Introduction: Human prion diseases are progressive neurodegenerative disorders caused by accumulation of aggregates of an abnormally folded normal cellular protein, the prion protein (PrP)\(^\text{\textsuperscript{1}}\). Most cases occur sporadically but inherited, iatrogenic and dietary transmission can occur. It has previously been shown that global and regional measures of non-DTI determined mean diffusivity (MD) measured at high and low b values are significantly different between symptomatic patients with prion disease and controls, correlating with disease severity \(\text{2,3}\) suggesting sensitivity to microstructural changes that are associated with prion pathology. However, few studies have used the enhanced microstructural sensitivity of diffusion tensor imaging (DTI) to examine brain tissue alterations in patients with prion disease \(\text{4}\). We hypothesized that fractional anisotropy (FA) could reveal brain abnormalities in prion disease even in the absence of volumetric changes. To test this hypothesis, we performed voxel-based analysis of DTI (VBA-DTI) in a large cohort of patients with a range of different forms of prion diseases and compared the findings to VBM.

Methods: Patients: 72 subjects comprising: 17 asymptomatic PRNP gene mutation carriers (AsIPD) (median age 44 years, range 21-57, 8 males); 14 symptomatic patients with inherited prion disease (sIPD) (median age 48 years; range 26-61, 8 males); 7 patients with sporadic Creutzfeldt-Jakob disease (sCJD) (median age 61 years, range 53-70, 5 males) and 24 healthy controls (Co) (median age 48 years, range 23-75, 12 males) were recruited as part of the 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, 55 slices of thickness 2.5mm with b value = 1000s/mm\(^2\) were collected (TR/TE 6800/91ms, FoV 24x24cm\(^2\), matrix 96x96, 1 average) with 7 images with b value = 0s/mm\(^2\). B0 field maps were acquired for geometric distortion correction (TR/TE1/TE2 688/4.92/90ms, 55 slices of thickness 3mm, FoV 192x192mm\(^2\), matrix 64 x64). 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 \(\text{5}\) (SPM 8 \(\text{\textsuperscript{6}}\)) 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) 6mm smoothing (Gaussian kernel); (v) mask generation using the ‘optimal threshold’ method. DTI data was first unwarped based on the acquired B0 field maps using the SPM Fieldmap toolbox \(\text{7}\). The FDT tool in FSL \(\text{8}\) was used to process the data: motion/eddy current correction and generation of mean diffusivity (MD) and fractional anisotropy (FA) maps. DTI-VBA involved: (i) affine registration of DTI data to T1 datasets (with transformations estimated using b0 images); (ii) warping (without ‘modulation’) of individual MD and FA maps to the VBM template; (iii) 6mm smoothing; (iv) mask generation by summing GM and WM masks. A group level random effect model ANCOVA consisting of diagnostic grouping (AsiPD, sIPD, sCJD, Co) with individual age and total intracranial volume as covariates, was performed. For multiple comparison correction we used voxel-wise false discovery rate (FDR) with p <0.01 or family wise error (FWE) with corrected p <0.05.

Results: Voxels surviving the threshold of FDR p<0.01 are overlaid onto the averaged warped T1 for visualization. sIPD: Significant difference in GM voxels between sIPD and Co were found in the following cortical areas bilaterally: cingulate, posterior frontal, superior temporal, medial occipital and superior parietal lobule (Fig1a). Significant WM atrophy was seen in the right cortico-spinal tract, left anterior-temporal WM and right inferior and superior frontal gyri. Numerous clusters of reduced FA were seen 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 (Fig1b). None of the regions overlapped the areas of GM or WM atrophy AsIPD: No significant differences were found at any threshold compared to Co for VBM and DTI-VBA. sCJD: GM atrophy was only seen in the ventrolateral nucleus of thalamus compared to Co (Fig 2a), even for FDR p<0.05, and no WM differences were seen, even with the much less stringent threshold: FDR p<0.05. In comparison, significant decreases in FA were observed in genu and body of the corpus callosum, anteromedial and pulvinar nuclei of the thalamus and cerebellar white matter (Fig 2b).

Conclusions: Although volume loss in prion diseases has previously been reported \(\text{10}\), this analysis demonstrates the regional specificity of the atrophy, which varies in different forms of prion disease and is in keeping with the clinical presentation (for example, parietal GM volume loss and apraxia in sIPD). The DTI-VBA analysis shows that WM alterations can be detected in the absence of volumetric changes. Significant WM volume loss, suggesting that FA may be more sensitive to microstructural changes than atrophy measures in this disease and WM changes may occur secondary to cortical GM degeneration. With attention focussed on therapeutic agents, DTI measures may be relevant for the selection of patients and the targeting of therapeutic agents to specific brain regions before the onset of atrophy.