Asymmetry of post-mortem neuropathology in behavioural-variant frontotemporal dementia

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Antemortem behavioural and anatomic abnormalities have largely been associated with right hemisphere disease in behavioural-variant frontotemporal dementia, but post-mortem neuropathological examination of bilateral hemispheres remains to be defined. Here we measured the severity of post-mortem pathology in both grey and white matter using a validated digital image analysis method in four cortical regions sampled from each hemisphere in 26 patients with behavioural-variant frontotemporal dementia, including those with frontotemporal degeneration (i.e. tau = 9, TDP-43 = 14, or FUS = 1 proteinopathy) or Alzheimer’s pathology (n = 2). We calculated an asymmetry index based on the difference in measured pathology from each left-right sample pair. Analysis of the absolute value of the asymmetry index (i.e. degree of asymmetry independent of direction) revealed asymmetric pathology for both grey and white matter in all four regions sampled in frontototemporal degeneration patients with tau or TDP-43 pathology (P ≤ 0.01). Direct interhemispheric comparisons of regional pathology measurements within-subjects in the combined tauopathy and TDP-43 proteinopathy group found higher pathology in the right orbitofrontal grey matter compared to the left (P < 0.01) and increased pathology in ventrolateral temporal lobe grey matter of the left hemisphere compared to the right (P < 0.02). Preliminary group-wise comparisons between tauopathy and TDP-43 proteinopathy groups found differences in patterns of interhemispheric burden of grey and white matter regional pathology, with greater relative white matter pathology in tauopathies. To test the association of pathology measurement with ante-mortem observations, we performed exploratory analyses in the subset of patients with imaging data (n = 15) and found a direct association for increasing pathologic burden with decreasing cortical thickness in frontotemporal regions on ante-mortem imaging in tauopathy (P = 0.001) and a trend for TDP-43 proteinopathy (P = 0.06). Exploratory clinicopathological correlations demonstrated an association of socially-inappropriate behaviours with asymmetric right orbitofrontal grey matter pathology, and reduced semantically-guided category naming fluency was associated asymmetric white matter pathology in the left ventrolateral temporal region. We conclude that pathologic disease burden is distributed asymmetrically in behavioural-variant frontotemporal dementia, although not universally in the right hemisphere, and this asymmetry contributes to the clinical heterogeneity of the disorder. The basis for this asymmetric profile is enigmatic but may reflect distinct species or strains of tau and TDP-43 pathologies with propensities to spread by distinct cell- and region-specific mechanisms. Patterns of region-specific pathology in the right hemisphere as well as the left hemisphere may play a role in antemortem clinical observations, and these observations may contribute to antemortem identification of molecular pathology in frontotemporal degeneration.

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Received April 28, 2017. Revised August 18, 2017. Accepted October 14, 2017.
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Introduction

The behavioural-variant of frontotemporal dementia (bvFTD) (Rascovksy et al., 2011) is a clinical syndrome characterized by a social comportment disorder often associated with cognitive difficulties, largely in executive functioning. BvFTD is the most common clinical phenotype caused by frontotemporal lobar degeneration (FTLD) neuropathology (Forman et al., 2006) and is a common form of ‘young-onset’ dementia in patients under the age of 65 (Rossor et al., 2010). Less commonly, atypical variants of Alzheimer’s disease neuropathology, can also present with a bvFTD clinical syndrome (Forman et al., 2006; Osenkoppole et al., 2015).

Neuroimaging studies in FTLD report varying degrees of asymmetry in cortical disease burden in patients with a specific molecular pathology (Whitwell et al., 2005; Rohrer et al., 2011), including those with underlying tauopathy (FTLD-Tau) or TDP-43 proteinopathy (FTLD-TDP) (Mackenzie et al., 2010) and those constrained to clinical bvFTD suggest that the disease may have a propensity for affecting the right hemisphere, particularly ventral frontal regions (Seeley et al., 2008; Whitwell et al., 2009). Further, measures of social cognition in bvFTD, such as behavioural disinhibition (Mychack et al., 2001; Liu et al., 2004; Massimo et al., 2009; Grossman et al., 2010; Eslinger et al., 2011; Powers et al., 2014), loss of empathy and perspective-taking (Rankin et al., 2006; Eslinger et al., 2011; McMillan et al., 2013a; Healey et al., 2015), hyperorality (Woolley et al., 2007) and apathy (Massimo et al., 2009, 2015; Powers et al., 2014) have neuroimaging correlates in frontotemporal regions largely in the right hemisphere. However, patients with bvFTD also frequently have clinical deficits in executive functioning such as category naming fluency, and these impairments appear to be less clearly lateralized, with findings associated with frontal and temporal regions in both right and left hemispheres, depending on the specific task (Rascovksy et al., 2007; Libon et al., 2009; Cook et al., 2014).

Since there are no validated measures to define pathology in bvFTD during life, neuropathological examination and clinical-pathological correlation remain the gold standard to study the anatomic distribution and clinical consequences of abnormal protein aggregations within the nervous system that characterize the neuropathological substrate of this clinical syndrome. With rare exception, neuropathological examination typically assesses a single hemisphere, and moreover uses ordinal ratings of the

Keywords: frontotemporal degeneration; tauopathy; TDP-43 proteinopathy; digital image analysis histology; behavioural variant frontotemporal dementia

Abbreviations: %AO = per cent area occupied; AI = asymmetry index; bvFTD = behavioural-variant frontotemporal dementia; FTLD = frontotemporal lobar degeneration; FTLD-Tau = FTLD with tau pathology; FTLD-TDP = FTLD with TDP-43 pathology; FTLD-FUS = FTLD with FUS pathology; INS = insula; OFC = orbitofrontal cortex; PPA = primary progressive aphasia; SPL = superior parietal lobule; VLT = ventral-lateral temporal lobe
severity of pathology. These factors significantly limit the ability to validate reported asymmetries in antemortem disease burden in FTLD. Thus, there exists a substantial gap in the medical literature regarding the distribution of microscopic FTLD neuropathology within the CNS.

We hypothesize that the microscopic density of FTLD pathology associated with clinical bvFTD is not evenly distributed between cerebral hemispheres and these asymmetries may relate to antemortem clinical features. We also hypothesize that FTLD-Tau has higher white matter pathology compared to FTLD-TDP and this may influence hemispheric asymmetry of disease burden. Here, we provide novel examination of the interhemispheric distribution of post-mortem grey matter and white matter pathology in a relatively large cohort of well-characterized bvFTD patients using a validated method of digital image analysis of histopathology in FTLD (Irwin et al., 2016b). We find that regional pathological analysis of both grey and white matter is asymmetric in bvFTD, but the asymmetry of pathology does not always implicate the right hemisphere, with significant interindividual variation for some regions. Moreover, we find preliminary data that suggest differences in asymmetry in grey and white matter between FTLD-Tau and FTLD-TDP, and we associate these lateralized pathological findings with antemortem clinical and imaging measures associated with bvFTD.

Materials and methods

Patients

Patients followed longitudinally by experienced bvFTD neurologists (H.B.C., R.H., G.A., M.G.) were followed to autopsy at the Penn Center for Neurodegenerative Disease Research (CNDR). Eligible subjects had a clinical diagnosis of bvFTD (CNDR). Subjects with a clinical diagnosis of bvFTD (CNDR). All procedures were performed with informed consent in accordance with the Penn Frontotemporal Degeneration Center. All procedures were performed with informed consent in accordance with local Institutional Review Board guidelines.

Neuropathological examination

Fresh tissue was sampled at autopsy in standardized regions for diagnosis according to a neuroanatomical atlas and fixed overnight in 10% neutral buffered formalin as described (Toledo et al., 2014). Tissue was processed as described (Irwin et al., 2016b), embedded in paraffin blocks and cut into 6 μm sections for immunohistochemical staining for tau, amyloid-β, TDP-43 and α-synuclein with well-characterized antibodies (Toledo et al., 2014). Neuropathological diagnosis was performed by expert neuropathologists (J.Q.T., E.B.L.) using formal criteria (Mackenzie et al., 2010; Montine et al., 2012). We had 25 bvFTD patients with available tissue from both hemispheres (FTLD-TDP = 14, FTLD-Tau = 9, Alzheimer’s disease = 2; Table 1). One additional case with ubiquitin-positive, TDP-43-negative inclusions was additionally stained for fused-in-sarcoma (FUS) protein and found to have FUS proteinopathy (FTLD-FUS). FTLD-Tau group included the following subtypes: Pick’s disease (n = 4), progressive supranuclear palsy (n = 3), corticobasal degeneration (n = 1) and an unclassifiable tauopathy with MAPT mutation (FTDP-17) (Table 1). We analysed the less common neuropathology groups separately (Alzheimer’s disease = 2, FTLD-FUS = 1) and FTLD-FUS was analysed using traditional ordinal assessment only due to the inability to validate digital methods in a single case. Seven FTLD-TDP cases and four cases with Pick’s disease tauopathy were previously reported in models of disease progression in bvFTD (Brettzschneider et al., 2014; Irwin et al., 2016a) and an additional seven FTLD-TDP cases were assigned stages according to this model (Table 1).

Genetic analysis

DNA was isolated from peripheral blood or frozen brain using standard methods. The FTLD-TDP group was genotyped for pathogenic mutations in GRN using PCR as described (Yu et al., 2010) or a custom targeted next-generation sequencing panel for neurodegenerative diseases (MiND-Seq) (Toledo et al., 2013) and the C9orf72 repeat expansion using a modified repeat-primed PCR (Suh et al., 2015). The FTLD-Tau group was genotyped for pathogenic mutations in MAPT using PCR (Irwin et al., 2013) or MiND-Seq. One FTLD-Tau patient had a p.P301L MAPT mutation, three FTLD-TDP patients had a pathogenic GRN mutation and four FTLD-TDP patients had a pathogenic C9orf72 repeat-expansion mutation (Table 1).

Bilateral hemisphere sampling

Fresh tissue was sampled from each hemisphere at the time of autopsy in regions previously associated with bvFTD (Liu et al., 2004; Woolley et al., 2007; Seeley et al., 2008) including the insula (INS), orbitofrontal (OFC), and ventral-lateral temporal (VLT) lobes, as well as a more posterior region in the superior parietal lobule (SPL) expected to have mild pathology to capture the full spectrum of interhemispheric disease burden in bvFTD. Missing data in a minority of cases was due to limited sampling at the time of autopsy (INS = 3, OFC = 3, SPL = 2, VLT = 7). An additional patient (Case 19) had insufficient insula grey matter on one section and was included only for white matter analysis for insula. Sections were stained with immunohistochemistry for the specific primary proteinopathy: phosphorylated TDP-43 for FTLD-TDP (rat monoclonal TAR5P-1D3, p409/410; Ascension) (Neumann et al., 2009), phosphorylated tau for FTLD-Tau or Alzheimer’s disease (AT-8; ThermoScientific) (Mercken et al., 1992) and FUS for FTLD-FUS (anti-FUS; ProteinTech). We also stained Alzheimer’s disease tissue for amyloid-β pathology (Nab228; CNDR) (Lee et al., 2003). To reduce ‘run-to-run’ variation in staining, the left/right hemispheric pair for each region was stained in the same immunohistochemical run.

Digital image analysis of histology

Digital images of histology slides at ×20 magnification were obtained using a Lamina (Perkin Elmer) slide scanning system.
Digital image analysis was performed with Halo digital image software v1.90 (Indica Labs) with validated grey matter sampling and thresholding algorithms for FTLD-Tau and FTLD-TDP (Irwin et al., 2016b). Briefly, a transect-belt sampling method is performed to manually segment representative cortex to reduce bias from under- or over-representation of FTLD pathology in tangentially-oriented tissue (Armstrong and Cairns, 2012). To further reduce sampling bias, we used a user-independent random sampling strategy using random tile placement feature of the program to include 175 m region of interest tiles for 30% of the total area (Supplementary Fig. 1). Finally, intensity thresholding algorithms were applied to regions of interest to detect the total % pixel area occupied (%AO) for tau, TDP-43 or amyloid pathology and the final measurement for each slide was derived from the average of the randomly selected regions of interest. We used a similar approach to sample white matter regions of interest with manual segmentation of adjacent deep white matter tracts on each slide with a rectangular selection tool and then created user-independent random sampling with 175 μm region of interest tiles for 30% of the total area (Supplementary Fig. 1). Since Alzheimer’s disease pathology is largely restricted to grey matter we did not analyse white matter for these cases. Validation of %AO pathology measurements were performed by comparing these data with traditional ordinal scores obtained blinded to quantitative measurements as described (Irwin et al., 2016b) in bvFTD; NA = no staging scheme available for pathological subtype; P/D = Pick’s disease; T/P = progressive supranuclear palsy; R = right-handed; UNCLASS = FTLD-TDP pathology not conforming to FTLD subtype criteria; UNK = unknown handedness.

Clinical data

The clinical records were reviewed by an experienced clinician (D.J.I.), blinded to the pathology data, and the onset of core clinical features of bvFTD (behavioural disinhibition, apathy, loss of empathy, ritualistic behaviour and hyperorality) were extracted, as described (Irwin et al., 2016a). Patients were
evaluated at baseline on average 3.3 years (±2.8) after the reported onset of disease with an average of 5.1 (±3.8) longitudinal visits at our FTD Centre. The average time from last visit to death was 2.1 years (±2.7). All cases met published criteria for bvFTD at presentation (Rascovsky et al., 2011) except three cases, which had a prominent social disorder with additional clinical features (Supplementary Table 1). Case 6 with sporadic FTLD-TDP type-A had prominent apathy and dysexecutive syndrome with parkinsonism and was evaluated only late in the disease course and prior to modern criteria, thus there was insufficient social history to meet full bvFTD criteria; Case 14 with C9orf72-positive FTLD-TDP type-A had a prodromal period with atypical parkinsonism for several years prior to developing a prominent social disorder consistent with bvFTD; and Case 15 with post-mortem CBD tauopathy presented with mixed expressive language and comprehension difficulties at first visit with core bvFTD features emerging soon after and within the first 3 years that remained the most prominent feature throughout disease course. The remaining patients with pathologic mutations (Table 1) all met clinical criteria for bvFTD (Supplementary Table 1) and Case 8 with C9orf72 expansion had co-occurring motor neuron symptoms of amyotrophic lateral sclerosis.

Available neuropsychological test scores for baseline evaluation were obtained from the Penn integrated neurodegenerative disease database (INDD) (Xie et al., 2011). These include Folstein Mini-Mental Status Examination (MMSE), letter-guided category naming fluency (i.e. number of “F” words generated in 60 s), semantically-guided category naming fluency (i.e. number of animals generated in 60 s) and digit-span (i.e. longest number of digits repeated in forward and backward sequences). We obtained normative scores for these tests from an age- and education-matched cohort of healthy control patients in INDD (n = 23) to derive z-scores for patient performance.

**Neuroimaging**

Fifteen patients had antemortem T1 structural MRI data available for analysis. Mean MRI-autopsy interval was 2.8 years (range 0–6 years) (Supplementary Table 5). All volumetric data were acquired on a Siemens 3.0 T Trio scanner at a resolution of 0.98 × 0.98 × 1 mm. MRI data were processed using the ANTS cortical thickness pipeline as previously reported (Tustison et al., 2014). We then computed mean cortical thickness in robust, neuroanatomically-defined regions of interest from a publicly-available atlas (Klein and Tourville, 2012) that map onto the anatomical regions sampled at autopsy, as described previously (Irwin et al., 2016a). To determine the degree of cortical thinning in each region of interest we derived a z-score based on a demographically comparable ageing cohort (54 males, 97 females) without psychiatric or neurological history (age mean = 62.9 ± 7.3, mean education = 15.5 ± 2.9).

**Statistical analysis**

**Direction-independent asymmetry index**

Interhemispheric differences in pathologic burden were compared using a ratio (i.e. ‘asymmetry index’) of the difference in pathologic burden to the average burden between hemispheres in each region to account for absolute differences in %AO between FTLD subtypes:

Asymmetry index (AI) = (%AO pathology left hemisphere – %AO pathology right hemisphere) / (average %AO pathology left plus right hemispheres) × 100.

AI data were found to be normally distributed. To test for overall asymmetry of pathology across patients, independent of left/right directionality, we performed one-sample t-tests for the absolute value of AI for the null-hypothesis = 0. For a more conservative measure of asymmetry, we also compared the lower limit of the 95% confidence interval (CI) of each region of interest absolute value AI compared to the null hypothesis = 0.

**Direction-dependent interhemispheric %AO measurement**

Quantification of tau and TDP-43 pathology (%AO values) were not normally distributed. To test for group-level asymmetry of pathology to a particular hemisphere we performed a Wilcoxon matched-pair signed rank test comparing the left-right difference in %AO pathology per individual for each region.

**Grey matter/white matter and clinicopathological correlations**

A linear mixed-effects model procedure (Laird and Ware, 1982) was used to test the association of individual slide grey matter %AO as the dependent variable and white matter %AO as an independent variable to account for correlations among grey matter %AO in multiple regions sampled within the same patient. A random intercept term was included in the mixed-effects model. Fixed-effects independent variables included region and hemisphere sampled while controlling for fixed-effects covariates for the presence of pathogenic mutation and disease duration. A similar model was used to test the association of regional MRI z-scores of CT on the dependent variable, pathology %AO measurement, including region and hemisphere as fixed effects independent variables and sex, presence of a pathogenic mutation, age and time to death (years) from MRI as fixed-effects covariates.

To quantify social disorder associated with bvFTD, we took the total number of three categories of behavioural disinhibition according to diagnostic criteria record (i.e. socially inappropriate behaviour, loss of manners/decorum and impulsivity; (Rascovsky et al., 2011) reported in the record and categorized into severe (≥2 features present) or mild (<2 features present). As detailed in the ‘Results’ section below, patients were characterized as right-predominant if grey matter pathology AI values were <0.5 standard deviation (SD) of the absolute value of AI for grey matter (i.e. for OFC, grey matter AI ≤ −18.5) and left-predominant if white matter pathology AI values were greater than 0.5 SD of the absolute value of AI for white matter (i.e. for VLT, white matter AI ≥ 20.4) for clinicoanatomical correlations.

All other comparisons and correlations were performed using parametric and non-parametric analyses as appropriate. All analyses were performed using SPSS version 21.0 (IBM Corp., Armonk NY, USA) with two-tailed statistics (P < 0.05), except for individual region absolute value AI analyses, for which we used a more conservative P ≤ 0.01 to control for multiple comparisons.
**Results**

**Patients**

Demographic data are presented in Table 1. The mean (±SD) post-mortem interval was 14.4 h (±5.6) and brain weight averaged 1174.7 g (±195.8). The mean age at onset = 58.5 years (±8.2), mean age at death = 66.0 years (±9.0) and disease duration = 7.5 years (±4.3). There was no significant difference between FTLD-TDP and FTLD-Tau groups in these variables (P > 0.3), which were similar to the FTLD-FUS and Alzheimer’s disease cases.

Patients were impaired on all cognitive measures compared to a cohort of age- and education-matched healthy controls (P < 0.001) with a range in global cognitive performance at baseline (mean MMSE z = −8.1 ± 7.5, range = 0, −20) and universal impairment in executive-mediated tasks (mean letter-guided verbal fluency z = −1.6 ± 0.7, mean semantically-guided category fluency z = −2.6 ± 0.9, mean digits backwards z = −1.8 ± 0.8) and attention (mean digits forward z = −2.3 ± 1.1) consistent with a clinical diagnosis of bvFTD (Supplementary Table 2).

Subsequent analyses focus on the majority of our patients who had either FTLD-Tau or FTLD-TDP pathology.

**Overall hemispheric asymmetry of pathology in post-mortem tissue**

Individual patient asymmetry may obscure measures of interhemispheric asymmetry in group-wise comparisons (e.g. equal numbers of right > left and left > right patients can create an average AI close to 0) (Rohrer et al., 2011). Therefore, we first examined the absolute value of AI (i.e. a measure of asymmetry without left/right directionality) and found significant overall asymmetry in each individual sampled region (i.e. INS, OFC, SPL, VLT) (P < 0.01) (Fig. 1 and Table 2). Subset analyses of both sporadic (i.e. exclusion of patients with pathogenic mutation) and patients with pathogenic mutations alone found similar significant laterализation in both grey and white matter for each group in each region (all P ≤ 0.01, data not shown).

**Direction-specific regional hemispheric asymmetry in post-mortem tissue**

Regional pathology measurement finds a higher median %AO of both tau and TDP-43 pathology burden for both right and left hemispheres in the frontotemporal regions sampled, with the area of lowest pathology in SPL (Fig. 2 and Supplementary Table 3). While we found little consistent evidence of direction-specific asymmetry between regions within an individual patient (see Supplementary Figs 3 and 4 that depict individual patient %AO and AI data for grey and white matter, respectively), we assessed group-level asymmetry of post-mortem pathology to a particular hemisphere in bvFTD for each region. In the total FTLD cohort, we found evidence for right greater than left hemisphere pathology in the OFC grey matter (Z = 2.7, P < 0.01) and left greater than right hemisphere pathology in VLT grey matter (Z = 2.4, P = 0.02) (Figs 2 and 3).

In preliminary pathological subgroup analyses, we found that the FTLD-Tau group has right greater than left OFC grey matter tau pathology (Z = 2.5, P = 0.01), and the FTLD-TDP group has right greater than left OFC white matter TDP-43 pathology (Z = 2.4, P = 0.02). There was a trend for increased left greater than right VLT grey matter pathology in the FTLD-TDP subgroup (Z = 1.9, P = 0.06) and FTLD-Tau subgroup (Z = 1.8, P = 0.08). There was no difference in right and left hemisphere pathology on group-
wise comparisons of SPL and INS (Supplementary Figs 3 and 4). Previous work has emphasized relatively greater white matter degeneration in FTLD-Tau than FTLD-TDP (McMillan et al., 2013b), and we examined whether this is equally evident in regions of both hemispheres. First, we examined the association between grey matter and white matter pathology. Overall, we found increased individual slide grey matter %AO was associated with increased white matter %AO in both the FTLD-TDP ($\beta = 2.9$, SE = 0.3, df = 1, t = 9.8, $P < 0.001$) and somewhat less so for the
FTLD-Tau subgroup ($\beta = 0.5, \ SE = 0.1, \ df = 1, \ t = 4.4, \ P < 0.001$). Next, we examined the %AO in paired grey matter-white matter samples from each slide and found in the FTLD-TDP group there was higher grey matter TDP-43 pathology %AO than in white matter for all regions in both hemispheres ($Z = 2.5–3.1, \ all \ P < 0.02$), while there was no significant difference in tau %AO in grey matter compared to white matter in any region for both hemispheres in FTLD-Tau ($Z < 1.6, \ all \ P > 0.05$).

**Neuroimaging analysis**

First, we examined the regional atrophy patterns in four regions of interest from antemortem MRI scans that approximate the same regions sampled at autopsy ($n = 15$). Similar to our pathology results, we found a high burden of atrophy in the frontotemporal regions (INS, OFC, VLT), with the more posterior SPL region having the least cortical atrophy (Supplementary Table 4). We next examined the relationship between antemortem cortical thinning and post-mortem %AO for tau and TDP-43 pathology for all available MRI-region of interest pairs in each hemisphere, and found a negative correlation of decreasing cortical thickness with increasing %AO of tau and TDP-43 pathology. This association appeared to be significant for FTLD-Tau ($\beta = -3.1, \ SE = 0.8, \ df = 1, \ t = 3.7, \ P = 0.001$), while we observed a trend in FTLD-TDP ($\beta = -0.1, \ SE = 0.05, \ df = 1, \ t = 1.9, \ P = 0.06$) (Fig. 4).

**Clinicopathological correlation**

To compare our pathology findings to cognitive/behavioural data, we tested the *a priori* hypothesis that more severe right OFC asymmetry would correspond to more severe behavioural disinhibition (Liu et al., 2004; Viskontas et al., 2007) and left VLT lateralization would be associated with worse performance on executive measures of category fluency (Libon et al., 2009).

Patients with right-predominant pathology in OFC grey matter had a high degree of behavioural disinhibition (behaviour sum score $\geq 2$) (9/10 patients) compared to those with more symmetric or left-predominant OFC grey matter pathology (5/10 patients; $\chi^2 = 3.8, \ P = 0.05$). These groups did not differ in the time interval from disease onset to first visit or from last visit to death ($t = 0.5–1.4, \ P > 0.1$). All but one patient with category fluency data available had left-predominant pathology in VLT grey matter so we examined VLT white matter for clinicopathological correlation. Comparison of patients with available neuropsychological test scores found those with left-predominant VLT white matter pathology ($n = 5$) had a lower score in semantically-guided category fluency (mean = $3.4 \pm 1.9$) compared to patients with more symmetric or right-predominant VLT white matter pathology ($n = 4$, mean $= 10.5 \pm 5.3; \ P < 0.03$). Further, we found an inverse correlation with increasing VLT white matter AI values (increasing left-predominant pathology) with reduced semantically-guided category fluency scores ($n = 9$, $r = -0.66, \ P = 0.05$), but not letter-guided fluency scores ($n = 6$, $r = -0.03, \ P > 0.1$), indicating specificity for worse performance on category fluency related to the degree of asymmetry of VLT pathology to the left hemisphere. Further, FTLD-Tau and FTLD-TDP subgroups did not differ in category fluency scores ($t = 0.6, \ P > 0.5$) and VLT white matter asymmetry groups did not differ in

**Figure 3 Photomicrographs of interhemispheric differences in the severity of FTLD neuropathology.** Representative images depict raw images and %AO measurement (yellow/orange/red overlay) of lateralization of grey matter (GM) pathology in FTLD-Tau to the right orbitofrontal cortex in a progressive supranuclear palsy patient and left ventrolateral cortex in a patient with Pick’s disease. FTLD-TDP type A case with more severe right orbitofrontal grey matter and white matter (WM) pathology than left hemisphere orbitofrontal cortex and more severe left ventrolateral temporal cortex grey matter and white matter compared to the right hemisphere homologous region. There is a higher proportion of total pathology in white matter in FTLD-Tau compared to FTLD-TDP. Scale bar = 100 μm.
MMSE score, letter-guided fluency scores, age at testing or the interval from onset to testing (t = 0.1–1.3, P > 0.05).

Analysis of less common pathologies associated with bvFTD

Measurement of interhemispheric burden of pathology in our two patients with Alzheimer’s disease found variable asymmetry that was largely congruent in directionality between tau and amyloid (Supplementary Table 4). SPL regions had similar or higher levels of tau and amyloid pathology compared to corresponding frontotemporal regions within each hemisphere. Qualitative examination of the single FTLD-FUS case found a higher severity of FUS-positive inclusion burden in the right (ordinal score = severe) > left (ordinal score = moderate) in OFC, while the VLT had severe FUS inclusion burden in the left hemisphere compared to moderate burden in the right hemisphere (Fig. 5). Finally, there was severe burden of pathology in the bilateral INS, while the SPL had lower burden of FUS pathology, which was at a moderate level in the right hemisphere compared to mild amounts in the left SPL.

Discussion

We report a novel digital neuropathological assessment of interhemispheric differences in pathology causing clinical bvFTD. We examined both grey and white matter pathological burden of tau and TDP-43 inclusions, and directly correlated pathological burden with antemortem imaging and clinical features. Pathology studies are critical because in vivo imaging methods to assess asymmetry in tauopathy (Chien et al., 2013; Maruyama et al., 2013) remain to be validated (Marquie et al., 2015; Lowe et al., 2016; McMillan et al., 2016), and imaging ligands for FTLD-TDP or FUS are lacking. We discovered several novel findings. First, we found significant asymmetry of pathology overall in both grey and white matter. While bvFTD has been associated with more prominent right hemisphere disease, asymmetries in our pathology study implicated both right and left hemisphere regions in these patients. Regional
comparisons thus revealed asymmetry of end-stage pathology to the right in OFC and left in VLT on group-level analysis, and increasing severity of post-mortem pathology measurement directly correlated with reduced antemortem cortical thickness on MRI. Moreover, we found converging evidence from exploratory clinicopathological observations in this bvFTD autopsy sample, where asymmetry in these regions was associated with antemortem clinical presentation in hemisphere-specific neuroanatomical substrates. Ratings of disinhibited behaviour were related to right-predominant OFC pathology, while performance on a measure of executive functioning that utilizes a temporal lobe-dependent semantic strategy was associated with left-predominant VLT pathology. These findings have important implications for future histopathological studies of FTLD.

The convention for neuropathological studies of neurodegenerative disease is microscopic examination of a single hemisphere. Very few studies have examined bilateral sampling in neurodegenerative disease and find minimal effects on diagnostic accuracy of Alzheimer’s disease or dementia with Lewy bodies using qualitative ratings, but these studies included only rare FTLD patients (Stefanits et al., 2010; Kim et al., 2016). Quantitative assessments of interhemispheric burden of TDP-43 neuropathology in PPA patients with FTLD-TDP due to GRN mutations (Gliebus et al., 2010; Kim et al., 2016) found a predominance of pathology in language-related regions of the left hemisphere, but quantitative histopathological assessments have not been performed in bvFTD. In the present study, both FTLD-Tau and FTLD-TDP patients in our cohort had a higher burden of pathology in frontal and temporal regions than the more posterior parietal region sampled in both hemispheres (Supplementary Table 3 and Figs 2). While our observations are largely consistent with previous models of progressive histopathological burden in bvFTD performed within a single hemisphere (Brettschneider et al., 2014; Irwin et al., 2016a), the relative burden of pathology differed across hemispheres in frontal and temporal regions. Examination of regional pathological burden in a manner sensitive to interhemispheric sampling is not currently a component of FTLD neuropathological criteria (Mackenzie et al., 2010). While more data are needed to make recommendations on the optimal regional sampling strategy across hemispheres for diagnostic purposes, and independent replication of our observation is required, we contend that regional asymmetry of pathology has important implications for FTLD research.

In the context of greater anterior disease burden in FTLD spectrum pathology, we found significant asymmetry of pathology in this cohort. Examination of the absolute value of AI revealed significant direction-independent asymmetry in all regions sampled, while direction-specific analyses found greater right than left grey matter pathology in OFC, and greater left than right grey matter pathology in VLT (Figs 2 and 3). These brain regions are thought to play a crucial role in the phenotypic expression of disease in FTD, as we discuss below. The basis for lateralization of pathology is unclear. The genetic and/or environmental aetiologies that influence the regional onset and progression of neurodegenerative disease are still speculative; however, premorbid neuroanatomic structure and intellectual skills or experiences also may influence the phenotypic expression of FTLD (Rogalski et al., 2013; Placek et al., 2016). It is possible that pathology accumulates in a lateralized manner as a unique property of these FTLD pathologies; however, left-lateralized neuropathology has been described in PPA patients with underlying Alzheimer’s disease pathology as well (Mesulam et al., 2014). In our two Alzheimer’s disease patients with bvFTD we found variable asymmetry of pathology. Intra-hemispheric comparisons found SPL had similar or higher burden of pathology than frontotemporal regions sampled (Supplementary Table 4), which differed from our FTLD patients but is similar to previous neuroimaging findings in bvFTD with Alzheimer’s disease pathology (Ossenkoppele et al., 2015). Further study with larger groups of patients with both bvFTD and PPA due to Alzheimer’s disease pathology are needed to fully compare interhemispheric patterns of disease in Alzheimer’s disease with FTLD.

While we found significant group-level asymmetry in this small cohort, it is important to keep in mind individual-patient variability in the regional pattern of asymmetry of post-

Figure 5 Photomicrographs of inter-hemispheric differences in the severity of FTLD-FUS patient. FTLD-FUS case shows some increase pathologic burden in the right orbitofrontal grey matter (GM) and left ventrolateral temporal cortex with rare white matter (WM) pathology overall. Scale bar = 100 μm.
mortem pathology (Supplementary Figs 3 and 4). Beyond heterogeneity associated with different pathologies, there is also heterogeneity on directionality of asymmetry depending on the anatomic region that was sampled. Thus, there was group-level asymmetric pathology in OFC and VLT, but we did not find consistent lateralization of pathology to a particular hemisphere in both INS, which is thought to be an early site of structural degeneration in bvFTD (Seeley et al., 2008), or SPL, which has minimal neurodegeneration on antemortem neuroimaging in most bvFTD patients (Seeley et al., 2008; Rohrer et al., 2011; Whitwell et al., 2015). There may be regional differences in the hemisphere-specific progression of disease, and strains of pathological TDP-43 and tau may have different regional predilections also depending on the cell types affected, so these and other factors could influence the heterogeneity observed at end-stage disease. Likewise, neuroimaging studies of clinical bvFTD have reported several subgroups of patients with varying patterns of interhemispheric regional cortical atrophy (Whitwell et al., 2009; Ranasinghe et al., 2016), congruent with our pathological observations here. We did not find an association with overall hemispheric asymmetry and disease duration or pathology stage (data not shown); although we had only two patients who died with low (phase II) pathology (Table 1) and disease duration may not capture differences in rates of progression between individuals. Indeed, longitudinal neuroimaging data find differing rates of progression among molecular subtypes of FTLD across various regions (Rohrer et al., 2011; Mahoney et al., 2015; Whitwell et al., 2015). Thus, our group-level analyses here may have limited generalizability to all forms of bvFTD.

We provide preliminary neuropathological data that show the proportion of overall pathology in white matter is higher for FTLD-Tau compared to FTLD-TDP in both hemispheres. Previously, in a cohort of autopsied FTLD patients with antemortem neuroimaging data, we found FTLD-Tau has a higher burden of white matter degeneration on MRI and in post-mortem evaluation compared to FTLD-TDP (McMillan et al., 2013b) and studies of non-autopsied genetic forms of these diseases find more prominent white matter degeneration in MAPT patients compared to hereditary forms of FTLD-TDP (Mahoney et al., 2014, 2015). While we accounted for influence of patients with pathogenic mutations, replication is needed in larger samples of each subtype of sporadic FTLD-Tau and FTLD-TDP to confirm these observations.

We emphasize that bvFTD is a clinically heterogeneous syndrome with diverse underlying neuropathological substrates. Previous studies found varying group-wise associations of clinical (Hu et al., 2007; Mendez et al., 2013) or neuroimaging features (Whitwell et al., 2005) in rare autopsy-confirmed studies. Thus, there is no reliable way to differentiate these molecular pathologies during life (Irwin et al., 2015). Here, we found preliminary evidence that distinct asymmetric patterns of tau and TDP-43 pathology across hemispheres may contribute to clinical heterogeneity on presentation, and thus detection of these features could potentially improve in vivo diagnostic accuracy of molecular pathology in bvFTD. Exploratory clinical-pathological correlations examined more closely the relationship between FTD phenotype during life and asymmetric FTLD pathology. Neurodegeneration in the right OFC has been associated with early symptoms of behavioural disinhibition (Liu et al., 2004; Viskontas et al., 2007; Seeley et al., 2008; Grossman et al., 2010) and may be one of the earliest sites for the deposition of TDP-43 pathology in bvFTD (Brettschneider et al., 2014) and an early region of pathological involvement in Pick’s disease (Irwin et al., 2016a). Our finding of increased social disinhibition during life in patients with more right-predominant pathology in OFC grey matter at autopsy suggests these patients may have had right-predominant OFC dysfunction during life, which remained evident microscopically at end-stage disease.

We also found a direct association of the degree of asymmetry with left-predominant VLT pathology with antemortem performance on a category-fluency task, which has neuroanatomical correlates in the temporal regions in the left hemisphere in bvFTD (Libon et al., 2009; Cook et al., 2014). This observation may reflect that some bvFTD patients have a phenotype that includes some features of semantic-variant PPA, especially those with a temporal-predominant presentation (Seeley et al., 2005); however, only three of our patients developed single-word and object knowledge loss, as seen in semantic-variant PPA, and these patients had missing sampling for VLT so they were unfortunately not included in our analysis. Further, in our subset of patients with antemortem MRI, no patients had isolated VLT atrophy (Supplementary Table 5) suggestive of a temporal-predominant of bvFTD (Whitwell et al., 2009; Ranasinghe et al., 2016). These findings begin to suggest the contribution of left hemisphere pathology to the clinical phenotype of bvFTD patients with temporal disease. Recent work has begun to emphasize the presence of subtle but statistically reliable language deficits in non-aphasic patients with a bvFTD phenotype, and these deficits have been associated with both left hemisphere and right hemisphere changes in MRI regression analyses (Ash et al., 2006; Charles et al., 2014; Healey et al., 2015; Cousins et al., 2017). Future studies comparing the interhemispheric distributions of neuroanatomical variants of bvFTD, including patients with right-temporal predominant bvFTD and semantic-variant PPA (Gorno-Tempini et al., 2011), which has predominant left temporal pathology, will be helpful to clarify the relationship between asymmetry of temporal lobe pathology at end-stage disease to antemortem origins of pathology.

Previous neuroimaging studies of autopsy-confirmed FTLD have not directly examined the relationship between severity of post-mortem pathology and antemortem grey matter atrophy (Whitwell et al., 2005; Rohrer et al., 2011), and quantitative study of FTLD-TDP in PPA has not examined antemortem MRI data (Kim et al., 2016). Here, we found a novel direct association of antemortem quantitative MRI and post-mortem quantification of pathology. An important caveat is that MRI data were obtained...
in our sample at a wide range of time intervals before the time of autopsy (<1 year to 6 years), and although we included time from MRI to death as a covariate in our model, we cannot exclude the possibility that differences in time of scanning and rate of progression of individual patients could contribute to the strength of association between antemortem imaging and post-mortem pathology.

The detection of these clinical-pathological correlations, as well as discovery of region-specific patterns of asymmetric pathological burden, was significantly facilitated by the digital pathological analysis used in this study (Irwin et al., 2016b). Fine-grained ascertainment of pathological burden as a continuous variable, rather than a traditional ordinal scale, allowed subtler characteristics of histopathological observations to emerge as significant factors. Further, density of protein inclusions can be variable along the cortical ribbon and variation across adjacent tissue sections could influence assessments of asymmetry (King et al., 2015). As such, we performed a user-independent, transect-belt sampling method (Supplementary Fig. 1) to reduce bias from variability in pathology density (Armstrong, 2003), which is not possible using subjective visual rating scales.

Several additional caveats should be considered in the interpretation of these results. First, despite our focus on clinical bvFTD to constrain neuroanatomical associations for group comparisons, our FTLD cohort was pathologically and genetically heterogeneous (Table 1), and molecular aetiologies of FTLD may have differing patterns of regional pathology and progression across hemispheres (Whitwell et al., 2005; Rohrer et al., 2011). Our bilateral sampling data are novel and represent over a decade of consecutive autopsy recruitment; however, due to the relative rarity of this disorder compared to more common neurodegenerative disease, the sample size does not permit analysis of individual subtypes of FTLD (i.e. A, B, C) (Mackenzie et al., 2011) or FTLD-Tau (i.e. 4-repeat, 3-repeat) (Mackenzie et al., 2010). Thus, we cannot generalize our specific regional findings of lateralization of right OFC and left VLT to all forms of bvFTD but instead we provide evidence of unequal inter-hemispheric distributions of microscopic pathology at end-stage disease. Although we sampled critical regions related to core clinical elements in bvFTD, we studied only a small number of regions and additional work is needed to study a larger sampling. Our clinical data were retrospective in nature, as such we had missing data for some neuropsychological measures and we cannot exclude the possibility that an unreported symptom in the record may have been present during the disease course.

With these caveats in mind, FTLD neuropathology associated with clinical bvFTD is asymmetric, although not always biased to the right hemisphere, and appears to have some regional specificity. These characteristics appear to contribute to the clinical heterogeneity of bvFTD. Further understanding of the divergent patterns of disease in molecular subtypes of FTLD are critical for the development of diagnostic biomarkers for aetiology-specific therapies in bvFTD.

Acknowledgements

We thank Manuela Neumann and Elisabeth Kremmer for providing the phosphorylation specific TDP-43 antibody p409/410. We thank Mary Leonard, Matthew Byrne, Felicia Cooper and Mendy Liang for their technical assistance. We thank Dr Gabor Kovacs for his thoughtful feedback on the manuscript. We would also like to thank the patients and their families, for without their meaningful contributions to medical research this work would not be possible.

Funding

This study was supported by NIH grants NS044266, AG038490, AG015116, AG010124, P01NS053488, P01A G032953, P01AG017356, AG043503, NS088341, Penn Institute on Aging and the Wyncote Foundation.

Supplementary material

Supplementary material is available at Brain online.

References


Asymmetric pathology in bvFTD

BRAIN 2018: 0; 1–14


