

# Absence of Pittsburgh Compound B Detection of Cerebral Amyloid $\beta$ in a Patient With Clinical, Cognitive, and Cerebrospinal Fluid Markers of Alzheimer Disease

## A Case Report

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**Background:** To date, there have been no reports of individuals who have been characterized longitudinally using clinical and cognitive measures and who transitioned from cognitive normality to early symptomatic Alzheimer disease (AD) during a period when both cerebrospinal fluid (CSF) markers and Pittsburgh Compound B (PiB) amyloid imaging were obtained.

**Objective:** To determine the temporal relationships of clinical, cognitive, CSF, and PiB amyloid imaging markers of AD.

**Design:** Case report.

**Setting:** Alzheimer disease research center.

**Participant:** Longitudinally assessed 85-year-old man in a memory and aging study who was cognitively normal at his initial and next 3 annual assessments.

**Main Outcome Measures:** Serial clinical and psychometric assessments over 6 years in addition to PiB imaging with positron emission tomography (PET) and CSF biomarker assays before autopsy.

**Results:** Decline in measures of episodic memory and, to a lesser degree, working memory began at about age 88 years. PiB PET amyloid imaging was negative at age 88½ years, but at age 89½ years there was reduced amyloid  $\beta$  42 and elevated levels of tau in the CSF. Beginning at age 89 years, very mild cognitive and functional decline reported by his collateral source resulted in a diagnosis of very mild dementia of the Alzheimer type. After death at age 91 years, the autopsy revealed foci of frequent neocortical diffuse amyloid  $\beta$  plaques sufficient to fulfill Khachaturian neuropathologic criteria for definite AD, but other neuropathologic criteria for AD were not met because only sparse neuritic plaques and neurofibrillary tangles were present. Postmortem biochemical analysis of the cerebral tissue confirmed that PiB PET binding was below the level needed for in vivo detection.

**Conclusion:** Clinical, cognitive, and CSF markers consistent with AD may precede detection of cerebral amyloid  $\beta$  using amyloid imaging agents such as PiB that primarily label fibrillar amyloid  $\beta$  plaques.

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THE IDENTIFICATION OF SENSITIVE and specific biomarkers of Alzheimer disease (AD) may improve its early diagnosis and may help to evaluate the efficacy of potential therapeutic interventions.<sup>1-4</sup> In particular, cerebrospinal fluid (CSF) assays of amyloid  $\beta$  42 ( $A\beta_{42}$ ) and tau and amyloid imaging tracers such as Pittsburgh Compound B (PiB)<sup>5</sup> may identify the AD pathologic process in the brain regardless of clinical status (ie, whether cognitive impairment or dementia is present).<sup>6-12</sup> To our knowledge, there have been no reports of

individuals who have been characterized longitudinally using clinical and cognitive measures and who transitioned from cognitive normality to early symptomatic AD during a period when both CSF markers and PiB amyloid imaging were

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obtained. Herein, we report such a case with clinicopathologic evidence for AD to provisionally examine the sequence of biomarker and neuropathologic abnormalities in AD.

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## METHODS

### PARTICIPANT

The case reported herein comes from a sample of community-dwelling volunteers enrolled in a longitudinal study of healthy aging and AD conducted by the Washington University Alzheimer's Disease Research Center, St Louis, Missouri. Participants in this longitudinal study are 60 years or older and in good general health and have (other than AD) no neurologic, psychiatric, or systemic medical illness that could contribute substantially to dementia. They also have no medical contraindication to lumbar puncture or structural or functional neuroimaging. All procedures were approved by the university's human subjects committee, and written informed consent was obtained from the participants and their collateral sources. Data from this case have been included in other publications from our center, including the article in this issue of the *Archives* titled "Pittsburgh Compound B Imaging and Prediction of Progression From Cognitive Normality to Symptomatic Alzheimer Disease" by Morris et al.<sup>13</sup>

### ASSESSMENTS

At enrollment and annual follow-up, experienced clinicians determine the cognitive status of participants based solely on semi-structured interviews with the individual and their observant collateral sources (typically the spouse or adult child), followed by a neurologic examination of the participant. Impaired cognition is detected when there is decline from previously attained cognitive abilities that interferes to at least some degree with the individual's performance in everyday activities.<sup>14</sup> The clinical battery includes the administration of the Mini-Mental State Examination.<sup>15</sup> Based on all information, the clinician determines the Clinical Dementia Rating (CDR),<sup>16</sup> where 0 indicates no dementia and excludes even minimal cognitive impairment, and 0.5 indicates very mild dementia. In individuals with dementia, a diagnosis of AD is made in accord with standard definitions and criteria.<sup>17</sup>

The CDR and dementia diagnosis are completed without reference to psychometric performance, which is obtained approximately 2 weeks after the clinical assessment. The 1½-hour psychometric test battery is administered annually. The tests include the following 3 measures of episodic memory: Logical Memory and Associate Learning from the Wechsler Memory Scale<sup>18</sup> and the Free and Selective Reminding Test (sum of 3 free-recall trials).<sup>19</sup> There were 2 measures of semantic memory, namely, Information<sup>20</sup> and the Boston Naming Test.<sup>21</sup> Working memory measures included Mental Control and Digit Span (forward and backward)<sup>18</sup> and word fluency for /s/ and /p/.<sup>22</sup> Visuospatial ability was assessed using Block Design,<sup>20</sup> Digit Symbol,<sup>20</sup> and the Trailmaking Test A.<sup>23</sup> Scores can be converted to *z* scores using means (SDs) from the first time of assessment of a reference group of 310 individuals who were enrolled with a CDR of 0 and remained that way as long as they were followed up (for ≥2 assessments).<sup>24</sup> The *z* scores from each cognitive domain are then averaged to form composites. At enrollment, a blood sample is obtained for determination of apolipoprotein E (APOE) (OMIM 107741) genotype. The method is as previously described.<sup>25</sup>

### CSF COLLECTION, PROCESSING, AND ASSESSMENT

Cerebrospinal fluid (20-30 mL) is collected at 8 AM after overnight fasting. Samples are gently inverted to avoid possible gradient effects, briefly centrifuged at low speed, and aliquoted into

polypropylene tubes before freezing at -84°C. The samples are analyzed for total tau, phosphorylated tau<sub>181</sub>, and Aβ<sub>42</sub> by enzyme-linked immunosorbent assay (ELISA) (Innotest; Innogenetics, Ghent, Belgium) as previously described.<sup>7</sup>

### IN VIVO AMYLOID IMAGING

Positron emission tomography (PET) with PiB is obtained as previously described.<sup>10</sup> A positive PiB PET image, denoting the presence of cerebral Aβ deposits, is defined by a mean cortical binding potential for PiB of 0.2 or more (averaging the binding potentials in prefrontal cortex, precuneus, laterotemporal cortex, and gyrus rectus).<sup>10</sup>

### NEUROPATHOLOGIC ASSESSMENT

Formalin-fixed tissue samples from 15 standard cortical and subcortical regions were embedded in paraffin wax and sections cut at 7 μm as previously described.<sup>26</sup> Hematoxylin-eosin stain and a modified Bielschowsky silver impregnation were used on representative brain areas. Immunohistochemistry was performed using the following antibodies: anti-ubiquitin (1:1000, rabbit polyclonal antibody; DAKO, Glostrup, Denmark), anti-TDP-43 (1:4000, rabbit polyclonal antibody; ProteinTech Inc, Chicago, Illinois), anti-tau (PHF-1), anti-α-synuclein (1:500, mouse monoclonal antibody LB-509; Zymed, San Francisco, California), and anti-Aβ (1:100 000, mouse monoclonal antibody, 10D5). Additional immunohistochemical and histologic analyses used the 6E10 antibody targeting amino acids 1 through 16 (N-terminus) of Aβ (1:3000, mouse monoclonal antibody; Signet, Emeryville, California) and beta-sheet markers 6-CN-PiB and X-34 (the highly fluorescent derivatives of PiB<sup>27</sup> and Congo red,<sup>28</sup> respectively).

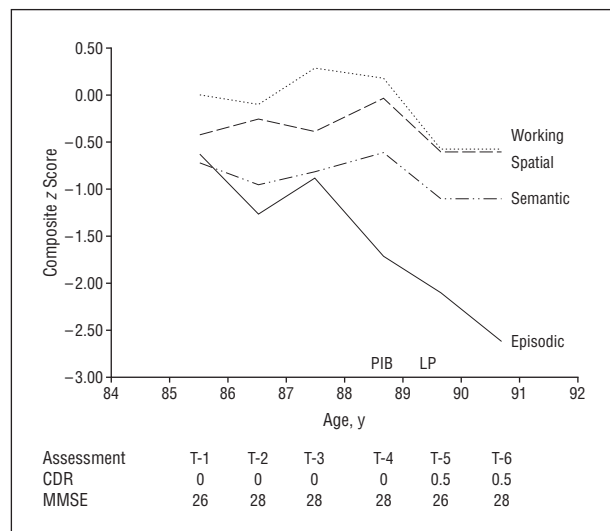
The neuropathologist (N.J.C.) was aware of the clinical diagnoses of autopsied participants. However, the clinical, psychometric, CSF, and PiB PET imaging assessments were completed by investigators (T.B., M.S., A.M.F., D.M.H., M.A.M., and J.C.M.) who were unaware of the results from the other assessments.

Biochemical analyses of frozen postmortem tissue were performed. These included quantitative assessment of radiolabeled tritium [<sup>3</sup>H] PiB binding to brain homogenates and ELISA of Aβ<sub>1-42</sub> using the antibodies and procedures described in detail by Ikonomic et al.<sup>27</sup>

## REPORT OF A CASE

An 85-year-old male civil servant with 12 years of education and without a family history of dementia had a CDR of 0 (cognitively normal) at study enrollment and at the next 3 annual assessments through age 88 years (**Figure 1**). At his fifth and sixth annual assessments (ages 89 and 90 years, respectively), his collateral source reported declining cognitive abilities with forgetfulness, poor decisional capacity, and mild interference with daily function (eg, impaired driving abilities causing a motor vehicle collision). The participant could not reliably recall recent events in which he had participated. The Mini-Mental State Examination score at age 90 years was 26 (normal range 24-30). He was diagnosed as having very mild (CDR of 0.5) dementia of the Alzheimer type (DAT). He died of congestive heart failure shortly after his 91st birthday. His APOE genotype was homozygous for ε3.

The longitudinal performance of this participant on composites of measures of episodic memory, semantic



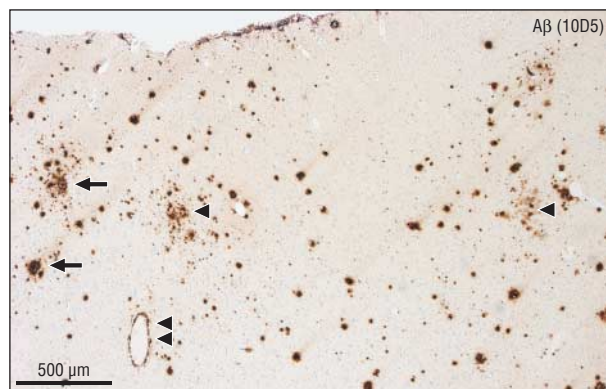
**Figure 1.** Clinical and cognitive course of the Pittsburgh Compound B (PiB) positron emission tomography amyloid-negative case reported herein. CDR, Clinical Dementia Rating; LP, lumbar puncture; MMSE, Mini-Mental State Examination; and T-1, first clinical assessment. Composite z scores are given for episodic memory, semantic memory, working memory, and visuospatial ability.

memory, working memory, and visuospatial ability is shown in Figure 1. The most striking feature of the figure is the 2-SD decrease on the episodic memory composite accompanied by a less precipitous decline in working memory (0.5 SD), with maintained semantic memory and visuospatial ability.

At age 88½ years, a PiB PET image was unremarkable (PiB mean cortical binding potential, -0.006). At age 89½ years, CSF assays showed the following: an elevated total tau level of 575 pg/mL (>500 pg/mL is abnormal), a phosphorylated tau<sub>181</sub> level of 83 pg/mL (>80 pg/mL is abnormal), a reduced Aβ<sub>42</sub> level of 303 pg/mL (<500 pg/mL is abnormal), and an Aβ<sub>40</sub> level of 12 943 pg/mL (in the normal range).<sup>7,29</sup>

The autopsy was performed 2½ years after the PiB PET imaging. The unfixed brain weighed 1310 g (reference range, 1250-1400 g). External examination showed no cerebral atrophy. Coronal sectioning revealed mild to moderate dilatation of the lateral ventricle with rounding of the angle and only modest increase in space in the inferior horn; the hippocampus appeared only slightly smaller than normal. The substantia nigra and locus ceruleus were well pigmented.

Microscopy of hematoxylin-eosin-stained tissue sections revealed some neuronal loss and gliosis throughout the frontal neocortex (data not shown). There were sparse to focally numerous diffuse Aβ plaques (**Figure 2**) but only infrequent neuritic plaques. Isolated neurofibrillary tangles were seen using Bielschowsky silver staining and PHF-1 (data not shown). There was no α-synucleinopathy and no TDP-43 proteinopathy. There was mild amyloid angiopathy and modest arteriosclerosis. Moderate neuronal loss and gliosis in the cornu ammonis 1 (CA1) subfield of the hippocampus were accompanied by modest granulo-vacuolar degeneration and several neurofibrillary tangles. There was also neuronal loss, gliosis, and a few



**Figure 2.** Microscopy of the left frontal lobe. A, There are numerous diffuse amyloid β plaques (arrowheads) but few ring-with-core plaques (arrows) and modest cerebral amyloid angiopathy (double arrowhead).

neurofibrillary tangles but no neuritic plaques in the parahippocampal gyrus.

The AD lesions met the criteria for neurofibrillary stage III for tangles and amyloid stage C for plaques in the staging system by Braak and Braak<sup>30</sup> and Braak et al.<sup>31</sup> The densities of diffuse plaques were sufficient to meet the age-adjusted neuropathologic criteria for AD according to Khachaturian,<sup>32</sup> but the low densities of neuritic plaques and tangles were consistent only with possible AD according to the Consortium to Establish a Registry for Alzheimer's Disease<sup>33</sup> criteria, and there was only a low probability that the very mild dementia was caused by AD according to criteria by the National Institute on Aging and Reagan Institute.<sup>34</sup> There was no evidence of any other neurodegenerative or clinically meaningful vascular disease.

Biochemical analysis of frozen brain tissue included [<sup>3</sup>H]PiB binding to brain tissue homogenates and ELISA for Aβ<sub>1-42</sub> (**Table**). The [<sup>3</sup>H]PiB binding was below the value of approximately 350 pmol/g required for in vivo detection by PiB PET as shown in a previous study,<sup>27</sup> although the ELISA revealed high levels of Aβ<sub>1-42</sub> in the frontal, occipital, and precuneus regions (**Table**). Levels of fibrillar Aβ<sub>1-42</sub> exceeding 750 pmol/g generally are detectable by in vivo PiB PET.<sup>27</sup> Hence, the lack of detection by PiB PET and [<sup>3</sup>H]PiB binding in this case suggests that the cerebral Aβ<sub>42</sub> deposition largely was nonfibrillar, consistent with the observation that X-34 and 6-CN-PiB histofluorescence staining revealed only scarce fibrillar Aβ plaques (**Figure 3**).<sup>27,28</sup>

## COMMENT

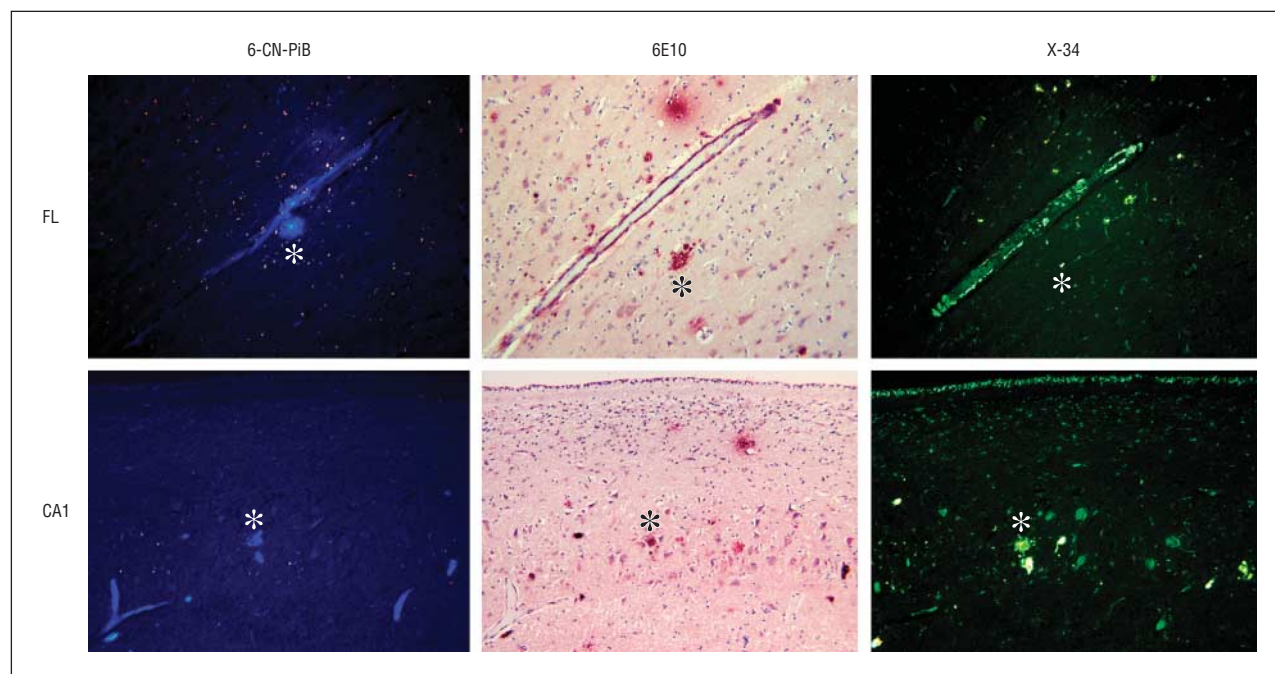
This case met conventional criteria for mild cognitive impairment.<sup>35</sup> Our clinicians are trained to use the observations of a collateral source to detect an individual's impaired ability to conduct accustomed activities (eg, driving a motor vehicle) caused by a decline from the individual's previously attained level of cognitive function. The clinician determines the CDR based on these observations and the clinician's examination of the individual but without knowledge of the individual's psychometric performance or imaging or CSF results. When an individual is determined to have cognitive impairment even



**Table. In Vivo Pittsburgh Compound B (PiB) Regional Binding Potentials and Postmortem Amyloid  $\beta$  ( $A\beta$ ) Measures**

$A\beta$ Measure	Superior Frontal Gyrus of the Frontal Lobe	Anterior Cingulate Gyrus	Superior Temporal Gyrus	Inferior Parietal Lobe	Precuneus	Calcarine Sulcus of the Occipital Lobe
PiB binding potential <sup>a</sup>	0.14	0.03	0.19	0.18	0.15	0.07
Area fraction of 10D5, %	4.4	1.6	2.0	2.9	5.4	0.9
[ <sup>3</sup> H]PiB bound, pmol/g	249	124	161	116	225	295
$A\beta_{1-42}$ enzyme-linked immunosorbent assay, pmol/g	1582	837	722	687	1785	1525

<sup>a</sup>As calculated by the distribution volume ratio minus 1.0 (obtained 2½ years before death). Regional binding potentials are not to be compared with the cortical binding potential (mean, -0.006) for this case because these specially drawn regions are less rigorous in matching the gray matter–white matter balance of the reference region (cerebellum).



**Figure 3.** Fluorescent beta-pleated sheet stains label a spectrum of amyloid  $\beta$  ( $A\beta$ ) structures in the frontal lobe (upper panels) and cornu ammonis 1 (CA1) subfield of hippocampus (lower panels) of postmortem brain of the Pittsburgh Compound B (PiB) positron emission tomography amyloid-negative case reported herein. Amyloid is visible using 6-CN-PiB and X-34, highly fluorescent derivatives of PiB and Congo red, respectively; the monoclonal antibody 6E10, targeting amino acids 1 through 16 (N-terminus) of  $A\beta$ , identifies similar structures as denoted by the asterisks.

at the CDR of 0.5, the clinician then judges the likely cause or causes of that impairment. If that cause is judged on clinical grounds to be AD, the individual is diagnosed as having DAT. Although this diagnosis may occur at an earlier stage of symptomatic AD than is common elsewhere and even at a stage where psychometric performance is insufficiently impaired to meet criteria for mild cognitive impairment, it is supported by subsequent progressive cognitive and functional decline and by the histopathologic confirmation of AD in 92% of those individuals who undergo autopsy.<sup>14,36</sup>

The clinical diagnosis of very mild DAT (CDR of 0.5) in this case was independently supported by cognitive decline in measures of episodic and working memory and by a CSF biomarker phenotype for AD, namely, elevated tau and phosphorylated tau<sub>181</sub> levels and reduced  $A\beta_{42}$  levels. Neuropathologic examination showed increased densities of diffuse plaques, but more mature neuritic plaques were scarce. These findings suggest that sub-

stantial densities of diffuse plaques, which may be downstream of more toxic species of  $A\beta$ ,<sup>37</sup> are not benign, as they can be associated with early symptomatic stages of AD. Hence, neuropathologic criteria such as the recommendations by the National Institute on Aging and Reagan Institute<sup>34</sup> that do not incorporate diffuse  $A\beta$  plaques into their algorithms may overlook an important early pathogenic feature of AD.

Amyloid tracers such as PiB bind strongly to fibrillar  $A\beta$  in compact or cored plaques and cerebral amyloid angiopathy but only weakly to amorphous cortical  $A\beta$  plaques.<sup>27,38</sup> Therefore, these tracers may be unable to detect AD variants that are characterized predominantly by diffuse  $A\beta$  plaques. Reports from our center indicate that the elevated mean cortical binding potential for PiB is almost always associated with low CSF  $A\beta_{42}$ <sup>6</sup> but that low levels of CSF  $A\beta_{42}$  can occur in the absence of elevated mean cortical binding potentials for PiB,<sup>8</sup> possibly because diffuse plaques can first appear without a substan-

tial amount of fibrillar A $\beta$ , as suggested by the present case. For individuals with substantial amounts of diffuse nonfibrillar A $\beta$  deposits, reduced levels of CSF A $\beta_{42}$  may reflect the AD pathologic process before the presence of sufficient numbers of fibrillar plaques to allow detection by PiB. Although this is a single case, it implies that a CSF profile of reduced A $\beta_{42}$  and elevated tau can serve as an antecedent biomarker for AD. These CSF abnormalities also may develop before the detection of fibrillar A $\beta$  deposits by PiB.

A limitation of this case report is the temporal dissociation between the PiB and CSF assessments. However, the scarcity of fibrillar A $\beta$  plaques at autopsy suggests that it is unlikely that the results of PiB PET would have been positive even if imaging occurred closer to the time of lumbar puncture (1 year later) or time of death (2½ years later). Another limitation is that the battery of psychometric tests was chosen many years ago for the longitudinal study in which this man participated; it did not include the more sensitive measures of working memory that are available today. Newer instruments might have detected earlier and more profound decline in this domain. The battery also did not include measures of attention that may also be affected early in the course of the disease.<sup>39</sup>

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