

## MRI Relaxometry Correlation against Iron in Alzheimer's Disease

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**Target Audience:** This work is relevant for scientific researchers interested in neurodegenerative diseases and the basis of MR relaxometry measurements, more specifically, against iron.

**Purpose:** Iron dysregulation is being increasingly identified as a supporting mechanism for oxidative stress and cell death of neurons during neurodegenerative diseases<sup>1</sup>, such as Alzheimer's disease (AD). Iron is highly paramagnetic and detection of iron *in vivo* may provide a viable method for non-invasive diagnosis or assessment of disease progression. Studies have shown clinical correlation of T2 and T2\* measurements with iron in neurodegeneration, however iron quantities can only be correlated against published post-mortem results<sup>2</sup>. In animal experiments, relaxometry measurements can be correlated against Perl's staining, but whilst this method highlights iron accumulation, it remains a qualitative assessment<sup>3</sup>. The purpose of this study was to therefore correlate MR relaxometry measurement in human AD and control brain samples, against a quantitative method for iron assessment, using synchrotron radiation X-ray fluorescence (SR-XRF) elemental mapping.

**Methods:** Formalin-fixed post-mortem medial temporal gyri from AD (n=9) and control (n=11) human subjects were immersed in perfluoropolyether and positioned in a 7T MRI scanner. R1 and R2 relaxometry were performed using a spin-echo sequence with varying TR (300-4000ms) and TE (12-60ms), respectively. R2\* relaxometry was performed using a gradient-echo sequence with varying TE (5-50ms). In-plane resolution was 0.11x0.15mm, thickness of 0.50mm. Following MRI, samples were processed and sectioned at 7µm for SR-XRF elemental iron mapping at 100µm resolution. Relaxometry and SR-XRF maps were manually registered in ImageJ and regions of interest (ROIs) drawn around the grey (GM) and white matter (WM), using a T2-weighted image as reference. Furthermore, for each sample, 20 small ROIs of 5x5 pixels were placed in each co-registered map to assess correlation of iron to relaxometry measurements (Fig.1).

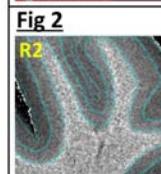
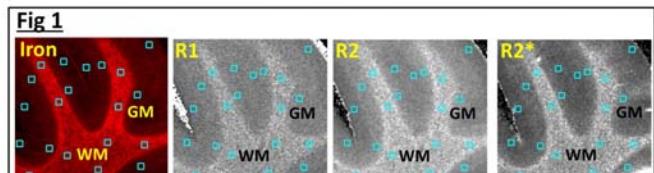
**Results and Discussion:** Mean WM iron concentrations, R1, R2 and R2\* values were significantly increased compared to GM ( $P<0.001$ ) (Fig.1). A mid-layer of cortex was identified within the GM of each sample adjacent to the WM and an ROI of this region (Fig.2) showed higher amounts of iron and increased R1, R2 and R2\* values compared to GM, but were lower than that in WM ( $P<0.001$ ). This highlights the sensitivity of relaxometry measurements to different iron concentrations in human tissue<sup>4</sup>. Similar iron and relaxometry values were observed between control and AD samples in both WM and GM.

R1, R2 and R2\* relaxometry correlated strongly to iron concentrations in individual samples (mean  $r^2$   $0.70\pm0.18$ ,  $0.81\pm0.12$  and  $0.80\pm0.13$  respectively). However, pooling all the ROIs across all samples showed poor iron correlation against R1 ( $r^2=0.002$ ), but remained good for R2 and R2\* ( $r^2=0.480$  and  $0.567$ , respectively) (Fig.3). R1 values were subsequently found to be significantly affected by long term fixation of the samples ( $P<0.001$ ), potentially driving the low R1 correlation against iron.

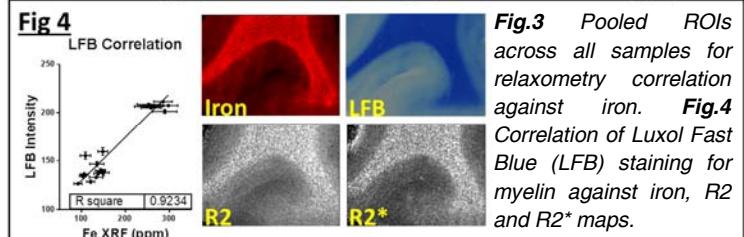
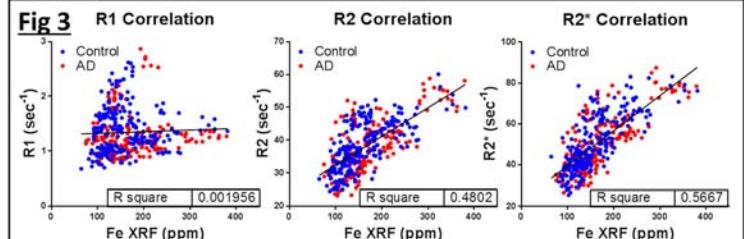
Good correlation of iron, R2 and R2\* values with Luxol Fast Blue (LFB) staining for myelin was observed in both AD and control samples (Fig.4). Our results, taken together, are consistent with increased concentrations of iron within white matter myelin.

**Conclusion:** Iron levels and MR relaxometry were similar between control and AD, however R2 and R2\* were shown to correlate with iron in both healthy and AD tissue, supporting their use in the non-invasive assessment of brain iron.

**References:** (1) Zecca L, et al. *Nature Reviews: Neuroscience*. 2004; 5:863-873. (2) Rodrigue KM, et al. *NeuroImage*. 2011; 54:750-759. (3) Wengenack TM, et al. *NeuroImage*. 2011; 54:113-122. (4) Fukunaga M, et al. *PNAS*. 2010; 8:3834-3839.



**Fig.1** Typical elemental iron and relaxometry maps with 20 small 5x5 pixel ROIs. **Fig.2** Representative R2 map showing the GM, mid-cortical GM and WM ROIs.



**Fig.3** Pooled ROIs across all samples for relaxometry correlation against iron. **Fig.4** Correlation of Luxol Fast Blue (LFB) staining for myelin against iron, R2 and R2\* maps.