Improved Correlation of Iron to R2 and R2* in Alzheimer's Disease-Affected White Matter

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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: Non-invasive assessment of iron may provide a feasible method for diagnosis of neurodegenerative diseases, such as Alzheimer's disease, due to increasing evidence highlighting iron dysregulation during pathology[1]. The paramagnetic properties of iron also provide an ideal candidate for detection by MRI, with iron shortening of T2 relaxation times within tissues. Thus, T2 and T2* measurement have been used in clinical studies as surrogate methods for iron detection[2], however measurement of iron by MRI has yet to be validated against a gold standard method. Iron quantities have been correlated against published post-mortem results in current clinical literature[3], whilst animal studies predominantly utilise MRI measurements correlated against Perl's staining for iron. Whilst this staining method highlights iron accumulation, it remains a qualitative assessment[4]. The purpose of this study was to identify factors that may influence the correlation of MR relaxometry measurements in human AD brain samples, by utilising gold-standard, quantitative methods for iron assessment, using synchrotron radiation X-ray fluorescence (SR-XRF) elemental mapping.

Methods: Formalin-fixed post-mortem medial temporal gyri from AD (n=15) and control (n=15) 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. Magnetisation Transfer Ratio (MTR) was acquired at TR=1100ms, TE=4ms, offset at 100000Hz and 4500Hz. Following MRI, samples were processed and sectioned at 7µm for SR-XRF elemental iron mapping at 100µm resolution. Relaxometry and SR-XRF maps had 20 small ROIs of 5x5 pixels placed in each co-registered map to assess correlation of iron to MRI measurements using r^2 linear regression analysis (Fig. 1). 14µm thick sections were used for luxol fast blue (LFB) staining of myelin.

Results and Discussion: For each sample, individual r^2 correlations were calculated across the 20 ROIs for iron against R1, R2, R2*, MTR and LFB (representative graphs shown in Fig. 2). The correlation between iron and R1, R2 and R2* were similar between AD and control samples (Fig. 3). However, the correlation between iron and MTR or LFB differed between AD and control (MTR mean r^2 is 0.74±0.12 and 0.54±0.23 for control and AD, respectively, P=0.022; LFB mean r^2 is 0.83±0.06 and 0.66±0.25 for control and AD, respectively, P=0.050; Fig3). This indicates a breakdown between the relationship of white matter (WM) to iron content in AD compared to control. Further investigation indicates better correlation of R2* with iron in WM of AD compared to control (r^2=0.403 and r^2=0.163, respectively; Fig. 4). Grey matter (GM) regions show similar correlation values between control (r^2=0.349) and AD (r^2=0.335) samples, therefore highlighting the specificity of this correlation disparity to WM.

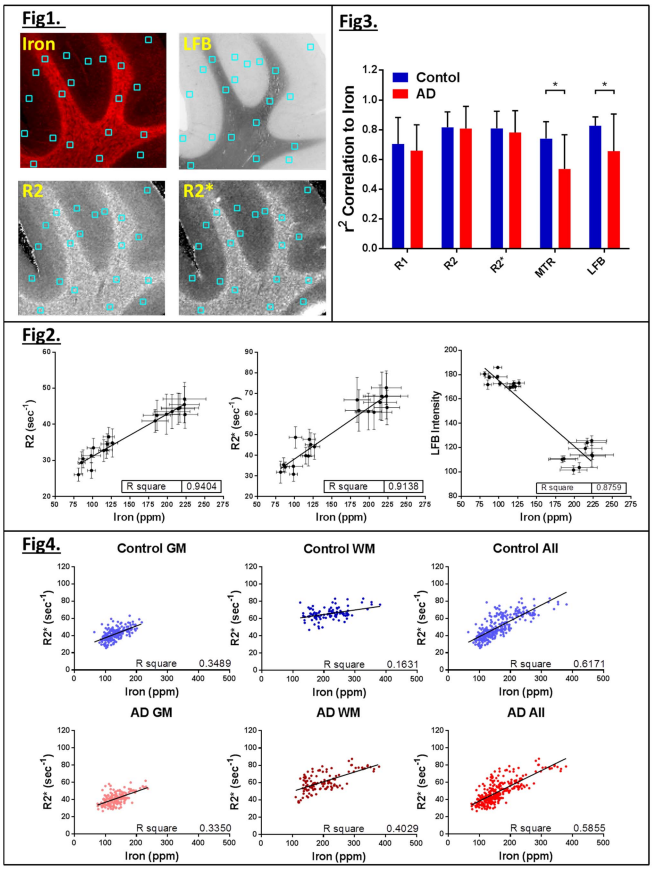

Fig.1. *Representative elemental iron, LFB and relaxometry maps.* ***Fig.2.*** *Representative correlation graphs of iron to R2, R2* and LFB staining in one tissue sample.* ***Fig.3.*** *Comparison of correlations between AD and control tissue.* ***Fig.4.*** *Correlation of R2* with iron changes in WM regions potentially due to changes in myelin.*

Conclusion: R2 and R2* correlated well with iron content in the GM, irrespective of disease. Their correlation in normal WM was poorer than that in AD-affected WM and appears to be dependent on myelination, therefore may have clinical relevance when applying R2 and R2* relaxometry to assess iron *in vivo*. **References:** (1) Zecca L, et al. *Nature Reviews: Neuroscience.* 2004; 5:863-873. (2) Langkammer C, et. al. *Radiology* 2010; 257:2 :455-462. (3) Rodrigue KM, et al. *NeuroImage.* 2011; 54:750-759. (4) Wengenack TM, et al. *NeuroImage.* 2011; 54:113-122.