

## Anti-inflammatory treatment in AD mice protects against neuronal pathology

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### ABSTRACT

Prior studies suggest that non-steroidal anti-inflammatory drugs (NSAIDs) may lower the incidence of Alzheimer's disease (AD) and delay onset or slow progression of symptoms in mouse models of AD. We examined the effects of chronic NSAID treatment in order to determine which elements of the pathological features might be ameliorated. We compared the effects of the NSAIDs ibuprofen and celecoxib on immunohistological and neurochemical markers at two different ages in APPxPS1 mice using measurements of amyloid plaque deposition, A $\beta$  peptide levels, and neurochemical profiles using magnetic resonance spectroscopy (MRS). At 6 months of age, few neurochemical changes were observed between PSAPP mice and WT mice using MRS. Ibuprofen, but not celecoxib, treatment significantly decreased the A $\beta$ <sub>42/40</sub> ratio in frontal cortex at 6 months, but overall amyloid plaque burden was unchanged. Consistent with prior findings in mouse models, at 17 months of age, there was a decrease in the neuronal markers NAA and glutamate and an increase in the astrocytic markers glutamine and *myo*-inositol in AD mice compared to WT. Ibuprofen provided significant protection against NAA and glutamate loss. Neither of the drugs significantly affected *myo*-inositol or glutamine levels. Both ibuprofen and celecoxib lowered plaque burden without a significant effect on A $\beta$ <sub>1–42</sub> levels. NAA levels significantly correlated with plaque burden. These results suggest that selective NSAIDs (ibuprofen and possibly celecoxib) treatment can protect against the neuronal pathology.

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### Introduction

Definitive diagnosis of Alzheimer's disease (AD) can be established only through postmortem examination of brain tissue. Development of markers to diagnose AD thus represents a major challenge. Advances on this front will also have critical implications for testing potential therapeutics. Finding markers that can reliably monitor progression of the disease with high sensitivity will be useful to get patients earlier in the disease process as well as to obtain good statistical power in a shorter period of time. Finding potential markers has been rendered more efficient through development of mouse models of AD. Development of mouse models makes it possible to work on the mechanisms of disease as well as to efficiently test therapeutics. A number of different mouse models of AD have been developed based upon genes associated with human familial AD (reviewed in McGowan et al., 2006). These mice replicate various features of the human disease including senile plaques and cognitive

deficits and tau pathology, thus providing the potential to investigate various potential markers for these elements of the pathology.

Magnetic resonance spectroscopy (MRS) is a noninvasive technique that permits the quantification of metabolic biomarkers *in vivo* and *in vitro* and has been used to characterize Alzheimer's disease (Kantarci et al., 2000; Shonk et al., 1995). Studies in humans show decreased *N*-acetylaspartate (NAA, a marker for neuronal number and health) and increased *myo*-inositol, which may either be a marker for osmotic stress or astrogliosis in AD (Valenzuela and Sachdev, 2001). The *myo*-inositol/NAA ratio was proposed, with some success, as a means for distinguishing between AD and other dementias (Shonk et al., 1995). We previously reported that the metabolic profile of APP<sub>Tg2576</sub> transgenic mice is altered (Dedeoglu et al., 2004). Taurine is increased and NAA, glutamate, and glutathione are decreased in the cerebral cortex of APP transgenic mice at 18 months of age when beta amyloid (A $\beta$ ) deposits are widespread. T2 images of the brains suggest that ventricular size is increased. Other studies in double transgenic Alzheimer mouse models that express both APP and human presenilin show parallel findings: decreased NAA and glutamate (Marjanska et al., 2005; von Kienlin et al., 2005) and increased *myo*-inositol (Marjanska et al., 2005).

Many studies suggest that non-steroidal anti-inflammatory drugs (NSAIDs) may either delay onset or slow progression of Alzheimer's

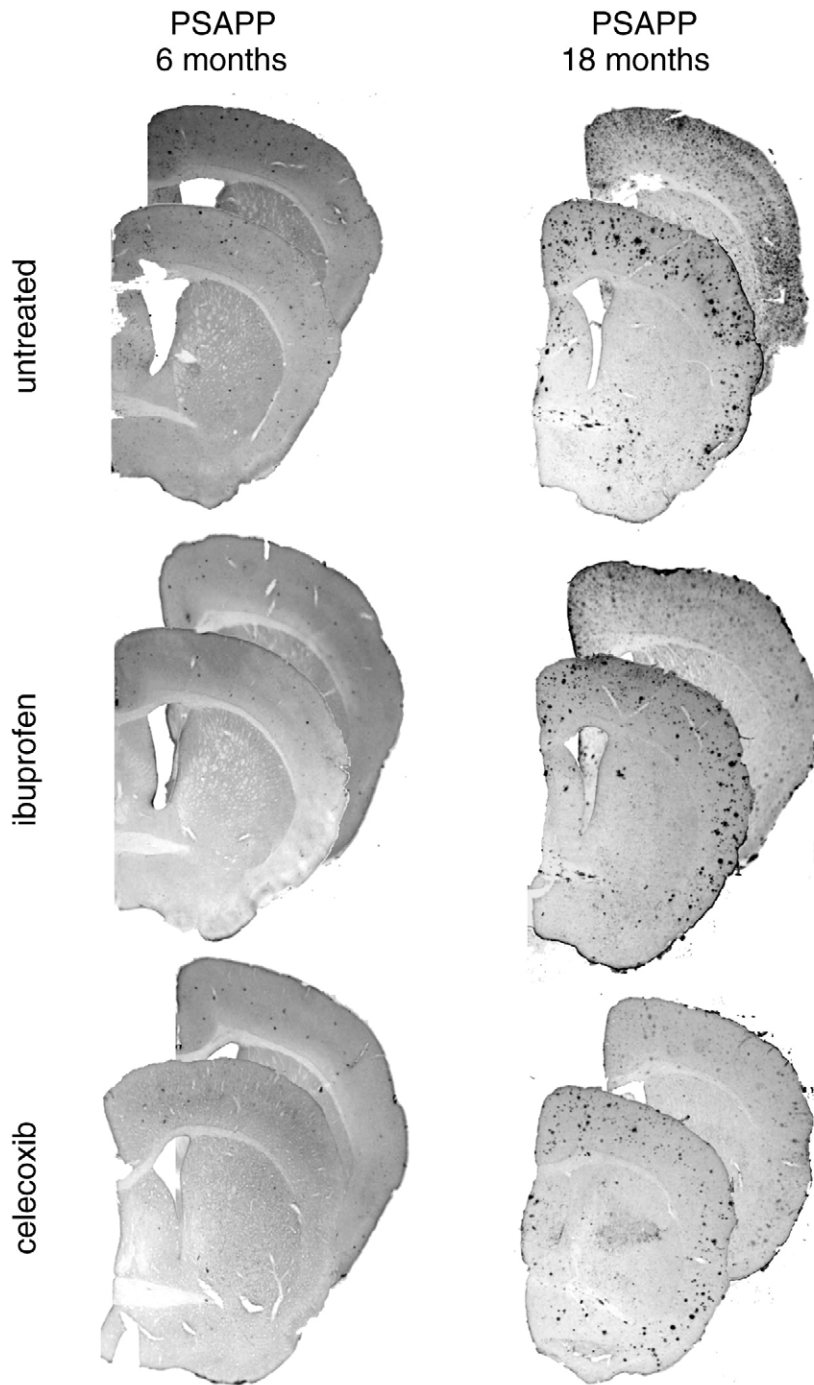
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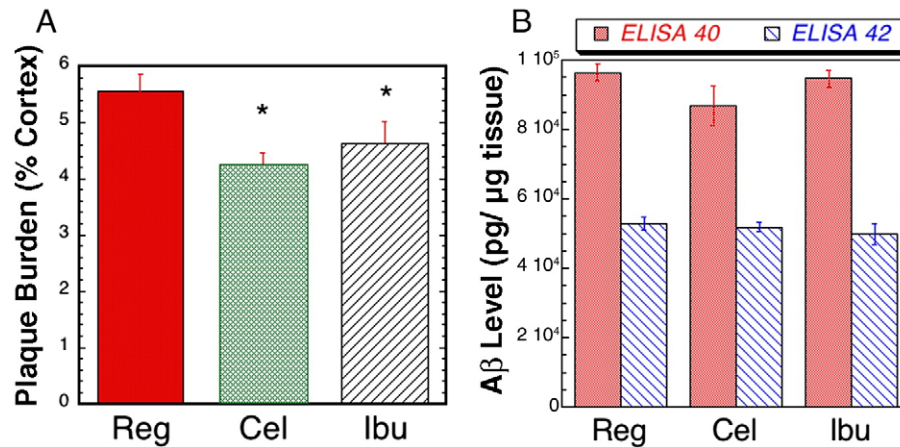
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disease (in t' Veld et al., 2001; Jantzen et al., 2002; Kopito, 2000; Weggen et al., 2001) and reduce A $\beta$  deposition in transgenic mice overexpressing human amyloid precursor protein (APP<sub>Tg2576</sub>) (Dedeoglu et al., 2004; Kopito, 2000; McGeer and McGeer, 2007). Ibuprofen reduces the generation of A $\beta$ <sub>1–42</sub>, the most toxic A $\beta$  species, in addition to its well-known role as a cyclooxygenase (COX) inhibitor (Weggen et al., 2001). We recently found that ibuprofen reduces A $\beta$  and hyperphosphorylated tau in a triple transgene AD model (McKee et al., 2008).

In spite of extensive study of AD using both histological methods and magnetic resonance spectroscopy, there are little data relating the two modalities. This study was, therefore, designed to examine the relationship between cortical levels of neurochemicals detected by MRS (i.e., NAA, *myo*-inositol) and with neuropathological changes (amyloid plaque burden detected by immunohistochemistry and measured by morphometric methods) and A $\beta$ <sub>1–40</sub> and A $\beta$ <sub>1–42</sub> levels detected by ELISA. We then examined the effects of ibuprofen and celecoxib treatment on these parameters. Brain tissue was collected



**Fig. 1.** Representative pictures from 6- and 18-month-old PSAPP mice. Front plane: the sections that are immunostained with A $\beta$ <sub>1–40</sub>. Back plane: sections stained with A $\beta$ <sub>1–42</sub>. Three 50- $\mu$ m sections per brain were used for quantification of plaque burden. Most anterior section was at the bregma level (anterior commissure) and 0.3 and 0.6 mm posterior to bregma covering approximately 0.6-mm thickness. This area is comparable to the cortex that we dissected out for *in vitro* MRS and ELISA for neurochemical profiling and A $\beta$  measurements.



**Fig. 2.** Effects of NSAID treatments on A $\beta$  and plaque levels. (A) Plaque burden (expressed as a fraction of cortical areas) at 16–18 months measured as a function of NSAID treatment. Both celecoxib and ibuprofen were significantly different from untreated animals. (B) Effects of NSAID treatment on A $\beta$  as measured using ELISA—there were no effects of treatment.

from the same mouse for histopathology, A $\beta$  levels, and in vitro MRS for accurate correlations.

## Materials and Methods

### Mice

Heterozygous transgenic male mice overexpressing the human A $\beta$  with the Swedish mutation (Hsiao et al., 1996), APP<sub>Tg2576</sub> were crossbred with homozygous transgenic female mice expressing mutant human presenilin-1 (PS-1<sub>M146V</sub>) to generate double transgenic mice heterozygous for APP and PS-1 (PSAPP). Littermates wild type for APP and heterozygous for PS-1 were used as control and referred to as wild type (WT) throughout the manuscript. Mice were studied at either 6 months of age or between 16 and 18 months of age. The average ages of the animals were WT  $n=10$ ,  $580 \pm 11$  days; celecoxib  $n=9$ ,  $500 \pm 6$  days; ibuprofen  $n=9$ ,  $536 \pm 3$  days; regular diet  $n=10$ ,  $540 \pm 10$  days of age.

### Diet and drug protocol

Ibuprofen (Sigma, 375 ppm) or celecoxib (120 ppm) was formulated into animal diets (Research Diets Inc., NJ, USA). Both drug treatments were started at 1 month of age. Mice were monitored for general well being and had weekly body weights and daily food consumption measured.

### Tissue collection

Mice were euthanized, and brains were removed. The left hemisphere was post-fixed with the periodate–lysine–paraformaldehyde solution for 24 h and cryoprotected in a graded series of 10% and 20% glycerol/2% DMSO solution for histological analysis. The right hemisphere was dissected coronally at the bregma level and 1 mm of cortical tissue, anterior to bregma was saved for the ELISA assay, and 1 mm thick posterior to bregma was saved for in vitro MRS.

### Histology/Immunohistochemistry

Brains were serially cut at 50  $\mu$ m on a freezing microtome and were immunostained for A $\beta$ <sub>1–40</sub> and A $\beta$ <sub>1–42</sub> to define A $\beta$  deposits. Immunohistochemical procedures were performed as previously described (Kowall et al., 2000). In brief, free-floating sections were incubated overnight in primary antibody followed by PBS washes and incubation in peroxidase-conjugated secondary antibody followed by development using DAB as a chromagen.

### Quantitative analysis of A $\beta$ deposits

Three serial sections per mouse brain were analyzed blindly using Stereo Investigator v. 6.55 (MicroBrightField Inc.) at 40 $\times$  magnification. The most rostral section analyzed was at the anterior commissure level ( $\approx 0.1$  mm anterior to bregma), and each successive section was at 0.3 mm increments caudal to the first. In each section, the cortex was manually traced and then plaques over a threshold diameter of 7.6  $\mu$ m were traced automatically after manual verification. Cortex area, total plaque area, plaque area fraction, and plaque count were then exported to Microsoft Excel for each section using NeuroLucida Explorer v. 4.50.4 (MicroBrightField Inc.) and custom software.

### ELISA assay

Dissected cortical brain tissue was homogenized with 8:1 (vol: mass) in cold 5M guanidine HCl/50 mM Tris–HCl and diluted 1:1500 with Dulbecco's phosphate-buffered saline (DPBS) containing 5% BSA and 0.03% Tween-20 supplemented with Protease Inhibitor Cocktail (Calbiochem) and a solid phase sandwich ELISA for A $\beta$ <sub>40</sub> and A $\beta$ <sub>42</sub> was

**Table 1**

Neurochemical concentrations in frontal cortex in the WT, AD, and AD-treated mice.

Chemical	WT ( $n=10$ )	AD Reg ( $n=10$ )	AD Cel ( $n=9$ )	AD Ibu ( $n=9$ )	Significance
Alanine	0.79 $\pm$ 0.06	0.79 $\pm$ 0.03	0.55 $\pm$ 0.07	0.63 $\pm$ 0.07	c,d,e
Aspartate	0.87 $\pm$ 0.11	0.88 $\pm$ 0.14	0.79 $\pm$ 0.08	0.94 $\pm$ 0.12	NS
Choline	1.21 $\pm$ 0.05	1.30 $\pm$ 0.04	1.22 $\pm$ 0.04	1.39 $\pm$ 0.07	NS
Creatine	8.26 $\pm$ 0.29	8.03 $\pm$ 0.21	8.15 $\pm$ 0.22	8.41 $\pm$ 0.27	NS
GABA	2.71 $\pm$ 0.37	2.62 $\pm$ 0.27	2.98 $\pm$ 0.35	3.33 $\pm$ 0.51	NS
Glutamate	7.78 $\pm$ 0.20	6.78 $\pm$ 0.22	6.71 $\pm$ 0.33	7.53 $\pm$ 0.31	a,c
Glutamine	4.12 $\pm$ 0.22	4.84 $\pm$ 0.20	5.06 $\pm$ 0.28	5.15 $\pm$ 0.24	a,b,c
Glutathione	0.96 $\pm$ 0.15	1.12 $\pm$ 0.15	1.20 $\pm$ 0.11	1.27 $\pm$ 0.14	NS
Glycine	1.92 $\pm$ 0.20	1.79 $\pm$ 0.11	1.92 $\pm$ 0.11	1.96 $\pm$ 0.11	NS
Lactate	9.55 $\pm$ 1.14	9.26 $\pm$ 0.44	8.50 $\pm$ 0.48	9.46 $\pm$ 0.22	NS
myo- inositol	4.89 $\pm$ 0.23	5.82 $\pm$ 0.14	5.67 $\pm$ 0.13	5.87 $\pm$ 0.19	a,b,c
Inositol					
NAA	4.90 $\pm$ 0.10	4.21 $\pm$ 0.07	4.58 $\pm$ 0.06	4.54 $\pm$ 0.08	a,b,c,d,e
Succinate	0.85 $\pm$ 0.05	0.81 $\pm$ 0.04	0.80 $\pm$ 0.05	0.90 $\pm$ 0.04	NS
Scyllo- inositol	0.09 $\pm$ 0.01	0.11 $\pm$ 0.01	0.13 $\pm$ 0.02	0.11 $\pm$ 0.01	NS
Taurine	8.54 $\pm$ 0.17	8.53 $\pm$ 0.14	8.35 $\pm$ 0.16	8.67 $\pm$ 0.20	NS

All concentrations reported as  $\mu$ mol/g wet weight.

Reg, regular diet; Cel, celecoxib-fed; Ibu, ibuprofen-fed.

Significance (Tukey's HSD): a, Reg different than WT; b, Ibu different than WT; c, Cel different than WT; d, Reg diff than cel; e, Reg different than Ibu; f, Cel different than Ibu.

performed following the manufacturer's instructions (BioSource). Briefly, homogenized samples were added into the wells of a 96-well plate precoated with a monoclonal antibody specific to the NH<sub>2</sub>-terminus of Hu A $\beta$  to capture APP and all A $\beta$  processed forms. Samples were then mixed with a cleavage-specific antibody to either A $\beta$ <sub>40</sub> or A $\beta$ <sub>42</sub>. After overnight incubation at 4 °C, plates were washed and incubated with the secondary antibody for 30 minutes at 25 °C. Washed wells were developed by the addition of a substrate. The substrate reaction was then stopped and color intensity was measured at 450 nm.

#### MR spectroscopy and imaging

MR of the mice was performed at 9.4T (Bruker, Billerica MA) using dual concentric surface coils of diameter 5 cm for transmit and 1.5 cm for receive. Animals were kept warm with a 38 °C circulating water blanket. Animals were anesthetized using 1.5% halothane in an NO<sub>2</sub>/O<sub>2</sub> mixture. T<sub>2</sub>-weighted RARE spin echo MR images (TR 5000; effective TE 100 ms; RARE factor 8) were collected with spatial resolution of 0.15 mm in plane and consecutive 0.6 mm slices. MR spectroscopy was performed as previously reported (Dedeoglu et al., 2004; Jenkins et al., 2000). Briefly, voxels were placed centered over the hippocampus (subiculum). Voxel sizes were tailored to the size of the brain. The average voxel size was 2×2.2×2 mm (ca. 10  $\mu$ L). Spectra were recorded using a PRESS technique with a TR of 2.2 s and TE values of 15 and 144 ms. Data were processed using curve fitting of the spectra, and intensities were integrated. Resonance integrals were normalized to the creatine peak. Collection of the two TE values allowed for corrections in the T<sub>2</sub>s of the metabolites that might occur because of differences between the transgenic and wild-type animals. In vitro MRS

measurements were made from PCA brain extracts of cortical brain tissue and were run on a 14-T Bruker spectrometer. Samples were prepared in 99.9% D<sub>2</sub>O (pH 7.2) and run at 26 °C and 3-trimethylsilylpropane sulfonic acid (DSS; 0.5 mM) was used as an internal standard. Spectra were analyzed as previously published (Dedeoglu et al., 2004; Jenkins et al., 2000).

#### Data analysis

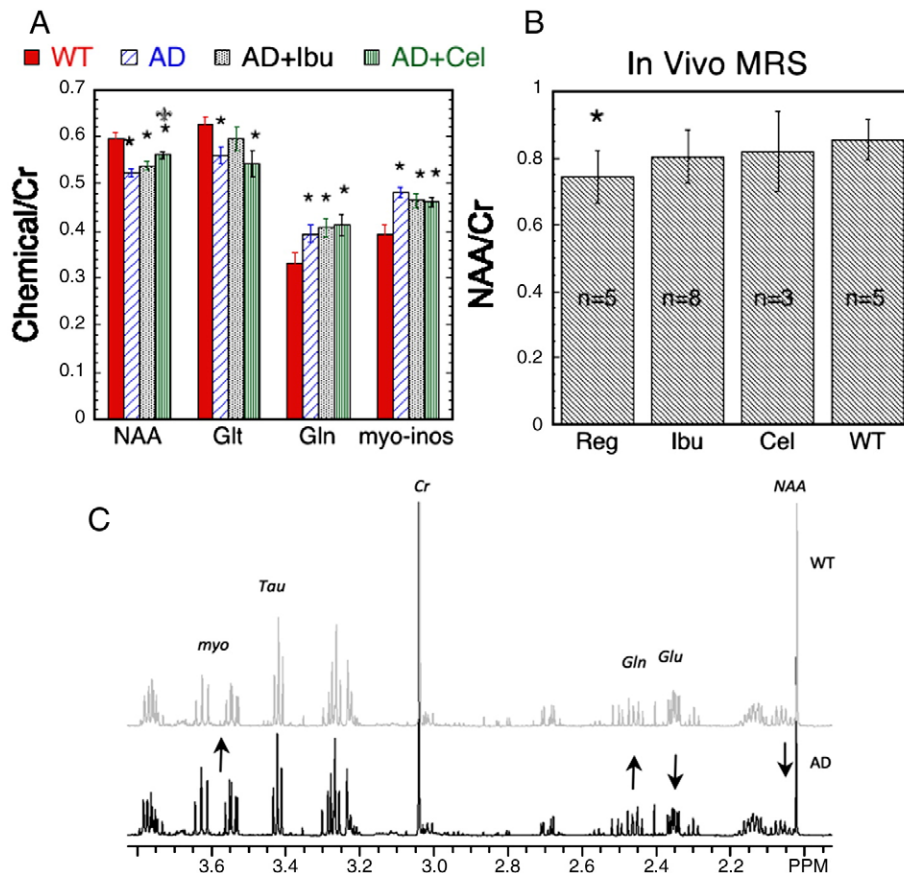
We performed ANOVA factorial analysis for comparisons of the different animal groups and the various metabolite, biochemical, and histological measures. The Tukey (honest significant difference, HSD) test was used for post hoc analysis of the variables when the omnibus group one-way ANOVA was significant. We constructed receiver operator curves to determine which of the various spectroscopic markers generated optimal sensitivity for discrimination between the groups. We also performed linear discriminant analysis to determine the ability of the markers to differentiate between the groups. These data were subjected to holdout analysis. In addition, we performed a series of regression analyses and Pearson product moment correlations to compare the different metabolite and histopathological variables.

All studies were conducted in accordance with local and national standards for treatment of laboratory animals.

### Results and Discussion

#### Young mice at 6 months of age

At 6 months of age, when there is small but significant plaque accumulation in the PS1×APP mice (see Fig. 1), there were no



**Fig. 3.** Effects of NSAID treatment on MRS measures. (A) Plots of the effects of NSAID treatment on NAA, glutamate, glutamine, and *myo*-inositol. (B) Effects of NSAID treatment on NAA measured in vivo. (C) Typical in vitro MRS spectrum in WT and AD regular diet mice at 18 months of age. \**p* < 0.05 by ANOVA.

**Table 2**

In vivo neurochemical concentrations in hippocampus in the WT, AD, and AD-treated mice.

Chemical	WT (n = 5)	AD Reg (n = 4)	AD Cel (n = 3)	AD Ibu (n = 8)
Choline	1.09 ± 0.20	1.02 ± 0.20	0.88 ± 0.10	1.19 ± 0.17
Glutamate	0.27 ± 0.07	0.27 ± 0.09	0.26 ± 0.08	0.33 ± 0.27
Glutamine	0.08 ± 0.03	0.12 ± 0.05	0.07 ± 0.03	0.15 ± 0.13
myo-Inositol	0.07 ± 0.02	0.07 ± 0.01	0.10 ± 0.05	0.11 ± 0.07
NAA	0.86 ± 0.05	0.74 ± 0.16*	0.82 ± 0.13	0.81 ± 0.20
Taurine	0.38 ± 0.09	0.31 ± 0.09	0.19 ± 0.11	0.29 ± 0.12

All concentrations reported as ratios to creatine.

Reg, regular diet; Cel, celecoxib-fed; Ibu, ibuprofen-fed.

Significance (Tukey's HSD).

\* Reg different than WT.

significant neurochemical changes in any of 16 metabolites analyzed using MRS under any treatment or compared to the wild-type mice (regular diet  $n = 8$ ; ibuprofen-treated  $n = 9$ ; celecoxib  $n = 7$ ; wt = 5) consistent with prior MRS reports in both PS1xAPP mice (Marjanska et al., 2005) and in PS2xAPP mice (von Kienlin et al., 2005). Similarly, no differences on amyloid plaque burden were observed as a result of the treatment with the NSAIDs. The only change noted was that ibuprofen treatment significantly decreased the ratio of cortical levels of  $A\beta_{42/40}$  measured using ELISA (the ratios were as follows: regular diet  $1.94 \pm .08$ ; ibuprofen  $1.54 \pm .07$ ; celecoxib  $1.94 \pm 0.19$ ;  $p < 0.05$  for ibuprofen vs. regular diet), although the effect on the absolute levels was not significant.

#### Mice at 16–18 months of age

#### Effects of AD and NSAID treatment

At 16–18 months of age, there is a large plaque burden in the mice, covering cortex and hippocampal regions (see Fig. 1), as well as a large increase in the  $A\beta$  content measured using ELISA. Both celecoxib and ibuprofen significantly lowered the plaque burden in the 16- to 18-month-old mice compared to the regular diet as measured by either the absolute plaque area or the percent of cortical area (celecoxib  $4.3 \pm 0.2\%$  vs.  $4.6 \pm 0.4\%$  in ibuprofen and  $5.6 \pm 0.3\%$  for regular diet  $F_{2,24} = 6.2$ ;  $p < 0.01$ ; see Fig. 3). There is a huge increase in the absolute levels of  $A\beta_{1-40}$  and  $A\beta_{1-42}$  as the AD animals age, and this large increase was unaffected by treatment with any of the NSAIDs (Fig. 2).

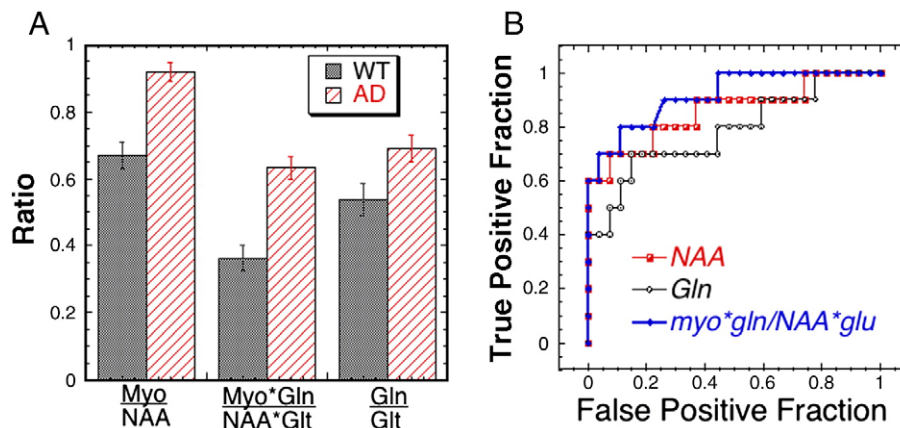
The changes noted are reflected in a number of different spectroscopic markers as shown in Table 1 and in Fig. 3. The

most prominent changes are increases in myo-inositol and glutamine and decreases in NAA and glutamate between the AD and WT animals. ANOVA factorial analysis between groups (WT, AD regular diet, or celecoxib or ibuprofen treated) showed a significant protection against the NAA loss for celecoxib and ibuprofen whereas only ibuprofen produced a significant protection against the glutamate decrease. Neither NSAID had an effect on either glutamine or myo-inositol. Interestingly, there were no increases in taurine unlike in the APP alone mice as observed in two prior studies (Dedeoglu et al., 2004; Marjanska et al., 2005). In vivo MRS was performed in mice between 17 and 20 months of age in the hippocampus. The results paralleled the in vitro results, although the only significant finding was that NAA loss was protected by ibuprofen. The data, reported as ratios to creatine, are reported in Table 2.

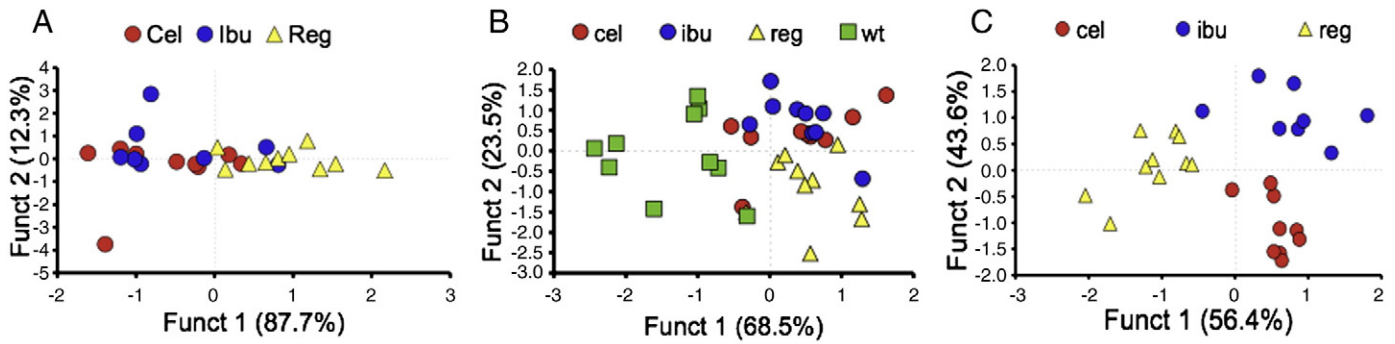
#### Classification into groups

A prior study in humans claimed that there was increased sensitivity and specificity for the ratio of myo-inositol/NAA for separation of AD from other dementias (Shonk et al., 1995). We thus investigated the myo/NAA ratio as well as two other ratios. Since myo-inositol and glutamine increase and glutamate and NAA decrease in the AD mice, we constructed a ratio of myo\*gln/NAA\*glu as well as the ratio of gln/glu to characterize the mice using a neural network classifier. The best classifiers were the latter two ratios, both of which were 100% correct for AD and 70% correct for the WT animals. Both the myo/NAA and the myo\*gln/NAA\*glu markers were significantly better than the simple metabolite ratios at differentiating AD from WT. None of the metabolites alone were as good, and even using all four of the chemicals in the ratios still only yielded 90% sensitivity. Unfortunately, the ratios were not as good at discriminating between treated or not treated with NSAIDs since both myo-inositol and glutamine were not affected by NSAID treatment. These data suggest that more work needs to be performed on determining the histopathological and molecular bases for the changes observed in the MRS-visible chemicals.

We found that using either NAA or myo-inositol alone yielded values that were close to those of the ratios. Inclusion of more metabolites in the classifier served to decrease the sensitivity and specificity of the test. Likewise, the myo/NAA ratio and the myo\*gln/NAA\*glu ratios yielded receiver-operator curves for distinguishing between AD and WT that had 0.896 and 0.917 areas under the curve, respectively, but these were not significantly different from the curves for NAA or myo-inositol alone. They were



**Fig. 4.** ROC curves for classification of metabolite ratios. (A) Ratios constructed using NAA/myo-inositol; NAA\*glt/myo-inositol\*gln or gln/glt. All the ratios were significantly different in AD and WT mice. (B) On the right is shown a receiver operator characteristic curve for three difference metrics used to characterize the mice as AD or non-AD. The area under the curves are significantly different for myo\*gln/NAA\*glu vs. NAA vs. gln, thus showing the ability to characterize the animals better using the combined ratio metrics. The ratio of myo\*gln/NAA\*glu was not significantly better than myo/NAA (not shown).



**Fig. 5.** Classification of groups using linear discriminant analysis. (A) Classification of groups using just the plaque areas and ELISA Ab 40 + 42 values. Using holdout analysis indicated 48% correct classification. (B) Classification using five MRS chemicals (NAA, gln, myo-inositol, alanine). There was 69% correct classification. Alanine adds significant power to discriminate between NSAID treated and untreated. (C) Linear discriminant analysis of the transgenic AD mice combining the MRS and histopathology data. The variables included were myo-inositol, glutamine, glutamate, NAA, alanine (MRS),  $A\beta_{40}$ , and  $A\beta_{42}$  levels using ELISA and the plaque burden expressed as the fraction of cortical area. There was excellent separation between the three different treatment groups (Wilks' lambda for functions 1 and 2 was 0.077 and 0.334, respectively;  $p < 0.001$ ). All the discriminant plots are normalized to one standard deviation.

better than an ROC constructed using, for instance, just gln (see Fig. 4).

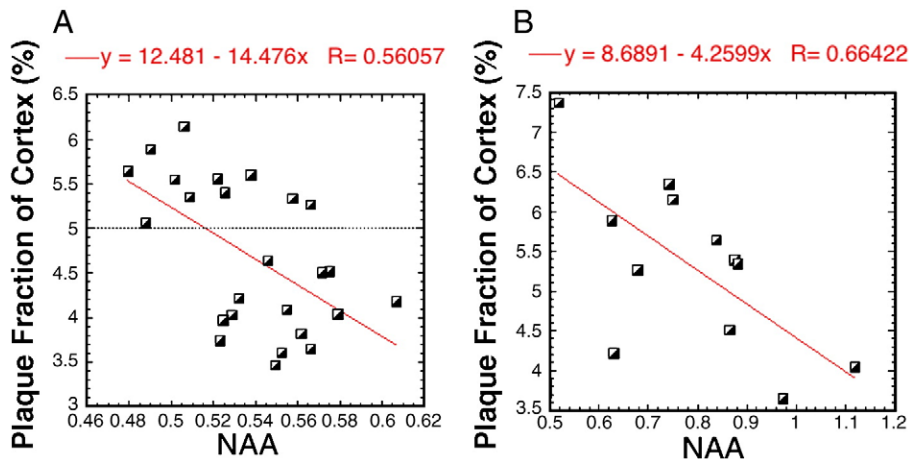
We also performed linear discriminant analysis using either metabolites alone or combinations of metabolites and pathological data. Shown in Fig. 5 are the results. We used the five metabolites myo-inositol, glutamine, glutamate, NAA, and alanine. The latter was included since it shows a large difference between the NSAID treated and other animals, although no apparent effects of AD (see Table 1). If one uses all the metabolites, there are too many variables for the number of experimental points. Nonetheless, we found that the classification (using holdout analysis) went from 84% with all the metabolites to 79% using just the five metabolites above. Furthermore, removing alanine changes the classification from 79% to 63%. Use of just the pathology data (ELISA and plaque fraction of cortex) allowed correct classification of just 48% of the animals (Fig. 5B). Combining the five metabolites and two pathological variables together allowed for classification of 93%. Clearly, larger numbers of animals will be needed to put these types of classifiers on a surer footing, but combining just five MRS markers and two pathological markers provided significantly better classification than using either alone—this was especially true for the pathology data. Since ELISA showed no effects of treatment,

then it is likely that it is not adding much in the way of classification accuracy.

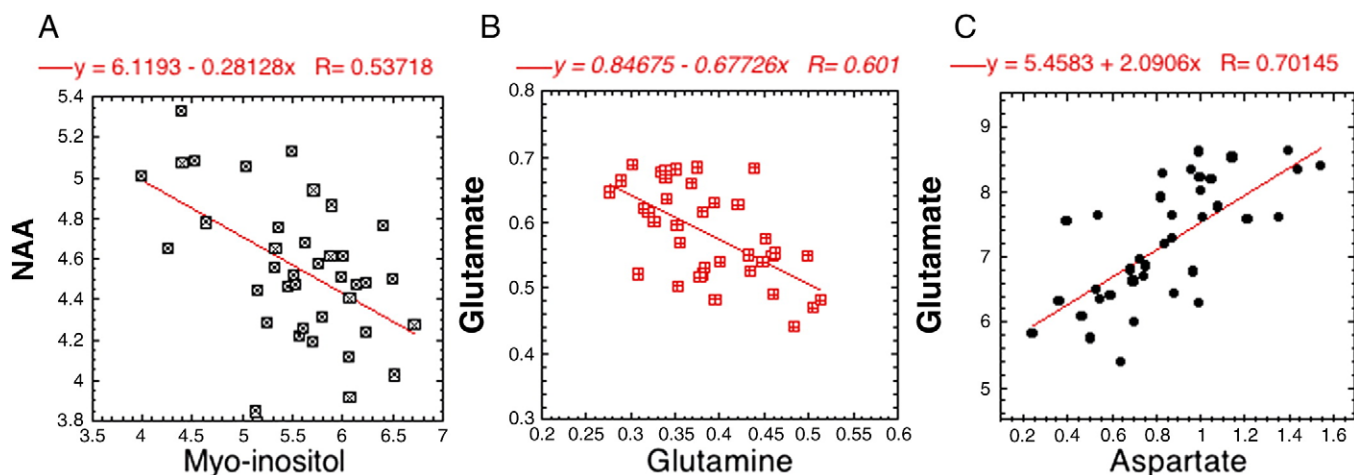
#### Relations between neurochemicals and neuropathology

For all the animals, there was a reasonable correlation between NAA and plaque burden that was highly significant for both the in vitro and in vivo data (see Fig. 6). It would appear, however, from the in vitro data, that this is largely the result of a threshold-like event where NAA decreases after the plaque burden reaches 5%. Interestingly, a similar 5% threshold can be seen in the data collected from APPxPS2 mice (von Kienlin et al., 2005). The correlation of myo-inositol with plaque burden was not significant.

There was a significant (though not very compelling) correlation between NAA and myo-inositol (Fig. 7A). This likely reflects only the fact that, at 18 months, both myo-inositol and NAA reflect the progression of the disease—even though these two metabolites likely reflect different pathological processes. Data from individuals with Down's syndrome suggest that the increase in myo-inositol precedes the decrease in NAA (Shonk and Ross, 1995). Myo-inositol is also elevated in patients with mild cognitive impairment, often considered a precursor to AD (Kantarci et al., 2000). There was a highly significant inverse correlation between glutamate and



**Fig. 6.** Correlations between NAA and plaque areas for all animals except WT. (A) Plot of the plaque area as a fraction of cortex vs. NAA from the in vitro data. There is a weak but significant correlation that seems to have a threshold at around 5% plaque area. (B) Plot of plaque area as a fraction of cortex vs. NAA from the in vivo data. The correlation is also significant.



**Fig. 7.** Relations between MRS markers for all animals. (A) Plot of NAA vs. *myo*-inositol—there is a weak but significant inverse correlation. (B) Plot of glutamate vs. glutamine, again showing an inverse correlation. (C) Plot of glutamate vs. aspartate. There is a positive correlation.

glutamine (Fig. 7B). This is driven by the fact that there was an increase in glutamine and a decrease in glutamate in the AD mice at 18 months of age. This pattern is similar to what we have observed in Huntington's disease mice (Jenkins et al., 2000) as well as single transgenic APP mice (Dedeoglu et al., 2004) and to that seen in toxin models of neurodegeneration (Tkac et al., 2001). The change reflects altered glial/neuronal cycling as well as, potentially, a shift in the glial/neuronal volume balance (Choi et al., 2007). There was also a very strong correlation between glutamate and aspartate (Fig. 7C), which may reflect the fact that both of these are excitatory neurotransmitters. Interestingly, aspartate showed no alterations in the AD models. Studies from cultured neurons and astrocytes indicate that astrocytes have nearly undetectable levels of NAA whereas neurons have nearly undetectable levels of *myo*-inositol (Brand et al., 1993; Urenjak et al., 1992; Zwingmann et al., 2000). Likewise, in cultured astrocytes, there is about 2× more glutamine than in neurons whereas for glutamate there is 3–4× more in neurons than astrocytes.

These data suggest that ibuprofen and possibly celecoxib can protect against some of the neuronal pathology in these mice. This fact is evidenced by the protection of NAA and glutamate loss by ibuprofen and the protection of NAA loss by celecoxib. Neither compound protects against the increases in *myo*-inositol or glutamine.

There are a few caveats to our data. First, there was a small but significant difference in age between the celecoxib and regular diet animals (500 days vs. 540, respectively,  $p = 0.02$ ), that was not true for ibuprofen treated animals (536 days mean). Since our data suggest a weak correlation between plaque area and age between 16 and 18 months of age (based upon comparisons in 16 AD mice regular diet between 470 and 590 days of age, there was 1.6% per day increase in plaque areas as a fraction of cortex,  $R = .50$ ;  $p = 0.05$ ), this would tend to favor celecoxib as far as showing protection against plaque build up. Data from Marjanska et al. (2005) show little change in NAA in this age range so it may be that celecoxib does protect the NAA loss. Other studies in AD mice have shown inconclusive effects using celecoxib (reviewed in McGeer and McGeer (2007)). Another limitation of our study for both the in vivo and in vitro studies was the relatively large voxels sampled. Since the pattern of plaque deposition is not necessarily uniform (for instance, there is more plaque in cingulate than other areas of cortex (see Fig. 1)), it would be advantageous to use smaller sampling volumes. Nonetheless, these data show that the combination of MRS and pathology can provide excellent markers for following disease progression.

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## References

- Brand, A., Richter-Landsberg, C., Leibfritz, D., 1993. Multinuclear NMR studies on the energy metabolism of glial and neuronal cells. *Dev. Neurosci.* 15, 289–298.
- Choi, J.K., Dedeoglu, A., Jenkins, B.G., 2007. Application of MRS to mouse models of neurodegenerative illness. *NMR Biomed.* 20, 216–237.
- Dedeoglu, A., Choi, J.K., Cormier, K., Kowall, N.W., Jenkins, B.G., 2004. Magnetic resonance spectroscopic analysis of Alzheimer's disease mouse brain that express mutant human APP shows altered neurochemical profile. *Brain Res.* 1012, 60–65.
- Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., Cole, G., 1996. Correlative memory deficits, Abeta elevation, and amyloid plaques in transgenic mice. *Science* 274, 99–102.
- in t' Veld, B.A., Ruitenber, A., Hofman, A., Launer, L.J., van Duijn, C.M., Stijnen, T., Breteler, M.M., Stricker, B.H., 2001. Nonsteroidal antiinflammatory drugs and the risk of Alzheimer's disease. *N. Engl. J. Med.* 345, 1515–1521.
- Jantzen, P.T., Connor, K.E., DiCarlo, G., Wenk, G.L., Wallace, J.L., Rojiani, A.M., Coppola, D., Morgan, D., Gordon, M.N., 2002. Microglial activation and beta-amyloid deposit reduction caused by a nitric oxide-releasing nonsteroidal anti-inflammatory drug in amyloid precursor protein plus presenilin-1 transgenic mice. *J. Neurosci.* 22, 2246–2254.
- Jenkins, B.G., Klivenyi, P., Kustermann, E., Andreassen, O.A., Ferrante, R.J., Rosen, B.R., Beal, M.F., 2000. Nonlinear decrease over time in *N*-acetyl aspartate levels in the absence of neuronal loss and increases in glutamine and glucose in transgenic Huntington's disease mice. *J. Neurochem.* 74, 2108–2119.
- Kantarci, K., Jack Jr., C.R., Xu, Y.C., Campeau, N.G., O'Brien, P.C., Smith, G.E., Ivnik, R.J., Boeve, B.F., Kokmen, E., Tangalos, E.G., Petersen, R.C., 2000. Regional metabolic patterns in mild cognitive impairment and Alzheimer's disease: a 1H MRS study. *Neurology* 55, 210–217.
- Kopito, R.R., 2000. Aggregates, inclusion bodies and protein aggregation. *Trends Cell Biol.* 10, 524–530.
- Kowall, N.W., Hantraye, P., Brouillet, E., Beal, M.F., McKee, A.C., Ferrante, R.J., 2000. MPTP induces alpha-synuclein aggregation in the substantia nigra of baboons. *NeuroReport* 11, 211–213.
- Marjanska, M., Curran, G.L., Wengenack, T.M., Henry, P.G., Bliss, R.L., Poduslo, J.F., Jack Jr., C.R., Ugurbil, K., Garwood, M., 2005. Monitoring disease progression in transgenic mouse models of Alzheimer's disease with proton magnetic resonance spectroscopy. *Proc. Natl. Acad. Sci. U. S. A.* 102, 11906–11910.
- McGeer, P.L., McGeer, E.G., 2007. NSAIDs and Alzheimer disease: epidemiological, animal model and clinical studies. *Neurobiol. Aging* 28, 639–647.
- McGowan, E., Eriksen, J., Hutton, M., 2006. A decade of modeling Alzheimer's disease in transgenic mice. *Trends Genet.* 22, 281–289.
- McKee, A.C., Carreras, I., Hossain, L., Ryu, H., Klein, W.L., Oddo, S., LaFerla, F.M., Jenkins, B.G., Kowall, N.W., Dedeoglu, A., 2008. Ibuprofen reduces Abeta, hyperphosphorylated tau and memory deficits in Alzheimer mice. *Brain Res.* 1207, 225–236.
- Shonk, T., Ross, B.D., 1995. Role of increased cerebral *myo*-inositol in the dementia of Down syndrome. *Magn. Reson. Med.* 33, 858–861.

- Shonk, T.K., Moats, R.A., Gifford, P., Michaelis, T., Mandigo, J.C., Izumi, J., Ross, B.D., 1995. Probable Alzheimer disease: diagnosis with proton MR spectroscopy [see comments]. *Radiology* 195, 65–72.
- Tkac, I., Keene, C.D., Pfeuffer, J., Low, W.C., Gruetter, R., 2001. Metabolic changes in quinolinic acid-lesioned rat striatum detected non-invasively by in vivo  $(^1\text{H})$  NMR spectroscopy. *J. Neurosci. Res.* 66, 891–898.
- Urenjak, J., Williams, S.R., Gadian, D.G., Noble, M., 1992. Specific expression of *N*-acetylaspartate in neurons, oligodendrocyte-type-2 astrocyte progenitors, and immature oligodendrocytes in vitro. *J. Neurochem.* 59, 55–61.
- Valenzuela, M.J., Sachdev, P., 2001. Magnetic resonance spectroscopy in AD. *Neurology* 56, 592–598.
- von Kienlin, M., Kunnecke, B., Metzger, F., Steiner, G., Richards, J.G., Ozmen, L., Jacobsen, H., Loetscher, H., 2005. Altered metabolic profile in the frontal cortex of PS2APP transgenic mice, monitored throughout their life span. *Neurobiol. Dis.* 18, 32–39.
- Weggen, S., Eriksen, J.L., Das, P., Sagi, S.A., Wang, R., Pietrzik, C.U., Findlay, K.A., Smith, T.E., Murphy, M.P., Bulter, T., Kang, D.E., Marquez-Sterling, N., Golde, T.E., Koo, E.H., 2001. A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. *Nature* 414, 212–216.
- Zwingmann, C., Richter-Landsberg, C., Brand, A., Leibfritz, D., 2000. NMR spectroscopic study on the metabolic fate of  $[3-(^{13}\text{C})]$ alanine in astrocytes, neurons, and cocultures: implications for glia-neuron interactions in neurotransmitter metabolism. *Glia* 32, 286–303.