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REVIEW ARTICLE

Molecular imaging of dementia

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Abstract

Diagnosis and treatment strategies for dementia are based on the sensitive and specific detection of the incipient neuropathological characteristics, combined with emerging treatments that counteract molecular processes in its pathogenesis. Positron emission tomography (PET) is used for diverse clinical and basic studies on dementia with a wide range of radiotracers. Approaches to visualize amyloid deposition in human brains non-invasively with PET depend on imaging agents reacting with amyloid fibrils. The most widely used tracer is [^{11}C]-6-OH-BTA-1, also known as Pittsburgh Compound-B, which has a high affinity to amyloid β peptide (Aβ) aggregates. Some ^{18}F-labeled amyloid ligands with a longer radioactive half-life have also been developed for broader clinical applications. In addition, there have been demonstrated advantages of tracers with high specific radioactivity in the sensitive detection of amyloid, which have indicated the significance of Aβ-N3-pyroglutamate as a new diagnostic and therapeutic target. Furthermore, beneficial outcomes of Aβ and tau immunization in humans and mouse models have highlighted crucial roles of immunocompetent glia in the protection of neurons against amyloid toxicities. The utility of PET with a radioligand for translocator protein as a biomarker for tau-triggered toxicity, and as a complement to amyloid and tau imaging for diagnostic assessment of tauopathies with and without Aβ pathologies, has also been demonstrated. Meanwhile, brain cholinergic function can be estimated by measuring acetylcholinesterase activity in the brain with PET and radiolabeled acetylcholine analogues. It has been reported that patients with early Parkinson's disease exhibit a reduction in acetylcholinesterase activity in the cerebral cortex, and this decline is more profound in patients with Parkinson's disease with dementia and dementia with Lewy bodies than in patients with Parkinson's disease without dementia. The Alzheimer's Disease Neuroimaging Initiative was a multicentre research project conducted over 6 years that studied changes in cognition, brain structure, and biomarkers in healthy elderly controls and subjects with mild cognitive impairment and Alzheimer's disease. An international workgroup of the National Institute on Aging-Alzheimer's Association has suggested that Alzheimer's disease would be optimally treated before significant cognitive impairment, defined as a 'presymptomatic' or 'preclinical' stage. Therefore, PET will be of technical importance for both clinical and basic research aimed at prodromal pathologies of Alzheimer's disease.

Key words: *Alzheimer's disease (AD), amyloid, Aβ, dementia, microglia, positron emission tomography (PET).*

INTRODUCTION

Japanese society is rapidly ageing, and the increasing number of dementia patients is resulting in social and economic problems. Dementia, progressive cognitive decline with various psychiatric symptoms, leads to a gradually increased restriction of daily activities. About 6% of the population 65 years and older suffers from dementia, and worldwide the number of new

cases in 2000 was estimated to be 4–6 million.[1] Dementia not only affects patients themselves but also places substantial burdens on their caregivers and society, as most patients require long-term care, which can often last 5 years or longer after symptom onset, at their homes or nursing homes. Therefore, a solution to these critical social issues is intimately associated with the establishment of diagnostic and therapeutic approaches to this condition. Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by gradual deterioration in cognition and behaviour, and it accounts for about 60% of all cases of dementia. Earlier detection of AD risk would enable preventive and more effective treatment, resulting in a delay in symptom onset that could significantly decrease the prevalence of the disease.[3] This achievement must rely on the sensitive and specific detection of the incipient neuropathological characteristics of AD, combined with emerging treatments that counteract molecular processes in its pathogenesis. Conventional neuroimaging-based measures as exemplified by morphometric indices, regional cerebral blood flow and glucose metabolism have offered informative adjuncts in predicting symptomatic conversion of minor memory disturbance, termed mild cognitive impairment (MCI), to clinically diagnosable AD. Meanwhile, recent advances in molecular imaging research have enabled visualization of brain amyloidosis, the core pathology of AD, potentially allowing diagnosis and initiation of disease-modifying therapies at a presymptomatic stage.

Positron emission tomography (PET) is used for many clinical studies because of its high sensitivity, low invasiveness and quantification capability. Non-invasive assessment of glucose utilization by means of PET with 2-[^{18}F] fluoro-deoxy-glucose has revealed a metabolic deficit in several neurodegenerative disorders, including AD and MCI.[4] Moreover, a broad range of molecular targets can be imaged with various PET tracers in both humans and small animals, owing to flexibility of designing PET probes. In this review, we summarize PET imaging biomarkers applied to research on dementia.

PATHOLOGICAL AND MOLECULAR ETIOLOGY OF ALZHEIMER'S DISEASE

The presence of amyloid lesions, comprised of senile plaques and neurofibrillary tangles, is a neuropathological hallmark of the AD brain.[5] Amyloid β peptide (Aβ) and tau protein constitute plaques and tangles, respectively, and these amyloidogenic molecules have been mechanistically implicated in the pathogenesis of AD and related neurodegenerative dementias. Numerous investigations have supported the notion that cascade reactions of molecular processes triggered by aberrancy of amyloidogenic components involve synaptic loss and neurotransmitter deficits, leading to neuronal death and cognitive impairments. Moreover, studies on the initialization of a neurotoxic cascade by amyloid fibrils have reinforced the rationale for applying *in vivo* amyloid imaging to the detection of AD pathology at an asymptomatic stage. Appearances of amyloid deposits precedent to symptomatic onset have been demonstrated by neuropathological analyses of post-mortem AD brains. As Braak and Braak proposed on the basis of their pathological staging of brain amyloidosis, amyloid deposits spread from the basal neocortex to all cortical regions.[6] In consideration of this putative spatiotemporal profile, the observation that tangles and plaques are already present in widespread areas of preclinical AD brains decisively indicates that the occurrence of amyloid aggregates long precedes cognitive deterioration.[7] In addition to the clinicopathological findings in human subjects, genetically engineered mouse models of familial dementias have provided an insight into the pathophysiological significance of amyloid fibrillogenesis in the devastating effects of AD and other degenerative disorders on neuronal integrities.[8,9] Moreover, studies have also focused on the application of genetically engineered disease models and small animal-dedicated PET devices to the development of amyloid probes and anti-amyloid treatments, as comparative evaluation of multiple candidate tracers and longitudinal assessments of neuropathology can be conducted in the same animals.

AMYLOID IMAGING

Approaches to non-invasive visualization of amyloid deposition in human brains with PET have been based on the development of imaging agents that can efficiently react with amyloid fibrils. The β-pleated sheet is a secondary structure commonly shared by amyloid assemblies, and chemicals that bind to β-sheets would make good candidates for a tracer compound. Thioflavin-S and Congo red are well-known dyes that stain a wide range of amyloid pathologies, and

thioflavin-T is also a chemical capable of binding to amyloid fibrils in a test tube. However, these compounds are not applicable as intravenously administrable tracers for amyloid imaging, as they cannot pass through the blood-brain barrier. Therefore, small and uncharged derivatives of these compounds with blood-brain barrier permeability have been developed as imaging probes, and their radiolabeled versions are used for PET.

With its binding increased in brain areas known to contain both amyloid plaques and neurofibrillary tangles, 2-(1-{6-[(2-[^{18}F]fluoroethyl)(methyl)amino]-2-naphthyl}ethylidene)malononitrile (FDDNP) is the first PET molecular imaging probe successfully applied to *in vivo* visualization of AD pathology in the brain of living patients.[10] However, specific signals of [18F]FDDNP in amyloid-rich regions of AD brains were only 0.3-fold higher than those in the cerebellar reference region. The most widely used amyloid tracer at present is [^{11}C]-6-OH-BTA-1, or Pittsburgh Compound-B (PIB), which was developed by a research group at the University of Pittsburgh (Pittsburgh, PA, USA).[11] [^{11}C]PIB has a favourable combination of a high affinity for Aβ and a moderate log *P*-value of 1.2 (Fig. 1), which accords a high initial uptake to and a fast clearance from non-AD brains. *In vitro* studies have demonstrated a consistency between the amounts of PIB binding sites and biochemically measured Aβ in frontal cortex samples from autopsied AD brains.[12] Since the first report on the successful application of PIB to the differentiation between normal subjects and AD patients, PIB and several other radioligands have been shown to be of great utility for the detection of amyloid at early symptomatic and possibly presymptomatic stages of AD.[13] Indeed, retention of [^{11}C]PIB in the AD neocotex with abundant plaque deposits is 1.5–2-fold higher than that in the cerebellum, much exceeding the performance of [18F]FDDNP. In the first autopsy case report of a patient who underwent an [^{11}C]PIB-PET scan and died 3 months later, a positive correlation between regional patterns of PIB binding and immunohistochemically measured Aβ burden was proven. This study also demonstrated that amyloid deposited as cerebral amyloid angiopathy can be a significant source of radioprobe signals.[14] In a recent study, [^{11}C]AZD2184 was shown to provide higher sensitivity for Aβ amyloid than [^{11}C]PIB,[15] suggesting the capability of this new radioligand in detecting brain amyloidosis at a very early stage. In addition, [^{11}C]-labelled 2-(2-[2-dimethylaminothiazol-5-yl]ethenyl)-6-(2-[fluoro]ethoxy)benzoxazole ([11C]BF-227) was developed and indicated to be a PET probe for *in vivo* detection of dense-cored amyloid deposits in AD patients.[16]

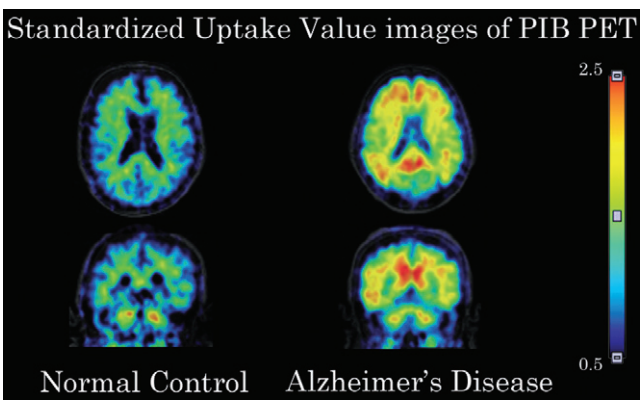

Figure 1 Representative amyloid PET images showing standardized uptake value for PIB. Increased uptake values reflect greater PIB binding in many cortical areas of AD than in normal control. AD, Alzheimer's disease; PET, positron emission tomography; PIB, Pittsburgh Compound-B.

The utility of ^{11}C-labeled tracers is limited by its short radioactive half-life (about 20 min), and they necessitate an on-site production by cyclotron and radiochemistry modules. In contrast, ^{18}F-labeled tracers, with their radioactive half-life of 110 min, can be distributed from a production site to local hospitals running a PET scanner. Given this advantage, [^{18}F]flutemetamol, which is structurally identical to [^{11}C]PIB apart from the presence of [^{18}F]fluorine attached to the core benzene ring, was developed and tested in a phase II clinical trial.[17] In addition, it is reported that florbetaben ([^{18}F]-BAY94-9172),[18] a stilbene derivative with some structural similarities to PIB, has shown high affinity and specificity for Aβ. Also, clinical studies of florbetapir ([^{18}F]AV-45) and [^{18}F]AZD4694 are in progress (Fig. 2).[19,20] A phase III trial for [^{18}F]-BAY94-9172 is ongoing, and data in a clinical trial for [^{18}F]AV-45 were submitted to the US Food and Drug Administration (FDA). In 2008, a FDA advisory committee noted that the detection of amyloid plaque in the brain could have clinical utility. The committee suggested that a new study should use a post-mortem pathological measure as a reference standard and, therefore, be designed to test intra-individual relationships between levels of neuritic amyloid plaques assessed by ante-mortem PET

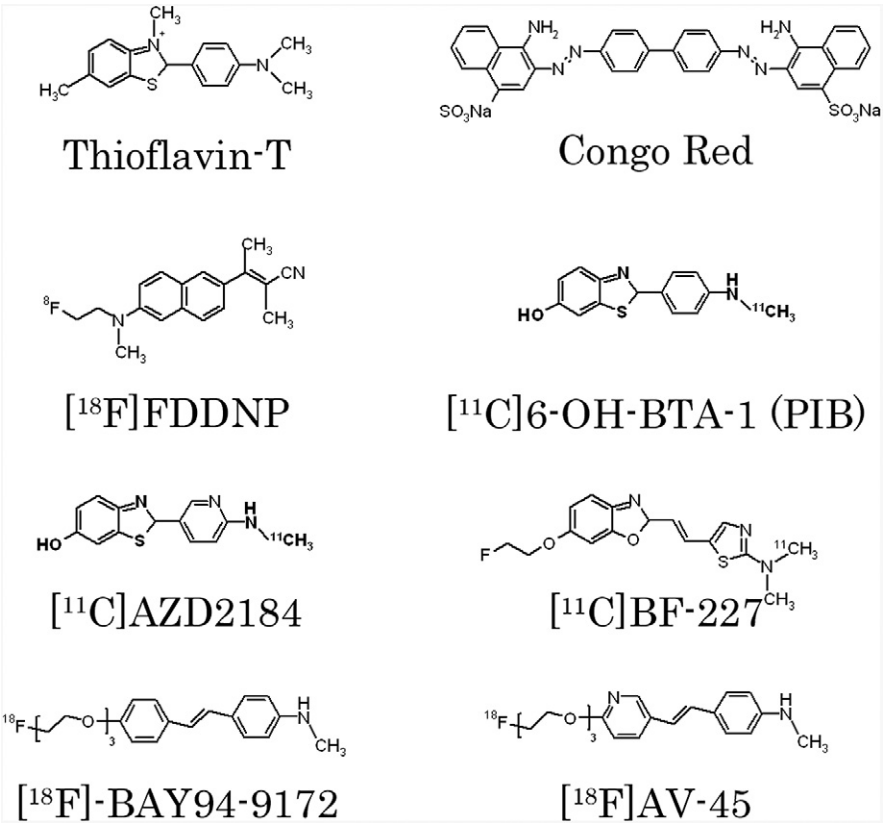

Figure 2 Chemical structures of amyloid ligands. [^{18}F]AV-45, florpiramine; [^{18}F]-BAY94-9172, florbetapir; [^{18}F]FDDNP, 2-(1-{6-[(2-[^{18}F]fluoroethyl)(methyl)amino]-2-naphthyl}ethylidene)malononitrile; PIB, Pittsburgh Compound-B.

imaging and post-mortem histopathology.[21] Following this, in a prospective clinical evaluation conducted from February 2009 through March 2010, [^{18}F]AV-45 PET data were acquired from 35 subjects, including AD patients, and were compared with immunohistochemical and silver stain measures of brain Aβ pathologies after their death. It was demonstrated that [^{18}F]AV-45-PET data was correlated with the presence and density of Aβ lesions.[22]

In contrast to the clinical data on PIB, PET experiments using animal models have not provided unequivocal evidence that radiolabeled compounds administered at an imaging dose bind specifically to brain amyloid, as Aβ plaques were only marginally captured in PET analyses of amyloid precursor protein transgenic (Tg) mouse brains.[23,24] To overcome the insensitivity of amyloid detection in mice, we have recently attempted to use PIB synthesized with high specific radioactivity, which supposedly increased the detectability of non-abundant binding sites, and successfully performed PET visualization of progressively depositing Aβ aggregates in the brains of amyloid precursor protein Tg mice (Fig. 3).[25] In addition, our *in vitro* assays revealed preferential binding of PIB to N-terminally modified Aβ, Aβ-N3-pyroglutamate (Fig. 4).[25] Interestingly, Aβ-N3-pyroglutamate is known to be a major constituent of pathological Aβ deposits in AD brains,[26] is more prone to fibrillization than unmodified Aβ species,[27] and has been critically implicated in Aβ-induced neurotoxicity.[28] These findings possibly support the advantages of tracers with high specific radioactivity in the sensitive detection of mouse amyloid and highlight the significance of Aβ-N3-pyroglutamate as a new diagnostic and therapeutic target that can be specifically monitored by amyloid PET imaging. In the interim, interspecies differences in levels of Aβ-N3-pyroglutamate indicate requirements for the generation of a better mouse model recapitulating AD-characteristic Aβ plaque compositions.[29]

IMAGING OF MICROGLIAL ACTIVATION

It is well known that amyloid deposition triggers microglial and astroglial activation in AD brains.[30] Moreover, beneficial outcomes of Aβ and tau immunization in humans and mouse models have highlighted the

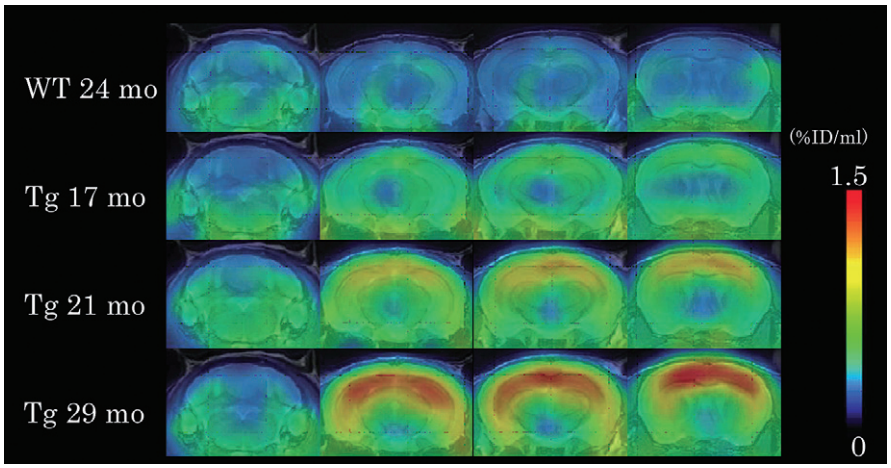

Figure 3 *In vivo* detection of amyloid plaques in wild-type (WT) and APP Tg mice at different ages. PET images were generated by averaging dynamic scan data at 30–60 min after administration of PIB and overlaid on the magnetic resonance imaging template. From left to right, panels represent coronal images at 7, 3, 2 and 0 mm posterior to the bregma. APP, amyloid precursor protein; PET, positron emission tomography; Tg, transgenic.

crucial roles that immunocompetent glia play in protecting neurons against amyloid toxicities.[31] In contrast, our study of tau Tg mice demonstrated attenuated tau accumulation and neuronal loss by suppressing neuroinflammation with an immunosuppressant,[32] suggesting two distinct roles of activated glia involving either detrimental action or protective action on neurons. Upregulation of 18-kDa translocator protein (TSPO), also known as peripheral benzodiazepine receptor, in activated glia is of diagnostic importance in neurological diseases,[33] and several different PET radioligands for this molecule are now available or are under development.[34] Elevated TSPO levels in living AD brains were initially detected with [^{11}C] 1-[2-chlorophenyl]-N-methyl-N-[1-methyl-propyl]-3-isoquinoline carboxamide,[35] and compounds with improved blood-brain barrier permeability and affinity for TSPO, including [^{11}C] N-5-fluoro-2-phenoxyphenyl) -N-(2,5-dimethoxybenzyl)acetamide (DAA1106) and [^{18}F]fluoroethoxy-DAA1106 ([^{18}F]FEDAA1106),[36,37] were developed and applied to neuroimaging of AD patients.[38–40] Autoradiographic assays of model mice with [^{18}F]FEDAA1106 combined with immunohistochemistry have also indicated that microglial TSPO expression is linked to toxic injuries of neurons and may herald neuronal death.[32,41,42] Furthermore, we have developed a novel class of TSPO ligands, [^{11}C] N-benzyl-N-ethyl-2-(7-methyl-8-oxo-2-phenyl-7,8-dihydro-9H-purin-9-yl)acetamide ([11C]AC5216) and its analogues,[43–45] which exhibit faster kinetics in the brain than DAA1106 families. These newer probes potentially produce high-contrast TSPO images and are likely to allow assessment of neurotoxic insults from an early pathogenic stage. Additionally, despite notably enhanced *in vivo* binding of the amyloid radiotracer [^{11}C]PIB, only modest TSPO elevation was observed in aged amyloid precursor protein Tg mice as compared to the tau Tg mice. In these mice, [11C]AC5216 yielded better TSPO contrasts than [^{18}F]FEDAA1106, supporting the possibility of capturing early neurotoxicity with high-performance TSPO probes.[46] An additional line of mouse modelling intraneuronal Aβ accumulation displayed markedly elevated TSPO signals, but this followed noticeable neuronal loss, unlike TSPO upregulation preceding massive neuronal death in tau Tg mice. These data corroborate the utility of TSPO-PET imaging primarily as a biomarker for tau-triggered toxicity and as a complement to amyloid scans for diagnostic assessment of tauopathies with and without Aβ pathologies.[32,47–49] Longitudinal TSPO-PET could also serve to stringently regulate microglial activity within an adequate range during the course of anti-amyloid treatments.

IMAGING OF CHOLINERGIC FUNCTION

Parkinson's disease (PD) is frequently associated with cognitive deficits that can range from subtle deficiencies to dementia.[50] These cognitive deficits are attributed mainly to Lewy body and Lewy neurite pathologies in the cerebral cortex and limbic structures, and partly to AD-type pathology in the forebrain and to the core PD pathology in the substantia nigra and brainstem. Alterations of ascending cholinergic systems from the basal forebrain and the brainstem have been implicated as pivotal players in cognitive

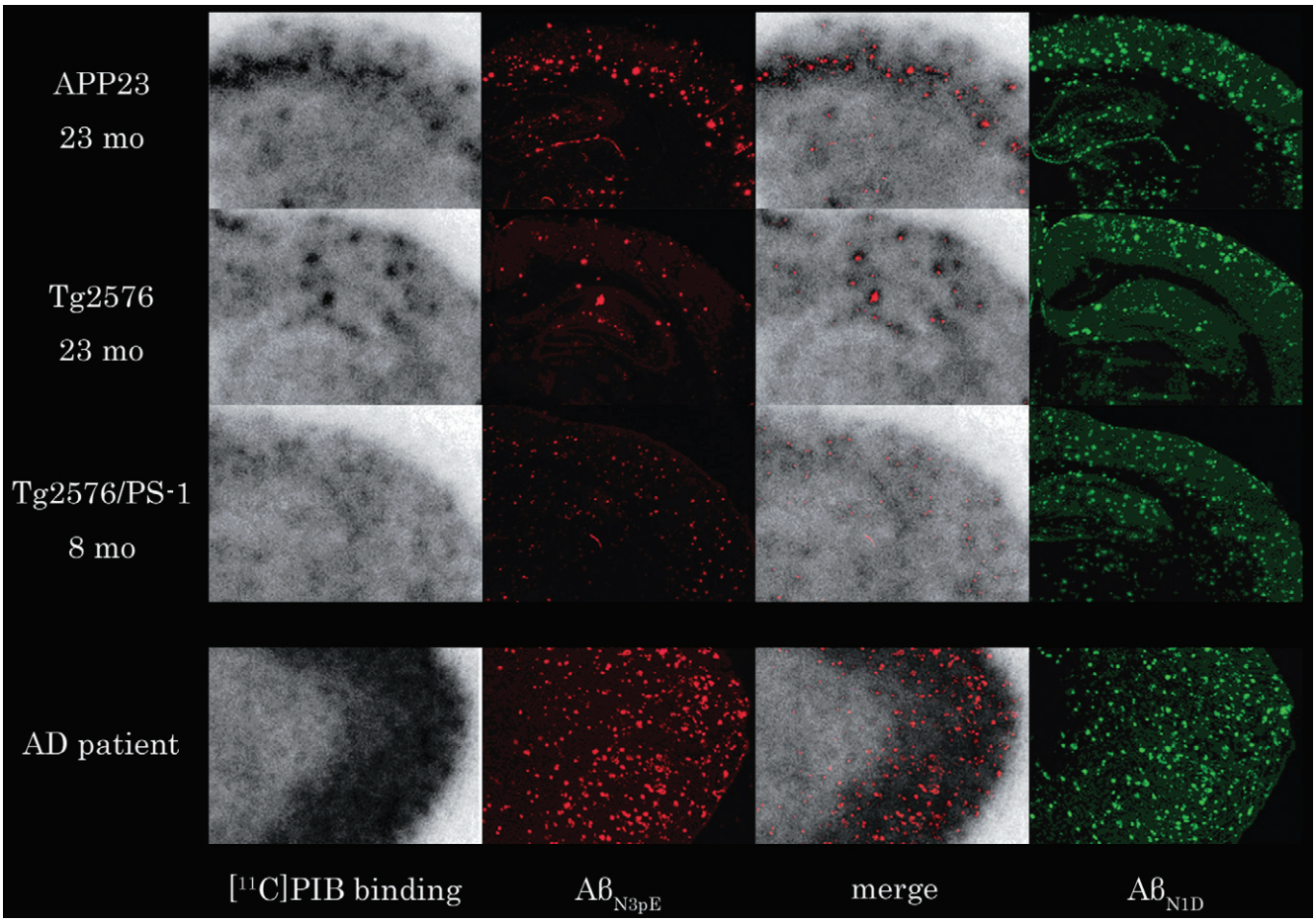

Figure 4 Search for Aβ subtypes accumulating in close relation to [^{11}C]PIB binding sites and association between *in vitro* radiolabelling of amyloid with [^{11}C]PIB and N-terminal truncation/modification of Aβ. Brain sections from a 23-month-old APP23 mouse (top row), 23-month-old Tg2576 mouse (second row), 8-month-old presenilin-1(PS-1)/APP double Tg mouse (third row), and AD patient (bottom row) were used for autoradiography with [^{11}C]PIB (autoradiograms are shown in the first column to the left), which were then immunostained with a polyclonal antibody against Aβ-N3-pyroglutamate ($Aβ_{N3pE}$) (second column). Co-localization of PIB radiolabelling with $Aβ_{N3pE}$ deposition was assessed by merging autoradiograms with immunohistochemical data (third column). Sub-adjacent sections were also immunolabeled with a polyclonal antibody against N-terminally unmodified Aβ, $Aβ_{N1D}$ (fourth column). Aβ, amyloid β peptide; AD, Alzheimer's disease; APP, amyloid precursor protein; PIB, Pittsburgh Compound-B; Tg, transgenic.

dysfunctions, especially impaired attention and consciousness.[51] Loss of cholinergic neurons in the nucleus basalis of Mynert has been observed in postmortem PD brains and has been thought to contribute to cognitive decline in PD.[52] PD and dementia with Lewy bodies (DLB) are regarded as two ends of the spectrum of Lewy body disease. Cholinergic deficits may play a role in these diseases, and widespread and profound reduction of choline acetyltransferase (AChE) activity has been observed in postmortem brains with DLB. Brain cholinergic function can be assayed by measuring AChE activity in the brain with PET and radiolabeled acetylcholine analogues as exemplified by N-[^{11}C]-methyl-4-piperidyl acetate ([11C]MP4A) and N-[^{11}C]-methyl-4-piperidyl propionate.[53–55] Some PET studies showed a significant reduction in cortical AChE activity in PD,[56,57] and this decrease was more sizeable in PD with dementia and DLB than in AD. Using [11C]MP4A, we demonstrated that an early PD group exhibited a reduction in AChE activity in the cerebral cortex, particularly in the medial occipital cortex. Moreover, we revealed that the widespread and pronounced reduction of AChE activity in the cerebral cortices, particularly in the posterior cortical regions, in patients with PD with dementia and DLB. The decline in cerebral cortical

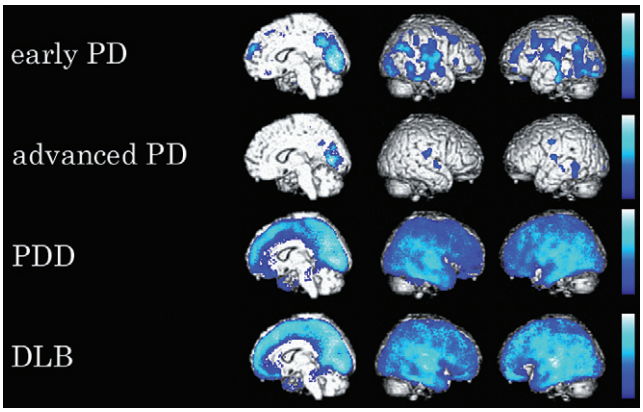

Figure 5 Regions showing a reduction in k^3, a rate constant for AChE activity, observed in early-stage PD, advanced-stage PD, PDD, and DLB, compared with healthy controls. The white-to-blue colour gradient indicates the locations and magnitude of the decrease in k^3 values. AChE, acetyltransferase; DLB, dementia with Lewy bodies; PD, Parkinson's disease; PDD, Parkinson's disease with dementia.

AChE activity was more profound in PD with dementia/DLB patients than in PD patients without dementia (Fig. 5).[58] We also found that cerebral cortical AChE activity was moderately reduced in corticobasal syndrome and mildly reduced in progressive supranuclear palsy, while thalamic AChE activity was remarkably reduced only in supranuclear palsy. Unlike these illnesses, there was no AChE activity deficit in the cerebral cortex and thalamus of patients with frontotemporal dementia.[59,60] In AD patients, reduced AChE activity after treatment with donepezil was shown in our PET study with [11C]MP4A,[61] implying potential usefulness of cholinergic PET imaging for evaluation of symptomatic treatments for dementia.

THE ALZHEIMER'S DISEASE NEUROIMAGING INITIATIVE (ADNI)

The ADNI, initiated in October 2004, was a 6-year research project focusing on studies of changes in cognition, brain structure and biomarkers in cognitively healthy elderly and subjects with MCI and AD. This project was aimed at determining and validating standardized protocols of magnetic resonance imaging, PET imaging and cerebrospinal fluid/blood biomarker measurements, and evaluating outputs of these assays as predictors of disease onset and progression and outcomes in future clinical trials of anti-AD treatments in the USA (http://adni.loni.ucla.edu/). One report in this study demonstrated that a decline in episodic memory may be mechanistically associated with Aβ-induced hippocampal atrophy in PIB-positive MCI subjects and even in cognitively normal elderly individuals, strengthening the implication that levels of PIB uptake in non-demented populations is biologically meaningful.[62] Another arm of the study reported a dissociation between the rate of amyloid deposition and the rate of neurodegeneration late in life, with amyloid deposition proceeding at a constant slow rate while neurodegeneration accelerates; this section also reported that clinical symptoms are coupled to neurodegeneration but not amyloid deposition.[63] Moreover, this project suggested that the earliest detectable changes are those related to Aβ, as detected by cerebrospinal fluid tests and amyloid PET imaging. The establishment of the ADNI stimulated many other related efforts, including the Japanese ADNI (http://www.j-adni.org/), which was started as a part of the worldwide ADNI.

PRECLINICAL STAGES OF AD

On the basis of the above-mentioned evidence, the use of AD-specific biomarkers for *in vivo* staging of the disease has been proposed.[64] These biomarkers exhibit abnormality in a temporally ordered manner as the disease progresses. Changes in Aβ-related biomarkers, such as cerebrospinal fluid Aβ42 and amyloid PET imaging, become prominent before the appearance of clinical symptoms and nearly reach a plateau by the time clinical symptoms emerge.[64] In addition, an international workgroup of the National Institute on Aging-Alzheimer's Association suggested that AD should be optimally treated at a 'presymptomatic' or 'preclinical' stage before the occurrence of significant cognitive impairments.[65] It is possible that individuals with abnormal biomarker levels already develop initial neurodegeneration, and amyloid-modifying therapies may be less efficacious after the downstream pathological process is set in motion.

CONCLUSION

We have reviewed molecular imaging research on dementia by focusing on PET studies. Establishment of radiolabeled tracers optimized for *in vivo* imaging of Aβ plaques could permit pathology-based diagnosis of AD at an asymptomatic stage and allow early immunotherapeutic intervention. Imaging agents for microglial activation and cholinergic function are also useful

research tools, and thus, PET will be in even greater demand as a clinical and basic research technology.

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