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Neuropathological heterogeneity in frontotemporal lobar degeneration with TDP-43 proteinopathy: a quantitative study of 94 cases using principal components analysis

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Abstract

Studies suggest that frontotemporal lobar degeneration with transactive response DNA-binding protein of 43 kDa (TDP-43) proteinopathy (FTLD-TDP) is heterogeneous with division into four or five subtypes. To determine the degree of heterogeneity and the validity of the subtypes, we studied neuropathological variation within the frontal and temporal lobes of 94 cases of FTLD-TDP using quantitative estimates of density and principal components analysis (PCA). A PCA based on the density of TDP-43 immunoreactive neuronal cytoplasmic inclusions, oligodendroglial inclusions, neuronal intranuclear inclusions, and dystrophic neurites, surviving neurons, enlarged neurons, and vacuolation suggested that cases were not segregated into distinct

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subtypes. Variation in the density of the vacuoles was the greatest source of variation between cases. A PCA based on TDP-43 pathology alone suggested that cases of FTLT-DTP with *progranulin* (*GRN*) mutation segregated to some degree. The pathological phenotype of all four subtypes overlapped but subtypes 1 and 4 were the most distinctive. Cases with coexisting motor neuron disease (MND) or hippocampal sclerosis (HS) also appeared to segregate to some extent. We suggest: (1) pathological variation in FTLT-DTP is best described as a 'continuum' without clearly distinct subtypes, (2) vacuolation was the single greatest source of variation and reflects the 'stage' of the disease, and (3) within the FTLT-DTP 'continuum' cases with *GRN* mutation and with coexisting MND or HS may have a more distinctive pathology.

Keywords

Frontotemporal lobar degeneration with TDP-43 proteinopathy; FTLT with ubiquitin-positive inclusions; TAR DNA-binding protein of 43 kDa; Neuronal cytoplasmic inclusions; Neuropathologic heterogeneity; Principal components analysis

Introduction

Frontotemporal lobar degeneration (FTLT) is the second commonest form of cortical dementia of early-onset after Alzheimer's disease (AD) (Tolnay and Probst 2002; Josephs 2008). The disorder is associated with a heterogeneous group of clinical syndromes including frontotemporal dementia (FTD), FTD with motor neuron disease (FTD/MND), progressive non-fluent aphasia (PNFA), semantic dementia (SD), and progressive apraxia (PAX) (Snowden et al. 2007).

Frontotemporal lobar degeneration with transactive response (TAR) DNA-binding protein of 43 kDa (TDP-43) proteinopathy (FTLT-TDP), previously called FTLT with ubiquitin positive inclusions (FTLT-U), is characterized by variable neocortical and allocortical atrophy principally affecting the frontal and temporal lobes. In addition, there is neuronal loss, microvacuolation in the superficial cortical laminae, and a reactive astrocytosis (Cairns et al. 2007a). A variety of TDP-43 immunoreactive lesions are present in FTLT-TDP including neuronal cytoplasmic inclusions (NCI), neuronal intranuclear inclusions (NII), dystrophic neurites (DN), and oligodendroglial inclusions (GI).

There are several sources of pathological heterogeneity within FTLT-TDP. First, a number of genetic defects have been identified in these cases; many being caused by mutations of the *progranulin* (*GRN*) gene (Baker et al. 2006; Cruts et al. 2006; Mukherjee et al. 2006; Mackenzie et al. 2006a; Behrens et al. 2007; Rademakers and Hutton 2007). A less prevalent disorder, FTLT with valosin-containing protein (*VCP*) gene mutation (Forman et al. 2006), also has TDP-43 immunoreactive inclusions and recently, variants in the ubiquitin associated binding protein 1 (*UBAP1*) gene (Luty et al. 2008; Rollinson et al. 2009) were shown to have TDP-43 inclusions. FTLT caused by *charged multivesicular body protein 2B* gene mutations (*CHMP2B*), however, has ubiquitinated but no TDP-43 immunoreactive inclusions (Van der Zee et al. 2007). Second, four, or five pathological subtypes of FTLT-TDP/FTLT-U have been proposed based on the predominant type of inclusion present as detected with anti-ubiquitin immunohistochemistry (IHC), and the distribution and density of the pathological changes in the cortex (Mackenzie et al. 2006b; Sampathu et al. 2006; Cairns et al. 2007b; Josephs 2008; Mackenzie et al. 2009). Patterns of FTLT-U histology based solely on cortical pathology include the systems of Sampathu et al. (2006) and Neumann et al. (2007) whereas Mackenzie et al. (2006b) propose a system that includes both cortical and dentate gyrus (DG) inclusions. More recently, Josephs (2008) has proposed five subtypes of FTLT-TDP and Mackenzie et al. (2009) four subtypes plus a group

containing unclassifiable cases. The same descriptors are often used to define subtypes, but the numbering of each subtype varies between schemes. Using a composite system proposed by Cairns et al. (2007b): type 1 cases (Mackenzie-type 2) are characterized by long DN in superficial cortical laminae with few or no NCI or NII, type 2 (Mackenzie-type 3) by numerous NCI in superficial and deep cortical laminae with infrequent DN and sparse or no NII, type 3 (Mackenzie-type 1) by pathology predominantly affecting the superficial cortical laminae with numerous NCI, DN, and varying numbers of NII, and type 4 is characterized by numerous NII, and infrequent NCI and DN especially in neocortical areas. A consensus regarding the validity and robustness of these schemes remains to be established. Third, FTLT can occur in combination with MND (FTLT-MND) and such cases are often associated with a more localized pattern of frontal lobe atrophy (Whitwell et al. 2006). Fourth, a proportion of FTLT-TDP cases have coexisting hippocampal sclerosis (HS) in which there is neuronal loss in the subiculum and sector CA1 of the hippocampus (Josephs et al. 2006). A significant degree of AD pathology, viz., senile plaques (SP) and neurofibrillary tangles (NFT) are also present in a proportion of cases.

The present study investigated the degree of heterogeneity within the frontal and temporal lobes of 94 cases of FTLT-TDP obtained from several centres using quantitative estimates of lesion density and principal components analysis (PCA) (Armstrong et al. 2000). The main objectives were: (1) to determine whether heterogeneity within FTLT-TDP was continuously distributed or consistent with the presence of distinct subtypes, (2) to identify the most important variables that could account for the heterogeneity, and (3) to establish whether familial cases and those with coexisting MND, HS, or AD had a distinctive phenotype.

Materials and methods

Cases

Ninety-four cases of sporadic and familial FTLT-TDP (see Table 1) were obtained from dementia centres in the USA and Canada: Washington University School of Medicine, St Louis, MO, USA (32 cases), University of California, Davis, CA, USA (15 cases), University of Pittsburgh, Pittsburgh, PA, USA (15 cases), Vancouver General Hospital, Vancouver, BC, Canada (11 cases), Harvard Brain Tissue Resource Center, Belmont, MA, USA (9 cases), Emory University, Atlanta, GA, USA (6 cases), University of Washington, Seattle, WA, USA (2 cases), Columbia University, New York, NY, USA (2 cases), University of California, Irvine, CA (1 case), and University of Michigan, Ann Arbor, MI, USA (1 case). All cases exhibited FTLT with neuronal loss, microvacuolation in the superficial cortical laminae, and reactive astrogliosis (Fig. 1) consistent with the proposed criteria for FTLT-TDP (Mackenzie et al. 2006b; Cairns et al. 2007b). A variety of TDP-43-immunoreactive lesions was present in these cases including NCI, NII, DN, and GI (Figs. 2,3) consistent with a diagnosis of TDP-43 proteinopathy (Cairns et al. 2007b). Of the 94 cases, 37 were identified as familial (at least one or more first degree relatives affected) and of these, 14 cases had *GRN* mutations (Baker et al. 2006; Cruts et al. 2006), one had a *VCP* gene mutation (Forman et al. 2006), and one case was associated with *UBAP1* (Rollinson et al. 2009), a presumptive gene on chromosome 9 (9p21). The genetic defects in the remaining familial cases have not been identified to date. The proportion of familial cases of FTLT-TDP in this sample is high and does not reflect the proportion in the general population. Nine of the cases had coexisting motor neuron disease (FTLT-MND) (Josephs et al. 2005; Kersaitis et al. 2006) and seven were identified as having combined FTLT-TDP and HS. A majority of cases within the age-range of FTLT-TDP will exhibit AD pathology, and 17 cases were identified as having densities of SP/NFT greater than expected from normal aging. Cases were assigned to the four subtypes based on the composite scheme of

Cairns et al. (2007b). Braak stage of the cases was based on the density and distribution of NFT (Braak et al. 1993).

Histological methods

After death, the consent of the next-of-kin was obtained for brain removal, following local Ethical Committee procedures and the 1995 Declaration of Helsinki (as modified in Edinburgh 2000). To enable the quantification to be accomplished by a single observer within a reasonable time-frame, sampling was confined to regions of the frontal and temporal lobe and to areas that were most consistently available across the different centres. Hence, tissue blocks were taken from the frontal lobe at the level of the genu of the corpus callosum to study the middle frontal gyrus (MFG) and the temporal lobe at the level of the lateral geniculate body to study the inferior temporal gyrus (ITG), parahippocampal gyrus (PHG), CA1/2 sectors of the hippocampus, and DG. TDP-43 pathology in the frontal lobe appeared to be more consistently present in the MFG of our cases compared with the more usual Brodmann area (B8). Tissue was fixed in 10% phosphate buffered formal-saline and embedded in paraffin wax. IHC was performed on 4–10 μ m sections with a rabbit polyclonal antibody that recognizes TDP-43 epitopes (dilution 1:1,000; ProteinTech Inc., Chicago, IL, USA), anti-ubiquitin (1:1,000, rabbit polyclonal antibody (PAB; Dako, Glostrup, Denmark), anti-tau (PHF-1; a gift from Dr P Davies, Albert Einstein School of Medicine, New York, NY, USA), anti-A β -synuclein [1:500, mouse monoclonal antibody (MAB) LB-509; Zymed, San Francisco, CA, USA], and anti-A β (1:100,000, mouse MAB, 10D5; gift of Eli Lilly, Indianapolis, IN, USA) antibodies. Sections were counterstained with haematoxylin. Additional stains included: haematoxylin and eosin.

Morphometric methods

In the MFG, ITG, and PHG of each case, histological features were counted along strips of tissue (1,600–3,200 μ m in length) located parallel to the pia mater, using 250 μ m $\times$ 50 μ m sample fields arranged contiguously. The sample fields were located both in the upper and lower cortex, the short edge of the sample field being orientated parallel with the pia mater and aligned with guidelines marked on the slide. Between 32 and 64 sample fields were usually necessary to sample each region. Hence, a standard sample field was used regardless of the degree of brain atrophy and this will inevitably effect which laminae are included in the sample. In the majority of cases, the upper and lower sample fields quantified lesions in laminae II and part of lamina III and in laminae V/VI respectively. In some of the longer duration cases, however, with a greater degree of brain atrophy, the upper sample fields could include a greater proportion of lamina III and part of lamina IV. Similarly in laminae V/VI of longer duration cases, the field would extend into the adjacent white matter and density counts were corrected for the smaller field size. In the hippocampus, the features were counted in the cornu ammonis (CA) in a region extending from the prosubiculum/CA boundary to the maximum point of curvature of the pyramidal layer before it extends to join the dentate fascia via CA3 and CA4. Hence, the region sampled encompassed approximately sectors CA1 and CA2, the short dimension of the contiguous sample field being aligned with the alveus. Very little pathology was observed to extend into CA3/4 in the cases studied and these areas were not sampled. NCI have been commonly observed in the DG fascia in FTLTDP (Mackenzie et al. 2006b; Woulfe et al. 2001; Kovari et al. 2004) and the sample field was aligned with the upper edge of the granule cell layer. The NCI (Fig. 2) are rounded, spicular, or skein-like in shape (Yaguchi et al. 2004; Davidson et al. 2007), while the GI (Figs. 2, 3) morphologically resemble the ‘coiled bodies’ reported in various tauopathies such as corticobasal degeneration, progressive supranuclear palsy, and argyrophilic grain disease. The NII (Fig. 3) are lenticular or spindle-shaped (Pirici et al. 2006) and the DN (Fig. 2) are characteristically long and contorted (Hatanpaa et al. 2008). There are significant qualitative differences in the morphology of inclusions in FTLTDP,

but a preliminary study suggested such differences could not easily be quantified within the time frame and were not carried out in this study. Surviving neurons were identified as cells containing at least some stained cytoplasm in combination with larger shape and non-spherical outline (Armstrong 1996). Small spherical or asymmetrical nuclei without cytoplasm but with the presence of a thicker nuclear membrane and more heterogeneous chromatin were regarded as glial cells. Abnormally enlarged neurons (EN) (Fig. 1) had enlarged perikarya, lacked NCI, had a shrunken nucleus displaced to the periphery of the cell, and the maximum cell diameter was at least three times the diameter of the nucleus (Armstrong 1996). The number of discrete vacuoles (Fig. 1) greater than 5 μm in diameter was also recorded in each sample field (Armstrong et al. 2009). It can be difficult to differentiate microvacuolation of the neuropil from vacuolation around neurons and blood vessels attributable to artefacts of processing. Hence, vacuoles clearly associated with such structures were not counted. To obtain an estimate of the agreement between two successive counts made on the tissue (count–recount variability), a second series of quantifications were undertaken by the same observer in the upper cortex of the MFG using the 32 cases from Washington University School of Medicine.

Data analysis

Count–recount variability was tested using the ‘method of agreement’ described by Bland and Altman (1986). The essential feature of the Bland and Altman method is that for a particular histological feature, the difference between the two counts are subtracted for each case. The mean of these differences is known as the degree of ‘bias’ or ‘agreement’ and the standard deviation of the mean can be used to calculate the ‘limits of agreement’ in which it would be expected that 95% of the differences between two successive counts would fall.

Variations in the densities of pathological features in frontal and temporal lobe in the 94 cases of FTLD-TDP were studied using PCA (Statistica Software, Statsoft Inc., Tulsa, OK, USA). PCA enables the similarity and dissimilarity between the cases to be studied based on their neuropathological characteristics (Armstrong et al. 2000; Armstrong 2003). Two separate analyses were carried out using the FTLD-TDP cases as variables and the histological measurements as defining features: (1) PCA-1 based on the spectrum of histological features: NCI, GI, NII, DN, surviving neurons, EN, and vacuolation and (2) PCA-2 based on the TDP-43 pathology alone. Preliminary analysis of the data suggested a significant degree of skew and kurtosis was present suggesting non-normality. Hence, each PCA was carried out on the raw data and on log transformed data. Initially, all PC were extracted which had eigenvalues (λ) > 1 but usually in a PCA, only the first three PC account for significant proportions of the original variance (Armstrong et al. 2000). The result of each PCA is a scatter plot of the FTLD-TDP cases in relation to the extracted PC in which the distance between cases reflects their similarity or dissimilarity, based on the defining histological features. Such a plot can reveal whether neuropathological variation is continuously distributed or whether discrete clusters of cases are present. Correlations (Pearson's ‘ r ’) were calculated between the ‘loadings’ (the coordinates of the case in relation to the PCs) of each FTLD-TDP case on the PC and each demographic and neuropathological variable to determine which features could account best for the distribution of the cases. To avoid Type 1 errors, only correlations with the PCs significant at $P < 0.01$ were discussed in this article.

Results

The degree of agreement between two successive counts of each histological feature in the upper cortex of the MFG applied to the Washington University School of Medicine cases only is shown in Table 2. There was a good agreement between successive counts of the various aspects of TDP-43 immunoreactive pathology. For example, the mean difference

between the two counts of the NCI, averaged over the 32 cases is 0.02 lesions per $50\ \mu\text{m} \times 250\ \mu\text{m}$ sample field and the limits of agreement ± 0.15 . Hence, 95% of all differences between successive counts of the NCI in the MFG would be expected to be in the range -0.13 to $+0.17$ lesions per field. There was less agreement between surviving neuron counts (mean difference 0.42 neurons per $50\ \mu\text{m} \times 250\ \mu\text{m}$ sample field, limit of agreement = ± 2.98) while the estimates of the vacuolation were the least reliable (mean difference 1.61 vacuoles per $50\ \mu\text{m} \times 250\ \mu\text{m}$ sample field, limit of agreement = ± 4.08).

Similar PCA results were obtained using untransformed and transformed data and only the results of the analysis of the original data are reported. In neither PCA-1 nor PCA-2 did cases segregate according to the centre of origin. In PCA-1, the first three PCs accounted for 86.7% of the total variance (PC1 = 80.7%, PC2 = 4.1%, PC3 = 1.9%). Most of the variance was associated with PC1 suggesting the presence of a single 'dominant' defining feature. A plot of the FTLT-DTP cases in relation to PC1 and PC2 is shown in Fig. 4. The majority of cases cluster towards the left of the plot with the remaining cases scattered at varying distances from the main cluster. Examination of the cases with high loadings on PC1 showed them to have especially high levels of vacuolation. The degree of correlation between the extracted PC and the demographic and neuropathological features is shown in Table 3. Vacuole density in the upper and lower laminae of the MFG, and PHG, lower laminae of the ITG, and in CA1/2 was positively correlated with PC1. In addition, neuronal density in a number of regions was negatively correlated with PC1. A number of histological features were correlated with PC2, most notably the density of the vacuoles was positively correlated with PC2 in the upper and lower laminae of the MFG, ITG, and PHG, and in CA1/2 and the DG, while the TDP-43 immunoreactive lesions were negatively correlated with PC2. Few features were correlated with PC3, but the density of vacuoles was negatively correlated with PC3 in both the upper and lower laminae of the MFG. Of the demographic features, only disease duration was positively correlated with PC1.

In PCA-2, based on the TDP-43 pathological features alone, the first three PCs accounted for 30.35% of the total variance (PC1 = 16.98%, PC2 = 13.37%, PC3 = 10.51%). A plot of the cases in relation to PC1 and 2 is shown in Fig. 5. Cases were scattered uniformly in relation to the extracted PC suggesting that heterogeneity was essentially continuously distributed. The familial cases as a group were also scattered throughout the plot suggesting that they did not have a pathological phenotype distinctly different from the sporadic cases. However, FTLT cases with *GRN* mutation, and the case with *VCP* mutation had higher loadings on PC1 and cluster towards the right of the plot. In addition, a number of cases with associated MND or HS clustered either towards the upper or lower region of the plot respectively (Fig. 6). By contrast, cases with significant AD pathology were spread more evenly in relation to the extracted PC. Several TDP-43 pathological features were significantly correlated with the PC (Table 3). First, the densities of NCI in the upper and lower laminae of the ITG, PHG, and DG were negatively correlated with PC1 while the densities of the NII in the lower laminae of the MFG and in the upper laminae of the PHG were negatively correlated with PC1. Second, the densities of the DN in the upper and lower laminae of the MFG, ITG, PHG, and DG were negatively correlated with PC2. Third, a variety of TDP-43 immunoreactive features were correlated with PC3, most notably the density of the NII. Of the demographic features, PC2 was negatively correlated with disease duration and age at death was positively correlated with PC3.

A plot of disease subtype from PCA-2 in relation to PC1 and 2 is shown in Fig. 7. There is some clustering of the cases; type 1 cases being located towards the bottom of the plot and type 4 cases towards the upper right of the plot. In addition, there was a significant negative correlation between disease subtype and factor loadings on PC2.

Discussion

Neuropathological heterogeneity is common in neurodegenerative disease (Armstrong et al. 2000; Cairns et al. 2004). Three hypotheses have been proposed to explain this heterogeneity, viz., the presence of distinct subtypes of the disease, variation in the 'stage' of the disease present at death, and variation in the brain region of origin and subsequent progression of the disease throughout the brain (Ritchie and Touchon 1992). A PCA of the FTLT-DTP cases based on all histological features identified the degree of vacuolation as the greatest single source of pathological variability. There can be considerable variation in vacuolation depending on how a tissue is fixed and processed. However, vacuoles likely to be attributable to artefacts, i.e. those associated with neuronal cell bodies and blood vessels were not counted and there was no evidence that cases from different centres segregated in relation to PC1/2 suggesting that differences in processing did not account for the variations in vacuolation. In sporadic forms of CJD (sCJD), clustering of the vacuoles is often correlated with the presence of neuronal perikarya (Armstrong et al. 2001). Moreover, in the cerebellum of the variant form of CJD (vCJD), clusters of vacuoles in the molecular layer are negatively correlated with surviving Purkinje cells (Armstrong et al. 2009). In FTLT-DTP, both the density of vacuoles and duration of disease were positively correlated with PC1 while neuronal density was negatively correlated with PC1. These results suggest that vacuole density in FTLT-DTP is a reflection of the extent of neuronal loss and therefore, may reflect the 'stage' of the disease at death (Ritchie and Touchon 1992).

Heterogeneity in TDP-43 pathology in FTLT-DTP could be attributable to a number of factors. First, there are familial and sporadic cases of FTLT-DTP, the majority of familial cases being caused by defects in the *GRN* gene (Baker et al. 2006; Cruts et al. 2006; Mukherjee et al. 2006; Mackenzie et al. 2006a; Behrens et al. 2007; Rademakers and Hutton 2007), and smaller numbers of cases by *VCP* mutations (Forman et al. 2006; Van der Zee et al. 2007). Cases with and without *GRN* mutations have similar demographics but *GRN* cases often have greater language deficits (Van Deerlin et al. 2007). Pathologically, NCI and DN may be more frequent in the frontal cortex but less frequent in the DG in *GRN* cases (Hatanpaa et al. 2008; Davion et al. 2007). Moreover cases lacking *GRN* mutations may have a less severe pathology affecting the neocortex and striatum while NII are usually absent or infrequent (Mackenzie et al. 2006b). By contrast, cases with *VCP* mutation exhibit NII and abundant DN in the neocortex (Forman et al. 2006). A PCA carried out using TDP-43 pathology alone suggested that the familial cases as a whole did not have a pathological phenotype that was distinct from that of the sporadic cases. However, cases with *GRN* mutation and the FTLT cases with *CH9* or *VCP* mutation, clustered to the right of the PCA1/2 plot. Since the density of the NII is positively correlated with PC1, at least some familial cases are associated with higher densities of NII and lower densities of NCI as previously reported (Forman et al. 2006; Mackenzie et al. 2006b) and therefore may have a more distinctive pathology.

Second, a proportion of cases of FTLT-DTP have coexisting MND (Kersaitis et al. 2006; Josephs et al. 2005) or HS (Josephs et al. 2006). FTLT-DTP lacking MND is associated with a more widespread pattern of atrophy affecting the frontal and temporal lobes while FTLT-MND has more localized frontal lobe atrophy (Josephs et al. 2005). In addition, NCI are frequently present in the DG in FTLT-MND with 66% of cases having NCI in this region compared with 58% in the temporal lobe and 32% in the frontal lobe (Kovari et al. 2004). Nine of the present cases were identified with associated MND and several of these cases are located together to the left of the PC1/2 plot. Since the densities of the NCI and DN were negatively correlated with PC1, the data suggest that increased density of these lesions could be a feature of FTLT-MND. HS cases may also cluster in relation to PC1, most cases being located towards the bottom left of the plot suggesting a reduced density of

NII but increased density of NCI and DN in these cases. The status of HS in relation to FTLT-DTP has been questioned, but the pathology of the present cases suggests they can be accommodated within the 'spectrum' of FTLT-DTP. Cases with AD pathology were spread more randomly in relation to the extracted PC and hence such pathology can potentially be found in any type of FTLT-DTP case.

Third, a number of authors have divided FTLT-DTP into four or five subtypes (Mackenzie et al. 2006b, 2009; Sampathu et al. 2006; Cairns et al. 2007b; Josephs 2008). Using the composite scheme proposed by Cairns et al. (2007b), cases ascribed to these subtypes segregate to some degree in relation to PC2, type 1 cases having low loadings on PC2 and type 4 cases having higher loadings on PC1 and PC2. Apart from the type 1 cases which do appear to more distinct, the FTLT-DTP cases form a 'continuum' and there is broad overlap between the subtypes, especially between subtypes 2 and 3. The original classification schemes were based on ubiquitin IHC, on the relative numbers of lesions, and on their laminar distribution. Hence, pathological information based on TDP-43 IHC may have added complexity to the set of pathological variables thus obscuring the original classification. Moreover, even if a degree of overlap between subtypes is present, it may be useful to have a classificatory system for TDP-43 subtypes however imperfect. As a consequence, the data from the frontal and temporal lobe suggest that the classifications as proposed require a degree of subjectivity in their application when based on TDP-43 pathology; the neuropathologist deciding what is important in the individual case. It is also possible that examination of other regions such as the motor cortex, basal ganglia, amygdala, midbrain, and medulla may facilitate division into subtypes. To clarify this question further, it would be useful to conduct a further large-scale study of FTLT-DTP cases to assess inter-rater reliability in assigning the four subtypes.

In conclusion, the data suggest that pathological variation within a group of FTLT-DTP is essentially continuously distributed without the presence of distinct subtypes. The degree of vacuolation is the single greatest source of heterogeneity between these cases, increased vacuolation reflecting increasing duration of disease and neuronal loss. The densities of TDP-43 immunoreactive lesions are also important sources of variation and are associated with disease phenotype. There may be no pathological phenotype characteristic of familial FTLT-DTP cases as a whole but cases caused by *GRN* mutations may have a distinctive pathology. FTLT-DTP with coexisting MND or HS may also be distinctive but coexisting AD pathology can potentially occur in association with any of the FTLT-DTP cases.

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Fig. 1.
a Neuronal loss, microvacuolation, and gliosis in lamina II of the superior temporal gyrus and **b** abnormally enlarged neuron (*arrow*) in lamina III of the superior temporal gyrus in a case of frontotemporal lobar degeneration with TDP proteinopathy (FTLD-TDP) (H/E, bars **a, b** = 20 μ m)

Fig. 2. TDP-43 immunoreactive lesions in the frontal cortex (**a**) and granule cells of the dentate fascia (**b**) showing neuronal cytoplasmic inclusion (NCI) (*arrow*), dystrophic neurite (DN) (*double arrow*), and glial inclusion (GI) (*arrow head*) (TDP-43 immunohistochemistry, *bars a, b* = 50 μ m)

Fig. 3.
a Neuronal intranuclear inclusion (NII) (*arrow*) in lamina III of the superior temporal gyrus and **b** glial inclusion (GI) (*arrow*) in underlying white matter (TDP-43 immunohistochemistry, bars **a**, **b** = 50 µm)

Fig. 4. Principal components analysis of 94 cases of frontotemporal lobar degeneration with TDP proteinopathy (FTLD-TDP) based on all histological variables (PCA-1). Identified on the plot are the subtypes of disease based on the system of Cairns et al. (2007b). Cases labelled ND could not be ascribed readily to disease subtype

Fig. 5.

Principal components analysis of 94 cases of frontotemporal lobar degeneration with TDP proteinopathy (FTLD-TDP) based on TDP-43 pathology only (PCA-2). Identified on the plot are familial and sporadic cases, those with progranulin (*GRN*), valosin containing protein (*VCP*), and chromosome 9p21 (*CH9*) mutation. The familial/sporadic status of cases labelled ND has not been determined to date

Fig. 6. Principal components analysis of 94 cases of frontotemporal lobar degeneration with TDP proteinopathy (FTLD-TDP) based on TDP-43 pathology only (PCA-2). Identified on the plot are cases with coexisting motor neuron disease (MND), hippocampal sclerosis (HS), and Alzheimer's disease (AD)

Fig. 7.

Principal components analysis of 94 cases of frontotemporal lobar degeneration with TDP proteinopathy (FTLD-TDP) based on TDP-43 pathology only (PCA-2). Identified on the plot are the subtypes of disease based on the system of Cairns et al. (2007b). Cases labelled ND could not be ascribed readily to disease subtype

Summary of demographic features, familial status, gross brain weight, Braak score, and pathological subtype of the 94 cases of frontotemporal lobar degeneration with TDP proteinopathy (FTLD-TDP) from various centres

Table 1

C	Case	M/F	Onset	Dur	Death	BW	Fm/S	Braak	AP	ST
WUS	1.	M	57	8	65	960	S	0	N	2
	2.	F	72	12	84	900	S	0	HS	2
	3.	F	61	16	77	950	S	1	N	3
	4.	F	68	6	74	975	GRN	0	N	4
	5.	M	52	13	65	1,300	GRN	4	AD	1
	6.	M	66	16	82	–	S	–	HS	3
	7.	F	69	15	84	970	GRN	4	AD	3
	8.	M	60	6	66	–	GRN	4	AD	1
	9.	F	65	12	77	810	GRN	4	N	1
	10.	M	52	15	67	960	GRN	0	N	4
	11.	M	74	6	80	1,270	Fm	4	N	4
	12.	F	–	–	67	990	S	1	N	2
	13.	F	59	9	68	650	Fm	0	N	2
	14.	M	74	1	75	1,360	S	1	AD	1
	15.	M	60	11	71	1,450	Fm	–	HS	4
	16.	M	43	7	50	1,060	Fm	–	N	3
	17.	M	55	11	66	1,005	GRN	1	HS	2
	18.	F	63	3	66	950	S	–	N	1
	19.	F	58	9	67	880	GRN	2	AD, HS	2
	20.	F	64	19	83	720	Fm	1	N	1/2
	21.	F	69	4	71	1,070	S	1	N	4
	22.	M	38	9	47	1,185	VCP	0	N	4
	23.	F	–	–	73	720	S	–	N	1
	24.	M	50	18	68	1,170	S	0	HS	1
	25.	M	58	8	66	1,080	Fm	2	AD	4
	26.	F	65	13	78	960	Fm	0	N	–
	27.	M	57	6	63	1,080	GRN	1	N	–
	28.	M	51	11	62	880	CH9	4	AD	2

C	Case	M/F	Onset	Dur	Death	BW	Fm/S	Braak	AP	ST
UCD	29.	F	71	13	84	960	S	2	AD	1
	30.	F	73	9	82	–	GRN	6	AD	2
	31.	F	58	8	66	–	Fm		N	4
	32.	M	71	8	79	1,150	GRN	–	AD	3
	1.	M	59	9	69	1,050	S	0	N	–
	2.	F	46	7	53	870	Fm	0	AD	2
	3.	M	72	6	78	1,190	S	1	N	2
	4.	M	77	7	77	1,170	S	0	N	4
	5.	F	68	14	81	1,020	S	1	N	–
	6.	F	58	9	67	1,080	S	1	N	1
	7.	M	78	10	88	1,020	S	0	N	4
	8.	F	57	16	73	834	S	0	N	2
UP	9.	M	63	12	75	1,160	S	2	N	1
	10.	F	55	4	59	1,050	S	0	N	1
	11.	F	68	2	70	1,354	Fm	0	N	4
	12.	F	78	13	91	1,016	S	1	AD	–
	13.	F	67	6	73	1,010	S	0	N	3
	14.	F	65	4	69	1,010	Fm	–	N	–
	15.	F	62	3	65	1,180	S	0	N	–
	1.	M	61	4	65	1,100	S	2	N	2
	2.	F	60	10	70	980	S	2	N	2
	3.	M	55	10	65	1,070	S	1	N	2
	4.	M	69	6	75	1,000	Fm	3	N	2
	5.	F	66	9	75	1,070	Fm	0	N	1
	6.	M	61	3	64	1,330	S	1	N	2
	7.	F	67	1	68	1,180	S	–	MND	–
	8.	F	59	6	75	880	S	1	N	–
	9.	F	68	13	81	810	–	5	AD, LBD	2
	10.	M	–	–	76	1,440	S	3	N	1
	11.	M	27	10	37	1,160	S	0	N	–
	12.	M	–	–	80	1,200	S	1	MND	3

C	Case	M/F	Onset	Dur	Death	BW	Fm/S	Braak	AP	ST
VH	13.	F	48	8	56	800	S	3	AD	4
	14.	M	47	1	48	1,370	S	0	MND	—
	15.	F	56	6	62	900	S	0	N	—
	1.	M	66	5	71	1,230	Fm	2	MND	3
	2.	F	—	—	66	940	S	0	N	1
	3.	M	69	3	72	—	S	0	MND	2
	4.	F	65	5	70	1,013	S	0	N	—
	5.	F	56	5	61	940	Fm	0	N	1
	6.	M	61	5	66	—	GRN	0	N	1
	7.	F	55	15	70	—	S	3	N	1
	8.	M	68	3	71	1,400	S	1	N	1
	9.	M	70	1	71	1,140	S	4	MND	2
	10.	M	55	14	69	—	GRN	0	N	1
	11.	F	66	5	71	1,050	GRN	0	HS	1
	1.	M	55	4	59	1,440	—	0	N	2
HV	2.	M	72	18	90	1,280	S	3	N	3
	3.	M	73	6	79	1,180	Fm	2	N	3
	4.	F	80	8	88	1,035	—	3	N	4
	5.	M	—	—	75	1,065	—	3	N	1
	6.	F	—	—	55	1,300	—	1	N	2
	7.	F	65	5	70	—	Fm	4	N	—
	8.	M	57	9	66	1,530	Fm	1	N	2
	9.	F	—	—	84	—	S	3	N	—
	1.	M	—	—	59	1,100	S	0	N	—
	2.	F	70	3	73	1,140	Fm	2	N	4
	3.	F	56	3	59	1,160	S	0	MND	2
	4.	F	58	5	63	1,090	Fm	1	N	3
	5.	F	69	2	71	1,510	S	3	N	—
	6.	F	80	3	83	—	S	0	N	—
	1.	F	67	12	79	870	S	0	—	—
	2.	M	67	6	73	970	Fm	0	N	—
EU	1.	M	—	—	59	1,100	S	0	N	—
	2.	F	70	3	73	1,140	Fm	2	N	4
	3.	F	56	3	59	1,160	S	0	MND	2
	4.	F	58	5	63	1,090	Fm	1	N	3
	5.	F	69	2	71	1,510	S	3	N	—
	6.	F	80	3	83	—	S	0	N	—
	1.	F	67	12	79	870	S	0	—	—
	2.	M	67	6	73	970	Fm	0	N	—
	1.	M	—	—	59	1,100	S	0	N	—
	2.	F	70	3	73	1,140	Fm	2	N	4
	3.	F	56	3	59	1,160	S	0	MND	2
	4.	F	58	5	63	1,090	Fm	1	N	3
	5.	F	69	2	71	1,510	S	3	N	—
	6.	F	80	3	83	—	S	0	N	—
	1.	F	67	12	79	870	S	0	—	—
	2.	M	67	6	73	970	Fm	0	N	—

C	Case	M/F	Onset	Dur	Death	BW	Fm/S	Braak	AP	ST
CU	1.	M	60	1	61	1,344	S	0	MND, AD	3
	2.	M	88	1	89	1,278	S	2	MND, AD	–
UCI	1.	M	58	2	60	1,297	S	2	N	–
UM	1.	M	70	11	81	1,340	S	–	AD	1

WUS Washington University, School of Medicine, UCD University of California, Davis, UP University of Pittsburgh, VH Vancouver Hospital, HU Harvard University, EU Emory University, UW University of Washington, CU Columbia University, New York, UCI University of California, Irvine, UM University of Michigan, M/F male/female, Dur disease duration, Death age at death, Fm/S familial/ sporadic, AP additional pathology, – indicates data not available, ST subtype, GRN cases caused by granulin gene mutations, VCP case caused by valosin-containing protein, CH9 case associated with putative gene on chromosome 9, N no associated pathology identified, HS hippocampal sclerosis, AD Alzheimer's disease

Table 2

Degree of agreement between pairs of counts (count–recount variability) made of each histological feature in the middle frontal gyrus (MFG) in the 32 Washington University, School of Medicine cases using the Bland and Altman method

Feature	Mean density	Mean difference between counts	SD	Limits of agreement
NCI	0.20	0.02	0.18	±0.15
GI	0.05	0.01	0.04	±0.07
NII	0.20	0.02	0.12	±0.24
EN	0.12	0.06	0.11	±0.21
N	5.76	0.42	1.52	±2.98
V	10.99	1.61	2.08	±4.08
DN	0.35	0.04	0.07	±0.13

SD standard deviation

Table 3

Correlations (Pearson's 'r') between the pathological features, demographic variables, and Braak score based on the distribution of neurofibrillary tangles (NFT) and the first three principal components (PC) in various brain regions in cases of frontotemporal lobar degeneration with TDP-43 proteinopathy (FTLD-TDP)

Region	Lesion	PCA-1: all variables			PCA-2: TDP-43 pathology		
		PC1	PC2	PC3	PC1	PC2	PC3
MFG (U)	NCI	0.06	-0.29*	-0.16	-0.16	0.02	-0.11
	GI	-0.17	0.05	0.04	-0.01	-0.12	0.12
	NII	-0.05	-0.26	0.07	0.21	0.07	0.39*
	EN	0.04	-0.13	0.16	-0.02	0.02	-0.08
	N	0.14	-0.55*	0.06	-0.16	-0.14	0.08
	V	0.46*	0.25	-0.82*	-0.08	-0.12	-0.08
	DN	0.07	0.06	0.06	-0.06	-0.62*	0.32*
MFG (L)	NCI	0.03	-0.31*	-0.14	-0.18	0.08	-0.14
	GI	-0.04	-0.06	0.11	-0.11	-0.12	-0.16
	NII	-0.04	-0.31*	0.10	0.30*	0.24	0.54*
	EN	0.18	-0.12	-0.14	-0.08	0.14	-0.14
	N	0.12	-0.34*	-0.04	-0.08	-0.05	0.14
	V	0.31*	0.24	-0.67*	-0.16	-0.17	-0.20
	DN	0.11	0.06	-0.02	0.03	-0.41*	0.22
ITG (U)	NCI	0.07	-0.20	-0.14	-0.47*	0.06	-0.49*
	GI	0.06	0	-0.24	-0.25	0	-0.38*
	NII	0.02	-0.20	0.06	0.17	0.28*	0.29*
	EN	0.07	-0.04	0.04	0.11	-0.02	0.11
	N	0.11	-0.29	-0.06	-0.08	-0.10	-0.05
	V	0.43*	0.66*	-0.12	-0.12	-0.34*	0.05
	DN	0.10	0.14	-0.06	-0.27*	-0.68*	0.02
ITG (L)	NCI	-0.05	-0.09	-0.14	-0.26	-0.10	-0.41*

Region	Lesion	PCA-1: all variables			PCA-2: TDP-43 pathology		
		PC1	PC2	PC3	PC1	PC2	PC3
PHG (U)	GI	-0.03	0.03	0.08	-0.14	-0.22	-0.33 *
	NII	0.13	-0.09	0.10	0.16	0.16	0.48 *
	EN	-0.01	-0.18	-0.16	0.01	0.14	0.02
	N	0.18	-0.22	-0.14	-0.05	-0.10	0.05
	V	0.22	0.57 *	0.10	0	-0.29	0.10
	DN	0.10	0.22	-0.05	-0.10	-0.64 *	0.04
	NCI	-0.08	-0.17	-0.05	-0.51 *	0.19	-0.43 *
	GI	0.05	-0.03	-0.17	-0.18	-0.17	-0.22
	NII	0.15	-0.20	-0.04	0.28 *	0.18	0.40
	EN	0.06	-0.08	-0.04	0.07	0.07	0.08
PHG (L)	N	0.07	-0.29 *	-0.03	-0.02	-0.07	0.13
	V	0.55 *	0.67 *	0.02	0	-0.31 *	0.14
	DN	0.03	0.01	0.14	0.05	-0.44 *	0.14
	NCI	0.09	-0.20	-0.06	-0.27 *	-0.05	-0.34 *
	GI	0.21	-0.11	-0.18	-0.28 *	-0.27	-0.32 *
	NII	0.16	-0.18	0.11	0.12	0.32 *	0.41 *
	EN	-0.01	-0.03	-0.09	0.01	-0.03	0.08
	N	-0.04	-0.46 *	-0.02	-0.02	0.07	0.02
	V	0.57 *	0.54 *	-0.11	0.06	-0.10	0.10
	DN	0.07	0.15	0.18	0.03	-0.40 *	0.07
CA1/2	NCI	-0.07	-0.03	-0.07	-0.08	0.04	-0.39 *
	GI	0.15	-0.20	0.01	-0.05	-0.19	-0.24
	NII	-0.08	-0.11	0.25	0.09	0.38 *	0.44 *
	EN	-0.02	-0.06	0.11	-0.07	-0.15	-0.05
	N	-0.02	-0.24	-0.01	0.03	0.15	-0.13
	V	0.33 *	0.41 *	0.01	0.14	-0.02	-0.16

Region	Lesion	PCA-1: all variables			PCA-2: TDP-43 pathology		
		PC1	PC2	PC3	PC1	PC2	PC3
DG	DN	0	-0.10	0.03	0.13	-0.29*	0.04
	NCI	0.17	-0.22	-0.11	-0.65*	0	-0.34*
	GI	-	-	-	-	-	-
	NII	0.12	-0.04	0.08	0.12	0.14	0.22
	EN	-0.03	0.03	0.03	0	-0.12	-0.07
	N	-0.05	-0.62*	-0.07	-0.02	0.01	0.09
	V	0	0.64*	-0.13	0.11	-0.29*	-0.03
	DN	0.14	0.08	-0.13	-0.16	-0.05	-0.12
	Onset	0.06	0.24	0.05	-0.07	-0.01	0.25
Duration		0.28*	0.09	-0.11	0.09	-0.35*	0.20
	Age at death	0.17	0.26	-0.01	-0.03	-0.19	0.29*
Brain weight		-0.26	0.04	0.14	-0.06	0.24	0.09
	F/S	-0.10	-0.02	0.10	0.30*	-0.14	0.17
Braak score		-0.06	-0.02	0.10	0.11	-0.17	0.14

All significant correlations, i.e. those significant at $P < 0.05$ are indicated in bold

Pathological features—*NCI* neuronal cytoplasmic inclusions, *GI* oligodendroglial inclusions, *NII* neuronal intranuclear inclusions, *DN* dystrophic neurites, *EN* abnormally enlarged neurons, *N* surviving neurons, *V* vacuolation; various brain regions—*MFG* superior frontal gyrus, *ITG* inferior temporal gyrus, *PHG* parahippocampal gyrus, *CA1/2* sectors CA1 and 2 of the hippocampus, *DG* dentate gyrus, *III* laminae II/III, *V/VI* laminae V/VI

* $P < 0.01$