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 an age-related disorder associated with both biochemical and genetic causes. At present, the diagnosis of AD relies primarily on cognitive symptoms and clinical criteria with neuropathological 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 biochemical 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 °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 °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 then normalized against 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 Figure 2, that appeared in AD samples but not in control samples. Our data also reveal newly observed resonances rising at 3.71 ppm and 5.85 ppm in AD samples in comparison to those of controls.

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 sample and a control brain. We found the statistically significant decreases of NAA/Cr, Ace/Cr, GABA/Cr, Asp/Cr, Tau/Cr and MDCr, as well as increases of PC/Cr and GPC/Cr 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. The new peak that appeared has been 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 Figure 2, that appeared in AD samples but not in control samples. Our data also reveal newly observed resonances rising at 3.71 ppm and 5.85 ppm in AD samples in comparison to those of controls. The increases in PC/Cr, GPC/Cr and the presence of newly observed resonances rising at 3.71 ppm were more pronounced in AD samples 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/Cr, GPC/Cr 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 MRS methods.

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 ppm and 5.85 ppm in AD samples more often 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/Cr, GPC/Cr 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 MRS methods.

References