

Atrophy and Microglial Distribution in Primary Progressive Aphasia With Transactive Response DNA-Binding Protein-43 kDa

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Objective: To quantitatively determine the density and distribution of activated microglia across cortical regions and hemispheres in the brains of primary progressive aphasia (PPA) participants with pathological diagnoses of frontotemporal lobar degeneration with transactive response DNA-binding protein-43 (TDP-43) inclusions and to examine the relationships between microglial densities, patterns of focal atrophy, (TDP-43) inclusions, and clinical phenotype.

Methods: Activated microglia and TDP-43 inclusions were visualized in whole-hemisphere brain sections using immunohistochemical methods from five participants with PPA-TDP. Unbiased stereology was used to bilaterally quantify human leukocyte antigen/D related-positive activated microglia and TDP-43 inclusions across five language-related regions. Density and distribution of both markers were compared across cortical regions and hemispheres, and their relationships to patterns of focal atrophy and clinical phenotype were determined.

Results: Activated microglia displayed asymmetric distribution favoring the language-dominant hemisphere, consistent with greater postmortem and/or in vivo atrophy in that hemisphere, in PPA-TDP. In one participant with no asymmetric atrophy, quantitative distribution of microglia also lacked asymmetry. Patterns of microglial activation also showed variation that favored areas of high atrophy in regions affiliated with language function, demonstrating concordance between patterns of microglial activation, atrophy, and clinical phenotype. TDP-43 also showed higher inclusion densities in areas of high atrophy than in regions with low atrophy, but no clear relationship with microglia density at a regional level.

Interpretation: The initial activation of microglia is most likely a response to cortical abnormalities in PPA-TDP, which contribute to atrophy. The patterns of microglial activation, TDP-43 inclusion deposition, atrophy, and clinical phenotype suggest that activated microglia may make unique contributions to cortical thinning and TDP-43 inclusion formation.

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Primary progressive aphasia (PPA) is a clinical dementia syndrome characterized by a gradual dissolution of language, asymmetric cortical atrophy favoring the language network of the dominant hemisphere,¹ and a multitude of underlying pathologies that include Alzheimer's disease (AD) and frontotemporal lobar degeneration (FTLD).^{1–3} The major molecular forms of FTLD comprise of tauopathies (FTLD-tau) and transactive response DNA-binding protein-43 (TDP-43) proteinopathies (FTLD-TDP).² Although deposition of abnormal

protein aggregates in cortical gray matter in FTLDs has received substantial experimental attention, the anatomical distribution of microglial activation and its potential role in disease progression remain largely unknown.

Microglia are the resident macrophages of the brain known for their roles in immune-surveillance and homeostatic maintenance in the central nervous system (CNS).⁴ Whereas microglia in their resting state regulate brain development and maintain neuronal networks, activated microglia are responsible for eliminating microbes,

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cellular debris, protein aggregates, and other soluble antigens that may endanger the CNS.⁵ They have been shown to secrete cytokines, chemokines, and reactive oxygen species and to stimulate other inflammatory processes, with particular links to neuronal damage.⁶ Activated microglia are often present in close proximity to pathological inclusions in neurodegenerative disorders, including neurofibrillary tangles and amyloid- β plaques in AD^{7–11} and hyperphosphorylated TDP-43 inclusions in nonamnestic clinical dementia syndromes.^{12–14}

We previously reported concordance between microglial activation and cortical atrophy in brains of PPA patients with mutations in the progranulin gene (*GRN*) and underlying pathology of FTLTDP.¹⁴ Quantitative assessment revealed consistent asymmetric distribution of activated microglia favoring the more atrophied (language-dominant) hemisphere. However, we did not find regional differences in distribution of activated microglia or any notable relationship with TDP-43 inclusions. Progranulin (*PGRN*) deficiency has been shown to promote neuroinflammation through microglial activation^{15,16} and to cause exaggerated inflammation in mice,¹⁷ leading us to speculate that the haploinsufficiency caused by *GRN* mutations contributed to an overall exacerbated activation of microglia that masked subtle differences across cortical regions that may be detectable under normal *PGRN* levels.

The goal of this study was to determine, using unbiased stereological methods, whether the extent of microglial activation in a cohort of PPA-TDP participants without *GRN* or

any other known genetic mutations shows concordance with cortical atrophy, pathological inclusions of TDP-43, and disease phenotype.

Patients and Methods

Study Participants

Five participants were included in this study. Four were right-handed, and one was left-handed (participant 3). Further characteristics of the participants, including clinical course, are summarized in Table 1. Postmortem brain tissue from all participants was obtained from the Northwestern University Alzheimer's Disease Center Brain Bank.

The clinical diagnosis of PPA was rendered based on previously described criteria.^{1,18–20} Two participants (1 and 2) were diagnosed with the agrammatic variant of PPA (PPA-G) with more than minimal motor-speech impairments (PPA-Gsp). PPA-G is clinically characterized by a distortion of word order and sentence construction, whereas word comprehension is typically spared.³ In those with PPA-G, peak atrophy sites commonly include the left inferior frontal gyrus (IFG).²¹

One participant (participant 3) was diagnosed with the logopenic variant of PPA (PPA-L), characterized by significant word-finding difficulties (anomia) with relatively intact grammar and comprehension.³ Peak atrophy sites in PPA-L include the left temporoparietal junction (TPJ), where the inferior parietal lobule (IPL) borders the posterior regions of the superior and middle temporal gyri.²²

Another participant (participant 4) initially presented with logopenic symptoms (PPA-L), but a year later progressed to display agrammatism (PPA-G). Participant 5 showed a mixed clinical subtype (Table 1). The “mixed” subtype (PPA-M) designates patients with agrammatism and comprehension deficits

TABLE 1. Demographic Features of PPA-TDP Participants

Participant No.	1	2	3	4	5
Sex	Male	Male	Male	Male	Female
Handedness	Right	Right	Left	Right	Right
Age at death, y	55	70	65	59	74
Disease duration, y	4	4	8	2	7
Education, years	18	16	NA	NA	11
Last MRI obtained before death, y	2.3	2.1	NA	1.5	2.5
Postmortem interval, hours	29	4	6	12	10
Brain weight, g	1,090	999	1,040	1,490	1,035
PPA clinical subtype	Gsp	Gsp	L	L → G	M
Pathological subtype	TDP-Type B	TDP-Type A	TDP-Type A	TDP-Type B	TDP-Type A

PPA clinical subtypes: G = agrammatic; Gsp = agrammatic with additional motor-speech deficits; L = logopenic; M = mixed. Participant 1 progressed from logopenism to agrammatism.

MRI = magnetic resonance imaging; PPA = primary progressive aphasia; NA = not available.

TABLE 2. Summary of Clinical Presentations

Participant	Clinical Presentation
1	At age 51, the patient began to notice changes in speech. On initial examination 1 year later, he was fully oriented, but speech was effortful and dysfluent. There was no agrammatism, but he had paraphasias in naming. The patient had a history of poor spelling and his father had a history of dyslexia. A paternal great grandmother had a late-life dementia and paternal grandfather developed Parkinsonian symptoms at age 70. The diagnosis was PPA with apraxia of speech. Three years later, his symptoms had progressed to the point where he required 24-hour care and he began having difficulty breathing and swallowing.
2	Severe speech, abnormalities mild right leg motor difficulties and ideomotor apraxia were apparent in the initial examination of this 68-year-old man with a 2-year history of symptoms. Memory was initially not impaired, and he was independent in activities of daily living. He was diagnosed with PPA with suspected underlying atypical Alzheimer's or some form of FTLD-related tauopathy. One son was delayed in talking and required speech therapy, and 1 sibling and a niece were diagnosed with dyslexia. Four years after initial evaluation, he was placed in a nursing home.
3	This patient was previously reported. ³⁰ Briefly, at age 57, this left-handed man had a 3-year history of difficulties in word-finding, working with numbers, and spelling. Activities of daily living were intact, and memory was relatively preserved. The patient's father had a dementia. There were no behavioral symptoms. The patient was diagnosed with PPA. Neuropsychological scores were not available for this participant.
4	At age 59, the patient presented with symptoms of slurred speech and anomia. There were no other motor or sensory symptoms. There were no significant behavioral disturbances. Retentive memory was preserved, although difficult to test given speech output limitations. Nonverbal memory was in the high average range. He was given a diagnosis of PPA. Neuropsychological scores were not available for this participant.
5	At age 69, the patient presented to clinic with a 2-year history of progressive decline in word-finding and other language skills, but also changes in behavior, including anxiety and irritability. Memory was initially preserved. She was largely independent in ADL, but was making errors in check writing. There was no family history of dementia. The initial diagnosis was progressive nonfluent aphasia. Symptoms of aphasia worsened as did behavior, but she was able to perform activities of daily living. Four years after initial evaluation, she was placed in a skilled nursing facility.

ADL = activities of daily living; FTLD = frontotemporal lobar degeneration; PPA = primary progressive aphasia.

of comparable magnitude, or when language output is too limited for specific characterization.¹⁹ Peak atrophy sites are generally observed in the anterior temporal lobe (ATL).²²

Additional information on clinical presentation, course of disease, and neuropsychological test scores are presented in Tables 2 and 3.

Standard Protocol Approvals and Patient Consent

Written informed consent for research was obtained from each participant or guardian. This study was approved by the Northwestern University Institutional Review Board.

Assessment of Cortical Atrophy and Pathology

The location and extent of cortical atrophy were assessed using photographs of the postmortem brain and corroborated with *in vivo* magnetic resonance imaging (MRI) data when available (Fig 1). Postmortem pathology and focal atrophy were assessed in each case. Structural MRI data were collected on a 3 Tesla scanner from two participants (1 and 2) within 2.3 years of death. FreeSurfer Software Suite (version 5.1.0; <http://surfer.nmr.mgh.harvard.edu>) was used to process the MRI scans and visualize peak cortical atrophy in comparison to a group of previously described age-matched healthy controls (n = 35)¹ (Fig 1). Clinical MRI scans collected from 2 other participants (3 and 5) within 2.5 years preceding death were used to examine the regions and extent of atrophy. Based on these assessments, one language region of high atrophy and another with low atrophy were chosen for analyses within the language-dominant hemisphere (Table 2).

Three of the participants were pathologically diagnosed as FTLD-TDP type A and the remaining 2 as FTLD-TDP type B (Table 1). FTLD-TDP type A is characterized by abundance of short dystrophic neurites and neuronal intranuclear inclusions of phosphorylated TDP-43 aggregates, whereas FTLD-TDP type B presents with predominance of neuronal cytoplasmic inclusions.²³

Tissue Processing

Formalin-fixed whole-hemisphere slabs containing regions of interest (ROIs) were cut on a sliding microtome at a thickness of 40 μ m. Every 1 in 24 sections was immunohistochemically

TABLE 3. Behavioral and Cognitive Assessment

Assessment at Initial Visit	Participant 1	Participant 2	Participant 5
MMSE (1–30)	29	29	22
CDR Global (0–3)	0	0	0
CDR Behavior (0–3)	0	0	0.5
Trail Making A (time in sec)	20	32	47
Trail Making B (time in sec)	62	74	27
Boston Naming Test (0–60)	28	27	4
RAVLT Delay (0–15)	8	5	NA
WMS-R Logical Memory Delay (0–25)	8	9	6
CERAD Constructions (0–11)	10	6	10
GDS (1–30)	5	0	2

Neuropsychological scores were not available for participants 3 and 4.

MMSE = Mini Mental State Examination; CDR = Clinical Dementia Rating; RAVLT = Rey Auditory Verbal Learning Test; CERAD = Consortium to Establish a Registry for Alzheimer's Disease; GDS = Geriatric Depression Scale; NA = not available.

stained using the avidin-biotin peroxidase method, with an antibody to human leukocyte antigen/D related (HLA-DR) protein (mouse monoclonal, 1/1,000; Dako, Carpinteria, CA), a class II cell-surface glycoprotein of the major histocompatibility complex that is upregulated in neurodegenerative diseases and serves as a marker of activated microglia. A series of adjacent sections were additionally stained for phosphorylated TDP-43 (pS409/410-2, rabbit polyclonal, 1:3,000; CosmoBio, Carlsbad, CA) to visualize inclusions and were counterstained with 0.05% cresyl violet to visualize the subcellular localization of the inclusions in neurons.

Unbiased Stereological Quantitation and ROIs

Densities of HLA-DR-positive activated microglia in the cortical gray matter were quantified across five bilateral regions affiliated with language function, namely inferior frontal gyrus (IFG), middle frontal gyrus (MFG), inferior parietal lobule (IPL), superior temporal gyrus (STG), and inferior/medial temporal gyrus (ITG/MTG), using the optical fractionator probe of the StereoInvestigator software (MBF Biosciences, MicroBrightfield, Inc., Williston, VT). In addition, intracytoplasmic, intranuclear, and neuritic TDP-43 inclusions were quantified in the same regions. These inclusions are considered fully mature and have been shown to be ubiquitinated.²⁴ Each ROI was delineated from the cortical surface to the white matter at 1× magnification, and counting was completed at 60× magnification. Section thickness was measured at each counting site, and all cortical layers were collectively assessed.

Counts were expressed as mean activated microglia or TDP-43 inclusions per cubic millimeter (mm³) based on the estimated population calculated using weighted section

thickness and the planimetric determination of volume by the optical fractionator software. The stereological parameters used resulted in a coefficient of error below 0.1.

Statistical Analysis

To assess hemispheric differences, overall densities of HLA-DR-immunoreactive microglia were compared between the language-dominant and non-language-dominant hemispheres

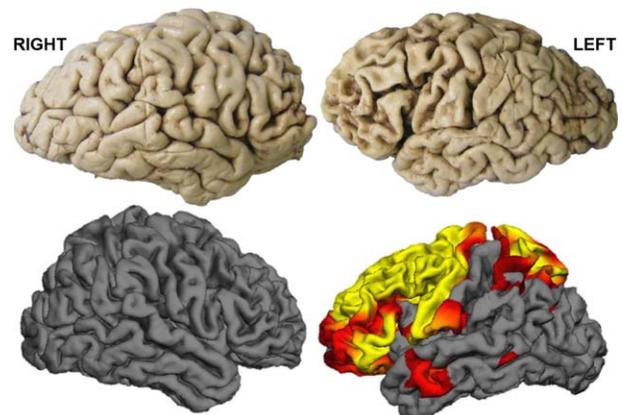


FIGURE 1: Location and extent of cortical atrophy were determined using postmortem assessments and corroborated with in vivo structural MRI acquired close to death. Postmortem photographs and MRI of participant 2 (PPA-Gsp) are shown as an example. Both postmortem brain and structural MRI scan show visible left-sided asymmetric atrophy, particularly in IFG. Yellow in MRI scans denotes areas of highest atrophy. IFG = inferior frontal gyrus; Gsp = agrammatic with additional motor-speech deficits; MRI = magnetic resonance imaging; PPA = primary progressive aphasia.

TABLE 4. High and Low Atrophy Regions from Language-Dominant Hemisphere of Each PPA Case

Participant No.	1	2	3	4	5
High atrophy region	IFG	IFG	STG	IFG	IFG
Low atrophy region	IPL	IPL	IPL	IPL	IPL

IFG = inferior frontal gyrus; IPL = inferior parietal lobule; PPA = primary progressive aphasia; STG = superior temporal gyrus.

using a paired t test. The same test was also conducted for TDP-43 inclusion densities. Hemispheric asymmetries within each cortical region were assessed at the group level using Wilcoxon matched-pairs signed-rank tests (did not pass tests of normality).

Microglial densities in a language region of high atrophy and another with low atrophy (Table 4) within each hemisphere were also compared using the Wilcoxon matched-pairs signed-rank test. The test was repeated for TDP-43 inclusion densities. Last, the microglia and TDP-43 inclusion densities across all regions were combined and assessed for correlation using Pearson correlation analysis. Statistical analyses were performed using GraphPad Prism (version 5.03; GraphPad Software Inc., La Jolla, CA) or RStudio (version 1.0.143; RStudio, Boston, MA).

Results

HLA-DR-positive staining was visualized across all cell layers in our ROI bilaterally across the 5 cases. Qualitative observations at high magnification revealed dominance of the hypertrophic morphology of microglia in all regions and cases examined.

Asymmetric Distribution of Activated Microglia Favors the Language-Dominant Hemisphere

To assess whether there was an asymmetric distribution of activated microglia favoring the language-dominant, and more atrophied hemisphere, we combined the stereological estimates across all regions and cases, distinguishing the language-dominant and non-language-dominant hemispheres. Analysis revealed significantly higher densities of activated microglia in the language-dominant hemisphere than the non-language-dominant hemisphere ($p < 0.0001$; Fig 2A).

To determine whether such asymmetric distribution is observed in individual cortical regions at the group level, we conducted Wilcoxon matched-pairs signed-rank tests to compare mean microglial densities in the language-dominant and non-language-dominant hemispheres. The analyses revealed significant asymmetry in the MFG and

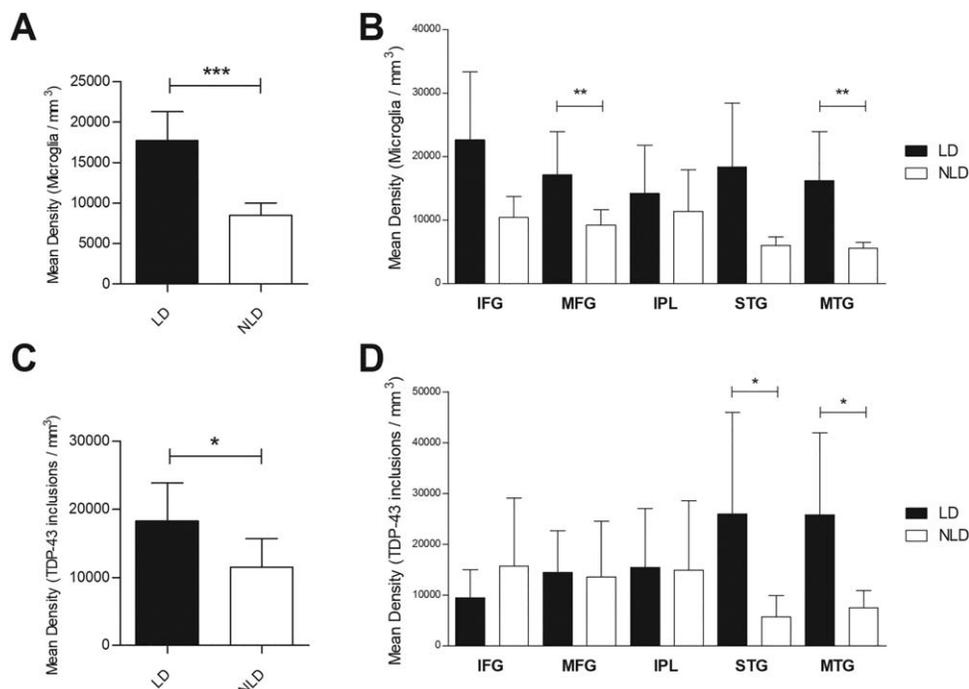


FIGURE 2: (A) Microglia density shows significant asymmetry favoring the language-dominant hemisphere ($p < 0.0001$). Bars represent mean \pm SEM. **(B)** The asymmetric pattern of microglia distribution is maintained within individual cortical regions. **(C)** TDP-43 inclusion density also shows significant asymmetry favoring the language-dominant hemisphere ($p = 0.0106$). Bars represent mean \pm SEM. **(D)** Whereas the asymmetric distribution of TDP-43 inclusions were maintained in the STG and MTG, the IFG and MFG did not share the same pattern of distribution when compared with activated microglia. Bars represent mean \pm SEM. IFG = inferior frontal gyrus; MFG = middle frontal gyrus; IPL = inferior parietal lobule; STG = superior temporal gyrus; MTG = middle temporal gyrus; LD = language-dominant hemisphere; NLD = non-language-dominant hemisphere; TDP-43 = transactive response DNA-binding protein-43. *** $p < 0.0001$; ** $p < 0.01$; * $p < 0.05$.

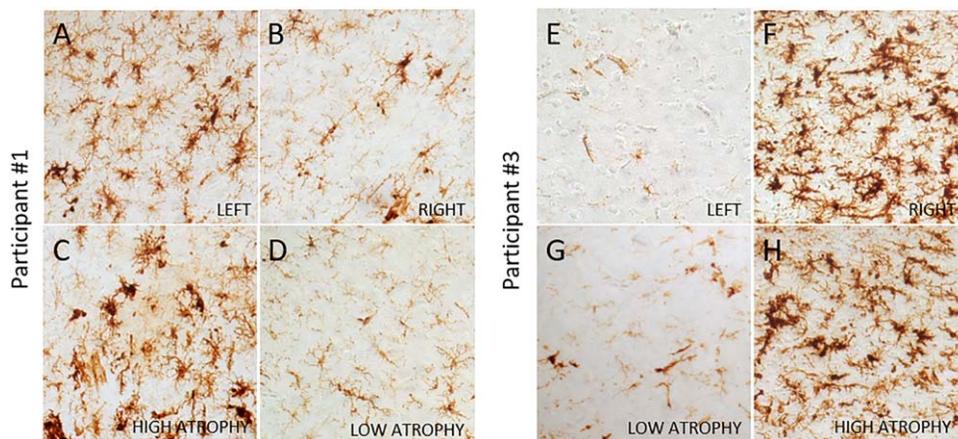


FIGURE 3: Hemispheric and regional differences of microglia densities in participant 1 (A–D) and participant 3 (E–H). Examples of asymmetric distribution of *HLA-DR*-immunoreactive activated microglia is seen in (A) left IFG and (B) right IFG of participant 1 (PPA-Gsp). This pathological distribution shows concordance with the asymmetric atrophy in participant 1, with greater atrophy in the language-dominant hemisphere. (C) Photomicrographs demonstrate differences in microglial density based on severity of atrophy in left IFG (severe atrophy) and (D) left IPL (mild atrophy) in participant 1 (PPA-Gsp). (E) Left STG and (F) right STG in participant 3 (PPA-L). Note the reverse asymmetry in participant 3, consistent with the right-hemisphere–dominant language function and atrophy as observed in clinical MRI. (G) Left MFG (mild atrophy) and (H) right STG (significant atrophy) in participant 3 (PPA-L). IFG = inferior frontal gyrus; MFG = middle frontal gyrus; IPL = inferior parietal lobule; STG = superior temporal gyrus; PPA-Gsp = agrammatic subtype of PPA with additional motor-speech impairments; PPA-L = logopenic subtype.

MTG ($p = 0.0313$) favoring the language-dominant hemisphere and a trend of asymmetry across all other regions ($p = 0.0625$; Fig 2B).

In 4 of the 5 cases, activated microglia displayed an asymmetric distribution favoring the language-dominant hemisphere across all regions, consistent with greater atrophy in that hemisphere (Fig 3A,B,E,F). In the one case that lacked asymmetry of microglial distribution (participant 4), there was also absence of asymmetric atrophy.

Microglial Activation Is Greater in More Atrophied Regions Within the Language-Dominant Hemisphere

Within the language-dominant hemisphere, the IPL was identified as a language region of low atrophy in all 5 cases, whereas the IFG was a highly atrophied language region in all cases except in participant 3, whose peak atrophy was in the STG (Table 4). When all 5 cases were examined as a group, mean density of activated microglia was significantly higher in the high-atrophy language region in the language-dominant hemisphere when compared to the low-atrophy language region ($p = 0.0313$; Fig 4A). In the same regions of the non-language-dominant hemisphere, the differences were not significant ($p > 0.05$; Fig 4B).

Relationship Between Distribution of TDP-43 Inclusions and Activated Microglia Remains Unclear

Similar to activated microglia, an analysis comparing combined TDP-43 inclusion densities (across all regions and cases) between the language- and non-language-dominant

hemispheres revealed significantly higher densities in the language-dominant hemisphere ($p = 0.0106$; Fig 2C). When asymmetry was assessed at the individual cortical region level, the analyses revealed significant asymmetry in the STG and MTG ($p = 0.0313$ in both regions) and no asymmetry in the IPL, similar to the distribution patterns of activated microglia (Fig 2D). However, densities of TDP-43 inclusions in the IFG and MFG showed patterns that drastically differed from those of activated microglia, with reversed or no asymmetry ($p > 0.05$).

Examination of only the high- and low-atrophy language areas (Table 4) for TDP-43 inclusion density revealed a pattern that was nearly identical to that of activated microglia in the same regions (Fig 4C,D). When all cases were combined, mean density of TDP-43 inclusions was higher in the high-atrophy language region when compared to the low-atrophy language region in the language-dominant hemisphere (Fig 4C). A slightly reversed pattern was observed in the non-language-dominant hemisphere (Fig 4D). However, neither of these patterns were statistically significant, likely attributed to the small sample size, high variability of absolute number of TDP-43 inclusions across cases, and inconsistent trends across regions.

To assess the overarching relationship between activated microglia and TDP-43 despite regional and case-by-case variabilities, a correlation analysis was performed in which all data points across regions and hemispheres were combined. This analysis revealed a significant, although not strong, relationship between the densities of

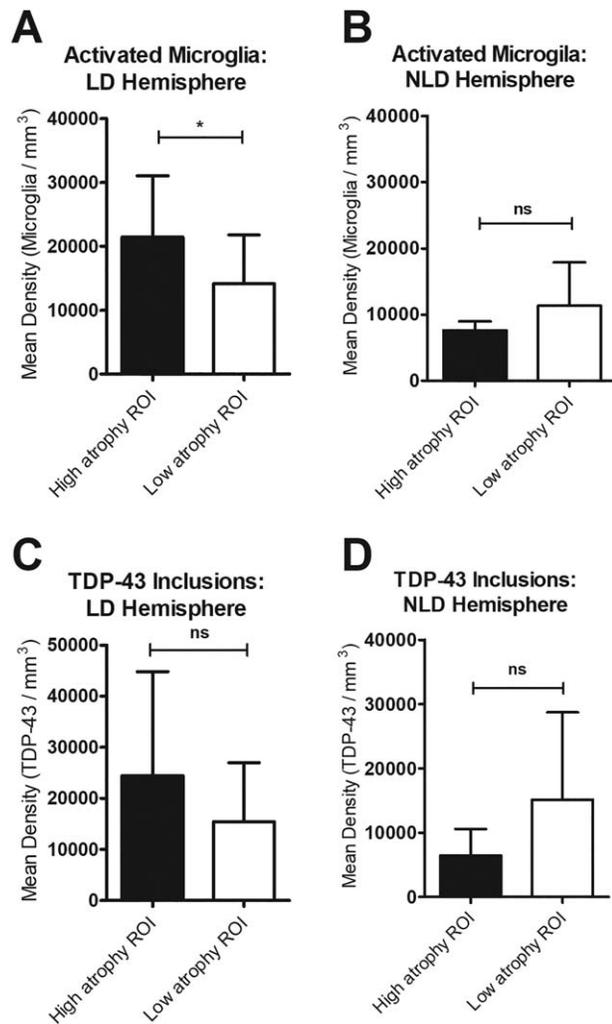


FIGURE 4: (A) Comparison of high- and low-atrophy language regions within the language-dominant hemisphere reveal significantly greater densities of activated microglia in the more atrophied region ($p = 0.0313$). (B) The differences were not significant in homologous regions of the non-language-dominant hemisphere ($p > 0.05$). (C) TDP-43 inclusion densities displayed similar patterns to microglia densities in the high- and low-atrophy language regions within the language-dominant and (D) non-language-dominant hemisphere, but the differences did not reach statistical significance. A correlation analysis revealed a highly significant correlation between the density of mature TDP-43 inclusions and microglia density (see text). Bars represent mean \pm SEM. LD = language-dominant hemisphere; NLD = non-language-dominant hemisphere; TDP-43 = transactive response DNA-binding protein-43.

activated microglia and TDP-43 inclusions, with a correlation coefficient of 0.6121 ($p = 1.558 \times 10^{-13}$).

Microglial Densities Show Concordance With Known Patterns of Atrophy in PPA Clinical Subtypes

PPA can be categorized into three major clinical subtypes—agrammatic, logopenic, or semantic—each of which is characterized by a set of predominant clinical symptoms and

distinguishing pattern of focal atrophy. Specifically, MR images from PPA-G patients in earlier stages of disease course generally show peak atrophy in IFG and MFG as well as in STG and MTG of the left hemisphere, whereas PPA-L patients most often display peak atrophy in left temporal and parietal lobes.^{1,25,26} Whereas the atrophy spreads over the course of the disease to encompass greater portions of the perisylvian language network, the hemispheric asymmetry typically remains.^{1,25}

When each case was analyzed individually, microglial densities showed peak activation in a language region of high atrophy corresponding to the known pattern of atrophy for each clinical subtype²² in all cases (Fig 3C,H). For instance, in the two PPA-Gsp cases, the IFG and MFG had among the highest densities of activated microglia, a pattern that was consistent with the phenotypes and atrophy associated with PPA-G (focal atrophy in IFG). Likewise, the PPA-M case displayed the highest densities of activated microglia in the IFG, MFG, and STG, matching the atrophy of the IFG and anterior temporal lobe (ATL) atrophy observed in the brain and typical of PPA-M.

Interestingly, in 4 of the 5 participants, the lowest density of activated microglia was found in the IPL. This finding shows concordance with lack of IPL atrophy in those 4 subjects. It is noteworthy that the 1 case that did not follow this trend was the only one in our cohort that showed significant atrophy in the IPL in addition to peak atrophy in STG and IFG (the PPA-M participant).

Discussion

In the current stereological study, we quantitatively examined the density and distribution of activated microglia across language-related neocortical regions and hemispheres in PPA-TDP brains in an effort to determine the relationships between cortical atrophy, microglial activation, and clinical phenotype. We hypothesized that activated microglia, as a marker of neuroinflammation and possibly a correlate of neurodegeneration, accumulate more prominently in the language-dominant, and more atrophied, hemisphere, especially within the compromised language network. This investigation was prompted by an earlier study in which high densities of activated microglia were observed without significant regional variations in the brains of PPA patients with *GRN* mutations and FTLD-TDP pathology.¹⁴ To determine whether these trends of microglial activation were attributable to the PGRN deficiency or to the nature of the TDP-43 pathology in PPA, we pursued the current study in a cohort of PPA subjects without any known genetic mutations. Similar to our findings in the previous study, there was an overall abundance of microglial activation and a consistently asymmetric distribution that favored the language-dominant, and more atrophied, hemisphere.

Whereas 4 of our 5 participants displayed a clear asymmetric distribution of activated microglia across all language regions, participant 4 stood out as distinctly lacking both asymmetry and variability in microglia density across areas and hemispheres. Interestingly, this participant not only showed the least atrophy and lowest densities of activated microglia among our cohort, but also had the shortest disease duration and highest brain weight (1,490g), suggesting a close link between microglial activation and cortical thinning.

Within the language-dominant hemisphere, the peak densities of activated microglia generally coincided with cortical regions that typically show greater atrophy in each clinical subtype of PPA. In the agrammatic subtype with additional speech-motor complications (participants 1 and 2), the highest densities of activated microglia were found in IFG or MFG. This pattern aligns with both the atrophy patterns characteristic of PPA-G and with the particular atrophy observed in the individual cases, as determined by examination of the brain, and structural and clinical MRI. In the logopenic participant, the highest microglial densities were noted in the STG and IFG of the language-dominant hemisphere, regions of severe atrophy, further supporting the hypothesis that microglial activation and cortical thinning are tightly linked in PPA-TDP. Lastly, consistent with the atrophy of the IFG and anterior temporal lobe characteristic of PPA-M, the participant with mixed subtype showed the highest densities of activated microglia in the IFG, MFG, and STG.

The IPL almost always had the lowest density of activated microglia relative to the other regions in the language-dominant hemisphere, and also showed the least asymmetry in distribution across hemispheres. These findings demonstrate concordance with the general lack of IPL atrophy in FTLTDP; atrophy in the IPL is usually a characteristic of PPA brains with underlying pathology of AD (PPA-AD).¹⁹ However, IPL atrophy is also observed in PPA-TDP, particularly in cases with severe, global atrophy and subjects with *GRN* mutations.¹⁹

There was notable variability in the overall densities of activated microglia between subjects. For instance, the density of activated microglia was significantly less in participant 2 than in participant 3. In our cohort and existing literature, there seems to be no apparent correlation between variations in overall activation of microglia and clinical phenotypes, including disease duration, severity of atrophy, and age at death.

The exact mechanisms underlying microglial activation and its relation to disease processes remain unknown. Lack of a consistent relationship between TDP-43 inclusion and activated microglia densities in our study suggests that the relationship may not be direct. Our findings do support the

notion that an upregulation of activated microglia in neurodegenerative diseases occurs in close proximity to pathological inclusions.^{7,9–11,13,14} In the context of FTLTDP, a number of studies have shown a direct effect of pathological proteins on microglial activation. For instance, one study using microglial cultures prepared from 7- to 8-day-old C57BL/6 mice investigated the extracellular effects of truncated and mutant forms of TDP-43 proteins on microglial activation, and found significant upregulation of microglial activity when compared with exposure to the injection of wild-type TDP-43.²⁷ Another study investigated the opposite effect, by inducing chronic inflammation in a transgenic mouse model expressing a genomic fragment of the human TDP-43 gene, and demonstrated enhanced cytoplasmic mislocalization and aggregation of TDP-43.¹² In PPA-TDP cases with *GRN* mutations, we found high densities of activated microglia, but failed to detect a strong correlation between burden of pathological inclusions and microglial densities.¹⁴ Similar to our findings in PPA-TDP cases with *GRN* mutations, the present study of PPA-TDP cases without known mutations revealed only a modest correlation between densities of microglia and mature TDP-43 inclusions. Other findings linking microglial activation to cellular injury, loss, and cortical atrophy have also been demonstrated.^{11,14} In spite of seemingly conflicting findings—inflammatory activity being the cause of TDP-43 pathology in some instances and a result of it in others—these studies share a common characteristic: They all demonstrate a link between neuroinflammatory processes, such as microglial activation, and TDP-43 proteinopathy.

Inflammation and microglial activation are features of nearly all neurodegenerative disorders. Microglia activation has also been reported in tauopathies. Microglia activation in the most common disorder with tau pathology, AD, has been extensively documented. Studies have also shown significant microglia activation in other tauopathies characteristic of FTLTDP, such as Pick disease, progressive supranuclear palsy, and corticobasal degeneration, which partly overlap with tau pathology.^{7,28,29} Thus, it does not appear that microglia activation is specific to TDP-43 pathology in FTLTDP.

Consistent with their normal function, microglia seem to share close links to pathological abnormalities and damage in PPA-TDP. Given what is known about the inflammatory response of microglia to neurodegenerative insults, it seems possible that activated microglia contribute to cortical thinning and TDP-43 inclusion formation in PPA-TDP.

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Author Contributions

G.K., M.M.M., and C.G. contributed to the design and conceptualization of the study. G.K., K.B., S.W., E.B., E.R., and C.G. contributed to acquisition and analysis of data. G.K., K.B., T.G., S.W., E.B., E.R., M.M.M., and C.G. contributed to drafting the manuscript and/or revising the manuscript for intellectual content.

Potential Conflicts of Interest

Nothing to report.

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