

Published in final edited form as:

Arch Neurol. 2007 March ; 64(3): 371–376. doi:10.1001/archneur.64.3.371.

Voxel-based morphometry in frontotemporal lobar degeneration with ubiquitin-positive inclusions with and without progranulin mutations

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Abstract

Background—Mutations in the progranulin gene (*PGRN*) have recently been identified as a cause of frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U) in some families.

Objective—To determine whether there is a difference in the patterns of atrophy in cases with FTLD-U with and without a mutation in *PGRN*.

Design—Case control study

Setting—Brain bank of a tertiary care medical center

Patients—All subjects that had screened positive for mutations in *PGRN* and had a volumetric MRI were identified (n=8, *PGRN* (+)). Subjects were then matched by clinical diagnosis to a group of eight subjects with a pathological diagnosis of FTLD-U that had screened negative for mutations in *PGRN* (*PGRN* (-)). All subjects were then age and gender-matched to a control subject.

Main outcome Measures—Voxel-based morphometry was used to assess the patterns of grey matter atrophy in the *PGRN* (+) and (-) groups compared to controls, and compared to each other.

Results—The *PGRN* (+) group showed a widespread and severe pattern of grey matter loss predominantly affecting the frontal, temporal and parietal lobes. In comparison, the *PGRN* (-) group showed a less severe pattern of loss restricted mainly to the temporal and frontal lobes. On direct comparison the *PGRN* (+) group showed greater loss in the frontal and parietal lobes compared to the *PGRN* (-) group.

Conclusions—This study suggests that *PGRN* mutations may be associated with a specific and severe pattern of cerebral atrophy in subjects with FTLD-U.

Keywords

Frontotemporal dementia; Voxel-based morphometry; Ubiquitin; Dentate; Progranulin

INTRODUCTION

There are three well recognized clinical variants of frontotemporal lobar degeneration: behavioral variant frontotemporal dementia (bvFTD); progressive non-fluent aphasia (PNFA); and semantic dementia (SD)¹. The bvFTD is characterized by behavioral and personality changes and executive dysfunction, while the other two variants are aphasic syndromes. Pathologically the frontotemporal lobar degenerations can be separated in those with tau-positive inclusions and those with ubiquitin-positive inclusions². Two recent studies have demonstrated that mutations in the progranulin gene (*PGRN*) are associated with frontotemporal lobar degeneration with ubiquitin-positive inclusions (FTLD-U)^{3, 4}.

The aim of this study was to determine if subjects with FTLD-U and a mutation in *PGRN* have a signature pattern of grey matter atrophy. We hypothesized that the pattern of atrophy in FTLD-U subjects with a mutation in *PGRN* would be no different from the pattern of atrophy of those without a mutation.

DESIGN AND METHODS

Subjects

We identified six subjects with a pathological diagnosis of FTLD-U from our autopsy database located at the Mayo Clinic in Rochester that had frozen brain tissue or stored DNA blood which had screened positive for mutations in *PGRN* (*PGRN* (+)) and which had a useable volumetric head MRI scan. Two additional cases without pathological confirmation were included: each had screened positive for mutations in *PGRN* and had pathological confirmation in multiple siblings.

All cases had been prospectively studied in our Alzheimer's Disease Research Center (ADRC) or Alzheimer's Disease Patient Registry (ADPR). The historical records of all cases were reviewed by an expert in neurodegenerative diseases (KAJ) for the abstraction of data, including gender, age at onset, illness duration, and short test of mental status (STMS)⁵, and the signs and symptoms recorded in the historical records by the treating physician up to the time of MRI scan (Table 1). We also abstracted information on whether or not behavioral features, parkinsonism, or significant language impairment developed prior to death. Behavioral features was defined as any change in the subjects behavior or personality; parkinsonism as the presence of two or more of tremor, bradykinesia, rigidity, or postural instability; and language impairment as any loss of syntax, fluency, comprehension, word-finding difficulties, or anomia. Each of the *PGRN* (+) subjects was then matched by clinical diagnosis to a subject that had screened negative for mutations in *PGRN* (Table 1). Six of the *PGRN* (+) subjects were diagnosed with bvFTD and were therefore matched to *PGRN* (-) subjects with a clinical diagnosis of bvFTD; one *PGRN* (+) subject had SD and was matched to a *PGRN* (-) subject with SD; and one *PGRN* (+) subject had left-sided corticobasal syndrome (CBS) and was therefore matched to a *PGRN* (-) subject with left-sided CBS. This matching was important since the different clinical phenotypes of FTLD-U may themselves have different patterns of grey matter atrophy⁶. The aim of this study was to assess the effects of the *PGRN* mutation independent of clinical phenotype. Each of the *PGRN* (+) and (-) subjects were age and gender matched to a healthy normal control.

Genetic analysis

The 12 coding exons of *PGRN* were amplified by PCR using our previously published primers and protocol³. Novel PCR primers flanking the non-coding exon 0 and the 3'untranslated region (UTR) of *PGRN* were also developed. Standard 25 μ l PCR reactions were performed using Qiagen PCR products (Qiagen, Valencia, CA) and a final concentration of 0.8 μ M for each primer. The *PGRN* exon 0 and 3'UTR fragments were cycled using touchdown protocols of 70–55°C and 58–48°C respectively. PCR products were purified with Multiscreen plates (Millipore, Billerica, MA) and sequenced in both directions on an ABI 3730 with the Big Dye chemistry following manufacturer's protocol (Applied Biosystems, Foster City, CA). Genomic characterization of *PGRN* was performed as previously described⁷.

Pathological analysis

All cases had undergone extensive neuropathological examination with routine and immunohistochemical stains as previously described in detail². A pathological diagnosis of FTL-D-U was made on the basis of finding neuronal loss, spongiosis and gliosis in frontotemporal neocortex consistent with frontotemporal lobar degenerative changes, as well as, the presence of ubiquitin-positive intraneuronal inclusions and neurites in the neocortex or the dentate granule cell layer of the hippocampus. Any case with evidence of motor neuron disease was excluded as previously described⁸.

Image analysis

MR studies were performed at 1.5T with a standardized imaging protocol that included a coronal T1-weighted 3-dimensional volumetric spoiled gradient echo (SPGR) sequence with 124 contiguous partitions and 1.6mm slice thickness (22 \times 16.5cm FOV, 25° flip angle). Voxel-based morphometry (VBM) was used to assess group differences, implemented using SPM2 (<http://www.fil.ion.ucl.ac.uk/spm>)^{9, 10}. The image processing steps have previously been described in detail^{10, 11}. Briefly, all images were spatially normalized to a customized template created from all subjects in the study. The spatial normalization of each image was optimized by matching the initial grey matter segmentation with the grey matter prior and applying the parameters to the whole head. Images were then segmented using customized prior probability maps, modulated, and smoothed with a 10mm FWHM smoothing kernel. Grey matter differences were assessed between both the *PGRN* (+) and (–) groups and the control group at a statistical threshold of $p < 0.05$ after correction for multiple comparisons using the false discovery rate (FDR). Direct comparisons between the *PGRN* (+) and (–) groups were performed at a less stringent threshold of $p < 0.01$ uncorrected for multiple comparisons.

RESULTS

Subjects

Table 1 details the clinical features of the *PGRN* (+) and (–) subjects. The majority had a clinical diagnosis of bvFTD, although one subject in each group also had a diagnosis of SD and CBS. The demographic features in each of the groups are compared in Table 2. There was no significant difference in the gender ratio or age at scan across all three subject groups. In addition, there was no significant difference between the *PGRN* (+) and (–) subjects in age at onset, total illness duration, time from onset to scan, or STMS. All subjects were Caucasian and non-Hispanic in origin. The subjects with a mutation in *PGRN* all had an autosomal dominant pattern of inheritance while only two of the subjects that tested negative for *PGRN* mutations had any suggestion of a family history. Additional clinical features developed after the time of the MRI scan (Table 1). The *PGRN* (+) subjects were more likely to develop language impairment (90% vs. 20%; $p = 0.03$) and parkinsonian symptoms (90% vs. 30%; $p = 0.05$) compared to the *PGRN* (–) subjects prior to death.

Mutations

Sequencing analyses showed that five different *PGRN* mutations were present in our cohort: c.154delA (p.Thr52HisfsX2), c.910_911insTG (p.Trp304LeufsX58), c.1395_1396insC (p.Cys466LeufsX46), c.1145delC (p.Thr382SerfsX30), c.138+1G>A (IVS1+1G>A).

VBM analysis

The *PGRN* (+) group showed a widespread and severe pattern of grey matter loss compared to the control group (Figure 1). The most severe regions of loss were observed in the inferior and middle frontal gyri, the medial frontal lobe, the temporal lobes, and the parietal lobes. The patterns of loss were bilateral, although the frontal and parietal lobe loss appeared to be slightly greater in the right hemisphere. In comparison, the regions of grey matter loss identified in the *PGRN* (-) group were relatively restricted to the temporal and frontal lobes (Figure 1). It is also noticeable from Figure 1 that the patterns of grey matter loss were less severe than those observed in the *PGRN* (+) subjects. The temporal lobe loss spread from the anterior temporal pole, back to involve the medial and inferior temporal regions, and the posterior inferior temporal gyri. The frontal lobe loss was observed in the medial frontal cortex, the inferior and middle frontal gyri, and the orbitofrontal cortex. The insula was also involved. As in the *PGRN* (+) group the frontal lobe loss was slightly greater on the right.

A direct statistical comparison was performed between the two disease groups (Figure 2). The *PGRN* (+) group showed significantly greater grey matter loss in the superior frontal lobes and the parietal lobes, predominantly on the right, than the *PGRN* (-) group (uncorrected, $p < 0.01$). No regions showed greater involvement in the *PGRN* (-) group compared to the *PGRN* (+) group.

COMMENT

This study has demonstrated differences in the severity and distribution of grey matter atrophy, as well as clinical differences, in FTL-D-U subjects with a mutation in *PGRN* compared to those without a mutation.

Since both groups had an identical pathological diagnosis of FTL-D-U and were matched for clinical diagnosis we had hypothesized that the patterns of atrophy would be similar in those with and without a mutation in *PGRN*. However, our findings do demonstrate differences between the *PGRN* (+) and (-) groups. The *PGRN* (-) subjects showed a pattern of temporal and frontal lobe volume loss as we have previously reported in FTL-D-U^{12, 13}. However, the *PGRN* (+) subjects showed a strikingly more severe and widespread pattern of grey matter loss than the *PGRN* (-) subjects. The most severe regions of loss were identified in the temporal and frontal lobes, but also in the parietal lobe which was relatively spared in the *PGRN* (-) group. A direct comparison between the two groups showed that the *PGRN* (+) subjects had a greater degree of frontal and parietal atrophy than the *PGRN* (-) subjects. The fact that these results did not survive a correction for multiple comparisons most likely reflects the heterogeneity present in each group. Similarly, our recent pathology study demonstrated significantly lower brain weights at death in *PGRN* (+) subjects compared to negative subjects (902 ± 160 g vs. 1030 ± 140 g ($p < 0.01$)), and demonstrated greater atrophy of the frontal and parietal lobes in the *PGRN* (+) subjects (manuscript in submission). Furthermore, another pathology study also found that the frontal lobes were the most severely affected region in *PGRN* (+) subjects, although the temporal and parietal lobes were also affected¹⁴. Single subject MRI scans have also shown frontal, temporal and parietal atrophy in subjects with *PGRN* mutations¹⁴⁻¹⁷. While VBM provides a group-level analysis and should not be generalized to single subjects these results could suggest that a severe pattern of atrophy affecting the temporal and parietal and especially frontal lobes may be suggestive of an

underlying mutation in *PGRN*. It is therefore possible that the mutation results in a more malignant form of FTLD. A recent case study did indeed report a large rate of brain atrophy of over 3.3% in a *PGRN* (+) subject¹⁸. This in turn may reflect differences between familial and sporadic forms of FTLD-U since the majority of the *PGRN* (-) cases had no family history. Indeed, greater severity of Alzheimer's disease (AD) pathology is found in genetically determined familial AD¹⁹, and MRI studies have also shown that younger onset familial cases of AD show a more widespread pattern of atrophy compared to older onset sporadic cases^{20, 21}.

While there were differences between the *PGRN* (+) and (-) groups it is notable that they both showed involvement of the temporal lobe, particularly the posterior temporal gyri. In two previous VBM studies from separate brain banks we identified a region of severe loss affecting the posterior temporal lobe in FTLD-U and hypothesized that this may be a signature pattern of this pathology^{12, 13}. This study concurs with those results and further suggests that the signature pattern is related to the pathology of FTLD-U and is perhaps independent of the genetic cause of the disease.

The pattern of loss in the *PGRN* (+) subjects was relatively symmetric, although with slightly greater involvement of the right frontal lobe. However, the lateralization observed in the *PGRN* (+) subjects may be due to the particular subjects, and particular clinical diagnoses, included in this analysis. Ongoing MRI studies on *PGRN* at our institution with a larger number of *PGRN* (+) subjects show greater involvement of the right hemisphere in some, while others show the opposite pattern. Indeed, previous studies reporting on single subject MRI have noted both left-sided¹⁷ and right-sided^{15, 18} *PGRN* (+) cases. Therefore although asymmetry does appear to be a feature of the mutation there appears to be no particular dominant hemisphere.

In our series of *PGRN* (+) subjects bvFTD was more prevalent than other syndromes, occurring in 75% of the subjects. This is quite similar to the frequency typically reported in FTLD (Miller). These patients presented with personality change, abulia, loss of attention and executive dysfunction¹. In addition, bvFTD has been described in other clinical series on *PGRN*^{15, 17} and was found to be the most prevalent clinical diagnosis¹⁵. One of our subjects was found to have a diagnosis of CBS²² at the time of scan which has also been described in another family with *PGRN* mutations¹⁶. This subject had been diagnosed with idiopathic Parkinson's disease before presentation at our institution. This is important and suggests that in patients with an initial diagnosis of idiopathic Parkinson's disease, and autosomal dominant family history, a mutation in *PGRN* should be considered. The one aphasia subject in our cohort had features most in-keeping with the SD syndrome¹. This subject had a fluent aphasia with prominent anomia and comprehension problems. This is different from previous *PGRN* studies in which the aphasia was more in-keeping with the PNFA variant of FTLD^{14, 17}. The *PGRN* (-) subjects were matched to the *PGRN* (+) subjects for syndromic diagnosis and therefore 75% were bvFTD, with one each of CBS and SD. As in the bvFTD *PGRN* (+) subjects, the *PGRN* (-) subjects had personality changes, apathy, anhedonia and executive dysfunction. The *PGRN* (-) SD subject had anomia, circumlocutions, and behavioral changes¹.

Although our subjects were matched by clinical syndromes at the time of MRI scan, and showed no differences in clinical severity and illness duration, the *PGRN* (+) subjects were more likely to develop language impairment (90%) and parkinsonism (90%) later in the disease course than the *PGRN* (-) subjects (20% language, 30% parkinsonism). However, the prevalence of behavioral symptoms was similar between the *PGRN* (+) (75%) and *PGRN* (-) (88%) groups. Therefore the later development of significant parkinsonism and language impairment in subjects with an autosomal dominant family history may be a clue to a mutation in the *PGRN* gene and should prompt the clinician to consider genetic screening. Language impairment and parkinsonism was also found to be more common in the *PGRN* (+) subjects

in our pathological study that included a somewhat different cohort (manuscript submitted). In some of those subjects Lewy Bodies were found which further supports the importance of considering *PGRN* mutations in autosomal dominant familial Parkinson's disease or in subjects in which parkinsonism is a significant feature. The language impairment was variable with the development of features of both expressive and receptive deficiencies. One feature of note was that the subjects with *PGRN* (+) mutations frequently became mute. Given the more widespread volume loss seen in the *PGRN* (+) subjects these developments should be of no surprise. We hypothesize one of two scenarios: Firstly, anatomical structures involved with the development of parkinsonism and aphasia eventually become affected in the *PGRN* (+) subjects, or they are already affected given the widespread atrophy, but there exists a threshold effect whereby there has to be a certain amount of pathway or anatomic destruction present before the clinical features emerge.

The major strengths of this study were the fact that the *PGRN* (+) and (–) groups were matched in terms of both clinical and pathological diagnosis, and demographic features. The technique of VBM is also unbiased and allows an assessment of grey matter loss across the whole brain without having to make any a priori assumptions concerning which structures to assess. The limitations include the fact that the number of subjects in each group was relatively small. It is however extremely difficult to find subjects with a mutation in *PGRN*, artifact free MRI acquired with a standardized protocol, and an autopsy confirmed diagnosis of FTLD-U. Despite the small numbers we still observed well defined patterns of grey matter loss that correlate closely to our previously published findings in larger groups of subjects with FTLD-U^{12, 13}. The results also closely match our recent pathological findings in *PGRN* (manuscript in submission). However, our cohort represented only five different *PGRN* mutations and therefore the results may not be generalizable to subjects with other *PGRN* mutations.

In summary, this study suggests that *PGRN* mutations may be associated with more severe patterns of cerebral atrophy, particularly involving the frontal and parietal lobes, in subjects with FTLD-U. The subjects with *PGRN* mutations also developed more parkinsonism and language impairments than the *PGRN* (–) subjects. It will be important for these results to be validated and correlated to detailed clinical information in a larger group of subjects.

ACKNOWLEDGEMENTS

This study was supported by the NIH Roadmap Multidisciplinary Clinical Research Career Development Award Grant (K12/NICHD)-HD49078 (KAJ), by grants P50 AG16574, U01 AG06786 and R01 AG11378 from the National Institute on Aging, Bethesda MD, NIRG-03-4842 from the Alzheimer's Association, and the generous support of the Robert H. and Clarice Smith and Abigail Van Buren Alzheimer's Disease Research Program of the Mayo Foundation, U.S.A. RR is a postdoctoral fellow of the Fund for Scientific Research Flanders (FWO-F).

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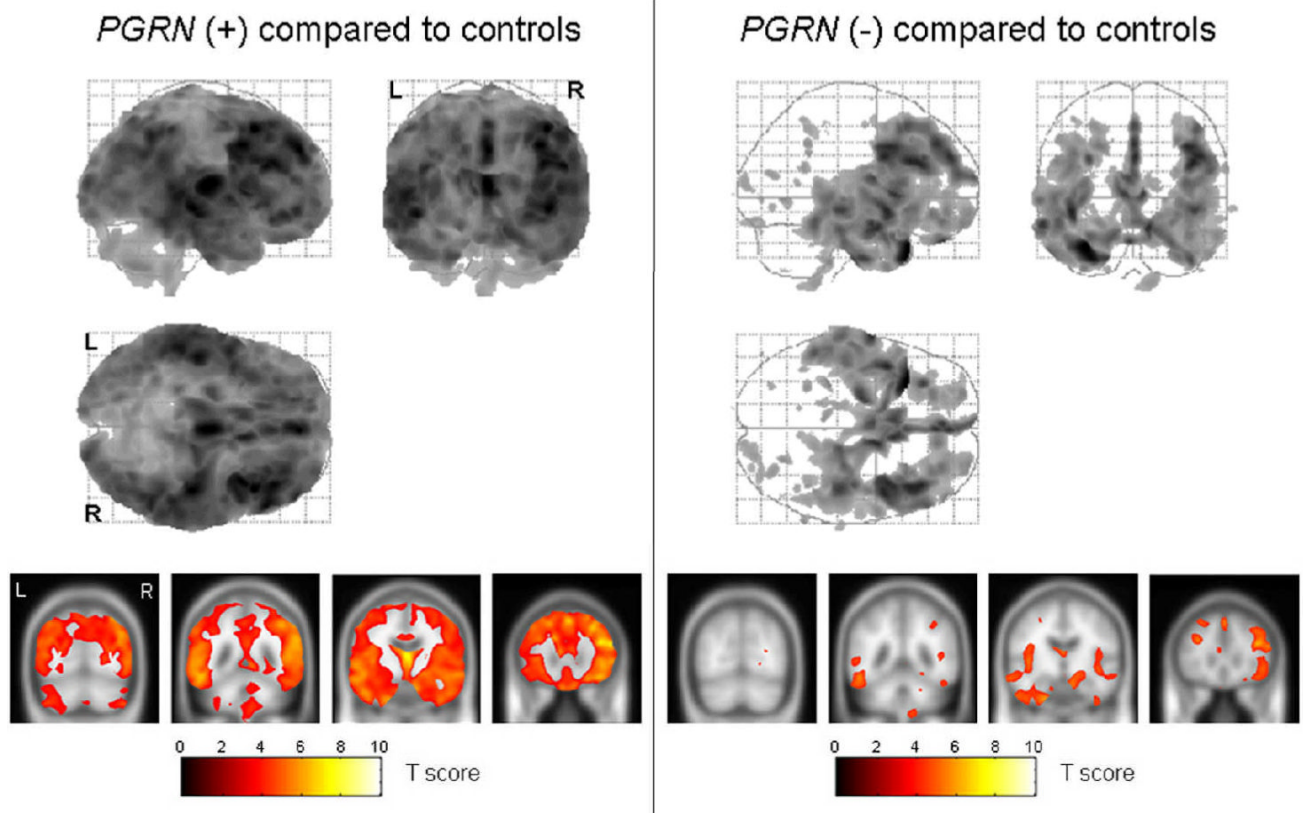
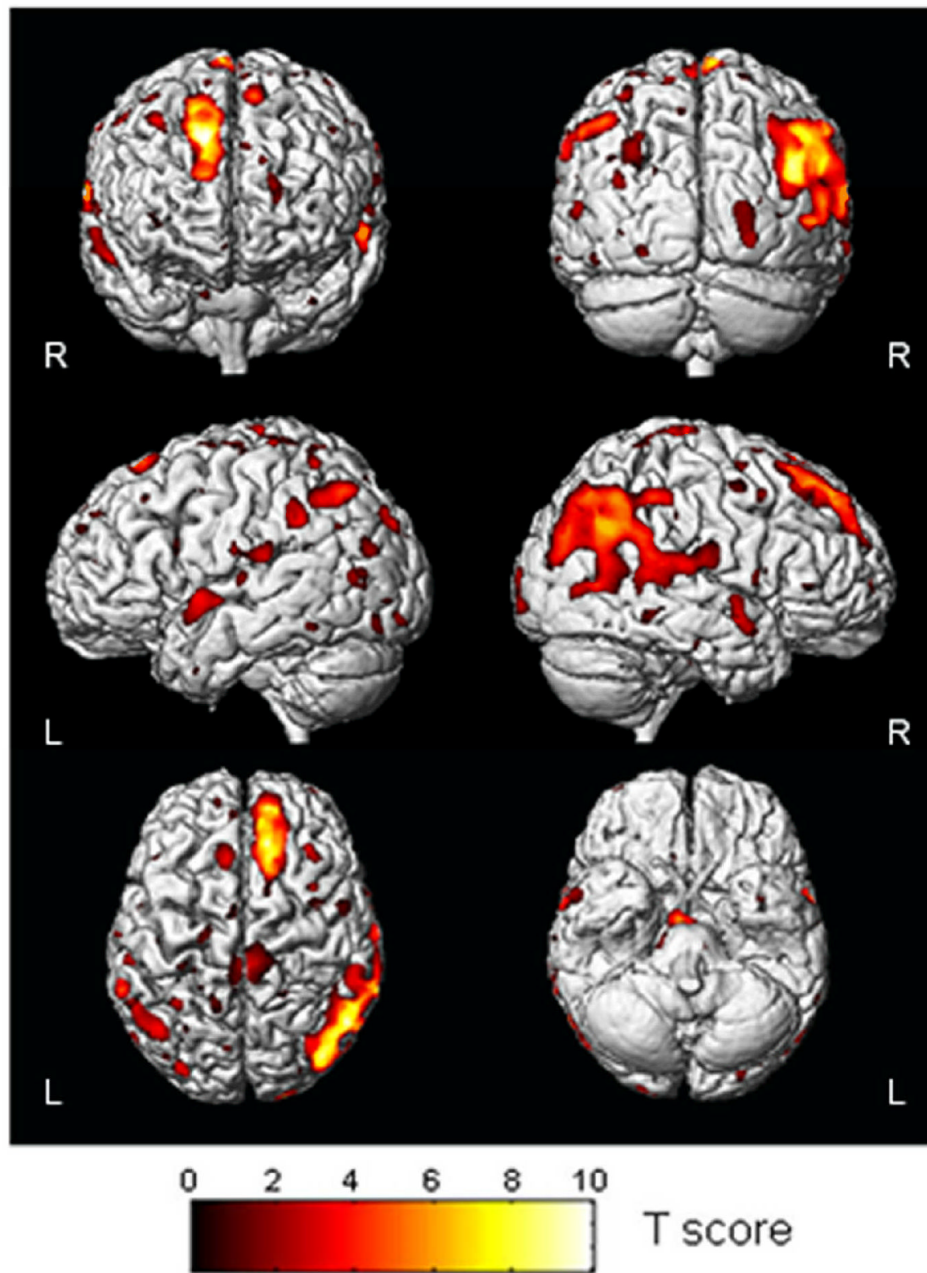


Figure 1.

Grey matter volume differences were assessed between both the *PGRN* (+) and (-) groups and the control group using VBM at a statistical threshold of $p < 0.05$ after correction for multiple comparisons. The patterns of grey matter loss in the *PGRN* (+) group compared to controls are shown on the left, while the patterns of grey matter loss in the *PGRN* (-) group compared to controls are shown on the right. The results of each comparison are shown both on a 3D glass brain render (top) and overlaid on the smoothed customized template at representative slices through the frontal ($y=30$), temporal ($y=-10$ and $y=-45$) and parietal lobes (-70) (bottom). The results show a more widespread and severe pattern of grey matter loss in the *PGRN* (+) group when compared to controls, than the *PGRN* (-) group when compared to controls.

PGRN (+) compared to *PGRN (-)***Figure 2.**

3D surface renders showing the results of a direct VBM statistical comparison performed between the *PGRN (+)* and *(-)* subjects. The highlighted areas show the regions of the brain that have reduced grey matter volume in the *PGRN (+)* group compared to the *PGRN (-)* group. No regions of the brain showed reduced grey matter volume in the *PGRN (-)* group compared to the *PGRN (+)* group. The results are shown at a statistical threshold of $p < 0.01$, uncorrected for multiple comparisons, and show greater grey matter loss in the frontal and parietal lobes in the *PGRN (+)* group than the *PGRN (-)* group.

Table 1

Clinical features of *PGRN* (+) and (-) subjects

Sex	Age at onset	Illness duration (years)	STMS at scan	*Diagnosis at the time of Scan	Prominent symptoms up to the time of MRI scan	Symptoms present over entire disease course (+ = present, 0 = absent)		
						Parkinsonism	Language	Behavior
<i>PGRN</i> (-)								
1	F	64	25	bvFTD	Personality change, apathy, loss of interest	0	0	+
2	F	62	34	bvFTD	Anhedonic, lack of initiative, poor judgment	0	0	+
3	M	45	26	bvFTD	Personality change, withdrawn, lack of initiative	0	0	+
4	F	68	NA	CBS	Parkinsonism, L>R limb apraxia	+	0	0
5	F	64	34	bvFTD	Changes in behavior and personality	+	0	+
6	M	51	30	bvFTD	Executive dysfunction, decreased personal hygiene, inappropriate laughter, compulsive behaviors	+	+	+
7	F	59	26	SD	Difficulty with naming of people and objects, and circumlocutions, behavioral changes	0	+	+
8	F	77	31	bvFTD	Personality change, executive dysfunction, & paranoia	0	0	+
<i>PGRN</i> (+)								
9	M	69	28	bvFTD	Executive dysfunction, loss of attention, & memory loss	+	+	+
10	M	60	31	bvFTD	Personality change, executive dysfunction & memory loss	+	+	+
11	F	52	23	bvFTD	Personality change, executive dysfunction, abulia, memory loss, and decrease word output & fluency	+	+	+
12	M	49	27	CBS	Parkinsonism, L>R limb apraxia	+	+	0
13	M	56	34	bvFTD	Personality change	+	0	+
14 x	F	56	32	bvFTD	Personality change, executive dysfunction & memory loss	+	+	+
15 x	F	61	NA	SD	Difficulty with naming of people, places, and things and comprehension problems	0	+	0
16	F	56	22	bvFTD	Executive dysfunction	+	+	+

STMS = Short Test of Mental Status Score

Using Fishers Exact test $p = 0.05$ for parkinsonism $PGRN (-)$ v $PGRN (+)$ (30% vs. 90%) and $P = 0.02$ for language (20% vs. 90%) using a one tail test (assuming that the feature would be more frequent in the $PGRN (+)$ group).

* Diagnosis based on Consensus criteria^{1,22}

χ^2 Subjects still alive

Table 2

Demographics of all subjects

	<i>PGRN</i> positive (n=8)	<i>PGRN</i> negative (n=8)	Controls (n=16)
Gender ratio M/F	4/4	2/6	6/10
Age at disease onset	57.4 (6.1)	61.3 (9.9)	NA
Total illness duration	6.7 (1.0) [*]	7.5 (4.0)	NA
Age at scan	60.1 (6.6)	64.5 (9.5)	62.4 (6.9)
Time from disease onset to time of MRI scan	2.7 (1.0)	3.2 (1.9)	NA
STMS at scan [†]	28.1 (4.5)	29.4 (3.8)	36.3 (2.2)

Results are shown as mean (standard deviation). Total illness duration represents time from disease onset to time of death. STMS = Short Test of Mental Status Score. Patient demographics were compared using the Mann Whitney U test with significance set at $p < 0.05$. Gender ratios were compared using Chi-square test.

* Total illness duration was only available for the six subjects that have died

[†] STMS data was available at the time of scan for seven *PGRN* positive and seven *PGRN* negative subjects