

厚生労働科学研究費補助金

認知症対策総合研究事業

『認知症治療を目的とした変異型オリゴマーアミロイド  
ペプチドを抗原とする神経免疫療法の開発』に関する研究

平成23年度 総括研究報告書

研究代表者 森 啓

大阪市立大学・大学院医学研究科・教授

平成24（2012）年 3月

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# I. 総括研究報告

厚生労働科学研究費補助金（認知症対策総合研究事業）  
（総括・分担）研究報告書

認知症治療を目的とした変異型オリゴマーアミロイドペプチドを抗原とする  
神経免疫療法の開発に関する研究

研究代表者 森 啓 大阪市立大学・大学院医学研究科・教授

研究要旨

アルツハイマー病の発症原因として議論されている A $\beta$ オリゴマー仮説を基に神経免疫療法について検証した。A $\beta$ オリゴマー仮説の病因論としての正当性と重要性について、遺伝性アルツハイマー病の分子基盤を解明し、分子機構について培養細胞やモデルマウスを作製して、神経細胞内に A $\beta$ オリゴマーが異常蓄積することが発症原因である証拠を発見した。今後、A $\beta$ オリゴマーの検査と治療薬開発によりアルツハイマー病医療が展開すると考えられる。

研究分担者氏名・所属研究機関名及び所属研究機関における職名

富山 貴美・大阪市立大学・大学院医学研究科・准教授

A. 研究目的

A $\beta$ オリゴマーについては、諸説が提唱され、その分子構成および意義については確定していなかった。1998年に Kleinらによって発表された ADDLs の分子量は 34kDa と記載されていたが、2003 年になると 55kDa と表記されるようになった。類似の分子量をもつ A $\beta$ オリゴマーが Asheらによって A $\beta$ \*56 と命名された。

2002 年には、Selkoe らが A $\beta$ オリゴマーは二量体であると発表し、シナプス障害からアルツハイマー病が生じるとした。

本研究課題は、A $\beta$ オリゴマーの意義そのものを検証すること、その病理学的な意義を調べることを目的としているが、同時に猶も結論のついていない A $\beta$ オリゴマーについての概念を整理することも視野においた研究によって、神経免疫療法の可能性を検証することを研究目的とした。

B. 研究方法

(1) アミロイドイメージング

PiB を当医学部付属病院のサイクロトロンにて  $^{11}\text{C}$  標識し、島津製 PET カメラにて撮像し

た。画像は、PiB 注入後 30 分から 60 分のデータを処理して得ることとした。

(2) A $\beta$ オリゴマーモデルマウスの作製

A $\beta$ オリゴマーをコードする大阪変異を持つ APPcDNA をマウスプリオンプロモーター下流に導入した遺伝子コンストラクトをもつ Tg モデルマウスを作製した。

(2) 培養細胞を用いた A $\beta$ オリゴマー発現の分析  
リポフェクトアミン法により上記の大阪変異を含む APP 遺伝子を培養細胞に導入した。

(3) 共焦点レーザー顕微鏡観察

培養細胞の固定後にブロッキング処理をし、1 次抗体処理をした。洗浄後、蛍光標識 2 次抗体処理をし、洗浄後に蛍光退色保護材の入った包埋剤で封入したプレパラートをライカ社製共焦点レーザー顕微鏡観察によって観察した。

(4) SDS 電気泳動とウェスタンブロット

12%アクリルアミドゲルにて電気泳動後に、PGDF 膜状にドライブロットした。PBS 中にて 10 分間の熱処理により抗原性の賦活化をさせ、3%ミルク、0.1% Triton、TBS にてブロッキングした。1 次抗体処理後に 0.1% Tween20 を含む TBS にて洗浄後、ペルオキシダーゼ標識 2 次抗体処理をし、蛍光基質による染色をイルミネーターにて検出した。

(倫理面への配慮)

本研究は、人権擁護上の配慮、不利益、危険性の排除と説明と同意（インフォームド・コンセント）を、外部委員を含む本学医学部の倫理委

員会で承認されている（受付・承認番号#1310）。

本研究は、実験動物に対する動物愛護上の配慮についても、本学の動物実験審査委員会で承認されています（受付・承認番号#310）。本研究は、動物実験の中で遺伝子実験を含むことから、組み替えDNA実験についても、安全等への配慮をした上で、本学の組換えDNA実験委員会で承認されています（受付・承認番号#269）。

### C. 研究結果

#### (1) Aβオリゴマーマウスの作製

APP E693Δマウスは、現在主要原因分子として注目されているAβオリゴマーのみを発現するように工夫した新規アルツハイマー病モデルマウスである。これは、大阪変異（E693Δ）をもつ患者分析から得られた遺伝変異を、脳組織とりわけニューロン選択性の特異性が高いプロンプモーターの作用で発現するコンストラクトを作製し、マウスに導入したものである。

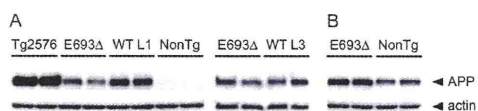


図1 Tgマウスに導入されたヒトAPP発現

図1にみられるようにアミロイド老人斑を形成しない大阪変異をもつAPPを導入されたモデルマウスにおけるAPP発現量は、通常のアミロイド老人斑モデルマウスTg2576や今回新たに作製した非変異型APPを導入された野生型モデルマウスよりも低い発現量であることがわかる。この低い発現量にもかかわらず、Aβオリゴマー形成能の高い大阪変異効果により、この新規モデルマウスは空間認知能力、学習能力の点で、Tg2576に比べて、さらには野生型Tgマウスと比べても大きな認知症症状を示すことが明らかとなった。

#### (2) モデルマウスの臨床（空間記憶試験）

モデルマウスの認知諸機能、神経活動を実施した。まずwater mazeでの学習記憶試験をした結果、APP E693Δマウスでは記憶力の学習記憶障害が8ヶ月例以後に、経時的に顕著に確認された。また、記憶保持力に相当するプローブ試験でも有意に低下していることを観察した（図2）。

#### (3) モデルマウスのシナプス機能

次にシナプスでの長期増強作用（LTP）を検討した結果、non-Tgマウスでは正常であったLTP作用が、APP E693Δマウスではほぼ完全に障害されていることが観察された（図3）。図2Aは、試行回数による標的まで辿り着く時間を測定したものであり、空間認知能が障害されているモデルマウスでは、時間の短縮が劣っていることがわかる。

図2Bでは、標的水域に存在する割合を見たものであり、学習により標的近傍での滞在確率が有意に高い。

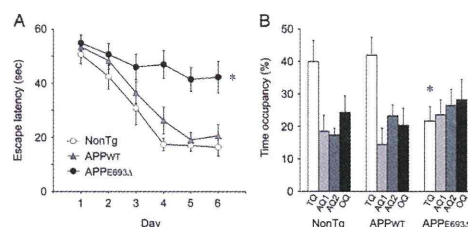


図2 Morris水迷路による認知機能テスト

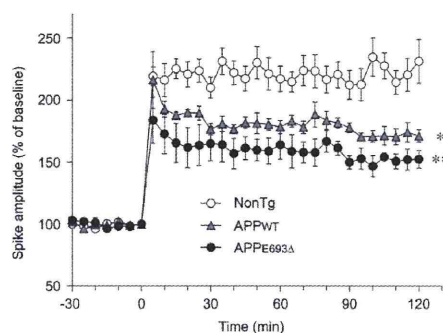


図3 モデルマウスにおける長期増強作用でのシナプス機能（○非Tgマウス、△野生型Tgマウス、●Aβオリゴマーマウス）

#### (4) モデルマウスの脳神経病理変化

脳組織変化について脳病理学的検索をした。通常のアミロイド老人斑は、24ヶ月齢に至るまで観察できなかったし、micro-PETを用いたPib-PETでも検出できなかった。一方、ニューロン内には、少ないAPP発現量にも関わらずAβの細胞内異常集積が観察された。しかも、この集積Aβは、Aβオリゴマー特異抗体（NU1；ノースウェスタン大学Klein博士よりご恵与）で認識されるADDLs様Aβオリゴマーであり、華麗に依存して蓄積することが判明した。

凍結脳組織を用いた生化学的検索であるSDSポリアクリルアミドゲル電気泳動および転写後の抗体検出をしたところ、Aβオリゴマーの有意な上昇が認められた。脳神経病理でも、ノースウェスタン大学Klein博士より供与された

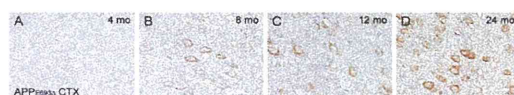


図4 ニューロン内に異常蓄積するAβオリゴマー



抗オリゴマー抗体による免疫組織化学的検索を実施した結果、APP E693ΔマウスではAβオリゴマーのニューロン細胞内の異常蓄積が加齢と共に、行動異常の出現時期と同じくして確かめられた。

脳萎縮は、神経脱落に起因するニューロン細胞死が原因で生じることが知られている。本研究では、その原因が細胞内Aβオリゴマーの異常蓄積であることを示し、その作用機構が、小胞体ストレス、細胞器の輸送機構の破綻だけではなく、ミトコンドリア機能障害に由来する可能性を強く示唆することができた。さらに、記憶障害という臨床症状については、同じく細胞内Aβオリゴマーの異常集積によってシナプス機能の破綻が生じることが原因であることを示した。このことは、シナプス特異分子であるシナプトフィジン免疫染色によって形態学的に、準定量的に示すことができた。最後に最近、生活習慣病によってアルツハイマー病の発症リスクが上昇することが議論されはじめ、いくらかの疫学的調査は脂質異常症、高血圧、糖尿病のリスクを示唆してきている。本研究では、アルツハイマー病モデルマウスに高コレステロール食餌を与えた場合、アルツハイマー病の最初期変化である細胞内Aβオリゴマーの異常蓄積が加速されることを示すことができた。その結果、記憶障害のテストである空間学習能力、シナプスフィジン低下、異常リン酸化タウの上昇が生じることを明らかにした。

#### (5) モデルマウスの免疫療法による認知症治療法の開発検討



図 2 モデルマウスを用いた免疫療法の試行実験

今回開発されたAβオリゴマーモデルマウスの病因であるAβオリゴマーを除去する治療法の1つとして、免疫療法が考えられる。現在我々が手にすることができるAβオリゴマー抗体であるNU-1モノクローナル抗体を投与したマウス脳に蓄積するAβオリゴマーが抗体投与によって減弱していることがわかる。つまり、間接免疫療法が有効であることが示唆された。ただ、この結果には、空間認知能力の試験、シナプス増強反応などの複合的な検索が必要であるし、今後より多数のマウスによる確認作業が必要である。

#### D. 考察

今回確立したAβオリゴマーモデルマウスは、現在考えられているAD所見を、ほぼ満たしていると考えられる。ただし、老人斑が存在しない点は、このモデルマウスに導入した大阪変異特有の性質であることを念頭に考慮しなければならない。同時に、大阪変異モデルマウスであっても、野生型Aβオリゴマーに対する抗体であるNU-1抗体などのアミロイド抗体との交差反応も確認されていることから、大阪変異に特異的なモデルではなく、Aβオリゴマーモデルマウスとして考えることができると結論した。

このモデルマウスは、その発現量は、生理的発現量に近く過剰発現による異常性がない点で、大きな意味がある。また、アミロイドタンパクによる病態変化が、アミロイド繊維老人斑に起因するのか、Aβオリゴマーに起因するのかについては、黑白をつける対象としては、この上ない有力なモデルであることがわかる。今後、ノックインマウスの開発により、さらに生理的な条件での検討も必要であると考えられる。

残された課題は、免疫療法を実施する対象としてのモデルマウスの確立は解決したが、治療薬となるワクチンと抗体の開発が待たれている。さらに、Aβオリゴマーから異常リン酸化タウ反応が惹起されているが、神経原線維変化の形成には至っていない理由の解明がある。

数多くの課題の底流には、Aβオリゴマーの脳内分子の単離精製という単純かつ本質的な問題を解決しなければならないことは、言うまでもない。

#### E. 結論

Aβオリゴマーモデルマウスを確立した。AD病態を発症するには、Aβオリゴマーだけでも十分であることが強く示唆され、アミロイド老人斑の病態の意義について疑問を投げかけることができた。Aβオリゴマー抗体を用いた免疫療法の有効性を示唆する前向きな実験結果を得ることができたことで、今後のAD根本治療薬開発への方向性が確立した。

#### F. 健康危険情報 とくになし。

#### G. 研究発表

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H. 知的財産権の出願・登録状況  
(予定を含む。)

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし



## II. 研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Shimada H, Ataka S, Tomiyama T, Takeuchi H, Mori H, & Miki T.	Course of Patients with Familial Early-Onset Alzheimer's Disease Potentially Lacking Senile Plaques Bearing the E693delta Mutation in Amyloid Precursor Protein.	Dement Geriatr Cogn Disord	32	45-54	2011
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書籍

著者氏名	論文タイトル名	書籍全体の 編集者名	書 籍 名	出版社名	出版地	出版年	ページ
なし							

### III. 研究成果の刊行物・別刷

# Clinical Course of Patients with Familial Early-Onset Alzheimer's Disease Potentially Lacking Senile Plaques Bearing the E693Δ Mutation in Amyloid Precursor Protein

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## Key Words

Alzheimer's disease · Clinical features · Amyloid  $\beta$  · Magnetic resonance imaging · Positron emission tomography · Mutation

## Abstract

**Background/Aims:** Oligomeric amyloid  $\beta$  ( $A\beta$ ) is currently considered to induce Alzheimer's disease (AD). We examined 2 patients with familial AD who possessed the Osaka (E693 $\Delta$ ) mutation in amyloid precursor protein. To the best of our knowledge, these patients are the first AD cases presumably affected with  $A\beta$  oligomers in the absence of senile plaques, and they support the  $A\beta$  oligomer hypothesis. **Methods:** We evaluated the clinical course, neuropsychological data, cerebrospinal fluid biomarker levels, magnetic resonance imaging (MRI) scans, fluorodeoxyglucose-positron emission tomography (PET) scans, and Pittsburgh compound B (PiB)-PET images of these patients. **Results:** In the early stages, these patients developed memory disturbances in a similar rate to patients with sporadic AD. Despite their memory disturbances, both patients showed only limited brain atrophy on MRI and little amyloid accumulation on PiB-PET. Subsequent to the development of memory disturbances, both

patients suffered from motor dysfunction, probably due to cerebellar ataxia, and, within a few years, the patients fell into an apallic state. **Conclusions:** Familial AD patients with Osaka (E693 $\Delta$ ) mutation show severe dementia, cerebellar ataxia, and gait disturbances. Copyright © 2011 S. Karger AG, Basel

## Background

The oligomer hypothesis of AD suggests that the soluble oligomers of amyloid  $\beta$  ( $A\beta$ ), but not the amyloid fibrils, initiate synaptic and cognitive dysfunction in patients with Alzheimer's disease (AD) [1–6]. However, there is no direct evidence that these mechanisms cause dementia in humans. Recently, we identified E693 $\Delta$ , which is a novel amyloid precursor protein (APP) mutation, in patients with AD and of Japanese lineage [7]. This mutation consists of the deletion of codon 693 (GAA) in the APP gene, which encodes glutamate-22 in the  $A\beta$  sequence. The resultant mutant  $A\beta$  (E22 $\Delta$ ) showed a unique aggregation property of enhanced oligomerization but no fibrillization. In support of the finding that the mutant  $A\beta$  failed to form a fibrillar structure, patients with this

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mutation were amyloid-negative on Pittsburgh compound B (PiB)-positron emission tomography (PET) imaging. In addition, the mutant A $\beta$  inhibited hippocampal long-term potentiation more potently than did wild-type A $\beta$  in rats *in vivo*. These findings suggest that the Osaka (E693 $\Delta$ ) mutation contributes to AD by enhancing the formation of synaptotoxic A $\beta$  oligomers, and not of amyloid fibrils, providing genetic evidence for the oligomer hypothesis described above.

In our opinion, patients with the Osaka (E693 $\Delta$ ) mutation are therefore expected to exhibit pure features of the A $\beta$  oligomer-induced neuropathology in their brains and of the A $\beta$  oligomer-associated clinical symptoms because the pathophysiology underlying their disease is free from the influence of amyloid plaques. However, the patients with this mutation presented in this paper are still alive, and, therefore, no autopsy data is available to date. Thus, in the present study, we describe the detailed clinical features of these patients and discuss the possible contribution of A $\beta$  oligomers to the clinical symptoms of AD. We believe that the precise clinical description of these patients will provide useful insights into dementia.

## Methods

### *Positron Emission Tomography*

[<sup>11</sup>C]PiB was synthesized using 2-(4'-aminophenyl)-6-hydroxybenzothiazole as the labeled precursor molecule. After an intravenous injection of [<sup>11</sup>C]PiB (150–300 MBq), a dynamic 60-min list-mode emission scan was acquired in the three-dimensional mode without arterial sampling using an Eminence-B PET scanner (Shimadzu Corp., Kyoto, Japan).

Magnetic resonance imaging (MRI) scans were coregistered with the respective PET images using the PMOD image-fusion tool (PMOD Technologies Ltd., Zurich, Switzerland). The PET images were reconstructed using a filtered back-projection algorithm for attenuation and scatter corrections. According to the study performed by Lopresti et al. [8], in which the frame summation of the dynamic images was recorded for 40–60 min, Logan graphical analysis was used for determining the regional counts (distribution volume ratio, DVR = binding potential + 1) using the cerebellum as the reference region. For this purpose, the cortical lesions occurring in the frontal, parietal, and lateral temporal lobes, the gyrus rectus, and the precuneus regions were selected. The mean cortical DVR (MCDVR) was the mean of the DVR values of these lesions [9]. Positive PiB binding indicated that the visible cortical PiB accumulation was higher than that of the white matter or that the MCDVR of the cortex was larger than the cutoff index obtained at our institute.

### *Neuropsychological Evaluations*

Neuropsychologists administered a set of neuropsychological test batteries that included a Mini-Mental State Examination (MMSE), a Rivermead Behavioral Memory Test (RBMT), a Clock

Drawing Test (CDT) [10], a Category Cued Memory Test (CCMT) [11], a Verbal Fluency Test (VFT) [12], a Scenery Picture Memory Test (SPMT) [13], a Block Design Test (BDT), a subtest of the Wechsler Adult Intelligence Scale-Revised, and the Trail Making Test (TMT) part A and B. For the CDT, the subject was asked to draw a clock with all of its numbers on a blank piece of paper and indicate the time as 10 after 11. We used the 10-point scoring system by Rouleau et al. [10] to score the exam. The CCMT, a modified version of Buschke's Double Memory Test that was reported previously, is a verbal memory test that contains 16 words from 8 categories and uses category cues during encoding and recall. In the CCMT, only cued recall, and not free recall, was tested. The score consisted of the number of items recalled correctly with the maximum score being 16. In the VFT, the subject was instructed to name as many vegetables, animals, and words that start from the letter 'ka' as possible, each within 1 min. The score was the total number of items mentioned. The SPMT is a newly developed, quick, and effective memory test that uses a line drawing of a scene of a living room in a house where 23 objects that are commonly observed in daily life are drawn on a piece of A4-sized paper. The test consisted of 2 trials (Pict-1 and Pict-2).

## Results

### *Patient 1*

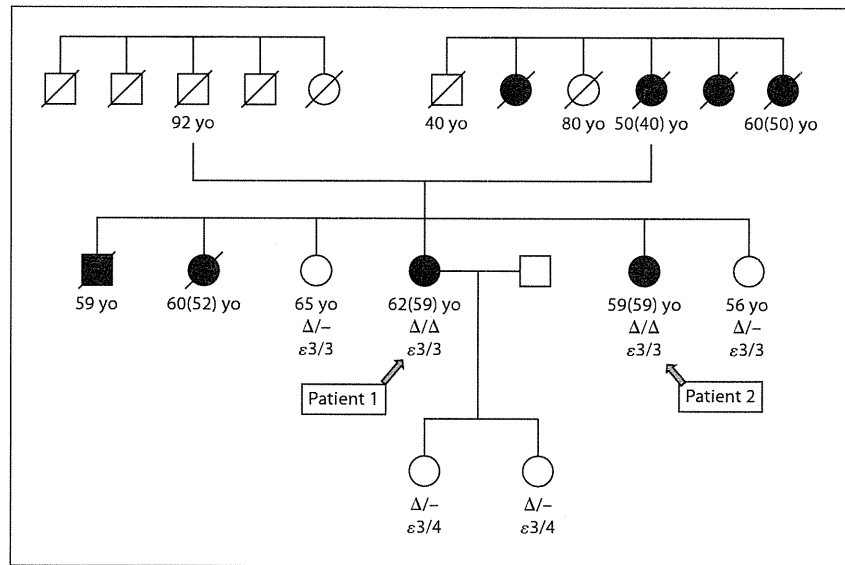
Patient 1 was a Japanese woman who was the proband of a highly consanguineous pedigree with hereditary dementia (fig. 1). She first experienced memory disturbances at the age of 55 and visited our hospital at the age of 57. Her MMSE scores (fig. 2) and MRI (fig. 3) and fluorodeoxyglucose (FDG)-PET (fig. 4a) images were rather normal. However, the three-dimensional stereotactic surface projection (3D-SSP) analysis showed hypometabolism of FDG in the posterior cingulate cortex, which is similar to that seen in patients with sporadic AD (fig. 4a) [14]. The levels of A $\beta$ 1–42 and A $\beta$ 1–40 in the patient's cerebrospinal fluid (CSF) were 22 and 390 pg/ml, respectively (normal values in our institute: A $\beta$ 1–42, >450 pg/ml). Based on a very small drop in her MMSE score and her own claims, the patient was diagnosed with mild cognitive impairment.

However, familial AD was suspected in the patient because of her pedigree's history of hereditary dementia. We therefore performed a genetic analysis on her and her family members under the agreement of informed consent and identified a novel mutation (E693 $\Delta$ ) in APP. She had homozygous alleles of this mutation, whereas her unaffected elder and younger sisters, who were 62 and 53 years old, respectively, bore heterozygous alleles.

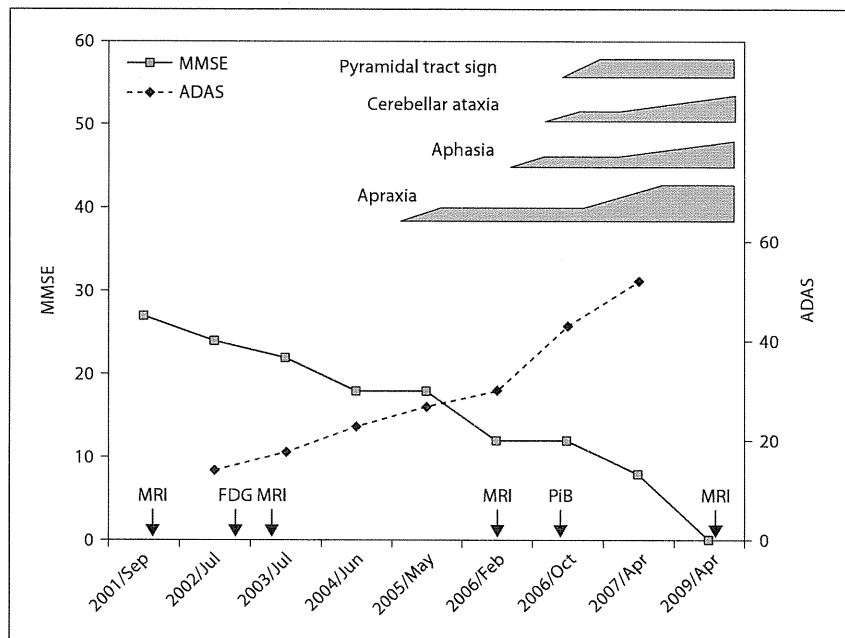
At the age of 59, patient 1's MMSE score decreased to 22. According to the Diagnostic and Statistical Manual for Mental Disorders, 3rd edition-revised, and the Na-



**Fig. 1.** Pedigree chart of the family carrying the E693 $\Delta$  mutation. The numbers indicate the ages of the family members at the time of death, at the time of the investigation of the gene, or at the time of the onset of the disease. The  $\Delta$  denotes the deletion of E693. The affected patients were homozygous for this mutation. The  $\epsilon$  denotes the Apo E genotype. yo = Years old.



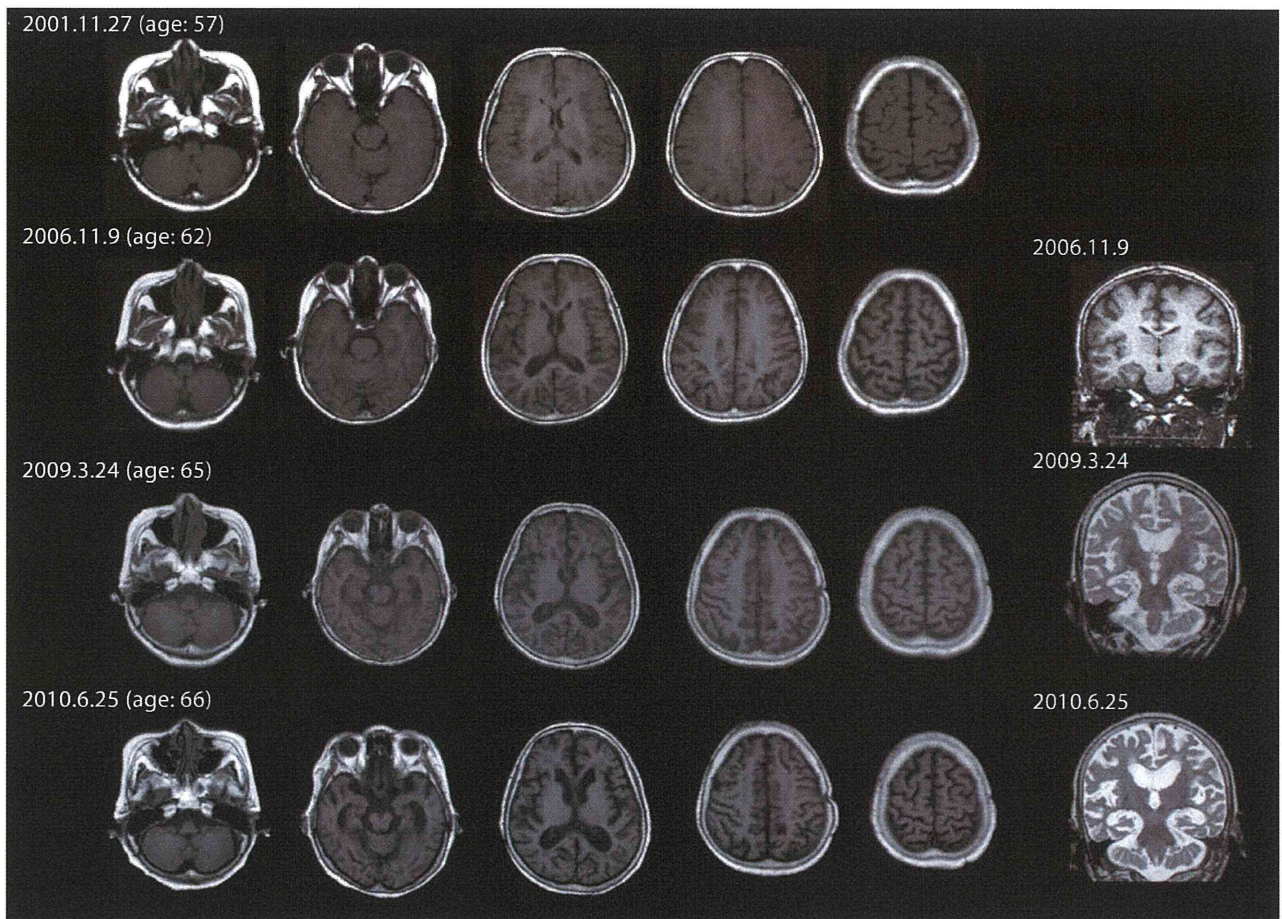
**Fig. 2.** Chart of the clinical course of patient 1. MMSE scores are graphed against the Alzheimer's Disease Assessment Scale (ADAS) scores. After the patient was diagnosed with mild cognitive impairment, her cognitive level declined gradually. Three years later, the patient developed apraxia and gait disturbance due to ataxia.



tional Institute of Neurological and Communicative Disorders and Stroke-AD and Related Disorders Association (NINCDS-ADRDA) criteria, she was diagnosed with AD, and treatment with donepezil was started.

Two years later, she showed dramatic changes in her clinical symptoms at the age of 61. Her MMSE score decreased to 12. She developed cerebellar ataxia, gait distur-

bances, apraxia, positive signs of pyramidal tract disturbances, including hyperreflexia of the patellar and Achilles tendons, and a positive sign of pathological reflex and spasticity in the lower extremities, none of which had been observed before. These features are unusual for patients with AD.

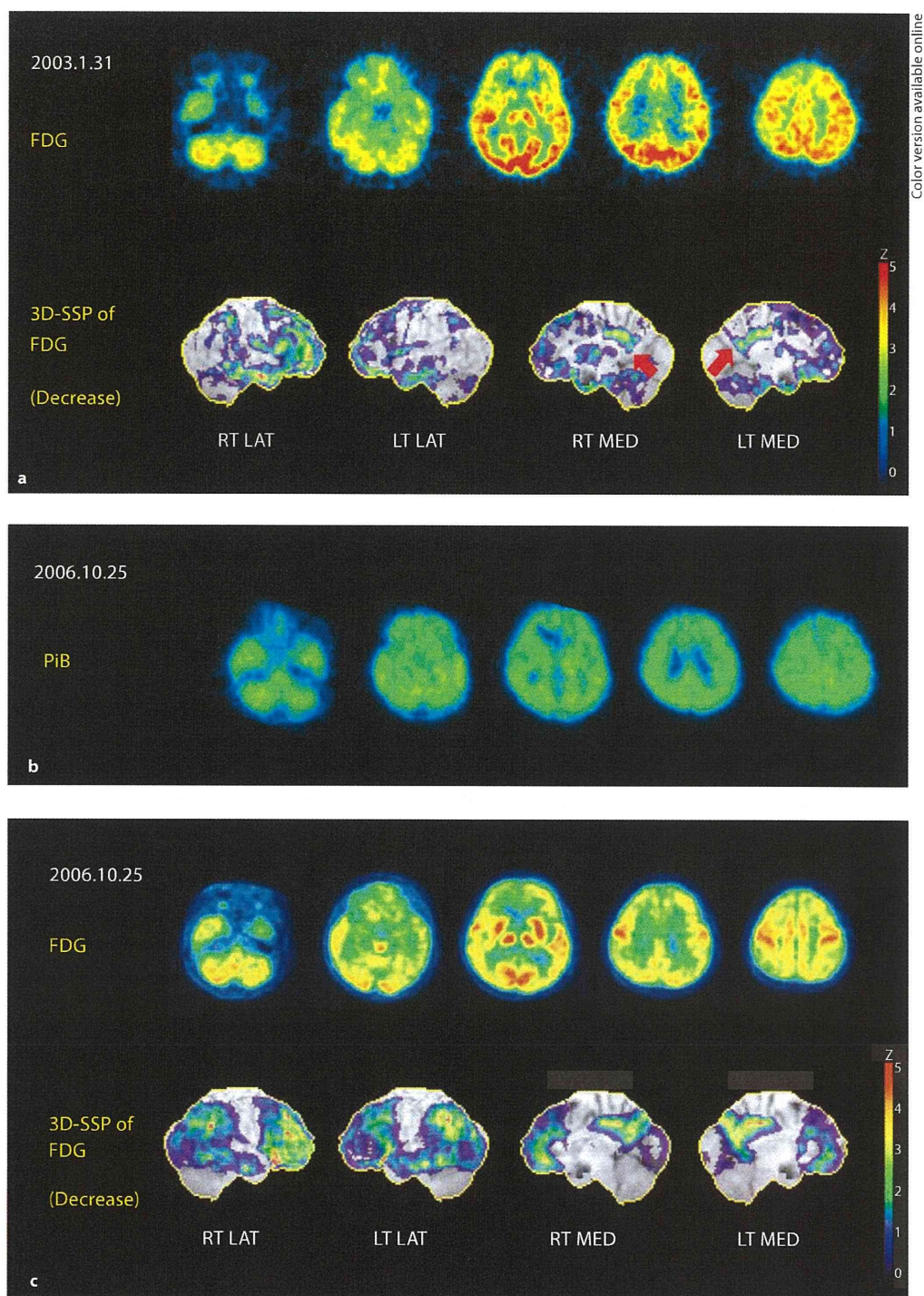


**Fig. 3.** MRI scans for patient 1 at the ages of 57, 62, 65, and 66. At the first visit, the MRI scans revealed no atrophy of the hippocampus. At the mid-stage of the disease (when the patient was 62), MRI images revealed slight worsening of the atrophy. After the patient entered the apallic state, atrophies of the hippocampus and the cerebral cortex were evident, but atrophy of the cerebellum was not detected.

Her cognitive impairment became very severe at the age of 62 when she had an MMSE score of 5. Nevertheless, an MRI scan revealed only mild parietal lobe atrophy (fig. 3) and no significant atrophy in the hippocampus. In addition, a [ $^{11}\text{C}$ ]PiB-PET scan revealed almost no amyloid accumulation in the cortex (fig. 4b). An FDG-PET scan showed decreased glucose metabolism throughout the brain of patient 1 except in the motor and sensory cortices and in the cerebellum (fig. 4c). At the age of 63, she became unable to walk by herself, and she fell in her house several times. After a chronic subdural hemorrhage due to a fall, she became bedridden, and her cognitive dysfunction worsened.

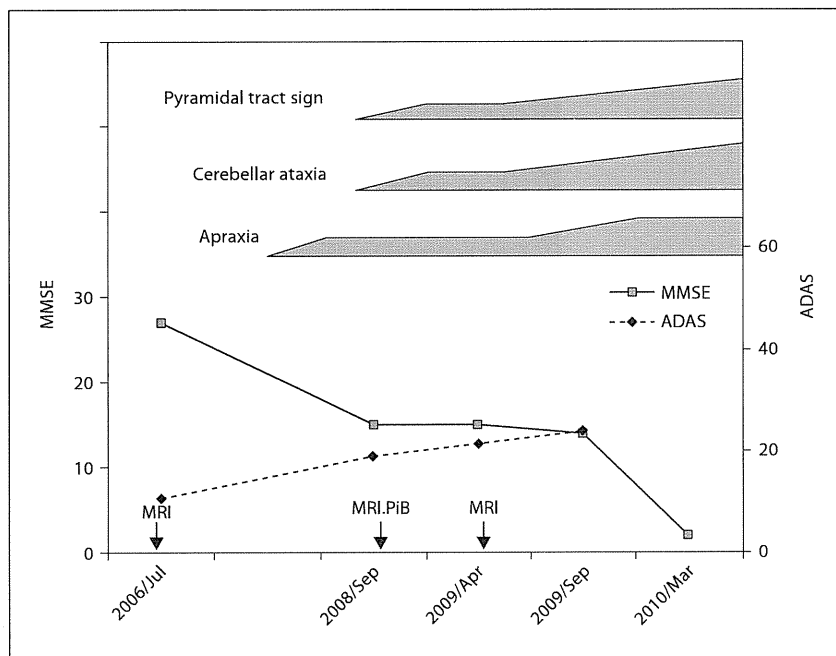
At the age of 65, her general status was comparable to that of a patient with apallic syndrome. MRI scans revealed moderate atrophy of the hippocampus, mild atrophy of the cerebrum, and mild dilatation of the third and lateral ventricles (fig. 3). It is noteworthy that, despite the severity of her cognitive dysfunction, only mild atrophy was detected in the cerebrum, which is in contrast to patients with sporadic AD who usually show severe atrophy in this region. CSF biomarker levels of  $\text{A}\beta$  and tau were highly indicative of AD. For instance, her CSF levels of  $\text{A}\beta_{1-42}$  and  $\text{A}\beta_{1-40}$  were 4.1 and 242.8 pg/ml, respectively, which were very low compared with published values in the US AD Neuroimaging Initiative study [15] and in a Japanese cohort study [16]. Moreover, the levels of





**Fig. 4.** **a** FDG and the 3D-SSP analysis of patient 1 showed FDG hypometabolism in the posterior cingulate cortex, which was similar to that seen in patients with sporadic AD (arrow). **b** PiB-PET images of patient 1. No accumulation of amyloid is seen in her brain. **c** FDG and 3D-SSP analysis images of patient 1, 3 years later than those in **a**. 3D-SSP reveals decreased glucose metabolism throughout the patient's brain, except in the motor and sensory cortices and in the cerebellum.

**Fig. 5.** Chart of the clinical course of patient 2. MMSE scores are graphed against the Alzheimer's Disease Assessment Scale (ADAS) scores. After the patient developed cerebellar ataxia, apraxia, and positive signs of pyramidal tract disturbances, her cognitive level declined rapidly.



total tau and phosphorylated tau in her CSF were 628 and 87.2 pg/ml, respectively (normal values in our institute: tau, <400 pg/ml; p-tau, <75 pg/ml). These values alone suggest that patient 1 was in an advanced phase of AD.

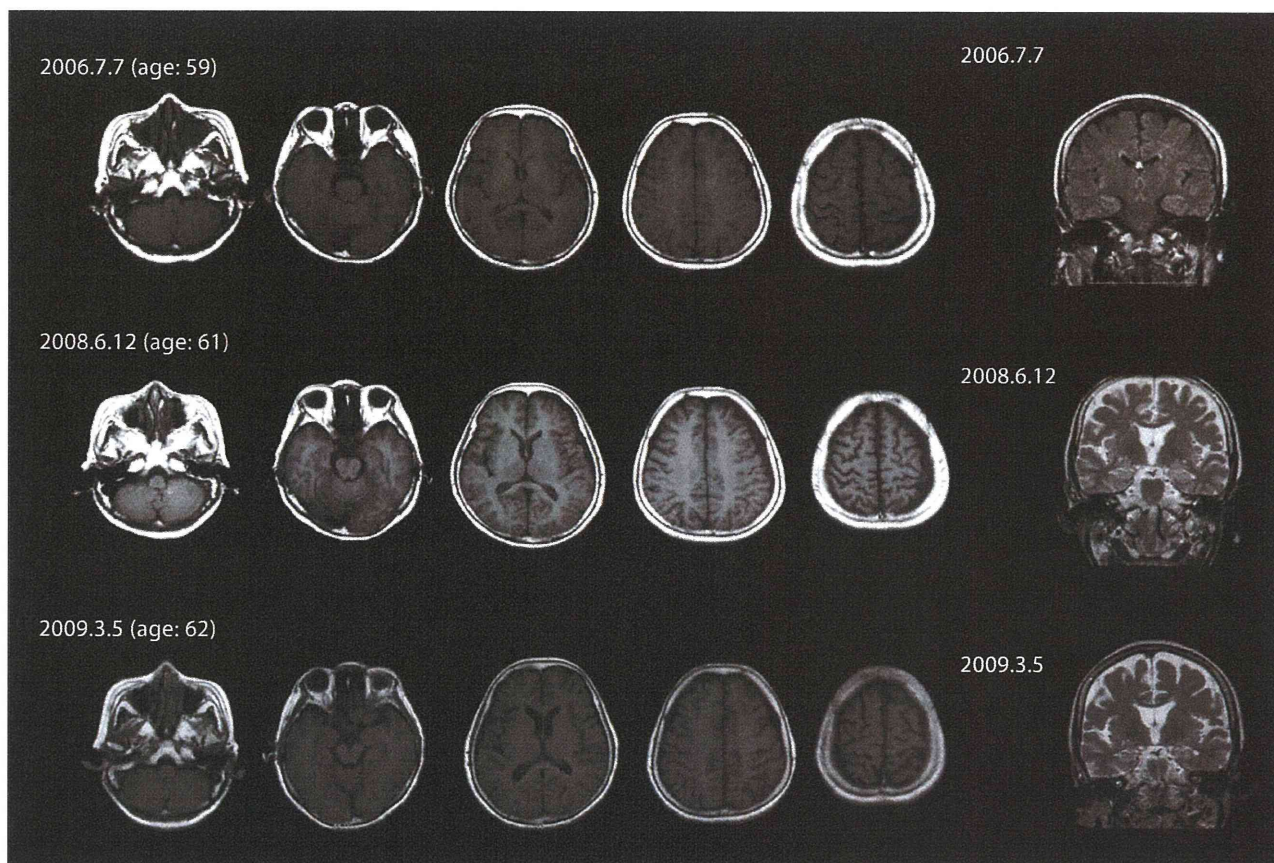
#### Patient 2

Patient 2 was the younger sister of patient 1 (fig. 1). She was carefully followed because she had homozygous alleles of the Osaka (E693Δ) mutation. When we found her homozygous mutation, she was 55. At that time, she was very thoughtful and polite to us and did not show any mental or behavioral abnormalities.

She experienced memory disturbances at the age of 59 and, hence, underwent a checkup at our hospital. However, her MMSE was 27 at that time. After that, her memory gradually worsened, and she was prescribed donepezil by her home doctor half a year later. Her condition worsened, and she again visited our hospital at the age of 61. She could speak fluently, but her MMSE score was 15 (fig. 5). A domain-specific cognitive evaluation was performed. Her recent memory was decreased to a score of 1 on the CCMT, 3 on the Pict-1, and 4 on the Pict-2 (table 1). The patient's visuospatial function showed mild to moderate decline, and her scores on the CDT and BDT decreased to 9 and 10, respectively. Her answer on the CDT showed distortion of the hour-hand of the clock, and

both hands were the same length. She showed an apparent decrease of her executive function with the results of 113 s on the TMT-A, and she was unable to do the TMT-B. The category fluency test showed relative preservation, and she could name 13 vegetables and 11 animals in a minute. She had a mild depressive mood with a score of 7 on the Geriatric Depression Scale. Her naming ability was relatively preserved with a score of 16 out of 17 on the RBMT naming subtest. These results suggest that her cognitive decline was compatible with moderate AD due to the obvious abnormalities in her recent memory and her general cognitive decline without predominant decline in executive and visuospatial functions. Similar to patient 1, she exhibited cerebellar ataxia, gait disturbances, and signs of disturbances in the pyramidal tract at the age of 61. An MRI scan revealed only mild cortical atrophy (fig. 6). A [<sup>11</sup>C]PiB-PET scan revealed no amyloid accumulation in the cerebral cortex (fig. 7). These clinical features were very similar to those of patient 1. The levels of Aβ<sub>1-42</sub> and Aβ<sub>1-40</sub> in her CSF were 9.5 and 285.2 pg/ml, respectively, and those of total tau and phosphorylated tau were 856 and 152 pg/ml, respectively. Again, these values alone suggested that patient 2 was in an advanced phase of AD similar to patient 1. At the age of 62, she could barely walk, even with the assistance of her family, and her MMSE score was 12.



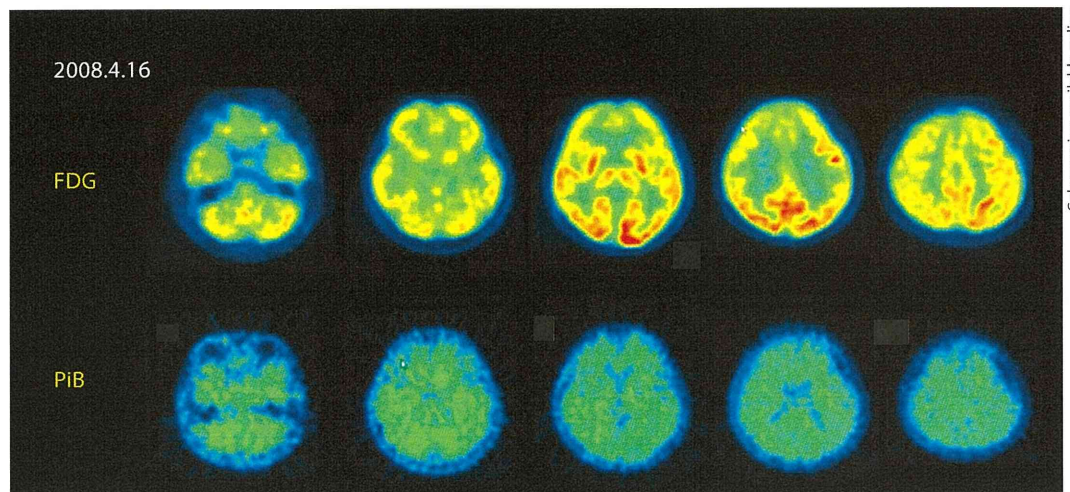


**Fig. 6.** MRI of patient 2 at the ages of 59, 61, and 62. The first MRI scan showed no atrophy of the brain. After her cognition decreased, her MRI images revealed slight cortical atrophy and no atrophy of the hippocampus.

**Table 1.** Neuropsychological evaluation in patient 2

Cognitive function	Test	Her score/maximum	Normal range
Recent memory	MMSE	15/30	≥24
	CCMT	1/16	≥9
	Pict-1	3/23	≥12
Visuospatial function	Pict-2	4/23	≥15
	CDT	9/10	≥10
	BDT	10/61	≥31
Attention, executive function	Category fluency (vegetable)	13	≥11
	Category fluency (animal)	11	≥11
	Letter fluency ('ka')	7	≥11
	TMT-A	113	≤43
	TMT-B	NA	
Depressive state assessment	Geriatric Depression Scale	7	<5

The data suggest that the patient developed memory disturbances and a loss of visuospatial function and executive function similar to that seen in patients with sporadic AD.



**Fig. 7.** FDG- and PiB-PET images of patient 2. PiB-PET images show no accumulation of amyloid in her brain, similar to that seen in patient 1.

## Discussion

We report here on 2 cases who were sisters in the same pedigree, who were both carrying an APP E693Δ mutation, and who possessed the same genotype of apolipoprotein E ( $\epsilon 3/3\epsilon 3$ ). Both of these cases had a variant type of AD. They developed very similar symptoms and showed a similar progression of the disease. In the early stages of the disease, they exhibited only memory disturbances, but they soon lost their visuospatial cognitive and executive functions. However, neither evident brain atrophy nor evident amyloid deposition was seen in the cortex. In the late stages, these patients displayed mild to moderate brain atrophy and unexpected motor dysfunction. These clinical features are unique and distinctive compared to patients with typical AD in several aspects.

First, our patients showed an apparent discrepancy between their cognitive impairment and apparent brain atrophy on MRI images. They first exhibited memory disturbances without any distinct atrophy. Similar observations were unexpectedly limited from our experiences in patients with AD when we considered their clinical dementia scales. The 3D-SSP analysis of the FDG-PET of patient 1 showed glucose hypometabolism in the posterior cingulate cortex, which is similar to that seen in patients with sporadic AD at the very early stages. This means that the mechanisms underlying the neuronal damage caused by the oligomers mimicked what happens in sporadic AD patients, and it caused the AD-like symp-

toms of these patients in their early stages. After the early stage, the rate of memory disturbance progression in these patients was apparently faster than that seen in sporadic AD cases. However, at this stage, brain atrophy was not apparent.

In our opinion, we speculate that both the memory disturbances and the subsequent brain atrophy were caused by A $\beta$  oligomers due to synaptic failure, but the resulting neuronal death required a longer period of time of cellular exposure to the A $\beta$  oligomers. Compared with typical AD, we expect that our patients possess higher levels of A $\beta$  oligomers in their brains, and they therefore exhibited memory disturbances at earlier ages. However, since the A $\beta$  oligomer-induced neurodegeneration required a long time, brain atrophy occurred only in the late stages. The second 3D-SSP analysis of FDG-PET of patient 1 showed severe hypoglycose metabolism throughout the brain except in the motor and sensory cortices and cerebellum, even though she could not walk due to cerebellar ataxia. In typical AD, the concentrations of brain A $\beta$  oligomers increase gradually, and neurons are exposed to A $\beta$  oligomers for a long time before the disease onset, which leads to eventual neurodegeneration. Once A $\beta$  oligomers reach a certain level, patients display memory disturbances, and in the clinic, their brain atrophy is observed simultaneously. However, we cannot exclude the possibility that amyloid fibrils, rather than A $\beta$  oligomers, primarily contribute to brain atrophy, and, thus, our patients did not possess significant