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Prominent microglial activation in cortical white matter is selectively associated with cortical atrophy in primary progressive aphasia

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Abstract

Aims—Primary progressive aphasia (PPA) is a clinical syndrome characterized by selective language impairments associated with focal cortical atrophy favouring the language dominant hemisphere. PPA is associated with Alzheimer's disease (AD), frontotemporal lobar degeneration (FTLD), and significant accumulation of activated microglia. Activated microglia can initiate an inflammatory cascade that may contribute to neurodegeneration, but their quantitative distribution in cortical white matter and their relationship with cortical atrophy are unknown. We investigated white matter activated microglia and their association with grey matter atrophy in 10 PPA cases with either AD or FTLD-TDP pathology.

Methods—Activated microglia were quantified with optical density measures of HLA-DR immunoreactivity in two regions with peak cortical atrophy, and one non-atrophied region within the language dominant hemisphere of each PPA case. Non-atrophied contralateral homologues of the language dominant regions were examined for hemispheric asymmetry.

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Results—Qualitatively, greater densities of activated microglia were observed in cortical white matter when compared to grey matter. Quantitative analyses revealed significantly greater densities of activated microglia in the white matter of atrophied regions compared to non-atrophied regions in the language dominant hemisphere ($p<0.05$). Atrophied regions of the language dominant hemisphere also showed significantly more activated microglia compared to contralateral homologues ($p<0.05$).

Conclusions—White matter activated microglia accumulate more in atrophied regions in the language dominant hemisphere of PPA. While microglial activation may constitute a response to neurodegenerative processes in white matter, the resultant inflammatory processes may also exacerbate disease progression and contribute to cortical atrophy.

Keywords

PPA; Alzheimer's disease; TDP-43; Activated microglia; Cortical atrophy; White matter pathology

INTRODUCTION

Primary progressive aphasia (PPA) is a clinical dementia syndrome characterized by selective and gradual dissolution of language function, with preservation of other cognitive domains in the initial stages of disease progression [1]. Consistent with this clinical phenotype, brains of PPA patients display focal and asymmetric cortical atrophy [2] in language regions of the language dominant hemisphere (typically left), as determined by structural magnetic resonance imaging (MRI) scans. Diverse neuropathologic changes occur in the grey matter of PPA brains, but the neuropathologic mechanisms of cortical atrophy remain poorly understood.

PPA is associated with Alzheimer's disease (AD) and frontotemporal lobar degeneration (FTLD). FTLD includes non-Alzheimer's tauopathies (FTLD-tau) and transactive response DNA-binding protein of 43-kD (TDP-43) proteinopathies (FTLD-TDP) [3]. Regardless of the underlying molecular pathology, there is extensive activation of microglia in PPA, which may serve as a potential inflammatory marker for pathology and disease severity that also plays a role in neurodegenerative processes [4]. We have previously shown that activated microglia accumulate in grey matter of PPA brains [5], but their density and distribution in white matter, and their relationship to neurodegenerative processes such as cortical grey matter atrophy are unknown in PPA.

The precise roles of microglia in health and disease are still under investigation. Microglia are the resident macrophages of the brain that engage in immune surveillance to maintain homeostasis. They exist in a continuum of shapes and sizes, ranging from 'ramified' microglia which tend to be small, thin, and highly branched, to 'activated' microglia which appear larger due to their hypertrophy and shorter, thicker processes [4]. The morphologic heterogeneity of microglia is believed to reflect a spectrum of functions. While ramified microglia appear motile and survey the local environment for danger, activated microglia may serve roles in wound healing, host defense, and regulatory processes [6]. When activated, microglia undergo a change in morphology and an upregulation of cell-surface

receptors and cytoplasmic proteins that can be readily targeted by immunohistochemical techniques to reveal their presence and distribution. In the activated state, microglia have the capacity to secrete harmful cytotoxic mediators including cytokines, reactive oxygen species, and other factors that stimulate inflammatory processes linked to neuronal damage [7]. Activated microglia also appear in close proximity to pathologic insults such as amyloid- β plaques and neurofibrillary tangles in AD [8–11], demyelinating pathology in multiple sclerosis [12], Pick bodies in frontotemporal dementia (FTD) patients with underlying FTLD-tau [13, 14], and TDP-43 inclusions in FTD and PPA patients with underlying FTLD-TDP [5, 14]. Together, inflammatory processes and co-localization with pathologic proteins have implicated activated microglia in disease pathogenesis.

The experimental study of microglial activation in white matter has been sparse in diseases such as AD and FTLD. One report showed that activated microglia increased linearly with age in the white matter of the superior frontal gyrus, with no obvious difference between AD patients and non-AD elderly controls [15]. Similar observations were reported in the white matter of hippocampus and superior frontal gyrus, with comparable patterns of activated microglia between AD patients and non-demented elderly controls [9]. Other studies have reported high densities of activated microglia in the white matter of the hippocampus [10] and entorhinal cortex in AD brains [16, 17], and prominent microglial activation in white matter when compared to grey matter in the frontal cortex and superior temporal gyrus in FTLD-tau and FTLD-TDP brains [13]. These findings suggest that microglial activation corresponds to pathologic changes in grey and white matter across disease phenotypes. However, the limited number of regions examined, and the relatively qualitative nature of these studies, make it difficult to determine the extent of activated microglia in human white matter and its potential regional selectivity.

The purpose of this study was to characterize the regional distribution and hemispheric asymmetry of activated microglia in cortical white matter of the brains of PPA patients with AD or TDP-43 pathology, and to determine their relationship with regional cortical grey matter atrophy. As a marker for neuroinflammation and possibly a correlate of neurodegeneration, we hypothesized that activated microglia accumulate more in white matter of cortical regions with prominent grey matter atrophy.

MATERIALS AND METHODS

PPA cases

Ten recent PPA cases with either AD or FTLD-TDP pathology and available structural MRI scans were included in the study. Eight out of 10 PPA cases were enrolled through the PPA Research Program at the Cognitive Neurology and Alzheimer's Disease Center at Northwestern University Feinberg School of Medicine. Two PPA cases (#6 and #7) were not enrolled in the PPA Research Program but were clinically evaluated at our Center. The diagnosis of PPA was based on at least a two year history of progressive, isolated impairments of speech or language functions; clinical subtypes (logopenic, agrammatic, and semantic) were classified based on previously described diagnostic guidelines [1, 18, 19]. Case characteristics are included in Table 1.

Assessments of cortical atrophy and regions of interest

The location and extent of cortical atrophy were determined by post-mortem assessments and corroborated with *in vivo* structural MRI data (Figure 1). Photographs of intact, post-mortem brains were collected from each PPA case and visually inspected to determine regions of principal atrophy and areas with undetectable atrophy. Six regions were selected and subsequently examined in each PPA case, three per hemisphere (Table 2). The three regions within the language dominant hemisphere included two regions with peak cortical atrophy, and one non-atrophied region. Non-atrophied contralateral homologues of each region were assessed to determine if activated microglia displayed asymmetric densities between hemispheres.

Structural MRI data were acquired on a 3T scanner from 35 previously described [2] healthy controls and 8/10 PPA participants. The remaining two PPA cases had clinical MRI scans acquired on a 1.5T scanner. FreeSurfer software (v5.1.0, <http://surfer.nmr.mgh.harvard.edu>) was used to analyse the 3T structural MRI in 7/10 PPA cases (#1–5, 9, 10). FreeSurfer generated cortical surface heat maps (false discovery rate correction set to 0.05 [20]) on the native space for each PPA case to detect areas of peak cortical thinning (i.e., grey matter cortical atrophy) in comparison to the control group. The cortical surface heat maps were visually inspected to validate the regions of peak cortical atrophy (areas in yellow, Figure 1) identified from post-mortem assessments. Movement artefacts precluded a reliable FreeSurfer analysis of structural MRI collected from case #8, prompting a visual inspection of atrophy from coronal MRI slices. Similarly, clinical MRI scans were visually inspected to corroborate post-mortem identification of atrophy for PPA case #6 and 7. The mean interval between MRI and death was 2.77 ± 1.5 years; range 0.6–6 years (Table 1).

Tissue processing

Brains were fixed in either 10% formalin (F) or 4% paraformaldehyde (P) for 30–36 hours at 4°C, and then submerged into increasing gradients of sucrose (10–40%) for cryoprotection (Table 1). A 1-in-24 series of 40 µm-thick coronal sections was collected from each region (six sections per region). Tissue was processed immunohistochemically without antigen retrieval using the avidin-biotin peroxidase complex method employing the Vectastain Elite Kit and 3,3'-diaminobenzidine as the chromogen. Activated microglia were visualized immunohistochemically using an antibody against the human leukocyte antigen-D related (HLA-DR) protein (mouse monoclonal; Dako; 1/1000) (Figure 2, Figure 3a) and an antibody against CD68 (mouse monoclonal; Dako; 1/500) (Figure 3b). HLA-DR is a cell surface glycoprotein present in microglia in class II of the major histocompatibility complex that mediates the presentation of foreign antigens in normal and diseased brains [21–23]. CD68 is a macrophage-specific lysosomal-associated protein that labels microglia in resting and activated states [14, 24–26].

For anatomic specification in each case, a series of sections was processed with the cresyl violet Nissl stain or immunohistochemically for neuronal nuclear protein (NeuN, mouse monoclonal; EMD Millipore; 1/2000).

Assessments of activated microglia

HLA-DR-immunoreactive microglia were assessed qualitatively and measured quantitatively in each PPA case. The current study restricted quantitative analyses to HLA-DR staining, but CD68-immunoreactive microglia were also assessed qualitatively in a subset of PPA-AD and PPA-TDP cases to determine if another, less specific marker of activated microglia displayed similar patterns to HLA-DR staining. The qualitative assessment encompassed regions of interest and neighbouring areas (grey matter and white matter) to document the full extent and distribution of activated microglia. The quantitative measurement included quantification of HLA-DR-immunoreactive microglia in the cortical white matter toward the gyral crown of each region using photomicrographs acquired at a final magnification of 4x. The cortical white matter was sampled once in every gyrus that comprised a region (1–4 photomicrographs per coronal section; 6–19 photomicrographs per region) to ensure that a representative sampling of the anatomy was acquired for analyses. The number of gyri in each region varied based on anatomical location regardless of degree of atrophy. For example, frontal and parietal regions typically had more than one gyrus compared to temporal regions which typically had one gyrus. Therefore, more photomicrographs were acquired in regions with more gyri, while still maintaining a similar mean number of photomicrographs sampled per region, regardless of whether it was an atrophied (10 ± 4.7 photomicrographs) or a non-atrophied (9 ± 3.9 photomicrographs) region. Irrespective of region, photomicrographs rarely contained grey matter, but when they did contain a small area of grey matter, the image was cropped to ensure that the quantification of activated microglia was limited to the white matter only. Photomicrographs containing only white matter were imported into ImageJ software (version 1.51) to measure the optical density (OD) of HLA-DR immunoreactivity. OD was calculated using the following formula:

$$OD = \log (\text{maximum light intensity}/\text{mean light intensity})$$

where maximum intensity is 255 for 8-bit images. OD was averaged across photomicrographs and used as a single data point in each region. The OD values acquired from the two atrophied regions were averaged to obtain one OD value used in analyses. Images displaying greater concentration and/or size of HLA-DR-immunoreactive microglia equated to lower light transmission, resulting in higher OD values.

The acquisition and subsequent post-processing of photomicrographs were conducted by two independent investigators (D.T.O. and G.K.). Photomicrographs were collected systematically from an investigator with strong anatomical knowledge (D.T.O.) to ensure sampling was drawn from appropriate anatomical locations consistently and accurately before the images were cropped and analysed by a second investigator (G.K.) blinded to the atrophy patterns specific to that region or PPA case.

Statistical Analyses

Each PPA participant with available quantitative MRI close to death was statistically compared to a control group of 35 healthy participants using spherical surface maps of the whole brain. A general linear model on every vertex along the cortical surface was used to calculate the significant differences in cortical thickness between groups.

Mean optical densities of activated microglia passed normality for all groups compared. Paired *t*-tests were used to determine differences in mean optical densities of activated microglia between atrophied and non-atrophied regions within the language dominant hemisphere. To assess asymmetry, paired *t*-tests determined the difference in mean optical density between hemispheres within atrophied and non-atrophied regions. Two-tailed Pearson correlation determined the relationship between age and mean optical densities of activated microglia. Significance was set to $p < 0.05$ for all comparisons. All statistical analyses of optical densities were performed using GraphPad Prism (version 7.0 for Mac, GraphPad Software, La Jolla California USA, www.graphpad.com).

RESULTS

Clinical and neuropathologic findings

Ten PPA cases were confirmed to have either AD pathology (PPA-AD; $n=5$) or FTLN-TDP pathology (PPA-TDP; $n=5$) at death based on published consensus criteria [27–30]. Each PPA-AD case was diagnosed with “high” AD neuropathologic change. Two PPA-TDP cases (#6 and 8) had FTLN-TDP type B pathology, and three PPA-TDP cases (#7, 9, and 10) had FTLN-TDP type A pathology. While additional pathologic features (e.g., cerebrovascular disease, arteriosclerosis) were present in most cases, they were minor and secondary to the primary pathologic diagnoses (i.e., AD and FTLN-TDP). Myelin rarefaction of the subcortical white matter varied in severity (i.e., mild to moderate) and was asymmetric in select PPA cases.

Of the five PPA-AD participants, two were clinically diagnosed with the logopenic variant (PPA-L), one with the agrammatic variant (PPA-G), and two were unclassifiable (PPA-U) due to the severity of their condition at the time of examination (see Table 1). In the PPA-TDP cohort, two were agrammatic with a speech disorder (PPA-Gsp), one had a mixture of agrammatism and comprehension deficits (PPA-M), one was logopenic, and one was logopenic before progressing to agrammatic (L→G; see Table 1). The 35 healthy participants did not differ in education or age compared to the PPA participants with available quantitative MRI. The PPA-AD and PPA-TDP groups did not differ in sex, education, age at death, disease duration, or PMI. While 9/10 PPA participants were right-handed with likely left hemisphere language dominance, PPA-TDP case #7, which was previously described [31], was left-handed and displayed right hemisphere language dominance based on a language task that produced fMRI activations in right perisylvian cortical regions.

Grey matter cortical atrophy

Each PPA case was evaluated individually to determine areas of greatest atrophy and undetectable atrophy. General patterns of cortical atrophy were observed: PPA-AD typically displayed the greatest atrophy in lateral temporal and parietal lobes, while PPA-TDP showed the most atrophy in the frontal and lateral anterior temporal lobes.

Peak atrophy sites varied by individual and by pathologic diagnosis but were most consistently found in language regions of the language dominant hemisphere (Figure 1). The

language dominant hemisphere contained the atrophied regions in all PPA cases, and the non-atrophied region included primary visual cortex in PPA-AD and areas of the parietal lobe in PPA-TDP (Table 2). The contralateral homologues to all regions examined in the language dominant hemisphere had undetectable cortical atrophy (non-atrophied). Post-mortem assessments of PPA case #4 revealed symmetric and mild cortical atrophy throughout the cerebrum, and asymmetric atrophy in the temporal lobe (pSTG) where it was moderate on the left and mild on the right. However, the MRI cortical thinning map for case #4 could not confirm this pattern of atrophy as it revealed no significant cortical thinning bilaterally.

Qualitative patterns of activated microglia in cortical white matter versus grey matter

HLA-DR staining was consistently present in white and grey matter of all PPA cases, but the intensity of staining in white matter varied greatly between regions and within brain sections (Figure 2). Qualitative observations of the post-mortem tissue revealed HLA-DR staining dark enough for the naked eye to readily discern the grey/white matter junction and regional asymmetries. Relative to the grey matter, the white matter tended to have a more homogeneous distribution of activated microglia, but topographic patterns were observed in relation to the gyral crown and fundus. Staining was often more prominent toward the gyral crown and in the short association U-fibres found in the white matter directly adjacent to the grey matter (Figure 2, red insets). High power magnification of the more prominent staining reflected a greater density of activated microglia, larger hypertrophic microglia, and often a combination of the two (Figure 2, black insets). The higher densities of activated microglia were occasionally due to round clusters of larger, overlapping hypertrophic microglia that ranged in diameter between 30–100 μm (Figure 3). Activated microglia typically displayed the hypertrophic morphology in the white matter, while the grey matter showed a greater spectrum of morphologies that were distributed unevenly across the cortical layers (Figure 2). Microglia of the ramified type were virtually absent in the white matter in all regions examined. When large clusters of activated microglia were present, they were observed more often in the U-fibre tracts (Figure 2, red insets). In direct contrast to the atrophied regions in the language dominant hemisphere, non-atrophied regions displayed sparse and lightly stained activated microglia in the white matter bilaterally such that the boundary between white and grey matter could only be determined at high magnification.

CD68 staining largely reflected the HLA-DR staining patterns observed in the grey matter and white matter across PPA-AD and PPA-TDP. Qualitative comparisons clearly demonstrated greater densities of CD68-immunoreactive microglia in the white matter of atrophied regions in the language dominant hemisphere when compared to non-atrophied regions (Figure 3b). Additionally, CD68-immunoreactive microglia showed asymmetry such that regions contralateral to the language dominant hemisphere displayed less staining. In contrast to HLA-DR staining, however, CD68-immunoreactive microglia had less robust staining that was mostly restricted to the somas and relatively absent in the dendritic processes. CD68-immunoreactive microglia were less dense throughout all regions of interest in comparison to HLA-DR staining. Nevertheless, the overall staining patterns for CD68 were strikingly similar to HLA-DR and consistent with our hypothesis that more activated microglia in white matter are associated with regions of greater atrophy.

Neuropathologic subtypes of PPA displayed notable differences in HLA-DR staining. Staining was often more prominent in the white matter of PPA-TDP compared to PPA-AD (Figure 2). While PPA-TDP displayed more activated microglia in anterior regions of the frontal and temporal white matter, PPA-AD showed more activated microglia in posterior regions of the temporal and parietal white matter. Despite less intense staining of the white matter in PPA-AD, the delineation between white and grey matter was accentuated by a relative absence of staining in cortical layer six across all brain regions in PPA-AD (Figure 2, PPA-AD—red insets). High magnification of layer six revealed that the reduced staining was due to several factors including a lower density of activated microglia, relatively lighter stained activated microglia, little to no clusters of activated microglia, and a greater proportion of ramified microglia (Figure 2; PPA-AD—green insets). In contrast, brain regions in PPA-TDP displayed more homogeneous staining across the cortical layers (Figure 2, red insets). The densities of activated microglia were also more homogenous in the cortical white matter in PPA-TDP, but many regions displayed darker staining in the short association U-fibres of white matter due to clusters and/or larger, more densely packed activated microglia.

The PPA-TDP subgroup was comprised of FTLTDP Type A (n=3) and Type B (n=2) pathologic diagnoses (Table 1) that appeared to reflect small differences in activated microglia densities. While visual inspection of the patterns of HLA-DR-immunoreactivity showed a similar range in intensity within the small cohort, Type B cases tended to have a 1.3–2.3x greater mean optical density of activated microglia compared to Type A cases across the regions examined. Despite these differences, asymmetric densities of activated microglia were consistently found in the atrophied regions regardless of FTLTDP type.

Quantification of HLA-DR staining in cortical white matter regions

Analysis within the language dominant hemisphere—The densities of activated microglia were examined in the greatest atrophied regions within the language dominant hemisphere and compared to a non-atrophied region within the same language dominant hemisphere. Analyses of activated microglia were carried out in 9 PPA cases; PPA case #4 was excluded due to the relative absence of detectable cortical atrophy and lack of corroboration between post-mortem and MRI assessments.

Within the language dominant hemisphere of all PPA cases combined, the mean optical density of activated microglia was greater in atrophied regions compared to non-atrophied regions (Figure 4; $p<0.05$). An analysis of each PPA neuropathologic subtype revealed a non-significant trend for a greater mean optical density of activated microglia in the atrophied regions compared to the non-atrophied regions in PPA-AD ($p=0.075$). The difference between atrophied and non-atrophied regions was not statistically different in PPA-TDP ($p=0.113$). Despite these results, individual differences were notable. In one case (PPA-TDP case #7), the mean optical density of activated microglia was 5.5x greater in the language dominant atrophied region compared to the non-atrophied region. On average, the mean optical densities in the atrophied regions were almost 3x greater than that in the non-atrophied regions of 7/9 PPA cases (three PPA-AD; four PPA-TDP). The remaining 2/9 PPA

cases (PPA-AD case #1; PPA-TDP case #6) displayed similar optical densities in the atrophied and non-atrophied regions of the language dominant hemisphere.

Analysis of asymmetry—The contralateral homologues of the atrophied and non-atrophied regions in the language dominant hemisphere were also examined in this study to establish whether activated microglia accumulate asymmetrically. An asymmetric distribution of activated microglia was only anticipated in the language dominant atrophied regions given the asymmetric cortical atrophy and the prominent language deficits characteristic of PPA.

When PPA cases were combined, atrophied regions in the language dominant hemisphere showed a significantly greater mean optical density of activated microglia than their contralateral counterparts (Figure 4, $p < 0.05$). A non-significant trend for greater mean optical density in the language dominant atrophied regions was observed in PPA-AD ($p = 0.082$) and PPA-TDP ($p = 0.088$). As expected, non-atrophied regions of all PPA cases combined did not display asymmetric differences in mean optical densities (Figure 4, $p = 0.307$). This pattern was preserved when examining the PPA-AD ($p = 0.574$) and PPA-TDP cohorts ($p = 0.226$) separately. In addition, optical densities of activated microglia did not correlate with variables such as age or PMI.

DISCUSSION

The present study investigated the density and distribution of activated microglia in the white matter of PPA participants, and their relationship to grey matter cortical atrophy. Consistent with our hypothesis, there were more white matter activated microglia in atrophied regions than non-atrophied regions within the language dominant hemisphere. Activated microglia were also more pronounced in atrophied regions of the language dominant hemisphere in comparison to the contralateral hemisphere. Little is known about the distribution and magnitude of white matter microglial activation in neurodegenerative diseases such as AD or FTL. Our findings suggest that white matter activated microglia accumulate with regional and hemispheric selectivity, which corresponds to patterns of grey matter neurodegeneration in PPA with either AD or FTL pathology.

The predilection of HLA-DR-immunoreactive microglia to regions of peak cortical atrophy suggests that activated microglia could be contributing to neurodegenerative processes by possibly secreting cytotoxic molecules that can exacerbate the inflammatory response. At the same time, it is well known that activated microglia can also provide protective benefits. The diverse roles of activated microglia make it difficult to tease apart the functional consequences of the prominent microglial activation observed in the current study, and this topic warrants further exploration. Nevertheless, our findings indicate that white matter activated microglia have a close relationship with grey matter neurodegeneration not previously reported.

Age, fixation, PMI, and clinical factors such as pre-agonal conditions could influence the HLA-DR-immunoreactivity of microglia present in the white matter [32]. In our well-characterized cohort of PPA cases, we did not find any link between HLA-DR antigenicity

and these potential confounding variables. HLA-DR-immunoreactivity produced a range of staining intensity that did not vary systematically across the PPA cases examined. More specifically, cases with PMI >6 hrs (n=6) presented HLA-DR staining comparable to the cases with PMI <6 hrs (n=3), leading to the conclusion that the PMI did not influence the HLA-DR antigenicity in our cohort. In addition, the type of fixative did not noticeably impact the HLA-DR-immunoreactivity since the PPA cases with paraformaldehyde fixation (n=2) showed a range of HLA-DR staining intensity that paralleled the staining intensities found in the PPA cases with formalin fixation (n=7). Neither age nor PMI correlated with optical densities of activated microglia. However, given that microglial activation may scale with age [15], it is possible that age-related factors could have contributed, at least in part, to the activated microglia observed in non-atrophied regions. The only factors we found that influenced the pattern of HLA-DR-immunoreactivity in our cohort are reported as the main findings in this study—grey matter cortical atrophy and underlying neuropathology.

The white matter tended to have a greater accumulation of activated microglia in PPA-TDP in comparison to PPA-AD. A recent report on CD68-positive activated microglia in patients with FTLT-DTP compared to typical AD and controls showed that the frontal white matter was the only cortical region that differentiated the two pathologic groups, with greater microglial scores in FTLT than AD [26]. Similarly, findings from a study of patients with underlying FTLT or AD pathology reported that there was greater microglial activation in the frontal white matter of FTLT brains compared to AD brains, while temporal grey matter showed more microglial activation in AD than FTLT [14]. Our qualitative assessment also found anatomic selectivity in the accumulation of activated microglia. More specifically, the frontal white matter showed greater microglial activation in PPA-TDP than PPA-AD, and the parietal white matter tended to display greater microglial activation in PPA-AD than PPA-TDP.

It is not currently known what could be contributing to the prominent microglial activation in the white matter of patients with PPA. Even though AD and TDP-43 pathology mostly accumulate in the grey matter, TDP-43-positive inclusions have been found in white matter glia [33] and may specifically deposit in oligodendrocytes of patients with FTD [34]. All five PPA-TDP cases examined in this study displayed sparse TDP inclusions in the white matter of the brain regions examined. In addition, 4/5 PPA-TDP cases had mild to moderate myelin rarefaction of the white matter that was occasionally more severe in regions where more atrophy was detected in the language dominant hemisphere. Although the present PPA-TDP cohort did not have progranulin gene (*GRN*) mutations, it has been shown that the haploinsufficiency caused by *GRN* mutations results in significantly elevated serum levels of the proinflammatory cytokine interleukin 6 compared to patients without the *GRN* mutation [35]. In addition, the progranulin (PGRN) protein associated with *GRN* mutations is known to be involved in immunity and has anti-inflammatory properties such that when there is a PGRN deficiency, neuroinflammation is enhanced through microglial activation [36]. A higher rate of autoimmunity has been linked to greater neuroinflammation in FTD, semantic variant PPA, and *GRN* mutation carriers [37–39], but this association is not well understood in PPA patients with underlying FTLT-DTP or AD pathology.

Despite a close link with neuroinflammation [4, 7, 8, 40], AD pathology does not accumulate in the white matter of PPA-AD where the activated microglia are densest. However, soluble amyloid-beta has been shown to accumulate in greater concentrations in the white matter versus grey matter in clinically typical AD [41]. Furthermore, post-mortem examinations of amnesic AD patients have revealed multiple forms of white matter change, including reductions in axons [42] and myelin [43], and increases in glial levels that scale with white matter disease [44]. Similar to the PPA-TDP cases, most of the PPA-AD cases included in this study had mild to moderate myelin rarefaction of the subcortical white matter. It is also important to note that in PPA, language network connectivity has been shown to be compromised in early stages of the syndrome before significant cortical atrophy, implying reductions in synaptic activity or axonal degeneration could be early root causes of language impairments [45]. Additional studies that measure the changes in myelin, axons, and other glial populations will help determine the potential causes of microglial activation in the white matter, and how the microglial response is related to the grey matter atrophy observed in PPA.

Recent advances in *in vivo* neuroimaging techniques have contributed to new insights into white matter degeneration in PPA. For example, proton magnetic resonance spectroscopy has demonstrated that abnormalities occur in the arcuate fasciculus in PPA [46]. Diffusion tensor imaging (DTI) studies have linked white matter changes to language impairments specific to the clinical subtypes of PPA [47, 48]. However, the extent to which grey and white matter degeneration occur in parallel or sequentially is not yet known in PPA. Importantly, longitudinal MRI studies have shown that white matter atrophy can be severe in PPA [49], and white matter changes can progress more rapidly than grey matter atrophy in as little as one year [50]. Other DTI investigations have reported that white matter abnormalities in PPA can be more extensive than the changes in grey matter [51, 52]. In addition, several DTI studies have indicated that patients with typical AD or PPA display reductions in white matter structural integrity that precede or are independent of grey matter cortical atrophy and clinical symptoms [53–56]. Discordant degeneration between white and grey matter has also been shown in a study of typical amnesic AD patients where grey and white matter changes did not always overlap anatomically [57]. It remains to be determined if factors such as neuroinflammation or prion-like propagation contribute to these patterns of degeneration. Interestingly, the PPA case excluded from our study due to undetectable atrophy still had prominent white matter activated microglia that unexpectedly appeared greater in regions of the non-language dominant hemisphere. Future longitudinal studies coupled with positron emission tomography ligands specific to neuroinflammatory markers will be critical for uncovering the relationships between atrophy, inflammation, and pathology in the grey and white matter.

While the PPA profile might suggest that all language regions would be the most atrophied regions associated with the greatest densities of activated microglia, this was not the case. For example, some language regions such as the IPL in PPA-TDP cases were often not atrophied and harboured very low densities of activated microglia. These patterns of staining and atrophy are noteworthy because they provide evidence that activated microglia do not necessarily have a predilection to all language regions. Instead, they suggest that activated microglia are more closely related to cortical atrophy as we originally hypothesized, which

in PPA is usually concentrated to the language network, but not necessarily all language regions. It is also a reminder that the determinants of selective vulnerability in neurodegenerative diseases remain mostly unknown. Elucidating these factors will be important for identifying therapeutic targets for neurodegenerative diseases.

The abnormal proteinopathies of AD (amyloid-beta [plaques] and tau [neurofibrillary tangles]) and FTLT-TDP (truncated and translocated TDP-43) are associated with focal neurodegenerative changes in specific clinical syndromes such as amnesic AD, FTD, and PPA. Except for neurofibrillary tangles, the relationship between the proteinopathy and neurodegeneration (i.e., regional atrophy) is poorly understood. We showed that microglial activation was more prominent in the white matter, although the burden of abnormal disease-specific markers in both AD and FTLT-TDP is more prominent in the grey matter. There was also regional selectivity that mirrored the anatomy of atrophy and the corresponding clinical syndrome. For example, activated microglia accumulated more in regions of greater cortical atrophy and were more numerous in the language dominant hemisphere in PPA. Given this correspondence between white matter activated microglia and cortical atrophy, this study raises the intriguing possibility that microglia-initiated inflammatory processes may influence the distribution of neurodegeneration. Microglial activation could also constitute a response to the neurodegeneration but could further exacerbate the disease process through local inflammatory reactions.

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ABBREVIATIONS

PPA	primary progressive aphasia
FTD	Frontotemporal Dementia
AD	Alzheimer's disease
FTLD	Frontotemporal Lobar Degeneration
TDP	transactive response DNA-binding protein
OD	optical density
HLA-DR	human leukocyte antigen-D related
NeuN	neuronal nuclear protein
MRI	magnetic resonance imaging
L	logopenic

G	agrammatic
Gsp	agrammatism with a speech disorder
M	mixture of agrammatism and comprehension deficits
U	unclassifiable
V1	primary visual cortex
IFG	inferior frontal gyrus
aSTG	anterior superior temporal gyrus
aMTG	anterior middle temporal gyrus
pSTG	posterior superior temporal gyrus
aIPL	anterior inferior parietal lobule
pIPL	posterior inferior parietal lobule
PrC	precuneus
P	paraformaldehyde
F	formalin

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Fig. 1. Cortical atrophy is focal and asymmetric in PPA

The location and extent of cortical atrophy were determined by post-mortem assessments and corroborated with *in vivo* structural MRI data acquired close to death. Gross pictures of the post-mortem brain and MRI are shown from PPA-TDP case #10 as an example. Regions of interest were selected based on areas with peak cortical atrophy (red arrows; IFG and aSTG) and areas with undetectable cortical atrophy (black arrow; PrC). Red and yellow (yellow > red) represent significant cortical thinning (i.e., atrophy).

Fig. 2. Activated microglia accumulate with regional and hemispheric selectivity in PPA
 Representative HLA-DR staining in bilateral whole-hemisphere sections from PPA-AD case #3 and PPA-TDP case #8, including photomicrographs taken at 0.5x (red), 4x (green), and 60x (black) magnifications. Across PPA neuropathologic subtypes, HLA-DR staining often appeared greater in white matter compared to grey matter, and high magnification revealed that the intensity of the staining was due to larger, denser populations of activated microglia. Scale bars = 2mm, 200µm, 20µm for 0.5x, 4x, 60x magnifications, respectively.

Fig. 3. Activated microglia display more prominent staining in atrophied regions that often favour the language dominant hemisphere

3a) Photomicrographs of representative HLA-DR staining in an atrophied region (pSTG) and a non-atrophied region (V1) within the language dominant hemisphere, along with their non-atrophied contralateral homologues from PPA-AD case #3.

3b) Photomicrographs of representative CD68 staining in an atrophied region (IFG) and a non-atrophied region (aIPL) within the language dominant hemisphere, along with their non-atrophied contralateral homologues from PPA-TDP case #8.

Atrophied regions have a red border; non-atrophied regions have a black border.

Photomicrographs acquired at 10x magnification. Scale bars = 100µm

Fig. 4. Activated microglia show a predilection for atrophied regions and the language dominant hemisphere

4a) A *t*-test showed that the mean optical density of activated microglia is greater in atrophied regions compared to non-atrophied regions within the language dominant hemisphere of all PPA cases combined. * $p < 0.05$

4b) In the assessment of hemispheric asymmetry, a *t*-test indicated that atrophied regions in the language dominant hemisphere have a greater mean optical density of activated microglia than their contralateral homologues. * $p < 0.05$

Table 1

Demographics and inclusionary criteria for PPA participants.

Case #	Pathologic Diagnosis	Clinical Subtype	Sex	Handedness	Education (Y)	Age at Death (Y)	Symptom Duration (Y)	Scan/Death Interval (Y)	Post-mortem Interval (H)	Fixative
1	AD	L	Male	Right	16	61	4.7	0.6	6	F
2	AD	U	Male	Right	12	63	7.9	2.1	14	F
3	AD	L	Male	Right	15	75	8.6	2.0	8	P
4	AD	U	Male	Right	14	61	9.4	2.5	19	P
5	AD	G	Male	Right	18	78	6.2	2.5	28	P
6	TDP, Type B	L→G	Male	Right	13–15	59	2	1.7	12	F
7	TDP, Type A	L	Male	Left	18	65	8	6	6	F
8	TDP, Type B	Gsp	Male	Right	18	55	4.4	2.6	29	F
9	TDP, Type A	Gsp	Male	Right	16	70	4.6	3.1	4	F
10	TDP, Type A	M	Female	Right	11	74	6.7	4.6	10	F

AD = Alzheimer’s disease; TDP = transactive DNA-binding protein; L = logopenic; G = agrammatic; Gsp = agrammatic with motor speech deficits; M = mix of agrammatic and semantic deficits; U = unclassifiable due to severity of impairments; Y = years; H = hours; P = paraformaldehyde; F = formalin.

Table 2

Bilateral regions assessed per PPA case.

PPA neuropathologic subtype	AD				TDP					
	1	2	3	4 ^a	5	6	7	8	9	10
Non-atrophied regions	V1	V1	V1	--	V1	aIPL	pIPL	aIPL	aIPL	PrC
	aIPL	IFG	pSTG	--	aSTG	IFG	IFG	IFG	IFG	IFG
Atrophied regions	pIPL	aSTG	aIPL	--	pSTG	aMTG	aSTG	aSTG	aMTG	aSTG

Primary visual cortex (V1); inferior frontal gyrus (IFG); anterior inferior parietal lobule (aIPL); posterior inferior parietal lobule (pIPL); anterior superior temporal gyrus (aSTG); posterior superior temporal gyrus (pSTG); anterior middle temporal gyrus (aMTG); precuneus (PrC)

^a PPA-AD case #4 was excluded from quantitative analyses due to lack of consensus between postmortem and MRI assessments of peak cortical atrophy (see Results).