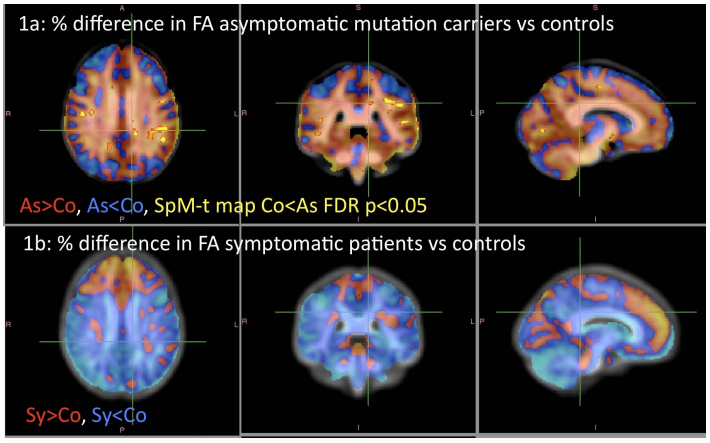
Presymptomatic altered white matter diffusivity in inherited prion disease

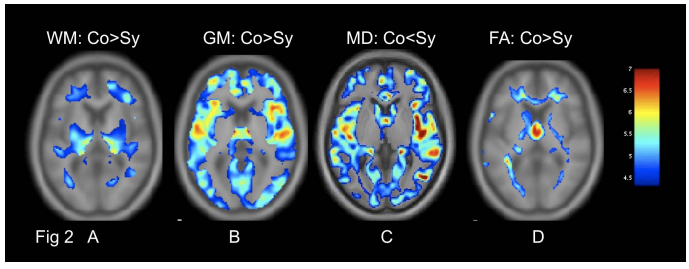
Harpreet Hyare[1], Enrico De Vita[2], Gerard Ridgway[1], Marie-Claire Porter[1], Peter Rudge[1], Tarek Yousry[2], Simon Mead[1], John Collinge[1], and John Thornton[2]
[1]UCL - Institute of Neurology, London, United Kingdom, [2]Lysholm Department of Neuroradiology, London, United Kingdom

Introduction: Human prion diseases remain a major public health concern but their early pathophysiological evolution is not well understood. The inherited form, although rare, offers a unique opportunity to study the earliest disease changes in asymptomatic carriers of prion protein gene (*PRNP*) mutations. As treatments are developed[1], this group would be most likely to benefit and neuroimaging could assist in the optimal timing of such intervention. Previous neuroimaging studies in presymptomatic mutation carriers have not detected changes attributable to prion pathology[2,3] except for one non-DTI study reporting reduced mean diffusivity (MD) in presymptomatic E200K mutation carriers[4]. Diffusion Tensor Imaging (DTI) allows assessment of alterations in tissue microstructure including anisotropy changes in specific brain regions and can interrogate the integrity of specific white matter pathways. We hypothesized that DTI metrics could reveal brain abnormalities in asymptomatic mutation carriers of prion disease. To test this hypothesis, we performed voxel-based analysis of DTI (VBA-DTI) and TBSS in a large cohort of asymptomatic mutation carriers and patients with a range of different forms of prion diseases.

Methods: *Patients:* 85 subjects comprising: 16 asymptomatic *PRNP* gene mutation carriers (As) (median age 44 years, range 21-57, 8 males); 32 symptomatic patients with prion disease (Sy) (median age 48 years; range 26-61, 8 males, 21 inherited, 7 sporadic CJD, 4 growth hormone, 1 variant CJD) and 35 healthy controls (Co) (median age 48 years, range 23-75, 12 males) were recruited as part of the United Kingdom National Prion Monitoring Cohort. *MRI:* Imaging was performed at 3T (Siemens Tim Trio) with structural (T1) data obtained by 3D-MPRAGE (repetition (TR)/echo time (TE)/inversion time 2200/2.9/900ms, flip angle 10°, 208 1.1mm partitions, field of view (FoV) 28.2x28.2cm^2, matrix 256x256). For DTI imaging, 75 slices of thickness 2.0mm with b value = 1000s/mm^2 in 64 non-colinear directions were collected (TR/TE 9500/93ms, FoV (19.2cm)^2, matrix 96x96, 1 average) with 8 images with b value = 0s/mm^2. *Data Processing and Statistical Analysis:* Spatial processing for VBM involved: (i) 'unified segmentation', generating grey, white matter and cerebrospinal fluid (GM, WM, CSF) segments; (ii) DARTEL [5] (SPM 8 [6]) to obtain cohort-specific GM, WM templates at 1.5mm isotropic resolution; (iii) Warping (with 'modulation') of individual GM and WM segments to this template; (iv) smoothing with 6mm Gaussian kernel; (v) mask generation using the 'optimal threshold' method[7]. The FDT tool in FSL [9] was used to process the data: motion/eddy current correction plus generation of mean diffusivity (MD), fractional anisotropy (FA) as well as radial (RD) and axial diffusivity (AD) maps. DTI-VBA involved: (i) affine registration of DTI data to T1 datasets (with transformations estimated using b=0 images); (ii) warping (without 'modulation') of individual MD and FA maps to the VBM template using the transformations calculated for VBM; (iii) 6mm smoothing; (iv) mask generation by summing GM and WM masks from VBM. A group level random effect model ANCOVA consisting of diagnostic grouping (As, Sy, Co) with individual age and total intracranial volume (GM+WM+CSF segments) as covariates, was performed. For multiple comparison correction we used voxel-wise false discovery rate (FDR) with p <0.05. Effect size maps, were calculated as 100*(FA_As-FA_Co)/FA_Co for visual assessment. Voxelwise statistical analysis of the FA data (as well as MD, AD, RD) was carried out using TBSS (Tract-Based Spatial Statistics[10]), part of FSL[11]. TBSS projects all subjects' FA data onto a mean FA tract skeleton, before applying voxelwise cross-subject statistics.



Results: As: No significant As *vs.* Co differences were found for VBM. However DTI-VBA revealed significantly *increased* FA in numerous white matter areas but predominantly left superior temporal gyrus white matter, bilateral inferior frontal gyrus white matter (left>white) and left superior parietal white matter. The significant clusters (FDR p<0.05) are displayed as red/yellow overlaid on the effect size maps in Fig 1a. No significant As *vs.* Co differences were observed for MD. Sy: On VBM significant Sy *vs* Co differences were found predominantly in the perisylvian cortex and thalamus (Fig 2a and b, FDR p<0.001). With DTI-VBA significantly *decreased* FA was observed in the genu and body of the corpus callosum, peri-callosal frontal WM, fornix, posterior limb of the internal capsule, optic radiation and cerebellar cortex (Fig 2d). The reduced FA extends consistently over most of the brain (except for the frontal lobes) as can be observed in the effect size maps in Fig. 1b (light blue/cyan). Significantly increased MD was seen in the perisylvian cortex and basal ganglia (Fig 2c). These observations were confirmed on TBSS where significant increases in radial but not axial diffusivity were seen (data not shown).



Discussion and Conclusions: Our findings of decreased FA in symptomatic patients is supported by a study in symptomatic E200K mutation carriers where decreased FA in functionally relevant white matter pathways including the corticospinal tract, internal capsule, external capsule, fornix and posterior thalamic radiation were observed[12]. Decreased FA is thought to reflect loss of integrity of cellular structures and is commonly observed in neurodegenerative diseases. Our presymptomatic mutation carriers, on the other hand, showed *increased* FA in specific white matter areas. Animal models of prion disease demonstrate white matter deposition of PrP^{Sc} in the early stages and it is likely that PrP^{Sc} propagates along white matter pathways[13]. It is therefore possible that PrP^{Sc} propagation causes increased anisotropy before the other pathological hallmarks of prion disease: spongiform degeneration and neuronal loss become evident. Further developments in DTI acquisition and analysis may allow us to further investigate the earliest microstructural changes in human prion disease and the optimal timing for therapeutic intervention.

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