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Voxel based analysis in neuroferritinopathy expands the phenotype and determines radiological correlates of disease severity

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Abstract

Neuroferritinopathy is an autosomal dominant adult-onset movement disorder which occurs due to mutations in the ferritin light chain gene (FTL). Extensive iron deposition and cavitation are observed post-mortem in the basal ganglia, but whether more widespread pathological changes occur, and whether they correlate with disease severity is unknown.

3D-T1w and quantitative T2 whole brain MRI scans were performed in 10 clinically symptomatic patients with the 460InsA FTL mutation and 10 age-matched controls. Voxel-based morphometry (VBM) and voxel-based relaxometry (VBR) were subsequently performed. Clinical assessment using the Unified Dystonia Rating Scale (UDRS) and Unified Huntington's Disease Rating Scale (UHDRS) was undertaken in all patients.

VBM detected significant tissue changes within the substantia nigra, midbrain and dentate together with significant cerebellar atrophy in patients (FWE, $p < 0.05$). Iron deposition in the caudate head and cavitation in the lateral globus pallidus correlated with UDRS score ($p < 0.001$). There were no differences between groups with VBR.

Our data show that progressive iron accumulation in the caudate nucleus, and cavitation of the globus pallidus correlate with disease severity in neuroferritinopathy. We also confirm sub-clinical

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cerebellar atrophy as a feature of the disease. We suggest that VBM is an effective technique to detect regions of iron deposition and cavitation, with potential wider utility to determine radiological markers of disease severity for all NBIA disorders.

Keywords

neuroferritinopathy; voxel; morphometry; relaxometry; iron

Introduction

Neuroferritinopathy (MIM 606159, also called hereditary ferritinopathy or neurodegeneration with brain iron accumulation type 2, NBIA2) is an autosomal dominant, adult-onset progressive movement disorder which occurs due to mutations in the ferritin light chain gene (FTL) [1]. Neuroferritinopathy is one of the 10 distinct neurogenetic conditions which together comprise neurodegeneration with brain iron accumulation (NBIA) [2]. Despite their genetic heterogeneity, all NBIA disorders have iron deposition within the basal ganglia, and are invariably characterised clinically by an extrapyramidal movement disorder and neurodevelopmental or cognitive impairment.

Magnetic Resonance Imaging (MRI) has become the principle investigation in the diagnosis of NBIA due to the propensity of iron to cause rapid signal dephasing in surrounding neural tissue [3]. Iron deposition is suggested by hypointensity on T2 and T2*-weighted imaging accompanied by hyperintensity or isointensity on T1-weighted sequences, with the former suggested to be most accurate [3, 4]. Over recent years, characteristic patterns of iron deposition for each of the major NBIA disorders, including neuroferritinopathy have been described [3]. With reference to neuroferritinopathy in particular, symptomatic cases show T2* hypointensity in the dentate nuclei (95 % of cases), followed by the substantia nigra (81 %), globus pallidus (38 %), putamen (28 %), thalamus (19 %) and caudate (14 %). In 52 % of cases, cavitation of the globus pallidus and putamen is also seen later in the disease course [3]. Whilst such clinical imaging findings are of marked diagnostic importance, our understanding of the regional distribution of iron, non-iron related pathological changes, and radiological correlates of disease severity remains poorly understood both for neuroferritinopathy, and almost all NBIA disorders [5].

Voxel-based morphometry (VBM) [6], and voxel-based relaxometry (VBR) [7] are techniques employing statistical analysis of both T1 and T2 imaging data. VBM utilises T1-weighted (T1w) images, and VBR utilises quantitative relaxation time maps of T1 or T2 (here termed qT1 and qT2, respectively). Of particular importance to NBIA disorders, is qT2 VBR, which has been demonstrated by several investigators to correlate strongly with iron deposition [8, 9], providing a potential non-invasive method to quantitatively monitor tissue iron content. In contrast, despite the perceived lack of subjective T1w imaging changes with iron deposition [10], VBM has been utilised to detect regional atrophy in neurodegenerative conditions such as Alzheimer's disease [11] and Parkinson's disease [12].

In this study, we utilised VBM and VBR in neuroferritinopathy and age-matched controls. We sought to determine previously unrecognised iron deposition or regional tissue changes

outside the basal ganglia and define radiological markers of disease severity. If successful, this would significantly expand our understanding of the neuropathology and clinic-pathological correlates within neuroferritinopathy and the utility of voxel-based imaging techniques for NBIA disorders.

Methods

The study was performed at a single time point in 10 patients (5 men and 5 women) with neuroferritinopathy whose mean (SD) age was 48.5 (9.2) years (Table 1). All patients were clinically symptomatic with positive molecular genetic testing for the 460InsA FTL mutation. Patients underwent a full general neurological examination as performed by one of three experienced Consultant clinical neurologists (GG, RH, PFC) and a further clinical assessment at the time of MR imaging using two validated rating scales: the Unified Huntington Disease Ratings Scale (UHDRS) for chorea, and the Unified Dystonia Rating Scale (UDRS) (Table 1). All studies were approved by the local ethics committee. Data were available for comparative purposes from 10 (7 male, 3 female) age-matched controls [Mean age 46.2 (SD 9.9), $p = 0.56$ (unpaired t test)] without history of any neurological disease and were, therefore, not clinically assessed.

Data acquisition

Patients were scanned in a Philips 3T Achieva system using an 8-channel head coil. The protocol comprised both qualitative and quantitative scans. A standard 3D-T1-weighted whole brain volumetric sequence (3D MPRAGE, sagittal acquisition, 1 mm isotropic resolution and 240 9 216 9 180 matrix; repetition time (TR) = 9.6 ms; echo time (TE) = 4.6 ms; SENSE factor = 1.7; flip angle = 8°) was acquired from an angulated volume with the axial slice orientation aligned with the AC-PC line. Routine clinical T1w and T2w anatomical scans were also collected in the axial and coronal planes.

Quantitative T2 relaxation time maps were acquired using a purpose-written CPMG style imaging sequence with 8 spin echoes and segmented EPI readout (TR = 10 s, TE = 20–160 ms in 20 ms steps, in-plane resolution 2 mm, slice thickness 2 mm). Imaging slices were angulated and aligned to match the 3D-T1 weighted volume scan.

VBM preprocessing

VBM analysis was performed on the 3D-T1w scans using SPM8 (<http://www.fil.ion.ucl.ac.uk/spm>) and MATLAB 7.2 (Math-Works, Natick, MA, USA). MR images were visually inspected for gross anatomical abnormalities or artefacts. Using the 'segment' option in SPM8, images were then spatially normalised into Montreal Neurological Institute (MNI) space (<http://mcin-cnim.ca/atlas/>) using non-linear transformation and segmented into grey matter (GM), white matter (WM) and cerebrospinal fluid [11]. Images were modulated before smoothing with an 8-mm full-width at half-maximum (FWHM) isotropic Gaussian kernel.

VBR preprocessing

Relaxation maps were calculated on a pixel by pixel basis by using a 2 parameter (M_0 and T_2) monoexponential fit to the acquired data. Maps were spatially normalised to MNI space via transformations determined by nonlinear registration of one T2-weighted image (3rd echo of the qT2 series, $TE = 60$ ms) to the standard T2w template in SPM8. The spatially normalised qT2 maps were subsequently smoothed using a Gaussian filter (8 mm, full-width half-maximum). Standard brain masking within SPM was used to restrict analysis to neural tissue.

Statistical analysis of VBM and VBR data

VBM and VBR comparison of patients against controls were performed using an unpaired t-test in SPM8 controlling for global differences in voxel intensity by including the overall mean of voxel intensity as a confounding covariate in the design matrix. Results were thresholded with a family-wise error (FWE) threshold of $p < 0.05$ or subsequently at an uncorrected value of $p \leq 0.001$ if no association was present. A cluster level of at least 20 voxels was applied to VBM and VBR. MNI co-ordinates were subsequently converted to Talaraich co-ordinates using GingerAle [13].

Analyses to investigate the relationship between imaging changes and age, disease duration, the Unified Huntington Disease Ratings Scale (UHDRS) for chorea, and the Unified Dystonia Rating Scale (UDRS) were performed using voxel-wise linear regression analysis in SPM8. Results were thresholded as done in VBM, with a family-wise error (FWE) threshold of FWE $p \leq 0.05$ or subsequently with an uncorrected value of $p \leq 0.001$ with a cluster level of at least 20 voxels.

Manual ROI assessment

To examine difference in tissue characteristics and relaxation times and to aid in the interpretation of findings from the VBM and VBR analysis within the structures of the basal ganglia, manual region of interest (ROI) analysis was performed for caudate, putamen, and globus pallidus. ROI were defined (MJK) blinded to patient clinical scores. Regions were manually defined using MRICro (<http://www.mricro.com>) on raw T1w and T2w MRI scans, qT2 maps, segmented and smoothed images. Minimum, mean and maximum image intensities within each ROI were recorded, and statistical comparison between groups was performed using unpaired t tests (SPSS v.21). The intra-rater reliability for the ROI assessment of raw T1 imaging was also determined by repeating the ROI definition and measurements (in triplicate) of mean image intensity in the dentate and GPi and then calculating the intraclass correlation coefficient (ICC) [14] (SPSS v.21). Clinical assessment of T1w and T2w scans were also assessed by an experienced neuroradiologist (DB) for features consistent with iron deposition and/or cavitation within the dentate and globus pallidus internus (Online Resource Table 3). Measurement of cerebellar volume was also performed (ET). Axial sections of the 3D-T1w scan were viewed and the cerebellum was manually outlined (ImageJ, <http://imagej.nih.gov/ij/>). Total cerebellum volume was determined in each subject by summing the area of the cerebellum on each slice and multiplying by the slice thickness. To account for differences in brain size between subjects, cerebellar volumes were normalised by the total intracranial volume (TICV) derived from

SPM segmentation (GM ? WM ? CSF volumes). Group differences in volumes were tested using unpaired t test (SPSS v.21).

Results

VBM analysis of segmented grey matter images revealed significant differences in the substantia nigra, mid brain, globus pallidus and the dentate nuclei and surrounding posterior lobe of the cerebellum (FWE, $p < 0.05$) (Fig. 1; Online Resource Table 1), corresponding to those areas with the most prominent iron deposition and cavitation in neuroferritinopathy [1, 3] (Online Resource Table 5). VBM analysis of segmented white matter images showed changes suggestive of cerebellar atrophy in patients (Fig. 2; Online Resource Table 2). ROI volumetric analysis of the whole cerebellum confirmed smaller cerebellar volumes in patients than controls ($p < 0.001$) (Online Resource Figure 1). Significant differences between patients and controls were also seen in the thalamus, and basal ganglia grey matter nuclei regions, in addition to the lingual gyrus of the occipital lobe and pre-central motor gyrus (FWE, $p < 0.05$) (Fig. 2; Online Resource Table 2). There were no group-wise differences with quantitative qT2 voxel-based relaxometry (VBR) between patients and controls with a family-wise error (FWE) corrected threshold of $p < 0.05$.

To aid in the interpretation of the VBM and VBR findings, the dentate gyrus and globus pallidus internus (GPi) were selected for manual ROI assessment due to (a) their established neuropathological involvement in neuroferritinopathy, and (b) as significant differences between patients and controls were identified with VBM but not VBR (Online Resource Figure 3). The appearance of the dentate and GPi on T1w and T2w images were assessed by an experienced neuroradiologist (DB) to confirm evidence of iron accumulation and/or cavitation (Online Resource Table 3). Significant T1w hypointensity in the paradentate region indicative of cavitation in the basic images and during subsequent spatial transformation (warping), segmentation and smoothing was seen with VBM (Online Resource Figure 2, 5). VBM, therefore, identifies the loss of deep GM. No differences were seen between groups in either ROI with qT2 images at any stage of processing (Online Resource Figure 4, 6).

Clinical correlates

Regression analysis showed a significant inverse correlation between the T1w GM segmented image signal intensity in the caudate head and lateral globus pallidus internus with Unified Dystonia Rating Scale score (UDRS, $p < 0.001$, Fig. 3; Online Resource Table 4). No correlations were found with age, disease duration or Unified Huntington Disease Ratings Scale (UHDRS).

To determine whether the association between T1w GM signal intensity in the caudate head and lateral GPi occurred secondary to the segmentation and smoothing process, manual assessment of caudate head and lateral globus pallidus was performed. This showed that the association between T1w signal intensity and UDRS score was present in raw images (caudate: $r = 0.752$, $p = 0.019$, lateral GPi: $r = 0.735$, $p = 0.015$), and these associations strengthened with subsequent segmentation and smoothing (Online Resource Figure 7). ROI analysis of the caudate head and lateral GPi on qT2 maps failed to show any correlation ($r =$

0.28, $p = 0.465$ and $r = 0.1$, $p = 0.78$, respectively—data not shown). In addition, repeating ROI measurements in the dentate and GPi showed that intra-rater variability was unlikely to have contributed to these findings with the ICC being 0.942 (95 % CI 0.847, 0.984) and 0.945 (95 % CI 0.855, 0.985), respectively.

Discussion

This study significantly expands our knowledge of the neuropathology of neuroferritinopathy. It also provides vital data on the utility of voxel-based imaging techniques which may inform further studies aiming to determine desperately needed radiological markers of disease severity for other NBIA disorders.

Despite being a T1-based technique, grey matter (GM) segmented VBM showed increased T1 voxel intensity within deep grey matter structures such as the thalamus and globus pallidus, extending into the extranuclear white matter together with the dentate nucleus and surrounding region of the posterior cerebellar lobe suggestive of iron deposition; the same regions identified by previous T2 and T2* manual clinical assessments [3, 15, 16] (Online Resource Table 5). We also detected significant T1w changes within the lingual gyrus, a region which has not previously been associated as a region of iron deposition in neuroferritinopathy. Abnormalities within the lingual gyrus are particularly interesting given their role and relationship to tasks involving verbal fluency [17], a cognitive domain particularly impaired in patients with neuroferritinopathy [18]. This finding supports clinical observations that iron deposition can occur in some discrete cortical regions in neuroferritinopathy [3,19].

An inverse VBM analysis for decreased grey matter voxel intensity (suggestive of regional atrophy) revealed no differences between groups, confirming that cortical atrophy is not a feature of the 460InsA genotype of neuroferritinopathy. White matter segmented VBM did detect cerebellar atrophy, which we confirmed through a detailed manual ROI assessment of the cerebellum (Online Resource Figure 1). Cerebellar atrophy has been described in other genotypes of neuroferritinopathy, though clinical, radiological, and pathological features suggestive of cerebellar involvement have not been described in the 460InsA genotype before [22], and clinically our patients had no clinical features of ataxia. Our data show that cerebellar atrophy is a feature of the 460InsA genotype of neuroferritinopathy, further expanding the phenotype.

Clinical correlates

Regression analysis revealed a correlation between T1w signal intensity in the caudate and lateral globus pallidus with UDRS score. Importantly, manual ROI analysis of raw T1-weighted images revealed this association to be due to a positive correlation in the caudate and a negative correlation in the lateral GPi (Online Resource Figure 7). There was no association with any region and UHDRS score, which may have been due to only a minority of patients ($n = 3$) having chorea as a dominant phenotype. Alternative explanations may also include that chorea may have been masked by medication (not recorded), or speculatively, our data may suggest that the pathogenesis of chorea in neuroferritinopathy is not directly mediated by focal iron accumulation or cavitation. Increasing T1w intensity in

the caudate nucleus is highly suggestive of increased iron or another paramagnetic agent such as manganese or methaemoglobin [20]. McNeil et al. in a previous study failed to detect an association between UDRS score and caudate $R2^*$ values, which is a more sensitive method of detecting iron deposition [15], suggesting that the T1w signal in our study may be indicative of additional paramagnetic substances; further neurochemical and pathological assessment is vital to define this. The correlation between T1w signal and UDRS score was, however, only present after removing the single patient with caudate cavitation. Cavitation indicates loss of the main tissue structure, representing a very different tissue state to the rest of the cohort, significantly confounding the data. Cavitation of the caudate is rare in neuroferritinopathy, and seen in only a single case of over 50 MRI scans of performed in our Centre [15] and is therefore unlikely to alter the utility of this finding.

Progressive T1w hypointensity in the lateral globus pallidus suggestive of cavitation also correlated to UDRS score. Cavitation of the globus pallidus in contrast to the caudate is common, and seen in 52 % of patients [3]. This cavitation has hindered the assessment of grey matter tissue changes in previous ROI based studies [15], and its association with clinical disease severity is, therefore, a further novel finding of our study (Online Resource Table 5). The globus pallidus functions as a control centre for basal ganglia function, and is directly implicated in the pathology of dystonia [21]; the dominant motor feature of neuroferritinopathy [22]. Given that iron accumulation begins in the globus pallidus in infancy in neuroferritinopathy, prior to the onset of symptoms, and before the onset of cavitation [16], our findings support the need for early treatment to prevent iron accumulation and subsequent cavitation in patients.

We also detected significant cerebellar atrophy in our cohort, without any patient exhibiting clinical features of ataxia. Understanding the temporal relationship of cerebellar atrophy to disease may help rectify this association: If the development of cerebellar atrophy echoes that of iron deposition (occurring decades before clinical presentation) [16], then it may suggest that the slow degeneration of the cerebellum may be compensated for by telencephalic connections during development. Alternatively, if cerebellar atrophy occurs alongside the development of symptoms, then it may further support the role of cerebellar connections in the development of dystonia [23]. Longitudinal imaging studies over coming years are likely to help rectify this association. We also sought to determine why VBR failed to detect any significant changes between groups. VBR utilises $R2$ data ($R2 = 1/T2$), which was expected to be far more sensitive method than qualitative T1w imaging in detecting iron within the brain [24–26]. As there is a greater difference in $T2$ relaxation times between grey matter and CSF than between the $T1$ relaxation times of the same tissues [27], our data suggest that the CSF incorporated into grey matter regions through cavitation negates the $T2$ signal hypointensity caused by iron deposition. In contrast, signal changes within the dentate and GPi were apparent in raw T1w images, persist through segmentation and smoothing and are detected by VBM (Online Resource Figure 2, 4). These findings are highly likely to be of assistance for future studies using voxel-based techniques in NBIA disorders.

Summary

We conclude that neuroferritinopathy can be classified as a focal brain iron accumulation disorder. We determine that cerebellar atrophy is a feature of the 460InsA genotype, and that the lingual gyrus is affected in the disease with potential implications for the recently described cognitive impairment in patients. We also show that T1w hyperintensity in the caudate, and T1w hypointensity in the lateral globus pallidus correlate with clinical disease severity, which generate important questions with regards to the role of iron in disease severity, and suggest regions and processes of critical importance in the pathogenesis of the disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Grey matter segmented VBM in neuroferritinopathy; patients vs controls

Images are in neuroradiological orientation. Analysis performed for patients > controls at thresholded at a FWE $p < 0.05$. (a) Changes in grey matter are shown on a T1w smoothed template brain. (b) Glass brain view. Corresponding Talaraich co-ordinates are provided in Online Resource Table 1.

Figure 2.

White matter segmented VBM; pts vs controls. Images are in neuroradiological orientation. Analysis performed for patients > controls at thresholded at a FWE $p < 0.05$. (a) Changes in white matter are shown on a smoothed T1w template brain. (b) Glass brain view. Corresponding Talairach co-ordinates are provided in Online Resource Table 2.

Figure 3.

Grey matter segmented VBM correlated with UDRS score. Images are in neuroradiological orientation. Analysis performed with an uncorrected threshold of $P < 0.001$. (a) Changes in grey matter are shown on a smoothed T1w template brain (B) negative correlation with UDRS scores. Corresponding Talairach co-ordinates are provided in Online Resource Table 4.

Table 1

Summary of subject information of the 10 patients with the 460InsA genotype of neuroferritinopathy in the study (UDRS Unified Dystonia Rating Scale, UHDRS Unified Huntington's Disease Rating Scale)

Patient	Sex	Age onset	Disease duration (years)	Dominant phenotype	Cerebellar ataxia	UDRS score	UHDRS Score	Serum iron	Serum ferritin
1	F	41	7	Parkinsonism and dystonia	None	9.5	19	18	21
2	F	41	15	Dystonia	None	6.5	10	15	9
3	F	42	7	Dystonia	None	5	7	16	6
4	F	59	3	Dystonia	None	11.5	17	16	11
5	M	57	5	Chorea	None	4.5	34	17	5
6	M	57	7	Chorea	None	3	22	9	38
7	M	39	8	Dystonia	None	3.5	9	15	36
8	F	41	19	Dystonia	None	27.5	60	14	11
9	M	62	8	Chorea	None	9.5	30	9	4
10	M	58	12	Dystonia	None	21	33	12	15
Mean		48.5	9.1 (4.8)			10.2 (8.1)	24.1 (16.0)	14.1 (3.1)	15.6 (12.3)