# Ultra-high field in vivo localized two dimensional correlated MR spectroscopy to probe membrane degradation during progression of Alzheimer's disease

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## Introduction

Alzheimer's disease (AD) is the most common form of dementia, afflicting mainly the elderly. In addition to the main hallmarks of AD, namely: amyloid β (Aβ) containing plaques and neurofibrillary tangles, the breakdown of neuronal membrane phospholipids is gaining increasing importance as significant pathological events of AD progression[1]. The increase in glycerophosphocholine (GPC) an indicator of increased breakdown of phospholipids has been shown to increase in CSF of AD patients[2]. Till now the detection of phosphocholine and GPC in disease affected regions in AD brain has not been possible due to lack of sensitive detection methods. In vivo localized MR spectroscopy cannot separate the signal of GPC and phosphocholine (PC). In the present study we have optimized and employed, for the first time, two dimensional correlated spectroscopy (L-COSY) in transgenic (TG) mouse model of AD to separately detect resonances of phosphocholine and GPC. In addition we imaged amyloid plaques *in vivo* by μMRI to monitor relationship between plaque deposition and membrane breakdown in AD mouse brain. Our results show that critical time window for membrane breakdown is linked with severity of plaque deposition.

## Methods

The transgenic mice (n=10) used in this study ($APP_{Tg2576}$) contain as transgene the Swedish double mutation of the human amyloid precursor protein ($APP_{695}$), as developed and described previously[3]. Age-matched non-transgenic littermates (n=10) served as controls. All MR measurements were performed using a 9.4-T vertical wide-bore imaging systems equipped with a Bruker Avance console and 1000-mT/m gradients. Images for voxel positioning and Aβ plaque visualization were acquired using the RARE sequence[4]. Aβ plaques load and numerical density in MR images were quantified by SCIL image software as described earlier[4]. The MRS voxel was located in the hippocampus/cortex region (4x4x1.7 $mm^3$; 27 μl – Fig. 1b). Field homogeneity was optimized using the Fastmap sequence, which typically yielded a water linewidth of ~16-20 Hz in live mouse brain. For 2D MRS, a localized 2D shift correlated spectroscopic sequence (L-COSY) was used[5,6] (Fig. 1a). 2D spectra were recorded using TR=1500 ms, TE=15 ms, 2048 complex points along F2, and 192 points along F1, with a spectral width of 11 ppm, and 20 averages per excitation step. Total scan time for a typical 2D measurement was ~96 minutes.

## Results and Discussion

Figure 1 (c,d) show high resolution 2D MR spectra obtained from the 27μl voxel placed in cortex/hippocampus region of control and TG mouse. The combination of the high field strength, strong gradient system, efficient water suppression and the use of short echo time allowed clear detection of cross-peaks of GPC, PC allowing their direct unambiguous chemical shift assignments *in vivo* (Fig. 1e). Comparison of 2D MRS spectra in control and TG mice show ~4 fold increase in GPC at 18 month of age. Aβ plaques were clearly detected in the cortex and hippocampus of the living transgenic mouse using $T_2$ weighted RARE sequence at 9.4T with a scan time of as short as 25 minutes (f). The distribution of plaques identified by MRM was in good agreement with that found in the immunohistochemically stained brain sections of the same animal (Fig. 1f,g). Monitoring the Aβ plaques over age in the same animals showed that plaque burden increased markedly with age between 12 and 18 months in the hippocampus and cortex.

## Conclusion

The L-COSY method allows the clear and unambiguous identification of GPC signal *in vivo* and provides a clear means to differentiate AD mouse brain from control brain. Increase in GPC reflects membrane breakdown which was correlated with the severity of β-amyloid plaque deposition.

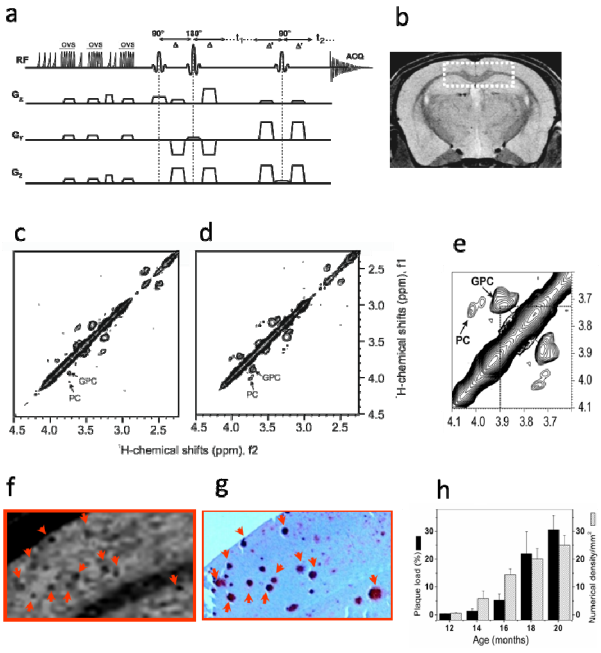


Fig. 1. (a) 2D L-COSY pulse sequence, (b) Voxel position in cortex/hippocampus of mouse brain for MRS. (c) 2D MRS data from WT and (d) Tg2576 mouse, (e) separation of GPC and PC is shown in enlarge view, (f) Aβ-plaque visualization by MRI and (g) co-registration with immunohistology, (h) quantitative analysis of plaque load and numerical density of plaques with age in TG mice (n=5).

**References:** [1]Farber SA, Slack BE et al FASEB J. 2000, 14:2198-2206; [2]Walter A, Korth U, Hilgert M et al Neurobiol Aging 2004, 25:1299-1303; [3]Hsiao K, Chapman P, Nilsen S, et al. *Science* 1996, 274:99-103; [4]Braakman et al, J Magn Reson Imaging 2006, 24:530; [5]Thomas MA, Yue K, Binesh N, et al. Magn Reson Med 2001, 46:58-67; [6]Braakman N, Oerther T, de Groot HJM, Alia A. Magn Reson Med 2008, 60:449-456.

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