Challenging the limits of Medical Science: Dementia

Aika Tahara

1. The Introduction

Dementia is a neurodegenerative disease which is said to have multiple factors. It includes many symptoms such as memory loss, disorientation, difficulty in communication, and so affects patients' lives by making it difficult for them to look after themselves.

1.1 Dementia

Dementia can mainly be separated into four kinds: Alzheimer's disease (AD), dementia with Lewy bodies (DLB), frontotemporal dementia (FTD) and vascular dementia (VaD). Each type of dementia has different properties. Alzheimer's disease can be found from damage in the parietal lobe and the temporal lobe, with especially harsh atrophy in the hippocampus (Medical science information institute, 2011, p.344). From a pathological point of view, senile plaques and neurofibrillary tangles are seen. The common symptoms are memory disturbance, disorientation and delusions (Medical science information institute, 2011, p.341). Dementia with Lewy bodies' is identified by a decrease in blood flow around the occipital lobe. This can be found by using single photon emission computed tomography (SPECT) or positron emission tomography (PET) (Medical science information institute, 2011, p.350). It is similar to Parkinson's disease but DLB has Lewy bodies widely spread in the cerebral cortex. In addition, there is a buildup of α-synuclein (Medical science information institute, 2011, p.341). The main symptom is visual hallucinations (Medical science information institute, 2011, p.341). Frontotemporal dementia is identified by damage to the temporal and frontal lobes. Four-fifth of FTD have symptom of pick diseases and other symptoms are personality changes and behavioral abnormalities such as stereotyped behavior (Medical science information institute, 2011, p.351). Vascular dementia can be found after cerebrovascular disorders. It is often the case that cognitive functions decline step-by-step (Medical science information institute, 2011, p.348).

In Japan, one person out of every seven over 65 years old was diagnosed with dementia in 2012 and it is speculated that, by 2025, this will rise to one person out of five (Cabinet Office, Government of Japan, 2016, http://www8.cao.go.jp/kourei/whitepaper/w-2016/html/gaiyou/s1_2_3.html). According to Alzheimer's disease International (ADI), there are about 46,800,000 patients who were suffering from dementia in the Worldwide in 2015. Though the numbers of patients are increasing, the cause and the processes have not been found and it is even said that dementia may be an incurable disease. This is because it is difficult to find out when the disorder starts.

1.2 Alzheimer's disease

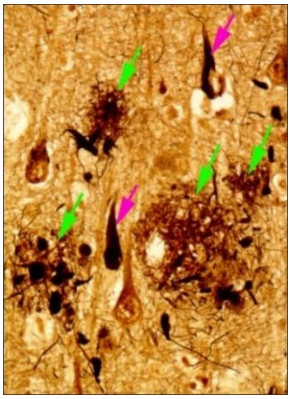
This research focuses on Alzheimer's disease. AD represent around 50% of dementia cases. It is said to be caused by senile plaque and neurofibrillary tangle (Medical science information institute, 2011, p.344). These are usually found in healthy people's brains; too, however, people with AD have this all over their brains, which can cause brain atrophy (Medical science information institute, 2011, p.344). Senile plaque is produced by the aggregation of amyloid β (Aβ) which is toxic. Amyloid precursor proteins (APP) turn into Aβ when interacting with β-secretase and γ-secretase (Crump, JC. *Et al.* 2013, p.2). The process is unknown but suggests that there are some toxic substances forming during this interaction. As for neurofibrillary tangle, tau proteins are hyperphosphorylated and aggregate. Currently, the cause of AD has not been solved.

What actually is AD? Is there any way to prevent it? Why is it so difficult to understand?

➢Objective

This study aims to greater understand of dementia, especially Alzheimer's disease, by introducing and discussing the latest research and treatments. Furthermore, through interviewing a variety of researchers it is hoped that ideas for future research can be discussed and brought to light.

Figure 1

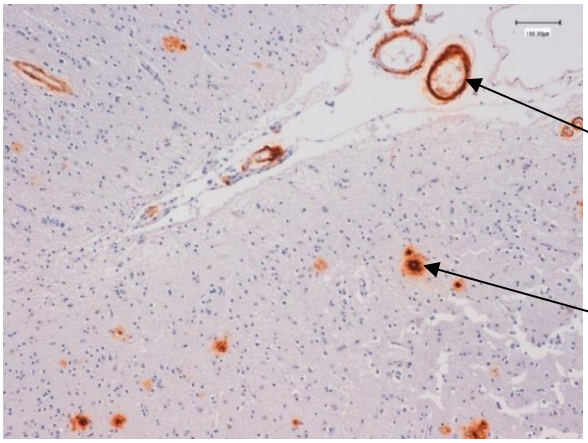


Patient's Brain

Black dots-death of neuron cells, red arrow-fibrillary tangled tau proteins, green arrow- amyloid ß

Reference(http://www.u-tokyo.ac.jp/ja/utokyo-research/feature-stories/aiming-for-a-future-without-tangles/)

Figure 2



Amyloid ß deposition in plaques and blood vessels

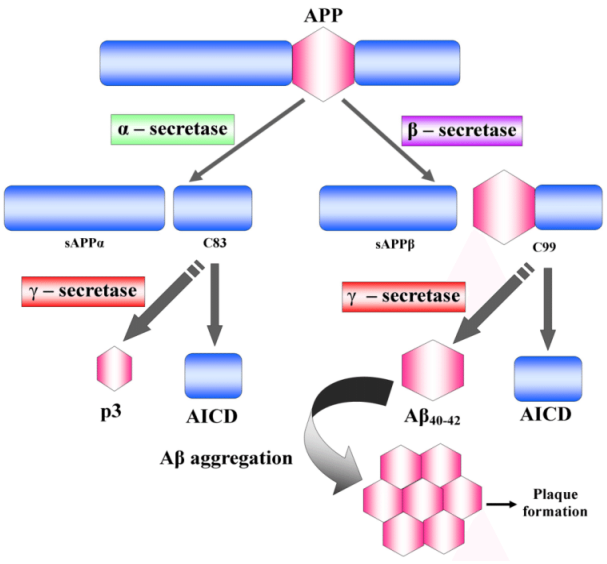
Amyloid ß deposition in plaques and blood vessels

➢ Picture shown from prof. Hunter, pers. comm.

2. Literature review

The core publications on dementia show that Aß is the main cause of AD and it is produced from APP (Crump, JC. *Et al.* 2013, p.2). On the one hand, APP is cut by α-secretase which is usual, on the other hand APP is cleaved by ß-secretase and then γ-secretase which produces toxic substances called Aß (Figure 3). The base sequence of amyloid where γ-secretase cleaves C99 can change the kinds of Aß like Aß40 or Aß42 etc. To stop this process, many scientists focused on stopping either ß-secretase or γ-secretase to restrain this process. Though there has been much research, no one has succeeded in making licensed medication because of the side effects. Gamma secretase inhibitors (GSIs) have been produced to stop γ-secretase's functions though inhabitation of APP processing could

Figure 3



Reference(https://www.researchgate.net/figure/308946170_fig5_Fig-4-Working-of-beta-secretase-in-the-formation-of-Amyloid-beta-and-Plaque)

actually aggravate AD pathology (Crump, JC. *Et al.* 2013, p.2, 3) and production stopped. After, scientists tried to find a way to stop γ-secretase's production Aß. These are called Gamma Secretase Modulators (GSMs). Many animal experiments showed reduced Aß42 and Aß40 levels while elevating levels of Aß38 and Aß39 (Crump, JC. *Et al.* 2013, p.37, 38). It demonstrated that GSMs could reduce plaque density and amyloid load in a transgenic mouse models of AD, however, increased in Aß38 reduced all brain Aß peptides (Crump, JC. *Et al.* 2013, p.7).

According to Funamoto *et al.* (2013, p.1), γ-secretase can distinguish the length of substrates and preferentially captures and cleaves substrates contacting a short proteins. The research team assumed that side effects were mainly caused by stopping the activation of the Notch signals, these are consequently, and proteins for Notch signals were made longer by creating C99-binding peptide to reduce the cutting rate. (Figure 4) However, it is still unclear how and why γ-secretase favours short substrates (Funamoto *et al.* 2013, p.8).

Figure 4

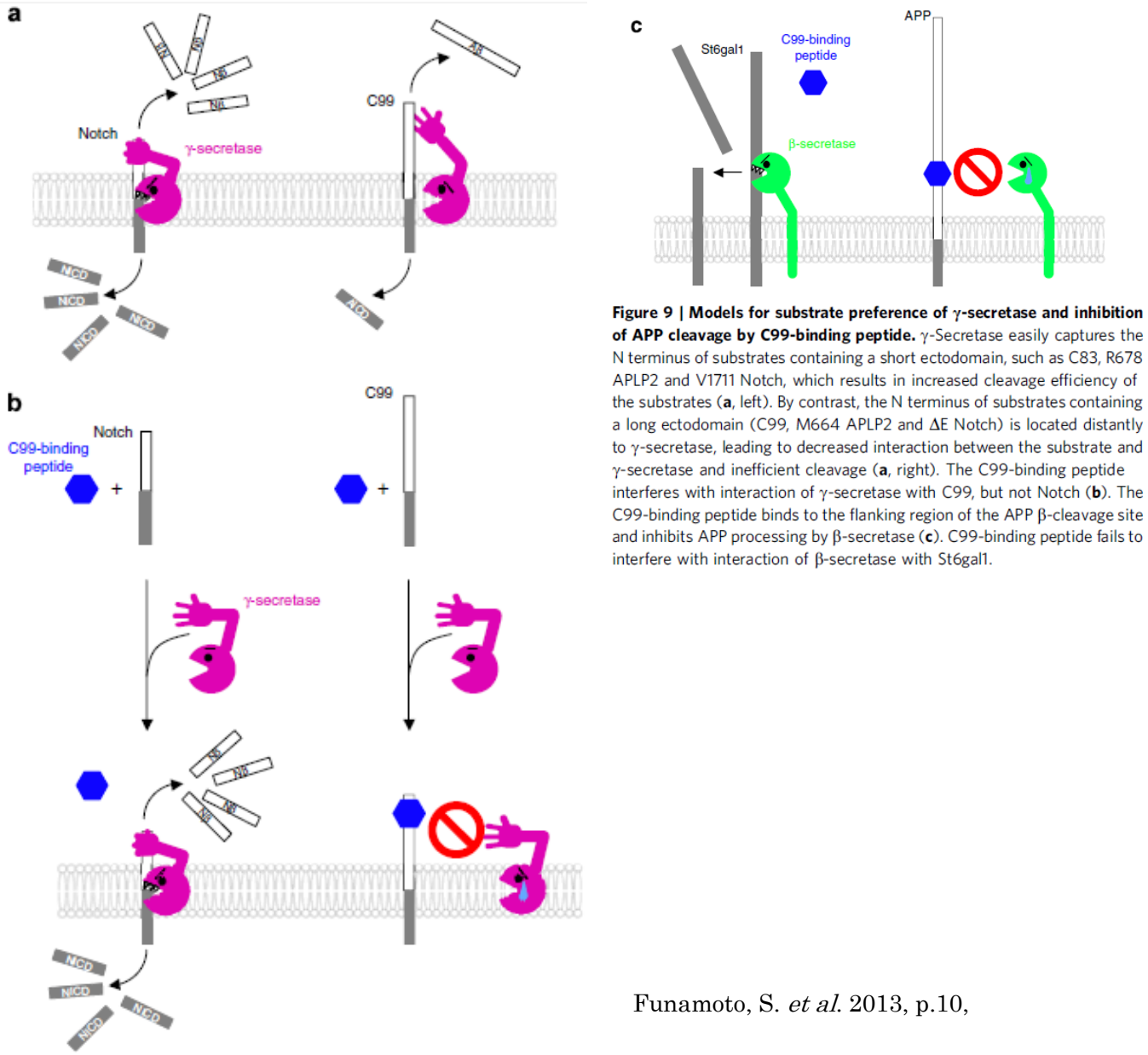


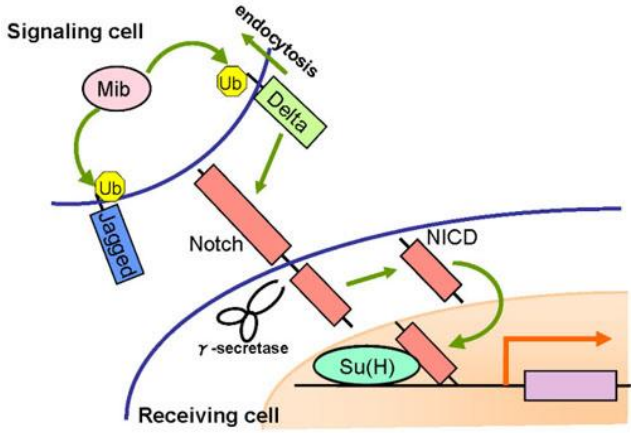
Figure 9 | Models for substrate preference of γ-secretase and inhibition of APP cleavage by C99-binding peptide. γ-Secretase easily captures the N terminus of substrates containing a short ectodomain, such as C83, R678 APLP2 and V1711 Notch, which results in increased cleavage efficiency of the substrates (**a**, left). By contrast, the N terminus of substrates containing a long ectodomain (C99, M664 APLP2 and ΔE Notch) is located distantly to γ-secretase, leading to decreased interaction between the substrate and γ-secretase and inefficient cleavage (**a**, right). The C99-binding peptide interferes with interaction of γ-secretase with C99, but not Notch (**b**). The C99-binding peptide binds to the flanking region of the APP β-cleavage site and inhibits APP processing by β-secretase (**c**). C99-binding peptide fails to interfere with interaction of β-secretase with St6gal1.

Funamoto, S. *et al.* 2013, p.10,

Notch signals can transmit information cell-to-cell. Delta and Jagged are signaling proteins and Notch is a receiving protein. When combined, the Notch Intracellular Domain (NICD), which is located inside Notch, becomes separated and eventually joins the Suppressor of Hairless (Su (H)) in order to activate the

production of targeted genes (Figure 5, http://www.p.chiba-u.jp/lab/seika/notch.html). The Notch signals control cellular differentiation. Therefore, without Notch signals, cells may suffer negative changes to their characteristics. Out of control Notch signals may cause some cells to turn into nerve cells unexpectedly, or cause mutations that promote the development of cancer cells (http://www.p.chiba-u.jp/lab/seika/notch.html). Notch signals have important roles in human bodies and are essential.

Figure 5



Reference (http://www.p.chiba-u.jp/lab/seika/notch.html)

Familial Alzheimer's disease (FAD) is also important. FAD is caused by some risk factors inherited from generation to generation. ApoE4 is said to be one of them (Ikota, 2012, p.79). According to Ikota (2012, p.79), among people who have ApoE4, about 50 to 60 percent of them diagnosed AD and about 13.5 percent of others are healthy. Many other studies have shown different statistics but Apo4E showed high possibility of diagnosing FAD. A lot of risk factors were gradually found, though none of the risk factors are certain to cause FAD.

The main symptom of dementia is cognitive disorders. Memories in the human brain are said to be kept in the hippocampus. However, some people suffer from dementia without any hippocampus damage (Ikota, 2012, p.97, 98). The more research there is, the more complex and confusing the problem becomes. The various causes of dementia make it difficult to find the diseases' sequence.

3. Method

3.1 Predictions

① the amyloid cascade hypothesis is true.

➢The amyloid cascade hypothesis is the theory that AD is caused by the amyloid proteins folding incorrectly and aggregating (Crump, JC. *Et al.* 2013, p.2). APP gets cleaved by two enzymes such as ß-secretase and γ-secretase, this then forms the amyloid beta peptide which aggregates in the nerve cells and produces toxic substances.

② the problem with Aß is the aggregation and not the increase, decrease or removal of Aß.

③ side effects of inhibiting γ-secretase can be reduced just by not stopping Notch signals.

④ tau aggregates after Aß, so handling Aß is essential to stopping AD.

3.2 Interviews in Cambridge

Four Cambridge University researchers were interviewed. These were:

Dr Minkoo Ahn-Department of Chemistry, University of Cambridge

Dr Tatsuya Ikenoue-Department of Chemistry, University of Cambridge

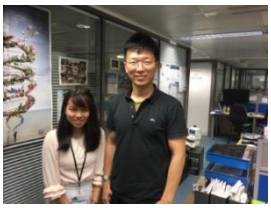
Dr Michel Goedert-MRC laboratory of Molecular biology

Prof. Sally Hunter-Institute of Public Health

A Japanese professor called Maho Yagi in the Okazaki Institute for Integrative Bioscience, Department of Bioorganization Research was interviewed on the recommendation of Dr Ahn. (Figure 6)

In the interview, some of the questions were repeated to compare the different opinions. In addition, questions were asked specific to the researchers' own work and the papers they have written.

Figure 6



Dr Minkoo Ahn

Dr Tatsuya Ikenoue

Dr Michel Goedert

Prof. Sally Hunter

Prof. Maho Yagi

- photography by the author

4. Results

Some of the questions asked have no conclusive answer yet and it is difficult to make a decision about the predictions. The only opinion that can be definitely stated is that the preparatory research before going to Cambridge was not enough to make a prediction. Dementia is not simple and a lot of hard work must be done to understand it.

5. Discussion

5.1 Prediction①: amyloid cascade hypothesis

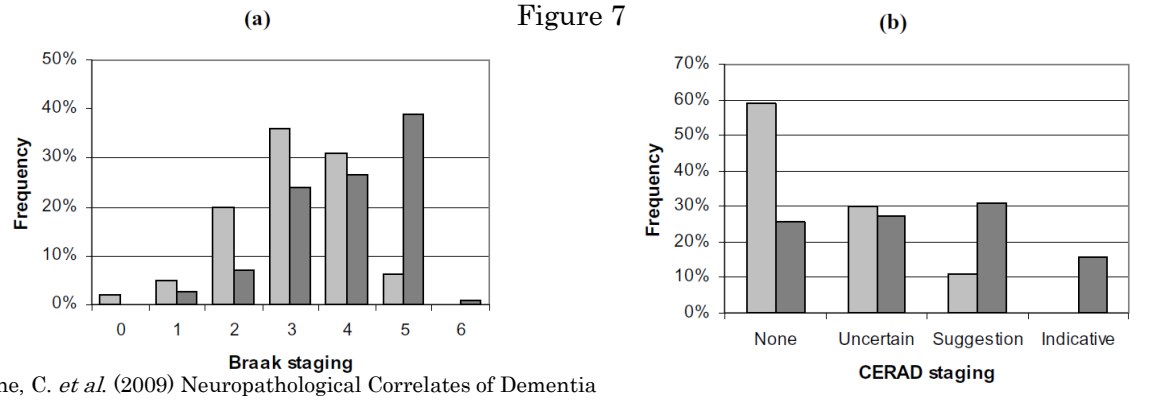
Looking at many papers, a lot of scientists have different opinions and it is not easy to say which particular explanation is right. Therefore, from this research, there was not enough information to decide whether the amyloid hypothesis is incorrect or not.

According to the literature review, the amyloid hypothesis is considered correct and many people are working as if the hypothesis is proven. From the interviews, except for Prof. Hunter, the researchers said that Aß is one of the causes and especially Dr Ahn said that there is an argument about at which point the toxic substance is made, but no one argues about the amyloid hypothesis.

However Prof. Hunter's group examined population-based longitudinal studies by brain donation from people aged 75 and over in Cambridge which casts doubts on the amyloid hypothesis (Brayne, C. *et al.* 2009). The purpose of this experiment was to step back and think again about the process and the cause of dementia. They checked 213 people both with and without dementia. As can be seen from the graph (Figure 7), ratings for tau reactive plaques and tangles were estimated according to Braak staging and CERAD staging which shows ratings for neurotic plaques. By looking at Braak staging, we can find out from participants with clinical dementia that there is higher risk of diagnosing dementia when neurofibrillary tangles are severe. Whereas the participants without clinical dementia have been measured as better stage

but it is not completely nothing. In addition, no matter how CERAD staging is, the frequency of the people who are diagnosed with dementia is the same. Therefore, we cannot say that neurofibrillary tangles and senile plaques are the cause for sure. Also, Table 1 shows neurotic plaques and neurofibrillary tangles which are said to be the cause of AD in three parts of the brain. Severe neurotic plaques do not correlate with more patients of AD. As for neurofibrillary tangles, some people have severe neurofibrillary tangles with no clinical dementia. From these statistics, it is difficult to find out tendency of dementia and it is not right to say that Aß and tau are the only causes. According to her experiments, she found that neurotic plaques, diffuse plaques, neurofibrillary tangle, hippocampal atrophy, white matter pallor and Lewy bodies were strongly associated with dementia, however it cannot be assumed to be the same for all people. From these studies, she thinks that dementia is caused by a complex of many factors, some of which may as yet be unknown.

Figure 7



Reference: Brayne, C. *et al.* (2009) Neuropathological Correlates of Dementia in Over-80-Year-Old Brain Donors from the Population-Based Cambridge City over-75s Cohort (CC75C) Study. J Alzheimer's Dis 18 645-658, p.648

Fig. 1. Distributions of (a) Braak staging and (b) CERAD staging in participants without clinical dementia (light grey) and with clinical dementia (dark grey).

Table 1

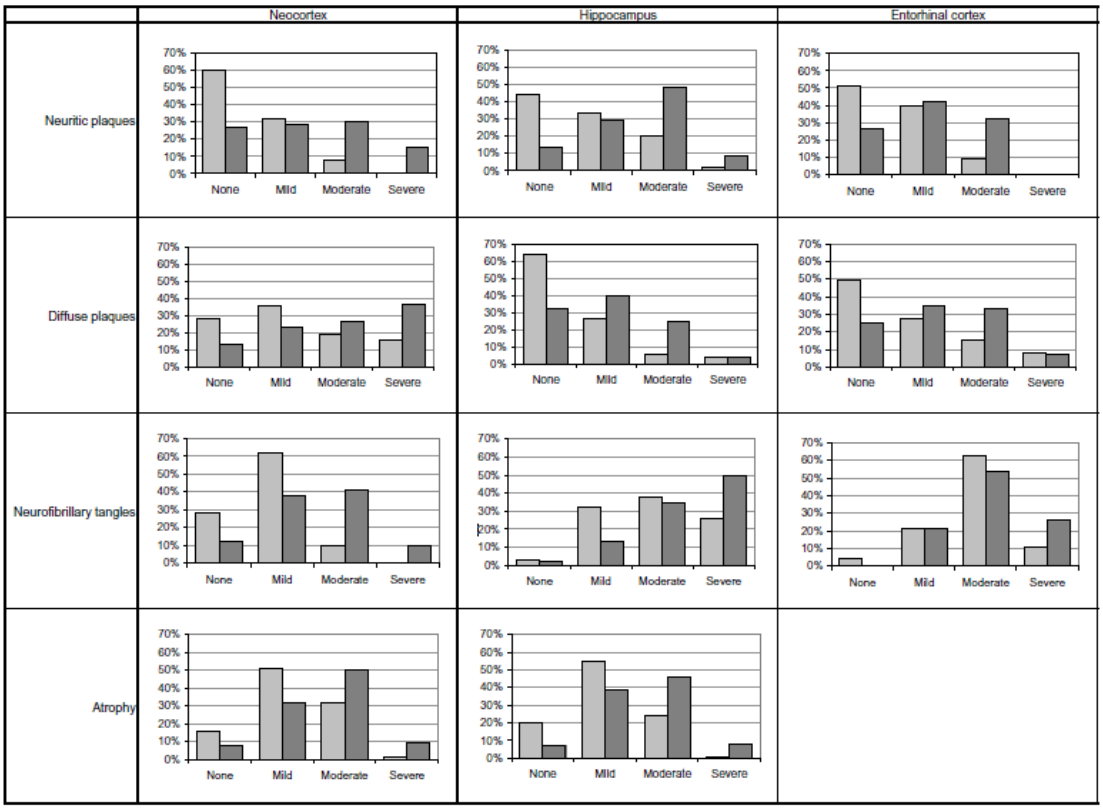


Fig. 3. Frequencies of severity of Alzheimer's Disease pathology and atrophy in the neocortex, hippocampus and entorhinal cortex in participants without clinical dementia (light grey) and with clinical dementia (dark grey).

Reference: Brayne, C. *et al.* (2009) Neuropathological Correlates of Dementia in Over-80-Year-Old Brain Donors from the Population-Based Cambridge City over-75s Cohort (CC75C) Study. J Alzheimer's Dis 18 645-658, p.651

5.2 Prediction②: the problem with Aß is the aggregation and not the increase, decrease or removal of Aß

Aß aggregates in human brains.

Many of the researchers agreed with prediction②. Dr Ahn, talked about his research group and how they have been looking for ways to stop the process of aggregation. There are many ways to approach this research. For example, they are trying to find a small molecule to add which can block the process of aggregation. Also, he is researching how to change the structure of Aß. He found that if they oxidized Aß, they could reduce Aß aggregation. However, it has been extremely difficult with little progress. According to Dr Ikenoue, he believes that stopping aggregation is almost impossible, because the increasing rate of Aß is nearly equal to aggregation. He recommended focusing on preventing the process that forms Aß.

Dr Goedert had a different opinion still. He does not believe that Aß is the certain cause of AD, but that the Amyloid cascade hypothesis is the predominant theory. Even if Aß is one of the causes and the prediction is correct, he thinks that it is not realistic to target Aß because it is difficult to know at what stage it should be treated, which makes it difficult to apply the findings to therapy. He assumes that many of the experiments have failed because it was too late to target Aß, and even if it was targeted earlier, we do not know when to start treatment. They cannot estimate whether a person will be diagnosed with AD in the future, so it is not easy to think about it for therapy.

This prediction was made because the amount of Aß was not proportionate to the probability of causing dementia. Although I did not know the process of Aß aggregating, I made this prediction though it is not clear if this prediction is correct. Therefore, an understanding of the process of forming Aß must be developed.

5.3 Prediction③: side effects of inhibiting γ-secretase can be reduced by not stopping Notch signals.

γ-secretase was said to have no substrate specificity. However, from the literature review, Funamoto *et al.* 2013 argued that γ-secretase cuts shorter substances easier than longer ones. Therefore, if there is a substance which can be easily combined to something before Aß, it can cut this substance for Notch signals without forming Aß. However, there was no mention of such a substance before going to Cambridge.

Dr Goedert said that γ-secretase has a lot of functions and the Notch signal is one of the main parts, but it also works for many other proteins which mean that the substance must be specific. Therefore, it is currently too complicated. Many pharmaceutical companies tried to make γ-secretase inhibitors (GSIs), but all of them failed because the side effects were too serious (Crump, JC. *Et al.* 2013, p.2, 3). From these experiences, many companies stopped producing GSIs and started to work on γ-secretase modulators (GSMs), however, not a single successful medication has been made. GSMs have not been used on human bodies yet, so it is unknown whether they work. Many kinds of GSMs were studied and Table 2 and 3 shows that most of the GSMs decreased Aß40 and Aß42. However, many other amyloids such as Aß37, Aß38, Aß39 increased. An example test of various GSMs carried out in animal experiments can be seen in (Table 2 and 3). Do these increasing Aß cause problems in the human body? The answer from Dr Goedert was that it could possibly turn into a toxic substance, so some side effects may appear. There was an experiment on mice without presenilin 1 to check the effect and the result was that the mouse died. He told me that γ-secretase is too complicated to work on, so many people gave up on it and research about it is not so active. According to Dr Goedert, recently, more people are focusing on ß-secretase rather than γ-secretase because there was

an experiment using mice without ß secretase to prove that inhibiting ß secretase does not have a negative effect. Prof. Yagi also believes that human bodies are well developed, so it is almost impossible to stop enzymes in them.

γ-secretase cuts a lot of proteins so looking at only Notch signals was not enough. Also no one has shown the process of how γ-secretase works, so it is very difficult to approach γ-secretase.

Table 2

Effect of 2nd generation acid GSMs on the production of Aβ peptides

Acid GSM	Cell-based Aβ42 IC_{50}	In vivo studies	Aβ profile		
			38	40	42
GSM-1	348 nM[123]	guinea pig[124]	↑	-	↓
GSM-10h	300 nM[114]	rat[115], mouse[125]			↓
GSM-2	65 nM[41]	mouse[41] [126]			↓
EVP-0015962	67 nM[117]	mouse[117]	↑	-	↓
JNJ-40418677	200 nM[118]	mouse[118]	↑	-	↓
BIIB042	170 nM[119]	mouse, rat, monkey[119]			↓

Increasing Aß (green arrow)

Reference (Crump, JC. *Et al.* 2013, p.37)

Table 3

Effect of 2nd generation non-acid GSMs on the production of Aβ peptides

Non-Acid GSM	Cell-based Aβ42 IC_{50}	In vivo data	Aβ profile				
			37	38	39	40	42
E2012	92 nM[130], 143 nM[137]	rat [130], dog[128], guinea pig[137]	↑	↑	↓	↓	↓
GSM-A	8–33 nM[124]	guinea pig[124]		↑		↓	↓
NGP-555	10–29 nM[127],	mouse[127]	↑	↑		↓	↓
GSM-53	33 nM[133]	rat, dog, monkey[134]	↑	↑	↓	↓	↓
GSM-35	44 nM[135]	rat[135]					
RO-02	~14 nM[136]		↑	↑		↓	↓
AZ4800	26 nM[129]	mouse[129]	↑	↑	↓	↓	↓
AZ3303	74 nM[129]	mouse[129]	↑	↑	↓	↓	↓
AZ1136	990 nM [129]		↑	↓	↑	↓	↓
JNJ-42601572	16 nM[138]	rat & mouse[138], dog[139]	↑	↑		↓	↓

Increasing Aß (green arrow)

Reference
(Crump, JC. *Et al.* 2013, p.38)

5.4 Prediction④:Tau aggregates after Aß, so handling Aß is essential to stop AD

Handling Aß faster than tau was predicted to be important, because when Aß aggregated, more and more Aß will spread. Stopping this process as fast as possible may provide a potential solution.

Dr Ikenoue and Prof. Yagi assumed the same but they were not sure, and also Dr Ahn said that no one clearly understands the process. It is a difficult argument because researchers cannot see the brain to know when aggregation starts. Dr Goedert argued that tau and Aß will become independent at a late stage at certain times, but he is not sure about which starts aggregating earlier.

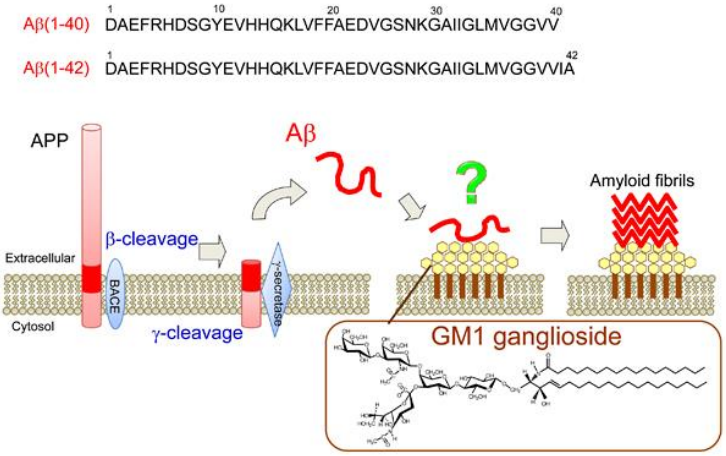
It is difficult to answer this prediction because no one even knows when the aggregation happens. The evidence is too inconclusive to make a statement.

5.5 Interviewers' Research

Prof. Yagi

- ✓ finding out the structure of Aß by using NMR
- ✓ NMR characterization of the interactions between GM1 and Aß via the hydrophilic/hydrophobic interface of ganglioside clusters

Figure 8



Yanagisawa *et al.* (2011) proved that production of Aß is tightly associated with GM1 ganglioside. Also, Kakio *et al.* (2002) demonstrated that Aß transits from an α-helix structure to a ß-sheet-structure when Aß density increases on liposomes containing GM1 ganglioside. These studies have formed the core of research into future medications for AD (Figure 8).

Reference (Nanotechjapan Bulletin, Vol.4 No.2 2011)

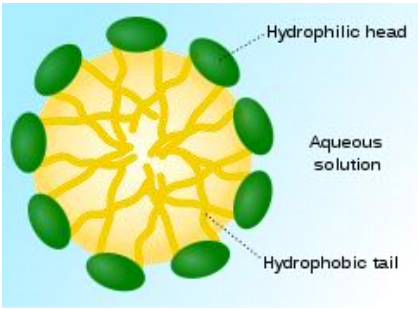
Figure 9

NMR stands for Nuclear Magnetic Resonance and Prof. Yagi said that it is used to find out the three dimensional structure and movements of molecules as well as determining unknown compounds (Figure 9). Prof. Yagi used this technology instead of X-ray crystallography, for example, because NMR enables proteins to be examined. To help this process the proteins are dissolved in water in order to make the conditions closer to those in the human body. Both NMR and X-ray crystallography have positives and negatives, so both techniques are used for different purposes. As for Aß, it is difficult to make crystals to use in X-ray crystallography so NMR is the most suitable method for this research.

Reference(http://www.ch.cam.ac.uk/analytical/nmr/700-mhz-txo-cryoprobe-spectrometer-arran-room-b14)

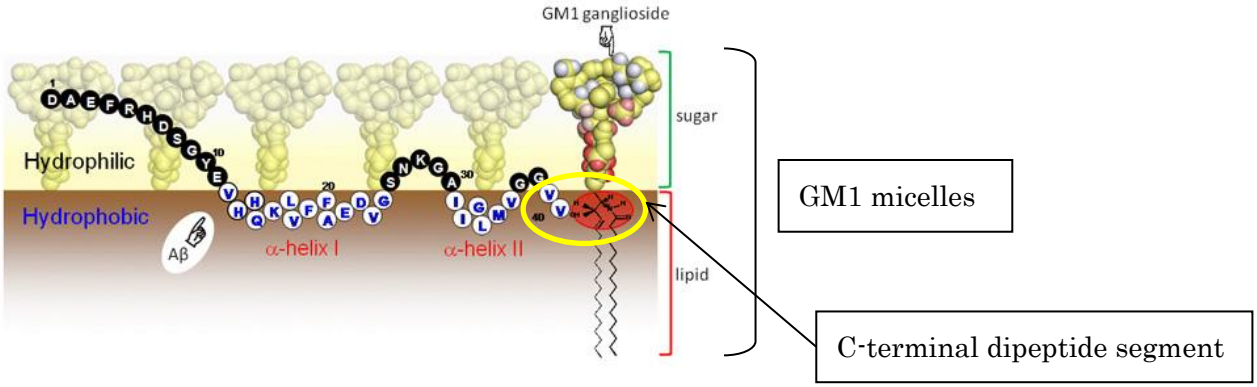
According to prof. Yagi, many people assumed that Aß has a random structure, however, by looking at the results of NMR analysis, Aß (1-40), which does not have particular structure, changes into a structural substance. It is Aß with two helix structures and it is formed when it is combined with GM1 micelles (Figure 10). In addition, it was demonstrated (Maho Utsumi *et al*, 2008, p.999) that Aß (1-40) resides on a ganglioside cluster, and both the two helices and the C-terminal dipeptide segment exist in the hydrophobic interior, whereas the others exist in the hydrophilic areas (figure 11). Therefore, when induced by the ganglioside cluster, the non-structural Aß changes into an α-helix structure then a ß-sheet structure and is then fixed in its position (Figure 12). This is why much research is being carried out on the interactions between GM1 and Aß.

Figure 10



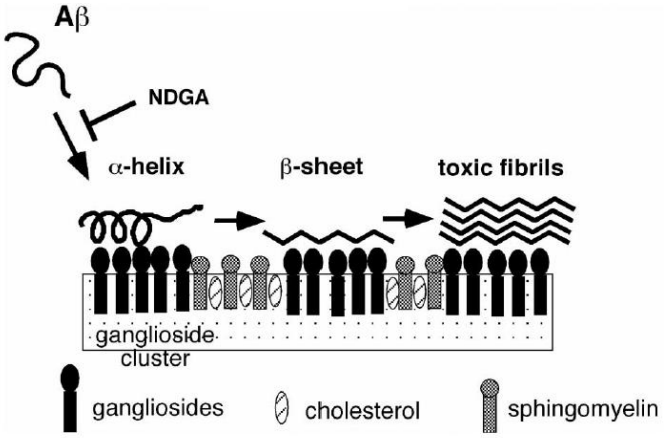
Micelle -An aggregate of molecules in colloidal solution that contains both hydrophobic regions as well as hydrophilic regions.
Reference(https://upload.wikimedia.org/wikipedia/commons/4/4d/Micelle_scheme-en.svg)

Figure 11



Reference (https://www.ims.ac.jp/en/news/2011/02/17_2202.html)

Figure 12



Reference (Katsumi Matsuzaki, 2010)

Dr Tatsuya Ikenoue

- ✓ Find out the mechanism of amyloids miss-folding in molecular point of view
- ✓ Trying to produce a small compounds which selectively work to amyloid fibrils and stop their increase.

He has been researching how to target Aß42 aggregation with small molecules. At the interview he insisted on advantages and disadvantages of antibodies which targets Aß. From these points of view, he is trying to make the best antibody by taking only suitable parts of both antibodies.

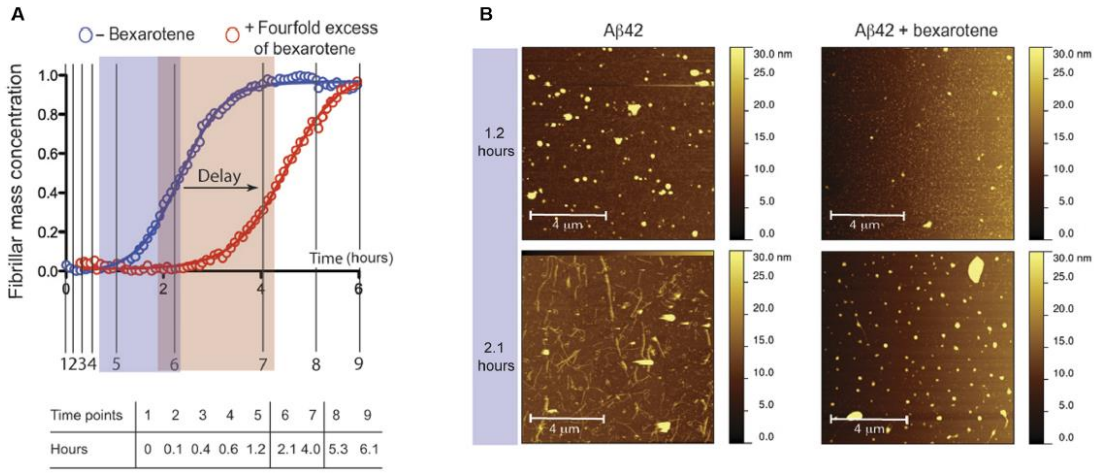
Antibody	Advantages	Disadvantages
small	• Gets combined strongly • Cheap • Easier to go through cell membrane	• It is not specific • Difficult to stop wide spread combination

big	• It is more specific →side effects are smaller • Good for stopping aggregation	• Difficult to go through cell membrane • Harder to reach to the target • Expensive

Another study that Dr Ikenoue talked about at the interview is about bexarotene. Bexarotene is an anticancer drug approved by the U.S. Food and Drug Administration. According to Habchi *et al.* (2016), all clinical trials have failed because of lack of understanding of the molecular mechanism, to be specific, the way of aggregation and inhibition. They have been trying to find small molecules to apply for drug discovery. From a lot of compounds they used chemical compounds called bexarotene and tramiprosate. From their experiments, the effect of bexarotene on Aß42 aggregation was substantial but not tramiprosate.

Figure 13 shows a fourfold excess of bexarotene delaying the fibril concentration than bexarotene. Also they monitored the fibril formation and fibrillary structures which can only be observed 2.1 hours after in the absence of bexarotene.

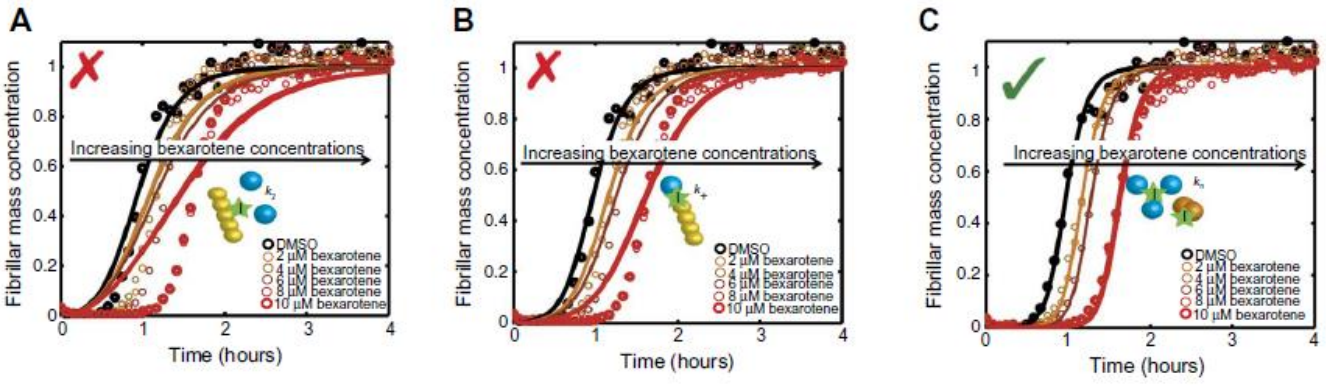
Figure 13



Reference (Habchi *et al.* 2016, p.5)

Figure 14 shows each of the proteins are inhibited by bexarotene in secondary nucleation (A), fibril elongation (B), or primary nucleation (C). As can be seen, only primary nucleation alone is inhibited. In conclusion, though tramiprosate has no effect on Aß42 aggregation, bexarotene interferes effectively with the earliest stage of Aß42 aggregation by reducing the rate of primary nucleation.

Figure 14



Reference (Habchi *et al.* 2016, p.6)

5.6 Other Information

(1) The latest research

Many professors (Dr Goedert, Dr Ahn and Dr Ikenoue) said that there has not been much progress on this topic so it is difficult to introduce the research, however Dr Ahn and Dr Goedert discussed the studies they are interested. Firstly, Dr Ahn talked about studying the structure of tau by using cryo-EM from MRC in Cambridge (Fitzpatrick, 2017). There are three ways to study structures, by x-ray, NMR and cryo-EM. They used this technology to find the structure in three dimensional ways by using the patient's brain directly. He told me that tau was previously payed attention to by many people and it is now a very hot topic.

<Comparison among methods for determining structure of the molecules>

	X-ray Crystallography	NMR	cryo-EM
Brief knowledge about each method	1. Make pure sample and crystallize it. 2. Irradiate crystal with x-ray beam. 3. A detector records the diffraction pattern and a computer reconstructs the atomic structure of the molecule. 4. Rotate the crystal to collect more diffraction patterns 5. Combining many 2D electron density maps gives a 3D map	1. Label protein with 13C and/or 15N 2. Make concentrated protein solution in water 3. Apply external magnetic field to sample. 4. Computer determines the J coupling constant and NOE value between every pair of NMR-active nuclei. (These values provide a set of estimates constraints which are distances between specific pairs of atoms.) 5. Build a model for the structure that is consistent with the set of constraints	1. Dilute the sample to make it only a few macromolecules appear 2. Put a droplet of the sample onto a carbon support film with holes in it 3. Blot away droplet with tissue, leaving just a thin film of liquid 4. Freeze sample in liquid ethane 5. Place sample in cooled EM instrument 6. Irradiate with electron beam 7. Collect projected 2D image and repeat 8. Computer reconstructs 3D structure of molecule
Advantages	• Best resolution • Broad molecular weight range • easy for model building	• High resolution (better than cryo-EM) • Native like conditions. (Sample is hydrated, not frozen, or a crystal) • Can get dynamic information • 3D structure in solution	• Preserves the native structure of the sample • Need only tiny amount of the sample • Large molecules can be used • Can get kinetic snapshots of various conformational states • Low temperature reduces radiation damage

Disadvantages	• Difficult for crystallization (Heterogeneous samples, membrane proteins and complexes of proteins) • Snapshot only and no temporal information • Solid structure preferred • Difficult for diffraction	• It needs to be very concentrated samples • Structure of mixed species cannot be determined • Difficult for sample preparation • Difficult for computational simulation	• Resolution not as high as NMR or x-ray • Expensive • Applicable to samples of high molecular weights only • Sample preparation is difficult (needs perfect ice thickness, no contamination) • Sample is frozen, not at room temperature • Sample can be damaged by intense electron beam

Reference (Massachusetts institute of technology, 2004,https://ocw.mit.edu/courses/chemistry/5-08j-biological-chemistry-ii-spring-2004/recitations/TD5.pdf
https://www.creative-biostructure.com/comparison-of-crystallography-nmr-and-em_6.htm)

Secondly, Dr Goedert talked about antibodies. According to Florence, C. *et al.* (2013, p.9539), "the inhabitation of cell-to-cell transmission of pathological aggregates, for instance by passive immunotherapy, may constitute an effective mechanism based therapeutic strategy for most human neurodegenerative disease." Passive immunotherapy is to give antibodies directly to the cell. He has been using tau antibodies for experiments but it has not been checked in human bodies yet. Moreover, Dr Ikenoue and Prof. Hunter have also been using antibodies. Antibodies could be a good way to approach this problem.

(2)Familial Alzheimer's disease (FAD)

Though many studies such as Ogawa (1999, p.83) and Ikota (2012, p.79) say that apolipoprotein E4 (ApoE4) is one of the risk factors of FAD, it does not mean that all people with ApoE4 will diagnose FAD. What is the criteria for these kinds of risk factors? Dr Goedert said that risk factor means that it is more likely that the person will get the disease. In contrast, changes in amyloid sequences such as presenilin 1 or APP, can definitely cause dementia but are not risk factors.

(3)Applying Studies Efficiently

Before going to Cambridge, I found a study suggesting that CALM genes can reduce Aß42 (Kanatsu, K *et al.* 2014). The mechanism is that firstly γ-secretase goes inside cells by endocytosis. CALM genes are directly connected to γ-secretase and it works as an adapter molecule in endocytosis transmission of γ-secretase. They found that when CALM genes are inhibited, the speed of sending γ-secretase become slower. Dr Goedert said that it could be true but that it may not be a relevant study because the evidence is small and effect may be small. Many studies of this kind appeared but it is important not to focus on them much. He taught me a good lesson. I read only the title and got interest, but that is not good and I should have read inside more carefully to consider whether this study is worth reading or not. I should not have

been influenced that easily. Thanks to this insight, it shows that a critical mind is necessary for research.

(4) Other Questions and Answers

Q. There are Aß resulting enzymes in our body such as neprilysin. Can these enzymes be active more to slow down AD?

A. Usually medicines cannot activate enzymes. It can inhibit but not activate. (Dr Goedert)

Q. Could you tell me why patients of dementia are increasing all over the world?

A. Because life span became longer and aging has advanced. There is not any licensed medication yet and it is more important to emphasize prevention. (Dr Ikenoue)

Q. I heard that no one would die of dementia. Do you think it is true?

A. Maybe. Though damage to the brain may not lead to death directly, it is common that pneumonia infects lungs while patients lie on beds all the time and are then diagnosed with dementia, then die. It is difficult to answer this question. (Dr Goedert)

Q. Can iPS cells replace dead neurons to create new brains?

A. iPS cells are used to study processes of human bodies but are not actually used in human bodies yet. This is because it is unethical. Correcting body cells is difficult because it can in theory change the body on demand. For example, if a baby was born in a the parents do not like, they can use iPS cells and change some parts, this means that theoretically people can choose what kind of child they want. In addition, there is a risk of getting other diseases. It is very difficult and he thinks that no one is working on it. (Dr Goedert)

(5)The future of dementia

The question “Do you think that dementia can be cured completely in the future?” was asked to all of the scientists.

- Dr Ikenoue

He really hopes that dementia would be a disease that can be cured in the future. He believes that it will be clarified in molecule point of view to think about dementia more logically.

- Dr Ahn

He thinks that it will take a long time but there are many ways to approach dementia so he hopes that the mechanism will be clarified in the future.

- Dr Goedert

He thinks that to cure dementia is impossible because thinking about therapy for dead nerve cells is impossible because dead cells cannot be replaced. He hopes that there would be some methods to prevent it and lower the risk of it happening.

- Prof. Hunter

She thinks it is almost impossible. She suggests that some other substance which is the cause of dementia is still missing so it will be long way off.

- Prof. Yagi

To cure dementia is like an unrealistic dream but she hopes that there would be at least some way to prevent it.

6. Conclusion

Despite dementia having been studied for a long time, no one could figure out the cause and the process. The increase of patients is not stopping and treatments are needed immediately, however it is essential for researchers to do sound research. The reasons why each result appeared must be analyzed. Dementia is too complicated to cure and prevention must be the focus. Many scientists have various opinions and some think that dementia is impossible to cure completely even with many years of research. At present, researchers are doing their own research hoping that they can reach or contribute to the goal.

Abbreviations

Aß	amyloid beta	GSMs	gamma secretase modulators
AD	Alzheimer's disease	NICD	Notch Intracellular Domain
ApoE4	apolipoprotein E4	NMR	Nuclear Magnetic Resonance
APP	Amyloid precursor proteins	PET	positron emission tomography
DLB	dementia with Lewy bodies	SPECT	single photon emission computed tomography
FAD	Familial Alzheimer's disease	Su(H)	Suppressor of Hairless
FTD	frontotemporal dementia	VaD	vascular dementia
GSIs	gamma secretase inhibitors		

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Figure:

1) http://www.u-tokyo.ac.jp/ja/utokyo-research/feature-stories/aiming-for-a-future-without-tangles/

2) Picture shown from prof. Hunter, pers. comm.

3)https://www.researchgate.net/figure/308946170_fig5_Fig-4-Working-of-beta-secretase-in-the-formation-of-Amyloid-beta-and-Plaque

4) Funamoto, S. *et al.* (2013) Substrate ectodomain is critical for substrate preference and inhibition of γ-secretase. Nat. Commun. 4:2529 doi: 10.1038/ncomms3529, Figure 9

5) http://www.p.chiba-u.jp/lab/seika/notch.html

6) Photography by the author

7) Brayne, C. *et al.* (2009) Neuropathological Correlates of Dementia in Over-80-Year-Old Brain Donors from the Population-Based Cambridge City over-75s Cohort (CC75C) Study. J Alzheimer's Dis 18 645-658, Figure 1

8)http://nanonet.mext.go.jp/magazine/index.php?plugin=attach&refer=Vol.%204%2C%20No.%202%2C%202011%E5%B9%B43%E6%9C%8830%E6%97%A5%E7%99%BA%E8%A1%8C%2F%E3%83%95%E3%82%A9%E3%83%BC%E3%82%AB%E3%82%B926%EF%BC%9C%E7%AC%AC%EF%BC%92%EF%BC%92%E5%9B%9E%EF%BC%9E920MHzNMR%E3%82%92%E7%94%A8%E3%81%84%E3%81%9F%E7%A5%9E%E7%B5%8C%E5%A4%89%E6%80%A7%E7%96%BE%E6%82%A3%E9%96%A2%E9%80%A3%E3%82%BF%E3%8

3%B3%E3%83%91%E3%82%AF%E8%B3%AA%E3%81%AE%E6%A7%8B%E9%80%A0%E8%A7%A3%E6%9E%90&openfile=Fig01.jpg
9) http://www.ch.cam.ac.uk/analytical/nmr/700-mhz-txo-cryoprobe-spectrometer-arran-room-b14
10) https://upload.wikimedia.org/wikipedia/commons/4/4d/Micelle_scheme-en.svg
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13) Habchi, J. *et al.* (2016) An anticancer drug suppresses the primary nucleation reaction that initiates the production of the toxic Ab42 aggregates linked with Alzheimer's disease. Sci. Adv. 2, e1501244
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