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### Authors

Troncoso, Juan C

Cataldo, Anne M

Nixon, Rallph A

et al.

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## Neuropathology of Preclinical and Clinical Late-Onset Alzheimer's Disease

Juan C. Troncoso, MD,\*†‡§ Anne M. Cataldo, PhD,||#  
 Ralph A. Nixon, MD, PhD,||# Jody L. Barnett,||  
 Michael K. Lee, PhD,†‡ Frederic Checler, PhD,††  
 David R. Fowler, MD,\*\* John E. Smialek, MD,\*\*  
 Barbara Crain, MD, PhD,\*†‡ Lee J. Martin, PhD,\*†‡¶  
 and Claudia H. Kawas, MD\*§

**We report on the neuropathological examinations of a 74-year-old woman with Alzheimer's disease (AD) and of her 47-year-old nondemented daughter. The brain of the mother showed fully developed pathological changes of AD. By contrast, the brain of the daughter revealed only perineuronal deposition of diffuse amyloid in cerebral cortex and striking abnormalities of the endosomal-lysosomal system, without neurofibrillary, glial, or microglial changes. These observations suggest that amyloid deposition and endosomal-lysosomal changes are early events in late-onset AD and that they may precede the onset of dementia by several decades.**

From the \*Alzheimer's Disease Research Center, †Neuropathology Laboratory, and Departments of ‡Pathology, §Neurology, and ¶Neurosciences, Johns Hopkins University School of Medicine, Baltimore, MD; ||Laboratories for Molecular Neuroscience, McLean Hospital, and Departments of #Psychiatry and Neuropathology, Harvard Medical School, Belmont, MA; \*\*Office of the Chief Medical Examiner of the State of Maryland, Baltimore, MD; and ††Institut de Pharmacologie Moléculaire et Cellulaire du CNRS, Valbonne, France.

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Address correspondence to Dr Troncoso, Johns Hopkins University, Neuropathology Laboratory, 720 Rutland Ave/558 Ross Building, Baltimore, MD 21205-2196.

The major neuropathological lesions of Alzheimer's disease (AD) are diffuse  $\beta$ -amyloid (A $\beta$ ) deposits, neuritic senile plaques (SPs), neuropil threads, and neurofibrillary tangles (NFTs). Despite substantial progress in understanding the pathobiology of AD, the evolution of these lesions and their relevance to the pathogenesis of the disease remain unclear. For example, it is not known whether A $\beta$  deposits and NFTs appear simultaneously in AD; and if they do not, which one develops first. Moreover, the relationship of these changes to dementia and to the recently reported abnormalities of the endosomal-lysosomal system in AD<sup>1–3</sup> are also unresolved.

We report here the neuropathological examinations of a patient with late-onset AD (LOAD) and of her nondemented daughter, including assessment of the endosomal-lysosomal system. These studies provide insight into the progression and clinical correlations of neuropathological lesions in LOAD.

### Subjects and Clinical Examinations

Subject 1 was a 74-year-old woman with a 5-year history of progressive cognitive decline and family history of dementia. On examination 9 months before death, she had impairments in several cognitive domains and her Mini-Mental State Examination score was 14.<sup>4</sup> Her Clinical Dementia Rating (CDR) score was 1, consistent with mild dementia.<sup>5</sup> She had mild bradykinesia and slowing of gait. Computed tomography showed mild cerebral atrophy. Her ApoE genotype was 4/4. The patient received a clinical diagnosis of probable AD.<sup>6</sup>

Subject 2, the daughter of Subject 1, was a 47-year-old woman who was employed as a licensed practical nurse and provided live-in care for her mother. Although without formal testing, she appeared to be cognitively normal with no complaints about her memory or other cognitive abilities. Interviews with her family using the Dementia Questionnaire<sup>7</sup> verified her normal cognitive status. Her Apo-E genotype was 2/4.

### Neuropathology

Both subjects died of homicidal strangulation, and the postmortem interval was about 36 hours. Brain tissue sections were stained with hematoxylin-eosin and Hirano silver<sup>8</sup> and immunostained with three different A $\beta$  antibodies: A $\beta$  raised against peptide 1–28 and recognizing all forms of A $\beta$  (a gift from Athena Neuroscience) and FCA3542 and FCA3340, which recognize longer (x-42) and shorter (x-40) forms of A $\beta$ .<sup>9</sup> We also examined staining with antibodies for  $\tau$  (Sigma, St Louis), Alz-50<sup>10</sup> (a gift of Dr P. Davies), GFAP

(Dako, Carpinteria, CA), and HLA-DR for microglia (Dako). For assessment of the endosomal-lysosomal system, 40- $\mu\text{m}$  thick vibratome tissue sections from both subjects and 4 age-matched (47 and 50 years; 74 and 75 years) cognitively normal controls and 3 controls who died of strangulation (20, 58, and 67 years; 24- to 36-hour postmortem delay) were immunostained with polyclonal antibodies directed against rab 5, a Rab family-related guanosine triphosphate (GTP)-binding protein, to detect early endosomes<sup>1</sup> and against cathepsin D for detection of the major hydrolase-containing lysosomal compartments.<sup>2</sup> Sections were also stained by the Nissl method.

**SUBJECT 1.** The brain weighed 1,380 g and showed minimal hydrocephalus. With silver stain, widespread diffuse A $\beta$  deposits, frequent neuritic SPs (CERAD criteria),<sup>11</sup> and NFTs were noted in neocortex (Fig 1A), entorhinal cortex, and hippocampus. Cortical SPs were stained in comparable numbers by the A $\beta$  and FCA3542 antibodies, whereas only a small subset reacted with the FCA3340 antibody (see Fig 1B and C).  $\tau$  and Alz-50 antibodies recognized neuritic components in SPs, NFTs, and neuropil threads. Reactive astrocytes (GFAP+) and microglia (HLA-DR+) were abundant in the neuropil and were associated with SPs.

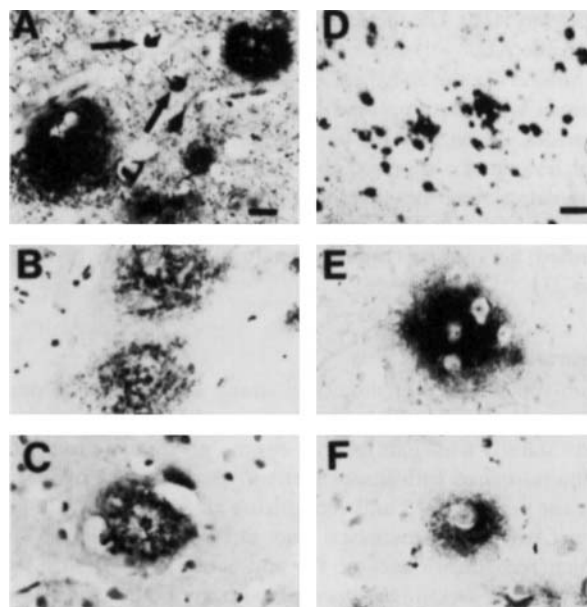
Examination of early endosomes revealed striking abnormalities. In age-matched controls, cortical pyramidal neurons

contained rab 5-positive early endosomes of uniform size distributed in the cell body predominantly close to the plasma membrane (Fig 2A). In Subject 1, pyramidal neurons in lamina III of the frontal cortex and most medium and large neurons in temporal cortex exhibited atypically large early endosomes (endosomal volume per neuron = 19.41  $\mu\text{m}^3$  vs control average = 5.61  $\mu\text{m}^3$ ) (see Fig 2B). These abnormally large profiles resembled those previously reported in AD.<sup>1</sup> Lysosomes, which contain cathepsin D, are typically 50 to 400  $\mu\text{m}$  in diameter and distributed uniformly throughout the cytosol (see Fig 2D). Most of the pyramidal neurons of Subject 1 showed a marked increase in cathepsin D-positive lysosomes (see Fig 2E), the major neuronal acidic vacuolar compartment.

**SUBJECT 2.** The brain weighed 1,350 g, and silver stain revealed diffuse A $\beta$  deposits, frequent in frontal lobes and moderate in parietal and temporal lobes, entorhinal cortex, and amygdala (see Fig 1D). These deposits stained with A $\beta$  and FCA3542 antibodies but not with the FCA3340 antibody (see Fig 1F). Most of these deposits surrounded morphologically normal neurons or groups of neurons (see Fig 1E) but were seldom noted around blood vessels and never in the subpial region. Despite the extensive A $\beta$  deposition, no significant astrocytic or microglial reaction was noted. Furthermore, neither silver stain nor  $\tau$  or Alz-50 antibodies recognized neuritic elements in SPs, neuropil threads, or NFTs. Hippocampus, entorhinal cortex, and the transtentorial region were free of NFTs or other  $\tau$ - or Alz-50-reactive lesions.

Although neurons in Subject 2 appeared normal in hematoxylin-eosin and Nissl stains, they showed large early endosomal profiles, similar to those observed in Subject 1 and in AD<sup>1</sup> (see Fig 2C). In most frontal and temporal pyramidal neurons, endosomes were clearly enlarged compared with those in neurons from control brains (endosomal volume per neuron = 19.35  $\mu\text{m}^3$  vs control average = 5.61  $\mu\text{m}^3$ ). Pyramidal neurons in these regions also exhibited a qualitative increase in cathepsin D immunoreactivity (see Fig 2F). No abnormality of endosomes or lysosomes was observed in the brains of controls who died of strangulation.

*Fig 1. A $\beta$  deposition, senile plaques, and neurofibrillary tangles in the early and advanced stages of late-onset AD. Tissue sections were taken from the middle temporal gyrus of Subject 1 (mother, A–C) and Subject 2 (daughter, D–F). Hirano silver stain identifies senile plaques and neurofibrillary tangles (arrows). Senile plaque (A) immunostained with FCA3542 (B) and with FCA3540 (C). Hirano stain of cerebral cortex showing many diffuse amyloid deposits in the neuropil (D) and surrounding neurons (E). Cortical neuron surrounded by diffuse FCA3542 immunoreactivity (F). Scale bar A (same for B, C, E, F) = 85  $\mu\text{m}$ ; scale bar D = 170  $\mu\text{m}$ .*



## Discussion

The lesions in these two subjects appear to represent the early and advanced neuropathological stages of LOAD. The neuropathological lesions readily confirmed the clinical diagnosis of AD in Subject 1<sup>11</sup> and suggest that Subject 2 may represent a very early, pre-clinical stage of LOAD.

Observations on Subject 2 suggest that the earliest pathological events in LOAD are perineuronal deposition of A $\beta$  x-42 and abnormalities of the neuronal endosomal-lysosomal system. It is remarkable that neurons in Subject 2 appeared morphologically normal, that A $\beta$  deposits were free of neuritic abnormalities, and that there was no microglial or astrocytic reaction to these early A $\beta$  deposits. This lack of reaction suggests that neurons and neuropil may tolerate A $\beta$  deposits for prolonged periods of time before the onset of

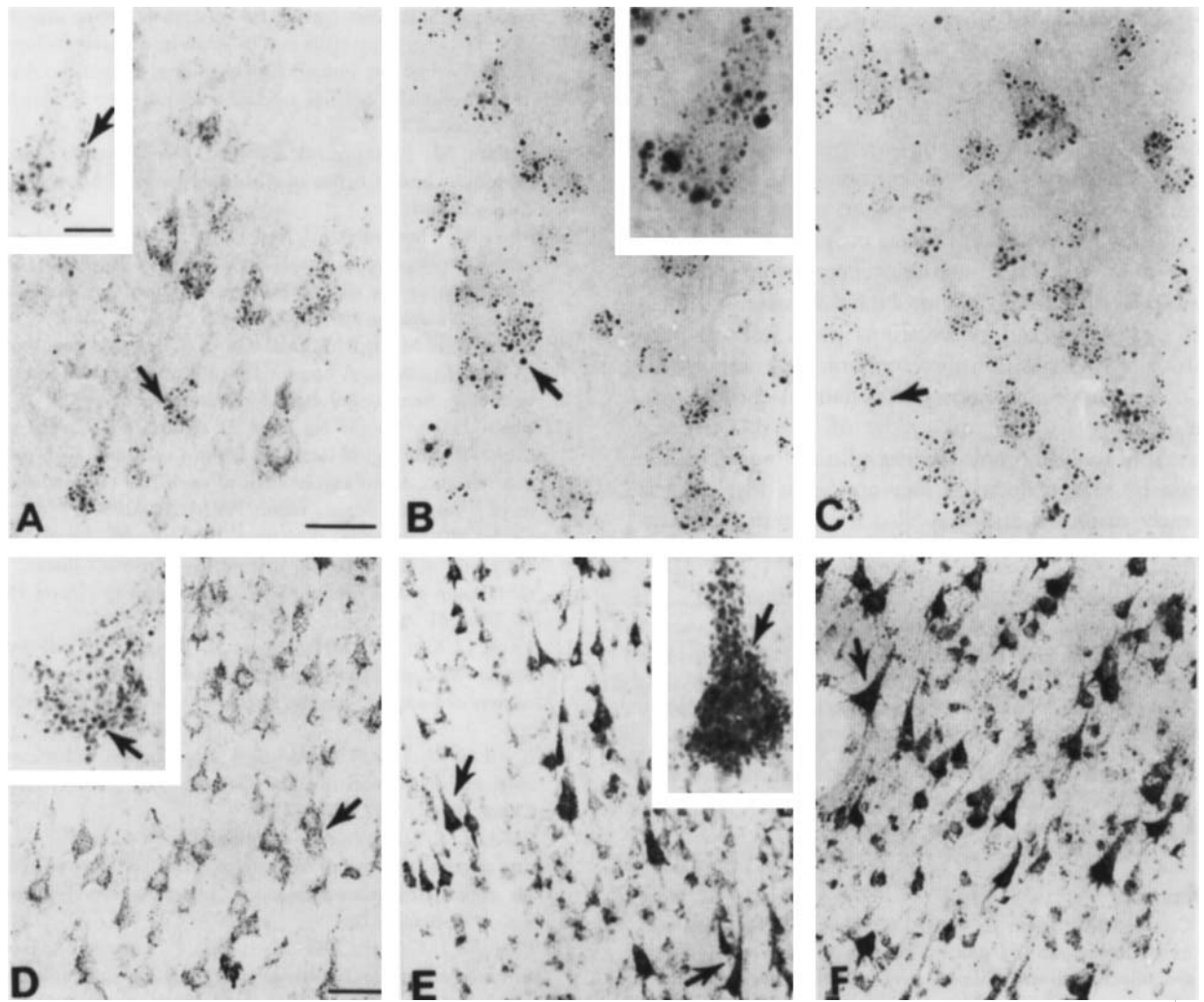


Fig 2. Alterations of endosomal-lysosomal compartments occur early in pyramidal neurons of vulnerable regions of late-onset AD brains. In frontal cortex from normal, control individuals (A and inset), anti-rab 5 antibodies immunolabeled a population of small vacuolar compartments (arrows) that were spherical and distributed close to the plasmalemma. In striking contrast, early endosomal compartments in cortical pyramids of Subject 1 (B and inset) and Subject 2 (C) were abnormally large (arrows). Antibody to cathepsin D showed an increase in the density and number of immunoreactive lysosomes (arrows), in pyramids of both Subject 1 (E and inset) and Subject 2 (F) compared with those of controls (D and inset). Scale bars for panels A–C = 5  $\mu\text{m}$ ; for A, B, D, E insets = 2  $\mu\text{m}$ ; for D–F = 10  $\mu\text{m}$ .

overt inflammatory or neurofibrillary changes. No NFTs were detected in Subject 2, nor was there any other evidence of  $\tau$ - or Alz-50-associated pathological changes, either within SPs or in the neuropil. Therefore, it appears that neuritic abnormalities, NFTs and  $\tau$ - and Alz-50-associated changes are not primary or early events in LOAD.

At more advanced stages of the disease, as represented by Subject 1, increasing numbers of SPs contain the shorter A $\beta$  x-40 peptide and reveal prominent astrocytic and microglial reactions and neuritic changes, as previously described elsewhere.<sup>12–14</sup> Diffuse amyloid deposits without concomitant astrocytic, microglial, or

neuritic changes have been described in Down's syndrome,<sup>13</sup> but no examination of neuronal endosomes has been reported in this syndrome.

Endosomal abnormalities identified in the two subjects, reflecting cellular activation of the endosomal-lysosomal system, are similar to those previously described in the same cell types in AD.<sup>1,2</sup> However, not only was the magnitude of these abnormalities larger than changes observed to date in AD,<sup>1</sup> but, more important, the abnormal endosomal-lysosomal system and A $\beta$  deposition were the only observed abnormalities found in Subject 2. Previous studies have implicated the endocytic<sup>15–17</sup> and secretory<sup>18,19</sup> pathways in the

normal processing of the amyloid precursor protein (APP) and A $\beta$  generation. Upregulation of endocytosis seen in neurons of AD brains<sup>1</sup> implies abnormal stimulation of normal endocytic uptake including processing of APP and the generation of A $\beta$ . Furthermore, acceleration of APP processing is expected if trafficking of appropriate proteases to endosomes is abnormally increased, which has been shown to occur in AD.<sup>1</sup>

Endosomal abnormalities have only been reported in AD,<sup>1,2</sup> not in other neurodegenerations, including Huntington's, Parkinson's, or Pick's diseases.<sup>1</sup>

In conclusion, our observations point toward perineuronal A $\beta$  deposition concomitant with abnormalities of the endosomal-lysosomal system in cortical neurons as early pathological events of LOAD. Because these abnormalities precede the clinical onset of the disease by several decades, they appear as ideal targets for early diagnosis and may also have therapeutic implications in AD.

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