SHORT REPORT

Diffusion tensor magnetic resonance imaging at 3.0 tesla shows subtle cerebral grey matter abnormalities in patients with migraine

M A Rocca, A Ceccarelli, A Falini, P Tortorella, B Colombo, E Pagani, G Comi, G Scotti, M Filippi

J Neurol Neurosurg Psychiatry 2006;**77**:686–689. doi: 10.1136/jnnp.2005.080002

Background and objective: Diffusion tensor (DT) magnetic resonance imaging (MRI) has the potential to disclose subtle abnormalities in the brain of migraine patients. This ability may be increased by the use of high field magnets. A DT MRI on a 3.0 tesla scanner was used to measure the extent of tissue damage of the brain normal appearing white (NAWM) and grey matter in migraine patients with T2 visible abnormalities.

Methods: Dual echo, T1 weighted and DT MRI with diffusion gradients applied in 32 non-collinear directions were acquired from 16 patients with migraine and 15 sex and age matched controls. Lesion load on T2 weighted images was measured using a local thresholding segmentation technique, and brain atrophy assessed on T1 weighted images using SIENAX. Mean diffusivity and fractional anisotropy histograms of the NAWM and mean diffusivity histograms of the grey matter were also derived.

Results: Brain atrophy did not differ between controls and patients. Compared with healthy subjects, migraine patients had significantly reduced mean diffusivity histogram peak height of the grey matter ($p=0.04$). No diffusion changes were detected in patients' NAWM. In migraine patients, no correlation was found between T2 weighted lesion load and brain DT histogram derived metrics, whereas age was significantly correlated with grey matter mean diffusivity histogram peak height ($p=0.05$, $r=-0.52$).

Conclusions: DT MRI at high field strength discloses subtle grey matter damage in migraine patients, which might be associated with cognitive changes in these patients.

conventional MRI.² This study, however, left a question unanswered: are normal appearing white matter (NAWM) and grey matter (GM) equally or differently affected in migraine? Clearly, understanding this is central to achieve a better definition of the pathological substrates of brain injury in migraine, which in turn might be difficult to be obtained histopathologically, given the nature of the condition. In addition, the use of a high field strength magnet and of parallel imaging technology, which are associated to increased image spatial resolution and signal to noise ratio (SNR),⁶ might further increase the sensitivity of DT MRI in disclosing subtle brain changes, which would go otherwise undetected.

In this study, using a 3.0 tesla scanner and sensitivity encoding (SENSE) technique, we acquired DT MRI to investigate whether "occult" damage can be detected in the brain NAWM and GM of patients with migraine.

METHODS

We studied 16 patients with migraine (15 women and one man; mean age = 42.7 years; range = 28–55 years; mean disease duration = 24.8 years, range = 2–48 years; mean number of episodes per year = 20.3, range = 12–244) according to the criteria of the Headache Classification Committee of the International Headache Society.⁷ The patients were recruited consecutively from the migraine population attending the outpatient clinics, department of neurology, Scientific Institute, and University Ospedale San Raffaele. Seven patients suffered with migraine with aura, and nine patients had migraine without aura. To have definite evidence of structural subcortical pathology in these patients, the presence of at least four discrete brain MRI abnormalities was an additional inclusion criterion. The cut off of four lesions was chosen to minimise the risk of including subjects with incidental MRI signal abnormalities.⁸ Patients with hypertension, hypercholesterolaemia, diabetes mellitus, vascular/heart diseases, and other major systemic and neurological conditions were excluded. At the time MRI was performed, five patients were taking prophylactic treatment for migraine. Fifteen sex and age matched right handed healthy volunteers, with no familiar history of migraine, no previous history of neurological dysfunctions (including migraine), and a normal neurological exam, served as controls (11 women and four men, mean age = 40.6 years, range = 24–52 years). All subjects were assessed clinically by a single neurologist, who was unaware of the MRI results. Local ethical committee approval and

Diffusion is the microscopic random translational motion of molecules, and water molecular diffusion can be measured in vivo using magnetic resonance imaging (MRI) based technology. Water diffusion in the brain is affected by the presence of barriers to translational motion such as cell membranes and myelin fibres. Pathophysiological processes, including ischaemia, can modify the integrity of the tissue microstructure, resulting in significant changes in its diffusion characteristics. For this reason, diffusion tensor (DT) MRI has become an established tool for providing more accurate in vivo pictures of the pathological changes associated with several neurological conditions, including vascular^{1–4} and demyelinating⁵ diseases.

Previous work using DT MRI at 1.5 T in patients with migraine has shown that, similarly to what has been seen in other chronic vascular affections, such as leucoaraiosis³ and cerebral autosomal dominant arteriopathy with subcortical infarcts and leucoencephalopathy (CADASIL),⁴ subtle brain damage extends beyond the abnormalities shown by

Abbreviations: DT, diffusion tensor; MRI, magnetic resonance imaging; NAWM, normal appearing white matter; GM, grey matter; MD, mean diffusivity; FA, fractional anisotropy; CSF, cerebral spinal fluid; SNR, signal to noise ratio

Figure 1 MD histograms of the normal appearing grey matter from healthy controls (black line) and patients with migraine (dotted line).

written informed consent from all subjects were obtained before the study.

Using a Philips Intera scanner 3.0 tesla with a gradient field strength of 30 mT/m (Philips Medical Systems, Best, Netherlands), the following brain scans were obtained from all the subjects: (1) T2 weighted turbo-spin echo images (TR/TE = 3000/120 ms, matrix size = 512×512, FOV = 230×230 mm², 28, four mm thick, contiguous, axial slices), (2) T1 weighted spin echo (TR/TE = 768/15, and the remaining acquisition parameters as for T2), and (3) pulsed gradient spin echo echo planar pulse sequence with SENSE (acceleration factor = 2.5; TR/TE = 8300/80 ms; acquisition matrix size = 96×96; FOV = 240×240 mm²; 55, 2.5 mm thick axial slices; after SENSE reconstruction, the matrix dimension of each slice was 256×256, with in-plane pixel size of 0.94×0.94 mm) and with diffusion gradients applied in 32 non-collinear directions, using a gradient scheme that is standard on this system (gradient overplus). To optimise the measurement of diffusion, only two b factors were used (b1 = 0, b2 = 1000 sec/mm²). Fat saturation was performed to avoid chemical shift artefacts.

All the structural MRI analysis was performed by a single experienced observer, unaware to whom the scans belonged. Lesion volumes were measured on T2 weighted images using a local thresholding segmentation technique.⁹ T1 weighted images were used to measure the normalised brain volumes (NBV), using the cross sectional version of the Structural Imaging Evaluation of Normalised Atrophy (SIENAX) software.¹⁰ SIENAX uses a brain extraction tool, to perform segmentation of brain from non-brain tissue in the head and to estimate the skull surface. Then, the extracted brain image is segmented into white matter (WM), GM, and cerebrospinal fluid (CSF), yielding an estimate of the absolute volumes of brain tissue compartments. The original MRI image is registered to a canonical image in a standardised space

(derived from the MNI152 standard space) to provide a spatial normalisation scaling factor for each patient. The estimated absolute volumes for each subject are then multiplied by the normalisation factor, to yield a normalised parenchymal volume of these tissue compartments. This procedure reduces within group variations, making between group comparisons more sensitive.¹⁰ From DT images, the diffusion tensor was estimated by non-linear regression (Marquardt–Levenberg method), assuming a mono-exponential relation between signal intensity and the b-matrix components.¹¹ After diagonalisation of the estimated tensor matrix, the two scalar invariants of the tensor, mean diffusivity (MD) and fractional anisotropy (FA), were derived for every pixel. Then, using the VTK CISG Registration Toolkit,¹² the rigid transformation needed to correct for position between the b = 0 images (T2 weighted, but not diffusion weighted) and T2 weighted images was calculated. Normalised mutual information¹³ was the similarity measure used for image matching. The same transformation parameters were then used to coregister the MD and FA images to the T2 weighted images. The final step consisted of automatic transfer of lesion outlines onto the MD and FA maps and calculation of average lesion MD and FA. Using statistical parametric mapping (SPM2) (Wellcome Department of Cognitive Neurology, Institute of Neurology, University College London, London, UK), brain GM, WM, and CSF were automatically segmented from DT images.¹⁴ Each pixel was classified as GM, WM, or CSF, dependent on which mask had the greatest probability (maximum likelihood) at that location. This generated mutually exclusive masks for each tissue. The resulting masks were superimposed onto the MD and FA maps (on which hyperintense lesions were masked out previously), and the corresponding MD and FA histograms of the NAWM and GM were produced (bin widths of 0.03×10⁻³ mm²/s for the MD histograms and of 0.01 for the FA histograms). FA histograms were derived only for the NAWM, as no preferential direction of water molecular motion is expected to occur in the GM, because of the absence of a microstructural anisotropic organisation of this tissue compartment. The original and segmented brain images were visually checked for quality assurance to avoid possible influences related to motion artefacts and to ensure correct compartments segmentation. To correct for the between subject differences in brain volume, each histogram was normalised by dividing the height of each histogram bin by the total number of pixels included. For each histogram, the average MD and FA and the corresponding peak heights were measured. Given the strong correlation existing between average histogram measures and histogram peak locations,¹⁵ the second quantity was not considered to reduce the number of comparisons and hence the risk of type I errors.

Table 1 Mean diffusivity (MD) and fractional anisotropy (FA) histogram derived metrics of the normal appearing white and grey matter from healthy volunteers and patients with migraine

	Control subjects	Migraine patients	p Values
NAWM average MD (SD)	0.79 (0.07)	0.80 (0.01)	0.8
NAWM MD peak height (SD)	142.0 (26.2)	133.5 (10.1)	0.3
GM average MD (SD)	1.00 (0.05)	1.04 (0.06)	0.07
GM MD peak height (SD)	65.8 (11.2)	57.0 (9.6)	0.04
NAWM average FA (SD)	0.38 (0.04)	0.38 (0.01)	0.7
NAWM FA peak height (SD)	24.6 (3.6)	24.0 (0.9)	0.6

NAWM, normal appearing white matter; GM, grey matter; SD, standard deviation. Average MD of the diffusivity histogram is expressed in units of mm²/s×10⁻³, FA is a dimensionless index, MD and FA peak heights are multiplied by ×10³. Peak heights' unit is always %. See text for statistical analysis and results.

Group comparisons were performed using two tailed Student's *t* test for non-paired data. Univariate correlations were explored using the Spearman rank correlation coefficient.

RESULTS

All healthy volunteers had normal brain T2 weighted scans. In patients with migraine, the mean T2 weighted lesion load was 2.1 ml (range = 0.02–11.2 ml), average lesion MD was 0.93×10^{-3} mm²/s (range = $0.85 - 1.10 \times 10^{-3}$ mm²/s) and average lesion FA was 0.34 (range = 0.24–0.42). There was no difference in NBV between patients and controls (1598 (SD 91) and 1659 (SD 79) ml).

Table 1 shows MD and FA histogram derived metrics of the brain NAWM and GM from migraine patients and controls. Compared with healthy subjects, migraine patients had significantly reduced MD histogram peak height of the GM ($p = 0.04$) (fig 1). This metric did not differ between patients with and without aura and between drug free patients and those receiving a prophylactic treatment for migraine. In migraine patients, no correlations were found between T2 weighted lesion load and brain DT histogram derived metrics, whereas age was significantly correlated with GM MD histogram peak height ($r = -0.52$, $p = 0.05$). No correlation was found between GM MD histogram peak height and duration of illness and number of yearly episodes. In healthy subjects, no correlation was found between age and GM MD histogram peak height.

DISCUSSION

In this study, we interrogated the status of the brain tissues appearing normal on conventional MRI using advanced DT technology and a 3.0 tesla scanner in patients with migraine. Given the preliminary nature of this study, we selected patients with evidence, albeit modest, of brain injury—in case of patients with completely normal brain MRI scans and negative results, it would have been indeed impossible to know whether additional subtle changes were not present or the technology used not sensitive enough to detect them. The main advantage of using a 3.0 tesla system is the gain in SNR over lower field MR scanners and, as a consequence, the possibility to achieve a higher scan resolution that permits a better visualisation of the different anatomical structures. The improvement in SNR is particularly important for DT MRI, because the application of diffusion gradients causes an attenuation of the signal and intrinsically limits the SNR available. Another limitation of DT MRI is that it requires fast acquisition protocols to make the acquisition independent from bulk motion. This is usually obtained with spin echo single shot EPI sequences, which however are affected by problems, such as image blurring and geometric distortions near air/tissue interfaces. Both these effects increase with the static magnetic field and become crucial at 3.0 tesla. These limitations have been recently addressed by combining the use of 3.0 tesla scanners with parallel acquisition techniques,¹⁶ which mitigate EPI related artefacts. By optimising the parallel imaging reduction factor, the inherent SNR loss is balanced by mitigating T2 decay and a better SNR along with reduced distortions is obtained.⁶

The main finding of this study was that brain GM of patients with migraine is not spared by the pathological process. This finding extends those of previous work at 1.5 tesla that showed subtle diffusion changes in the normal appearing brain tissue (NABT), without separating NAWM from GM.² Our results also fit with studies of GM density measurements with MRI, which have shown abnormalities in several GM regions from patients with different types of pain,¹⁷ including headache.¹⁸ In contrast, the absence of a reduced NBV in patients with migraine compared with controls, allows us to rule out a possible role of partial

volume effect from the CSF. Additional studies are now warranted to ascertain whether such changes also occur in migraine patients without T2 visible abnormalities and might be related to the cognitive abnormalities known to occur in these patients.^{19, 20}

Although this study cannot provide definitive answers about the pathological nature of the observed GM changes in patients with migraine (but definitive histopathological correlations in these patients are unlikely to be ever obtained), three plausible explanations, which are not mutually exclusive, are readily apparent. Firstly, GM changes might result from ischaemia caused by the blood flow reduction, which can persist for hours during a migraine episode.²¹ Secondly, as all patients had macroscopic T2 visible lesions, another explanation is that GM changes may be secondary to retrograde degeneration of axons passing through macroscopic WM lesions. This seems, however, unlikely given the paucity of T2 lesions in this patient sample and the lack of a correlation between DT MRI histogram metrics and T2 lesion load. Thirdly, GM abnormalities in people with migraines might reflect an increased susceptibility to age related changes, as suggested by the correlation with age and by what has been seen in other neurological conditions.^{22–24} This increased susceptibility to age related changes might be in turn secondary to repeated neuronal vascular insufficiency.

The second main result of this study is the finding that brain “occult” damage in migraine patients does not involve the NAWM. Albeit tiny and scarce NAWM changes might have been missed, we believe this not to be the case or, at any rate, to have only a marginal clinical significance when considering that we used a sensitive imaging approach based on advanced DT technology operating at 3.0 tesla. In addition, this agrees with the previous finding of normal magnetisation transfer ratio values from several NAWM regions of patients with migraine.²⁵ The sparing of the WM outside T2 visible lesions suggests that regional blood flow reductions, a plausible cause of WM damage in migraine,²¹ might preferentially involve vessels of a size large enough to cause lesions that can be seen on cMRI scans.

Admittedly, we recruited a selected group of patients with migraine—that is, patients with WM lesions and we used as controls a group of healthy people, as a consequence, our results might not be generalised to the entire population of people with migraines. Therefore, we acknowledge the need to replicate these findings in a population of migraine patients not selected for the presence of T2 brain abnormalities.

Authors' affiliations

M A Rocca, A Ceccarelli, P Tortorella, E Pagani, M Filippi, Neuroimaging Research Unit, Scientific Institute and University Ospedale San Raffaele, Milan, Italy

M A Rocca, A Falini, G Scotti, M Filippi, CERMAC, Scientific Institute and University Ospedale San Raffaele

A Falini, G Scotti, Department of Neuroradiology, Scientific Institute and University Ospedale San Raffaele

B Colombo, G Comi, M Filippi, Department of Neurology, Scientific Institute and University Ospedale San Raffaele

Funding: none

Competing interests: none declared

Correspondence to: Dr M Filippi, Neuroimaging Research Unit, Department of Neurology, Scientific Institute and University Ospedale San Raffaele, Via Olgettina, 60, 20132 Milan, Italy; massimo.filippi@hsr.it

Received 9 September 2005

In revised form 12 January 2006

Accepted 17 January 2006

REFERENCES

- 1 **Sotak CH**. The role of diffusion tensor imaging in the evaluation of ischemic brain injury—a review. *NMR Biomed* 2002;**15**:561–9.
- 2 **Rocca MA**, Colombo B, Inglese M, *et al*. A diffusion tensor magnetic resonance imaging study of brain tissue from patients with migraine. *J Neurol Neurosurg Psychiatry* 2003;**74**:501–3.
- 3 **O'Sullivan M**, Summers PE, Jones DK, *et al*. Normal-appearing white matter in ischemic leukoaraiosis: a diffusion tensor MRI study. *Neurology* 2001;**57**:2307–10.
- 4 **Chabriat H**, Pappata S, Poupon C, *et al*. Clinical severity in CADASIL related to ultrastructural damage in white matter: in vivo study with diffusion tensor MRI. *Stroke* 1999;**30**:2637–43.
- 5 **Cercignani M**, Bozzali M, Iannucci G, *et al*. Magnetisation transfer ratio and mean diffusivity of normal appearing white and grey matter from patients with multiple sclerosis. *J Neurol Neurosurg Psychiatry* 2001;**70**:311–17.
- 6 **Jaermann T**, Crelier G, Pruessmann KP, *et al*. SENSE-DTI at 3 T. *Magn Reson Med* 2004;**51**:230–6.
- 7 **Headache Classification Committee of the International Headache Society**. [The new IHS classification. Background and structure]. *Schmerz* 2004;**18**:351–6.
- 8 **Fazekas F**, Offenbacher H, Fuchs S, *et al*. Criteria for an increased specificity of MRI interpretation in elderly subjects with suspected multiple sclerosis. *Neurology* 1988;**38**:1822–5.
- 9 **Rovaris M**, Filippi M, Calori G, *et al*. Intra-observer reproducibility in measuring new putative MR markers of demyelination and axonal loss in multiple sclerosis: a comparison with conventional T2-weighted images. *J Neurol* 1997;**244**:266–70.
- 10 **Smith SM**, Zhang Y, Jenkinson M, *et al*. Accurate, robust, and automated longitudinal and cross-sectional brain change analysis. *NeuroImage* 2002;**17**:479–89.
- 11 **Basser PJ**, Mattiello J, LeBihan D. Estimation of the Effective self-diffusion tensor from the NMR echo. *J Magn Reson B* 1994;**103**:247–54.
- 12 **Harikens T**, Rueckert D, Schnabel JA, *et al*. *VTK CISC Registration Toolkit: an open source software package for affine and non-rigid registration of single- and multimodal 3D images*. BVM2002. Leipzig: Springer-Verlag, 2002.
- 13 **Studholme C**, Hill DLG, Hawkes DJ. An overlap invariant entropy measure of 3D medical image alignment. *Pattern Recognition* 1999;**32**:71–86.
- 14 **Ashburner J**, Friston KJ. Multimodal image coregistration and partitioning: a unified framework. *NeuroImage* 1997;**6**:209–17.
- 15 **Rovaris M**, Bozzali M, Santuccio G, *et al*. In vivo assessment of the brain and cervical cord pathology of patients with primary progressive multiple sclerosis. *Brain* 2001;**124**:2540–9.
- 16 **Pruessmann KP**, Weiger M, Scheidegger MB, *et al*. SENSE: sensitivity encoding for fast MRI. *Magn Reson Med* 1999;**42**:952–62.
- 17 **Apkarian AV**, Sosa Y, Sonty S, *et al*. Chronic back pain is associated with decreased prefrontal and thalamic gray matter density. *J Neurosci* 2004;**24**:10410–15.
- 18 **May A**, Ashburner J, Buchel C, *et al*. Correlation between structural and functional changes in brain in an idiopathic headache syndrome. *Nat Med* 1999;**5**:836–8.
- 19 **Mulder EJ**, Linssen WH, Passchier J, *et al*. Interictal and postictal cognitive changes in migraine. *Cephalalgia* 1999;**19**:557–65.
- 20 **Calandre EP**, Bembibre J, Arnedo ML, *et al*. Cognitive disturbances and regional cerebral blood flow abnormalities in migraine patients: their relationship with the clinical manifestations of the illness. *Cephalalgia* 2002;**22**:291–302.
- 21 **Olesen J**, Friberg L, Olsen TS, *et al*. Timing and topography of cerebral blood flow, aura, and headache during migraine attacks. *Ann Neurol* 1990;**28**:791–8.
- 22 **Ge Y**, Grossman RI, Babb JS, *et al*. Age-related total gray matter and white matter changes in normal adult brain. Part II: quantitative magnetization transfer ratio histogram analysis. *AJNR Am J Neuroradiol* 2002;**23**:1334–41.
- 23 **Helenius J**, Soine L, Perkio J, *et al*. Diffusion-weighted MR imaging in normal human brains in various age groups. *AJNR Am J Neuroradiol* 2002;**23**:194–9.
- 24 **Mezzapesa DM**, Rocca MA, Pagani E, *et al*. Evidence of subtle gray matter pathology in healthy elderly individuals with nonspecific white matter hyperintensities. *Arch Neurol* 2003;**60**:1109–12.
- 25 **Rocca MA**, Colombo B, Pratesi A, *et al*. A magnetization transfer imaging study of the brain in patients with migraine. *Neurology* 2000;**54**:507–9.