Degeneration in Different Parkinsonian Syndromes Relates to Astrocyte Type and Astrocyte Protein Expression

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Abstract
The reactive changes in different types of astrocytes were analyzed in parkinsonian syndromes in order to identify common reactions and their relationship to disease severity. Immunohistochemistry was used on formalin-fixed, paraffin-embedded sections from the putamen, pons, and substantia nigra from 13 Parkinson disease (PD) cases, 29 multiple-system atrophy (MSA), 34 progressive supranuclear palsy (PSP), 10 corticobasal degeneration (CBD), and 13 control cases. Classic reactive astrocytes were observed in MSA, PSP, and CBD, but not PD cases; the extent of reactivity correlated with indices of neurodegeneration and disease stage. Approximately 40% to 45% of subcortical astrocytes in PD and PSP accumulated α-synuclein and phospho-tau, respectively; subcortical astrocytes in MSA and CBD cases did not accumulate these proteins. Protoplasmic astrocytes were identified from fibrous astrocytes by their expression of parkin coregulated gene and apolipoprotein D, and accumulated abnormal proteins in PD, PSP, and CBD, but not MSA. The increased reactivity of parkin coregulated gene–immunoreactive protoplasmic astrocytes correlated with parkin expression in PSP and CBD. Non-reactive protoplasmic astrocytes were observed in PD and MSA cases; in PD, they accumulated α-synuclein, suggesting that the attenuated response might be due to an increase in the level of α-synuclein. These heterogenous astrogial responses in PD, MSA, PSP, and CBD indicate distinct underlying pathogenic mechanisms in each disorder.

Key Words: α-Synuclein, Astrocytes, Corticobasal degeneration, Multiple system atrophy, Parkin, Parkinson disease, Progressive supranuclear palsy, Tau

INTRODUCTION
There are differences in astrocytic reactions in different parkinsonian syndromes. In Parkinson disease (PD) cases, reactive astrogliosis is minimal (1–3), but abnormal α-synuclein deposition occurs in astrocytes and relates to the severity of neuronal Lewy body (LB) inclusions (4, 5). In contrast to PD, all other parkinsonian syndrome cases showed marked reactive astrogliosis (6, 7). Astrocytes in multiple system atrophy (MSA) do not accumulate α-synuclein (6), whereas in progressive supranuclear palsy (PSP) and corticobasal degeneration (CBD) cases, some reactive astrocytes accumulate phospho-tau (8). The corresponding morphologic changes in tau-accumulating astrocytes in PSP and CBD (i.e. tufted astrocytes and astrocytic plaques, respectively) are diagnostic for these disorders and are thought to directly contribute to the neurodegeneration (7, 8).

Reactive astrogliosis is associated with the upregulation of glial fibrillary acidic protein (GFAP) and cell enlargement (9). These changes occur after many types of brain injury in different astrocyte populations depending on the location of the injury or change. The gray matter is populated by protoplasmic astrocytes that have a mossy appearance with radiating branched processes and express apolipoprotein D (ApoD), a lipid transport protein involved in CNS repair and regeneration (10). Fibrous astrocytes populate CNS white matter and have longer, less branched processes; they do not express ApoD (10). Some PD-associated proteins (e.g. parkin [11], parkin coregulated protein [PACRG] [12], DJ-1 [13], and PINK-1 [14]) are also concentrated in astrocytes, but
the type of astrocyte has not been identified. As the expression of Parkin and PACRG are coregulated (15), both proteins were evaluated.

The determinants of the different types of astrocytic changes and whether the changes observed relate directly to the neurodegeneration in the different parkinsonian disorders are not clear. To our knowledge, there have been no studies assessing whether different reactions occur because of the type of astrocyte affected, whether changes in the expression of astrocytic proteins have pathologic correlates, and whether these changes relate to the neurodegeneration observed. We assessed reactive changes in different types of astrocytes in PD, MSA, PSP, and CBD to identify any relationships to the severity of the degenerative changes observed.

MATERIALS AND METHODS

Cases

Formalin-fixed brain tissue samples that fulfilled the pathologic criteria for PD (n = 13) (16), MSA (n = 29) (17, 18), PSP (n = 34) (19), CBD (n = 10) (20), or age-matched controls without significant neuropathologic abnormalities (n = 13) were provided by the Prince of Wales Medical Research Institute Human Tissue Resource Centre after study approval by the Australian Brain Donor Programmes’ Scientific Advisory Committee and by the Queen Square Brain Bank, UCL Institute of Neurology. All tissues were collected with appropriate consent from brain donors and/or their next of kin, and the collection programs approved by appropriate institutional or regional human ethics committees. Tissues from some of the cases from the Queen Square Brain Bank have been previously used and published in other studies (6, 21, 22). Unpaired t-tests (SPSS, Inc., Chicago, IL) showed no differences in the demographics (age at onset, age at death, disease duration, or postmortem delay) of the different types of cases from the 2 sources.

Tissue Preparation

In all cases, multiple brain regions were screened using immunohistochemical protocols for abnormal accumulations of pathologic proteins. Tissues blocks from the putamen (dorsolateral level), substantia nigra (SN; transverse level including the red nucleus), and the pontine base (transverse level, including the superior cerebellar peduncle) were analyzed. Sections (5-μm thick) were cut on a microtome, mounted on 3-aminopropyltriethoxysilane–coated slides, and deparaffinized.

The following antibodies were used for identifying pathologic inclusions: mouse monoclonal antibody (mAb) clone 42 to α-synuclein (1:200; BD Biosciences, San Jose, CA), rabbit anti-α-synuclein (1:2000; Abcam, Cambridge, UK), mouse anti-tau mAb (1:600; Autogen Bioclear, Calne, UK), and rabbit anti-tau (Abcam; 1:500). Astrocytic proteins were identified using mouse anti-GFAP mAb (1:1000; DAKO, Ely, UK); rabbit anti-GFAP (1:750; DAKO); mouse anti-ApoD mAb (1:50; Vector Laboratories, Burlingame, CA); mouse anti-proteoglycan NG2 mAb (1:100; Millipore, Billerica, MA); rabbit anti-parkin (1:200; Biosensis, Adelaide, Australia); rabbit anti-PACRG (MC1290, 1:100; kindly provided by Dr. Paul Lockhart). Immunohistochemical staining and counterstaining with cresyl violet (0.5%) or Mayer hematoxylin were performed as previously described (6, 12, 22). Various antigen retrieval methods (99% formic acid/pressure cooking/citrate buffer pH 6.0) were used before antibody incubation to maximize antigen detection. The specificity of the immunohistochemical reaction was tested by omitting the primary antibody, and no peroxidase reaction was subsequently detected.

FIGURE 1. Parkin immunoreactivity in mouse striatum. A control mouse (A) shows parkin immunoreactivity, whereas a quaking mouse with parkin and parkin coregulated gene deletions (B) is negative. KO, knockout.
observed in those sections. The specificity of the parkin antibody was confirmed by negative immunostaining using the same protocol in formalin-fixed, paraffin-embedded brain tissue from quaking mice that have deletions of both parkin and PACRG (Fig. 1) (23). The specificity of the PACRG antibody, that is, Western blot assessment of soluble extracts from human brain cortex to confirm a 29-kDa band and incubating it with purified recombinant PACRG protein, as well as with preimmune serum to show no peroxidase staining observed in these sections, has been previously detailed (12).

Double labeling immunofluorescence was performed as previously described (12) using the same antigen retrieval methods on additional slides. Rabbit anti-GFAP was mixed with either mouse anti-α-synuclein or anti-tau. Mouse anti-GFAP, anti-ApoD, anti-α-synuclein, or anti-tau mAb was mixed with either rabbit anti-PACRG or anti-parkin. Mouse anti-NG2 was mixed with rabbit anti-tau. The fluorescent probes used for the detection of primary antibodies were secondary antibody anti-mouse conjugated to Alexa 488 (1:500) or tetramethylrhodamine (1:500; Perkin Elmer, Waltham, MA) and anti-rabbit conjugated to Alexa 568 (1:250; Molecular Probes) or tetramethylrhodamine (1:500; Perkin Elmer). To ensure specificity of the immunohistochemical reactions and non-cross-reactivity of secondary fluorescent probes, a section without primary antibodies was included for each staining procedure as a negative control. Additionally, a mixture of the secondary antibodies was applied to sections with only 1 primary antibody incubated on each section. For primary antibodies from the same species (parkin and PACRG), a combined method of immunofluorescence and peroxidase visualization was used as previously described (24). Pretreatment methods and standard peroxidase immunohistochemistry were performed with the incubation of anti-PACRG (1:100) as the first primary antibody. After peroxidase visualization, slides were then incubated with anti-parkin (1:200) as the second primary antibody for 1 hour at 37°C and then incubated with the secondary antibody anti-rabbit conjugated to Alexa 568 (1:250) for 2 hours at room temperature. Similar controls to those previously described revealed no cross-reactivity using this method.

**Analyses**

The peroxidase-stained sections from all cases were viewed using a Zeiss Axioskop MC80 DX or an Olympus BX51 microscope to evaluate astrocyte morphology. The extent of reactive GFAP-, PACRG-, and parkin-immunoreactive astrocytes was scored in 3 random 100× magnification fields in each section from each case as follows: none, rare, mild (a sparse distribution); moderate (in close to half the field of view); or severe (densely spread throughout the field of view). Because parkin immunoreactivity was only observed in pathologic and not in control samples, further quantitative assessment with a stereologic approach was performed on these samples using an imaging analysis software program (Image Pro Plus, version 6.2; Media Cybernetics, Bethesda, MD). Using a motorized stage attached to the microscope (Nikon Eclipse 50i), the regions of interest were delineated using a computer mouse and random sites sampled at 400× magnification by the imaging software program to quantify parkin-immunoreactive astrocytes. Intrarater and interrater analysis was less than 5% variance using this method. Nonparametric Kruskal-Wallis and Mann-Whitney U tests (SPPS, Inc.) were performed to identify group differences.

To assess the degree of protein colocalization, double labeled sections were viewed and photographed using Zeiss Axioskop MC80 DX or Olympus BX51 microscopes (Axiocam camera and Axiovision software), and confocal images were taken using a Leica TCS4D confocal microscope with a 3-channel scan head and argon/krypton laser (University College London confocal imaging unit). The proportion of astrocytes with colocalizing proteins was determined in 10 random photographs of each section for each case taken at 200× magnification.

To establish correlates with pathologic severity, a number of indices were evaluated, including neuronal loss in the SN, LB severity (graded by sampling the whole region of interest at 100× magnification as none, 1, 2–6, and 7 or more LB) and Braak LB stage (25) for PD, glial cytoplasmic inclusion (GCI) severity (graded as for astrocytes) and MSA stage (26) for MSA, neurofibrillary tangle severity (graded as for astrocytes) and PSP stage (22) for PSP and overall tau severity (graded as for astrocytes) and disease stage (27) for CBD. Spearman rank tests (SPPS, Inc.) were performed to determine any relationships between the degree of astrocytic reactivity and indices of pathologic severity.

**RESULTS**

**Pathologic Changes in Astrocytes**

Glia fibrillary acidic protein–immunoreactive astrocytes in PD cases had normal stellate appearances (Fig. 2A, B), and approximately 45% also showed α-synuclein immunoreactivity using either α-synuclein antibody (Fig. 2C–E). Only the SN showed mildly increased GFAP immunoreactivity (Table 1).

In MSA cases, reactive astrocytes had enlarged cell bodies and distorted processes (Fig. 2F). Double labeling immunofluorescence showed no deposition of α-synuclein in GFAP-immunopositive astrocytes, as previously reported (5). The putamen in MSA cases had the most marked increase in GFAP immunoreactivity (Table).

In PSP cases, reactive astrocytes had enlarged stellate morphologies (Fig. 2G), and approximately 40% also had phospho-tau immunoreactivity (Fig. 2H). Tau-immunoreactive tuffed astrocytes were not GFAP (Fig. 2I), as previously reported (28). There was obvious reactive astroglialosis in all regions examined in PSP cases, but the most prominent was in the SN (Table).

Reactive astrocytes in CBD cases were similar to those in PSP (Fig. 2J). No tau-immunoreactive astrocytes or astrocytic plaques were observed in the regions examined, although all CBD cases had cortical astrocytic plaques and increased GFAP immunoreactivity in the putamen and SN. The pons was not available for analysis in CBD cases (Table).

In summary, typical astrocytic reactivity was observed in MSA, PSP, and CBD cases with no morphologic changes.
and only mild increases in numbers of GFAP-immunopositive astrocytes in the SN in PD cases. Approximately 40% to 45% of subcortical astrocytes analyzed accumulated pathologic proteins in PD and PSP but not in MSA or CBD cases.

The slight reactive astrogliosis in PD cases did not correlate with either the severity of SN cell loss or the severity of LB formation (Table). In MSA, the degree of GFAP-immunoreactive astrocytes in the putamen correlated with the degree of α-synuclein-immunoreactive GCI in thepons (Rho = 0.73; p = 0.007) and MSA pathologic stage (Rho = 0.67; p = 0.02). In PSP, the degree of GFAP-immunoreactive astrocytes in both the SN and putamen positively correlated with the degree of LB formation (Table). In MSA, the degree of GFAP-immunoreactive astrocytes also correlated with CBD stage (Rho = 0.97; p = 0.007) and CBD stage (Rho = 0.97; p = 0.007). In addition, astrocyte containing phospho-tau (green Alexa 488) in the pons of a PD case. (F) Brightfield photomicrograph of an enlarged and distorted GFAP-immunoreactive astrocyte in a white matter bundle of a multiple system atrophy (MSA) case. (G) Brightfield photomicrograph of an enlarged reactive GFAP-immunoreactive astrocyte in the putamen of a progressive supranuclear palsy (PSP) case. (H) Immunofluorescent merged image of a GFAP-immunopositive (red Alexa 568) reactive astrocyte containing phospho-tau (green Alexa 488) in the putamen of a PSP case. (I) Immunofluorescent merged image of a phospho-tau immunopositive (green Alexa 488), but GFAP-immunonegative (red Alexa 568) tufted astrocyte in the putamen of a PSP case. (J) A brightfield photomicrograph of an enlarged reactive GFAP-positive astrocytes in the putamen of a corticobasal degeneration (CBD) case. Brightfield photomicrographs are counterstained with cresyl violet.

Types of Astrocytes Affected
Apolipoprotein D is selectively expressed in protoplasmic astrocytes (10). All ApoD-immunoreactive astrocytes in controls were also immunoreactive for PACRG (Fig. 3A–C). Despite previous descriptions of parkin-immunoreactive glia (29), only PACRG immunoreactivity was observed in the typical stellate astrocytes in controls in the regions examined (Fig. 3D). Co-localization experiments indicated that approximately 80% of GFAP-immunoreactive astrocytes also contained PACRG immunoreactivity (Fig. 3E). This approximates the expected proportion of protoplasmic versus fibrous astrocytes in these regions and suggests that they all constitute PACRG and ApoD.

Different types of astrocytes had distinct reactions in the different parkinsonian syndromes. The distribution of ApoD-/PACRG-immunoreactive astrocytes did not differ

Table 1: Regional Severity of Nigral Cell Loss, Pathologic Inclusions, and Enhanced Astrocytic Immunoreactivity

<table>
<thead>
<tr>
<th>Substantia nigra</th>
<th>Mean Severity Grade (range)</th>
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<tbody>
<tr>
<td>Control PD MSA PSP CBD</td>
<td></td>
</tr>
<tr>
<td>Cell loss</td>
<td>0</td>
</tr>
<tr>
<td>Inclusion pathology</td>
<td>0</td>
</tr>
<tr>
<td>GFAP increase</td>
<td>1 (0–1)</td>
</tr>
<tr>
<td>PACRG increase</td>
<td>0 (0–1)</td>
</tr>
<tr>
<td>Parkin increase</td>
<td>0</td>
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<tr>
<td>Parkin-positive cells/mm²</td>
<td>—</td>
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Putamen

| Inclusion pathology | 0 | 0 (0–1) | 2 (1–3) | 0 (0–1) | 1 (0–3) |
| GFAP increase | 1 (0–2) | 0 (0–1) | 2 (0–3) | 1 (0–2) | 2 (1–3) |
| PACRG increase | 1 (0–1) | 1 (0–2) | 1 (0–2) | 2 (0–3) | 2 (1–3) |
| Parkin increase | 0 | 0 | 0 | 2 (1–3) | 2 (1–3) |
| Parkin-positive cells/mm² | — | — | — | 300 ± 192 | 147 ± 43 |

<table>
<thead>
<tr>
<th>Basis pontis</th>
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<tbody>
<tr>
<td>Control PD MSA PSP CBD</td>
<td></td>
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<tr>
<td>Inclusion pathology</td>
<td>0</td>
</tr>
<tr>
<td>GFAP increase</td>
<td>1 (0–1)</td>
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<tr>
<td>PACRG increase</td>
<td>1 (0–1)</td>
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<tr>
<td>Parkin increase</td>
<td>0</td>
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</tbody>
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CBD, corticobasal degeneration; GFAP, glial fibrillary acidic protein; MSA, multiple system atrophy; NA, not available; PACRG, parkin coregulated gene; PD, Parkinson disease; PSP, progressive supranuclear palsy.

FIGURE 2. Pathologic changes in astrocytes. (A, B) Brightfield photomicrographs of similar typical stellate astrocytes with finely branching processes immunostained for glial fibrillary acidic protein (GFAP) in the putamen of a control (A) and a Parkinson disease (PD) case (B). (C–E) Immunofluorescent images of a typical stellate GFAP-immunoreactive (red Alexa 568) (C, E) astrocyte containing α-synuclein (green Alexa 488) (D, E) in the pons of a PD case. (F) Brightfield photomicrograph of an enlarged and distorted GFAP-immunoreactive astrocyte in a white matter bundle of a multiple system atrophy (MSA) case. (G) Brightfield photomicrograph of an enlarged reactive GFAP-immunoreactive astrocyte in the putamen of a progressive supranuclear palsy (PSP) case. (H) Immunofluorescent merged image of a GFAP-immunopositive (red Alexa 568) reactive astrocyte containing phospho-tau (green Alexa 488) in the putamen of a PSP case. (I) Immunofluorescent merged image of a phospho-tau immunopositive (green Alexa 488), but GFAP-immunonegative (red Alexa 568) tufted astrocyte in the putamen of a PSP case. (J) A brightfield photomicrograph of an enlarged reactive GFAP-positive astrocytes in the putamen of a corticobasal degeneration (CBD) case. Brightfield photomicrographs are counterstained with cresyl violet.
from controls in PD or MSA. This suggests that the non-PACRG fibrous astrocytes may be selectively affected in MSA. In PD cases, many ApoD/PACRG-immunoreactive protoplasmic astrocytes also showed α-synuclein immunoreactivity (Fig. 3F) but did not show increased GFAP (Table) and were not associated with significant morphologic changes, whereas in PSP and CBD, most ApoD-/PACRG-immunoreactive protoplasmic astrocytes had enlarged reactive morphologies (Fig. 3G–I). Tau-immunoreactivity was found in approximately 25% of ApoD-/PACRG-immunoreactive and in approximately 20% of PACRG-/parkin-immunoreactive astrocytes only in PSP (Fig. 3J–L). These astrocytes did not have the typical morphologic appearance of tufted astrocytes. Tau-immunoreactive tufted astrocytes were also not GFAP-immunoreactive (Fig. 2I), suggesting they may not be astrocytic. Other glial cells with similar morphology are the NG2 cells, which do not express GFAP but express NG2 chondroitin sulfate proteoglycan and accumulate rapidly in glial scars after injury (30, 31). The tau-immunoreactive tufted astrocytes were also not NG2-immunoreactive (Fig. 3M). Protoplasmic astrocytes were abnormal in PD, PSP, and CBD but not MSA. They were reactive in PSP and CBD but not PD, and accumulated abnormal proteins in PD and PSP but not CBD.

**Astrocytic PACRG and Parkin**

There were no changes in the densities or morphology of PACRG-immunoreactive astrocytes in the putamen, SN, or pons in PD cases compared with controls (Fig. 4A), and there was no parkin immunoreactivity (Table). In MSA, there was only a mild increase in the density of PACRG-immunoreactive astrocytes in the putamen, no change from controls in the SN or pons, and no parkin immunoreactivity (Table). In PSP and CBD, the SN and putamen showed greater astrocytic PACRG immunoreactivity than in the pons (Table), with morphologic enlargement similar to that observed for GFAP. Enlarged reactive parkin-immunoreactive astrocytes were also observed in PSP and CBD (Fig. 4B). Double labeling experiments showed that approximately 70% of GFAP-immunoreactive astrocytes in PSP and CBD also showed parkin immunoreactivity (Fig. 4C–E), whereas approximately 80% of GFAP-immunoreactive astrocytes also showed PACRG immunoreactivity (Fig. 4F–H). Approximately 90% of PACRG-immunoreactive astrocytes also showed parkin immunoreactivity (Fig. 4I, J). As shown in the Table, the putamen and SN were regions with consistent astrogliosis in PSP and CBD. There was no significant difference observed in the SN, but significantly increased densities of parkin-immunoreactive astrocytes was found in the putamen of PSP compared with CBD (×2; U = 16; p = 0.03; Table). Overall, reactive PACRG-/parkin-immunoreactive astrocytes were observed only in PSP and CBD.

In PSP and CBD, the severity of PACRG-/parkin-immunoreactive astrocytes in the putamen positively correlated with the PACRG-/parkin-immunoreactive astrocytes in the pons (Rho = 0.55; p < 0.03) and neuronal loss in the SN (Rho > 0.56; p < 0.04) and in CBD with increasing CBD stage (Rho = 0.97; p = 0.007). The severity of PACRG-/parkin-immunoreactive astrocytes in the SN correlated with increasing neurofibrillary tangles in PSP pons (Rho = 0.72; p = 0.006) and overall tau severity in CBD SN (Rho = 0.92; p = 0.03). This astrocytic change directly correlated with indices of neurodegeneration in PSP and CBD.

**DISCUSSION**

Our data show significantly different astrocytic reactions among PD, MSA, PSP, and CBD cases. Different types of astrocytes are selectively involved and their reactions are dependent on the proteins they express and accumulate. In particular, α-synuclein accumulation in PACRG-expressing protoplasmic astrocytes does not seem to be related to neurodegeneration and seems to diminish the reactive capacity of astrocytes in PD. In contrast, astrocytic reactivity is directly related to neurodegeneration in PSP and CBD cases in which the protoplasmic astrocytes express parkin in addition to constitutively expressing PACRG. In MSA, neurodegeneration seems to be related to reactivity of fibrous astrocytes that do not express any of these proteins.

Protoplasmic astrocytes are the dominant glial cell type in CNS gray matter, whereas fibrous astrocytes are mainly found in white matter (9, 32). In addition to PACRG, ApoD (10) and α-serine (α ligand for synaptic NMDA receptors) (33) are expressed in protoplasmic astrocytes. Of these ligands, only PACRG seems to be restricted to protoplasmic astrocytes in human brain tissue, and it was not found in other cell types in the regions sampled. Because PACRG is a tubulin-binding structural component of microtubules (34, 35), it may be an important component of the cytoskeleton of protoplasmic but not fibrous astrocytes; its expression seems to increase along with morphologic reactive changes in these astrocytes.
Astrocytic parkin and parkin coregulated gene (PACRG) expression. J Neuropathol Exp Neurol
degeneration. Experimental studies using parkin knockouts
tau association with neuronal loss (42); in PSP, enlarged phospho-
syndromes that have cortical astrocytic degeneration in assoc-
iation is part of the spectrum of frontotemporal degeneration
phospho-tau in PSP but not in CBD. The tau isoform that
no NG2-positive cells (a separate type of glial cell) contained
would increase microtubule stability as the cell enlarges.

A minority of subcortical protoplasmic astrocytes and
no NG2-positive cells (a separate type of glial cell) contained
phospho-tau in PSP but not in CBD. The tau isoform that
deposits in astrocytes is 4-repeat tau with both PSP and CBD
sharing a common tau haplotype (41). Corticobasal degener-
ation is part of the spectrum of frontotemporal degeneration
syndromes that have cortical astrocytic degeneration in assoc-
iation with neuronal loss (42); in PSP, enlarged phospho-
tau–depositing tufted astrocytes dominate regions undergoing
degeneration. Experimental studies using parkin knockouts and
mixed parkin/tau vectors suggest that parkin expression is
a normal protective response that reduces tau levels and
phosphorylation (43–46). The different end result of such
increased astrocytic reactivity in CBD versus PSP points to
potential differences in these dynamic intracellular processes.
Of interest is the recent finding that there is a genetic
association for PSP with the valine-380-leucine polymor-
phism in the C-terminus region of the parkin gene (47), while
the pathology of CBD has many similarities to that observed
in parkin-null mice expressing 4-repeat tau (44–46, 48).
Certain wild-type parkin isoforms are prone to misfolding
under severe oxidative stress, and most types of misfolded
parkin are rapidly degraded (49).

Parkin folding is dependent on the C-terminus structure
(49), and we speculate that the parkin isoform in PSP
protoplasmic astrocytes may be more stable compared with
that found in CBD, although there are no functional data
to support this at this time. This would lead to the eventual
incorporation of misfolded phospho-tau into the cytoskeletal
structures of protoplasmic astrocytes in PSP in contrast to
their cytoskeletal instability under similar end-stage condi-
tions in CBD. Functional studies on these different parkin
isoforms in appropriate cell systems are needed.

In PSP, only a small proportion of protoplasmic ast-
rocytes accumulated phospho-tau, and these exhibited less
abnormal morphology than was observed in tufted astrocytes.
Although tufted astrocytes have been previously described
as protoplasmic based on their morphology and distribution
(50), they have also been shown to be GFAP-negative (28).
We found that they are also NG2-negative. All protoplasmic
astrocytes are GFAP-immunoreactive (9, 32), whereas NG2
glia do not express GFAP and have large, stellate morphol-
gegies (31). Based on this morphology, NG2 glia were
previously suggested to accumulate phospho-tau and form
tufted astrocytes (51). Because no NG2 glia colocalized with
tau but a proportion of protoplasmic astrocytes did colocalize
phospho-tau, our data support the idea that in PSP proto-
plasmic astrocytes further metamorphose to incorporate
phospho-tau rather than other elements into their enlarged
cytoskeleton. The recent identification of significant oxida-
tive damage leading to GFAP fragmentation in PSP (52) may
explain the loss of this cytoskeletal element. Our data support
the concept that this is a late event (52), and that it is
relatively independent of reactive astrogliosis (7), with the
loss of normal proteins suggesting significant dysfunction of
these protoplasmic astrocytes consistent with their associa-
tion with increasing neuronal dysfunction (7, 36).

The distribution of α-synuclein–immunoreactive astro-
ocytes parallels the spread of intraneuronal pathology in
PD (4, 5, 53). In the present study, however, this astrocytic
response was not associated with astrocytic reactivity, it
was not directly associated with the severity of neuronal
inclusion formation, and it was more widespread than the
PD neuronal abnormalities. As in PSP and CBD, the same
protoplasmic astrocytes were affected in PD, but their
responses differed. Protoplasmic astrocytes in PD do not
increase GFAP, PACRG, or parkin; rather, they accumulate
α-synuclein. Protoplasmic astrocytes normally express β-
synuclein (54), whereas α-synuclein expression occurs in
response to certain inflammatory cytokines (55). Increased
astrocytic α-synuclein enhances their susceptibility to oxida-
tive stress and induces apoptosis in cell culture (56–58). We
speculate that astrocyte apoptosis due to increased α-
synuclein might give the impression of a less reactive
astrocytic population in PD. In this regard, patients with
parkinsonism and mutations in the parkin gene do not
accumulate α-synuclein in neurons or glia (59–61) but have

**FIGURE 4.** Astrocytic parkin and parkin coregulated gene (PACRG) expression. (A) Brightfield micrograph of PACRG-immunoreactive protoplasmic astrocytes from a Parkinson disease (PD) case. The astrocyte morphology is similar to those in control cases. (B) Brightfield micrograph of enlarged reactive parkin-immunopositive protoplasmic astrocytes from a progressive supranuclear palsy (PSP) case. (C–E) Immunofluorescent photomicrographs of parkin immunoreactivity (red Alexa 568) (C, E) colocalizing with glial fibrillary acidic protein (GFAP) immunoreactivity (green Alexa 488) (D, E) in an astrocyte in a PSP case. (F–H) Immunofluorescent photomicrographs of PACRG immunoreactivity (red Alexa 568) (F, H) colocalizing with GFAP immunoreactivity (green Alexa 488) (G, H) in an astrocyte from a PSP case. (I, J) Double labeled photomicrographs of an enlarged protoplasmic astrocyte from a PSP case that shows colocalization of PACRG (brightfield immunoperoxidase) (I) and parkin (red Alexa 568) (J) immunoreactivity.

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a substantial microglial response associated with their neurodegeneration (61), a feature also found in parkin-null mice (48). In parkin-null mice, this is associated with early astrocytic degeneration (48).

The protoplasmic astrocytes in MSA cases did not seem to be affected despite considerable neurodegeneration; there was, however, marked reactivity of fibrous GFAP-positive, PACRG-negative astrocytes. This difference between the astrocyte response in gray versus white matter has recently been highlighted (62). The astrocytic reactivity in MSA correlates with the degree of α-synuclein–immunoreactive GCI, suggesting that oligodendroglial pathology and degeneration drive this process. Such a relationship may have been expected considering the association between fibrous astrocytes and myelinated fibers. What is more remarkable, perhaps, is the lack of reactivity of the protoplasmic astrocytes in regions undergoing neuronal cell death in MSA. Apart from an absence of α-synuclein, the response of protoplasmic astrocytes is similar to that observed in PD and may suggest their targeted degeneration by alternative means.

In summary, this is the first study to identify PACRG as a constituent protein in protoplasmic astrocytes; it is the first to show that different types of astrocytes are affected in different parkinsonian disorders; and it is the first to associate protoplasmic astrocytic parkin expression with reactivity and neurodegeneration in PSP and CBD. Although protoplasmic astrocytes were not spared in PD, a completely different reaction was observed. The protoplasmic astrocytes in PD abnormally accumulated α-synuclein but did not become reactive. A lack of reaction in protoplasmic astrocytes to significant neurodegeneration was also a feature of MSA, although in MSA, the oligodendroglial GCI caused significant reactivity in associated fibrous astrocytes. This suggests that both PD and MSA have an attenuated protoplasmic astrocytic response. Because increased astrocytic levels of α-synuclein increase their susceptibility to oxidative stress and induce apoptosis, degeneration of protoplasmic astrocytes may occur in both these disorders, giving the appearance of an attenuated response. Overall, these studies reveal significantly different astrogial responses in PD, MSA, PSP, and CBD, supporting different underlying pathogenic cellular mechanisms.

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REFERENCES