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Short communication

Plaque-associated α-synuclein (NACP) pathology in aged transgenic mice expressing amyloid precursor protein

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Abstract

Patients with the Lewy body variant (LBV) of Alzheimer's disease (AD) have ubiquitinated intraneuronal and neuritic accumulations of α-synuclein and show less neuron loss and tau pathology than other AD patients. Aged Tg2576 transgenic mice overexpressing human βAPP695. KM670/671NL have limited neuron loss and tau pathology, but frequent ubiquitin- and α-synuclein-positive, tau-negative neurites resembling those seen in the LBV of AD.

Keywords: α-Synuclein; Amyloid precursor; Transgenic; Alzheimer's; Parkinson's; Lewy

α-Synuclein is genetically implicated in Parkinson's disease (PD) and is a major filamentous component of eosinophilic, ubiquitin-positive Lewy bodies [2] and Lewy neurites found in PD, the Lewy body variant (LBV) of Alzheimer's disease (AD) and diffuse LB disease (DLB) [2,11,12]. α-Synuclein was cloned first as a synaptic protein and then as NACP, the precursor of a self-aggregating peptide, NAC (non-Aβ component), found in plaques and amyloid-enriched fractions from AD brain [17]. NACP/α-synuclein accumulates in select dystrophic neurites in AD [12] and is distinct from tau pathology, although Lewy bodies are occasionally found in neurons with tangles [11]. PD and AD share clinical and pathological features [14], raising the question of whether α-synuclein pathology also exists in APP transgenic mice.

Heterozygous outbred Tg2576 transgenic mice expressing high levels of human amyloid precursor protein (βAPP) with the ''Swedish'' KM670/671NL double mutation [8] show phospho-tau immunopositive dystrophic neurites, but lack fibrillar intracellular tau inclusions or major neuron loss [9]. Tg2576 transgene positive and negative (littermate) mice on a C57Bl6J/SJL background were aged to 12–18 months and perfused. Brains were fixed in 4% paraformaldehyde and coronal sections taken as previously described [5]. Immunocytochemistry was performed on adjacent 12 μm cryostat sections with monoclonal antibodies 10G4 (anti-Aβ) [5], synuclein-1 (anti-α-synuclein, Transduction Labs, Lexington, KY), AT8 (anti-phosphoserine 202 tau, Innogenetiks, Ghent, Belgium), and rabbit polyclonal antibodies to C-terminal (PQE3), N-terminal (MDV2) and NAC (EQV1) domains of NACP [1] or anti-ubiquitin (DAKO, Carpinteria, CA), followed by peroxidase/DAB or alkaline phosphatase/Vector blue with Vectastain Elite ABC kits (Vector Labs, Burlingame, CA). Additional 0.2–0.5 μm pseudoconfocal (Scanalytics) optical sections were made of immunofluorescent triple-labeling with ubiquitin, α-synuclein and biotinylated Aβ primary antibodies and anti-rabbit fluoroscein, anti-mouse rhodamine and avidin-AMCA blue (Molecular Probes, Eugene, OR).

Global α-synuclein staining was similar in transgene positive (Fig. 1A) and negative mice with punctate neuropil labeling resembling that of synaptophysin. In transgene positive mice, enlarged and increased punctate staining surrounded the perimeter of plaques. Neuropil and plaque staining was not seen on adjacent sections after preabsorption with 20 μg/ml of peptide antigen (Fig. 1B)

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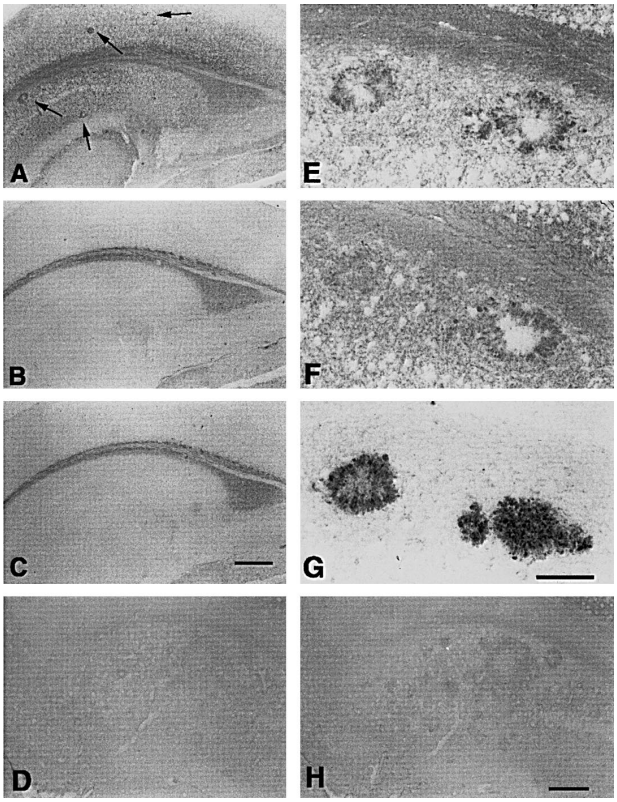

Fig. 1. (A) Neuropil and plaque (arrows) PQE3 α-synuclein staining. (B) Adjacent, PQE3 antiserum pre-absorbed with free peptide antigen. (C) Adjacent, pre-immune serum for PQE3 antiserum. (A–C) bar = 500 μm. Higher magnification, E–G, in three adjacent sections, staining with PQE3, Synuclein-1 and 10G4 (anti-Aβ), respectively. Bar = 100 μm. (D, H) Hippocampus, PQE3. (D) No pretreatment. (H) 70% formic acid, 5 min. Bar = 200 μM.

or after preimmune serum (Fig. 1C). At higher magnification, similar neuritic staining occurred on adjacent sections with PQE3 (Fig. 1E) and synuclein-1 (Fig. 1F), surrounding Aβ deposits (Fig. 1G). N-terminal α-synuclein antibodies (MDV2) produced weaker staining (not shown), as is the case with filamentous inclusions and isolated filaments from patients with Lewy body disease [1]. Formic acid pretreatment enhanced α-synuclein labeling around plaques more than in the neuropil (Fig. 1H), suggesting that these epitopes are hidden as they are in α-synuclein aggregates in Lewy body diseases [15]. Anti-NAC antibodies (EQV1) failed to label plaques without pretreatment, but after formic acid, labeled plaque neurites, not amyloid, in a pattern resembling that seen with anti-NACP antibodies (not shown).

Double α-synuclein/Aβ staining confirmed prominent α-synuclein plaque neurites after formic acid (Fig. 2A) and proteinase K (not shown) pretreatments. Very similar plaque neurite staining was seen on adjacent sections with ubiquitin (Fig. 2C). Pseudoconfocal reconstructions of triple immunofluorescence in 0.5 μm planes showed a large subset of α-synuclein-positive plaque neurites (red) were also ubiquitin labeled (green) and appeared (yellow) in β-amyloid (blue) plaques (2B), similar to Lewy neurite pathology in AD [12].

Although Lewy bodies are intraneuronal and filamentous like neurofibrillary tangles, they can be distinguished from the latter at both the light and ultrastructural levels [1]. Lewy neurites rarely overlap with neurites containing tau pathology [15]. In Tg2576, double labeling revealed numerous independent phospho-tau and α-synuclein labeled neurites as well as neurites with variable overlap

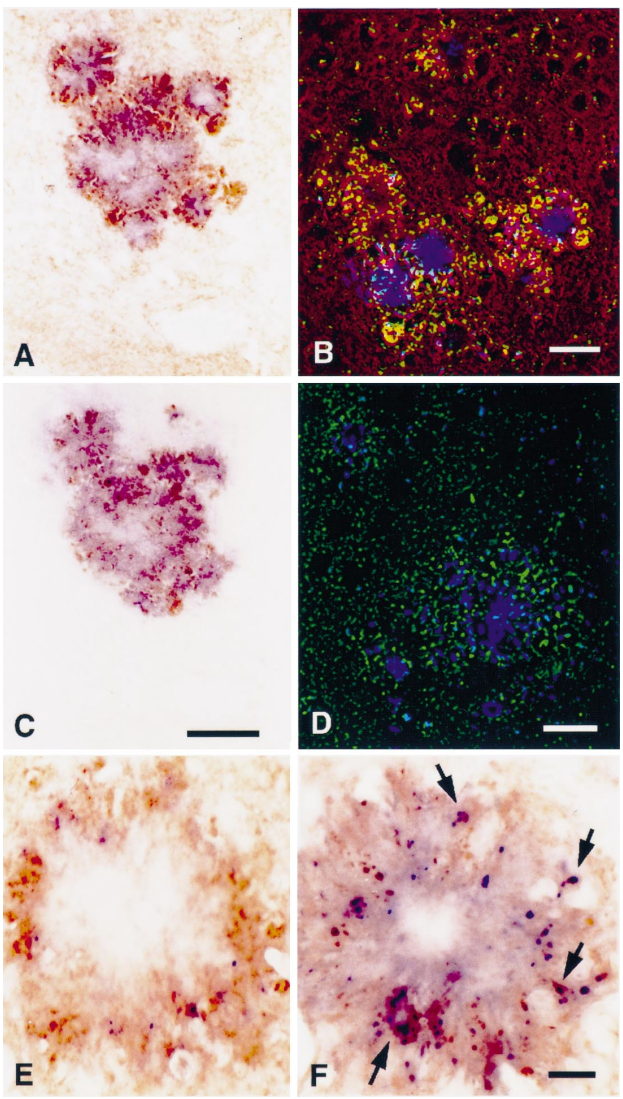

Fig. 2. Neuritic plaques in aged Tg2576(+). (A) PQE3 (DAB, brown) in enlarged, dystrophic neurites around an amyloid core in temporal cortex (10G4, blue). (B) Triple labeling of plaque in entorhinal cortex as in D with ubiquitin (green), α-synuclein (Synuclein-1, red) and anti-Aβ (blue). (A,C,D) Bar = 50 μm. Yellow indicates red and green overlap. (C) Plaque neurite labeling with anti-ubiquitin (brown) of the same plaque seen in panel A on an adjacent section, double-labeled with anti-Aβ (blue). (A–C) Bar = 50 μm. (D) Confocal double-labeling with PQE3-(FITC green) and biotinylated 10G4 anti-Aβ (avidin Oregon blue). (D) Bar = 80 μm. E, F show PQE3 labeled α-synuclein (DAB, brown) and AT8-labeled phospho-tau (blue) of the same plaque in adjacent sections with E and without F formic acid pretreatment. Arrows indicate points of overlap. Bar = 10 μm.

(Fig. 2F). Unlike α-synuclein, phosphoserine 202 tau labeling was reduced following formic acid pretreatment, especially in the hippocampus (Fig. 2E). No significant β-synuclein pathology was observed in aged Tg2576 transgenic mice (not shown).

Like the NAC peptide, the precursor α-synuclein can aggregate to form fibrils in vitro [6]. Intense α-synuclein labeling of plaque neurites could be due to α-synuclein aggregation caused by Aβ peptides [13] or oxidative damage [7]. That some α-synuclein neurites were ubiquitin-negative suggests α-synuclein accumulation may precede ubiquitin accumulation. A mutation in the proteasomal ubiquitin carboxy-terminal hydrolase L1 gene has been linked to PD [10]. Because α-synuclein is normally degraded by ubiquitin-dependent proteasomes, age- or plaque-related damage to the proteasome system could play a role in α-synuclein accumulation, even in the absence of aggregation.

In classical AD, tau is the major intracellular aggregating protein. Our results show that in Tg2576 transgenic mice, α-synuclein is a major intracellular accumulating protein. Since there is more α-synuclein than tau pathology in neurites in aged Tg2576 mice, these βAPP transgenic mice appear to bear a closer histopathological resemblance to the LBV of AD than to classical AD. The LBV of AD has limited tangles, but plaque pathology comparable to AD [3]. However, extensive tau-negative, neuritic α-synuclein pathology occurs in the LBV of AD [16], where synapse loss is comparable to that in AD, but Braak stages are lower and tangle number is insufficient to account for dementia [3]. The neuritic α-synuclein pathology observed in Tg2576 mice may contribute to synaptic degeneration in the absence of extensive fibrillar somatodendritic tau pathology and neuron loss [9]. Alternatively, α-synuclein accumulation could reflect a more fundamental problem related to synaptic dysfunction, independent of synaptic degeneration [4].

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