Positron Emission Tomography and Neuropathologic Estimates of Fibrillar Amyloid-\(\beta\) in a Patient With Down Syndrome and Alzheimer Disease

Marwan N. Sabbagh, MD; Adam Fleisher, MD; Kewei Chen, PhD; Joseph Rogers, PhD; Camryn Berk; Eric Reiman, MD; Michael Pontecorvo, PhD; Mark Mintun, MD; Daniel Skovronsky, MD; Sandra A. Jacobson, MD; Lucia I. Sue, BS; Carolyn Liebsack, BSN; Albert S. Charney, MD; Lauren Cole, BS; Christine Belden, PsyD; Thomas G. Beach, MD, PhD

**Background:** Down syndrome appears to be associated with a virtually certain risk of fibrillar amyloid-\(\beta\) (A\(\beta\)) pathology by the age of 40 and a very high risk of dementia at older ages. The positron emission tomography (PET) ligand florbetapir F18 has been shown to characterize fibrillar A\(\beta\) in the living human brain and to provide a close correlation with subsequent A\(\beta\) neuropathology in individuals proximate to and after the end of life. The extent to which the most frequently used PET ligands can be used to detect fibrillar A\(\beta\) in patients with Down syndrome remains to be determined.

**Objectives:** To characterize PET estimates of fibrillar A\(\beta\) burden in a Down syndrome patient very close to the end of life and to compare them with neuropathologic assessment made after his death.

**Design/Methods:** With the family's informed consent, florbetapir PET was used to study a 55-year-old Down syndrome patient with Alzheimer disease near the end of life; his brain was donated for neuropathologic assessment when he died 14 days later. Visual ratings of cerebral florbetapir uptake were performed by trained readers who were masked to the patient's diagnosis as part of a larger study, and an automated algorithm was used to characterize regional-to-cerebellar standard uptake value ratios in 6 cerebral regions of interest. Neuropathologic assessments were performed masked to the patient’s diagnosis or PET measurements.

**Results:** Visual ratings and automated analyses of the PET image revealed a heavy fibrillar A\(\beta\) burden in cortical, striatal, and thalamic regions, similar to that reported for patients with late-onset Alzheimer disease. This matched neuropathologic findings of frequent neuritic and diffuse plaques, as well as frequent amyloid angiopathy, except for neuropathologically demonstrated frequent cerebellar diffuse plaques and amyloid angiopathy that were not detected by the PET scan.

**Conclusions:** Florbetapir PET can be used to detect increased cerebral-to-cerebellar fibrillar A\(\beta\) burden in a Down syndrome patient with Alzheimer disease, even in the presence of frequent amyloid angiopathy and diffuse plaques in the cerebellum. Additional studies are needed to determine the extent to which PET could be used to detect and to track fibrillar A\(\beta\) and to evaluate investigational A\(\beta\)-modifying treatments in the presymptomatic and symptomatic stages of Alzheimer disease.

Arch Neurol. 2011;68(11):1461-1466

Author Affiliations are listed at the end of this article.
suited to the exploration of the relationship between early neurologic changes, dementia, and Aβ plaques. A better understanding of the early markers of AD would allow for earlier and more efficient treatment of AD in individuals with DS as well as a potentially broader understanding of the early signs of idiopathic AD in general. Here, we describe a case involving a DS patient with clinically confirmed AD who underwent florbetapir imaging proximate to death and autopsy evaluation, as part of a phase 3, multisite autopsy comparison study conducted by Avid Radiopharmaceuticals.

**REPORT OF A CASE**

The patient was a 55-year-old right-handed white man with DS and 8 years of formal education through special education. At the time of presentation at age 51 years, the patient was having difficulty maintaining occupational duties at his job at a gas station, difficulty multitasking, confusion with well-known tasks, word and naming difficulty, and a loss of pleasurable activities. He was having difficulty with doing his own cooking and maintaining his home environment. He also had become less fastidious regarding appearance. He had no change in his personality or mood, no weight loss, no reported hallucinations or delusions, and no change in gait or posture.

The patient's medical history at initial presentation included DS, cataracts, and gout, with no remarkable surgical history except for cataract extraction or medical allergies. The patient's medications included allopurinol and mouthwash for stomatitis. His family history indicated risk for diabetes mellitus, hypertension, and coronary disease, as well as a mother who died with AD. The patient's social history was unremarkable. He resided in a group home. The patient was not reported to have incontinence. The review of systems was not significant for notable change in appetite, weight, or sleep and was not indicative of anxiety or depression.

The patient's physical examination at initial evaluation revealed normal vital signs, including regular cardiovascular rate and rhythm as well as normotensive blood pressure. He exhibited a rash on his scalp and morphologic features consistent with a diagnosis of DS.

His neurologic examination revealed intact cranial nerves. His motor examination indicated normalcy in all muscle groups. The patient exhibited intact sensation peripherally and intact coordination and stood toe to heel in tandem. The patient's deep tendon reflexes were symmetric and brisk throughout.

The patient's initial cognitive examination indicated that his mentation was alert and his speech fluent. His premorbid IQ was difficult to gauge, but his medical records indicated that neuropsychologic testing performed 11 years before presentation put his full-scale IQ at 55, his verbal IQ at 53, and his performance IQ at 63, which are all consistent with mild retardation. His Mini-Mental State Examination score at the time of the initial neurologic evaluation for cognitive decline was derived and ascertained to be approximately 11/23. During the next few years, he declined in terms of cognitive ability and developed agitation and aggressive behavior. He was treated with Aricept (donepezil hydrochloride; Pfizer Inc), Namenda (memantine hydrochloride; Forest), and antidepressant and antipsychotic medications. In 2006, he had a seizure (not described in detail). Beginning in September 2007, he was noted to have a slow shuffling gait, and later his posture also became stooped. In the last year of life, he had visual hallucinations and multiple falls, as well as myoclonic jerking and tremors. He was using a wheelchair. Carbidopa-levodopa was added to his treatment regimen. A magnetic resonance imaging scan performed in March 2009 showed enlarged lateral ventricles and dystrophic calcifications of the head of the caudate nucleus and globus pallidus. The patient progressively declined in a manner typical of AD during the 4 years ensuing from the low initial assessment baseline until he was non-testable, with his last Mini-Mental State Examination score recorded as 0. The patient was enrolled in a florbetapir histopathology study.

**FLORBETAPIR IMAGING METHODS**

The patient received a 10-minute florbetapir positron emission tomography (PET) scan, beginning 50 minutes after a single intravenous bolus of 370 MBq (10 mCi) F-18 florbetapir. Images were acquired with a 128 × 128 matrix (zoom 2) and were reconstructed with an iterative reconstruction algorithm. Images were assessed using a semiquantitative visual image score ranging from 0 (no cortical amyloid) to 4 (high levels of amyloid). Three independent readers, masked to all clinical, demographic, and neuropathologic information, performed the visual rating. The median rating of these 3 readers served as the primary outcome variable. Independently, the images were first normalized to a standard template in Talairach space (SPM2, http://www.fil.ion.ucl.ac.uk/spm/), and then a semiautomated algorithm was used to calculate standard uptake value ratios (SUVRs) in predefined anatomically relevant cortical regions (frontal, temporal, parietal, anterior cingulate, posterior cingulate, and precuneus), with the whole cerebellum as the reference region.

To explore the Aβ deposition pattern, voxel-wise SUVRs (parametric imaging) were generated using the same reference region. The patient died 14 days after imaging. The brain was collected at autopsy for neuropathologic analysis. Neuropathologic studies were performed without knowledge of the clinical and PET data.

**NEUROPATHOLOGIC EVALUATION**

At the time of death, the brain was removed and processed according to the standard autopsy protocol for the Avid A07 clinical trial (NCT00857415). The brain was placed in fixative for 2 weeks before dissection at Banner Sun Health Research Institute, Sun City, Arizona.

Three independent methods were used to identify and to quantify cerebral amyloid burden. The first method used immunohistochemistry with Aβ antibody 4G8 (1:2000 dilution; antibody localization was amplified with an avidin-biotin peroxidase [VECTASTAIN Elite ABC System; Vector Laboratories, Inc, Burlingame, CA] and visualized with 3,3’-diaminobenzidine enhanced with a mixture of nickel and ammonium sulfate as horseradish peroxidase sub-
strate [Signet, Dedham, Massachusetts; provided by Covance, Princeton, New Jersey]) at Biospective Inc, Montreal, Canada. The stained slides were digitized using a Zeiss MIRAX high-resolution automated slide scanner (Carl Zeiss Canada, Toronto, Ontario). Image quantification was performed using the PERMITS image processing/analysis software (Biospective Inc). This automated quantification method segments chromogen-positive pixels based on red-green-blue intensity and generates a parametric map of Aβ/H9252 aggregation over the entire tissue section. The Aβ/H9252 burden (percentage of gray matter containing Aβ/H9252 aggregates) was calculated for each tissue section and averaged across multiple slides for each anatomical region.

The second method used a modified Bielchowsky silver stain applied to 6-µm sections cut from each region of interest and cerebellum. Two sections, separated by 300 µm, were evaluated from each region, and the average was used to represent the plaque density. Density of both neuritic and diffuse plaques was assessed by 2 independent experienced neuropathology raters using the Consortium to Establish a Registry for Alzheimer’s Disease templates. The results were reviewed by a senior neuropathologist (T.G.B.); all were masked to the clinical information and imaging results.

The third method of estimating plaque burden was a standard protocol at the Banner Sun Health Research Institute Brain and Body Donation Program. Standardized fixed and cryoprotected 4 × 5-cm tissue blocks were sectioned at 40 µm on a sliding freezing microtome, and sections were stained using the Campbell-Switzer Gallyas silver stains.12 Average total (neuritic and diffuse) plaque densities as well as average neurofibrillary tangle densities were estimated using the Consortium to Establish a Registry for Alzheimer’s Disease templates as zero, sparse, moderate, or frequent in the following regions: frontal lobe cortex at the coronal level of the genu of the corpus callosum, temporal lobe cortex at the coronal level of the amygdala and at the level of the lateral geniculate nucleus, and parietal lobe cortex at a level 1-cm caudal to the splenium of the corpus callosum. Density descriptive terms were converted to a 0-to-4 scale for statistical purposes. Braak neurofibrillary tangle stage was established according to the original publication.10

**FLORBETAPIR FINDINGS**

The DS patient’s florbetapir PET image (analyzed both in terms of raw counts and cerebral-to-whole cerebellar SUVRs) revealed a pattern of cortical fibrillar amyloid burden similar to that in patients with late-onset AD (Figure 1). All 6 cortical regions evaluated demonstrated significant cortical florbetapir uptake in both visual reads and SUVR analysis (Figure 2). The image also revealed fibrillar amyloid burden in the striatum and thalamus. On visual inspection, there was also more tracer uptake in the cerebellum than typically seen. However, whole cerebellar uptake quantitated within the normal range, resulting in cortical-to-whole cerebellar SUVRs in the expected range for florbetapir.13
AUTOPSY FINDINGS

On gross examination, the anterior part of the temporal lobes showed gyral atrophy with no herniation, as well as marked atrophy of the amygdala, head and body of the hippocampus, and parahippocampal gyrus.4 The cerebellum and brainstem were externally unremarkable. Cerebral slices revealed no cortical lesions but moderate to marked dilation of the lateral and third ventricles. Histology with hematoxylin-eosin showed substantial gliosis of the upper neocortical layers, with many Aβ plaques and neurofibrillary tangles.2 Mild depigmentation of the substantia nigra, without Lewy bodies, and calcification of the basal ganglia were observed; the latter is common in DS. Otherwise, sections of the basal ganglia, thalamus, subthalamic regions, cerebellum, brainstem, spinal cord, and paraspinal sympathetic ganglia were unremarkable. Amyloid staining revealed frequent diffuse, neuritic, and cored Aβ plaques and frequent neurofibrillary tangles in neocortex and limbic areas (Figure 3). The Braak stage was VI. Diffuse Aβ plaques were observed in the cerebellum, striatum, and thalamus, with frequent amyloidotic blood vessels in all cerebral lobes, thalamus, striatum, and cerebellum. Immunohistochemical staining for phosphorylated α-synuclein showed no evidence of neuronal cytoplasmic inclusions or neurites in multiple sections of the olfactory bulb, brainstem, amygdala, cerebral cortex, spinal cord, or paraspinal sympathetic ganglia. The final neuropathologic diagnosis, based on established National Institutes of Health Alzheimer Disease Center criteria,14 was AD with trisomy 21.

COMPARISON OF FLORBETAPIR AND AUTOPSY FINDINGS

Diffuse amyloid plaques, neuritic plaques, and percentage of cortical amyloid on immunohistochemistry on pathology were elevated in all 6 regions assessed. Amyloid pathology was consistent with regional quantitative florbetapir standard uptake value ratios and visual reads. AC indicates anterior cingulate; Frn, frontal; High, highest counts; IHC, amyloid-β immunohistochemistry; Par, parietal; PC, posterior cingulate; PreC, precuneus; rep Diff, representative diffuse plaque counts; rep NP, representative neuritic plaque counts; rep TP, representative total plaque counts; SUVR, cerebral-to-whole-cerebellum standard uptake value ratio; and Tem, temporal. Asterisk indicates percentage of gray matter containing amyloid.

COMMENT

To our knowledge, this is the first report of florbetapir imaging in a DS patient with AD. Because the images were gathered proximate to death, it was also possible to correlate imaging findings with pathologic features at autopsy. This case reveals an association between antemortem amy-
loid PET findings and postmortem neuropathologic findings in a DS patient with clinical and neuropathologic evidence of AD. Florbetapir PET revealed a characteristic pattern of fibrillar amyloid burden in the cerebral cortex in a DS patient with AD. Both visual reads and quantitative binding measures were consistent with amyloid seen on pathology. Quantification of florbetapir PET was useful even when using a cerebellar reference region found to have elevated florbetapir uptake and neuropathologic evidence of diffuse plaques and vascular amyloid compared with what is typically found in late onset. Although additional studies are needed, our findings suggest the promise of florbetapir PET to detect and to track AD-related fibrillar amyloid pathology and to evaluate amyloid-modifying treatments in DS patients.

Although this is a single case report, florbetapir has recently been shown to be highly accurate in identifying amyloid on pathology. An ongoing phase 3 study of 152 terminally ill participants and 74 healthy young controls is comparing florbetapir imaging and postmortem amyloid immunohistochemistry. This study recently demonstrated high levels of correlation between florbetapir imaging and postmortem amyloid pathology in the first 35 cases to come to autopsy, with high levels of correlation between visual reads of raw PET images ($r=0.78$) and quantitative measures with immunohistochemistry ($r=0.76$). Overall, florbetapir showed 99% accuracy, 98% positive predictive value, and 100% negative predictive value for determining underlying amyloid pathology. In the image-to-pathology study, there was a comparable correlation between image (SUVR) and either diffuse or neuritic plaques, with the best correlation for total plaque. The DS case gives a similar result. In addition, florbetapir binds to vascular amyloid in vitro. This supports the use of florbetapir as a true biomarker of underlying amyloid pathology and provides secondary validation for the results from our single case report.

Molecular imaging with florbetapir in DS subjects could be important for several reasons. First, life expectancy of the DS population has increased dramatically during the past few decades, and this population is at extremely high risk for AD even in middle age. As such, if early detection is critical in late-onset AD subjects, it may be as much or more so in DS. Second, the virtual universality of $\alpha\beta$ deposition in the DS population may make DS subjects optimal for tracking $\alpha\beta$ pathology over time. Whereas one would have to recruit and to scan hundreds of normal elderly to arrive at the few who develop $\alpha\beta$ pathology in a longitudinal study, all DS subjects will do so by middle age or younger. Third, despite the ubiquity of $\alpha\beta$ deposition in DS, many DS individuals reportedly do not develop overt AD. Thus, the DS population may also be optimal for research into the salience of $\alpha\beta$ pathology in AD. For example, differential distributions of $\alpha\beta$ over the various cortical areas might be found to account for discrepancies in the development of AD, although many more cases will be necessary to confirm the unusual $\alpha\beta$ distribution observed in the present study.

An effective path toward treatment of AD depends on the successful development of noninvasive methods for early diagnosis, including measurements of $\alpha\beta$ load in...
the brain. Current measures of Aβ load, such as Aβ levels in cerebrospinal fluid, may not be direct enough to accurately assess brain Aβ pathology. In typical late-onset AD, as well as in DS patients, florbetapir may offer a unique opportunity for presymptomatic identification of patients in the early stages of AD. Its half-life of 110 minutes makes it easier to store and to use than other radioactive materials used in PET scans, and it appears to have high selectivity and optimum kinetics for Aβ plaques. Trials using florbetapir have shown that healthy control patients exhibited minimal accumulation of florbetapir in white matter areas, whereas patients with AD exhibited high tracer uptake in areas of the brain where Aβ plaques are commonly observed at autopsy.

Accepted for Publication: January 24, 2011.

Author Affiliations: Banner Sun Health Research Institute, Sun City, Arizona (Drs Sabbagh, Rogers, Jacobson, Charney, Belden, and Beach and MsS Berk, Sue, Liebsack, and Cole); Banner Alzheimer’s Institute (Drs Fleisher, Chen, and Reiman), Translational Genomics Research Institute and Department of Psychiatry, University of Arizona (Dr Reiman), and Arizona Alzheimer’s Consortium (Drs Sabbagh, Fleisher, Chen, Rogers, Reiman, Jacobson, Charney, Belden, and Beach and MsS Sue and Liebsack), Phoenix, Arizona; Avid Radiopharmaceuticals, Philadelphia, Pennsylvania (Drs Pontecorvo, Mintun, and Skovronsky); and Department of Neurosciences, University of California, San Diego (Dr Fleisher).

Correspondence: Marwan N. Sabbagh, MD, The Cleo Roberts Center for Clinical Research, Banner Sun Health Research Institute, 101515 W Santa Fe Dr, Sun City, AZ 85351 (marwan.sabbagh@bannernhealth.com).

Author Contributions: Study concept and design: Sabbagh, Fleisher, Rogers, Pontecorvo, Skovronsky, Jacobson, and Cole. Acquisition of data: Sabbagh, Pontecorvo, Sue, Liebsack, Charney, Belden, and Beach. Analysis and interpretation of data: Sabbagh, Fleisher, Chen, Reiman, Pontecorvo, Mintun, Jacobson, and Charney. Drafting of the manuscript: Sabbagh, Fleisher, Rogers, Berk, Jacobson, Cole, and Beach. Critical revision of the manuscript for important intellectual content: Sabbagh, Fleisher, Chen, Reiman, Pontecorvo, Mintun, Jacobson, and Charney. Statistical analysis: Chen. Obtained funding: Sabbagh, Rogers, Pontecorvo, Skovronsky, and Beach. Administrative, technical, and material support: Sabbagh, Pontecorvo, Mintun, Skovronsky, Sue, Liebsack, Charney, Cole, Belden, and Beach. Study supervision: Sabbagh, Reiman, and Skovronsky.

Financial Disclosure: Dr Sabbagh has worked in an advisory capacity for Eisai Co, Ltd; Pfizer Inc; Amerisiences, LP; and GlaxoSmithKline plc; has received royalties from John Wiley & Sons, Inc and Amerisiences, LP; and has grants and contracts with Eli Lilly and Company; Baxter Healthcare Corporation; Bayer AG; General Electric Company; Bristol-Myers Squibb; Eisai Co, Ltd; Janssen Pharmaceuticals, Inc; Wyeth Pharmaceuticals/Elan Corporation, plc; Avid Radiopharmaceuticals, Inc; and Medivation, Inc. Dr Rogers is a consultant for Eisai Co, Ltd. Drs Pontecorvo and Skovronsky are employees and Dr Mintun is chief medical officer of Avid Radiopharmaceuticals, Inc, the maker of florbetapir. Dr Jacobson receives royalties from American Psychiatric Publishing and research support for clinical trials from Bristol-Myers Squibb; Avid Radiopharmaceuticals, Inc; Forest; General Electric Company; Bayer AG; Baxter Healthcare Corporation; Wyeth Pharmaceuticals; Janssen Pharmaceuticals, Inc; Eli Lilly and Company; and Medivation, Inc. Dr Beach has received scientific support from Avid Radiopharmaceuticals, Inc and Bayer Schering Pharma AG.

Funding/Support: This study was supported by grant P30 AG 019610 from the National Institute on Aging; the Banner Sun Health Research Institute; the Banner Alzheimer’s Institute; and Avid Radiopharmaceuticals, Inc.

Additional Information: We thank Paul Thompson, PhD, for allowing us to use the human brain template and cortical brain surface display technique from the Laboratory of Neuro Imaging (LONI) in our case report.

REFERENCES