positive  $\beta$ -amyloid plaques were visualized with a fluorescence microscope using filters for fluorescein isothiocyanate (6).

Although the data came from a treatment study, an analysis of treatment effects on MRS was not part of this analysis. Spearman's rank correlation was used to assess associations due to non-normal distributions in plaque counts and metabolite values.

### Results and Discussion

Figure 1 shows plots of concentrations of NAA and mIns obtained just prior to sacrifice from the voxel placed mostly in the hippocampus versus plaque counts in the hippocampus using Thio-S stained brain sections. A bimodal distribution of plaque counts with 18 of 49 mice (37%) showing counts in the range of 4 to 25 and the remaining 31 mice (63%) showing counts above 45 was observed. Higher plaque counts were associated with lower NAA values (Spearman rho = -0.54, p < 0.001) and higher mIns values (Spearman rho = +0.62, p < 0.001). As expected, the two metabolites themselves were negatively correlated (Spearman rho = -0.39, p = 0.006).

The  $^1\text{H}$  MRS concentrations of NAA and mIns correlate well with histological plaque count obtained in the same voxel.

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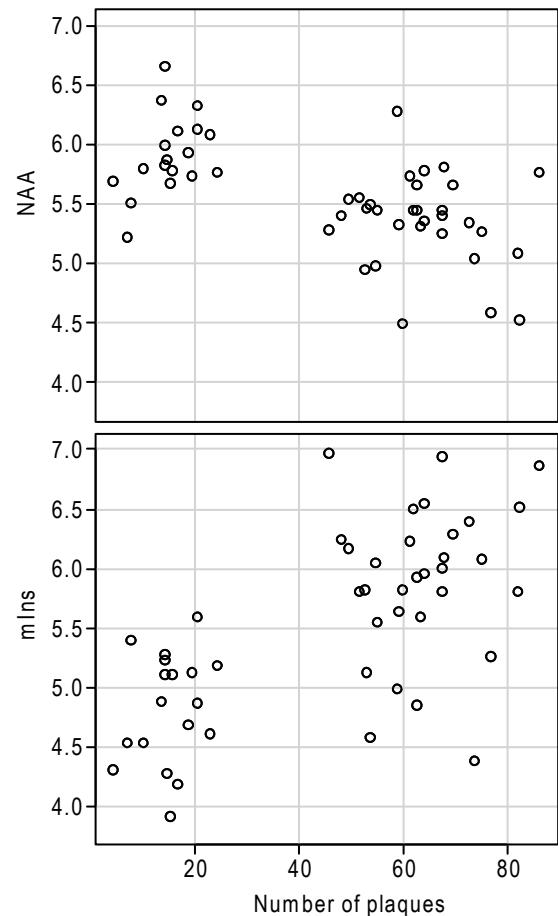


Figure 1. NAA and mIns concentrations versus plaque counts in the hippocampus using Thio-S stained brain sections for 49 animals.