Nigral and Cortical Lewy Bodies and Dystrophic Nigral Neurites in Parkinson's Disease and Cortical Lewy Body Disease Contain α-synuclein Immunoreactivity

MICHAEL C. IRIZARRY, MD, WHITFIELD GROWDON, TERESA GOMEZ-ISLA, MD, PhD, KATHY NEWELL, MD, JULIA M. GEORGE, PhD, DAVID F. CLAYTON, PhD, AND BRADLEY T. HYMAN, MD, PhD

Abstract. A mutation in the α-synuclein gene has recently been linked to some cases of familial Parkinson's disease (PD). We characterized the expression of this presynaptic protein in the midbrain, striatum, and temporal cortex of control, PD, and dementia with Lewy bodies (DLB) brain. Control brain showed punctate pericellular immunostaining. PD brain demonstrated α-synuclein immunoreactivity in nigral Lewy bodies, pale bodies and abnormal neurites. Rare neuronal soma in PD brain were immunoreactive for α-synuclein. DLB cases demonstrated these findings as well as α-synuclein immunoreactivity in cortical Lewy bodies and CA2-3 neurites. These results suggest that, even in sporadic cases, there is an early and direct role for α-synuclein in the pathogenesis of PD and the neurologically related disorder DLB.

Key Words: Lewy body; NACP; Neurite; Parkinson's disease; Substantia nigra; Synuclein; Synellin.

INTRODUCTION

Recently, Polymeropoulos et al described an Ala53Thr mutation in the α-synuclein gene associated with Parkinson's disease (PD) in four chromosome 4-linked families with autosomal dominant disease (1). Sporadic PD cases examined by these authors and other groups did not show linkage to this gene (2, 3). The question remains, however, whether the α-synuclein protein is involved in the pathophysiology of PD.

α-Synuclein is identical to NACP, the precursor protein of the non-αβ component of senile plaques (4, 5), and its homologue in songbirds has been termed synellin (6). The synuclein gene family includes β-synuclein, whose homologue is phosphoneuroprotein 14 kDa (PNP-14) in cows and rats (7), and γ-synuclein, which is identical to the breast cancer-specific gene-1 (BCSG1) (8). α-Synuclein is a 140 amino acid protein with 2 structural domains: an acidic C-terminal domain and an N-terminal domain organized around 7 copies of an 11 residue motif with a secondary structure predicted to be similar to the lipid-binding domain of exchangeable apolipoproteins (6). The mutation reported by Polymeropoulos et al replaces Ala53, which lies in a gap between the fourth and fifth 11 residue repeats. Interestingly, Thr is normally present at position 53 in rat and zebra finch α-synuclein, raising the concern that the polymorphism observed in familial PD is genetically linked to another disease-causing gene on chromosome 4, rather than being pathogenic. The finding of α-synuclein immunoreactivity in Lewy bodies and Lewy neurites in PD substantia nigra and cingulate cortex (9) suggests that α-synuclein itself, rather than an unrelated protein, is responsible for the genetic association of the mutant α-synuclein and Parkinson's disease.

Using the well-characterized monoclonal antibody H3C against the C-terminus of α-synuclein (5, 6), we further investigated the distribution of α-synuclein immunoreactivity and its association with ubiquitin, synaptophysin, and phosphorylated neurofilament immunoreactivity in the PD and dementia with Lewy bodies (DLB) midbrain, striatum, and temporal cortex by confocal and conventional microscopy.

MATERIALS AND METHODS

Materials

Postmortem adult human brain specimens were received from the Massachusetts Alzheimer's Disease Research Center and the Harvard Brain Tissue Resource Center. PD was established by standard clinicopathologic criteria, consisting of an extrapyramidal syndrome with normal striatum, neuronal loss in the substantia nigra, and Lewy bodies in the pigmented nuclei of the brainstem, substantia innominata, and hypothalamus, without Lewy bodies in the neocortex (10, 11). DLB brains met consensus neuropathologic diagnostic criteria and contained both mesencephalic and cortical Lewy bodies (12). Tissue was fixed in paraformaldehyde lysine metaperiodate for 24–48 hours before sectioning. Blocks from the midbrain containing substantia nigra, the temporal cortex including hippocampus, and the striatum from 5 cases of Parkinson's disease (age 72–87 years), 5 cases of dementia with Lewy bodies (age 63–87), and 6 control cases (age 72–86) were examined.

Immunohistochemistry

50 μm coronal temporal lobe, coronal striatal, and transverse midbrain sections were sequentially probed with primary antibody (1:5,000 H3C, mouse anti-α-synuclein [5, 6]; 1:300 rabbit anti-ubiquitin, Dako, Denmark; 1:1,000 mouse anti-phosphorylated...
Fig. 1. Confocal microscope images of immunohistochemistry in PD/DLB (A–H) and control (I) brain for α-synuclein (red) and ubiquitin (green), with double immunostaining in yellow. Abnormal α-synuclein immunoreactivity is present in ubiquitin-positive nigral (A; C, arrow) and cortical (B) Lewy bodies; in ubiquitin-negative pale bodies (C, arrowhead); in abnormal nigral neurites that are variably immunoreactive for ubiquitin (D), and in larger neuritic inclusions that are variably immunoreactive for ubiquitin (E, F). Rare neurons in the substantia nigra in PD contain α-synuclein immunoreactivity in the soma and proximal neurites (G, H). These are in contrast to the normal pattern of diffuse punctate pericellular α-synuclein immunoreactivity of the neuropil (I, substantia nigra). Scale bar = 25 μm.

RESULTS

Double immunofluorescent confocal microscopy on 5 PD, 5 DLB, and 6 control brains demonstrates that both cortical and nigral Lewy bodies identified by ubiquitin immunoreactivity robustly immunostain for α-synuclein (Fig. 1A–C). The area of α-synuclein immunostaining is greater than that of ubiquitin immunostaining, suggesting that only the center of the lesion is ubiquitinated.

α-Synuclein immunostaining reveals additional novel features of Parkinson’s disease neuropathology. Amorphous intracellular structures consistent with pale bodies, which may be precursors to Lewy bodies in PD (13), are...
α-synuclein immunoreactive (Figs. 1C, 2A, B). α-Synuclein immunoreactivity is also present in a previously underappreciated feature of PD pathology: enlarged neuronal processes (Figs. 1D, 2C) containing ovoid, fusiform, or club-shaped inclusions that are variably ubiquitin immunoreactive (Fig. 1E, F). These prominent abnormal neurites were found throughout the substantia nigra pars compacta in all PD and DLB cases studied, as well as within the Edinger-Westphal nucleus and nucleus basalis of Meynert. Ubiquitinated neurites in hippocampal subfields CA2–3 in the cases with cortical Lewy bodies also stained for α-synuclein. Within the PD nigra, rare neurons display punctate α-synuclein immunoreactivity in the soma and proximal neurites (Fig. 1G, H). The substantia nigra, cortex, and striatum in control cases demonstrated punctate pericellular α-synuclein immunoreactivity, without staining of discrete neurons or neurites (Figs. 1I, 2D). The pattern of α-synuclein immunoreactivity in the caudate, putamen, and globus pallidus in PD cases did not differ from control brain. Antibodies against synaptophysin and phosphorylated neurofilaments did not appreciably stain Lewy bodies or selectively immunostain the abnormal Lewy neurites.

**DISCUSSION**

PD pathology in the substantia nigra is characterized by dopaminergic neuronal loss and Lewy bodies, which are intraneuronal eosinophilic, ubiquitin immunoreactive inclusions that are also within cortical neurons in DLB. Lewy bodies in PD may also be identified in the dorsal motor nucleus of the vagus, the hypothalamus, the nucleus basalis of Meynert, locus ceruleus, Edinger-Westphal nucleus, raphé nuclei, cerebral cortex, olfactory bulb, and autonomic ganglia (14). In addition to ubiquitin, Lewy bodies are variably immunoreactive for neurofilament proteins (15, 16) and synaptic proteins (17). Spillantini et al demonstrated α-synuclein immunoreactivity in Lewy bodies and Lewy neurites in the substantia nigra and cingulate cortex (9). Our studies extend these results with a distinct c-terminal antibody to α-synuclein in additional cortical and basal ganglia regions, correlated with other synaptic and neuritic markers. Using double immunostaining and confocal microscopy to characterize in detail α-synuclein immunoreactivity in PD and DLB brain, we find that the pattern of abnormal α-synuclein immunoreactivity in these disorders is distinct from that of other synaptic or axonal proteins, and that α-synuclein immunostaining is more sensitive to pathological changes in PD than ubiquitin immunostaining.
Our results demonstrate that in PD and DLB brain there is a pathological accumulation of α-synuclein immunoreactivity in Lewy bodies, pale bodies, abnormal neuritic structures, and rare neuronal soma, whereas in normal brain α-synuclein immunoreactivity is localized only to synaptic structures (5, 18). Ubiquitination appears to be a subsequent modification of α-synuclein-containing Lewy bodies, given the greater immunoreactivity of Lewy bodies and neurites for α-synuclein than ubiquitin, and the presence of α-synuclein-positive, ubiquitin-negative neurites and pale bodies. The neuritic changes occur in the same subcortical brain regions as Lewy bodies (e.g., Edinger-Westphal nucleus, nucleus basalis of Meynert, substantia nigra) and spare the terminal fields of the nigral dopaminergic neurons in the striatum.

α-synuclein is a highly conserved pre-synaptic protein, a fragment of which (termed the non-ĄB component, NAC) is found in senile plaques of Alzheimer disease (19). The protein is highly expressed in cortical and subcortical structures (18). α-synuclein is found in the cytosolic fraction of brain homogenates, and is loosely associated with synaptic membrane structures (5). The expression of the full-length protein (also known as the NAC precursor, NACP) is regulated during development (20) and learning (6), and the protein is a substrate for phosphorylation (7). In familial PD, the Ala53Thr mutation presumably alters α-synuclein metabolism or subcellular distribution to predispose to Lewy body formation and neuritic changes, and perhaps disrupts the normal functions of α-synuclein. The natural occurrence of Thr53 in rodents and birds could indicate that additional differences in their primary sequences (compared with the human sequence) compensate for the otherwise detrimental effects of Thr53, or that the Thr53 effect in PD is a result of differential interactions with a specific human α-synuclein–binding protein. Together with the genetic data, the intimate relationship between α-synuclein and neuropathologic features of PD favor a pathogenic role for α-synuclein, even in sporadic PD and the neuropathologically related disorder DLB. The accumulation of α-synuclein protein in multiple abnormal neuronal structures—within both the soma and the neurites—in substantia nigra appears to be a striking early alteration in PD.

While this paper was in press, an Ala30Pro α-synuclein mutation was found to be associated with PD in another family (21), further favoring a pathogenic role for the protein.

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