# High-resolution Magnetic Resonance Imaging of β-amyloid plaques in transgenic mice

C. Tang[1,2], G. J. Aguinaldo[3], A. Hajianpour[1], L. Ho[2], Z. Zhang[2], T. Wisniewski[4], P. Hof[2,5], J. Morisson[5], D. Perl[6], G. Passinetti[2]

[1]Radiology, Mt. Sinai School of Medicine, New York, New York, United States, [2]Psychiatry, Mt. Sinai School of Medicine, New York, NY, United States, [3]Cardiology, Mt. Sinai School of Medicine, New York, NY, United States, [4]Neurology, Pathology and Psychiatry, New York University, New York, NY, United States, [5]Neurobiology, Mt. Sinai School of Medicine, New York, NY, United States, [6]Pathology, Mt. Sinai School of Medicine, New York, NY, United States

**Introduction**

There are currently no definitive diagnostic techniques for Alzheimer's Disease (AD). The neuropathological hallmark of AD are neuritic plaques and neurofibrillary tangles that can only be detected using histochemical staining techniques. Mouse models have been developed that overexpress beta-amyloid plaques. The APPswe transgenic mouse has robust plaque deposits in both cortical and hippocampal areas at the age of 12 months. Current research in treatment strategies can roughly be divided into methods that clear the plaque deposits, methods that prevent the buildup of plaques and techniques that compensate for the neuronal deaths dues to the plaques. The former two require the quantification of plaque burden. The current gold standard using histological stains is time consuming and too costly to apply to the whole brain. We explored the possibility of using Magnetic Resonance Microscopy (MRM) to visualize beta-amyloid in the transgenice APPswe. Previous attemps to visualize amyloid plaque have used T2* contrast on small human specimens at smaller resolutions[1].

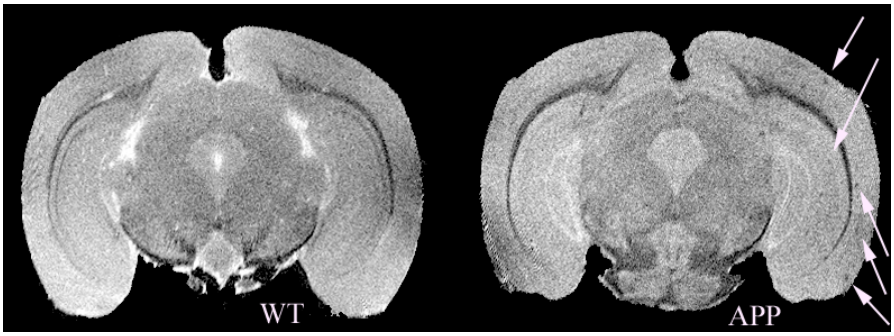
**Materials and methods**

Mice: Tg2576 mice carrying the Swedish mutation of APP were used as a mouse model for the b-amyloid hallmark of AD[2].

MRI: all MRM scans were performed on a 9.4T Bruker vertical bore magnet. An actively shielded gradient coil set was used with gradient strenghts of up to 100G/cm. A RF birdcage coil with 10mm inner diameter was used to scan the specimens. Both wild type and transgenic APPs were sacrificed using perfusion fixation. The mice were perfused intracardially first with heparinated (20unit/ml) phosphate buffered saline (PBS, pH 7.4) and then with 4% paraformaldehyde in PBS for 10min. Brains were extracted and post-fixed for at least 48 hours. It is then placed in a 10mm tube filled with Fomblin (perfluoropolyether, Ausimont, Morristown, NJ). Fomblin was used to seal the specimen to prevent dehydration and as the same time to prevent susceptibility effects. MR acquisition protocol: 3D RARE (Rapid Acquisition Relaxation Enhanced) sequence. To obtain 25 μm resolution we used the following parameters: TR=2s, TE=40ms, FOV=12.8mm, Matrix size=512x512x512, RARE factor=4 and averages=1. Total imaging time was approximately 36.4hrs. For 20μm resolution the same protocol was used with an FOV=10.24mm and NAV=4.

Standard staining procedures for βA were used (6E10, Congo Red, Thioflavin S). The histopathology slides were compared to the MRI scans by reslicing the high-resolution MRI volumetric data until a visually matching slice was obtained. This was performed with the Analyze software V5.0. For the 20μm resolution scans, scan times of more than100 hours were needed to obtain the desired SNR. In order to shorten the scan time we perfused the specimen in a Gadopentatate Dimeglumine (Magnevist, Berlex Laboratories, NJ). A dilution of 1:50 was sufficient to reduce the T1 relaxation rate of the specimen so that TR's of 200ms could be used[3]. Scan times were reduced to less than 15 hours with 4 averages.

**Results**




Beta-amyloid plaques were visualized on both the 20 and 25 micron resolution scans. We were also able to match the digitized pathology slides with the MRI data. The use of gadolinium as a T1 shortening agent did not alter the underlying structural contrast nor the contrast of the beta-amyloid plaque.

Fig 1: Micro-MRI at 25μm x 25μm x 25μm resolution. Left – wild type, Right – APP (12 months). Beta-amyloid plaque shown as hypointense lesions (arrows).

**Discussion**

The hypointense contrast of the plaques could be due to a variety of sources, including the fibrous structure, reduced water content and the presence of metals. We have proposed here the application of Micro MRI as an alternative to immunohistochemistry for measuring beta-amyloid plaques in transgenic mice for research in AD. Conventional pathology procedures require several days of processing in order to obtain a limited number of slides. It would be labor intensive to stain hundred of slices to cover the whole brain. Using micro MRI we can obtain a whole brain plaque measure in one overnight scan with resolutions sufficient to visualize plaques. For computerized quantitation of whole brain plaque burden, conventional slides have to be digitized and imported to the software to measure the plaques. The micro-MRI volumetric data has the advantage of already being in a digital format. An additional advantage is that one also obtains a high-resolution structural scan for volumetric measures of brain structures.

1. Benveniste, H., et al., Proc Natl Acad Sci U S A, 1999. **96**(24): p. 14079-84.
2. Hsiao, K., et al., Science, 1996. **274**(5284): p. 99-102.
3. Johnson, G.A., et al., Radiology, 2002. **222**(3): p. 789-93.