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Brain structural damage in Friedreich's ataxia

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ABSTRACT

Objective: Neuropathological descriptions of the brain in Friedreich's ataxia (FRDA) were obtained before availability of the current molecular genetic tests for this disease. Voxel-based morphometry (VBM) enables an unbiased whole-brain quantitative analysis of differences in gray matter (GM) and white matter (WM) volume.

Methods: Using VBM, we assessed the brain structural damage in 22 patients with genetically confirmed FRDA and 25 healthy controls. The results were correlated with the disease duration and the severity of the patients' clinical deficits—evaluated using the International Cerebellar Ataxia Rating Scale and Inherited Ataxia Clinical Rating Scale.

Results: In patients with FRDA, VBM showed a symmetrical volume loss in dorsal medulla, infero-medial portions of the cerebellar hemispheres, the rostral vermis and in the dentate region. No volume loss in cerebral hemispheres was observed. The atrophy of the cerebellum and medulla correlated with the severity of the clinical deficit and disease duration.

Conclusions: In patients with FRDA, significant GM and WM loss was observed only in the cerebellum and dorsal medulla. These structural changes correlate with the severity of the clinical deficit and disease duration.

Friedreich's ataxia (FRDA) is the most common inherited ataxia and, in most cases, is due to a GAA repeat expansion in a gene on chromosome 9q13, which codes for a mithocondrial protein named frataxin.[1]

Neurological features include progressive ataxia and impaired vibration or position sense, areflexia of the lower extremities, pyramidal-tract dysfunction, distal muscular atrophy and dysarthria, which are accompanied by skeletal deformities, cardiomyopathy and diabetes mellitus.[1] Atypical clinical variants are frequent, which makes molecular diagnostic testing fundamental for the diagnosis of FRDA.

Neuropathological descriptions of the brain in FRDA were obtained before the availability of the current molecular genetic tests.[2 3] Magnetic resonance imaging (MRI) demonstrates *in vivo* atrophy of the main CNS structures that are involved in FRDA—namely, the spinal cord and the medulla—whereas atrophy of the cerebellum and of the cerebral hemispheres were occasionally reported.[4–7]

Voxel-based morphometry (VBM) is an unbiased whole-brain method that assesses regional differences between brain gray matter (GM) and white matter (WM) volume in T1-weighted images,[8] and has been widely applied to the investigation of atrophy in patients with degenerative CNS diseases. We hypothesised that VBM could help to establish, *in vivo*, the structural damage of the brain in patients with genetically proven FRDA and correlated the findings with clinical features.

PATIENTS AND METHODS

Between March 2005 and March 2007, 22 (12 women and 10 men; mean age 33 ± 10 years, range 13–54 years) with genetically proven FRDA gave their informed consent to participate in this prospective study, which was approved by the Local Ethical Committee.

A molecular diagnostic method was previously reported[9] and the cut-off number of expansion of triplet repeats qualifying for diagnosis was 100 GAA on both alleles for FRDA. In the 22 patients, the mean number of triplets in the shorter alleles was 646 ± 22 and in the longer alleles 895 ± 212.

On the day of the MRI examination, the same neurologist (blind to the results of MRI) defined the patient's disease duration and computed her or his scores on the International Cooperative Ataxia Rating Scale (ICARS)[10] and on the Inherited Ataxia Clinical Rating Scale (IACRS).[11] The ICARS is a 0–100 semi-quantitative scale, with 100 corresponding to maximal clinical deficit; it evaluates 19 different features of the cerebellar syndrome. The IACRS is a 0–46 semi-quantitative scale, with 46 corresponding to maximal clinical deficit; it evaluates signs and symptoms related to ataxia, pyramidal-tract dysfunction, and impaired vibration or position sense. The mean disease duration in the patients was 14.5 ± 9.2 (range 5–39) years, their mean ICARS score was 54 ± 22 (range 17–96) and their mean IACRS score was 25 ± 7 (range 9–36).

Twenty-five healthy volunteers without personal or familial history of neurological disease (10 women and 15 men; mean age 37 ± 11 years, range 26–66 years) served as controls.

MRI examination

Patients and controls underwent MRI examination in a single centre on a 1.5T system (Philips Intera; Best, The Netherlands) with 33 mT/m maximum gradient strength and SENSE coil technology. After scout, axial 3D T1-weighted turbo gradient echo (repetition time = 25 ms, echo time = 4.6 ms, flip angle = 30°, field of view = 256 mm, matrix size = 256 × 256, 160 contiguous slices, slice thickness = 1 mm) images were obtained for VBM in all FRDA patients and controls.

Data processing

Image data processing was performed on a PC running MATLAB 7 (The Mathworks, Inc., Natik, MA, USA) and the statistical parametric mapping 2 (SPM2) software (Wellcome Department of Cognitive Neurology, London, UK).

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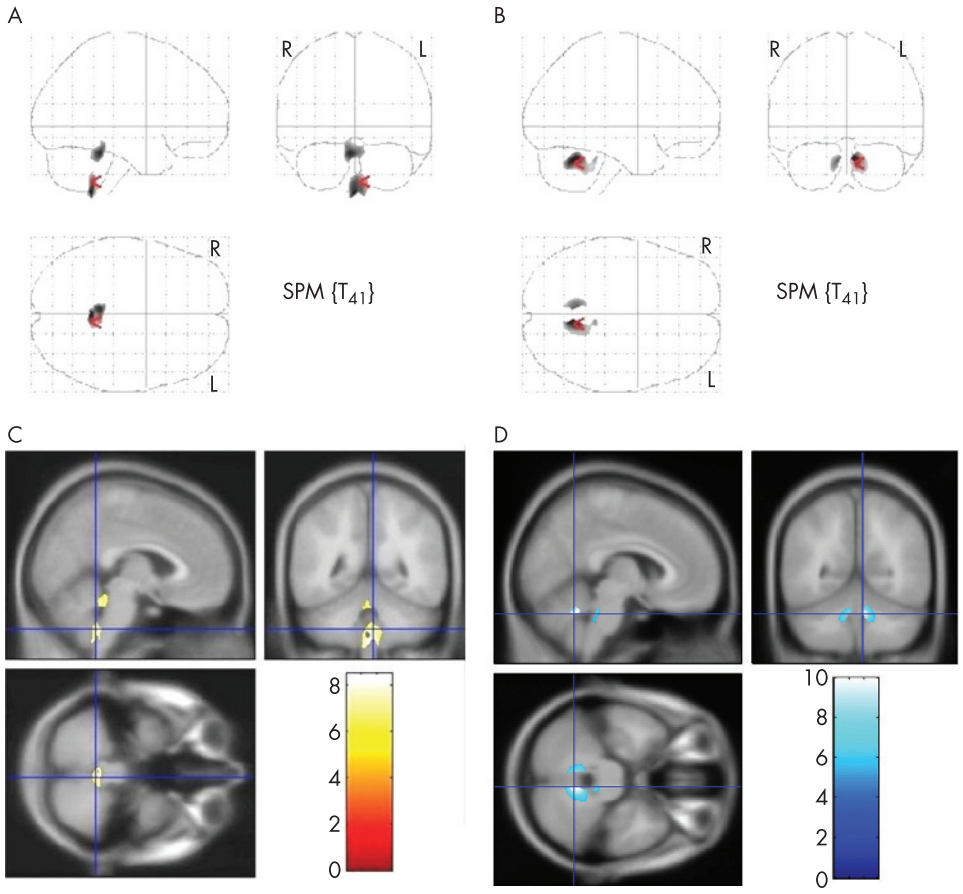

Figure 1 Maps of the t-value (voxel analysis at p<0.05 corrected for multiple comparisons) represented as glass brain (A, B) and superimposed on T1-weighted images (C, D), showing nearly symmetrical loss of the gray matter (GM) in the rostral vermis (lobules I–III), the dorsal medulla and infero-medial portions of the cerebellar hemispheres (lobules IX) (A and C) and of the white matter (WM) in the dentate region (B and D) in patients with Friedreich's ataxia (FRDA) compared with healthy controls. No supratentorial GM or WM volume loss is present.

The methodology of VBM closely followed that previously reported[8] and included seven steps: reorientation according to the antero-posterior commissure line; template creation to improve brain segmentation; normalisation; segmentation into three classes of tissue (GM, WM and CSF); modulation; smoothing with a 8 mm full-width half-maximum Gaussian kernel; voxelwise between-groups statistical analysis.

Statistical methods

VBM

Statistical analysis of the MRI data was based on the general linear model and the theory of Gaussian random fields. A voxelwise comparison of spatially normalised T1-weighted images was made using SPM2. Group comparisons were performed by means of analysis of covariance (ANCOVA) using the total volume of each segmented image (GM volume for GM analysis, WM volume for WM analysis) as confounding covariate. Age and gender were included as covariates of no interest to exclude the possible effects of these variables on regional GM or WM volumes.[8]

Voxel-level analysis with a significance threshold set at p-value <0.05 corrected for multiple comparisons across the whole brain (family-wise error correction) were applied to the resulting t-statistic maps of GM and WM.

To correlate the extent of GM or WM volume loss with severity of the clinical deficit, disease duration and GAA repeat number of the longer allele, the significant areas at group analysis were saved and applied as regions of interest (ROI) on the maps of local average volume of each patient. Extracted local average volumes for each region of decreased GM or WM were finally correlated to ICARS and IACRS scores, disease duration and GAA repeat number of the longer allele by means of the Spearman rank correlation test (p<0.05).

The Schmahmann *et al*. MRI atlas of the human cerebellum[12] was used as an anatomical reference to assess the exact localisation of significantly atrophic GM.

Table 1 Spearman's rank correlation coefficients between local average volume, clinical scores and GAA repeat number of the longer allele in 22 patients with inherited ataxias

	FRDA	
	GM	WM
ICARS	−0.71**	−0.66**
IACRS	−0.46*	−0.49*
Disease duration	−0.53*	−0.49*
GAA repeat number of the longer allele	−0.19	−0.19

*p<0.05; **p<0.001
FRDA, Friedreich's ataxia; GM, gray matter; IACRS, Inherited Ataxia Clinical Rating Scale; ICARS, International Cooperative Ataxia Rating Scale; WM, white matter.

RESULTS

Voxel-based morphometry showed symmetrical loss of the GM in the rostral vermis (lobules I–III) (cluster extent 1684; coordinates: −4, −41, −24; Z = 6.25) and in the dorsal medulla and infero-medial portions of the cerebellar hemispheres (lobules IX) (cluster extent 1458; coordinates 1, −48, −58; Z = 6.24) and of the WM in the dentate region (right cluster extent 2602; coordinates 6, −56, −30; Z = 7.06; left cluster extent 964; coordinates −8, −54, −31; Z = 8.0) in FRDA patients compared with healthy controls (fig 1).

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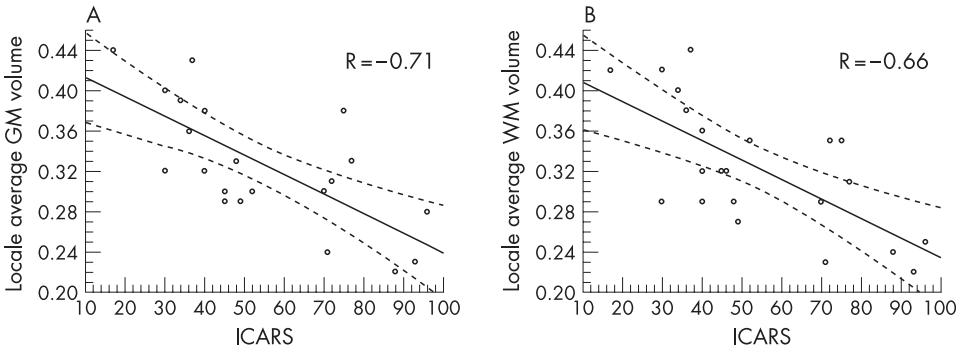

Figure 2 Spearman rank correlation coefficient (R) between loss of gray matter (GM; A) and white matter (WM; B) and the International Cooperative Ataxia Rating Scale (ICARS) score in patients with Friedreich's ataxia.

No significant cluster of GM or WM volume reduction was observed in the cerebral hemispheres.

The volume loss of the GM and WM correlated with all the clinical variables but not with the GAA repeat number of the longer allele (table 1, fig. 2).

DISCUSSION

The combination of molecular genetic diagnosis with VBM offers the possibility of performing a structural assessment, *in vivo*, of a given disease condition—without the limitations that are inherent to neuropathological examination. Such limitations include the usually small number of cases, technical problems (fixation artefacts, etc.), sample (a whole brain neuropathological examination is rarely performed) bias and the coexistence of agonal changes.

Voxel-based morphometry was applied to the investigation of patients with spinocerebellar ataxia type 2,[13] type 3, type 6[14] and type 17,[15] but no VBM study evaluated patients with FRDA.

The neuropathological hallmark of FRDA is neuronal loss and shrinkage in the spinal ganglion cells and in the Clarke column of the spinal cord, with degeneration of the gracilis and cuneatus tracts in the posterior columns and of the spinocerebellar tracts in the lateral columns of the spinal cord.[2 3] Secondary neuronal loss in the brainstem predominantly involves the accessory cuneate and gracilis nuclei in the dorsal medulla. The most striking feature in the cerebellum is loss of neurons in the dentate nuclei, whereas the cerebellar cortex is spared.[3] The cerebellar WM is diffusely gliotic and there is loss of the myelinated fibres in the hilum of the dentate nuclei.[2 3] Cell loss and astrocytosis occurs in the vestibular and cochlear nuclei and in the superior olives, whereas the inferior olives are spared. Most cases show degenerative changes in the corticospinal tracts at the level of the medulla and below, whereas pathological changes in the supratentorial compartment are usually restricted to optic nerve and tract damage.[3]

Magnetic resonance imaging studies in patients with FRDA, using visual assessment or measurement of the region of interest of CNS structures in the posterior cranial fossa and cervical spine, pointed out atrophy of the cervical spinal cord and medulla.[4] Atrophy of the superior vermian and paravermian areas (culmen and declive lobules) of the cerebellum and of the cerebral hemispheres were visually noted in some studies.[5–7]

In the present VBM study of patients with genetically proven FRDA, we demonstrated GM volume loss in the dorsal medulla and the rostral cerebellar vermis, inferomedial cerebellar hemispheres and of WM in the region of the dentate, but no atrophy of the cerebral hemispheres. The atrophy of the dorsal medulla, where the relais between the gracilis and cuneatus tracts of the spinal cord and the corresponding nuclei is located, and the atrophy of the cerebellar WM in the dentate region, match the neuropathological descriptions. In addition, the distribution of GM atrophy in the cerebellum is partially in line with that noted in a previous study.[5] In our opinion, the failure in our study to demonstrate atrophy of the dentate, which is a very small GM structure, and other small GM and WM structures of the brainstem, exemplifies the difficulties of using VBM to evaluate the CNS structures contained in the posterior cranial fossa (RDN, unpublished observation, 2007). These difficulties presumably arise from problems with spatial normalisation due to high variability between subjects of small structures and regional differences in the quality of GM and WM segmentation. In particular, the latter could be influenced by partial volume between CSF and GM secondary to atrophy or signal changes related to pathology, decreasing the GM/WM contrast. Diffusion tensor imaging represents an alternative tool for evaluation of the WM of the brainstem and cerebellum, but this was not investigated in this study.

A general expectation for imaging data in inherited ataxias and other neurodegenerative diseases of the CNS is that they could provide useful markers of severity of neurodegeneration.[15] In our study, atrophy of the dorsal medulla, rostral vermis, inferomedial cerebellar hemispheres and of the peridentate WM correlated with clinical severity and disease duration. As in previous MRI studies of inherited ataxias, including FRDA,[16] SCA1[17] and SCA2,[13] we found no correlation between the severity of atrophy and number of triplet expansions.

We recognise that our study was limited by the fact that the gender of the patients and controls was unbalanced, and that their age was similar but not matched.

Regarding the distribution of neuropathological changes, it is anticipated that atrophy of the spinal cord is likely to be equally or even more strictly correlated with disease severity and duration than brain atrophy in patients with FRDA. However, correlation analyses between clinical severity and the size (and, possibly, structure) of the spinal cord in patients with FRDA are lacking.

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