Magnetization transfer ratio may be a surrogate of spongiform change in human prion diseases

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Human prion diseases are fatal neurodegenerative disorders caused by misfolding of the prion protein. There are no useful biomarkers of disease progression. Cerebral cortex spongiform change, one of the classical pathological features of prion disease, resolves in prion-infected transgenic mice following prion protein gene knockout. We investigated the cross-sectional, longitudinal and post-mortem cerebral magnetization transfer ratio as a surrogate for prion disease pathology. Twenty-three prion disease patients with various prion protein gene mutations and 16 controls underwent magnetization transfer ratio and conventional magnetic resonance imaging at 1.5 T. For each subject, whole-brain, white and grey matter magnetization transfer ratio histogram mean, peak height, peak location, and magnetization transfer ratio at 25th, 50th and 75th percentile were computed and correlated with several cognitive, functional and neuropsychological scales. Highly significant associations were found between whole brain magnetization transfer ratio and prion disease (P < 0.01). Additionally, highly significant correlations were found between magnetization transfer ratio histogram parameters and clinical, functional and neuropsychological scores (P < 0.01). Longitudinally, decline in the Clinician’s Dementia Rating scale was correlated with decline in magnetization transfer ratio. To investigate the histological correlates of magnetization transfer ratio, formalin-fixed cerebral and cerebellar hemispheres from 19 patients and six controls underwent magnetization transfer ratio imaging at 1.5 T, with mean magnetization transfer ratio calculated from six regions of interest, and findings were followed-up in six variant Creutzfeldt–Jakob disease cases with 9.4 T high-resolution magnetization transfer imaging on frontal cortex blocks, with semi-quantitative histopathological scoring of spongiosis, astrogliosis and prion protein deposition. Post-mortem magnetization transfer ratios was...
Introduction

Human prion diseases are rapidly progressive, uniformly fatal neurodegenerative disorders affecting one to two people per million worldwide annually (Collinge, 2001). Histopathologically, they are characterized by spongiform change, neuronal loss, reactive astrogliosis and deposition of misfolded prion protein. Etiologically, prion diseases are subdivided into inherited prion disease, sporadic and acquired Creutzfeldt–Jakob disease (CJD), with sporadic CJD accounting for 85% of the incidence of human prion disease. In 1996, a new acquired form of human prion disease was described, termed variant CJD, caused by the transmission of bovine spongiform encephalopathy prions to humans (Collinge et al., 1996; Will et al., 1996; Hill and Collinge, 2003). Although the numbers succumbing to variant CJD are relatively modest at around 200 to date, remarkably long incubation periods (Collinge et al., 2006), a propensity for subclinical infection (Hill and Collinge, 2003) and secondary transmission by blood transfusion (Llewelyn et al., 2004; Wroe et al., 2006) cause ongoing concern. As there are no treatments for prion diseases, the development of these is a research priority and there is a need to complement clinical outcome markers with objective and quantifiable non-invasive measures.

Magnetization transfer imaging is a quantitative MRI technique that contrasts free protons with those bound to fixed macromolecules, and is thought to be a sensitive marker of histopathological change in vivo (Filippi et al., 2003; Symms et al., 2004). Abnormal magnetization transfer ratio parameters have been described in Alzheimer’s disease (Bozzali et al., 2001; van der Flier et al., 2002) and multiple sclerosis (van Waesberge et al., 1999; Filippi et al., 2003), in vivo as well as in post-mortem tissue (Schmierer et al., 2004) but there are, to our knowledge, no reports of magnetization transfer imaging findings in prion diseases.

The purpose of this study was to first assess whole brain, grey and white matter cerebral magnetization transfer ratio parameters derived from magnetization transfer imaging, and correlate these cross-sectionally and longitudinally with indices of clinical disease severity to determine their potential utility as biomarkers of disease progression. Once this was established, we used ex vivo MTR in intact cerebral and cerebellar hemispheres in various human prion diseases and in small frontal lobe blocks in variant CJD. Post-mortem quantitative MRI allows for correlations with semi-quantitative scoring of histopathology; the objective here was to determine utility of MTR as an in vivo surrogate marker for specific pathologies in human prion disease, facilitating a greater understanding of the mechanism of MTR as a biomarker.

Materials and methods

Ante-mortem studies

Subjects

Thirty-nine individuals were studied. 23 patients had a diagnosis of inherited prion disease (12 male, 11 female, mean age 45.5 years, range 32–59 years). All patients attended the National Prion Clinic at the National Hospital for Neurology and Neurosurgery, London, UK, and were recruited into the UK MRC (Medical Research Council) PRION-1 trial. The MRC PRION-1 trial was a partially randomized patient-preference trial to evaluate the activity and safety of quinacrine in adults with human prion disease (Collinge et al., 2009). Ethical approval was given by the Eastern Multi-Centre Research Ethics Committee (MREC), Cambridge, UK. Seventeen patients were symptomatic, with mean disease duration at the time of MRI scan of 43.9 months (range 3–138 months), and six were asymptomatic. Patients were considered symptomatic if they or a close informant reported clinical and neuropsychiatric symptoms and a clinician judged that they were affected on the basis of the clinical examination. PRNP analysis confirmed the causal mutation of inherited prion disease, six patients had P102L, nine had 6-octapeptide repeat insertion (OPRI), two had A117V, two had 5-OPRI and four had various other point mutations. Where possible, MRI examinations and clinical assessments were obtained at multiple time points. Sixteen healthy volunteers (eight male, eight female mean age 36.8 years, range 25–66 years) with no personal or family history of neurological disorder were also studied.

MRI acquisition

MRI scans were acquired on a 1.5-T Signa Echospeed Horizon system [General Electric (GE), Milwaukie, WI, USA] using the standard transmit/receive head coil. MTR was measured using an interleaved 2D-gradient-echo sequence, similar to the EuroMT sequence (Barker et al., 2005), with echo time of 15.4 ms; repetition time 1500 ms; flip angle 70°; matrix size 256 × 192; 0.75 averages; field of view 24 × 18 cm; 30 axial slices of thickness 5-mm with separation 1.5 mm with total acquisition time 12 min. The presaturation pulse was a Gaussian pulse with duration 12.8 ms giving a nominal bandwidth of 125 Hz and peak amplitude of 23.2 μT, applied 2 kHz off water-resonance. Scans with and without presaturation were interleaved for each repetition time period ensuring exact co-registration of the pixels on saturated and unsaturated images (Barker et al., 1996). In addition, 3D T1-weighted inversion-recovery prepared...
spoilt gradient-echo images, diffusion-weighted imaging and fluid-attenuated inversion recovery images were also acquired.

**Image analysis**

**Conventional**

A blinded, randomized assessment of signal abnormalities in the caudate nucleus, putamen, thalamus (including pulvinar), frontal, parietal, temporal, occipital, insular and cingulate cortices bilaterally were performed on conventional fluid-attenuated inversion recovery and diffusion-weighted imaging sequences by two consultant neuroradiologists to determine whether MTR changes were observed in areas with or without signal change on conventional MRI.

**Quantitative**

Image data were anonymized and transferred to a Sun Solaris workstation (Sun Microsystems, Mountain View, CA, USA) for off-line analysis. From the inversion-recovery prepared spoilt gradient-echo data, whole brain, grey matter and white matter masks were obtained and co-registered to the magnetization transfer imaging data using third-party software for image manipulation [JIM Version 4.0 (http://www.xinapse.com/software.html)], registration and tissue segmentation [FSL v3.2 (http://www.fmrib.ox.ac.uk/fsl), Fig. 1]. To minimize contamination from CSF, two morphological erosion operations with a $3 \times 3$ pixel restructuring element were applied to the white matter mask and one morphological erosion applied to the grey matter mask. The grey matter segment was too thin to support more than one erosion. MTR histograms were then generated for each tissue class using in-house software with bin widths of 1 percentile unit (pu), and normalized to the residual brain tissue volume by dividing the number of counts in each sampling bin by the total number of parenchymal voxels. For each normalized histogram, mean (average) MTR, peak height, peak location and MTR at 25th ($MTR_{25\%}$), 50th ($MTR_{50\%}$), 75th ($MTR_{75\%}$) percentile were calculated, which are simple robust statistics summarizing the shape of the histogram. In MTR histogram analysis, a histogram of pixel MTR values is formed from the brain parenchyma, with focal and diffuse tissue damage reflected in changes in the histogram shape.

**Clinical assessments**

Each patient was assessed at the same time as the MRI examination by experienced neurologists blinded to the MRI findings. Videoed assessment of cognitive, extrapyramidal, pyramidal and cerebellar impairment was based on a standardized neurological examination based on the Queen Square format, comprising a 15 point cognitive (memory, letter cancelling, line drawing, reading, spelling, fragmented letters, fragmented objects, calculation, miming, copying, frontal lobe sequencing, words beginning with a letter, proverbs, digit span, recall) and 11 point motor (eye movements, finger nose testing, rapid alternating finger movements, sequential index finger tapping, sequential opposition, primitive reflexes, one minute observation, walking, heel toe walking, Romberg’s and neurological examination of tone, power and reflexes). Overall impression of cognitive, extrapyramidal, pyramidal and cerebellar impairment was further independently scored by a consultant neurologist in a blinded randomized manner as follows: 0 (no impairment), 1 (mild impairment), 2 (moderate impairment), 3 (severe impairment), 4 (cannot assess impairment).

Non-videoed neurological rating scales used were the Clinician’s Global Impression of Disease Severity (CGIS) (Guy, 1976), Clinician’s Dementia Rating Scale (CDR) (Morris, 1997), Alzheimer’s Disease Assessment Scale (ADAS-COG) (Rosen et al., 1984), Barthel Activities of Daily Living (ADL) (Collin et al., 1988), Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962), Mini-Mental State Examination (MMSE) (Folstein et al., 1975) and Rankin scores (Rankin, 1957).

**Statistical analysis**

**Comparison of magnetization transfer ratio measures between control and patient groups**

Differences in MTR measures among control, asymptomatic gene-positive subjects, and symptomatic subjects were assessed using a Kruskal–Wallis test followed by post hoc Mann–Whitney U-tests comparing the asymptomatic and symptomatic groups with controls.

**Relationship between magnetization transfer ratio measures and disease severity at baseline**

The relationship between MTR measures and baseline clinical scores was assessed across the whole patient group (symptomatic and asymptomatic subjects) initially with Spearman rank correlation. In consideration of the number of multiple comparisons of related variables, the significance value was set at $P < 0.01$ for each test. Standard normal linear regression models were then fitted with outcome the baseline clinical score, considering whole brain, grey matter and white matter average MTR, peak height, peak location, $MTR_{25\%}$, $MTR_{50\%}$ and $MTR_{75\%}$ and age one at a time in a univariate analysis. Finally, a stepwise multivariate regression procedure was performed with each clinical score as the dependent variable and each of the MRI variables as the independent predictors. The aim of this...
model was to identify which of the above MTR histogram measures were independent predictors of clinical score as opposed to potential confounders. All of the predictor variables were rank transformed to avoid violations of the assumption of a linear relationship.

Relationship between decline in magnetization transfer ratio measures and decline in clinical score
The aim was to investigate whether changes in MTR measures over time were paralleled by changes in clinical score. Longitudinal changes in the MTR histogram measures were quantified by performing a standard linear regression of each MTR measure against time for each patient. This provided an indication of the magnitude and statistical significance of the longitudinal change in each magnetic resonance measure. An equivalent analysis was performed to quantify longitudinal changes for each clinical score. The relationships between significant MTR measure reduction and significant decline in clinical score were then assessed using Pearson correlation. All statistical tests were performed using Statistical Package for the Social Sciences for Windows (version 11.5, SPSS, Chicago, IL, USA).

Post-mortem studies
Subjects
Twenty-five individuals were studied. Post-mortem formalin-fixed cerebral and cerebellar hemispheres of 19 patients with sporadic, variant and inherited forms of prion disease (12 male, 7 female, mean age 41.6 years, range 19–76 years) were analysed. Seven patients had sporadic CJD, six had inherited prion disease (two had 6-OPRI, one had D178N, A117V, E200K and P102L) and six had variant CJD. Six subjects were non-prion disease controls who had died from causes which included Lewy body dementia, amyloid angiopathy and Alzheimer’s disease (one male, five female, mean age 68.6 years, range 47–86 years). Ethical approval was given by the Joint Ethics Committees of The National Hospital for Neurology and Neurosurgery and the Institute of Neurology, University College London, London, UK. Post-mortem specimens were obtained between 24–72 h after death. The mean duration of formalin-fixation was 46.1 weeks (range 4–88 weeks). Post-mortem hemispheres were stored in formalin at room temperature (~22°C) until immediately before scanning.

1.5 T image acquisition and analysis
Cerebral and cerebellar hemispheres were placed flat and immersed in formalin in a plastic container suitable for the standard birdcage head coil of a 1.5 T system (GE, Milwaukee, WI); these were scanned at a bore temperature of 24°C. Magnetization transfer imaging was performed using a similar interleaved 2D-gradient-echo sequence to that employed in vivo (Barker et al., 2005), with echo time 7 ms to reduce susceptibility artefacts. MTR maps were generated from the raw magnetization transfer imaging images, off-line on a dedicated workstation (Sun Microsystems, Mountain View, CA, USA). The segmentation methods (FSL v3.2) that were used for the in vivo analysis did not perform successfully for the post-mortem hemisphere images due to morphological and image-contrast differences. Therefore, region of interest analysis was performed by manually defining regions of interest using commercially available software (Jim Version 4.0) in a blinded randomized manner. Six regions of interest, namely cerebellar, frontal and occipital cortices, caudate nucleus, thalamus and frontal white matter, were manually defined on the MTR maps and the mean MTR for each region of interest extracted.

9.4 T image acquisition and analysis
Frontal cortex samples (20 × 20 × 7 mm) from each of the six variant CJD specimens and six non-CJD control specimens were excised and held in a standard histopathology cassette, positioned tightly in a 50 ml Vycon tube filled with Fomblin Perfluorosolv PFS-1 (Solvay Solexis, Milan, Italy) to minimize sample motion. MRI was performed with a horizontal bore 9.4 T/21 cm Varian Inova MRI system (Varian Inc., Palo Alto, CA, USA) using a 43 mm internal diameter quadrature volume coil (MR Laboratories, Oxford) at room temperature (bore temperature 22°C). MTR was measured using a 2D-gradient-echo sequence with echo time 5 ms; repetition time 186 ms; flip angle 70°; matrix size 256 × 256; 16 averages; field of view 40 × 40 mm; slice thickness 1 mm. Two data sets were acquired, one with and one without an off-resonance saturation pulse (offset frequencies 6 and 100 kHz, respectively). In each case, seven contiguous coronal slices were obtained. The total measurement time per specimen was ~10 h. All quantitative image processing was performed off-line on a dedicated workstation (Sun Microsystems) using commercially available software (JIM Version 4.0). For each specimen, regions of interest in the frontal cortex and white matter were defined manually on the MTR maps.

Histopathological analysis of samples imaged at 9.4 T
Following 9.4 T MRI acquisition, samples were re-immersed in 10% buffered formalin for one week (Pioneer Research Chemicals Ltd., Colchester, UK) followed by incubation in 98% formic acid for 1 h. Following further washing for 24 h in 10% buffered formalin, tissue samples were processed and paraffin wax embedded. Sections were cut at a nominal thickness of 4 μm and mounted on Superfrost UltraPlus charged glass slides. Following dewaxing in xylene and an alcohol gradient, the sections were stained with haematoxylin and eosin. For immunohistochemical staining, dewaxed sections were treated with 98% formic acid for 5 min and then boiled in a low ionic strength buffer (2.1 mM Tris, 1.3 mM EDTA, 1.1 mM sodium citrate, pH 7.8) for 20 min. Abnormal prion protein accumulation was examined using anti-prion protein monoclonal ICSM 35 followed by a biotinylated-anti-mouse IgG secondary antibody (iView Biotinylated Ig, Ventana Medical Systems Inc.) and an avidin-biotin horseradish peroxidase conjugate (iView SA-HRP, Ventana Medical Systems Inc.) before development with 3,3′-diaminobenzidine tetrachloride as the chromogen (iView DAB, Ventana Medical Systems Inc.). Haematoxylin was used as the counterstain and appropriate controls used throughout.

The immunoreactivity of astrocytes was tested using rabbit anti-gliial fibrillary acidic protein antiserum (DAKO). A biotinylated-anti IgG secondary antibody (iView SA-HRP, Ventana Medical Systems, Tuscon, AZ, USA) was applied before development with 3,3′-diaminobenzidine tetrachloride as the chromogen (iView DAB, Ventana Medical Systems, Tuscon, AZ, USA). Haematoxylin was used as the counterstain and appropriate controls used throughout. All slides were assessed by a single experienced consultant neuropathologist. Areas corresponding to the regions of interest on MRI were scored for the degree of spongiosis, gliosis and prion protein deposition in a semi-quantitative manner from 0 to 3 (0 = none, 1 = mild, 2 = moderate, 3 = severe; Fig. 1).

Statistical analysis
The Mann–Whitney U-test was used to assess differences in regions of interest between patients and controls in measurements at both field strengths. At 1.5 T, a Kruskal–Wallis test was performed to assess differences in mean MTR between the variant CJD, sporadic CJD and inherited prion disease subgroups in each of the regions of interest.
Spearman rank correlation analysis assessed relationships between MTR and histological measures at 9.4 T. All statistical tests were performed using Statistical Package for the Social Sciences for Windows (version 11.5, SPSS, Chicago, IL, USA).

Results

Ante-mortem studies

Conventional imaging
On conventional MRI, there was a consensus agreement that 1 of the 23 patients had hyperintensities in frontal, parietal and cingulate cortices bilaterally. This was a symptomatic patient with the 6-OPRI mutation. No pathological signal change was detected on the remaining cases.

Comparison of magnetization transfer ratio measures between control and patient groups
In our association study at baseline, all MTR histogram parameters were significantly lower in symptomatic patients compared with controls (Table 1). There were no significant differences in any of the MTR measures comparing the healthy control subjects and asymptomatic patients with inherited prion disease.

Table 1 Baseline MTR histogram measures in symptomatic patients (n = 17) and healthy volunteers (n = 16)

<table>
<thead>
<tr>
<th>Measure</th>
<th>A: Healthy control subjects (n = 16)</th>
<th>B: Asymptomatic gene-positive (n = 6)</th>
<th>C: Symptomatic patients (n = 17)</th>
<th>P-value</th>
<th>Post hoc tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>36.8 (11)</td>
<td>39.7 (4.8)</td>
<td>45.5 (2.3)</td>
<td>0.117</td>
<td></td>
</tr>
<tr>
<td>MMSE</td>
<td>30.0 (0.0)</td>
<td>29.2 (1.6)</td>
<td>20.5 (3.1)</td>
<td>&lt;0.001</td>
<td>0.696</td>
</tr>
<tr>
<td>Whole brain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVMTR</td>
<td>38.5 (0.8)</td>
<td>38.5 (1.5)</td>
<td>35.6 (0.90)</td>
<td>&lt;0.001</td>
<td>0.956</td>
</tr>
<tr>
<td>PH</td>
<td>8.1 (0.5)</td>
<td>8.0 (0.1)</td>
<td>7.0 (0.4)</td>
<td>&lt;0.001</td>
<td>0.985</td>
</tr>
<tr>
<td>PL</td>
<td>35.7 (1.4)</td>
<td>35.8 (2.1)</td>
<td>33.8 (1.3)</td>
<td>0.001</td>
<td>0.971</td>
</tr>
<tr>
<td>MTR25%</td>
<td>28.3 (1.0)</td>
<td>28.0 (1.7)</td>
<td>23.5 (1.7)</td>
<td>&lt;0.001</td>
<td>0.915</td>
</tr>
<tr>
<td>MTR50%</td>
<td>32.8 (1.0)</td>
<td>33.1 (1.6)</td>
<td>30.3 (0.98)</td>
<td>&lt;0.001</td>
<td>0.815</td>
</tr>
<tr>
<td>MTR75%</td>
<td>36.3 (1.1)</td>
<td>36.3 (2.0)</td>
<td>34.5 (0.99)</td>
<td>&lt;0.001</td>
<td>0.999</td>
</tr>
<tr>
<td>White matter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVMTR</td>
<td>42.7 (1.3)</td>
<td>43.1 (1.9)</td>
<td>40.5 (1.5)</td>
<td>&lt;0.001</td>
<td>0.754</td>
</tr>
<tr>
<td>PH</td>
<td>14.7 (1.4)</td>
<td>15.3 (0.2)</td>
<td>12.3 (0.6)</td>
<td>0.002</td>
<td>0.987</td>
</tr>
<tr>
<td>PL</td>
<td>36.7 (1.4)</td>
<td>37.2 (2.0)</td>
<td>34.5 (0.88)</td>
<td>&lt;0.001</td>
<td>0.746</td>
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<tr>
<td>MTR25%</td>
<td>34.4 (1.5)</td>
<td>35.0 (2.1)</td>
<td>31.4 (2.1)</td>
<td>&lt;0.001</td>
<td>0.723</td>
</tr>
<tr>
<td>MTR50%</td>
<td>36.6 (1.4)</td>
<td>36.8 (2.3)</td>
<td>34.0 (1.1)</td>
<td>&lt;0.001</td>
<td>0.901</td>
</tr>
<tr>
<td>MTR75%</td>
<td>38.2 (1.2)</td>
<td>38.3 (2.0)</td>
<td>36.4 (0.86)</td>
<td>&lt;0.001</td>
<td>0.958</td>
</tr>
<tr>
<td>Grey matter</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AVMTR</td>
<td>36.4 (1.3)</td>
<td>37.2 (1.6)</td>
<td>34.4 (1.4)</td>
<td>&lt;0.001</td>
<td>0.389</td>
</tr>
<tr>
<td>PH</td>
<td>8.8 (0.8)</td>
<td>89.0 (0.4)</td>
<td>06.8 (0.9)</td>
<td>&lt;0.001</td>
<td>0.902</td>
</tr>
<tr>
<td>PL</td>
<td>30.6 (1.6)</td>
<td>31.5 (2.0)</td>
<td>28.6 (1.1)</td>
<td>&lt;0.001</td>
<td>0.374</td>
</tr>
<tr>
<td>MTR25%</td>
<td>26.6 (1.5)</td>
<td>27.0 (1.7)</td>
<td>22.9 (2.1)</td>
<td>&lt;0.001</td>
<td>0.482</td>
</tr>
<tr>
<td>MTR50%</td>
<td>29.7 (1.4)</td>
<td>30.5 (2.0)</td>
<td>27.5 (1.5)</td>
<td>&lt;0.001</td>
<td>0.458</td>
</tr>
<tr>
<td>MTR75%</td>
<td>32.6 (1.4)</td>
<td>33.3 (2.0)</td>
<td>31.3 (1.1)</td>
<td>0.004</td>
<td>0.468</td>
</tr>
</tbody>
</table>

All values are mean (standard deviation). Magnetization transfer ratio (MTR) histogram measures: AVMTR = average MTR (p.u.); PH = normalized peak height (% vol/p.u.); PL = peak location (p.u.); MTR25% = 25th percentile (p.u.); MTR50% = median (p.u.); MTR75% = 75th percentile (p.u.); p.u. = percentage unit.

a Kruskal–Wallis test.
b Post hoc Mann–Whitney U-tests.

Relationship between magnetization transfer ratio measures and disease severity
In our cross-sectional study of patients with inherited prion disease at baseline, significant (P ≤ 0.01) bivariate Spearman rank correlations were found between multiple whole brain, white and grey matter MTR histogram parameters and clinical scores at baseline. Expected correlations, based on our association findings, were a decline in MTR histogram parameters as the patient’s disease severity increased, indicated by deterioration on our cognitive, neurological and functional rating scales. The strongest correlations were between whole brain MTR25% and cognitive impairment (P = 0.001, Fig. 2A), Mini-Mental State Examination (P = 0.008), Rankin score (P = 0.001), CDR (P < 0.001; Fig. 2B), Alzheimer’s Disease Assessment Scale (P = 0.008) and Clinician’s Global Impression of Disease Severity (P = 0.006). Whole-brain average MTR was strongly correlated with cognitive impairment (P < 0.001; Fig. 2C), extrapyramidal impairment (P = 0.008), Rankin score (P = 0.008), CDR (P = 0.003; Fig. 2D) and Alzheimer’s Disease Assessment Scale (P = 0.004). Grey matter average MTR was strongly correlated with cognitive impairment (P = 0.002), Rankin (P = 0.01), CDR (P = 0.01) and Alzheimer’s Disease Assessment Scale (P = 0.008).

Using a stepwise multivariate regression procedure (Table 2) with each clinical score as the dependent variable and each MTR variable that correlated strongly in the univariate analysis as the independent predictors, whole brain MTR25%
was an independent predictor of Mini-Mental State Examination ($P = 0.01$), Barthel Activities of Daily Living ($P = 0.04$) and Alzheimer’s Disease Assessment Scale ($P = 0.04$). Whole-brain average MTR was an independent predictor of cognitive impairment ($P < 0.001$) and CDR ($P = 0.009$). Whole brain MTR$50\%$, white matter average MTR and grey matter peak height were independent predictors of Rankin score ($P < 0.001$), cerebellar impairment ($P = 0.02$) and Clinician’s Global Impression of Disease Severity ($P < 0.001$), respectively.

**Relationship between decline in magnetization transfer ratio measures and decline in clinical score**

Eighteen patients were scanned on more than one occasion, the mean number of examinations being five, with a median interval

![Figure 2](image-url)
between the first and last scans of 11 months (range 2–29 months). For each MTR acquisition a clinical examination was obtained on the same visit. Although a large number of neurological, cognitive and functional scales were obtained, only four scales (Barthel Activities of Daily Living, Rankin, Clinician’s Global Impression of Disease Severity and CDR) showed statistically significant slopes of decline in the study period, and only two from these four scales (Barthel Activities of Daily Living and CDR) showed meaningful magnitudes of slope of decline. We observed correlations between the decline in MTR histogram measures and CDR. Specifically, whole brain MTR75% ($P < 0.001$; Fig. 2E), white matter MTR50% ($P = 0.03$) and MTR75% ($P = 0.002$, Fig. 2F); and grey matter MTR50% ($P = 0.02$) and MTR75% ($P = 0.01$) correlated with decline in CDR. No significant correlations were found between MTR measure decline and other rating scales.

### Post-mortem studies

#### 1.5 T image acquisition and analysis

Post-mortem region of interest MTRs at 1.5 T in both patients and controls were generally lower than average MTR values obtained in vivo (Table 3). In the group of 19 patients who had died from various forms of prion diseases, the mean MTR in each region of interest was significantly lower compared with non-prion controls in the grey matter regions of interest: cerebellum ($P = 0.003$), frontal ($P = 0.004$) and occipital cortices ($P = 0.03$), caudate nucleus ($P = 0.001$) and thalamus ($P = 0.001$), but not in the frontal white matter ($P = 0.35$). A Kruskal–Wallis test showed no significant differences in mean MTR between the variant CJD, sporadic CJD and inherited prion disease subgroups in each of the regions of interest.

#### 9.4 T image acquisition and analysis

We followed up this association with the presumed higher sensitivity available at 9.4 T in frontal blocks from six variant CJD patient brains, together with a blinded semi-quantitative assessment of histopathology (Fig. 3). In this follow-up study we found a significant association with frontal grey matter MTR with spongiosis ($r = 0.686$, $P = 0.02$; Fig. 4). No significant correlation was noted for gliosis ($r = 0.521$, $P = 0.1$) or prion protein deposition ($r = -0.374$, $P = 0.26$). In the white matter, no correlation was found between MTR and gliosis ($r = 0.309$, $P = 0.552$). Neither spongiosis nor prion protein deposition were detected in the white matter, so no correlation analyses for these histology measures were performed.

### Discussion

We propose that the MTR is a potential biomarker in human prion disease acting as a surrogate for cortical spongiform change. In the first study of its kind in prion disease, we show that reduced MTR histogram measures, particularly in whole brain and grey matter, were significantly associated with disease and correlated
with severity measures both cross-sectionally and longitudinally. The strongest associations were between whole brain MTR25% and cognitive impairment, Mini-Mental State Examination, Rankin, CDR, Alzheimer’s Disease Assessment Scale and Clinician’s Global Impression of Disease Severity, and this measure was also an independent predictor of Mini-Mental State Examination, Barthel Activities of Daily Living and Alzheimer’s Disease Assessment Scale. These findings are consistent with our understanding of prion disease as diffuse but affecting predominantly the cerebral and cerebellar cortices. Global and grey matter MTR measurements such as ours are particularly appropriate for detecting such generalized pathology. A possible reason for the difficulty in establishing significant associations between white matter MTR histogram parameters and measures of disease severity is that white matter involvement is usually only seen in the rare panencephalopathic variant of sporadic CJD (Matsusue et al., 2004).

Assessment of conventional diffusion-weighted imaging/fluid-attenuated inversion recovery MRI images showed cortical signal change in only one patient with a 6-OPRI mutation. Despite this, associations between MTR indices and clinical status were observed at baseline in the absence of radiological abnormalities on conventional MRI in the majority of our subjects. All whole brain, white and grey matter MTR histogram measures were significantly lower in symptomatic patients than in controls. We were unable to detect any differences in MTR measures between our asymptomatic gene positive subjects and the control group, possibly due to the small numbers in the asymptomatic patient group. A potential weakness of MTR as a pathological index is that absolute values are dependent upon field strength and

![Figure 3](https://example.com/figure3.png)

**Figure 3** A panel demonstrating the semi-quantitative scoring system used for the histology analysis.
implementation details making inter-study comparisons of absolute MTR values difficult (Barker et al., 2005; Tofts et al., 2006). Taking this into account, our average control whole brain, white matter and grey matter MTR data are consistent with control values previously reported with lower standard deviations in MTR measures than many larger cohorts (Supplementary Table 1) (Dehmeshki et al., 2003; Davies et al., 2005; Gallo et al., 2007; Ridha et al., 2007b; Rovaris et al., 2008).

Our observations suggest that reduced global and tissue-specific MTR measures may provide useful predictors of clinical disease onset at an early stage. However, MTR is not likely to be a useful diagnostic biomarker in prion disease as abnormalities have been described in Alzheimer’s disease, one of the main differential diagnoses. Further, sporadic and acquired prion diseases have conventional MRI abnormalities that are reasonably sensitive and specific (Collie et al., 2003; Young et al., 2005) and inherited prion disease is diagnosed by PRNP gene analysis. The purpose of our ante-mortem measurements was to establish the utility of MTR as a measure of disease severity and progression in inherited prion disease compared with a healthy control group. For clinical practice it would be highly beneficial to determine disease-specific cerebral MTR profiles: comparisons of MTR changes in patients with prion diseases with those in patients suffering from other neurodegenerative diseases is the subject of ongoing research.

Importantly, our longitudinal study showed MTR measurement may offer an objective means of monitoring progression in trials, for which there is no alternative at present. The analysis of high quality and longitudinal MRI data from a sizable patient cohort was a major achievement of the study considering the rarity of prion diseases. These data were acquired as part of the first therapeutic trial for prion disease in the UK, the MRC PRION-1 trial (Collinge et al., 2009), with contributions from a multidisciplinary team of researchers, with one investigator responsible for all MRI data analysis. Inter-rater variability was therefore not a confounding factor in calculating MTRs or segmentation. However, a large number of assessments were carried out in PRION-1; Mini-Mental State Examination, Alzheimer’s Disease Assessment Scale, Rankin, Barthel Activities of Daily Living, CDR and Clinician’s Global Impression of Disease Severity were used in all patients. Several of these measures showed no significant decline in the imaged cohort, others were missing in a large number of patients. The question of the best way to monitor clinical progression is to be addressed by the ongoing National Prion Monitoring Cohort study in the UK. The multiplicity of scales is problematic for our longitudinal study as only the CDR showed strong longitudinal correlations with MTR. The CDR is a clinician-rated scale based on an overall assessment of a persons cognitive abilities and how these impact on activities of daily living, with some theoretical advantages to other scales in that it flexibly captures global functional abilities. Investigation of relationships between cerebral MTR and the various clinical measures inevitably involved a large number of multiple comparisons, although this is partially mitigated by the expected inter-dependence between the individual clinical scores, and the MTR histogram measures. MTR baseline associations and longitudinal correlations were consistently significant, findings unlikely to arise from multiple testing alone. We view our longitudinal studies as hypothesis generating, and the correlations we report require confirmation in future, larger studies.

Knowledge of the profile of MTR changes in inherited prion disease, particularly at baseline, is consistent with MTR changes in other dementing disorders. A recent cross-sectional study in Alzheimer’s disease (Ridha et al., 2007a), comparing global and regional MTR changes in whole brain and hippocampus, showed significantly reduced whole brain average MTR (P=0.002), MTR25% (P=0.03) and mean hippocampal MTR (P<0.001) in patients compared with controls, although unlike the present study, no significant correlations could be established with severity measures and patients were not studied longitudinally. Magnetization transfer imaging has been used as a validated outcome measure for Alzheimer’s disease in a therapeutic trial with donepezil, with responders having less severe structural damage to hippocampus and parahippocampus, as evidenced by less severe MTR changes compared to non-responders (Tanaka et al., 2003). However, a rationalization for this finding is problematic as donepezil is a symptomatic therapy and not expected to modify histopathology.

Other limitations of the patient study include the heterogeneity of severity, PRNP mutation and extrapolation from inherited prion disease to other categories of prion disease. Clinical assessment in inherited prion disease is challenging due to the diverse range of symptoms and disease severity. Many of the cognitive and motor measures are affected by variable factors such as mood, intellectual ability, motivation or fatigue as well as neurological dysfunction; these factors may have confounded some of the correlations. MTR, and in view of the strongest correlations with clinical score whole brain MTR25%, have been shown to be an objective measure. Despite remarkable differences in clinical phenotype, particularly disease duration, many of the classical histopathological features of prion disease are common between aetiological categories. Most notably, spongiform change, the substrate, we argue, for MTR changes, is common to all categories of prion disease. We therefore argue that the findings are likely to be generalized. At present in the UK, with no available therapies,
sporadic CJD is often diagnosed at a very advanced stage and longitudinal MRI studies are challenging.

The post-mortem aspect of our study is important as this provides a rational basis to hypothesize that MTR might be a therapeutic biomarker. The generally reduced MTR observed post-mortem at 1.5T compared with the in vivo values may reflect genuine changes in MTR subsequent to disease progression and death, and also changes caused by fixation and differences in magnetization transfer imaging pulse sequence implementation. Nevertheless we were able to demonstrate that ante-mortem cerebral MTR differences between patients with prion disease and controls were preserved post-mortem and following formalin fixation.

Experiments with laboratory transgenic mice engineered to post-nata tally knock-out PRNP are informative of the possible response of prion pathology to therapeutics (Mallucci et al., 2003, 2007). When these mice are infected with prions at an early age they develop behavioural change, and hippocampal neuronal loss and spongiosis at 12 weeks. At this age, PRNP is programmed to be deleted, and subsequently, unlike control mice, they recover behavioural function and remain healthy until they die in old age. Remarkably, spongiform change seen in the hippocampus at 12 weeks also recovers in these mice. If MTR is a surrogate for spongiform change we might speculate that this will improve in successful human therapy trials. Post-mortem MTR has been shown to be a sensitive marker of neuropathological changes in multiple sclerosis (van Waesberghe et al., 1999; Schmierer et al., 2004, 2008) and schizophrenia (Kalus et al., 2005) but for the first time here in prion disease.

Future studies will establish whether magnetization transfer measurements may be used as a prognostic indicator in early prion disease or as an early diagnostic marker in variant and sporadic CJD. Prospective monitoring of asymptomatic at-risk individuals, such as those exposed through potentially variant CJD-infected blood transfusion (Wroe et al., 2006; Tofts et al., 2008), and the use of MTR in therapeutic trials may provide further evidence for the utility of this biomarker.

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Supplementary material

Supplementary material is available at Brain online.

References


