Fiber-specific micro- and macroscopic white matter alterations in progressive supranuclear palsy and corticobasal syndrome

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Abstract

Progressive supranuclear palsy (PSP) and corticobasal degeneration, which frequently present as a corticobasal syndrome (CBS), are characterized by progressive white matter (WM) alterations associated with the prion-like spreading of four-repeat tau. Considering the interplay of tau pathologies with clinical symptoms, capturing the disease-specific patterns of WM alterations might provide valuable clinical information; however, the mechanisms of loss of WM integrity and its involvement in the clinical deficits in tauopathies remain unknown, likely due to the difficulties in estimating complex WM structure. Here, a novel fibre-specific fiber density and fiber cross-section, and their combined measure estimated using voxel-based analysis (FBA), were cross-sectionally and longitudinally assessed in PSP (n = 20) and CBS (n = 17), and healthy controls (n = 20). Cross-sectional and longitudinal FBA indicated disease-specific progression patterns of fiber density loss and subsequent bundle atrophy consistent with the tau propagation patterns suggested in previous histopathological findings. Furthermore, longitudinal changes in fibre-wise metrics in WM tracts which control motor and cognitive functions exhibited strong correlations with changes in clinical dysfunction in both diseases. Our findings suggest that the FBA can be useful in determining the mechanisms of clinical deficits related to sequential WM alterations in PSP and CBS.

Introduction

Progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) are four-repeat (4R) tauopathies characterized by the accumulation of 4R-tau in the neurons and glia,\(^1,^2\) with general absence of beta-amyloid deposits, unlike in Alzheimer's disease.\(^3\) PSP is clinically associated with progressive gait failure, vertical gaze palsy, dysarthria, and dysphagia.\(^4,^5\) Moreover, PSP is characterized by vulnerability to tau toxicity and aggregation, not only in the midbrain and superior cerebellar peduncle (SCP), which is known as a hallmark, but also in the tracts under the premotor and motor cortices.\(^6,^7\) Although CBD has a variety of phenotypes, the most prototypical clinical phenotype is corticobasal syndrome (CBS), which presents with cognitive deficits and asymmetrical frontoparietal features such as limb rigidity and postural instability.\(^8,^9\) Previous studies reported the involvement of tau pathology in the motor cortex and subcortical structures in CBS, despite phenotype variation in cases that show clinical symptoms of CBS.\(^10,^11\)

Both PSP and CBS are characterized by selective white matter (WM) degeneration due to the patterns of prion-like spread of tau pathology through the axons.\(^12\) Changes in WM topology and connectivity are driven by the patterns of the spread of tau aggregation in neurodegenerative tauopathy\(^13\) that cause dysfunction of axons and glial cells.\(^14\)–\(^16\) Given this feature and the effect of 4R-tau distributions on clinical symptoms, understanding the micro- and macroscopic structures of WM that correspond to tau depositions in neurodegenerative tauopathies could be useful in diagnosis and follow-up of PSP and CBS. However, the mechanisms underlying the disturbances in the integrity of WM that lead to the progressions of clinical dysfunction are not fully elucidated; thus, hindering the development of therapies that could provide adequate functional improvement in both diseases. Furthermore, due to a high degree of overlapping symptomatology between the two diseases, differentiating PSP and CBS is often challenging using current in-vivo assessments, especially in the early stages of the disease. These facts emphasize the necessity for objective biomarkers that can be used to accurately classify PSP and CBS, capture patterns of disease progression, contribute to the refinement of clinical criteria, estimate intervention effects, and guide disease-modifying therapies.

Previous WM structural assessment studies in PSP and CBS have focused primarily on morphological atrophy based on structural MRI.\(^17,^18\) Recently, however, there has been increased focus on the importance of assessing microstructural degeneration that typically precedes macrostructural atrophy using diffusion tensor imaging.\(^19\)–\(^21\) Most of these are voxel-based analyses, which determine the average value of a quantitative index within a single voxel. Despite the evidence for different WM alteration patterns in PSP and CBS according to voxel-based analysis, sufficient knowledge has not been accumulated to establish WM alterations as an in-vivo biomarker, likely due to difficulties with interpretation derived from the partial volume effect that arises from the crossing fiber population (up to 90% of WM voxels).\(^22\) Considering this scenario, there are high expectations for fixel-based analysis (FBA), which can model complex fiber geometry in multiple directions within a single voxel and evaluate both micro- and macroscopic neural structures within a fiber-specific grid (i.e., “fixel“).\(^17,^23\) FBA can estimate fixel-wise parameters, including fiber density (FD; a microscopic parameter corresponding to the axonal density), fiber-bundle cross-
sections (FC; a macroscopic parameter of fiber-bundle cross-sections), and a combination of both denaturation processes (fiber density and bundle cross-section; FDC; a micro- and macroscopic parameter that is sensitive, especially in the fixel grids, wherein differences in both FD and FC appear).

Recently, a few studies adopted FBA to assess WM integrity in PSP and CBS. A previous study found both micro- and macroscopic WM changes in PSP, along the corpus callosum (CC) and descending fibers from the motor cortex, more conspicuous than Parkinson's disease. Another study reported a higher degree of FD reduction along the CC and bundle atrophy along the projection fibers from the motor cortex in CBS than Parkinson's disease. They also emphasized the contribution of the dentatorubrothalamic tract (DRTT), including the SCP in CBS. However, there has been no study that directly compared WM integrity in PSP and CBS using FBA or evaluated the association between WM changes and actual clinical dysfunction based on a longitudinal approach, even using voxel-based methods. Insights that contribute to the development of a useful biomarker and help to fully understand WM structural changes underlying rapid progressive motor and cognitive dysfunctions in PSP and CBS have been limited. Thus, it is important to evaluate the structural changes of WM in PSP and CBS cross-sectionally and longitudinally, including more detailed morphological information and changes in clinical indices.

Thus, in the current study, we aimed to investigate if FBA can serve as an imaging biomarker that: (1) grasps the progressive WM degeneration patterns in PSP and CBS that underlie these clinical dysfunctions from micro- and macroscopic perspectives, (2) accurately classifies patients with PSP and CBS in vivo, and (3) predicts future dysfunctions in the early stages.

Results

Participant’s demographic and clinical information

Table 1 shows the demographic information and the longitudinal changes in the clinical parameters. Age and sex were matched statistically among the three groups. The disease duration at baseline was also not significantly different between patient groups. Significant group differences at baseline between patients and HC were observed in the PSPRS Total, MMSE, MoCA, and the SEADL as shown in Table 1. Meanwhile, the PSPRS subscores of “Bulbar” and “Gait and midline” were significantly lower in CBS compared with that in PSP, and the PSPRS subscores for limb motor was significantly lower in PSP than that in CBS. Longitudinally, the clinical indices that were significantly worse over one year were: PSPRS Total, PSPRS subscores for “Oculomotor” and “Gait and midline,” UPDRS- , MMSE; and, in CBS, MoCA in PSP and PSPRS Total, PSPRS subscores of “Oculomotor,” “Limb motor” and “Gait and midline,” and UPDRS-. The degree of changes shown in the PSPRS subscores for “Gait and midline” was significantly severe in PSP compared with that in CBS. The percentage of cases included in the correlation analysis were as follows: PSPRS in 60% of PSP and 65% of CBS cases; UPDRS- in 70% of PSP and 59% of CBS cases; MoCA in 50% of PSP and 59% of CBS cases; and MMSE in 70% of PSP and 53% of CBS cases.
### Table 1
Participants demographics, clinical information, and magnetic resonance findings

<table>
<thead>
<tr>
<th></th>
<th>HC (N = 20)</th>
<th>PSP (N = 20)</th>
<th>CBS (N = 17)</th>
<th>Cross-sectional group differences</th>
<th>Longitudinal changes</th>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>Baseline</td>
<td>Follow-up</td>
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<tr>
<td>Age, y</td>
<td>64.7 ± 6.1</td>
<td>69.0 ± 6.5</td>
<td>65.6 ± 7.1</td>
<td>n.s.</td>
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<tr>
<td>Sex, % male</td>
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<td>55.0%</td>
<td>29.4%</td>
<td>n.s.</td>
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<tr>
<td>Disease duration, y</td>
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<td>4.4 ± 3.6</td>
<td>4.5 ± 2.4</td>
<td>-</td>
<td>n.s.</td>
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<tr>
<td>PSPRS Total</td>
<td>0.61 ± 1.42</td>
<td>30.4 ± 11.7</td>
<td>39.0 ± 12.8</td>
<td>28.3 ± 11.2</td>
<td>36.9 ± 15.7</td>
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<td>PSPRS History</td>
<td>-</td>
<td>7.42 ± 2.78</td>
<td>8.17 ± 2.51</td>
<td>6.45 ± 2.87</td>
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<td>PSPRS Mention</td>
<td>-</td>
<td>2.42 ± 1.55</td>
<td>3.42 ± 3.33</td>
<td>3.09 ± 1.83</td>
<td>3.82 ± 3.16</td>
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<td>PSPRS Bulbar</td>
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<td>2.67 ± 1.43</td>
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<td>7.42 ± 3.80</td>
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<td>7.09 ± 2.57</td>
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<td>UPDRS-II Total</td>
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<td>MMSE</td>
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<td>MoCA</td>
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Bold values denote statistical significance (P < 0.05). n.s., not significant

### Whole-brain FBA

At baseline comparisons (Fig. 1), the FBA metrics of the PSP revealed significantly (family-wise error corrected P-value < 0.05) decreased FD in the SCP, midbrain, pons, SLF, and body of the CC and log-FC in the SCP, midbrain, pons, CST, and SLF compared with that in the HCs. On the other hand, there was significantly decreased FD in the fornix, genu, body, and splenium of the CC and log-FC in the CST, SLF, cingulum, and body of the CC in CBS compared with that of the HCs. The reduced FDC, which is the
combined scores of FD and FC, generally overlapped on the tracts that were significantly changed in FD and log-FC. Compared
with that of the HCs, all FBA metrics were consistently decreased in PSP and CBS. Log-FC and FDC showed a significant loss
only in PSP along the SCP and midbrain.

Longitudinally, FBA detected further changes mainly in the log-FC, along the SCP, SLF, and WM projecting to the motor cortex in
PSP and along projection fibers and commissural fibers corresponding to the motor cortex and SLF in CBS (Fig. 2). The reduced
FD and FDC were observed in parts of the CC in both PSP and CBS and in the SCP, specifically, in PSP.

**Voxel-based analysis**

Figure 1 also shows the results of whole-brain voxel-based analysis at baseline. The VBM analysis detected significantly
atrophied WM in the midbrain and SCP in PSP versus that in the HCs as well as the WM under the motor cortex in CBS versus
that in the HCs. VBQ also detected significantly decreased FA in the right SCP and midbrain in a comparison between PSP and
HCs, and CBS also showed decreased FA in a part of the CC that appeared to connect the motor cortex compared with that in the
HCs. Both VBM and VBQ indicated no-significant changes between PSP and CBS.

No-significant changes in voxel-wise metrics were shown in both PSP and CBS over a one-year period.

**Fixel-wise tract-specific analysis**

In the baseline comparisons (Fig. 3 [A]), microscopic changes in WM were consistent with the whole-brain FBA and were observed
in the CC and striato-cortical pathways relating to the frontal, parietal, and occipital lobes and SLF in both patient groups
according to FD. In addition, decreased FD along the CST was observed only in CBS. On the other hand, macroscopic WM
changes (i.e., decreased log-FC) were observed mainly in CBS along the CST, SLF, and parts of the CC and striato-cortical
pathways, which were comparably more widespread than the whole-brain FBA. In PSP, the lower log-FC was detected only in the
SCP and CST. The significantly lower FDC had similar patterns in the FD and log-FC results, and the magnitude of changes in FD,
log-FC, and FDC were consistently noticeable in the WM tracts corresponding to the motor cortex.

Longitudinal FDC change patterns were similar in both diseases (Fig. 3 [B]) as well as the results of whole-brain analysis.
Significantly decreased log-FC was found in the subcortical WM, corresponding to the motor cortex in the PSP and relatively
extensive cortex in CBS and the SCP in both diseases. A particularly high degree of macroscopic degeneration over one year was
shown in the SCP and commissural and projection WM under the motor cortex, which were common trends in PSP and CBS.
Decreased FD was specifically shown in the association fibers corresponding to the motor cortex in PSP. Significantly lower FDC
was roughly congruent with the log-FC results and were more sensitive than the whole-brain results in CBS in contrast to
consistency in the PSP. Longitudinal FBA parameter changes were the greatest in the WM tracts under the motor cortex in PSP
and CBS, which was also indicated in the baseline comparisons and whole-brain analysis.

**A receiver operating characteristic analysis**

To verify the possibility that voxel-wise metrics can accurately diagnose PSP and CBS, the log-FC and FDC in the SCP that
significantly differed between the diseases were utilized for a receiver operating characteristic analysis. When using the SCP, the
area under the curve was 0.85 (specificity, 88%; sensitivity, 80%) in the FDC and 0.87 (specificity, 82%; sensitivity, 75%) in the log-
FC. The classical MRI results were: specificity of 70% and sensitivity of 65% in the hummingbirds sign; and specificity of 85% and
sensitivity of 70% in the asymmetrical frontoparietal atrophy.

**Correlation analysis**

Figure 4 shows that the changes in the fixel-wise indices along some tracts were associated with the actual changes in the
clinical scores. Significant correlations were observed between the degree of decrease in log-FC along the SCP ($r = -0.87$, FDR-
corrected $P = 0.023$) and the degree of severity over one year for subscores of "Gait and midline" in the PSP. In CBS, the degree of
decrease in FD and FDC were significantly correlated with the deterioration of PSPRS Total, MoCa, and MMSE in the WM tracts, as
follows: genu of CC (FD-MoCA, $r = 0.89$, FDR-corrected $P = 0.0031$; FDC-MoCA, $r = 0.87$, FDR-corrected $P = 0.0048$), rostral body
of CC (FD-MoCA, $r = 0.92$, FDR-corrected $P = 0.0012$; FDC-MoCA, $r = 0.96$, FDR-corrected $P = 0.0012$), SLF- (FD-PSPRS Total, $r = -0.85$, FDR-corrected $P = 0.0039$), striato-prefrontal pathway (FDC-MoCA, $r = 0.90$, FDR-corrected $P = 0.0021$), and striato-premotor
pathway (FD-PSPRS Total, $r = -0.86$, FDR-corrected $P = 0.0028$; FD-MMSE, $r = 0.93$, FDR-corrected $P = 0.0022$; FDC-MoCA, $r = 0.87$, FDR-corrected $P = 0.0048$).

**Linear regression analysis**

A forward selection in the linear regression model was used to test the possibility that pixel-wise metrics could predict future dysfunction in PSP and CBS (Fig. 5). The mean log-FC along SCP in PSP significantly predicted changes in the subscore “Gait and midline” ($F = 7.67, P = 0.02$ in ANOVA; $B = 14.47, P = 0.02$), and the variables estimated in the bootstrap procedure were significantly predictive ($B = 14.47, P = 0.005$, confidence interval = $7.10–22.92$, bias = 0.048). In contrast, there were no-significant measures along the tracts that were associated with the clinical measures in CBS. The ANOVA results were: genu of CC (FD-MoCA, $F = 1.23, P = 0.3$; FDC-MoCA, $F = 1.59, P = 0.24$), rostral body of CC (FD-MoCA, $F = 1.33, P = 0.28$; FDC-MoCA, $F = 2.03, P = 0.19$), SLF- (FD-PSPRS Total, $F = 4.08, P = 0.74$), striato-prefrontal pathway (FDC-MoCA, $F = 0.83, P = 0.39$), and striato-premotor pathway (FD-PSPRS Total, $F = 2.06, P = 0.19$; FD-MMSE, $F = 3.38, P = 0.11$; FDC-MoCA, $F = 0.86, P = 0.38$).

**Discussion**

We performed cross-sectional and longitudinal FBAs to provide insights into the progressive WM changes in PSP and CBS, considering the advantage that FBA can assess fiber-specific microscopic and macroscopic WM integrity. In the baseline comparisons with the HCs, PSP patients had lower FD and FDC along the SLF and widespread CC and projection fibers. Specifically, lower log-FC was found in the SCP and CST. In contrast, lower log-FC along WM under the motor cortex and lower FD and FDC were widely observed in the WM in CBS. These findings were consistent with the distribution patterns of 4R-tau recognized in patients with PSP and CBS. Next, a direct comparison between PSP and CBS indicated lower log-FC and FDC only in the SCP and midbrain, and the mean log-FC along these pixels differentially classified patients with PSP and CBS more than typical clinical findings. We also demonstrated longitudinal WM changes in PSP and CBS that indicated progressive WM degeneration in the midbrain and SCP, suggesting the possibility that FBA can be a biomarker of disease progression. Especially in PSP, the extent of atrophy in the SCP at baseline was shown to be a predictor of future dysfunction of stability. Therefore, a pixel-wise approach might be useful for classifying patients with PSP and CBS and estimate disease progression.

FBA enabled the assessment of both fiber-specific FD corresponding to the microscopic changes in the intra-axonal volume and fiber-specific FC, indicating macroscopic bundle atrophy subsequently occurred. In PSP at baseline, the lower pixel-wise metrics were especially localized on the SCP, midbrain, projection fibers, and CC corresponding to the frontal and motor cortices and the frontal part of association fibers of the SLF, which was consistent with previous histopathological studies focused on tau depositions. Prominent WM degeneration might reflect axonal degeneration and neuronal loss from neurofibrillary and globose tangles affected by hyperphosphorylated tau, which postmortem studies showed in the frontal and motor cortices, in addition to SCP neuronal loss, which is a hallmark of PSP pathology. CBS had severe bundle atrophy of projection and CC connections to the motor cortex and association fibers, which were observed in extensive WM areas corresponding to the frontal and parietal lobes, including the motor cortex. The distribution of significant pixels was consistent with the distribution of prominent tau in CBD pathology, which is the most frequent phenotype. Particularly severe atrophy in cross-sectional and longitudinal FBA along the WM descending to the motor cortex might support the hypothesis of the involvement of 4R-tau accumulation in the motor cortex at CBS symptom onset. This trend was demonstrated in previous studies indicating atrophy of WM under the motor cortex in subjects with CBD, which clinically presented with CBS symptoms despite variable phenotypes in CBD. This might indicate a disease mechanism of CBS; however, verification requires further studies with case-confirmed pathological diagnoses.

The longitudinal FBA indicated further WM atrophy along the SCP and CST and axonal degeneration and bundle atrophy along the WM under the motor cortex in PSP. CBS also showed consistent continuous changes in the WM under the cerebral cortex and even the SCP as a part of the midbrain. Recent pathological studies suggested continuous spreading of tau propagation in CBD and PSP (Supplementary Fig. 1A). In PSP, neurofibrillary tangles were observed in the following: (1) subthalamic nucleus, globus pallidus, and substantia nigra; (2) striatum, brainstem, and motor cortex; (3) dentate nucleus, and amygdala; (4) frontal lobe; (5) parietal, temporal, and occipital lobes. In contrast, CBD showed prominent tau distribution in the basal ganglia,
including the striatum, in the early stages, primary motor and frontal cortex in the mid-stages, and parietal, temporal, and midbrain nucleus in the later stages. Although these schemes in cortical lobes were largely based on tau distribution in the cortex, previous neuropathological and biochemical studies demonstrated similar patterns of tau load on WM in the corresponding lobes. In summary, the WM in PSP and CBS is continuously affected by tau pathology corresponding to the scheme of each disease. Our cross-sectional and longitudinal findings in the pixel-wise metrics showed axonal loss and atrophy as a result of tau toxicity and progressive WM alterations and variable clinical dysfunctions, which is consistent with the severity of both diseases.

Log-FC and FDC in the SCP and log-FC and FDC in the DRTT, located across the midline coursing toward the ventrolateral thalamic nucleus via the red nucleus, were also significantly reduced in PSP patients (Fig. 6). Despite previous neuroimaging results showing atrophy in the SCP based on VBM, this novel finding identified both micro- and macro-WM degeneration along bilateral DRTTs, as confirmed by constructing a pixel-decussation of the SCP, which suggests superiority of FBA in delineating crossing fibers. Incorporating recent work that demonstrated non-decussating DRTT and its different functions and endpoints in the thalamus from decussating DRTT, our results also provide new insights in the involvement of decussating DRTT, at least in the pathology of PSP. It is worth noting that bundle atrophy in the SCP progressed over one year in association with the PSPRS subscore of “Gait and midline.” PSP is characterized by the 4R-tau burden in neurons and oligodendrocytes with axonal loss and demyelination indirectly associated with axonal loss in the earliest stage. Pathology in the DRTT is commonly found and has been considered a hallmark of PSP pathology associated with frequent falls and postural instability. In summary, changes in pixel-wise metrics might reflect that bundle atrophy occurs following axonal degeneration, which is indirectly associated with neuronal and oligodendrocyte pathology. Progressive gait dysfunction and postural instability in PSP were specifically dependent on continuous neuronal degeneration along the DRTT. Furthermore, PSP-specific changes in log-FC and FDC in the DRTT could better classify both diseases than typical clinical features. Higher accuracy of FDC than log-FC in differentiating between PSP and CBS suggest that the combination of FDC and log-FC is better than each measurement for assessment due to the occurrence of both atrophy and axonal degeneration in PSP. Although the hummingbirds sign and asymmetrical frontoparietal atrophy have been generally recognized as typical imaging features, they are not specific. In this study, a high diagnostic performance of FBA was based on the clinical diagnostic criteria rather than pathological diagnosis. However, considering tau vulnerability in the midbrain tegmentum in PSP and a preserved brainstem structure from tau pathology in mid- to late stage CBS phenotypes, the pixel-wise metrics along brainstem and SCP might provide a potential target for understanding the disease mechanisms in the context of differentiating PSP and CBS. Thus, this non-invasive and expedient analysis might provide more accurate clinical diagnoses and may differentially classify both diseases, which overlap clinically, and may serve as a useful biomarker that can predict future progression.

There is an increasing demand for non-invasive and expedient biomarkers that predict clinical symptoms, especially in fast-progressing diseases such as PSP and CBS. Bundle atrophy along the SCP in PSP was specifically correlated with the PSPRS subscores of “Gait and midline,” and log-FC at baseline could precisely predict subsequent deterioration. This unique feature was also indicated in a previous study using diffusion tensor imaging. Another study that assessed the relationship between diffusion tensor metrics and gait dysfunction demonstrated the role of the SCP in stride length, length of gait phases, gait stability ratio, dynamic stability, and velocity. Postmortem studies also suggested degeneration of red nuclei and other nuclei bordered by the SCP, which could relate to dysfunctions of gait initiation and postural instability. The current results showed bundle atrophy along the SCP significantly predicted subsequent gait impairment and instability and emphasized the contribution of the SCP as a biomarker. Although the baseline WM structures did not predict future clinical impairment in CBS, the strong correlation between clinical impairment total scores and cognitive scores and the structural alterations along tracts corresponding to the motor cortex, including primary motor and sensory motor cortices, agreed with prominent tau pathology observed from mid-staged CBD. The rating scale of PSPRS was not originally designed to assess CBS; however, the current results might derive from various clinical dysfunctions such as impairments of mentation, limb, oculomotor and bulbar disfunctions, and stability in CBS. Furthermore, CBS showed progressive cognitive decline had a strong association with longitudinal changes in FD along the WM of the frontal and motor cortices. In line with previous findings on early involvement of the fronto-parietal network including the basal ganglia, frontal cortex, and parietal cortex related to cognitive dysfunction in CBD, our results might provide new insights on the microstructural WM changes associated with cognitive impairment in
CBS. Future longitudinal studies with histopathological information and more detailed cognitive information, including language, executive, and verbal memory and visuospatial functions, is expected to support these findings.

FBA is methodologically useful in that it can assess both progressive fiber structural changes and crossing fibers, especially in a voxel that has multi-directional fibers.\(^{26}\) Certainly, in addition to the detectability of fixel-decussation of SCP, the significantly changed fixels (i.e., reduced FD or log-FC) completely represented each tract beyond contamination by other voxels where the CC and projection fibers intersect. This showed high superiority over voxel-wise diffusion tensor imaging, which did not delineate the crossing region even in cases without consideration of multiple comparisons (Supplementary Fig. 2). Focusing on this region, which corresponds to subcortical WM under the motor cortex, bundle atrophy of projection fibers and reduced FD in the CC were observed in PSP, whereas bundle atrophy and further fiber damage already occurred as reduced FD in the CC in CBS at baseline (Fig. 3). These features were also shown along the striato-motor pathways as well as CC projections in both hemispheres (Supplementary Fig. 1). Incorporating the scenario that FBA can assess FD and subsequent bundle atrophy, which generally indicate WM alterations over time, our findings reflect WM alteration patterns as a result of early tau vulnerability of the motor cortex and striatum and subsequent patterns of tau propagation to other affected cortical areas in CBS\(^{27,29,34,35}\) and PSP's prominent tau pathology in the motor cortex, which occurs later but is the earliest affected compared to other cortical areas.\(^{3,7,33}\) These progressive WM degeneration patterns based on FBA methodology were also observed in longitudinal WM alterations as the most prominent WM changes along motor cortex tracts. In summary, the current study showed abnormal CCs with disease progression and differentiated between PSP and CBS, suggesting that the striato-cortical pathway can be a potential target for an imaging biomarker.

There are some limitations that should be considered. One is the lack of pathologic confirmation of diagnoses for all patients in our cohort. We expect future studies on postmortem confirmed cases will lead to a better understanding of the pathogenesis of PSP and CBS and will improve FBA to be a strong biomarker. We did not consider typical left-right differences in CBS because of the lack of clinical laterality information in the 4RTNI database. This may have resulted in reduced sensitivity to detect neurodegeneration underlying clinical disabilities in CBS. Nevertheless, our results indicated prominent WM alterations might produce clinical impairments, which is consistent with previous histopathological findings. In this regard, the lack of histopathological properties underlying the fixel-wise metrics should be noted. Technically, it should also be noted that single-shell DWI data \((b = 2,000\ \text{s/mm}^2)\) were obtained from 4RTNI and FTLDNI databases. Although, the deliberately chosen relatively high \(b\)-values from the databases to consider the characteristics of the SS3T-CSD algorithm-optimized high \(b\)-values, higher \(b\)-value data or multi-shell data might have the ability to eliminate the partial volume effect.\(^{47}\) Some studies have suggested superiority of the SS3T-CSD method compared with the multi-shell multi-tissue CSD algorithm.\(^{47}\) Furthermore, we did not correct susceptibility-induced distortion because the reverse-phase encoded images were not provided by 4RTNI and FTLDNI for all subjects, and absence of the distortion correction might have induced a greater variance to estimate the FOD model.\(^{48}\)

We performed FBA for PSP and CBS, and our results suggested that the clinical progression in both diseases is accompanied by loss of FD and subsequent bundle atrophy, which is congruent with the distribution of tau pathology. Additionally, fixel-wise metrics could be used to classify these diseases that overlap clinically. FBA could be a useful \textit{in-vivo} biomarker for predicting later clinical progression of PSP. Future studies are needed to validate the association between changes in fixel-wise metrics and the underlying histopathology in both diseases; however, FBA is likely to be highly useful for understanding the mechanisms of disease progression and differences in the characteristics of the two diseases, which are currently difficult to distinguish clinically.

### Materials And Methods

### Participants

CBS and PSP data were collected from the 4R Tauopathy Neuroimaging Initiative (4RTNI) and HC data were obtained from the Neuroimaging Initiative for Frontotemporal Lobar Degeneration (FTLDNI). All patients were selected using MRI acquisition criteria, which were diffusion-weighted images (DWIs) acquired with standardized parameters at two-time points with a one-year interval (range, 10 to 14 months) as a longitudinal approach. Patients with a history of significant psychiatric or neurological
disorders other than that associated with PSP or CBS were excluded from our dataset. All HCs were confirmed to have normal cognition with an MMSE score of \( \geq 27 \). Eventually, our dataset included 57 subjects consisting of 20 PSP and 17 CBS patients and 20 HCs. PSP patients were diagnosed with the National Institute of Neurological Disorders the Stroke/Society for PSP criteria for PSP-Richardson syndrome. All CBS patients met the requirements for the Armstrong criteria for CBS-CBD subtypes. Patients underwent neurological examinations, including the PSP Rating Scale (PSPRS) with constructed subscores for “Gait and midline,” “Limb motor,” “Oculomotor,” “Bulbar,” “History,” and “Mentation,” the Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Unified Parkinson's Disease Rating Scale part 3 (UPDRS- ), the Clinical Dementia Rating Sum of Boxes, the Schwab and England Activities of Daily Living (SEADL), and the Functional Activities Questionnaire.

**MRI data**

MRI data were acquired at two sites, namely, the University of California, San Francisco and the Martinos Center for Biomedical Imaging, using 3-Tesla TIM Trio MRI scanners with a 12-channel head coil. All DWIs were scanned with the following standardized parameters: echo-planner imaging, repetition time, 9,200 ms; echo time, 82 ms; thickness, 2.7 mm; matrix, 128 \( \times \) 128 (2.7 \( \times \) 2.7 mm); \( b \)-values, 0 and 2,000 s/mm\(^2\); and diffusion encoding directions, 41. The T1-weighted imaging data were collected using the sequence of three dimensional MPRAGE with the following parameters: repetition time, 2,300 ms; echo time, 2.98 ms; inversion time, 900 ms; matrix, and 160 \( \times \) 240 \( \times \) 256 (1 mm isotropic voxel). All DWI data were acquired with relatively high \( b \)-values (\( b = 2,000 \) s/mm\(^2\)), considering that higher \( b \)-values might have the ability to eliminate the partial volume effect using single-shell three tissue constrained spherical deconvolution (SS3T-CSD).

**Diagnostic evaluation based on classical imaging findings**

To assess the diagnostic ability of conventional MRI features for classifying both PSP and CBS, a neuroradiologist (K.K.) assessed the “hummingbirds sign” in PSP and asymmetrical frontoparietal atrophy in CBS as classical MRI findings by referring to the T1-weighted images of all participants.

**Missing data handling**

Patients with missing data at either baseline or follow-up were omitted from the correlation analysis because the longitudinal changes in the clinical indices could not be calculated.

**Imaging analysis**

**Cross-sectional FBA at baseline**

The following imaging analysis pipeline was performed using the MRtrix3Tissue (http://3tissue.github.io/) based on the recommendation by the developers. The pre-processing of DWI data involved denoising with Marchenko–Pastur principal component analysis and correction for Gibbs artifacts, eddy current-induced and motion-induced distortion, B1 field inhomogeneities, and up-sampling of the resolution with cubic b-spline interpolation to 1.3-mm isotropic voxels that were more than twice the resolution to improve the image alignment. The response functions corresponding to WM, gray matter, and CSF were estimated, and the group-averaged response functions were generated across all participants for each whole-brain FBA. Using group-averaged response functions, the fiber orientation distributions (FODs) for all subjects were estimated based on the SS3T-CSD algorithm. Also, the sum of intensities from each tissue component was normalized toward a constant value in all voxels. The group-specific averaged FOD templates were created by iterative nonlinear registration, and the FODs of all participants were normalized to the template based on FOD. Then, the fixels within all voxels were defined as peaks of FOD lobes that were specifically segmented. Finally, fixel-wise metrics (FD, fiber density; log-FC, log-transformed fiber-bundle cross-sections; and FDC, a product of FD and FC) were defined on the produced fixel grid. To perform a connectivity-based fixel enhancement statistical analysis, a whole-brain probabilistic tractography consisting of 20 million streamlines was estimated based on the FOD template and decreased to 2 million streamlines using the spherical deconvolution-informed filtering of tractograms algorithm, which can improve the quantitative nature and reduce biases in estimations of streamlines.
Longitudinal FBA for PSP and CBS

To accurately normalize the WM-FODs to group-specific templates in the longitudinal FBA for patient groups, FBA processing was performed as described in a previous study, that is: $^6$4 (1) the baseline and follow-up FODs were rigidly co-registered to the midways for all individuals; (2) the co-registered FODs were then averaged to create subject-specific FOD templates; (3) the subject-specific FOD templates were used for generating group-specific FOD templates; and (4) the baseline and 1-year follow-up FODs in the original space were subsequently normalized to a group-specific FOD template through the subject-specific templates for each individual.

Fixel-wise tract-specific analysis with a tract segmentation method

A fixel-wise tract-specific analysis was performed to comprehensively investigate selective WM integrity in PSP and CBS. The tract segmentation method based on TractSeg $^6$5 was adopted to obtain the averaged fixel-wise metrics without contamination by the crossing fibers. FOD template-based whole-brain tractograms were categorized for generating tract-specific fixel masks in a FOD template space. Subsequently, the averaged FBA parameters along the tracts at each time point were calculated and cross-sectionally and longitudinally compared across all groups. The longitudinal changes in the fixel-wise indices were defined by the formula: $\text{Followup} - \text{Baseline}$ for correlation analysis with the degree of severity based on clinical indicators. The 19 tracts were selected by considering the evidence suggesting that the misfolded tau exhibited self-propagation along axons $^{12}$ and involvement of tau in the cerebral neocortex, basal ganglia, especially in the striatum, and SCP observed in PSP and CBD $^{29,66}$ Finally, the following tracts were adopted: SCP, corticospinal tract (CST), rostrum, genu, rostral body, anterior midbody, posterior midbody, isthmus, and splenium parts of the CC, superior longitudinal fascicle I (SLF-I), SLF-II, SLF-III, and striato-cortical, including striato-fronto-oral, striato-prefrontal, striato-premotor, striato-precentral, striato-postcentral, striato-parietal, and striato-occipital pathways.

Conventional voxel-based analysis of morphometry and diffusion tensor imaging metrics

The voxel-based analysis was also performed to examine the cross-sectional and longitudinal WM changes using voxel-based morphometry (VBM) $^6$7 and voxel-based quantification (VBQ) $^6$8 for comparisons of fractional anisotropy (FA) derived from the DTIFIT (FMRIB Software Library v6.0, www.fmrib.ox.ac.uk/fsl). VBM and VBQ were conducted using the Statistical Parametric Mapping 12 software (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). In brief, these approaches included the following processes: (1) FA maps were co-registered into the 3D T1-weighted imaging space for each subject using a boundary-based registration; (2) the 3D T1-weighted images were segmented into WM, gray matter, and CSF; (3) the WM and FA maps were normalized to the Montreal Neurological Institute/International Consortium for Brain Mapping 152 standard space using the DARTEL (Diffeomorphic Anatomical Registration and Exponentiated Lie algebra); (4) the normalized maps were resampled to a 1-mm isolated voxel, and only WM maps were modulated; and (5) a kernel of 8-mm full width at half maximum was applied to the normalized maps to smoothen them.

Statistical analysis

Demographic and clinical assessments

The Statistical Package for the Social Sciences for Windows, Release 25.0 (SPSS, IBM Corporation, Armonk, NY, USA), was used for all analyses. First, we performed a Kolmogorov–Smirnov test to evaluate the normality of the data. All assessments of participants at baseline were evaluated using Student’s $t$-test or the Mann–Whitney $U$ test for group-wise comparisons, and ANOVA with Tukey–Kramer as a post-hoc analysis or Kruskal–Wallis test was applied to compare the three groups. For comparisons of the categorical variables, a $\chi^2$ test was used. $P$-values < 0.05 were considered significant.

Cross-sectional and longitudinal whole-brain FBA

To assess the fixel-wise metrics (i.e., FD, log-FC, and FDC), a general linear model was used, which included fiber-specific smoothing using whole-brain tractograms and statistical inference with default parameters ($C = 0.5; E = 2; H = 3$; and smoothing =
10 mm full width at half maximum). The non-parametric 10,000-permutation test was performed next to assign the family-wise error corrected $P$-value to each pixel. We performed a group comparison in pixel-wise metrics at baseline across all groups after adjusting age, sex, and intracranial volume (log-transformed intracranial volume for log-FC) measured using FreeSurfer 6.0.1 (http://surfer.nmr.mgh.harvard.edu/fswiki) as covariates. The family-wise error corrected $P$-value $< 0.05$ was considered significant.

**Voxel-based analysis**

The voxel-based analysis, including VBM and VBQ, was performed using an un-paired $t$-test for cross-sectional comparisons and a paired $t$-test for longitudinal changes. We adjusted age, sex, and intracranial volume in the cross-sectional analysis. The family-wise error corrected $P$-value of $< 0.05$ was considered significant.

**Fixel-wise tract-specific analysis**

All statistical analyses in the fixel-wise tract-specific analysis were performed with IBM SPSS Statistics version 27.0 (IBM Corporation, Armonk, NY, USA). ANOVA with Tukey–Kramer was performed to compare the mean FD, log-FC, and FDC across groups at baseline. Paired-$t$ test was used for pairwise comparisons of the longitudinal changes over one year of fixel-wise metrics in PSP and CBS. A receiver operating characteristic analysis was also performed based on classical MRI features (i.e., hummingbird sign for PSP and asymmetrical frontoparietal atrophy for CBS) and the mean of log-FC and FDC along SCP, which differed between CBS and PSP. The sensitivity, specificity, and area under the curve were then calculated. Partial correlations between the longitudinal changes of fixel-wise parameters and clinical indices were calculated with Spearman rank correlations accounting for age, sex, and disease duration at baseline. Changes across time points were calculated as baseline values minus the 1-year follow-up values. Additionally, a bootstrapping procedure of 1,000 samples was applied to estimate the 95% confidence interval. In all analyses, the false-discovery rate (FDR) corrected $P < 0.05$ was considered as significant for multiple comparisons of the number of WM tracts. Additionally, we utilized a forward selection linear regression model to assess the possibility that the baseline fixel-wise metrics, which were significantly correlated with clinical assessment parameters, could predict the changes in clinical indices over one year in PSP and CBS.

**Declarations**

**Data availability**

All subject’s imaging data and clinical information can be available in the 4RTNI and FTLDNI database (http://4rtni-ftldni.ini.usc.edu/) after agreeing to the data terms.

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Data used in preparation of this article were obtained from the 4RTNI and the FTLDNI database (http://4rtni-ftldni.ini.usc.edu). The investigators at 4RTNI and FTLDNI contributed to the design and implementation of 4RTNI and FTLDNI and/or provided data, but didn't participate in analysis or writing of this report (unless otherwise listed).

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**Author contributions**
WU, KK, CA, AH, and SA contributed to the conception and design of the study. WU, KK, CA, KT, YS, MO, SF, AW, TA, KS, and MH contributed to the analyses of data and preparing the figures. WU, KK, CA, AH, and SA contributed to drafting the manuscript. All authors have reviewed and approved the contents of the manuscript.

Competing interests

The authors report no competing interests.

References


### Figures

**Figure 1**

**Cross-sectional group differences in the fixel-based metrics.** In the upper panel, the streamlines cropped from template tractograms passing significant fixels are shown as colored by direction: red, left-right; green, anterior-posterior; and blue, inferior-superior, as the results of fixel-based analysis (family-wise error corrected $P < 0.05$). The lower panel shows the results of voxel-based analysis, including voxel-based morphometry (VBM) and voxel-based quantification comprising the fractional anisotropy. The significant voxels (family-wise error corrected $P < 0.05$) were scaled by the $t$-values, ranging from 3 to 8.
Figure 2

Longitudinal changes in the fixel-based metrics over one year in patients with progressive supranuclear palsy (PSP) and corticobasal syndrome (CBS). The streamlines that were cropped from template tractograms passing significant fixels (family-wise error-corrected \( P < 0.05 \)) are shown as colored by direction: red, left-right; green, anterior-posterior; and blue, inferior-superior, in the longitudinal fixel-based analysis of PSP shown in the left and CBS shown in the right.

Figure 3

Cross-sectional and longitudinal fixel-wise tract-specific analysis in the progressive supranuclear palsy (PSP) and corticobasal syndrome (CBS). (A) The cross-sectional mean percent changes and 95% confidence intervals in the fiber density (FD), log-transformed fiber cross-section (log-FC), and fiber density and cross-section (FDC) are displayed for PSP and CBS compared to those of healthy controls. The significantly decreased tracts are colored with blue for PSP and orange for CBS, and the tracts that
did not show significant changes are displayed as gray bars. (B) The longitudinal mean percent changes and 95% confidence intervals in the FD, log-FC, and FDC are displayed compared to the baseline metrics in PSP and CBS. The significantly altered tracts over one year are colored, and the no-significant tracts are shown as gray bars. Superior cerebellar peduncle, SCP; corticospinal tract, CST; corpus callosum, CC; superior longitudinal fascicle I, SLF-I; superior longitudinal fascicle II, SLF-II; superior longitudinal fascicle III, SLF-III; striato-fronto-orbital, ST-FO; striato-prefrontal, ST-PREF; striato-premotor, ST-PREM; striato-precentral, ST-PREC; striato-postcentral, ST-POSTC; striato-parietal, ST-PAR; and striato-occipital, ST-OCC.

Figure 4

Partial correlation between the longitudinal changes of the voxel-wise parameters and clinical indices. The partial correlation (Spearman's $\rho$) adjusted for age, sex, and disease duration are illustrated as heatmaps, which are colored red (positive; Spearman's $\rho$ range, 0 to 1) to blue (negative, Spearman's $\rho$ range, −1 to 0). The diameter of the circles in each grid are scaled with the absolute Spearman's $r$, which was set 1 as the maximum and 0 as the minimum. The asterisks on the circles represent a significant correlation (false-discovery ratio-corrected $P < 0.05$). Superior cerebellar peduncle, SCP; corticospinal tract, CST; corpus callosum, CC; superior longitudinal fascicle I, SLF-I; superior longitudinal fascicle II, SLF-II; superior longitudinal fascicle III, SLF-III; striato-fronto-orbital, ST-FO; striato-prefrontal, ST-PREF; striato-premotor, ST-PREM; striato-precentral, ST-PREC; striato-postcentral, ST-POSTC; striato-parietal, ST-PAR; striato-occipital, ST-OCC; Total progressive supranuclear palsy rating scale, PSPRS_TOTAL; PSPRS subscores of “History,” PSPRS_HISTORY; “Mentation,” PSPRS_MENTATION; “Bulbar,” PSPRS_BULBAR; “Oculomotor,” PSPRS_OCCULARMOTOR; “Limb motor,” PSPRS_LIMBMOVEMENT; and “Gait and midline,” PSPRS_GAITMIDLINE; Unified Parkinson’s Disease Rating Scale part 3, UPDRS; Mini-Mental State Examination; MMSE; and Montreal Cognitive Assessment, MoCA.
Figure 5

The association between longitudinal changes in the clinical index and the bundle cross-section. (A) The scatter plot represents the association between actual changes of a progressive supranuclear palsy rating scale subscore for "gait and midline" (PSPRS_GAITMIDLINE) and actual changes in log-transformed fiber cross-sections (log-FC) along the superior cerebellar peduncle (SCP). (B) The scatter plot represents the association between actual changes in PSPRS_GAITMIDLINE and the predicted changes of PSPRS_GAITMIDLINE derived from a linear regression analysis.
Figure 6

Decussation of the superior cerebral peduncle construction with the significant fixels. The shown fixels are significantly decreased in the log-transformed fiber cross-section in progressive supranuclear palsy compared to that in corticobasal syndrome (family-wise error-corrected $P < 0.05$) colored by direction: red, left-right; green, anterior-posterior; and blue, inferior-superior. The significant fixels construct the decussation of the superior cerebellar peduncle through the contralateral red nucleus (yellow arrowheads).

Supplementary Files

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