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Down syndrome: age-dependence of PiB binding in postmortem frontal cortex across the lifespan

Harry LeVine III [a,b], H. Peter Spielmann [b,c,d,e], Sergey Matveev [a], Francesca Macchiavello Cauvi [a], M. Paul Murphy [a,b], Tina L. Beckett [a], Katie McCarty [a], Ira T. Lott [f], Eric Doran [f], Frederick Schmitt [a,g], Elizabeth Head [a,h,*]

[a] Sanders-Brown Center on Aging, University of Kentucky, Lexington, KY, USA
[b] Department of Molecular and Cellular Biochemistry, University of Kentucky, Lexington, KY, USA
[c] Department of Chemistry, University of Kentucky, Lexington, KY, USA
[d] Center for Structural Biology, University of Kentucky, Lexington, KY, USA
[e] Markey Cancer Center, University of Kentucky, Lexington, KY, USA
[f] Department of Pediatrics, University of California at Irvine, Irvine, CA, USA
[g] Department of Neurology, University of Kentucky, Lexington, KY, USA
[h] Department of Pharmacology & Nutritional Sciences, University of Kentucky, Lexington, KY, USA

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ABSTRACT

Beta-amyloid (Aβ) deposition in brain accumulates as a function of age in people with Down syndrome (DS) with subsequent development into Alzheimer disease neuropathology, typically by 40 years of age. In vivo imaging using the Pittsburgh compound B (PiB) ligand has facilitated studies linking Aβ, cognition, and dementia in DS. However, there are no studies of PiB binding across the lifespan in DS. The current study describes in vitro ^{3}H-PiB binding in the frontal cortex of autopsy cases with DS compared to non-DS controls. Tissue from 64 cases included controls (n = 25) and DS (n = 39). In DS, ^{3}H-PiB binding was significantly associated with age. After age 40 years in DS, ^{3}H-PiB binding rose dramatically along with increasing individual variability. ^{3}H-PiB binding correlated with the amount of Aβ42. Using fixed frontal tissue and fluorescent 6-CN-PiB, neuritic and cored plaques along with extensive cerebral amyloid angiopathy showed 6-CN-PiB binding. These results suggest that cortical PiB binding as shown by positron emission tomography imaging reflects plaques and cerebral amyloid angiopathy in DS brain.

1. Introduction

Alzheimer disease (AD) is the most common cause of dementia in the elderly and affects 1 in 9 people over the age of 65 years (http://www.alz.org/facts/). There are currently no biomarkers for AD that can clearly distinguish people who will develop the disease from those who will not. The presence of beta-amyloid (Aβ) plaques and tau neurofibrillary tangles determined at autopsy are defining characteristics for a pathological diagnosis of AD. However, the development of in vivo ligands that selectively bind to Aβ, allows these lesions to be visualized and quantified in people using positron emission tomography (PET), significantly accelerating biomarker development (Cohen and Klunk, 2014; Johnson et al., 2012; Mintun et al., 2006; Sperling et al., 2014). The first of these ligands, Pittsburgh compound B (PiB) (Klunk et al., 2004) has now been used in a large number of clinical studies in patients with AD and can detect Aβ plaques in early disease (Cohen and Klunk, 2014).

^{3}H-PiB and 6-CN-PiB binding in vitro has been described in autopsy cases of AD in the general population (Bacskai et al., 2007; Beckett et al., 2012; Ikonomovic et al., 2008, 2012; Klunk et al., 2007). Aβ40 and Aβ42 positive plaques as well as vascular Aβ bind PiB in vitro. PiB binding was more robust in compact or cored plaques and less so with diffuse plaques. Typically neurofibrillary tangles did not bind PiB except for possible weak binding to extracellular “ghost” tangles, which may be due to associated Aβ (Ikonomovic et al., 2008). Finally, PiB binding correlates early in disease with postmortem insoluble Aβ measures and with plaque loads. In 1 case that was PET imaged in life with PiB and then came to autopsy, there was a significant overlap in the regional distribution of the in vivo plaque binding and in vitro PiB binding (Bacskai et al., 2007).

* Corresponding author at: Sanders-Brown Center on Aging, University of Kentucky, 800 South Limestone Street, Lexington, KY 40536, USA. Tel.: 859-218-3172; fax: 859-323-2866.
E-mail address: elizabeth.head@uky.edu (E. Head).

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Down syndrome (DS) or trisomy 21 is the most common genetic cause of intellectual disability and is associated with a neurologic phenotype that includes the development of AD neuropathology by the age of 40 years (Head et al., 2016; Lott, 2012; Lott and Dierssen, 2010). Further, autopsy studies indicate that in DS there is an age-associated progression of AD neuropathology with initial deposits of Aβ and subsequent formation of neurofibrillary tangles (Head et al., 2016; Hof et al., 1995; Leverenz and Raskind, 1998; Wisniewski et al., 1985). Even so, the age of onset of dementia may be delayed by almost a decade (over 50 years of age) after AD neuropathology is present and a subset of people with DS appear to not develop dementia even at very old ages (Lai and Williams, 1989; Schupf and Sergievsky, 2002).

PiB PET imaging studies in people with DS show that binding is age-dependent (Annus et al., 2016; Handen et al., 2012; Hartley et al., 2014; Landt et al., 2011; Lao et al., 2016). Two of these studies included measures of impaired cognition and dementia status and found a positive significant correlation with PiB load (Annus et al., 2016; Hartley et al., 2014). Striatal PiB is the earliest site of binding with age in DS, typically observed after 35 years of age (Annus et al., 2016; Handen et al., 2012; Lao et al., 2016) and is similar to reports of patients with presenilin-1 mutations (Klunk et al., 2007; Koivunen et al., 2008; Villemagne et al., 2009). Subsequently, with increasing age, more brain regions become affected including the neocortex (Annus et al., 2016).

The age-dependency of PiB binding in DS in vivo may be a biomarker for AD neuropathology that can be used as an outcome measure in clinical trials. We hypothesized that ^{3}H-PiB binding would increase as a function of age in DS in frontal cortex. Thus, we measured ^{3}H-PiB binding biochemically in frontal cortex homogenates from autopsy cases with and without DS. We used the highly fluorescent PiB derivative, 6-CN-PiB, which has similar binding properties as PiB (Ikonomovic et al., 2008; Mathis et al., 2003) in fixed tissue to visualize plaques and, if present, cerebral amyloid angiopathy (CAA) to determine what types of pathology PiB binding represent in vivo.

2. Materials and methods

2.1. Autopsy cases

Frozen frontal cortex (Brodmann area 46) was obtained from 64 cases in total from the University of Kentucky Alzheimer Disease Center, the Alzheimer Disease Research Center at the University of California at Irvine, and the NIH NeuroBioBank. Human tissue collection and handling conformed to each University's Institutional Review Board guidelines.

Cases ranged from 1 to 66 years of age (Table 1). Control cases were selected to match for age and postmortem interval (PMI) to match the DS cases. Both males and females were included in the study, but given the challenges of matching cases, we did not match for gender. The level of premorbid intellectual disability or cognitive status was not available in most cases and thus it was not possible to use these variables in the analysis.

Table 1
Case demographics

Characteristic	DS	Control
n	39	25
Mean age (range) y	44.1 (1–66)	36.4 (1–66)
PMI (h)	9.6	15.2
Female (%)	20 (59)	10 (40)

Key: DS, Down syndrome; PMI, postmortem interval.

The PMI was different across groups, with the control cases showing an overall longer PMI ($t(62) = 3.0$, $p = 0.004$) relative to DS cases. Correlation coefficients were thus adjusted for PMI when necessary.

2.2. ^{3}H-PiB binding

^{3}H-PiB binding was used to measure fibrillar Aβ and was assessed in homogenates of frontal cortex (Beckett et al., 2012; Matveev et al., 2014b). Instead of the 10 μM concentrations typically used for histological assay, which will also bind to low affinity sites, we used 1.2 nM PiB, a concentration closer to the K_d of the PiB analog CN-PiB for Aβ pathology and to the in vivo concentration of ^{11}C-PiB used to visualize high affinity PiB binding in human AD brain. For binding studies, 20 μL of a phosphate buffered saline (PBS) homogenate containing 0.166 mg wet weight tissue were added to each of 3 wells of a 96-well polypropylene plate (Costar 3365). Two hundred microliters of 1.2-nM ^{3}H-PiB (cat. VT 278 specific radioactivity = 70.2 Ci/mmol, Vitrax [Placentia, CA, USA]) in PBS +5% v/v EtOH was added to 2 of the wells (total binding) and to the third well 200 μL of 1.2 nM ^{3}H-PiB +1 μM unlabeled competitor BTA-1 (nonspecific binding) was added. The plate was sealed with plastic film. Samples were incubated for 2 hours at room temperature without shaking, transferred to a 96-well Millipore Multiscreen HTS Hi Flow FB (GF/B) filter plate, and filtered on a multiwell plate vacuum manifold (Millipore Corporation, Bedford, MA, USA). The filters were washed 3 times with 200 μL of PBS +5% v/v EtOH, dried, removed from the plate, and placed in scintillation vials with 2 mL of BudgetSolve scintillation fluid and counted for ^{3}H. Specific binding for each sample was calculated as total binding (mean counts per minute of the 2 filters from wells containing radioactive PiB) minus nonspecific binding (counts per minute value from the well containing radioactive PiB +1 μM nonradioactive BTA-1 competitor).

2.3. ^{3}H-X-34 binding

^{3}H-X-34 binding (Matveev et al., 2014a,b) was used to measure a combination of fibrillar Aβ and neurofibrillary tangles and was performed with 10 μL of a PBS homogenate containing 0.166 mg wet weight tissue similar to ^{3}H-PiB binding, with 5 nM ^{3}H-X-34, 23 Ci/mmol, (custom titrated by Vitrax) (Matveev et al., 2014a,b) with 10 μM Congo red as the nonradioactive X-34 competitor.

2.4. Aβ ELISA

To measure total soluble and insoluble Aβ40 and Aβ42 in frontal cortex, we used an enzyme linked immunosorbant assay (ELISA). The methods for tissue extraction and Aβ measurements have been published previously (Beckett et al., 2010). Briefly, frozen cortical samples were serially extracted to obtain fractions of different assembly states of Aβ. The tissue was homogenized in a Dounce homogenizer in ice cold PBS (pH 7.4) containing 1x complete protease inhibitor cocktail (Amresco, Solon, OH), and centrifuged at 20,800 × g for 30 minutes at 4 °C. Following centrifugation, the supernatant was collected for subsequent measures of PBS soluble Aβ and the pellets were sonicated (10 × 0.5 seconds pulses at 100W, Fisher Sonic Dismembrator) in room temperature 2% sodium dodecyl sulfate (SDS) in PBS with protease inhibitor cocktail followed by centrifugation (as above, but at 14 °C). The supernatant was again collected to measure SDS-soluble Aβ, and the remaining pellets were sonicated in 70% formic acid (FA) followed by centrifugation at 20,800 × g for 1 hour at 4 °C. The supernatant collected was used to measure insoluble Aβ. Aβ was measured in these extracts using a standard, well-characterized two-site sandwich ELISA as described