Metabolite Profiles in the Frontal and Occipital Cortices in Alzheimer's Disease as Analyzed by HRMAS 1H NMR

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Introduction Alzheimer's disease (AD) is age-related disorders associated with both biochemical and genetic causes. At present, the diagnosis of AD relies primarily on cognitive and behavioral criteria with neuropathologic confirmation. Thus non-invasive imaging methods that permit diagnosis of AD prior to significant cognitive impairment are highly desirable. Magnetic resonance spectroscopy (MRS) is capable of noninvasive detection of neurochemical abnormalities that reflect these pathologic processes. However, *in vivo* MRS method still suffers from low sensitivity and low spectral resolution in detecting and resolving critical metabolites that may be important to AD diagnosis. HRMAS 1H NMR spectroscopy has been developed to investigate metabolites using intact tissue specimens [1-2]. This approach offers enhanced spectral resolution and a quantitative method for discovering metabolite markers that can be useful for the development of *in vivo* MRS measurements of neurochemistry in AD. Here, high resolution magic angle spinning (HRMAS) 1H nuclear magnetic resonance (NMR) was applied to investigate the metabolite profiles of postmortem tissue samples from the frontal and occipital cortex of AD and non-demented age-matched controls. Our results showed abnormal metabolites and neurochemical changes in the frontal and occipital cortex in AD, including newly observed spectral features that are specific to the AD brain, which have strong correlation to the Apolipoprotein E (APOE) genotypes in frontal cortex.

Methods Postmortem tissue blocks from the frontal and occipital cortices of age-matched AD (n=11) and non-demented control (n=11) subjects were provided by the Tissue Bank of Alzheimer's Disease Research Center (ADRC) of this institution. Tissue blocks were stored in the -80 ^{o}C before NMR experiments. Three or four intact tissue samples (30 mg/each, typically) cut from the tissue block of each subject were analyzed ex vivo using 600 MHz HRMAS proton NMR (Bruker) at 4 ^{o}C and low sample spin rate of 2800 Hz. External reference (TSP) was added to each samples as the chemical shift reference of each resonance and for metabolite quantification. Measurements of peak integrals per proton for each resonance selected to represent each metabolite of interest were first normalized against that of creatine (Cre) and then averaged for the samples taken from each tissue block. Statistical analysis was carried out using independent two- tailed *t*-tests with SPSS 15.0 (LEAD Technologies, Inc). Any differences with $p \leq 0.05$ were considered to be statistically significant.

Results High resolution NMR spectra with effective water suppression were obtained successfully from all samples in this study. **Figure 1** shows the expanded region of the typical 1H HRMAS NMR spectra obtained from the frontal cortex of an AD and a control brain. We found the statistically significant decreases of NAA/Cre, Ace/Cre, GABA/Cre, Asp/Cre, Cho/Cre, Tau/Cre and MI/Cre, as well as increases of PC/Cre and GPC/Cre in AD samples in comparison to those of controls. Interestingly, our spectra showed a resonance rising at 3.71 ppm (noted with arrow) clearly increased in AD samples. This newly reported resonance appeared to have broadened line-width. 2D COSY experiments did not yield J-couple correlations with other resonances at the current sample concentration and resolution. The ratios of the average metabolite levels normalized to creatine and the changes in metabolite ratios were obtained. Given the improved sensitivity of HRMAS NMR, we also investigated the aromatic region (5.6 - 8.5 ppm) of the HRMAS NMR spectra, which has not been reported previously. There were noticeable changes of the metabolite profile in this region, as shown in the typical spectra of AD and control samples. The significant observations include a resonance at 5.85 ppm (as shown in **Figure 2**) that appeared in 11 AD samples but only two control samples. When using Descriptive Statistics followed by Crosstabs analysis of cases in which this aromatic resonance was present, we found this spectral marker has an 82% specificity and 91% sensitivity to AD. It also was exhibited in all seven AD samples with an APOE ε4 allele. This resonance was also detected in three of four APOE ε4- samples in the AD group. In contrast, in the control group this resonance appeared in only one of three APOE ε4+ samples and only one of eight APOE ε4-samples. Spectroscopic data were further analyzed to investigate the relationship between APOE genotype and metabolite levels in the samples both AD and control. The results indicated that the APOE ε4+ samples exhibited greater increases of PC/Cre, GPC/Cre and 3.87ppm peak of Unk/Cre and a greater decrease of Asp/Cre compared to the APOE ε4- samples(as shown in **Figure 3**). If only those samples with APOE ε4 allele in the AD group (n=7) are compared to samples without an APOE ε4 allele in the control group (n=8), the magnitudes of the observed metabolic differences between AD and controls were even greater with more pronounced statistical significance. When examining the metabolite profile differences between gray and white matters of AD and control brains, we found most of amino acids, such as Ala, Glu and Gln, increase in the gray matter comparing those in the white matter in the AD brains.

Discussion and conclusion Previous study using solid state NMR and intact tissue focused primarily on NAA and choline compounds. In this study, samples from AD and control groups were carefully matched for gender, age and PMI. In order to assess the reproducibility of the data and to evaluate the heterogeneity of the tissue samples, three or four samples from each 200 mg tissue block from each brain were analyzed and compared. High spectral resolution of HRMAS NMR enabled us to identify and assign additional resonances and metabolites that were not reported before and provides better resolved spectra to allow for differentiating different choline derivatives, Our data also reveal newly observed resonances rising at 3.71 and 5.85 ppm appear more often in AD than control samples, moreover are strongly associated with APOE-ε4 allele in AD. Inheritance of APOE-ε4 was associated with a significantly higher Aβ plaque burden than was observed in patients lacking APOE-ε4. The increases in PC/Cre, GPC/Cre and the presence of newly observed resonance at 3.71 ppm were more pronounced in APOE ε4+ AD samples as compared to APOE ε4- samples. An even stronger association with APOE ε4+ genotype was found with the newly observed aromatic metabolite. Such a strong correlation between abnormal metabolite profiles in AD and APOE genotype has not been reported previously and needs further validation with larger sample sizes. Nevertheless, this possible correlation suggests the importance of examining the influence of underlying genetic factors on the neurochemical and metabolic changes in AD. Since the MRS method has become a technique for in vivo or ex vivo evaluation of brain diseases in both clinical studies and diagnosis as well as experimental studies with animal models using specific metabolites as "surrogate markers" of particular cell types, the current results and further extension of this study may provide potential surrogate markers that may be applied in the noninvasive diagnosis and monitoring of AD using the clinically available MRS method.

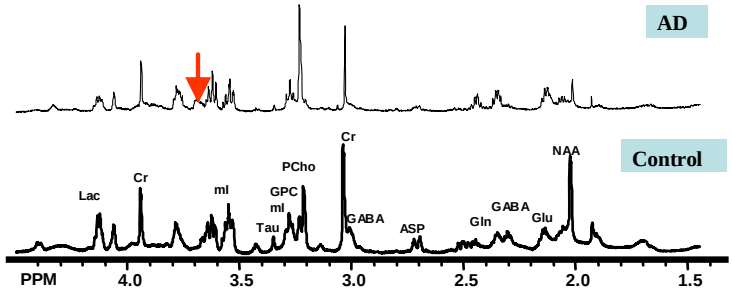

Fig 1. Expanded region of the HRMAS NMR, AD specific resonance (3.71 and 5.85 ppm) newly observed in this study is indicated (arrow).

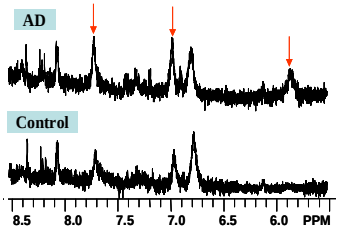

Fig 2. An expand region showed the aromatic resonances in AD and controls.

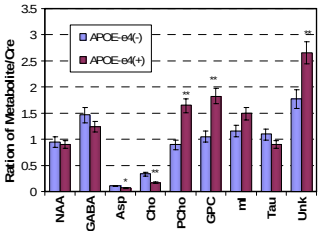

Fig 2. Comparison of metabolite/Cre ratios of samples with different APOE gentypes.

References

[1] Cheng, LL, Ma, MJ, at al. (1997). *Proc. Natl. Acad. Sci. U.S.A.* **94,** 6408-6413; [2] Cheng, LL, Newell, K., et al. (2002). *Magn. Reson. Imaging.* **20,** 527-533.