# 老年医学の基礎と臨床 II

——認知症学とマネジメント——

2009年6月25日 第1版第1刷

編集 浦上克哉
監修 大内尉義

●

発行者 吉岡正行
発行所 株式会社 ワールドプランニング
〒105-0001 東京都港区虎ノ門3-7-2
Tel：03-3431-3715 Fax：03-3431-3325
E-mail：world@med.email.ne.jp
http://www.worldpl.com
振替口座 00150-7-535934
表紙デザイン 星野鏡子（ケイ・デザインオフィス）
印刷 三報社印刷株式会社


ISBN978-4-86351-014-2

RESEARCH ARTICLE

International Journal of Geriatric Psychiatry

# Construction of a $^{18}$F-FDG PET normative database of Japanese healthy elderly subjects and its application to demented and mild cognitive impairment patients

Eizo Iseki[1], Norio Murayama[1], Ryoko Yamamoto[1], Hiroshige Fujishiro[1], Masaru Suzuki[1], Masato Kawano[2], Shuya Miki[2] and Kiyoshi Sato[1]

[1]PET/CT Dementia Research Center, Juntendo Tokyo Koto Geriatric Medical Center, Juntendo University School of Medicine, 3-3-20 Shinsuna, Koto-ku, Tokyo, Japan
[2]Nihon Medi-Physics, 3-4-10 Shinsuna, Koto-ku, Tokyo, Japan
*Correspondence to:* Dr E. Iseki, E-mail: iseki@juntendo.gmc.ac.jp

**Objective:** To construct a $^{18}$F-FDG PET normative database of Japanese healthy elderly subjects and to apply it to demented and mild cognitive impairment (MCI) patients.

**Methods:** Seventy-seven Japanese normal volunteers from 41 to 84 years of age (36 males and 41 females) who underwent clinical, neuropsychological, and MRI examinations were selected. In these subjects, $^{18}$F-FDG PET/CT scans were performed, $^{18}$F-FDG PET images were analyzed using the 3D-SSP program, and a normative database for cerebral glucose metabolism was constructed. Then, $^{18}$F-FDG PET images from 14 demented and MCI patients were evaluated based on the normative database.

**Results:** The 77 healthy elderly subjects were divided into three groups according to their age. In these subjects, the difference in glucose metabolism between males and females was minimal in contrast, glucose metabolism showed a weak reciprocal correlation with aging in several cerebral regions. The 3D-SSP images of 14 demented and MCI patients based on the age-matched $^{18}$F-FDG PET normative database showed decreased patterns of glucose metabolism similar to those of previous studies on dementia diseases and MCI.

**Conclusions:** An age-matched normative database can be applied to the evaluation of single subjects, and the application of a mixed database of males and females is viable. Normative databases are useful for detecting dementia diseases and their MCI. 

**Key words:** $^{18}$F-FDG PET; normative database; Alzheimer's disease; dementia with Lewy bodies; frontotemporal lobar degeneration; mild cognitive impairment

**History:** Received 24 December 2008; Accepted 26 May 2009; Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/gps.2346



## Introduction

Measurement of the cerebral metabolic rate of glucose (CMRglc) with $^{18}$F-FDG PET is being increasingly used to support the clinical diagnosis of several degenerative dementia diseases including Alzheimer's disease (AD). Compared with healthy elderly subjects, AD patients present with characteristic regional decreases of CMRglc in the posterior cingulate gyrus and parieto-temporal area (Minoshima *et al.*, 1994; Silverman *et al.*, 2001; Mosconi, 2005). In contrast, patients with dementia with Lewy bodies (DLB) and frontotemporal lobar degeneration (FTLD) show characteristic regional CMRglc decreases in the occipital lobe (Minoshima *et al.*, 2001; Small, 2004) and fronto-temporal area (Franceschi *et al.*, 2005; Drzezga *et al.*, 2008), respectively.

Mild cognitive impairment (MCI) is defined as suffering from a limited cognitive dysfunction with a clinical dementia rating (CDR) of 0.5 and is generally thought to be a preclinical stage of dementia diseases (Petersen *et al.*, 2001; Petersen and Negash, 2008). Among MCI patients, amnestic MCI patients usually progress to AD (Petersen and Negash, 2008) and show

a pattern of glucose hypometabolism similar to AD patients, although the magnitude and extent of the CMRglc decreases are milder than those observed in AD (Minoshima *et al.*, 1997; Drzezga *et al.*, 2005).

Automated methods of diagnostic image analysis such as three-dimensional stereotactic surface projections (3D-SSP) have been developed to facilitate $^{18}$F-FDG PET evaluation of single subjects with a normative database by producing observer-independent quantitative mapping of regional CMRglc abnormalities (Minoshima *et al.*, 1995; Signorini *et al.*, 1999; Herholz *et al.*, 2002). Whether regional CMRglc is considered abnormal is therefore related to the selection of appropriate reference subjects for the normative database.

Progressive CMRglc decreases in disease-affected brain regions are present in elderly subjects years before dementia diseases develop (De Leon *et al.*, 2001; Jagust *et al.*, 2006; Mosconi *et al.*, 2007). Therefore, a $^{18}$F-FDG PET normative database comprising of healthy elderly subjects with sufficient neuropsychological and neuroradiological data would make it possible to detect MCI and demented patients. To our knowledge, there are scarcely any currently available $^{18}$F-FDG PET normative databases containing sufficient numbers of Japanese healthy elderly subjects. The sample size of a normative database affects the diagnostic performance of $^{18}$F-FDG PET using automated image analysis, and the inclusion of 20 subjects may be preferable for constructing such a normative database (Chen W-P *et al.*, 2008).

The present study aims to construct a $^{18}$F-FDG PET normative database based on sufficient numbers of appropriate healthy elderly subjects from the Japanese population and to apply the normative database to demented and MCI patients.

## Materials and methods

### Subjects

We recruited 77 Japanese volunteers who were from 41 to 84 years of age (36 males and 41 females) to construct a $^{18}$F-FDG PET database of healthy elderly subjects at Juntendo Tokyo Koto Geriatric Medical Center between 2006 and 2007. These subjects underwent clinical, neuropsychological, and MRI examinations, as well as routine blood analysis before $^{18}$F-FDG PET examination. All 77 subjects met the following conditions: at least 6 years of education; no evidence of stroke, clinically uncontrolled diabetes, major head trauma, or depression; no use of cognitively active medications; no evidence of cognitive impairment based on extensive clinical interviews; a CDR of 0; a mini-mental state examination (MMSE) score of at least 27; and scores on the Alzheimer's disease assessment scale (ADAS), Wechsler memory scale-revised (WMS-R), and Wechsler adult intelligence scale-III (WAIS-III) suitable for their age. In addition, the conditions included brain atrophy and/or vascular change suitable for their age as measured by MRI examination (1.5 T scanner, MAGNETOM SYMPHONY, SIEMENS).

In addition to these subjects, 14 patients who consulted our memory clinic because of progressive cognitive dysfunction underwent $^{18}$F-FDG PET examination after clinical, neuropsychological, and MRI examinations.

All subjects and patients provided written informed consent for the present study.

### $^{18}$F-FDG PET scan Procedure

The $^{18}$F-FDG PET/CT scans were performed using a Discovery ST scanner [General Electric Healthcare; the mean trans-axial spatial resolutions (full width at half maximum) at 1 and 10 cm off axis were 6.2 and 6.7 mm, respectively]. The subjects fasted for at least 4 h before the scanning. After intravenous administration of 185 MBq/2 ml $^{18}$F-FDG, the subjects were kept in a dimly isolation room and instructed to remain still with their eyes open to avoid falling a sleep. A set of transaxial images was obtained with a standard 3D emission scan mode starting 40–60 min after the injection, and the scan duration was 15 min. The images were reconstructed using 3-dimensional iterative reconstruction including correction for attenuation as measured by the transmission scan. The matrix size, slice thickness, and number of $^{18}$F-FDG-PET images were 128 × 128 mm, 3.3 mm, and 47 slices, respectively.

### $^{18}$F-FDG PET data analysis

The $^{18}$F-FDG PET images were analyzed using the 3D-SSP program in NEUROSTAT developed by Minoshima *et al.* (1995). The original FDG-PET image data were realigned to the AC-PC line. The differences in individual brain size were removed by linear scaling, and regional anatomical differences were minimized by the nonlinear warping technique. After anatomical standardization, peak cortical activity in the brain was measured, and the peak value was projected onto the

standard brain surface. This procedure was repeated on a pixel-by-pixel basis covering the entire cortex of the brain, and 3D-SSP created images of the CMRglc found. As these CMRglc data were relative, the CMRglc image sets were normalized to the global activity.

### Construction of the $^{18}$F-FDG PET database

The pixel values of individual 3D-SSP images were normalized to mean whole brain CMRglc activity. Then, the 77 normalized images were averaged to construct a normative database.

### Evaluation of CMRglc in healthy elderly subjects

In Japan, elderly subjects are defined as follows: late-middle subjects are those from 40 to 64 years of age, early-elderly subjects are those from 65 to 74 years of age, and late-elderly subjects are those above 75 years of age. Therefore, the 77 healthy elderly subjects were divided into three groups according to their age: a group from 40 to 64 years of age, a group from 65 to 74 years of age, and a group above 75 years of age.

To demonstrate the regional patterns of CMRglc, a two-sample *t*-test was carried out on a pixel-by-pixel basis between the males and females in each group using 3D-SSP analysis. In addition, the correlation between CMRglc and age was examined using Pearson's correlation coefficient. The pixel value was normalized to mean whole brain CMRglc activity, and the correlation coefficient between the pixel value and age was calculated on a pixel-by-pixel basis.

For these, the *p*-value was calculated, translated to a Z-score, and superimposed onto 3D-SSP maps. The regional Z-score was examined using the SEE (stereotactic extraction estimation) method developed by Mizumura *et al.* (2003), which is an analytical method measuring regional Z-scores using a stereotactic ROI template from the Talairach daemon (Lancaster *et al.*, 2000). The null hypothesis was rejected at a Z-score $< 2.0$.

### Evaluation of CMRglc in patients

In order to evaluate the regional CMRglc decrease in 14 patients, the CMRglc in each patient was compared with the age-matched normative database using 3D-SSP analysis, and the CMRglc decrease was expressed as a Z-score (normal mean—individual value/normal SD) and superimposed onto 3D-SSP maps.

## Results

### Construction of the $^{18}$F-FDG PET database

The 77 healthy elderly subjects were divided into three groups: 26 subjects from 40 to 64 years of age, 30 subjects from 65 to 74 years of age, and 21 subjects above 75 years of age (Table 1). The overall neuropsychological data of the subjects were as follows: mean MMSE score: $28.7 \pm 1.5$, mean ADAS score: $2.5 \pm 1.7$, mean WMS-R score (general memory: GM): $115.4 \pm 12.1$, and mean WAIS-III score (full scale IQ: FIQ): $122.3 \pm 13.5$.

Table 1 Clinical and neuropsychological data of three age-groups of 77 healthy elderly subjects

| Age group | 40–64 (y.o.) | 65–74 (y.o.) | 75 (y.o.) | Total |
|---|---|---|---|---|
| Number (M:F) | 13:13 | 13:17 | 10:11 | 36:41 |
| Mean age | 52.6 ± 7.0 | 69.7 ± 2.8 | 78.0 ± 2.9 | 66.3 ± 14.1 |
| M | 51.5 ± 7.4 | 68.6 ± 2.7 | 77.0 ± 2.0 | 65.3 ± 14.7 |
| F | 54.1 ± 6.6 | 70.5 ± 2.7 | 78.8 ± 3.4 | 67.2 ± 12.5 |
| MMSE | 29.2 ± 1.5 | 28.5 ± 1.7 | 28.0 ± 2.0 | 28.7 ± 1.5 |
| M | 29.4 ± 1.2 | 28.7 ± 1.8 | 27.6 ± 2.2 | 28.6 ± 1.8 |
| F | 28.9 ± 1.8 | 28.4 ± 1.7 | 28.3 ± 1.9 | 28.5 ± 1.7 |
| ADAS | 2.0 ± 1.8 | 2.4 ± 1.5 | 3.2 ± 1.7 | 2.5 ± 1.7 |
| M | 2.4 ± 2.2 | 2.3 ± 1.2 | 3.4 ± 2.0 | 2.6 ± 1.9 |
| F | 1.7 ± 1.3 | 2.5 ± 1.7 | 2.9 ± 1.2 | 2.3 ± 2.0 |
| WMS-R (GM) | 115.1 ± 14.0 | 114.0 ± 12.1 | 117.8 ± 9.2 | 115.4 ± 12.1 |
| M | 116.2 ± 11.8 | 115.0 ± 13.6 | 116.5 ± 7.8 | 115.8 ± 11.3 |
| F | 114.2 ± 16.2 | 113.3 ± 11.3 | 119.0 ± 10.7 | 115.0 ± 12.8 |
| WAIS-III (FIQ) | 120.6 ± 13.5 | 121.9 ± 13.5 | 125.3 ± 13.8 | 122.3 ± 13.5 |
| M | 127.4 ± 10.5 | 126.6 ± 14.7 | 124.0 ± 17.1 | 126.1 ± 13.8 |
| F | 114.2 ± 13.1 | 118.4 ± 11.9 | 126.5 ± 10.3 | 119.1 ± 12.5 |

Mean ± SD M: male, F: female, MMSE: mini-mental state examination, ADAS: Alzheimer's disease assessment scale, WMS-R: Wechsler memory scale-revised, GM: general memory, WAIS-III: Wechsler adult intelligence scale-III, FIQ: full scale IQ

The $^{18}$F-FDG-PET 3D-SSP images in each group were examined for males only, females only, and mixed subjects, and then their mean and standard deviation images were constructed for the normative database.

## Characteristics of the $^{18}$F-FDG PET database

Differences between males and females. The regions showing a CMRglc decrease with a Z-score of over 2 in males compared to females were distributed in parts of the bilateral paracentral lobules, superior and inferior parietal lobules, and precuneus in the 40–64 years of age group, in parts of the right middle frontal and anterior cingulate gyri, and left superior parietal lobule in the 65–74 years of age group, and in parts of the right precuneus in the over 75 years of age group. The magnitude and extent of the differences were minimal, and there were almost no regions with a Z-score of over 3 (Figure 1A).

The regions showing a CMRglc decrease with a Z-score of over 2 in females compared to males were distributed in parts of the cerebellum in the 40–64 years of age group, in parts of the left inferior frontal, medial occipitotemporal, middle occipital and inferior occipital gyri and cuneus in the 65–74 years of age group, but none were present in the over 75 years of age group. The magnitude and extent of the differences were minimal, and there were almost no regions with a Z-score of over 3 (Figure 1B).

Change with advancing age. The 36 males showed a weak reciprocal correlation between CMRglc and age in the right middle frontal and anterior cingulate gyri, left inferior parietal lobule, thalamus, and the bilateral anterior and posterior cingulate gyri (Figure 2A). The 41 females showed a weak reciprocal correlation between CMRglc and age in the bilateral middle frontal gyri, right superior frontal gyrus, inferior parietal lobule, and the supramarginal and angular gyri (Figure 2B). The 77 mixed subjects showed a weak reciprocal correlation between CMRglc and age in the right inferior frontal and supramarginal gyri, the bilateral superior, middle frontal, and anterior

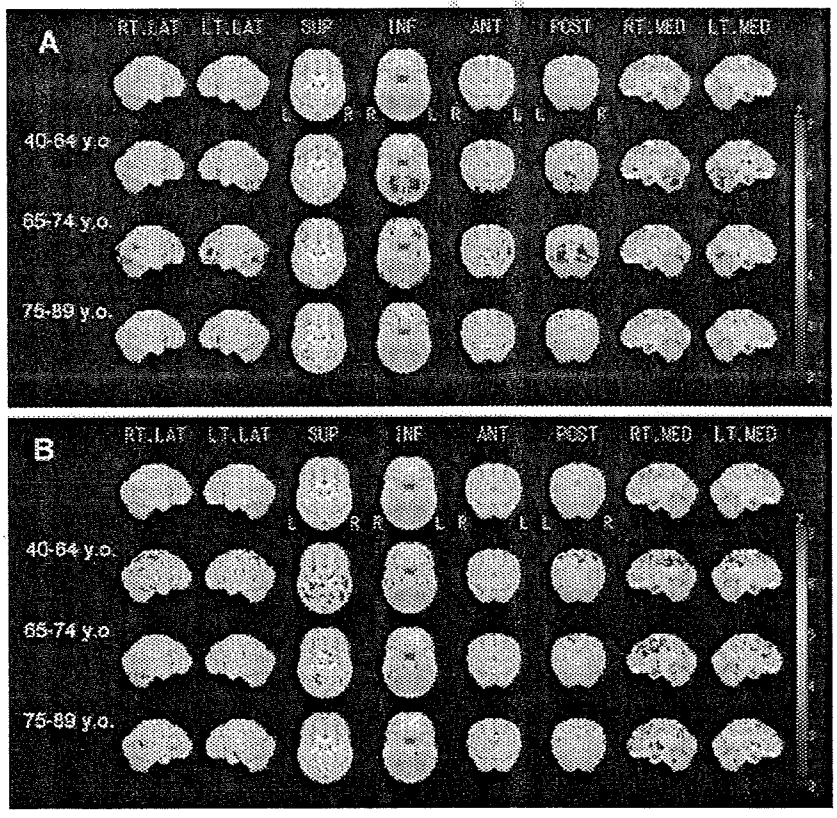


Figure 1 Gender-related differences in 3D-SSP Z-score images of glucose metabolism in three age-groups. (A) Decreased regions over Z-score 2 in males compared to females. (B) Decreased regions over Z-score 2 in females compared to males.

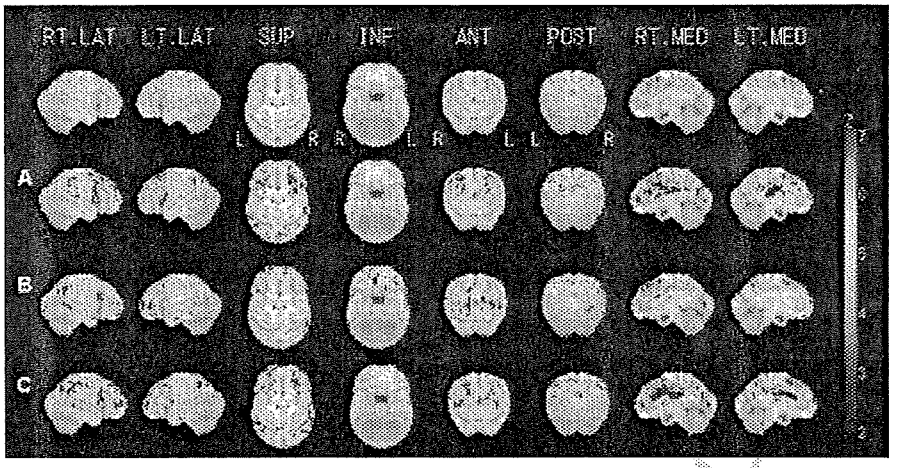


Figure 2 Change with advancing age in 3D-SSP Z-score images of glucose metabolism in healthy elderly subjects. (A) Regions showing a reciprocal correlation between glucose metabolism and age in males. (B) Regions showing a reciprocal correlation between glucose metabolism and age in females. (C) Regions showing a reciprocal correlation between glucose metabolism and age in mixed subjects.

cingulate gyri, and the inferior parietal lobule (Figure 2C).

## Clinical, neuropsychological, MRI findings, and $^{18}$F-FDG PET images of patients

The clinical and neuropsychological findings of 14 patients are shown in Table 2. Cases 1–5 showed general cognitive dysfunction including the impairment of recent memory, and fulfilled the diagnostic criteria for probable AD (McKhann *et al.*, 1984). Their MRI findings exhibited mild diffuse cerebral atrophy or mild atrophy of the hippocampus. On the other hand, Cases 6–10 showed impairment of recent memory without other cognitive dysfunctions, and did not fulfill the criteria for probable AD but corresponded to amnestic MCI (Petersen *et al.*, 2001; Petersen *et al.*, 2008). Their MRI findings exhibited mild atrophy of the hippocampus or no

Table 2 Clinical and neuropsychological data of 14 patients

| Case number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Age (y.o.) | 59 | 68 | 69 | 69 | 80 | 61 | 76 | 82 | 83 | 83 | 74 | 68 | 64 | 72 |
| Sex | F | F | F | F | F | F | F | F | M | M | M | M | F | M |
| Duration (y.) | 6 | 3 | 4 | 5 | 3 | 1 | 2 | 1 | 2 | 2 | 5 | 2 | 5 | 2 |
| CDR | 1 | 1 | 1 | 1 | 1 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 1 | 0.5 | 2 | 0.5 |
| MMSE | 21 | 22 | 20 | 21 | 19 | 25 | 27 | 24 | 25 | 27 | 18 | 25 | 17 | 28 |
| ADAS | 14.0 | 8.7 | 11.7 | 16.0 | 18.9 | 8.4 | 9.0 | 12.0 | 6.6 | 11.4 | 8.6 | 7.0 | — | 7.0 |
| WAIS-III | | | | | | | | | | | | | | |
| FIQ | 78 | 74 | 85 | 60 | 81 | 106 | 120 | 108 | 124 | 121 | 91 | 69 | — | 86 |
| VIQ | 95 | 77 | 86 | 70 | 91 | 109 | 124 | 107 | 123 | 128 | 112 | 75 | — | 81 |
| PIQ | 63 | 75 | 86 | 54 | 74 | 102 | 110 | 106 | 120 | 108 | 67 | 69 | — | 95 |
| VC | 99 | 82 | 80 | 69 | 90 | 100 | 124 | 102 | 120 | 116 | 114 | 73 | — | 76 |
| PO | 70 | 75 | 87 | 57 | 77 | 99 | 108 | 108 | 121 | 110 | 68 | 77 | — | 97 |
| WM | 72 | 76 | 88 | 85 | 92 | 109 | 111 | 111 | 111 | 126 | 103 | 83 | — | 94 |
| PS | 60 | 81 | 92 | 66 | 86 | 97 | 100 | 92 | 116 | 100 | 60 | 66 | — | 89 |
| WMS-R | | | | | | | | | | | | | | |
| GM | 57 | 63 | 63 | 54 | 50 | 88 | 73 | 78 | 65 | 82 | 65 | 73 | — | 91 |
| VeM | 64 | 73 | 65 | 68 | 59 | 94 | 75 | 82 | 65 | 89 | 75 | 77 | — | 81 |
| ViM | 61 | 57 | 73 | 50 | 50 | 81 | 75 | 77 | 70 | 75 | 57 | 75 | — | 114 |
| A/C | 81 | 78 | 88 | 83 | 71 | 131 | 94 | 94 | 110 | 120 | 72 | 98 | — | 99 |
| DR | <50 | <50 | <50 | <50 | 56 | 68 | 59 | 80 | 68 | 85 | 63 | 69 | — | 93 |

CDR: clinical dementia rating, VIQ: verbal IQ, PIQ: Performance IQ, VC: verbal comprehension, PO: perceptual organization, WM: working memory, PS: processing speed, WMS-R: GM: general memory, VeM: verbal memory, ViM: visual memory, A/C: attention/concentration, DR: delayed recall

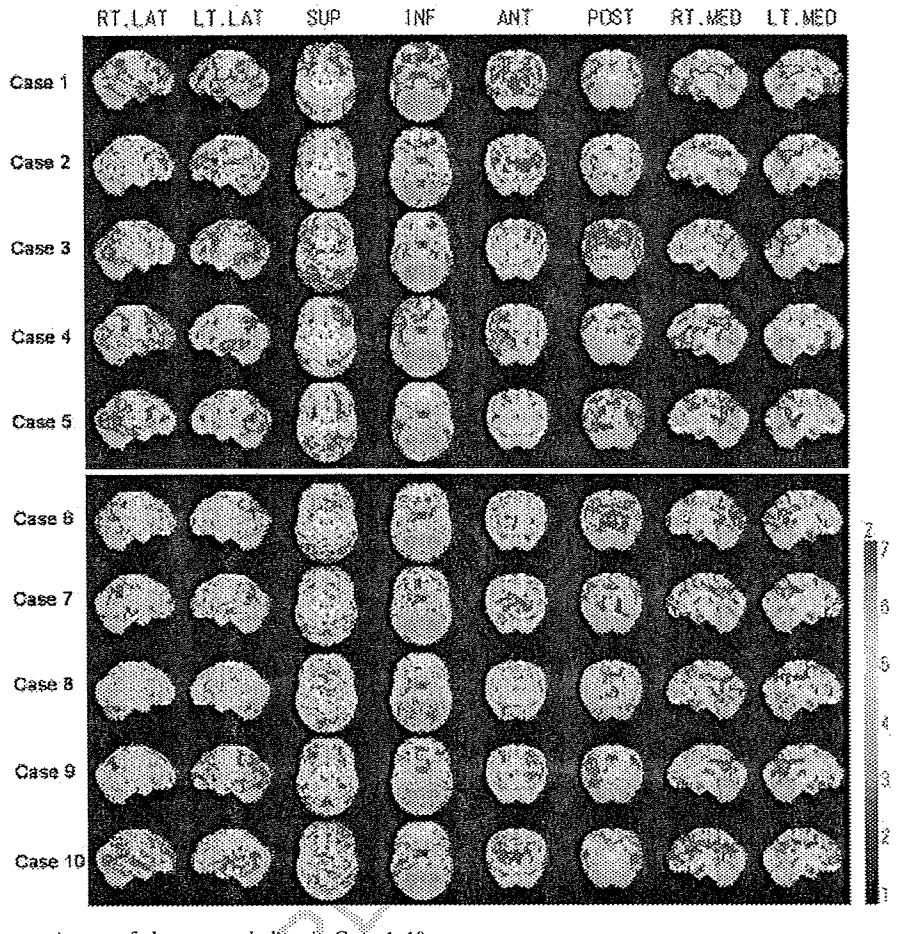


Figure 3 3D-SSP Z-score images of glucose metabolism in Cases 1–10.

significant brain atrophy. When compared with the age-matched $^{18}$F-FDG PET normative databases, Cases 1–5 exhibited a CMRglc decrease in the posterior cingulate gyrus and parietotemporal area, while Cases 6–10 did so in the posterior cingulate gyrus and/or part of the parietotemporal area (Figure 3).

Case 11 showed core features such as visual hallucinations, cognitive fluctuation, and parkinsonism in addition to general cognitive dysfunction, and fulfilled the diagnostic criteria for probable DLB (McKeith *et al.*, 1996, 2005). He revealed visuoperceptual disability in the Bender gestalt test (Murayama *et al.*, 2007). His MRI examination exhibited mild atrophy of the hippocampus. When compared with the age-matched normative database, he exhibited a CMRglc decrease in the lateral and medial occipital lobes (Figure 4). On the other hand, Case 12 developed REM sleep behavior disorder and impairment of recent memory, and showed mild general cognitive dysfunction with visuoperceptual disability, although he did not fulfill the diagnostic criteria for probable DLB. His MRI examination exhibited only mild atrophy of the hippocampus. When compared with the age-matched normative database, he exhibited a CMRglc decrease in the lateral and medial occipital lobes similar to that of Case 11 (Figure 4).

Case 13 showed semantic aphasia including loss of word meaning and comprehension as well as behavioral and personality changes, and fulfilled the diagnostic criteria for probable semantic dementia (SD) set out in the diagnostic criteria for FTLD (Neary *et al.*, 1998). She could not undergo WMS-R, WAIS-III, or ADAS because of semantic aphasia. Her MRI examination exhibited moderate atrophy of the temporal pole and base, predominantly on the left side. When compared with the age-matched normative database, she exhibited a CMRglc decrease in the anterior portion of the temporal lobes as well as in

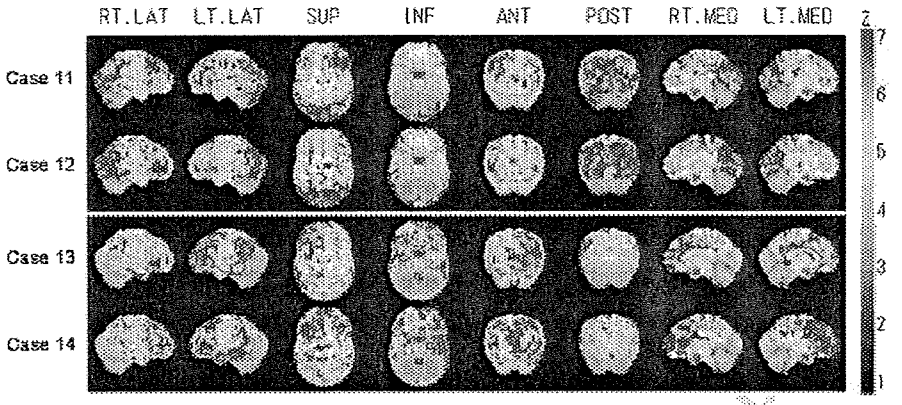


Figure 4 3D-SSP Z-score images of glucose metabolism in Cases 11–14.

the medial and basal frontal lobes with left predominancy (Figure 4). On the other hand, Case 14 developed mild loss of word meaning and mental rigidity and hypobulia, and showed low scores on verbal tasks and mild amnestic aphasia with the Western Aphasia Battery, although he did not fulfill the criteria for probable SD. His MRI examination exhibited mild atrophy of the left temporal pole and base. When compared with the age-matched normative database, he exhibited a CMRglc decrease in the anterior portion of the temporal lobes and medial frontal lobes with left predominancy similar to that of Case 13 (Figure 4).

## Discussion

The clinical usefulness of a $^{18}$F-FDG PET normative database for the assessment of demented patients depends on the selection of appropriate reference subjects to determine with confidence whether the regional glucose metabolism in single subjects is abnormal, particularly during the early stages of disease. In the present study, we selected 77 subjects that showed no significant but age suitable changes in detailed clinical, neuropsychological, and MRI examinations.

### Construction of the $^{18}$F-FDG PET database

The 77 subjects used in the present study were confirmed to be appropriate healthy elderly subjects before the $^{18}$F-FDG PET examination was performed. However, Mosconi *et al.* (2007) reported that 22 of the 77 healthy elderly subjects recruited for their $^{18}$F-FDG PET database developed MCI during a 4-year follow up study. This finding suggests that our $^{18}$F-FDG PET database may also require a follow up study to examine whether the present healthy elderly subjects would develop MCI or any dementia diseases later.

### Characteristics of the $^{18}$F-FDG PET database

In the 77 subjects, the difference in glucose metabolism between males and females was minimal, although the decreased regions of glucose metabolism tended to change with advancing age. It is unknown whether this difference depends on the sex or specificity of the present subjects. Previous studies about gender-related differences in the glucose metabolism of young volunteers have shown inconsistent results stating that the regional glucose metabolism was lower in males than in females in the orbital frontal area (Andreason *et al.*, 1994); that it was higher in males than in females in the temporal lobe, cerebral limbic area, and cerebellum, but higher in females than in males in the cingulate gyrus (Gur *et al.*, 1995); and that there were no gender-related differences (Miura *et al.*, 1990).

In contrast, glucose metabolism showed a weak reciprocal correlation with aging in the right inferior frontal and supramarginal gyri, the bilateral superior, middle frontal, and anterior cingulate gyri, and the inferior parietal lobules of the 77 subjects, confirming the change in glucose metabolism with advancing age. Previous studies have reported that glucose metabolism showed a reciprocal correlation with aging in several cerebral regions (Petit-Taboue *et al.*, 1998; Herholz *et al.*, 2002; Pardo *et al.*, 2007). Petit-Taboue *et al.* (1998), Herholz *et al.* (2002), and Pardo *et al.* (2007) reported that glucose metabolism showed a reciprocal correlation with normal aging in the bilateral perisylvian and insular regions, inferior and posterior-lateral frontal regions, anterior cingulate

gyri, the head of the caudate nucleus, and anterior thalamus; in the bilateral anterior cingulate gyri and fronto-lateral perisylvian regions; and in the bilateral anterior cingulate and medial prefrontal gyri, subgenual cingulate and basal forebrain, and dorsomedial thalamus, respectively. Our study and the other studies do not share regions of decrease in glucose metabolism except for the anterior cingulate gyrus. Pardo *et al.* (2007) suggested that the interaction of attention in the anterior cinglate gyrus with memory in the medial temporal lobe may explain cognitive dysfunction with aging. It remains unknown why there are differences in the regions of decrease other than in the anterior cingulate gyrus between these studies. 3D-SSP analysis decreases the bias caused by cortical atrophy compared to standard volume of interest (VOI) analysis (Ishii *et al.*, 2001). Some regions of decrease such as the perisylvian and insular regions, which the two former studies using standard VOI analysis showed, may be influenced by cortical atrophy. In addition, the differences in the regions of decrease may partly depend on differences in the age groups of the subjects between these studies.

In conclusion, the influence of aging on glucose metabolism should be considered, indicating that an age-matched database could be applied to the evaluation of single subjects. In contrast, the difference between males and females is minimal, suggesting that the application of a mixed database is viable.

## $^{18}$F-FDG PET Images of patients

It is well known that $^{18}$F-FDG PET normative databases are useful for diagnosing AD and differentiating MCI from AD. AD patients are clinically and neuropsychologically characterized by general cognitive dysfunction including progressive impairment of memory and other cognitive functions, and are radiologically characterized by diffuse cerebral atrophy accentuated in the hippocampus (McKhann *et al.*, 1984). In the present study, Cases 1–5 fulfilled the diagnostic criteria for probable AD (McKhann *et al.*, 1984) and were diagnosed as having early AD. In contrast, Cases 6–10 did not fulfill the criteria for probable AD and were diagnosed as having amnestic MCI (Petersen and Negash, 2008). When the age-matched $^{18}$F-FDG PET normative databases using 3D-SSP images were applied to these patients, Cases 1–5 exhibited a CMRglc decrease in the posterior cingulate gyrus and parietotemporal area, while Cases 6–10 did so in the posterior cingulate gyrus and/or part of the parietotemporal area. These two groups revealed an apparent continuity in regions of decrease, although the regions of decrease in Cases 6–10 were more limited than those in Cases 1–5. The decreased patterns of glucose metabolism in Cases 1–5 and Cases 6–10 are fundamentally coincide with those of previous studies on AD (Minoshima *et al.*, 1994; Silverman *et al.*, 2001; Mosconi, 2005) and MCI (Minoshima *et al.*, 1997; Drzezga *et al.*, 2005), respectively. These findings suggest that Cases 6–10 may progress to AD and that age-matched $^{18}$F-FDG PET normative databases are useful for diagnosing AD and differentiating amnestic MCI from AD.

In contrast, $^{18}$F-FDG PET images of MCI of DLB or FTLD are very rare, although a comparative diagnostic study between MCI, AD, DLB, and frontotemporal dementia (FTD) of FTLD has been performed using $^{18}$F-FDG PET (Mosconi *et al.*, 2008). This may be due to the fact that the clinical conditions corresponding to MCI of DLB or FTLD have not been defined. DLB patients show characteristic visuoperceptual and/or constructional disabilities (Moshiman *et al.*, 2004; Murayama *et al.*, 2007). In the present study, Case 11 fulfilled the diagnostic criteria for probable DLB (McKeith *et al.*, 1996, 2005), while Case 12 showed memory impairment and REM sleep disorder, one of the suggestive features of DLB, as well as visuoperceptual disability, suggesting that he may progress to DLB (MCI of DLB). When compared with the age-matched $^{18}$F-FDG PET normative databases, both Cases 11 and 12 showed a decreased pattern of glucose metabolism similar to probable DLB (Minoshima *et al.*, 2001; Small, 2004), although the magnitude and extent of this in Case 12 were milder than those of Case 11.

FTLD is composed of FTD, SD, and progressive nonfluent aphasia (Neary *et al.*, 1998). SD patients show semantic aphasia including loss of word meaning and comprehension as well as behavioral and personality changes in spite of having relatively mild memory impairment (Hodges *et al.*, 1992; Neary *et al.*, 1998). The MRI findings of SD exhibit atrophy of the anterior temporal lobes, predominantly on the left side (Rosen *et al.*, 2002). In the present study, Case 13 fulfilled the criteria for probable SD set out in the diagnostic criteria for FTLD (Neary *et al.*, 1998), while Case 14 showed loss of word meaning, mental rigidity, and hypobulia without memory impairment and revealed low scores on verbal tasks and mild amnestic aphasia as well as mild atrophy of the left temporal pole and base, suggesting that he may progress to SD (MCI of FTLD). When compared with the age-matched $^{18}$F-FDG PET normative databases, both Cases 13 and 14 showed a decreased pattern of glucose metabolism similar to probable SD (Drzezga *et al.*, 2008), although

the magnitude and extent of this in Case 14 were milder than those of Case 13. These findings suggest that age-matched $^{18}$F-FDG PET normative databases are also useful for detecting DLB, FTLD, and their MCI. However, the assumption that each of the MCI cases, especially Cases 12 and 14, will progress to the suggested dementia diseases has to be confirmed by definite data obtained from a longitudinal study using clinical, neuropsychological, and neuropathological methods.

In future, the normative database will be applied to patients with various degenerative dementia diseases and will facilitate their early clinical diagnoses by examining regional glucose abnormalities characteristic of each disease.

## Acknowledgements

This study was supported in part by the Anti-aging Research Center of Juntendo University School of Medicine, The Ogasawara Foundation for the Promotion of Science and Engineering, and Nihon Medi-Physics.

## References

Andreason PJ, Zametkin AJ, Guo AC, *et al.* 1994. Gender-related differences in regional cerebral glucose metabolism in normal volunteers. *Psychiatry Res* **51**: 175–183.

Chen W-P, Samuraki M, Yanase D, *et al.* 2008. Effect of sample size for normal database on diagnostic performance of brain FDG PET for the detection of Alzheimer's disease using automated image analysis. *Nucl Med Commun* **29**: 270–276.

De Leon MJ, Convit A, Wolf OT, *et al.* 2001. Prediction of cognitive decline in normal elderly subjects with 2-[$^{18}$F]fluoro-2-deoxy-D-glucose/positron-emmision tomography (FDG/PRT). *Proc Natl Acad Sci USA* **98**: 10966–10971.

Drzezga A, Grimmer T, Riemenschneider M, *et al.* 2005. Prediction of individual outcome in MCI by means of genetic assessment and $^{18}$F-FDG PET. *J Nucl Med* **46**: 1625–1632.

Drzezga A, Grimmer T, Henrikson G, *et al.* 2008. Imaging of amyloid plaques and cerebral glucose metabolism in semantic dementia and Alzheimer's disease. *Neuroimage* **39**: 619–633.

Franceschi M, Anchisi D, Pelati O, *et al.* 2005. Glucose metabolism and serotonin receptors in the frontotemporal lobar degeneration. *Ann Neurol* **57**: 216–225.

Gur RC, Mozley RH, Mozley PD, *et al.* 1995. Sex differences in regional glucose metabolism during a resting state. *Science* **267**: 528–531.

Herholz K, Salmon E, Perani D, *et al.* 2002. Discrimination between Alzheimer dementia and controls by automated analysis of multicenter FDG PET. *Neuroimage* **17**: 302–316.

Hodges JR, Patterson K, Oxbury S, Funnell E. 1992. Semantic dementia. Progressive fluent aphasia with temporal lobe atrophy. *Brain* **115**: 1783–1806.

Ishii K, Willoch F, Minoshima S, *et al.* 2001. Statistical brain mapping of $^{18}$F-FDG PET in Alzheimer's disease: Validation of anatomic standardization for atrophied brains. *J Nucl Med* **42**: 548–557.

Jagust W, Gitcho A, Sun F, *et al.* 2006. Brain imaging evidence of preclinical Alzheimer's disease in normal aging. *Ann Neurol* **59**: 673–681.

Lancaster JL, Woldorff MG, Parsons LM, *et al.* 2000. Automated Talairach atlas labels for functional brain mapping. *Hum Brain Mapp* **10**: 120–131.

McKeith IG, Galasko D, Kosaka K, *et al.* 1996. Consensus guidelines for the clinical and pathological diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. *Neurology* **47**: 1113–1124.

McKhann G, Drachman D, Folstein M, *et al.* 1984. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* **34**: 939–944.

Minoshima S, Foster NL, Kuhl DE. 1994. Posterior cingulate cortex in Alzheimer's disease. *Lancet* **344**: 895.

Minoshima S, Frey KA, Koeppe RA, *et al.* 1995. A diagnostic approach in Alzheimer's disease using three-dimensional stereotactic surface projections of fluorine-18-FDG PET. *J Nucl Med* **36**: 1238–1248.

Minoshima S, Giordani B, Berent S, *et al.* 1997. Metabolic reduction in the posterior cingulate cortex in very early Alzheimer's disease. *Am Neurol* **42**: 85–94.

Minoshima S, Foster NL, Sima AA, *et al.* 2001. Alzheimer' disease versus dementia with Lewy bodies; cerebral metabolic distinction with autopsy confirmation. *Ann Neurol* **50**: 358–365.

Miura SA, Schapiro MB, Grady CL, *et al.* 1990. Effect of gender on glucose utilization rates in healthy humans: a positron emission tomography study. *J Neurosci Res* **27**: 500–504.

Mizumura S, Kumita S, Cho K, *et al.* 2003. Development of quantitative analysis method for stereotactic brain image: assessment of reduced accumulation in extent and severity using anatomical segmentation. *Ann Nucl Med* **17**: 289–295.

Mosconi L. 2005. Brain glucose metabolism in the early and specific diagnosis of Alzheimer's disease: FDG-PET studies in MCI and AD. *Eur J Nucl Med Imaging* **32**: 486–510.

Mosconi L, Tsui WH, Pupi A, *et al.* 2007. $^{18}$F-FDG PET database of longitudinally confirmed healthy elderly individuals improves detection of mild cognitive impairment and Alzheimer's disease. *J Nucl Med* **48**: 1129–1134.

Mosconi L, Tsui WH, Herholz K, *et al.* 2008. Multicenter standardized $^{18}$F-FDG PET diagnosis of mild cognitive impairment, Alzheimer's disease, and other dementias. *J Nucl Med* **49**: 390–398.

Mosimann UP, Mather G, Wesnes KA, *et al.* 2004. Visual perception in Parkinson disease dementia and dementia with Lewy bodies. *Neurology* **63**: 2091–2096.

Murayama N, Iseki E, Yamamoto R, *et al.* 2007. Utility of the Bender Gestalt Test for differentiation of dementia with Lewy bodies from Alzheimer's disease in patients showing mild to moderate dementia. *Dement Geriatr Cogn Disord* **23**: 258–263.

Neary D, Snowden JS, Gustafson L, *et al.* 1998. Frontotemporal lobar degeneration. A consensus on clinical diagnostic criteria. *Neurology* **51**: 1546–1554.

Pardo JV, Lee JT, Sheikh SA, *et al.* 2007. Where the brain grows old: decline in anterior cingulate and medial prefrontal function with normal aging. *Neuroimage* **35**: 1231–1237.

Petersen RC, Doody R, Kurz A, *et al.* 2001. Current concepts in mild cognitive impairment. *Arch Neurol* **58**: 1985–1992.

Peterson RC, Negash S. 2008. Mild cognitive impairment: an overview. *CNS Spectr* **13**: 45–53.

Petit-Taboue MC, Landeau B, Desson JF, *et al.* 1998. Effects of healthy aging on the regional cerebral metabolic rate of glucose assessed with statistical parametric mapping. *Neuroimage* **7**: 176–184.

Rosen HJ, Gorno-Tempini ML, Goldman WP, *et al.* 2002. Patterns of brain atrophy in frontotemporal dementia and semantic dementia. *Neurology* **58**: 198–208.

Signorini M, Paulesu E, Friston K, *et al.* 1999. Rapid assessment of regional cerebral metabolic abnormalities in single subjects with quantitative and nonquantitative [$^{18}$F] FDG PET: a clinical validation of statistical parametric mapping. *Neuroimage* **9**: 63–80.

Silverman DH, Small GW, Chang CY, *et al.* 2001. Positron emission tomography in evaluation of dementia: regional brain metabolism and long-term outcome. *JAMA* **286**: 2120–2127.

Small GW. 2004. Neuroimaging as a diagnostic tool in dementia with Lewy bodies. *Dement Geriatr Cogn Disord* **17**: 25–31.

*Aging & Mental Health*
Vol. 13, No. 4, July 2009, 563–568

Routledge
Taylor & Francis Group

# Risk factors for delusion of theft in patients with Alzheimer's disease showing mild Dementia in Japan

N. Murayama[ab*], E. Iseki[a], T. Endo[c], K. Nagashima[c], R. Yamamoto[ad], Y. Ichimiya[ad], H. Arai[d] and K. Sato[a]

[a]*PET/CT Dementia Research Center, Juntendo Tokyo Koto Geriatric Medical Center, Juntendo University School of Medicine, 3-3-20 Shinsuna, Koto-ku, Tokyo 136-0075, Japan;* [b]*Doctor Course of Psychology, Graduate School of Literature and Social Sciences, Nihon University, Tokyo, Japan;* [c]*Department of Psychology, College of Humanities and Sciences, Nihon University, Tokyo, Japan;* [d]*Department of Psychiatry, Juntendo University School of Medicine, Tokyo, Japan*

(*Received 3 August 2008; final version received 10 November 2008*)

The mechanism underlying delusion in Alzheimer's disease patients has not been fully clarified; however, the occurrence of delusion is a critical issue for dementia patients and their caregivers. In Japan, delusion of theft is the most frequent delusion in AD patients. We examined the risk factors for delusion of theft in AD patients showing mild dementia. Fifty-six AD patients were administered HDS-R, MMSE and COGNISTAT, including the 'speech sample', to assess their neuropsychological and social cognitive functions. The age, years of education, presence of cohabiting family members and premorbid personality traits were obtained from family members. About 25.0% of AD patients showed delusion of theft (D-group), and 75% did not (non-D-group). About 33.3% of female patients and 5.9% of male patients were included in the D-group ($p < 0.05$). About 13.6% of patients who were cohabiting with family members and 66.7% of patients who were living alone were included in the D-group ($p < 0.05$). About 35.1% of patients who had a neurotic personality and 5.3% of patients who did not were included in the D-group ($p < 0.05$). There were no significant differences in scores on HDS-R, MMSE and COGNISTAT sub-scales, except for 'speech sample', between the two groups. In the 'speech sample', 38.7% of patients who understood a relationship between two boys and 12.0% of patients who did not were included in the D-group ($p < 0.05$). These results indicated that delusion of theft in AD patients was related to female gender, absence of cohabiting family members, neurotic personality and retained social cognitive function.

**Keywords:** Alzheimer's disease; psychological and behavioural symptoms; psychological and social aspects; delusion

## Introduction

In Japan, there are 26 million elderly people who are 65 years of age or older, accounting for 20% of the approximately 130 million population in 2005. One out of the 13 elderly people of age 65 years or older, and one out of the four elderly people of age 85 years of or older have dementia. The presence of dementia patients is an important social issue not only in Japan, but also throughout the world.

Especially, the occurrence of behavioural and psychological symptoms in dementia (BPSD) is a critical issue for dementia patients and their caregivers because it frequently leads to a reduction in quality of life (Deimling & Bass, 1986), early institutionalization (Colerick & George, 1986), and progression of cognitive dysfunction (Haupt, Romero, & Kurz, 1996; Jeste, Wragg, Salmon, Harris, & Thal, 1992; Ropacki & Jeste, 2005). Delusion is the most frequent BPSD in Alzheimer's disease (AD) patients, and its prevalence has been reported to be about 36%, ranging from 9.3 to 63% (Ropacki & Jeste, 2005). The mechanism underlying delusion in AD patients, however, has not been fully clarified (Lee et al., 2007; Mizrahi, Starkstein, Jorge, & Robinson, 2006). Some studies reported that delusion in AD patients was related to female gender (Bassiony & Lyketsos, 2003; Ikeda et al., 2003; Ozawa, 1997; Rao & Lyketsos, 1998), whereas other studies reported that males were more likely to have delusions than females (Burns, Jacoby, & Levy, 1990), or there was no significant difference in terms of gender (Migliorelli et al., 1995). The relations between delusion and age (Migliorelli et al., 1995; Ozawa, 1997), educational level (Jeste et al., 1992) and severity of dementia (Swearer, Drachman, O'Donnell, & Mitchell, 1988; Teri, Larson, & Reifler, 1988) have also been controversial in previous studies (Ballard & Oyebode, 1995; Bassiony & Lyketsos, 2003; Cummings & Victoroff, 1990; Kazui et al., 2006; Ropacki & Jeste, 2005).

As for cognitive function, some studies reported that there was a significant correlation between delusion and scores on generalized cognitive psychological tests such as the Mini Mental State Examination (MMSE) (Folstein, Folstein, & McHugh, 1975; Haupt et al., 1996; Jeste et al., 1992; Takechi, Yamada, Sugihara, & Kita, 2006), whereas other studies reported that there was no significant correlation (Burns et al., 1990; Ikeda et al., 2003; Migliorelli et al., 1995). Only a few studies have examined the relation between delusion and particular neuropsychological cognitive functions.

*Corresponding author. Email: murayama@juntendo.gmc.ac.jp

ISSN 1360-7863 print/ISSN 1364-6915 online

DOI: 10.1080/13607860902774402
http://www.informaworld.com

Jeste et al. (1992) reported that there were significant differences between AD patients with and without delusion in scores on the MMSE, blessed information memory concentration test and dementia rating scale, and that there were no significant differences in scores on the Boston naming test, number information test, verbal fluency test, block design subtest in WISC-R, clock drawing test, modified Wisconsin card sorting test and similarities sub-scales in WAIS-R. In Migliorelli's study (Migliorelli et al., 1995), there were no significant differences between AD patients with and without delusion in scores on various psychological tests, including the Boston naming test, Raven's progressive matrices test and several tests for executive function, in addition to the MMSE. It is, however, a critical point that severity of dementia among patients was not controlled in these studies, so it is not clear which delusion or severity of dementia is related to these findings. A previous study using patients with similar MMSE scores reported that there were no significant differences between delusion and cognitive functions in psychological tests estimating some verbal functions, non-verbal functions or executive functions (Burns et al., 1990). In addition, cognitive functions include not only neuropsychological cognitive functions such as memory, orientation, construction, language and executive functions, but also social cognitive functions. It is also important to examine the relation between delusion and social cognitive functions because delusion is frequently caused by interpersonal relations.

One reason why the mechanism underlying delusion in AD patients has not been clarified may be the fact that previous studies have examined delusion without distinguishing between different delusions such as delusion of theft, delusion of infidelity or jealousy, somatic delusions and delusions of grandeur. These delusions represent different notions; for example, in delusion of theft the patient believes 'People are stealing my things', in somatic delusions the patient feels 'There are subcutaneous worms in my arm', and in delusions of grandeur the patient believes 'I am the greatest man'. These delusions may also have different mechanisms, so it is necessary to study the mechanism of each delusion in dementia patients separately.

In Japan, delusion of theft is the most frequent delusion in AD patients (Ikeda et al., 2003; Ozawa, 1997), although there have been few studies concerning this delusion. Ikeda (2004) reported that there were no significant differences in sub-scale scores on the Alzheimer's disease Assessment Scale (ADAS) between AD patients with and without delusion of theft. Takechi et al. (2006) reported that there were no significant differences in scores on the word fluency test, category cued memory test and clock drawing test between AD patients with and without delusion of theft, although there was a significant difference in their MMSE scores. Other studies also reported that there was no significant correlation between delusion of theft and cognitive functions (Kazui et al., 2006; Terada et al., 2005).

Another reason why the mechanism underlying delusion in AD patients has not been clarified may be the fact that previous studies have underestimated the importance of psychosocial factors other than cognitive functions. The presence of cohabiting family members or premorbid personality traits may be general psychosocial risk factors for delusion, especially delusion of theft.

In the present study, we examined the risk factors for delusion of theft in AD patients showing mild dementia, focusing on social cognitive functions, cohabiting family members and premorbid personality traits, which have so far been rarely examined, in addition to gender, age, years of education and neuropsychological cognitive functions.

## Methods

Alzheimer's disease patients were recruited from a special dementia outpatient department at a general hospital in Tokyo, from May 2004 to April 2006. Fifty-six AD patients met the NINCDS-ADRDA criteria for the clinical diagnosis of probable AD (McKhann et al., 1984), had no previous history of psychiatric disorders, and had a score of one in the Clinical Dementia Rating (CDR) (Hughes, Berg, Danziger, Coben, & Martin, 1982), corresponding to mild dementia. These patients and/or their families gave informed consent.

Delusion of theft was defined as a 'People are stealing things' delusion based on the Behavioural Pathology in Alzheimer's Disease Rating Scale (BEHAVE-AD) (Reisberg et al., 1987). About 14 of the 56 AD patients (25.0%) showed delusion of theft (D-group), and the remaining 42 patients (75.0%) did not (non-D-group).

All 56 AD patients were administered the revised version of Hasegawa's dementia scale (HDS-R) (Kato et al., 1991), MMSE and the Japanese version of the Neurobehavioural Cognitive Status Examination (COGNISTAT) (Matsuda & Nakatani, 2004). The HDS-R and MMSE are the most popular screening measures for evaluation of neuropsychological cognitive functions in Japan and worldwide, respectively. The COGNISTAT consists of 11 sub-scales: 'orientation', 'attention', 'speech sample', 'comprehension', 'repetition', 'naming', 'constructional ability', 'memory', 'calculation', 'similarities' and 'judgement'. Scores on these sub-scales are calculated based on a $Z$ score, except for the 'speech sample'. The 'speech sample' requires patients to tell the story of a cartoon. The cartoon is usually explained as 'A fishing boy has fallen asleep. He does not realize that a fish is pulling his fishing line. Another boy riding a bicycle on a bridge is trying to wake him up.' In the present study, the responses to this task were classified into 1 or 0 based on whether the patients understood the relationship between the two boys or not. This task was used

as an indicator of social cognitive functions. Other tasks that have been used to evaluate social cognitive functions (Gregory et al., 2002; Maylor, Moulson, Muncer, & Taylor, 2002) were difficult to apply to AD patients showing mild dementia.

The age, years of education and presence of cohabiting family members were obtained from the patients and/or their family members. In addition, the family members were questioned about any premorbid personality traits of the patients and were required to answer with 'yes' or 'no' about responses of the patients to past events.

In statistical analyses, the quantitative data (age, years of education and scores on the MMSE, HDS-R and COGNISTAT excluding the 'speech sample') were analysed by the *t*-test, and the qualitative data (gender, presence of cohabiting family members, premorbid personality traits and 'speech sample') were analysed by Fisher's exact probability test and logistic regression analysis. In all tests, the null hypothesis was rejected at a significance level of $p < 0.05$.

**Results**

The data on the mean age, years of education and MMSE and HDS-R scores in the AD patients are shown in Table 1. There were no significant differences in these factors between the D-group and non-D-group by *t*-tests. No significant differences in MMSE and HDS-R scores between the two groups indicated that the severity of dementia was controlled as well as the same score on the CDR was found in the AD patients.

The data on gender, presence of cohabiting family members, premorbid personality traits and 'speech sample' are shown in Table 2. For gender, the female AD patients more frequently showed delusion of theft than the male AD patients ($p < 0.05$).

As for the presence of cohabiting family members, more AD patients were living alone in the D-group than in the non-D-group ($p < 0.05$). More AD patients were not cohabiting with their spouses or both their spouses and children in the D-group than in the non-D-group ($p < 0.05$), although there was no significant difference in cohabitation with their children. As for the

Table 1. Age, years of education, MMSE and HDS-R scores of the AD patients.

| | Mean (SD) | | |
|---|---|---|---|
| | D-group ($n=14$) | Non-D-group ($n=42$) | *p*-score |
| Age (years old) | 77.2 (6.1) | 78.6 (4.9) | 0.434 |
| Years of education (years) | 11.4 (2.6) | 10.1 (2.3) | 0.307 |
| MMSE | 20.6 (3.0) | 19.8 (2.2) | 0.531 |
| HDS-R | 17.7 (2.8) | 18.2 (2.7) | 0.359 |

Note: D-group: patients showing delusion theft; non-D-group: patients not showing delusion of theft. MMSE: Mini Mental State Examination; HDS-R: Hasegawa's dementia scale-Revised. Statistical analysis was conducted using the *t*-test.

Table 2. Qualitative data on the AD patients.

| | | D-group ($n=14$) | Non-D-group ($n=42$) | Total ($n=56$) |
|---|---|---|---|---|
| Gender | Female | 13 (33.3) | 26 (66.7) | 39 (100.0)* |
| | Male | 1 (5.9) | 16 (94.1) | 17 (100.0) |
| Presence of cohabiting family members | | | | |
| Any family member | Presence | 6 (13.6) | 38 (86.4) | 44 (100.0)* |
| | Absence | 8 (66.7) | 4 (33.3) | 12 (100.0) |
| Only spouse | Presence | 2 (11.1) | 16 (88.9) | 18 (100.0)* |
| | Absence | 10 (40.0) | 15 (60.0) | 25 (100.0) |
| Only children | Presence | 4 (26.7) | 11 (73.3) | 15 (100.0) |
| | Absence | 10 (33.3) | 20 (66.7) | 30 (100.0) |
| Both spouse and children | Presence | 0 (0.0) | 11 (100.0) | 11 (100.0)* |
| | Absence | 14 (31.1) | 31 (68.9) | 45 (100.0) |
| Premorbid personality traits | | | | |
| Neurotic personality | Presence | 13 (35.1) | 24 (64.9) | 37 (100.0)* |
| | Absence | 1 (5.3) | 18 (94.7) | 19 (100.0) |
| Extravert personality | Presence | 10 (30.3) | 23 (69.7) | 33 (100.0) |
| | Absence | 4 (17.4) | 19 (82.6) | 23 (100.0) |
| 'Speech sample' of COGNISTAT | Understand | 12 (38.7) | 19 (61.3) | 31 (100.0)* |
| the relation between two boys | Not understand | 3 (12.0) | 22 (88.0) | 25 (100.0) |

Note: D-group: patients showing delusion theft; non-D-group: patients not showing delusion of theft. Numerals in parentheses represent percentages. Statistical analysis was conducted using the Fisher's exact probability test; *$p < 0.05$.

Table 3. Sub-item scores on COGNISTAT.

| | Mean (SD) | |
|---|---|---|
| | D-group ($n=14$) | Non-D-group ($n=42$) |
| Orientation | 5.5 (3.5) | 6.2 (3.2) |
| Attention | 9.1 (2.6) | 8.9 (2.8) |
| Comprehension | 8.5 (1.6) | 7.3 (2.5) |
| Repetation | 9.9 (1.3) | 9.8 (1.6) |
| Naming | 8.5 (1.5) | 7.7 (2.4) |
| Construction | 7.0 (1.8) | 7.7 (1.8) |
| Memory | 5.9 (1.3) | 6.0 (1.6) |
| Calculation | 9.6 (1.6) | 8.3 (2.5) |
| Similarities | 8.9 (1.1) | 8.7 (1.0) |
| Judgement | 9.8 (1.3) | 9.6 (1.5) |

Note: D-group: patients showing delusion theft; non-D-group: patients not showing delusion of theft. Statistical analysis was conducted using the *t*-test.

cohabiting children, all AD patients lived with the son or daughter, and two of the four in the D-group and eight of the 11 in the non-D-group lived with the son and his wife or the daughter and her husband, although there were no significant differences.

As for premorbid personality traits, the AD patients who had a neurotic personality before AD developed more frequently showed delusion of theft than the AD patients who did not ($p < 0.05$). On the other hand, there was no significant difference between the AD patients who had an extrovert personality before AD developed and the AD patients who did not.

On COGNISTAT, both the D-group and non-D-group achieved low scores on 'orientation', 'constructional ability' and 'memory'. There were no significant differences on all sub-scales except for the 'speech sample' between the two groups (Table 3). For 'speech sample', the AD patients who understood the relationship between the two boys in the cartoon more frequently showed delusion of theft than the AD patients who did not ($p < 0.05$) (Table 2). The AD patients who did not understand the relationship between the two boys explained the cartoon as 'this is a boy, a fish and a bridge.'

Logistic regression analysis was used to identify significant predictors for delusion of theft (the independent variables were gender, presence of cohabiting spouse and/or children, neurotic and extrovert personalities, and 'speech sample'). As a result, this analysis was significant ($p < 0.01$), but only a neurotic personality tended to be related to delusion of theft (Odds ratio (OR) = 9.922, 95%-confidence interval (CI) = 0.720–136.826, $p < 0.10$), while the other variables showed no significant relations.

## Discussion

The risk factors for delusion in AD patients have not been fully clarified, although delusion is the most frequent symptom. In the present study, delusion was restricted to delusion of theft, because the variety of delusions may be one reason that risk factors for delusion have been uncertain in previous studies. Also, delusion of theft is the most frequent delusion in AD patients in Japan. Further, severity of dementia was controlled to mild dementia (CDR = 1) in all our AD patients, because the risk factors for delusion may be different according to the severity of dementia. The age and years of education between the D-group and non-D-group were also controlled.

In the present study, 25% of the AD patients showed delusion of theft, essentially in agreement with the previous results (Ozawa, 1997; Ropacki & Jeste, 2005). The female AD patients showed significantly more frequent delusion of theft than the male AD patients. This result confirms the results of previous studies, especially Japanese studies (Bassiony & Lyketsos, 2003; Ikeda et al., 2003; Ozawa, 1997; Rao & Lyketsos, 1998). The higher prevalence of delusion of theft in the female AD patients may be related to the fact that housework has been traditionally done by a wife in Japan, so housewives have more opportunities to deal with the household items like purses, clothes and tableware, and housewives with AD cannot find lost household items and can easily develop delusion of theft. Further studies are needed to clarify this relationship.

In the presence of cohabiting family members, the AD patients who were living alone showed significantly more frequent delusion of theft than the AD patients who were cohabiting with their families. The AD patients who were not cohabiting with their spouses or both their spouses and children showed significantly more frequent delusion of theft than the AD patients who were. In contrast, there was no significant correlation between cohabitation with children and prevalence of delusion of theft. The lower prevalence of delusion of theft in the AD patients who were cohabiting with their families may be explained by their families' support to find lost things and/or their families' management of important things such as bankbooks. The higher prevalence of delusion of theft in the AD patients without a spouse who were cohabiting with their children may be explained by the fact that AD patients often live alone because their children usually go out in the daytime.

As for the premorbid personality traits, the AD patients with a neurotic personality showed significantly more frequent delusion of theft than the AD patients without a neurotic personality. The AD patients with a neurotic personality may more frequently put away things where they cannot be easily found, and/or, may more seriously worry about the place where important things are kept. Further studies about other personality traits including agreeableness, conscientiousness and openness to experience in the Big Five Personality Factors (Goldberg, 1990) are needed to clarify the correlation between premorbid personality traits and delusion of theft.

There were no significant differences in MMSE and HDS-R scores on generalized cognitive psychological

tests between the two groups because severity of dementia was controlled to mild dementia by the CDR. In addition, there were no significant differences in neuropsychological cognitive sub-scales of COGNISTAT scores between the two groups. These results confirm the results of previous studies concerning the relations between delusion and neuropsychological cognitive functions (Burns et al., 1990; Ikeda, 2004; Migliorelli et al., 1995; Takechi et al., 2006).

Social cognitive functions construct representations of relationships between oneself and others, and cannot be generally assessed by neuropsychological cognitive tests (Adolphs, 2001). Some recent functional imaging studies have indicated the relations between social cognitive functions and the amygdala, medial prefrontal gyrus or superior temporal gyrus (Birbaumer et al., 1998; Gallagher et al., 2000; Grossman et al., 2000). Delusion, especially delusion of theft, is a psychiatric symptom with special reference to interpersonal relations, and may be related to social cognitive functions. The cognitive functions examined by the COGNISTAT include social cognitive functions beside neuropsychological cognitive functions such as memory, construction, calculation and language. In the present study, the AD patients with delusion of theft more frequently understood the interpersonal relation in the 'speech sample' of COGNISTAT than the AD patients without delusion of theft. This result suggests that mild AD patients without delusion of theft are more likely to have disturbance of social cognitive functions than mild AD patients with delusion of theft.

Most of the AD patients are likely to have some disturbance of social cognitive functions, although they often remain intact in AD patients with very mild dementia (Gregory et al., 2002). When AD patients cannot find lost things, those with relatively retained social cognitive functions may put the blame on others for lost things compared with the AD patients with disturbed social cognitive functions. The 'Speech sample' task used in the present study may not be an adequate indicator of social cognitive functions. However, other tasks used in previous studies for evaluation of social cognitive functions in non-demented elderly people (Maylor et al., 2002) and AD patients showing very mild dementia (the mean MMSE scores of 27.1) (Gregory et al., 2002) are difficult to apply to AD patients showing mild dementia. Further studies are needed to clarify the relation between social cognitive functions and delusion of theft using more appropriate tasks for AD patients.

There are some limitations in the present study. First, we should examine in detail the psychosocial factors such as the role of cohabiting family members in the care of AD patients, and the mechanism by which premorbid personality traits lead to delusion of theft. Second, the sample size was too small to do logistic regression analysis of psychosocial factors for generalization. Therefore, we could not discuss the inter-relationship among these factors. Third, cross-cultural studies about the relation between delusion of theft and psychosocial factors are needed in the future as the present study was performed using only Japanese AD patients.

Finally, the present study suggests the possibility of preventing and/or treating delusion of theft by psychosocial approaches. For example, it might be recommended that AD patients live with their family or enter an institution to treat delusion of theft for AD patients living alone. We often find that AD patients with delusion of theft do not show this delusion without medication after they enter a hospital. Further, psychosocial approaches considering premorbid personality traits of AD patients or maintaining their social cognitive functions are needed to prevent delusion of theft.

**Acknowledgements**

This study was supported by Grants-in-aid from the Research Support Foundation of Juntendo Institute of Mental Health.

**References**

Adolphs, R. (2001). The neurobiology of social cognition. *Current Opinion in Neurobiology, 11*, 231–239.

Ballard, C., & Oyebode, F. (1995). Psychotic symptoms in patients with dementia. *International Journal of Geriatric Psychiatry, 10*, 743–752.

Bassiony, M.M., & Lyketsos, C.G. (2003). Delusions and hallucinations in Alzheimer's disease: Review of the brain decade. *Psychosomatics, 44*, 388–401.

Birbaumer, N., Grodd, W., Diedrich, O., Klose, U., Erb, M., Lotze, M., et al. (1998). fMRI reveals amygdale activation to human faces in social phobics. *Neuroreport, 9*, 1223–1226.

Burns, A., Jacoby, R., & Levy, R. (1990). Psychiatric phenomena in Alzheimer's disease. I: Disorders of thought content. *The British Journal of Psychiatry, 157*, 72–76.

Colerick, E.J., & George, L.K. (1986). Predictors of institutionalization among caregivers of patients with Alzheimer's disease. *Journal of the American Geriatrics Society, 34*, 493–498.

Cummings, J.L., & Victoroff, J.I. (1990). Noncognitive neuropsychiatric syndromes in Alzheimer's disease. *Neuropsychiatry, Neuropsychology, and Behavioral Neurology, 3*, 140–158.

Deimling, G.T., & Bass, D.M. (1986). Symptoms of mental impairment among elderly adults and their effects on family caregivers. *Journal of Gerontology, 41*, 778–784.

Folstein, M.F., Folstein, S.E., & McHugh, P.R. (1975). 'Mini-Mental State': A practical method for grading the cognitive state for the clinician. *Journal of Psychiatric Research, 12*, 189–198.

Gallagher, H.L., Happe, F., Brunswick, N., Fletcher, P.C., Frith, U., & Frith, C.D. (2000). Reading the mind in cartoons and stories: An fMRI study of 'theory of mind' in verbal and nonverbal tasks. *Neuropsychologia, 38*, 11–21.

Goldberg, L.R. (1990). An alternative 'description of personality': The Big-Five factor structure. *Journal of Personality and Social Psychology, 59*, 1216–1229.

Gregory, C., Lough, S., Stone, V., Erzinclioglu, S., Martin, L., Baron-Cohen, S., et al. (2002). Theory of mind in patients with frontal variant frontotemporal dementia and Alzheimer's disease: Theoretical and practical implications. *Brain, 125*, 752–764.

Grossman, E., Donnelly, M., Price, R., Pickens, D., Morgan, V., Neighbor, G., et al. (2000). Brain areas involved in perception of biological motion. *Journal of Cognitive Neuroscience, 12*, 711–720.

Haupt, M., Romero, B., & Kurz, A. (1996). Delusions and hallucinations in Alzheimer's disease: Results from a two-year longitudinal study. *International Journal of Geriatric Psychiatry, 11*, 965–972.

Hughes, C.P., Berg, L., Danziger, W.L., Coben, L.A., & Martin, R.L. (1982). A new clinical scale for the staging of dementia. *British Journal of Psychiatry: The Journal of Mental Science, 140*, 566–572.

Ikeda, M. (2004). The relationship between delusions of theft and memory disturbance in Alzheimer's disease. *Higher Brain Function Research, 24*, 147–154, (in Japanese).

Ikeda, M., Shigenobu, K., Fukuhara, R., Hokoishi, K., Nebu, A., Maki, N, et al. (2003). Delusions of Japanese patients with Alzheimer's disease. *International Journal of Geriatric Psychiatry, 18*, 527–532.

Jeste, D.V., Wragg, R.E., Salmon, D.P., Harris, M.J., & Thal, L.J. (1992). Cognitive deficits of patients with Alzheimer's disease with and without delusions. *The American Journal of Psychiatry, 149*, 184–189.

Kato, S., Shimogaki, H., Onodera, A., Ueda, H., Oikawa, K., et al. (1991). Development of the revised version of Hasegawa's dementia scale (HDS-R). *Japanese Journal of Geriatric Psychiatry, 2*, 1339–1347, (in Japanese).

Kazui, H., Hirono, N., Hashimoto, M., Nakano, Y., Matsumoto, K., et al. (2006). Symptoms underlying unawareness of memory impairment in patients with mild Alzheimer's disease. *Journal of Geriatric Psychiatry and Neurology, 19*, 3–12.

Lee, E., Meguro, K., Hashimoto, R., Meguro, M., Ishii, H., Yamaguchi, S., et al. (2007). Confabulations in episodic memory are associated with delusions in Alzheimer's disease. *Journal of Geriatric Psychiatry and Neurology, 20*, 34–40.

Matsuda, O., & Nakatani, M. (2004). *The manuals for the Japanese version of the Neurobehavioral Cognitive Status Examination*. Tokyo: World Planning (in Japanese).

Maylor, E.A., Moulson, J.M., Muncer, A.M., & Taylor, L.A. (2002). Does performance on theory of mind tasks decline in old age? *British Journal of Psychology, 93*, 465–485.

McKhann, G., Drachman, D., Folstein, M., Katzman, R., Price, D., & Stadlan, E.M. (1984). Clinical diagnosis of Alzheimer's disease; Report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. *Neurology, 34*, 939–944.

Migliorelli, R., Petracca, G., Teson, A., Sabe, L., Leiguarda, R., & Starkstein, S.E. (1995). Neuropsychiatric and neuropsychological correlates of delusions in Alzheimer's disease. *Psychological Medicine, 25*, 505–513.

Mizrahi, R., Starkstein, S.E., Jorge, R., & Robinson, R.G. (2006). Phenomenology and clinical correlates of delusions in Alzheimer disease. *The American Journal of Psychiatry, 14*, 573–581.

Ozawa, I. (1997). The delusion of theft in elderly with dementia (1). Statistical data and psychopathology of the delusion. *Seishin Shinkeigaku Zasshi, 99*, 370–388, (in Japanese).

Rao, V., & Lyketsos, C.G. (1998). Delusions in Alzheimer's disease: A review. *The Journal of Neuropsychiatry and Clinical Neurosciences, 10*, 373–382.

Reisberg, B., Borenstein, J., Salob, S.P., Ferris, S.H., Franssen, E., & Georgotas, A. (1987). Behavioral symptoms in Alzheimer's disease: Phenomenology and treatment. *The Journal of Clinical Psychiatry, 48*(Suppl), 9–15.

Ropacki, S.A., & Jeste, D.V. (2005). Epidemiology of and risk factors for psychosis of Alzheimer's disease: A review of 55 studies published from 1990 to 2003. *The American Journal of Psychiatry, 162*, 2022–2230.

Swearer, J.M., Drachman, D.A., O'Donnell, B.F., & Mitchell, A.L. (1988). Troublesome and disruptive behaviors in dementia. Relationships to diagnosis and disease severity. *Journal of the American Geriatrics Society, 36*, 784–790.

Takechi, H., Yamada, H., Sugihara, Y., & Kita, T. (2006). Behavioral and psychological symptoms, cognitive impairment and caregiver burden related to Alzheimer's disease patients treated in an outpatient memory clinic. *Nippon Ronen Igakkai Zasshi, 43*, 207–216, (in Japanese).

Terada, S., Ishizu, H., Fujisawa, Y., Yokota, O., Kugo, A., Nakashima, H., et al. (2005). Delusion of theft and phantom intruder delusion in demented elderly patients in Japan. *Journal of Geriatric Psychiatry and Neurology, 18*, 142–148.

Teri, L., Larson, E.B., & Reifler, B.V. (1988). Behavioral disturbance in dementia of the Alzheimer's type. *Journal of the American Geriatrics Society, 36*, 1–6.

Biochemical and Biophysical Research Communications 379 (2009) 691–695

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Decrease of dynamin 2 levels in late-onset Alzheimer's disease alters Aβ metabolism

Eiichiro Kamagata [a], Takashi Kudo [a,*], Ryo Kimura [b], Hitoshi Tanimukai [a], Takashi Morihara [a], Md.Golam Sadik [a], Kouzin Kamino [c], Masatoshi Takeda [a]

[a] Department of Geriatric Medicine, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan
[b] Osaka General Medical Center, 3-1-56 Bandai-higashi, Sumiyoshi-ku, Osaka 558-8558, Japan
[c] Shoraiso National Hospital, 2815 Koizumi-cho, Yamatokoriyama, Nara 639-1042, Japan

ARTICLE INFO

*Article history:*
Received 1 December 2008
Available online 4 January 2009

*Keywords:*
Alzheimer's disease
Dynamin
Amyloid-β peptide
Amyloid precursor protein
Lipid raft

ABSTRACT

Late-onset Alzheimer's disease (LOAD) is significantly associated with a single nucleotide polymorphism located in the dynamin (DNM) 2 gene, especially in non-carriers of the apolipoprotein E-ε4 allele. In this study we used real-time PCR to show that *DNM2* mRNA is significantly reduced in the cortex of AD brains and in the peripheral blood of dementia patients. Neuroblastoma cells transfected with a dominant negative *DNM2* had increased amyloid beta protein (Aβ) secretion and most of the amyloid precursor protein (APP) in these cells was localized to the plasma membrane. In addition, these cells were rich in flotillin, which is a component of lipid rafts. These data suggest that *DNM2* expression is reduced in LOAD, which results in the accumulation of APP in lipid raft-rich plasma membranes. Consequently, Aβ secretion may increase in LOAD neurons.



Alzheimer's disease (AD) is the most common form of dementia in the elderly, characterized by cognitive decline and progressive neurodegeneration in the brain. In AD brains, two types of abnormal protein deposits are observed pathologically. One is neurofibrillary tangles (NFTs), which consist mainly of tau protein, forming paired helical filament (PHFs). The other is extracellular amyloid plaques, which are composed of amyloid beta protein (Aβ). The current consensus is that Aβ is a causative molecule in AD by disturbing synaptic function, which leads to neuronal death (for review, see [1,2]). Early-onset AD (EOAD) and late-onset AD (LOAD) exhibit the same neuropathology in the brain, however, LOAD, which represents the majority of AD cases, is genetically classified as a polygenetic disease and is characterized by more heterogeneous conditions compared with EOAD, some of which are autosomal dominant.

Aβ interacts with dynamin1 (DNM1) [3] and *DNM2*, a homologue of *DNM1*, is encoded on chromosome 19p13.2 where a susceptibility locus has been detected by linkage analysis. We previously examined the genetic association of LOAD with the *DNM2* gene and showed a significant association of LOAD with a single nucleotide polymorphism (SNP) marker of the *DNM2* gene, especially in non-carriers of the apolipoprotein E-ε4 allele [4]. Furthermore, the dynamin-binding protein gene on chromosome 10 has also been associated with LOAD [5].

The relationship between the function of DNM2 and amyloid pathology is largely unknown. To address this issue, we measured the level of *DNM2* mRNA in LOAD brains and in peripheral blood. Also, using *DNM1* or *DNM2* dominant negative neuronal cells we investigated the influence of DNM2 dysfunction on the metabolism of Aβ and on the localization of amyloid precursor protein (APP).

## Materials and methods

*Subjects.* Postmortem temporal cortex tissues were obtained from the Brain and Tissue Bank for Developmental Disorders of the National Institute of Child Health and Human Development. The samples were from 7 histopathologically confirmed AD patients (age 82.6 ± 3.4) and 7 aged individuals without neurological symptoms (age 65.0 ± 11.5). After obtaining written informed consent to participate in this study, peripheral blood was drawn from 82 dementia patients (49 AD, 14 mild cognitive impairment, 4 vascular dementia, 2 front temporal dementia, 13 other dementia; age 72.0 ± 8.1) and from 11 aged controls (age 63.7 ± 9.6). Total RNA was isolated from frozen temporal cortex tissues and from blood using Isogen (Nippon Gene), an acid guanidine–phenol–chloroform RNA extraction method, and purified using an RNeasy Mini kit (Qiagen). RNA samples with an $A_{260}/A_{280}$ absorption ratio over 1.9 were subjected to cDNA synthesis using a High-Capacity cDNA Archive kit (Applied Biosystems). The procedure to obtain the specimens was approved by the Genome Ethical Committee of Osaka University Graduate School of Medicine.

* Corresponding author. Fax: +81 6 6879 3052.
*E-mail address:* kudo@psy.med.osaka-u.ac.jp (T. Kudo).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.12.147

*Quantitative real-time PCR.* Primers and probe sets for the human *DNM2*, β-actin, and *GAPDH* genes were purchased from TaqMan Gene Expression Assay products (Applied Biosystems), and quantitative real-time PCR was carried out in an ABI PRISM 7900HT (Applied Biosystems). All quantitative PCRs were duplicated and the ratio of the amount of *DNM2* cDNA to that of β-actin or *GAPDH* internal control cDNA was determined at the cycle threshold (CT).

*Cell culture.* SH-SY5Y human neuroblastoma cells were stably transfected with Swedish mutant APP670/671(SY5Y/swAPP). Dominant negative pcDNA/dynamin 1K44A (*DNM1K44A*) and pcDNA/dynamin 2K44A (*DNM2K44A*) plasmids were obtained from ATCC. Transient transfections with DNM1K44A, DNM2K44A, or both in SY5Y/swAPP cells were performed using the Amaxa nucleofector system. To check viabilities of transfected cells, lactate dehydrogenase (LDH) levels were measured using an LDH kit (Promega).

*Levels of Aβ40 and Aβ42.* Culture media of SY5Y/swAPP cells were collected 72 h after transfection with dominant negative *DNM* plasmids. Using a human Aβ 1–40 ELISA kit and a human Aβ 1–42 ELISA kit (Biosource), the levels of Aβ 1–40 and Aβ 1–42 in culture media were quantified.

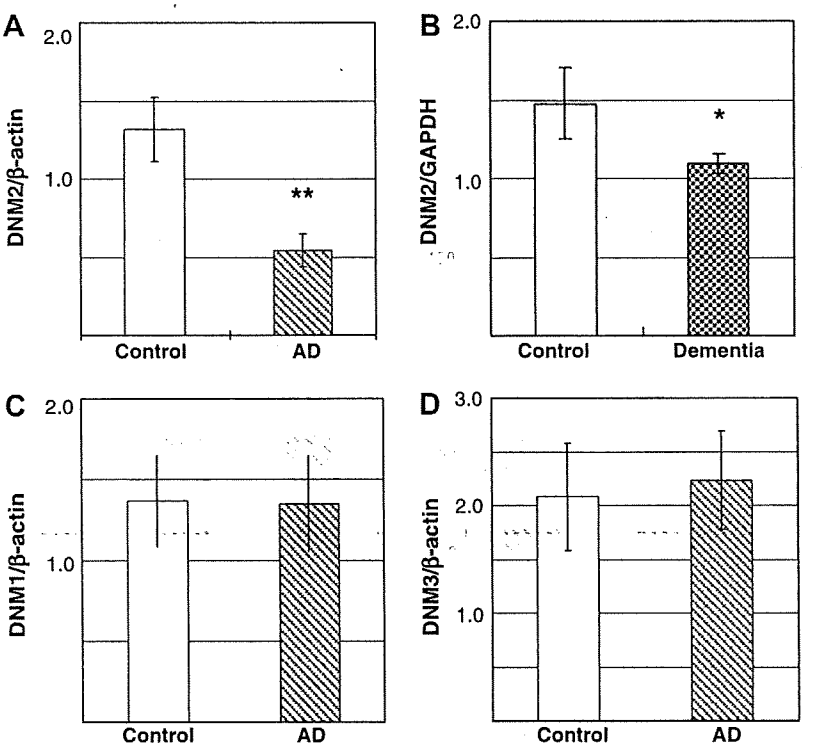


**Fig. 1.** Expression of *DNM* mRNA in the temporal cortex and in peripheral blood. Real-time PCR for *DNM2* (A), *DNM1* (C), and *DNM3* (D) mRNA from the temporal cortex of 7 AD and 7 control brains, normalized to β-actin mRNA, showed a significant reduction of *DNM2* mRNA levels in AD brains compared with control brains. In contrast, reduction of *DNM1* and *DNM3* levels were not observed. Analysis of *DNM2* mRNA, normalized to GAPDH mRNA, in peripheral leukocytes from 82 dementia patients and from 11 controls also revealed a significant reduction of *DNM2* mRNA (B). $^{*}p < 0.05$; $^{**}p < 0.01$.

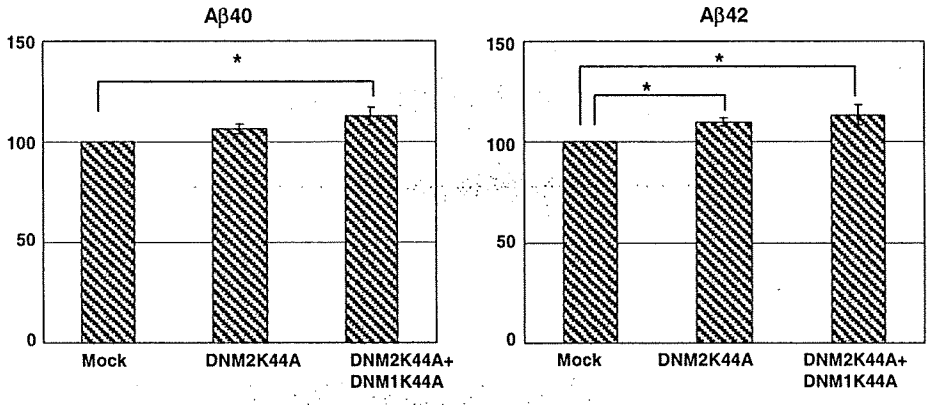


**Fig. 2.** Secretion of amyloid peptides (Aβ40 and Aβ42) in *DNM2* and *DNM1* dominant negative SY5Y/swAPP cells. ELISA of media 3 days after transfection showed that Aβ42 secretion was significantly increased in single *DNM2K44A* transfected cells and that both Aβ40 and Aβ42 secretion significantly increased in double *DNM2K44A* and *DNM1K44A* transfected cells. Levels of Aβ40 and Aβ42 in MOCK-transfected neuronal cells was combined. $^{*}p < 0.05$.

*Sample preparation and Western blot analysis.* Cell lysates were collected in PBS containing 2% Nonidet P-40, 0.2% SDS, protease inhibitor, and phosphatase inhibitor. Subcellular fractions of SY5Y/swAPP cells were collected using a ProteoExtract Subcellular Proteome Extraction Kit (Calbiochem). Biotinylated plasma membranes of SY5Y/swAPP cells were collected using a Cell Surface Protein Isolation Kit (Pierce). Equal amounts of each sample were separated by SDS–PAGE and electroblotted onto nitrocellulose membranes. Bands are visualized using a luminescent image analyzer Las-3000 and multi gauge software (Fujifilm).

*Immunohistochemistry.* SY5Y/swAPP cells transfected with dominant negative DNM were fixed in 4% paraformaldehyde, as described previously [6]. The samples were incubated with primary antibodies (see below) and then visualized with AlexaFluor568 goat anti-mouse IgG(H+L) (Invitrogen). APP staining and DAPI staining (Vector Laboratories) were examined using a Leica TCS SPE confocal microscope system (Leica Microsystems).

*Antibodies.* 22C11, APP monoclonal antibody, anti-presenilin 1 (Chemicon), Anti-flottillin-1 and anti-EEA1 (BD Transduction Laboratories), anti-pan-cadherin (Zymed Laboratories).

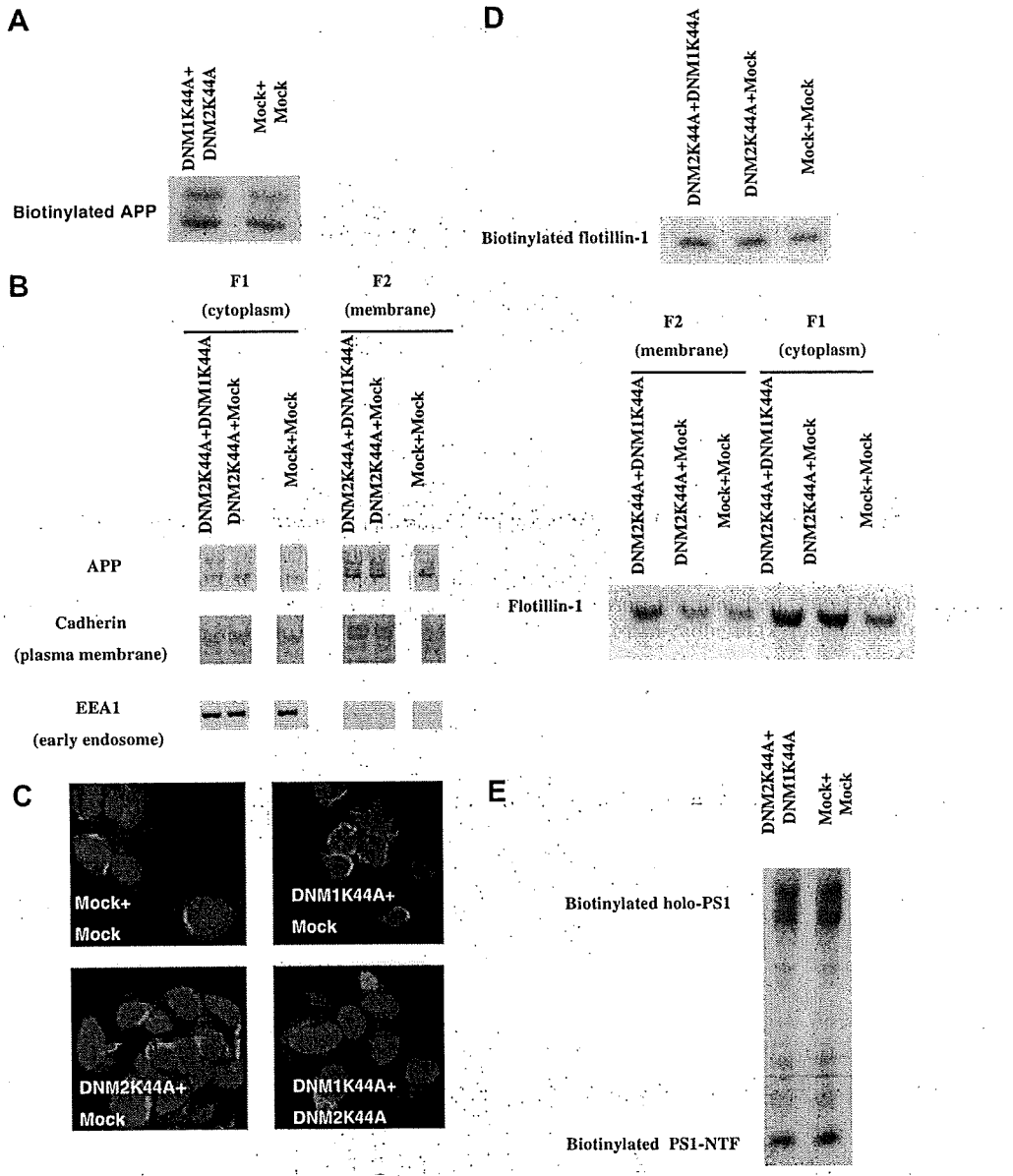


**Fig. 3.** Localization of APP, lipid raft, and PS1 in DNM dominant negative neuronal cells. (A) The cytoplasm and the membrane components of SY5Y/swAPP cells were fractionated using ProteoExtract Subcellular Proteome Extraction Kit, and each fraction was confirmed by immunoblotting with antibodies against specific marker proteins; cadherin for plasma membrane; EEA1 for early cytoplasmic endosome. Immunoblotting with anti-APP antibody showed that the membrane fraction of double *DNM2K44A* and *DNM1K44A* or of *DNM2K44A* transfected cells had increased amounts of APP, especially high molecular weight, mature APP. (B) Using a Cell Surface Protein Isolation Kit, the amount of biotinylated APP, especially high molecular weight, mature APP was increased in double *DNM2K44A* and *DNM1K44A* transfected cells compared with control. (C) Immunohistochemical examination showed that some APP staining (red) was observed on the cell surface of *DNM2K44A* transfected cells and of double transfected *DNM2K44A* and *DNM1K44A* cells, compared with MOCK-transfected cells. DAPI staining visualized nuclei in blue. (D) Using a Cell Surface Protein Isolation Kit, the amount of biotinylated flotillin-1, a marker for lipid raft, was shown to be increased in both single *DNM2K44A* transfected cells and in double *DNM2K44A* and *DNM1K44A* transfected cells. (E) Cell surface biotinylation showed that the plasma membrane of DNM dominant negative neuronal cells had enough amount of PS1-NTF and of holo-PS1 compared with MOCK-transfected cells. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)

## Results and discussion

To examine the expression of the *DNM* genes in the AD temporal cortex, we measured the amount of *DNM1*, *DNM2*, and *DNM3* mRNA, normalized to that of β-actin, using quantitative real-time PCR. Analysis of 7 AD tissues and 7 control tissues revealed that the amount of *DNM2* mRNA in the AD temporal cortex was significantly lower compared with that in controls (Fig. 1). On the other hand, the level of *DNM1* and *DNM3* mRNA in AD samples was not significantly different compared with that in controls (Fig. 1). Moreover, analysis of peripheral leukocytes from 82 dementia patients and from 11 controls revealed that the level of *DNM2* mRNA in dementia patients was significantly lower compared with that of controls (Fig. 1). These data suggest that *DNM2* may be one of the causal genes for LOAD. The dementia patients in the study of peripheral leukocytes consisted of patients with various forms of dementia, therefore, the analysis of *DNM2* mRNA in blood might be a biomarker for dementia.

It may be safely assumed that a decreased *DNM2* mRNA level causes dysfunction of DNM2. Because an increase of Aβ is the key event of AD pathology, we investigated the effects of DNM2 dysfunction on Aβ production using a neuronal cell line transfected with dominant negative *DNM* genes. We transiently transfected SY5Y/swAPP cells with either dominant negative *DNM2K44A or DNM1K44A*, or both. ELISA analysis of media 3 days after transfection showed that Aβ42 secretion significantly increased in *DNM2K44A* transfected cells and that both Aβ40 and Aβ42 secretion significantly increased in cells transfected with both *DNM2K44A* and *DNM1K44A* (Fig. 2). *DNM2K44A* transfected cells had a tendency of high Aβ40 levels, but the increase was not significant (Fig. 2). DNM1 has a similar structure to DNM2 [7], therefore, it was possible that DNM1 may compensate for DNM2 dysfunction in *DNM2K44A* cells. Therefore, it was suggested that the dysfunction of DNM2 may cause an increase of Aβ secretion.

A dominant negative DNM has been shown to cause an altered localization of amyloid precursor protein APP [8]. To elucidate the mechanism of increased Aβ production in the *DNM2* dominant negative neuronal cells, we focused on the localization of APP, 3 days after transfection. First, the cytoplasm and the membrane compartments of SY5Y/swAPP cells were fractionated, and each fraction was confirmed by immunoblotting with antibodies against specific marker proteins; cadherin for plasma membrane and EEA1 for cytoplasmic early endosomes [9,10]. Immunoblotting with anti-APP antibody showed that the membrane fraction of neuronal cells double transfected with *DNM2K44A* and *DNM1K44A* or singly transfected with *DNM2K44A* had increased amounts of APP, especially high molecular weight, mature APP (Fig. 3A). Because the membrane fraction contained high levels of cadherin, it mostly consisted of plasma membrane. Second we biotinylated the plasma membrane of SY5Y/swAPP cells using a Cell Surface Protein Isolation Kit. In neuronal cells double transfected with *DNM2K44A* and *DNM1K44A*, the amount of biotinylated APP, especially high molecular weight, mature APP, increased, suggesting that the amount of APP was increased in the plasma membrane of dominant negative neuronal cells (Fig. 3B). Third, immunohistochemical examination of SY5Y/swAPP cells was performed to investigate the localization of APP. In neuronal cells singly transfected with *DNM2K44A* and double transfected with *DNM2K44A* and *DNM1K44A*, higher levels of APP staining was observed on the cell surface compared with MOCK-transfected neuronal cells (Fig. 3C). Taken together, these data suggest that APP may accumulate in the plasma membrane in the *DNM2* dominant negative cells.

Recently, it has been reported that the lipid raft is a major location for Aβ generation [11–13]. Therefore, we examined the existence of lipid rafts in the plasma membrane of *DNM* dominant negative neuronal cells. The amount of biotinylated flotillin-1, a marker for lipid rafts, increased in both *DNM2K44A* transfected and *DNM2K44A* and *DNM1K44A* transfected neuronal cells suggesting that the amount of lipid raft was increased in the plasma membrane of *DNM2* dominant negative neuronal cells (Fig. 3D). This is consistent with recent reports of dominant negative *DNM* causing accumulation of lipid raft associated with invaginations of the plasma membrane [14,15]. We also examined the existence of presenilin 1 (PS1), a major component of the γ-secretase complex, in the plasma membrane of *DNM* dominant negative neuronal cells. Cell surface biotinylation showed that the plasma membrane of *DNM* dominant negative neuronal cells had enough amount of PS1-NTF and of holo-PS1 (Fig. 3E). These data suggest that Aβ generation may occur efficiently in the plasma membrane of *DNM* dominant negative neuronal cells.

APP is transported to the plasma membrane via the endoplasmic reticulum and the Golgi apparatus and is then taken up into endosomes by endocytosis [16], where Aβ generation occurs. In contrast, in neuronal cells that have lost DNM function APP accumulates in the plasma membrane. A major role of DNM is in vesicle endocytosis, and DNM function in vesicle budding is involved in the constriction of the lipid neck, fission of lipids and regulation of the scission reaction (for review, see [17]). According to our present data and to recent reports [13–15,18], lipid rafts or PS1 in the plasma membrane may be suitable locations for Aβ genera-

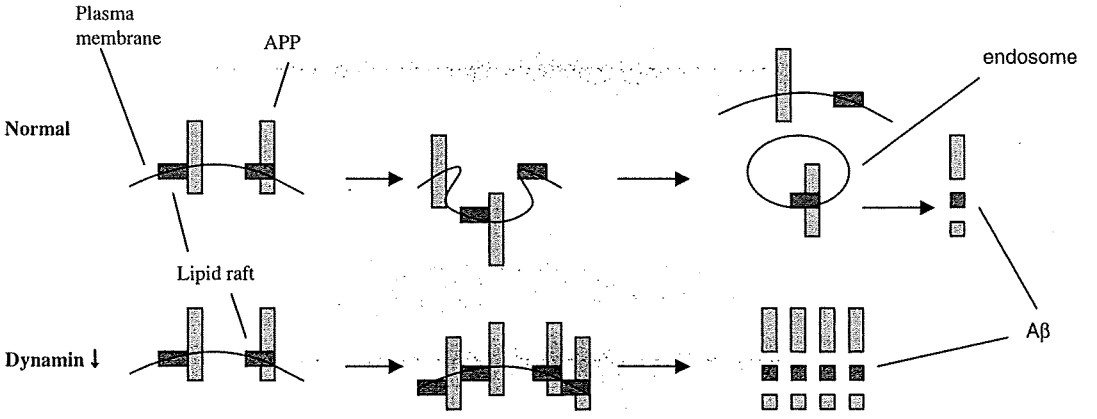


**Fig. 4.** Dysfunction of DNM2 increased Aβ generation as a result of the accumulation of APP in the plasma membrane. In normal conditions, APP is transported to plasma membrane through the endoplasmic reticulum and the Golgi apparatus and then is taken up in endosomes by endocytosis, where Aβ generation occurs. In contrast, neuronal cells that have lost DNM function may have an accumulation of APP in the plasma membrane, because the major role of DNM is in endocytosis. In DNM dominant negative neuronal cells increased Aβ production may occur due to the accumulation of APP and the presence of lipid raft or PS1 in the plasma membrane.