# Medial temporal lobe hypointense foci in Alzheimer's disease reflects accumulation of iron-containing microglia.

Michael Zeineh[1], Hannes Vogel[2], Yuanxin Chen[3], Hagen Kitzler[4], Scott Atlas[2], and Brian Rutt[2]

[1]*Radiology, Stanford University, Stanford, CA, United States,* [2]*Stanford University, Stanford, California, United States,* [3]*Robarts Research Institute, Ontario, Canada,* [4]*Universitätsklinikum Carl Gustav Carus an der Technischen Universität Dresden, Germany*

**Target Audience:** Alzheimer's clinicians/scientists, MR scientists correlating MRI with histology, inflammation

**Introduction:** Alzheimer's disease (AD) is characterized largely by the presence and distribution of beta-amyloid plaques and tau-containing neurofibrillary tangles. A less well-investigated pathway of neuronal destruction may involve inflammation led by iron-rich microglia. By the time AD is clinically diagnosed, there has been extensive neuronal loss, and there is no effective treatment. *Ex vivo* high-field 7.0 Tesla MR studies of human AD specimens have demonstrated that beta-amyloid plaques may be visualized as small hypointense foci, but this claim has been the subject of some controversy. The precise etiology of the signal changes is unclear, though it is suspected that microscopic iron contributes to this low signal intensity. The goal of this study was to delineate the contributions of iron, microglia, and beta-amyloid to MR signal voids.

**Methods:** Five AD and five normal formalin-fixed human brain specimens were obtained. Each specimen consisted of five 3cm square slabs that were 4mm in thickness, dissected from the frontal, parietal, medial temporal, temporal, and occipital lobes. These were immersed in Fluorinert (3M, USA) in a 4cm diameter sealed container, and scanned with a closely-coupled transmit/receive solenoid coil at 7T using a GRE sequence (TR 21, TE 10.5, FA 20, 8 NEX, 0.1mm isotropic voxels, FOV 3cm, 256 0.1mm slices, BW 8kHz, total scan time 3h35m).

The specimens were sectioned at 10um slice thickness. We performed serial triple staining on 5-10 contiguous sections in order to separately visualize DAB-enhanced iron, microglia via a CD163 immuno stain, and a beta-amyloid also via an immuno-stain. After each stain the slide was scanned with an Olympus microscope. An extremely high-fidelity image registration enabled the simple subtraction of prior stains from successive stains (e.g. the single-stain was subtracted from the double stain.) Adjacent histology sections were coregistered using manual landmark correspondences and a nonlinear moving least-squares alignment with Fiji. In order to colocalize with MRI, we acquired DAB-CD163 double stains approximately every 10 slices throughout the entire specimen. A histology volume was then composed consisting of every 10$^{th}$ section and the contiguous sections. Using MNI's register and a 9-parameter transformation, we manually identified landmarks to align the histological image volume with the MRI. This alignment was further refined again using landmark correspondences and a moving least-squares approach in Fiji. The MR was segmented for signal voids using an edge-detection filter in Fiji, and the histology was segmented using a color-threshold in ImageJ. Coregistered images were downsampled for a rank-correlation analysis. Additionally, individually segmented iron foci were automatically examined for greater than 10% pixel overlap, and if the center-of mass of the beta-amyloid and microglia were within the segmented iron foci.

**Results:** Hypointense foci were present at and immediately inferior to the subicular-CA 1 junction in 4/5 AD specimens but not in control specimens. These hypointense voids visually corresponded with the DAB-iron stains (rank correlation of 0.44). In contradistinction, the distribution of beta-amyloid did not correspond to the MR signal voids (rank correlation of 0.23). An analysis of individual iron-containing foci further suggests that a high-proportion of the iron resides within microglia (Table 1).

| | > 10 % Overlap | Centroid Overlap |
|---|---|---|
| CD 163 - Microglia | 50.0 ± 18.4 | 34.1 ± 15.7 |
| Beta Amyloid | 10.8 ± 11.0 | 2.2 ± 1.4 |

**Table 1:** Analysis of overlap of individual iron-containing foci with microglia and amyloid

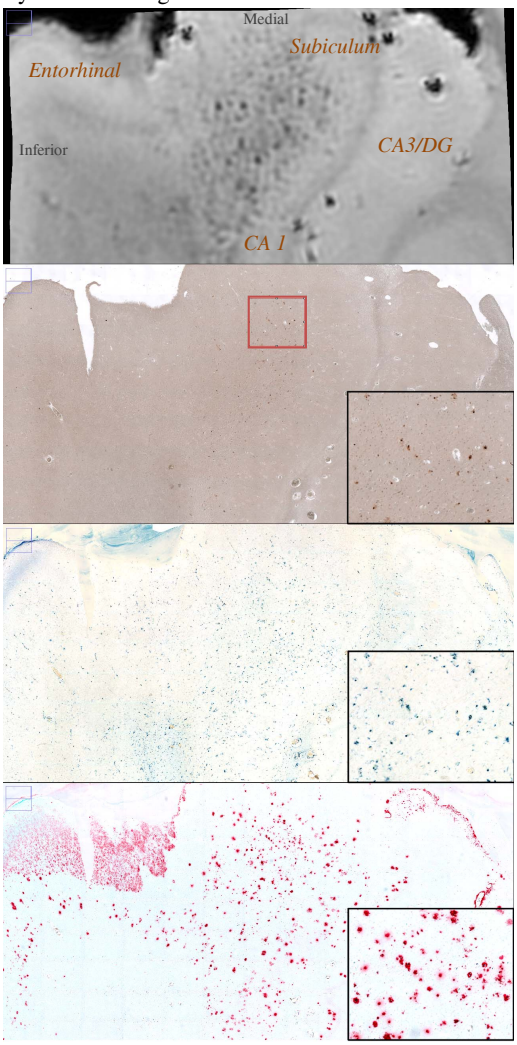


**Figure 1**: Coregistered MR, DAB-Iron, CD163-Microglia, and Beta-Amyloid stains. The red box on the DAB-Iron stain shows the region magnified in the inset at the bottom right.

**Conclusions:** *Ex vivo* MR can visualize hypointense foci associated with Alzheimer's disease, but these foci appear primarily related to iron-containing microglia, or siderophages, not beta-amyloid. The location of these MRI hypointensities along the undersurface of the subicular-CA 1 junction suggests this may be a marker of inflammatory destruction of the perforant pathway.

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