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Trends in Research on Dementia
— Discussion Centering on Alzheimer's Disease —

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2.1 Introduction

In these days when declining birth rates and graying are reported to be ongoing in Japan, many people desire not only to live a long life but also to spend their old days in good health. In this increasingly graying society, we might be obliged to not only receive care but also administer care to someone. According to the report on the "Basic Investigation of National Life" in 1998, published by the Ministry of Health, Labor and Welfare, about 81% (one million people) of the roughly 1.24 million Japanese citizens requiring care at home is represented by people aged 65 years or older. Furthermore, about 53% of the people who are administering care to those aged 65 years or older requiring at-home care is constituted by the elderly aged 60 years or more.

Under these circumstances, dementia has become a grave concern to many people. According to the "Report on Special Investigation of the Actual Conditions and Health of the Elderly" in 1997, published by the Tokyo metropolitan government, about 4% of the elderly aged 65 years or more living at home have developed dementia. Furthermore, according to the report on the "Projected future population of Japan" in 1997, published by the National Institute of Population and Social Security Research, Ministry of Health, Labor and Welfare, the number of elderly people with dementia in Japan reached 1.56 million in 2000, which is expected to increase sharply to 2.26 million in 2010, and further to 2.92 million in 2020.

Against this backdrop, in the Phase Two Technology Master Plan (adopted at the Cabinet meeting held in March 2001), elucidation of the mechanism of brain aging and suppression of nervous system diseases were selected as strategic challenges to be addressed with high priority in order to tackle national and social problems.

In the 7th Technology Foresight, whose results were published by the National Institute of Science and Technology Policy, survey of the current state of research on Alzheimer's disease, a common type of dementia, was conducted and the outlook for the challenges to be addressed through research on the disease was given. The challenges mentioned and year when each of those challenges is expected to be accomplished is as follows:

- "Elucidation of the mechanism of development of Alzheimer's disease"..... 2014
- "Inhibition of the progression of Alzheimer's disease"..... 2017
- "Development of therapy that completely cures Alzheimer's disease"..... 2020

Under circumstances where the Japanese government has taken various measures to cope with problems arising in this graying Japanese society such as the provision of nursing care services and establishment of facilities for nursing care, to what extent has research targeting the suppression of Alzheimer's disease been pursued?

In order to find the answer to this question, we invited Professor Takeshi Iwatsubo, Graduate School of Pharmaceutical Sciences, Faculty of Pharmaceutical Sciences, University of Tokyo to have him deliver a lecture about recent trends in research on dementia, especially on Alzheimer's disease, at the Institute of Policy for Science and Technology on December 11, 2001, and wrote this article as the summary of the contents of his

lecture while incorporating the data from our study.

This report discusses the "General description of dementia (section 2.2), "Characteristics of Alzheimer's disease" (section 2.3), "Current state of research on the mechanism of Alzheimer's disease development" (section 2.4), "Current state of development work on pharmaceutical products for Alzheimer's disease" (section 2.5), and "Strategies for promoting research on Alzheimer's disease in Japan" (section 2.6), and, in the last section, gives a summary of "Challenges to be addressed through research on Alzheimer's disease" (section 2.7).

2.2 General description of dementia

Dementia is a disorder of memory and intelligence (judgement, cognitive function, etc.) in adults. With aging, people become more forgetful but can live a normal life as a member of society if they still have normal judgement, etc. On the other hand, patients with dementia have not only memory disturbance but also intelligence disorder, so they cannot live a normal life as a member of society. In most cases, dementia occurs when nerve cells in a certain region of the brain are damaged from some cause and are lost.

Dementia can be classified into various groups including senile dementia, dementia caused by abnormality of proteins, etc. (e.g., Alzheimer's disease), that following the onset of cerebrovascular disease (cerebrovascular dementia), that associated with the displacement of a part of the brain (displacement of neurovascular structures), that caused by brain tumors, etc., as well as due to infectious diseases including prion disease, etc.

The two most common types of dementia are Alzheimer's disease and cerebrovascular dementia.

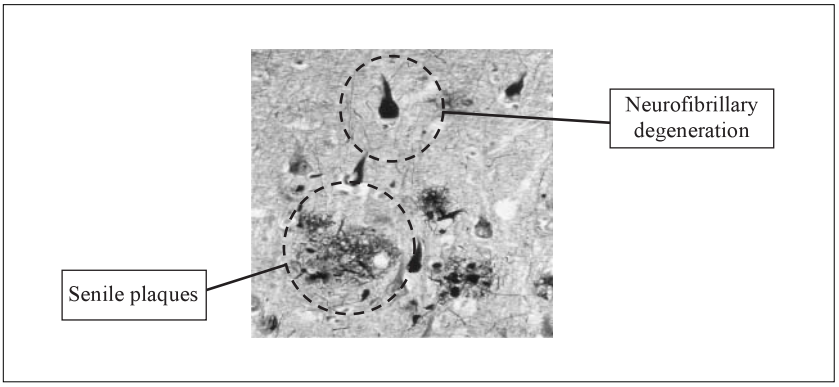
2.3 Characteristics of Alzheimer's disease

Dementia is diagnosed by step-by-step elimination of other diagnoses, which are conceivable in light of the patients' clinical history, as well as findings from physical examination or from diagnostic imaging, etc. However, in the last ten some odd years, studies on Alzheimer's disease have greatly advanced and produced a variety of scientific evidences.

The first report on a patient with Alzheimer's disease (AD) was made by Alois Alzheimer in 1906. The patient died at the age of 51. During autopsy, marked cerebral atrophy (shrinkage of the brain) was observed, and many granular structures and degenerated nerve cells with thick tangled fibrils were seen mainly in the cerebral cortex.

Principal symptoms of Alzheimer's disease include progressive and irreversible dementia, particularly memory disorder, orientation disturbance (impairment in the ability to recognize one's surroundings and their temporal and spatial relationship to oneself), as well as impaired judgement. In the brain of patients with Alzheimer's disease, characteristic histo-pathological changes are noted such as marked cerebral atrophy, as well as deposition of senile

Figure 1: Senile plaques and neurofibrillary degeneration



Brain tissue sample taken from a patient with Alzheimer's disease was stained using a special staining technique.

Source: Materials provided by Professor Takeshi Iwatsubo

plaques (Figure 1) and tangled bundles of fibers called neurofibrillary tangles (Figure 1) due to the accumulation of abnormal proteins. Other brain changes in people with Alzheimer's disease include death and loss of nerve cells (neuronal loss) in areas of the brain (cerebral neocortex and hippocampus) that are vital to higher-order cognitive functions such as memory and learning.

The senile plaque is histopathological change observed only in patients with Alzheimer's disease or Down's syndrome, or the elderly, while neurofibrillary degeneration is associated with various diseases. During the course of Alzheimer's disease, senile plaques appear at the earlier stage, and neurofibrillary degeneration takes place at the advanced stage of the disease.

After the first report by Dr. Alois Alzheimer, studies on these structures started. Electron microscopic studies and subsequent immunohistochemical studies have provided clues for clarifying the causes of the accumulation of abnormal proteins. Currently, the mechanism of the development of Alzheimer's disease is well on the way to becoming elucidated by studies also adopting molecular biological techniques.

A certain type of Alzheimer's disease (familial Alzheimer's disease) has been found to occur in specific families. Molecular generic studies in such families have yielded many significant findings. In around the early 1990s, patients with Alzheimer's disease were found to have mutation on genes from a protein called amyloid beta. In parallel with studies on such genes, research on causative agents of neurofibrillary degeneration (see section 2.4.3) had been pursued. Then, researchers trying to elucidate the causative agents of Alzheimer's disease had competed fiercely to identify the major constituents of the two histopathologically abnormal structures, i.e., senile plaques and neurofibrillary tangles. At present, based on the results of many studies, the theory that accumulation of amyloid beta is deeply involved in the development of Alzheimer's disease has gained strong support. On the other hand, it has been reported that the major constituent of neurofibrillary tangles seems to play a significant role in inducing nerve cell death.

2.4 Current state of research on the mechanism of Alzheimer's disease development

2.4.1 Major molecules involved in the deposition of senile plaques

Deposition of senile plaques, one of the characteristic histopathological findings seen in the brain of Alzheimer's disease patients, results from the extracellular accumulation of amyloid beta protein (Aβ), which is a protein rarely seen in normal brains. Generally speaking, amyloid refers to the bundle of protein fibrils. Amyloid is barely soluble and is apt to clump (aggregation) and accumulate. The letter "β" in the name of amyloid β, which appears in the brains of Alzheimer's disease patients, is derived from the fact that the protein has a characteristic conformational structure called β sheet. This β sheet structure contributes to the protein's predisposition to aggregate.

Aβ is processed from the amyloid β precursor protein (APP) (Figure 2). The role of APP, which is expressed in all organs and tissues of the body, has not been fully elucidated as of yet.

In the processing of Aβ, two enzymes, i.e., β and γ secretases, are involved; β secretase works first, and then γ secretase.

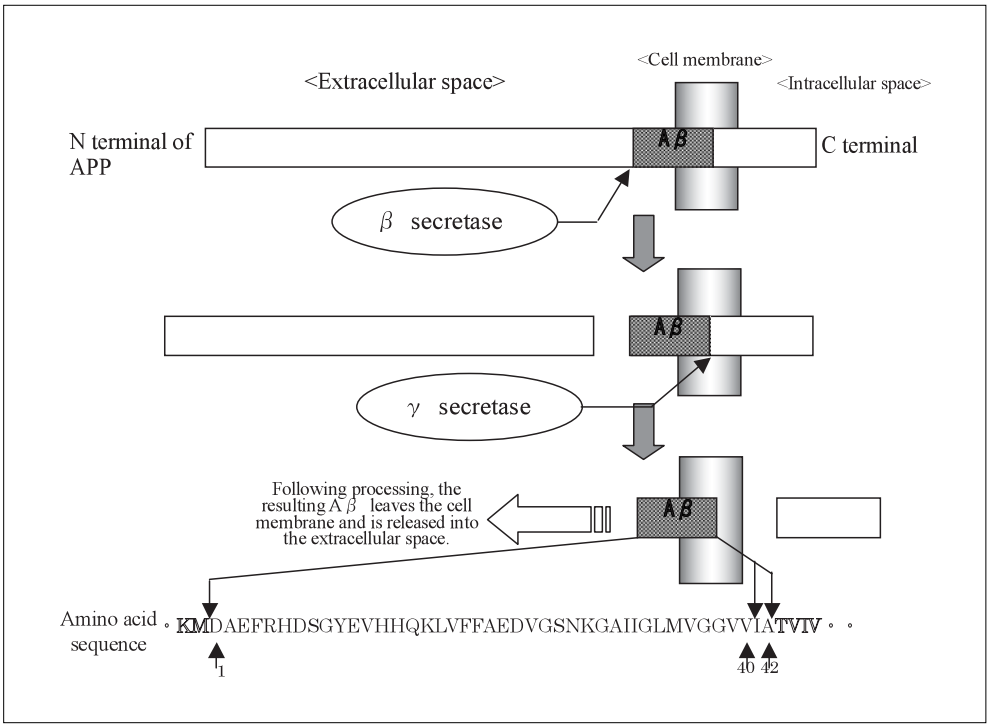
Aβ can be classified into two groups including Aβ40 and Aβ42 according to the number of constituent amino acids. Aβ42 has a stronger tendency to aggregate one another as compared with Aβ40. In addition, Aβ42 is closely involved in the formation and distribution of senile plaques.

2.4.2 Molecules involved in the degradation of Aß

In the human body, Aβ is steadily generated under normal conditions. However, under normal metabolic conditions, Aβ is thought to be rapidly degraded following its generation before it aggregates or accumulates. In studies on non-familial, sporadic Alzheimer's disease, it has been reported that decreased degradation of Aβ may lead to its accumulation.

While the mechanism of the degradation of Aβ has not been clarified, Japanese researchers recently

Figure 2: Processing from APP into Aß



reported new findings with regard to the mechanism. Nishimichi et al. at the Brain Science Institute, the Institute of Physical and Chemical Research has published an article entitled "Identification of the Metabolic Pathway involved in the Decrease of Aβ42 Predominantly Expressed in the Brain Tissue" in the journal Nature Medicine in 2000, which suggests for the first time that an enzyme called "neprilysin" is involved in the degradation of Aβ. Subsequently, in 2001, Nishimichi et al. published an article entitled "Control of Cerebral Metabolism of Aβ by neprilysin" in the journal Science. In the study reported in this article, he demonstrated that Aβ increases in the brain of neprilysin-knockout mice (mice whose genes for neprilysin are knocked out by genetic engineering) , showing that neprilysin is involved in the degradation of Aβ.

2.4.3 Mechanism of the development of neurofibrillary degeneration

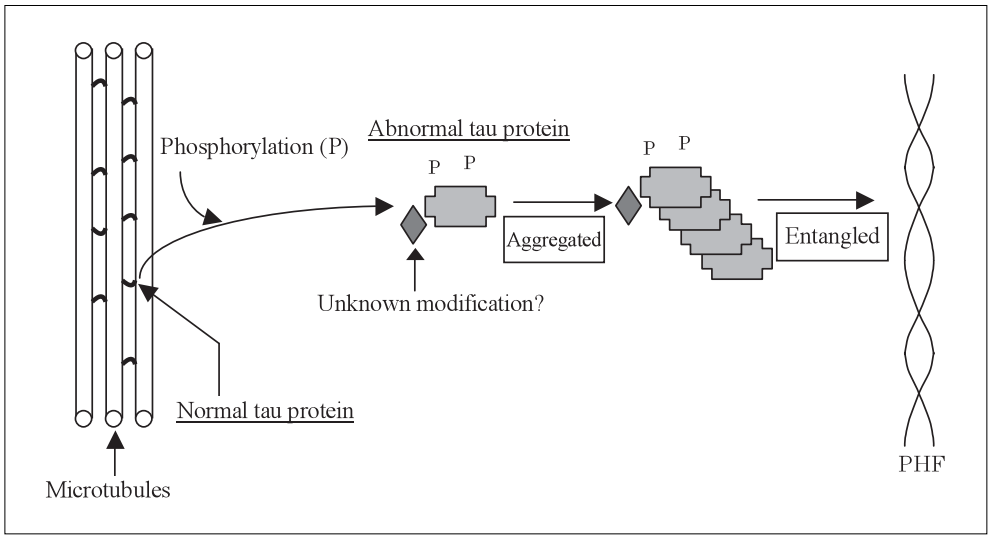
In addition to senile plaques, neurofibrillary tangles are characteristically seen in patients with Alzheimer's disease. As is the case with senile plaques, neurofibrillary degeneration is caused by the accumulation of abnormal proteins.

Neurofibrillary tangles are abnormal fibrous structures and are composed mainly of paired helical filaments (PHF) consisting of two helical proteins. In contrast to Aβ which forms senile plaques extracellularly, PHF is accumulated in nerve cells. Figure 3 shows the process of PHF generation. In nerve cells, there is an organelle called microtubule, whose major functions are to maintain cytoskeleton and transport substances intracellularly. Tau protein is a substance whose function is to place microtubules in a specific direction and at regular spatial intervals to stabilize them. When the tau protein is hyper-phosphorylated for some reason or another, it will leave the microtubule, and hyperphosphorylated tau proteins are believed to aggregate to form PHF. Moreover, it is speculated that PHFs also aggregate one another and accumulate in nerve cells, leading to neurofibrillary degeneration.

2.4.4 Challenges to be addressed through future research

With regard to the mechanism of the development of Alzheimer's disease, observational findings strongly support the hypothesis that onset of the disease is first triggered by the accumulation of Aβ, which is followed by the accumulation of hyperphosphorylated tau proteins, resulting in nerve cell death. Currently, investigation on the pathway from the accumulation of Aβ to the accumulation of hyperphosphorylated tau proteins is being actively conducted. In a recent

Figure 3: Normal tau protein and abnormal tau protein



Source: Authors' compilation by making reference to the materials provided by Professor Iwatsubo.

study using genetically engineered mice, it was reported that a certain relationship was actually noted between Aβ and hyperphosphorylated tau protein.

In addition, research on the mechanism of excessive phosphorylation of tau protein has also been performed. Such research will evolve into that aiming to find the mechanism through which development of Alzheimer's disease can be inhibited.

Furthermore, many studies on the ultimate mechanism of nerve cell death are also in progress. There are some diseases other than Alzheimer's disease, which are caused by excessive phosphorylation. herefore, it is expected that elucidation of the mechanism of the excessive phosphorylation will lead to advances in studies not only on Alzheimer's disease but also on some other diseases.

Concerning familial Alzheimer's disease, results of studies have indicated that there still exist Alzheimer's disease susceptibility genes, which have not been found so far, and the competition to identify such genes is becoming increasingly fierce.

2.5 Current state of development work on pharmaceutical products for Alzheimer's disease

No infallible remedy has been developed for Alzheimer's disease. Since therapeutic drugs for Alzheimer's disease with higher efficacy could produce large profits, research and development aiming at introducing such a drug into the market has been very actively conducted with the involvement of many companies. On the other hand, it is also important to develop prophylaxes, because histopathological changes in the brains of Alzheimer's disease patients have already become irreversible at the time when clinical symptoms of the disease appear.

The following paragraphs in this section briefly introduce major drugs currently used in the treatment of Alzheimer's disease, or are under research and development, as well as the current state of major studies targeting the development of therapeutic drugs.

2.5.1 Drugs for Alzheimer's disease

Currently, not many drugs are used in the treatment of Alzheimer's disease, and the drugs prescribed to patients with the disease are mostly represented by antipsychotic agents, anti-depressants, antianxiety agents, hypnotic drugs, etc., targeting symptoms associated with the disease including depression.

Donepezil hydrochloride (trade name: Aricept) is the only drug so far to have gained marketing approval in Japan for use in improving symptoms specifically seen in patients with Alzheimer's disease (approved in November 1999). It has been shown that acetylcholine, a neurotransmitter associated with memory, etc., decreases in the brains of patients with Alzheimer's disease.

Donepezil hydrochloride is an acetylcholinesterase inhibitor that interferes with the degradation of acetylcholine in the synapse to increase available acetylcholine. The drug can delay the progression of early-stage Alzheimer's disease to a certain degree.

2.5.2 Studies on therapies focusing on the accumulation of Aß

Aβ is thought to be closely involved in the development of Alzheimer's disease. At present, studies on the following five therapies aiming to inhibit the accumulation of Aβ are in progress:

Firstly, reports have been made on studies on "vaccine therapy" (administration of a segment of the Aβ peptide): Alzheimer's disease model mice in which APP is overexpressed were immunized with Aβ, leading to the generation of antibodies, which prevented the accumulation of Aβ within the mouse brain and even markedly decreased existing Aβ deposits, known as plaques. Secondly, results of studies on "antibody therapy" (passive immunization with antibody administration specific to the Aβ peptide) have also been reported. Studies on these two therapies have advanced rapidly and clinical studies on these therapies are underway in the United States. However, concerning the vaccine therapy, new problems to be tackled have arisen recently, including adverse reactions of the central nervous system.

Thirdly, several nonsteroidal anti-inflammatory drugs (NSAIDs) including indomethacin and ibuprofen have been reported to exert effects in the treatment of Alzheimer's disease. In addition, in studies using Alzheimer's disease model mice orally given NSAIDs and experiments using cultured cells, generation and accumulation of Aβ have been reported to decrease. While the mechanism of inhibition of Aβ generation and accumulation by NSAIDs are being clarified, further investigation is required.

Fourthly, studies on gamma-secretase inhibitors specifically targeting gamma secretase have been conducted. In experiments using Alzheimer's disease model mice, gamma secretase inhibitors have been reported to be effective in, for example, inhibiting the accumulation of Aβ. It is expected that gamma secretase inhibitors can be a class of the next-generation drugs, which are effective in preventing the onset and inhibiting the progression of Alzheimer's disease, and clinical studies on some gamma secretase inhibitors have started in the United States. However, gamma secretase has been found to have substrates other than APP, and there is a possibility that gamma secretase-mediated reaction with APP might be disturbed by such substrates, so there remain problems to be resolved before gamma secretase inhibitors are brought to the market as therapeutic drugs for Alzheimer's disease.

Lastly, studies focusing on beta secretase have been actively conducted recently. It has been reported that knockout mice missing genes for beta-secretase do not seem to exhibit any abnormality in general, and that accumulation of Aβ is not seen in the brains of such mice. Many pharmaceutical companies are reportedly competing fiercely to develop gamma secretase inhibitors as therapeutic drugs for Alzheimer's disease, but most of the results of studies they conducted have not been published. Introduction of gamma secretase inhibitors to the market requires further advances in research.

2.5.3 Studies on biochemical markers

Studies have also been performed on specific biochemical markers of Alzheimer's disease. As in the cases of other diseases whose severity or risk can be known from the results of blood tests including the quantitative evaluation of blood sugar and serum cholesterol, risk or stage of Alzheimer's disease might be known from the results of examination on specific biochemical markers, which may possibly lead to the prevention or delay of onset of the disease through adequate prophylactic treatment. Currently, studies are being pursued on the relationship between the level of Aβ or excessively phosphorylated tau protein in cerebrospinal fluid taken from Alzheimer's disease patients and the stage of Alzheimer's disease. However, many problems should be addressed before the utilization of such relationship for diagnosing the disease in that invasiveness of cerebrospinal fluid sampling is so high and that utilization of the levels of Aβ and excessively phosphorylated tau protein require data sampling not only from

patients with the disease but also from people without the disease. Up to this time, no specific molecular markers have been found other than Aβ42 and hyperphosphorylated tau protein in cerebrospinal fluid. Under such current realities, many problems have to be tackled before the preclinical diagnosis of Alzheimer's disease becomes utilizable in actual clinical practice, including the exploration of new molecular markers, determination of reference values and the development of less invasive diagnostic techniques.

2.5.4 Development of new materials for diagnostic imaging with PET

With regard to measures to investigate cerebral histopathological changes in living patients, development work is proceeding to develop techniques for detecting the distribution and the amount of Aβ in senile plaques and neurofibrillary tangles in the brain with the use of PET (positron emission tomography). The first isotope probe for use in such PET examination has been reportedly developed. Currently, fierce competition is being intensified to develop better isotope probes, which can pass through the blood-brain barrier and are specifically bound to Aβ for the intended time with unbound probes going out of the brain rapidly. The PET examination using such isotope probes will serve as a very useful technique in the diagnosis of MCI (mild cognitive impairment) discussed in the following section as well as in future preclinical diagnosis of Alzheimer's disease.

2.5.5 Methods for diagnosing very early dementia

Also under development are methods for diagnosing mild cognitive impairment (MCI: a condition characterized by mild recent memory loss without dementia or significant impairment of other cognitive functions), which is regarded as very early dementia but not clinically diagnosed as dementia. According to a report on Alzheimer's disease published in 1999 by the U.S. National Institute of Health, an epidemiological survey of a community cohort revealed that 40% of people who had been given the diagnosis of MCI in accordance with certain diagnostic criteria, developed Alzheimer's disease within 3 years of the diagnosis. In Japan, efforts are underway to make draft diagnostic criteria for MCI highly adaptable to Japanese people.

2.6 Strategies for promoting research on Alzheimer's disease in Japan

In March 1997, the Bioscience Section of the Scientific Council, Ministry of Education, Culture, Sports, Science and Technology submitted a report titled "Promotion of Brain Research at Universities, etc." and then, in May 1997, the Brain Science Committee, Life Science Section, Council for Science and Technology submitted a report titled "Long-term Prospects of Brain Research." In the latter report, three areas of brain research were set up including "Understanding the Brain," "Protecting the Brain" and "Creating the Brain." In addition, a strategic timetable was developed in the latter report, targeting the suppression of various diseases including Alzheimer's disease, which was planned to be overcome within 15 years (as of 1997). Moreover, also in the Second Science and Technology Basic Plan, elucidation of the mechanism of brain aging and suppression of nervous system diseases were adopted as challenges to be addressed.

Based on the above-mentioned policies, research in the field of brain science in Japan as a whole has been greatly encouraged and studies on dementia have also been promoted. In 1997, the "Brain Science Institute (BSI)" was established under the Institute of Physical and Chemical Research as the engine that will drive brain science research. At the BSI, the Laboratory for Alzheimer's disease and the Laboratory for Proteolytic Neuroscience of the Aging in Psychiatric Research Group take charge of studies on Alzheimer's disease and are investigating the mechanisms of the occurrence of neurofibrillary degeneration, nerve cell death, degradation of Aβ, etc.

Publicly funded studies aiming to address challenges to be dealt with for the suppression of Alzheimer's disease, which were invited from the public, have also been performed. Under the Scientific Research Subsidy System, various challenges to be addressed have been chosen as themes of fundamental studies (in the fields of

pathology, pharmacology, pharmaceutical sciences, medicine, neuroscience, etc.), which are to be promoted with public monetary support. In addition, studies on dementia (including those on Alzheimer's disease) have been conducted as studies in the fields with high priority or in special fields almost in succession since 1989. Furthermore, as part of the Millennium Project, "Studies in Special Fields C" has been set since 2000, and "Studies in Frontier Brain Science" including studies on Alzheimer's disease have been started to deal with one of the themes in the Studies in Special Fields C.

Under the Funds for the Coordination of Advancement of Technology System and under the Core Research for Evolutional Science and Technology (CREST) system set up by the Japan Science and Technology Corporation, brain-related themes have been selected for study to be publicly funded every year from 1997 and from 1995 to 2000, respectively. Under these systems, studies on Alzheimer's disease have been performed from 1996 to 2001.

In addition, also under the Scientific Research Subsidiary System established by the Ministry of Health, Labor and Welfare, studies on Alzheimer's disease have been conducted.

According to the Human Frontier Science Program (HFSP), monetary support has been provided to studies on brain functions within the international framework for the promotion of such studies. Within the framework, basic research on dementia including Alzheimer's dementia has also been performed.

2.7 Conclusion — Challenges to be addressed through research on Alzheimer's disease —

Now that several genes associated with the onset of Alzheimer's disease have been identified, postgenome research on the disease will assume more significance from this day forward. While elucidation of the pathways leading to abnormal protein accumulation is one of the important challenges to be addressed in future studies, as discussed in section 2.4, it is also significant to analyze conformational structures of proteins or complexes of proteins as functional units involved in such pathways. In addition, studies on SNPs (single nucleotide polymorphisms: a kind of genetic polymorphism) associated with sporadic (non-familial) Alzheimer's disease will become more important from now on. In order to promote such postgenome research, it is crucial to have collaboration among specialists in various fields including medicine, pharmaceutical sciences and science.

Since Alzheimer's disease will constitute a bigger concern with the increasing graying of Japanese society, studies on the disease need to be conducted more widely and deeply. In addition, since studies on Alzheimer's disease are not pure academic research but a sort of purpose-oriented research, it is desired that researchers who are not clinicians as well as experts in government, academia and industry should participate in such studies. Some people point out that Japan has no established system to introduce researchers in such fields as science, pharmaceutical sciences and agriculture into research on diseases.

Alzheimer's disease occurs "only in the highly developed human brains" and it is essential, in every study conducted by experts in the government, academia and industry, to use biological samples (tissue samples, DNA samples) taken from patients with the disease. Therefore, it is important to construct systems for appropriate collection and accessible supply of samples for research taken from Alzheimer's disease patients. Until now, even universities with attached hospitals have faced difficulty in obtaining samples for research from affected patients, and most institutes for research on Alzheimer's disease have purchased from overseas brain banks. In order to further facilitate studies on Alzheimer's disease from now on, it is required that brain banks be established in Japan to collect biological samples (samples of brain tissue, cerebrospinal fluid, blood, etc.) from a large number of patients with Alzheimer's disease and supply those samples to researchers with due consideration for the significance of informed consent, protection of private information as well as ethical implications of such sample collection and distribution.

Acknowledgement

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