1. Introduction

The number of patients with dementia has been increasing exponentially with the aging of societies in advanced and Asian countries. About 24,300,000 people are considered to have dementia worldwide. More than 2,500,000 people suffer from dementia in Japan. A half of the cases of dementia is caused by Alzheimer’s disease (AD). Over the last 10 years, AD research has clarified that the pathological process of AD is initiated by Aβ amyloidosis with secondary tauopathy, highlighting a strategy for developing drugs that may improve or cure AD. Mild cognitive impairment (MCI) as a prodromal stage of AD, and the pathogenesis of Dementia with Lewy bodies (DLB) and Frontotemporal lobar degeneration (FTLD) as a non-AD type dementia have also been elucidated. Currently, a consortium study by the Alzheimer Disease Neuroimaging Initiative (ADNI) is being performed to establish global standardization regarding neuropsychiatric tests, cerebrospinal fluid (CSF) biomarkers, and neuroimaging including magnetic resonance imaging (MRI), fluorodeoxy glucose (FDG) – positron emission tomography (PET), and amyloid PET to predict the progression from MCI to AD and to promote studies of basic therapy for AD (1).

In each advanced country, clinical guidelines for the diagnosis and treatment of dementia have been established. Studies to establish clinical evidence for symptomatic drugs, such as donepezil, rivastigmine, and memantine; cognitive rehabilitation; and care for AD are now ongoing. Based on this progress, the revision of diagnostic criteria for AD by the National Institute of Neurological Disorders and Stroke / Alzheimer’s Disease and Related Disorders Association (NINDS/ADRD) was proposed by National Institute of Aging (NIA) and Alzheimer’s Association (AA) work groups after an interval of 27 years (2, 3). Approximately 10 years have passed since the usage of donepezil, start of public nursing care insurance to care for patients and their families, and legislative preparations in Japan. Clinical guidelines for dementia treatment were reviewed by the Japanese Neurological Society and 5 related societies (4). Now are we involved in the new step of clinical molecular and genetic studies on AD.

Abstract. Recent advances in clinical molecular and genetic studies on Alzheimer’s disease (AD) are summarized here. Cerebrospinal fluid (CSF) Aβ42 and tau are the most sensitive biomarkers for the diagnosis of AD and prediction of its onset following mild cognitive impairment (MCI). Based on this progress, new diagnostic criteria for AD dementia, MCI due to AD, and preclinical AD were proposed by the National Institute of Aging (NIA) and Alzheimer’s Association (AA) in April 2011. In these new criteria, progress in CSF biomarker and amyloid imaging studies over the past 10 years has added to critical information. The marked contributions of basic and clinical studies have established clinical evidence supporting these markers. Based on this progress, essential curative therapy for AD is urgently expected.

Keywords: Alzheimer’s disease, mild cognitive impairment, amyloid β (Aβ), tau, biomarker
2. Advances in studies on pathogenesis of AD

In 1999, presenilin was shown to be the main component of γ-secretase by Selkoe (5), and the possibility of medical treatment using amyloid β (Aβ) vaccine or antibodies to Aβ was reported by Schenk in the same year (6). After these epochal findings, an enormous effort to elucidate the presenilin–γ-secretase complex, Aβ generation/metabolism, and its regulation system has been made to identify radical therapy for Aβ amyloidosis (Fig. 1). The first large-scale multicenter follow-up study of the decreased level Aβ42 and increased total tau level in CSF as diagnostic markers of AD was reported in 1998 (7). The first report on MCI by Petersen was published in 1999 (8), and the first report on Pittsburgh Compound B (PIB) amyloid PET by Klunk was published in 2004 (9). The usage of donepezil was initiated in 1999, and the nursing-care insurance system was started in 2000, leading to marked changes in the treatment and care systems for dementia in Japan.

In studies of AD-related genes, almost all familial gene variation in AD concentrates on APP, PSEN1, and PSEN2, and APOE genetic polymorphism is the strongest risk factor for AD. Associated genetic studies on AD have also revealed the presence of many associated genes such as DMP, SORL1, CTNNA3, PCDH11X, CLU, CR1, PICALM, BIN1, CNTN5, MS4A4A/MS4A6E, EPHA1, CD33, or CD2AP, involving large-scale genome-wide studies (10, 11). In contrast, large scale prospective studies to evaluate and standardize the natural course of the pathological and clinical development of AD have also been performed, such as the Braak and Braak stage classification of AD pathology (12), Nun study (13), and Hisayamamachi study (14). For evaluation of the cognitive function or efficiency of drug trials, global standardizations of neuropsychiatry tests, such as Mini-mental state examination (MMSE), clinical dementia rating (CDR), Wechsler memory scale-revised

![Fig. 1. Summary of advances in Alzheimer’s research over the last 10 years. The gain of function role of these responsible gene mutations finally causes an increased synthesis of Aβ42 or Aβ oligomers. Amyloid β protein precursor (βAPP) is cut by β-secretase (BACE1) to produce secretory forms of βAPPα/β and CTF (C-terminal fragments of βAPP) and then cut by γ-secretase (presenilin-1, 2/nicastrin/APH1/PEN2/TMP21 complex) to generate Aβ and AICD (βAPP intracellular domain). Based on these findings, possible anti-β amyloidosis therapies including β-, γ-secretase inhibitors and anti-Aβ antibodies and anti-Aβ oligomer antibodies have been studied.](image-url)
(WMS-R), and Wechsler adult intelligence scale-III (WAIS-III), and Alzheimer’s Disease Assessment Scale cognitive part (ADAS-Cog) have also been carried out (1). In neuroimaging diagnosis, 3 tesla MRI imaging, high-resolution single photon emission computed tomography (SPECT), and FDG-PET using statistical image processing such as three-dimensional stereotactic surface projections (3D-SSP) became clinically available, and the validity of these neuroimaging approaches was established (4). The rapid development and spread of amyloid PET images have provided a qualitative aspect to the diagnosis and discrimination of AD. Thus, many studies on the neuropsychiatric characteristics, risk factors, neuroimaging, biomarkers, natural course, and progression of MCI have been conducted over the last 10 years and the findings have led to the current ADNI study (1).

Immunotherapy for Aβ amyloidosis has received the attention of the entire world, leading to many interesting findings; however, markedly effective results have yet to be reported. Long-term observation results from a 5-year follow-up study involving a phase I trial of the Aβ42 vaccine AN1792 were reported in 2008, and the side effects, encephalitis, and failure to improve the cognitive function though brain amyloid accumulation disappeared. However, effective results employing the Disability Assessment for Dementia scale and Dependence Scale for antibody positive participant groups were reported in a follow-up survey lasting 4.6 years involving a phase II trial in 2009 (15). Improvement of the brain amyloid burden and neurite abnormality in the hippocampus and decreased phosphorylated tau-positive nerve cells were also shown, suggesting that Aβ antibody therapy may be effective for a tauopathy-associated pathology (16). At least 7 clinical trials of different anti-Aβ antibodies are now ongoing in phase II to III stages. As for semagacestat, a γ-secretase inhibitor, a clinical trial was canceled in phase II due to there being no improvement of cognitive dysfunction and disability levels of Activities of Daily Living (ADL) and the development of skin cancer. Since semagacestat was seen as a promising drug for Aβ amyloidosis among other γ-secretase inhibitors, cancellation of the clinical trial was very disappointing. It seems that the development of a radical therapeutic drug will take more time. Candidates for new disease-modifying drugs that reduce Aβ amyloidosis and improve the cognitive function were reported in Japan, such as apomorphine (17) or anti-Aβ oligomer antibody (18), although they remain at animal experimental stages.

3. CSF Aβ40, Aβ42, tau, and phosphorylated tau

Aβ levels in CSF are controlled by orexin, suggesting the presence of a daily change in the CSF Aβ level (19). Aβ is produced mainly in the nerve cells of the brain, and it is secreted about 12 h later into the CSF, then excreted through the blood–brain barrier 24-h later into blood (Aβ clearance), and finally degraded in the reticuloendothelial system (20). In AD brains, Aβ42 forms insoluble amyloids and accumulates as insoluble amyloid fibrils in the brain. The reason why Aβ42 levels are decreased in the CSF of AD patients is considered to be the deterioration of physiologic Aβ clearance into the CSF in AD brains (21). CSF total tau levels increase slightly with aging. However, CSF tau levels show a 3-fold greater increase in AD patients than in normal controls. The CSF tau level is related to the degeneration of axons and neurons and to severe destructive disease of the nervous system. Several diseases show slightly increased tau levels such as vascular dementia (VaD), multiple sclerosis, AIDS dementia, head injury, and tauopathy (22).

4. Clinical evidence, sensitivity, and specificity

In the first large-scale collaborative multicenter study of CSF Aβ40, Aβ42, and total tau, a total of 236 subjects were followed in Japan and showed a diagnostic sensitivity of 71% – 91% and specificity of 83% in AD in 1998 (7). A European and American large-scale multicenter study showed a diagnostic sensitivity of 85% and specificity of 86% (23). A European and American large-scale multicenter Japanese study of CSF total tau alone in 1,031 subjects reported a sensitivity of 59% and specificity of 90% for a diagnosis of AD (22). Practical guidelines for dementia proposed by the American Academy of Neurology in 2001 showed a diagnostic sensitivity of 78% – 92% and specificity of 81% – 83% (24).

In the examination of CSF phosphorylated tau, the first study of p-tau199 was reported as a large-scale multicenter collaborative study by a Japanese group and showed a diagnostic sensitivity of 85% and specificity of 85% in a comparison between AD and non-AD disease controls (25). Assay systems using different epitopes of phosphorylated tau (p-tau231, p-tau181, and p-tau199) have been internationally standardized. A systematic review of CSF biomarkers for AD in 2001 analyzed 41 studies of CSF total tau, 15 studies of Aβ42, and 11 studies of p-tau; 5 studies on the diagnosis of early AD; and 9 studies of MCI; it showed that the respective specificities and sensitivities were 90% and 81% for CSF total tau, 90% and 86% for CSF Aβ42, 92% and 80% for CSF phosphorylated tau, and 83% – 100% and 85% – 95% in the combination assay with CSF Aβ42 and total tau (26).
5. CSF biomarkers for prediction of the onset of AD from MCI

A study following 52 MCI subjects for 8.4 months found that 29 MCI subjects developed AD and the specificity of the CSF Aβ42 assay was 90% (27). A follow-up study of 273 subjects for 2 years showed that 38% of MCI already showed the alteration of at least 2 CSF biomarkers including Aβ42, total tau, and p-tau181 at the time of study initiation. Ten of 11 MCI patients who progressed to AD showed the alteration of at least 2 CSF biomarkers, and all 11 converters showed high p-tau181 levels in CSF. Conversely, 29 (88%) of the 33 stable MCI subjects did not show any alteration of CSF biomarkers (28). The longest prospective study (4 – 6 years) followed 137 MCI and 39 normal subjects and showed that the onset of AD was predicted with a sensitivity of 95% and specificity of 83% using CSF Aβ42 and total tau assays. The addition of the p-tau181 assay further improved sensitivity and specificity to 95% and 97%, respectively (29). In comparison studies between CSF biomarkers and amyloid imaging by PIB-PET, CSF Aβ42 levels were related to brain amyloid deposits in patients with or without dementia (30). In the United States-ADNI, 819 healthy subjects (229 normal, 398 MCI, 192 mild AD) were prospectively studied. The results one year later showed that the mean AD conversion/year was 16.5%. Among all CSF biomarkers, Aβ42 at the initial evaluation was the most reliable marker predicting conversion from MCI to AD and the progression of cognitive impairment. Using the CSF total tau/Aβ42 ratio, 33 converters (89%) were predicted before onset among 37 subjects who progressed from MCI to AD within one year (31).

6. Revised diagnostic criteria for AD

The NINCDS-ADRDA as the standard clinical criteria for AD discrimimates probable AD with a sensitivity of 81% and specificity of 70%. Based on progress in research during these 27 years, NIA and AA proposed new diagnostic criteria of AD in April 2011 (2). Conventional probable AD was called probable AD Dementia. The criteria were divided into core clinical criteria based on clinical symptoms and research criteria including CSF biomarkers, PIB amyloid PET, FDG-PET, MRI, and genetic tests. MCI due to AD is proposed as a possible converter to AD dementia with a decline in specific neuropsychiatric tests (1 – 1.5 SD) (3). A large prospective consortium study of MCI due to AD from preclinical AD is going to be performed in ADNI-II following ADNI, and further fruitful results are expected over the next 5 years.

7. Conclusion: Aβ cascade hypothesis or Aβ/tau cascade hypothesis?

Here, I summarized the advances in the clinical, molecular, and genetic studies over the last 10 years and suggested future prospects. However, when will we reach the stage of radical therapy and prevention of AD as the final aim? Studies on AD continuously showed essential approaches and methods to clarify the pathogenesis and how to evaluate clinical issues and develop radical therapy as crucial in the field of research on neurodegenerative disorders over the last 10 years. The development of many candidate drugs for radical therapy such as Aβ vaccines or γ-secretase inhibitors is now hampered by the lack of phase III clinical trials. A large degree of skepticism exists regarding the Aβ amyloid cascade hypothesis because aggravation of dementia is not stopped even if the Aβ vaccine removes Aβ amyloid deposits. Revisions of the Aβ cascade hypothesis have been announced by Hardy and Hymann, who initially proposed this hypothesis (32, 33). Aβ amyloidosis, tauopathy, and neuronal cell death were cardinal clues to clarify all pathological processes from the early to late stages of AD, and researchers changed the focus repeatedly depending on the trend of the times. It should be considered that many issues still remain to be clarified from Aβ amyloidosis to tauopathy leading to nerve cell losses. I support this thought based on the findings of associated genes detected by global genome-wide studies and the results using animal models that represent Aβ amyloidosis and tauopathy. It is necessary to elucidate all associated molecules in every step of the pathological cascades of AD and refine the target molecules to develop a therapy to cure AD.

References

5. Wolfe MS, Xia W, Ostaszewski BL, Diehl TS, Kimberly WT,


