cells could be changed by the compounds and mutants. GSMs drastically increased the relative levels of both VVIA and VVIAT, whereas an iGSMs decreased the relative levels of the two peptides (Figure 4I; Table S3F). Notably, because of the GSM effect, more than half of the Aβ38 was derived from Aβ43. The PS1 mutants decreased the relative levels of VVIA and VVIAT; however, the degree of the changes was much smaller than that observed for GSM (Figure 4J; Table S3G). The PS1 G384A mutant did not decrease the relative levels of VVIAT. Very similar data were obtained when the levels of VVIA and VVIAT were normalized to the level of each major cleavage in the previous step (i.e., TVI for VVIA and VIV for VVIAT; Figures S4B and S4C). Collectively, these results indicate that GSMs strongly affect Aβ42(43) cleavage, and the increase in Aβ38 production is largely attributed to the cleavage of Aβ43 into Aβ38.

We also performed an Aβ45 and Aβ46 cleavage assay. MALDI-TOF MS showed that both Aβ45 and Aβ46 were cleaved by PS/γ-secretase. Interestingly, Aβ41 and Aβ40, in addition to Aβ42, were produced from Aβ45. Aβ42, in addition to Aβ43, was produced from Aβ46 (Figures 4K and 4L). Combined with the results showing that considerable amounts of Aβ38 were derived from Aβ43, the data indicate that the proposed "Aβ38 product line" (from βAPP-CTF via Aβ48 and Aβ42) and the proposed "Aβ40 product line" (from βAPP-CTF via Aβ49 and Aβ43) (Takami et al., 2009) cross each other.

We studied how the CHAPSO concentration affects the rates of Aβ42(43) cleavage, when βAPP-CTF is cleaved in vitro (Figures S4D and S4E). Interestingly, CHAPSO affected the rates in a dose-dependent manner and in a similar way to the effect of GSMs.

Long Aβ-like Peptides Other Than Aβ Are Also Substrates of PS/γ-Secretase

Aβ-like peptides (Okochi et al., 2002), secreted by a process similar to that for Aβ secretion, include mNotch-1-derived Nβ (Okochi et al., 2006) and APLP1-derived APL1β (Yanagida et al., 2009). We found that PS/γ-secretase cleaved Nβ25 into Nβ21 (Figure 4M) and APL1β28 into APL1β25 (Figure 4N), suggesting that long Aβ-like peptides are generally intermediate products. This may explain why the relative levels of some longer secreted Aβ-like peptides, including Aβ42, change in parallel (Okochi et al., 2006; Yanagida et al., 2009). This finding also indicates that APL1β28 cleavage to APL1β25 is impaired in the sporadic AD brain (Yanagida et al., 2009).

DISCUSSION

In this study, we show that de novo Aβ42(43), a secreted species, is an intermediate of PS/γ-secretase in living cells, and this discovery affects the understanding of the nature of Aβ42(43) production. We suggest that Aβ42 production does not directly reflect the level of cleavage at the C terminus of Aβ42, but rather depends on how much newly produced Aβ42 dissociates from the PS/γ-secretase enzyme and thereby avoids further cleavage. Thus, competition between further cleavage and dissociation from the enzyme may be the key to determining the Aβ42(43) ratio. Importantly, our results also suggest that a new type of partial loss of function in PS/γ-secretase [e.g., reduction in Aβ42(43) cleavage or at the final step of PS/γ-secretase cleavage of βAPP] may cause a gain of function in AD [an increase in the Aβ42(43) ratio]. GSMs increase the relative k_{cat} for the further cleavage of Aβ42 to Aβ38 and decrease the relative k_b for the dissociation of Aβ42 from PS1/γ-secretase. This suggests a potential model to explain how GSMs can lower Aβ42 production.

Chávez-Gutiérrez et al., 2012 showed that PS1 mutations lower the relative levels of Aβ38 to Aβ42 and Aβ40 to Aβ43 compared with WT PS1. The GSMs tested increased both the level of Aβ38 relative to Aβ42 (Weggen et al., 2001) and the level of Aβ40 relative to Aβ43. Based on the hypothetical model proposed by Ihara and colleagues (Takami et al., 2009), those authors speculated that the PS1 mutants and GSMs decrease and increase, respectively, the rate of the fourth cleavage (i.e., Aβ43 cleavage to Aβ40 and Aβ42 cleavage to Aβ38, respectively), possibly because of the premature release of the Aβ42/Aβ43 peptides.

Figure 4. Cleavage of Aβ43, Nβ25, and APL1β28 by PS/γ-Secretase

(A) Representative MALDI-TOF MS spectrum from the Aβ43 cleavage assay (0.5% CHAPSO).

(B) Aβ-derived peptides in the Aβ43 cleavage assay. Addition of L685,458 abolished their generation. Note that Aβ43 cleavage produced much smaller levels of IAT and VVIAT than that of VVIA produced by Aβ42 cleavage. This may be due to the fact that the aggregation property of Aβ43 is higher than that of Aβ42 (Saito et al., 2011).

(C) Aβ species by the βAPP-CTF cleavage assay in the presence of 0.25% or 0.5% CHAPSO.

(D) Aβ species in lysates of HEK293 cells in a 10 cm dish stably expressing sw βAPP and WT PS1.

(E) Fold changes of the relative VVIAT levels in cell lysates treated with GSMs/iGSMs.

(F) Fold changes of the relative VVIAT levels in lysates from cells stably expressing PS1 mutants.

(G) Fold changes of the relative IAT levels in cell lysates treated with GSMs/iGSMs.

(H) Fold changes of the relative IAT levels in lysates from cells stably expressing PS1 mutants.

(I) The relative rates of VVIA (blue) and VVIAT (red) in cells treated with GSMs/iGSMs.

(J) The relative rates of VVIA (blue) and VVIAT (red) in cells stably expressing PS1 mutants.

(K) Representative MALDI-TOF MS spectrum of products from the Aβ45 cleavage assay (0.25% CHAPSO).

(L) Representative MALDI-TOF MS spectrum of products from the Aβ46 cleavage assay (0.75% CHAPSO).

(M) Nβ25 cleavage assay (0.5% CHAPSO).

(N) APL1β28 cleavage assay (0.5% CHAPSO). Insets show an enlargement of the part encircled by the dotted line. Note that APL1β28 cleavage was less efficient than Nβ25 cleavage and Aβ42 cleavage.

Asterisks in (A), (K), (L), and (N) indicate nonspecific peaks, and those in (E), (F), (G), (H), (I), and (J) indicate statistical significance. Error bars represent SD. See also Figures S1 and S4.

We found that, in living cells, ~40% of Aβ38 was derived from Aβ43. Moreover, Aβ40 and Aβ41 were produced in the Aβ45 cleavage assay, and Aβ42 was produced in the Aβ46 cleavage assay. Thus, the putative Aβ38 and Aβ40 product lines (Takami et al., 2009) turn out to overlap at several points.

Takami et al. (2009) showed that Sulindac sulfide decreased the levels of Aβ42 and Aβ43 in a βAPP-CTF cleavage assay, but did not significantly increase the levels of VVIA and IAT. This may be because Sulindac sulfide exerts a weaker GSM action than GSM1 and Eisai, which were used in this study.

We also found that Notch-1 and APLP1 transmembrane domains are cleaved in a similar way, which should help clarify the physiological process of intramembrane proteolysis by PS/γ-secretase. We showed previously that Aβ42 ratio changed in parallel with APL1β28 ratio, and that the APL1β28 ratio increases in the cerebrospinal fluid of AD patients (Yanagida et al., 2009). In this article, we demonstrated that both Aβ42 and APL1β28 were cleaved similarly into the shorter species (i.e., Aβ38 and APL1β25). Therefore, we suggest that Aβ42 cleavage may also decrease in AD brains.

Collectively, we speculate that the increase in the Aβ42 ratio simply reflects the accelerated dissociation of membrane-bound long Aβs (ie, Aβ44~49) from PS/γ-secretase. Because long Aβs on the membrane may perturb the physiological function of neuronal cells, further studies are necessary to investigate whether the prolonged stay of long Aβ at the membrane is pathologically relevant.

At present, inhibition of PS/γ-secretase activity using agents such as Notch-sparing inhibitors is the central approach to decreasing Aβ production specifically. Our results may shift the nature of new drugs for treating AD to repair or increase the ability of PS/γ-secretase to cleave Aβ42(43).

EXPERIMENTAL PROCEDURES

Aβ and Aβ-like Peptide Cleavage Assays

In vitro γ-secretase assays (Li et al., 2000; Osawa et al., 2008) using Aβ and Aβ-like peptides (Aβ42, 43, 45, 46, Nβ25, and APL1β28) were performed under the modified conditions described here with a modified reaction buffer (150 mM citrate buffer [pH 6.0], 0.25 M sucrose, 0.04%–1.5% CHAPSO, 0.1% phosphatidylcholine, 10 μM bestatin, 10 μM amastatin, 5 μM phenanthroline, 10 μM captopril, and 5× Roche protease inhibitor mix).

Extraction of Tri-, Tetra-, and Pentapeptides from Living Cultured Cells

HEK cells stably expressing sw βAPP and PS1 derivatives were cultured to confluence in 10 cm dishes, and 24 hr before collection, the cells were treated with GSM or iGSM. Proteasome inhibitors (1 μM lactacystin, 100 nM MG262, and 1 μM epoxomicin) were added, with or without GSM/iGSM, to the conditioned medium for the last 4 hr. The cells were washed quickly with ice-cold PBS and then immediately boiled for 2 min. The boiled samples were sonicated for 5 s three times and ultracentrifuged. The resultant supernatant was subjected to LC-MS/MS analysis to measure the tri-, tetra-, and pentapeptides.

For additional details, please refer to the Extended Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Extended Experimental Procedures, four figures, and three tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2012.11.028.

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REFERENCES

Chávez-Gutiérrez, L., Bammens, L., Benilova, I., Vandersteen, A., Benurwar, M., Borgers, M., Lismont, S., Zhou, L., Van Cleynenbreugel, S., Esselmann, H., et al. (2012). The mechanism of γ-Secretase dysfunction in familial Alzheimer disease. *EMBO J. 31*, 2261–2274.

De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Guhde, G., Annaert, W., Von Figura, K., and Van Leuven, F. (1998). Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. Nature *391*, 387–390.

De Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J.S., Schroeter, E.H., Schrijvers, V., Wolfe, M.S., Ray, W.J., et al. (1999). A presenilin-1-dependent gamma-secretase-like protease mediates release of Notch intracellular domain. Nature *398*, 518–522.

Gravina, S.A., Ho, L., Eckman, C.B., Long, K.E., Otvos, L., Jr., Younkin, L.H., Suzuki, N., and Younkin, S.G. (1995). Amyloid beta protein (A beta) in Alzheimer's disease brain. Biochemical and immunocytochemical analysis with antibodies specific for forms ending at A beta 40 or A beta 42(43). J. Biol. Chem. *270*, 7013–7016.

Gu, Y., Misonou, H., Sato, T., Dohmae, N., Takio, K., and Ihara, Y. (2001). Distinct intramembrane cleavage of the beta-amyloid precursor protein family resembling gamma-secretase-like cleavage of Notch. J. Biol. Chem. *276*, 35235–35238.

He, G., Luo, W., Li, P., Remmers, C., Netzer, W.J., Hendrick, J., Bettayeb, K., Flajolet, M., Gorelick, F., Wennogle, L.P., and Greengard, P. (2010). Gamma-secretase activating protein is a therapeutic target for Alzheimer's disease. Nature *467*, 95–98.

Kounnas, M.Z., Danks, A.M., Cheng, S., Tyree, C., Ackerman, E., Zhang, X., Ahn, K., Nguyen, P., Comer, D., Mao, L., et al. (2010). Modulation of gamma-secretase reduces beta-amyloid deposition in a transgenic mouse model of Alzheimer's disease. Neuron *67*, 769–780.

Kukar, T., Murphy, M.P., Eriksen, J.L., Sagi, S.A., Weggen, S., Smith, T.E., Ladd, T., Khan, M.A., Kache, R., Beard, J., et al. (2005). Diverse compounds mimic Alzheimer disease-causing mutations by augmenting Abeta42 production. Nat. Med. *11*, 545–550.

Kuperstein, I., Broersen, K., Benilova, I., Rozenski, J., Jonckheere, W., Debulpaep, M., Vandersteen, A., Segers-Nolten, I., Van Der Werf, K., Subramaniam,

V., et al. (2010). Neurotoxicity of Alzheimer's disease Aβ peptides is induced by small changes in the Aβ42 to Aβ40 ratio. EMBO J. *29*, 3408–3420.

Li, Y.M., Lai, M.T., Xu, M., Huang, Q., DiMuzio-Mower, J., Sardana, M.K., Shi, X.P., Yin, K.C., Shafer, J.A., and Gardell, S.J. (2000). Presenilin 1 is linked with gamma-secretase activity in the detergent solubilized state. Proc. Natl. Acad. Sci. USA *97*, 6138–6143.

Okochi, M., Steiner, H., Fukumori, A., Tanii, H., Tomita, T., Tanaka, T., Iwatsubo, T., Kudo, T., Takeda, M., and Haass, C. (2002). Presenilins mediate a dual intramembranous gamma-secretase cleavage of Notch-1. EMBO J. *21*, 5408–5416.

Okochi, M., Fukumori, A., Jiang, J., Itoh, N., Kimura, R., Steiner, H., Haass, C., Tagami, S., and Takeda, M. (2006). Secretion of the Notch-1 Abeta-like peptide during Notch signaling. J. Biol. Chem. *281*, 7890–7898.

Qi-Takahara, Y., Morishima-Kawashima, M., Tanimura, Y., Dolios, G., Hirotani, N., Horikoshi, Y., Kametani, F., Maeda, M., Saido, T.C., Wang, R., and Ihara, Y. (2005). Longer forms of amyloid beta protein: implications for the mechanism of intramembrane cleavage by gamma-secretase. J. Neurosci. *25*, 436–445.

Saito, T., Suemoto, T., Brouwers, N., Sleegers, K., Funamoto, S., Mihira, N., Matsuba, Y., Yamada, K., Nilsson, P., Takano, J., et al. (2011). Potent amyloidogenicity and pathogenicity of Aβ43. Nat. Neurosci. *14*, 1023–1032.

Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T.D., Hardy, J., Hutton, M., Kukull, W., et al. (1996). Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nat. Med. *2*, 864–870.

Shen, J., and Kelleher, R.J., 3rd. (2007). The presenilin hypothesis of Alzheimer's disease: evidence for a loss-of-function pathogenic mechanism. Proc. Natl. Acad. Sci. USA *104*, 403–409.

Sherrington, R., Rogaev, E.I., Liang, Y., Rogaeva, E.A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K., et al. (1995). Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature *375*, 754–760.

Struhl, G., and Greenwald, I. (1999). Presenilin is required for activity and nuclear access of Notch in Drosophila. Nature *398*, 522–525.

Suzuki, N., Cheung, T.T., Cai, X.D., Odaka, A., Otvos, L., Jr., Eckman, C., Golde, T.E., and Younkin, S.G. (1994). An increased percentage of long amyloid beta protein secreted by familial amyloid beta protein precursor (beta APP717) mutants. Science *264*, 1336–1340.

Takami, M., Nagashima, Y., Sano, Y., Ishihara, S., Morishima-Kawashima, M., Funamoto, S., and Ihara, Y. (2009). gamma-Secretase: successive tripeptide and tetrapeptide release from the transmembrane domain of beta-carboxyl terminal fragment. J. Neurosci. *29*, 13042–13052.

Weggen, S., Eriksen, J.L., Das, P., Sagi, S.A., Wang, R., Pietrzik, C.U., Findlay, K.A., Smith, T.E., Murphy, M.P., Bulter, T., et al. (2001). A subset of NSAIDs lower amyloidogenic Abeta42 independently of cyclooxygenase activity. Nature *414*, 212–216.

Winkler, E., Hobson, S., Fukumori, A., Dümpelfeld, B., Luebbers, T., Baumann, K., Haass, C., Hopf, C., and Steiner, H. (2009). Purification, pharmacological modulation, and biochemical characterization of interactors of endogenous human gamma-secretase. Biochemistry *48*, 1183–1197.

Wolfe, M.S. (2012). γ-Secretase inhibitors and modulators for Alzheimer's disease. J. Neurochem. *120(Suppl 1)*, 89–98.

Wolfe, M.S., Xia, W., Ostaszewski, B.L., Diehl, T.S., Kimberly, W.T., and Selkoe, D.J. (1999). Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and gamma-secretase activity. Nature *398*, 513–517.

Yanagida, K., Okochi, M., Tagami, S., Nakayama, T., Kodama, T.S., Nishitomi, K., Jiang, J., Mori, K., Tatsumi, S., Arai, T., et al. (2009). The 28-amino acid form of an APLP1-derived Abeta-like peptide is a surrogate marker for Abeta42 production in the central nervous system. EMBO Mol. Med. *1*, 223–235.

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ORIGINAL ARTICLE

Classification of delusions in Alzheimer's disease and their neural correlates

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Abstract

Background: Previous findings on neural correlates of delusion in Alzheimer's disease (AD) have been inconsistent because of methodological issues, such as treating multiple delusions as a single entity. In this retrospective study, we classified AD delusions and investigated their neural correlates by using single-photon emission computed tomography data.

Methods: We selected AD patients with delusions from our consecutive outpatients from 2004 to 2010. In this study, eight types of delusions were evaluated with Neuropsychiatric Inventory and classified by factor analysis. Twenty-five of the patients also had single-photon emission computed tomography data, which we used to assess the relationships between cerebral regions of hypoperfusion and hyperperfusion and each classified delusion. The relations were assessed using Statistical Parametric Mapping with normalization to the white matter cerebral blood flow.

Results: The delusions were classified into three factors. Factor 1 consisted of a belief that his/her house is not his/her home, phantom boarder symptom, delusion of abandonment, and belief that one's spouse or others are not who they claim to be. Factor 1 was related to hypoperfusion in the right temporal pole and hyperperfusion in the medial frontal and precentral regions. Factor 2 consisted of delusion relating to the television and delusion of persecution. Factor 2 was related to hypoperfusion in the precuneus and hyperperfusion in the insula and thalamus. Factor 3 consisted of delusion of abandonment and delusional jealousy. Factor 3 was related to hypoperfusion in the right inferior temporal and frontal regions and hyperperfusion in the middle frontal gyrus, insula and posterior cingulate gyrus. Delusion of theft was not included in any factors, and it was related to hypoperfusion in the bilateral thalami and left posterior cingulate gyrus and hyperperfusion in the left inferior frontal regions and anterior cingulate gyrus.

Conclusions: Delusions in AD were classifiable, and each classified delusion was related to different neural networks.

Key words: *Alzheimer's disease, delusions, factor analysis, neuroanatomical basis, regional cerebral blood flow, single-photon emission computed tomography.*

INTRODUCTION

A wide range of neuropsychiatric symptoms and behavioural changes, known as behavioural psychological symptoms of dementia (BPSD), can emerge in the course of Alzheimer's disease (AD).[1,2] BPSD can encompass delusions, hallucinations, agitation, dysphoria, anxiety, euphoria, apathy, disinhibition, irritability and aberrant motor behaviours.[1] Among BPSD, delusions are more likely to appear at the earlier stage and are one of the common symptoms.[3–6] The occurrence of delusions in AD is generally a sign of a worsening prognosis.[7,8] Delusions increase a patient's sense of distress and the burden on the caregivers,[9] and can be a predictor of the need for

institutionalization.[10–12] Current neuroleptic drugs for delusions are not very effective.[10,13] Designing more effective drugs would be easier if the mechanisms by which delusions in AD develop were better understood.

Although many studies have investigated the neural correlates of delusion in AD with single-photon emission computed tomography (SPECT) and positron emission tomography (PET), there is no consensus on these findings.[4,6,14–18] The lack of consensus could be the result of methodological differences, which fall into four categories.

First, previous AD delusion studies considered the delusions as a single entity. However, many types of delusions have been described in AD. The most common types are delusion of persecution,[4–6,11,14,16,17,19–21] delusion of theft,[4–6,11,17,19–22] delusion of abandonment,[4–6,19–21] phantom boarder symptoms (PBS) (belief that some people are in his/her house although the no one is actually there),[4–6,19,20,23] misidentification of people,[6,17,20] including Capgras phenomenon,[14,16,20,21,24,25] belief that his/her house is not his/her home,[4–6,11,17,20,21,24] delusions relating to the television (i.e. the belief that television or magazine images or reports are actually present in the home),[4–6,19,20,23,26] and delusional jealousy.[4–6,14,20,21] Other delusions, such as misidentification of mirror image and the belief that a deceased family member is still alive,[17,19,20,23] can be also observed in AD. Patients with AD often experience more than two types of delusions at the same time.[5] However, AD patients with delusions do not experience all kinds of delusion in the course of the disease, and the frequency of each type of delusion differs.[4–6] Therefore, we thought that delusions in AD may be classifiable and distinguishable neuropsychiatric symptoms.

Second, among the three accumulative radiopharmaceuticals used to assess regional cerebral blood flow (rCBF) with SPECT (technetium-99-labelled hexamethylpropyleneamine oxime, technetium-99-labelled ethyl cysteinate dimer, and iodine-123-labelled N-isopropyl-p-iodoamphetamine (^{123}I-IMP)),[27] ^{123}I-IMP shows the best linearity between the cerebral radioactivity and cerebral blood flow (CBF).[28] Furthermore, ^{123}I-IMP is more sensitive to abnormalities in brain perfusion than the others.[29] However, no studies have yet used ^{123}I-IMP SPECT to investigate the relationship between AD delusions and rCBF. We expected that ^{123}I-IMP SPECT would detect minor alterations of rCBF that previous studies have missed.

Third, most previous neuroimaging studies investigating AD delusions used the regions of interest (ROI) technique to evaluate alterations in rCBF or regional cerebral metabolic rate.[4,14,16,18,24] The ROI technique is not user-independent and cannot evaluate the whole brain. Moreover, the ROI technique does not take individual variations in brain size and shape into account, so it is not suitable for assessing AD brains, which are atrophic. Statistical parametric mapping (SPM) has recently supplanted the ROI technique. SPM analyzes the obtained spatially normalized brain images on a voxel-by-voxel comparison without any priori assumptions and evaluates the whole brain.[22]

Fourth, in statistical analyses, normalization is required to reduce intra-individual variation and to sensitively detect disease-dependent patterns of rCBF and regional cerebral metabolic rate in the SPM.[30,31] Usually, the counts per voxel are normalized to the global mean, which normalizes a global CBF for each subject to 50 mL/100 g/min.[30,31] However, the normalization to the global mean falsely increases CBF and cerebral metabolic rate, which are in fact unchanged.[30] Recently, it has been found that normalization to the white matter produced much less biased patterns of CBF than normalization to the global mean.[30,31]

The aims of this study were to classify delusions in AD with a factor analysis (Study 1) and to investigate the neural correlates of each classified delusion with ^{123}I-IMP SPECT (Study 2). We analyzed the SPECT data with SPM and normalized the counts per voxel to the white matter for statistical analyses.

STUDY 1 AND STUDY 2

These studies were carried out in accordance with the World Medical Association's Declaration of Helsinki (2008) and approved by the Research Ethical Committee of Osaka University (Suita, Japan).

Study 1: classification of delusions in AD

Methods

Participants. Eighty-seven AD patients with delusion were entered into this study. None of them lived in a nursing home. They were consecutive outpatients of the neuropsychological clinic in the Department of Neuropsychiatry of Osaka University Medical Hospital

between December 2004 and December 2010. All patients met the following criteria: (i) met the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association criteria for probable AD;[32] (ii) showed evidence of diffuse cerebral atrophy and possible atrophy in the medial temporal lobes on a cranial magnetic resonance imaging (MRI) or a cranial computed tomography; (iii) had no history of other neurological or psychiatric disorders, serious cerebral vascular disorders, brain tumours, brain injuries, or alcohol abuse; (iv) were at least 60 years old at the first visit; and (v) had a reliable caregiver who could evaluate the BPSD. This study carefully excluded patients who showed indication of dementia with Lewy bodies, such as notable fluctuating cognition with pronounced variations in attention and alertness, recurrent visual hallucinations, or spontaneous motor features of parkinsonism.[33]

The mean age of the patients with delusions was 75.7 ± 6.8 years (range: 63–90 years). The number of women exceeded the number of men (65 vs 22). The mean years of education were 11.5 ± 2.8 (range: 6–18 years). Fifty-five patients (63.2%) were receiving donepezil and nine others (10.3%) were receiving antipsychotics (risperidone: four; tiapride: two; quetiapine: two; sulpiride: one). The mean Mini-Mental State Examination score was 17.4 ± 5.3 (range: 3–26).[34] The Clinical Dementia Rating (CDR) was used to evaluate disease stage.[35] The CDR is a five-point scale with the following grades: 0, no symptoms; 0.5, very mild; 1, mild; 2, moderate; 3, severe.[35] The numbers of patients with CDR grades of 0.5, 1, 2 and 3 were 19, 37, 25 and 6, respectively.

Assessment of delusions. The Neuropsychiatric Inventory (NPI) was employed to evaluate BPSD.[36] The NPI has been frequently used in clinical settings and has been shown to be valid and reliable in both Western countries and Japan.[36,37] The NPI contains 10 subscales of BPSD, including delusion. For those symptoms, the caregiver was asked to rate severity from 0 to 3 and frequency from 0 to 4 for each subscale. The NPI composite scores were calculated by multiplying the severity and frequency scores, so the possible composite scores ranged from 0 to 12 for each subscale. In the delusion subscale, eight different types of delusion, which have been reported to be the most frequent in AD,[20,21] can be evaluated in a manner of present or absent. The eight delusions were: delusion of persecution, delusion of theft, delusional jealousy, PBS, belief that one's spouse or others are not who they claim to be, belief that his/her house is not his/her home, delusion of abandonment, and delusion relating to the television (i.e. the belief that television or magazine images and reports are actually present in the home).[36,37] Although the original NPI defines PBS as a belief that unwelcome guests are living in his/her house,[36,37] we also considered the complaint PBS if a patient complained that family members or acquaintances who had already died or left home were in his/her house. We evaluated the eight delusions within the preceding 30 days by interviewing each patient's main caregiver.

Statistical analysis for clinical data and classification of delusions. The eight types of delusions were analyzed with exploratory factor analysis. Before carrying out a principal component analysis, we assessed the suitability of data and the factorability of the correlation matrix by calculating the Kaiser-Meyer-Olkin measure of sampling adequacy and Bartlett's test of sphericity. The principal component analysis was used to analyze the inter-item relationship and to extract the initial factors, and then a Varimax rotation was performed. The number of factors to be retained was determined by examining the eigenvalues exceeding 1.0 and by examining a scree plot. Items with factor loadings ≥0.30 were entered as a factor. In general, dichotomous responses are not appropriate for a factor analysis. However, if a factor analysis is employed to investigate a general clustering of variables and if the underlying correlations among variables are moderate, a factor analysis for dichotomous variables is allowed.[38] A factor analysis accompanies factor scores, which can be used as variables in subsequent statistical modelling, for each factor. We used the factor scores for Study 2. All statistical analyses of the demographic and neuropsychological data were performed with SPSS v. 17.0 (SPSS Inc. Chicago, IL, USA). An alpha level less than 0.05 was considered to be significant for all statistical analyses.

Results

Frequencies of different types of delusion. The mean composite scores of NPI delusion was 4.4 ± 3.4 (range: 1–12). The most common type of delusion was delusion of theft (n = 47, 54.0%), followed by PBS

Table 1 Factor loadings for delusions according to Neuropsychiatric Inventory in patients with Alzheimer's disease

	Factor 1	Factor 2	Factor 3
Eigenvalues	1.83	1.28	1.10
Variance explained (%)	22.8	16.0	13.7
His/her house is not his/her home	**0.687**	0.069	−0.010
Phantom boarder symptom	**0.605**	−0.095	−0.113
Delusion of abandonment	**0.590**	0.004	**0.579**
Spouse or others are not who they claim to be	**0.352**	−0.676	−0.199
Delusion relating to the television	0.038	**0.579**	−0.469
Delusion of persecution	0.225	**0.487**	0.054
Delusional jealously	−0.221	0.087	**0.738**
Delusion of theft	−0.610	−0.416	0.188

Significant loadings (≥0.30) were entered into the factor and are displayed in boldface.

(n = 29, 33.3%) and belief that his/her house is not his/her home (n = 29, 33.3%), and delusion of persecution (n = 26, 29.9%). Delusion relating to the television (n = 11, 12.6%), belief that one's spouse or others are not who they claim to be (n = 8, 9.2%), delusional jealousy (n = 7, 8.0%), and delusion of abandonment (n = 6, 6.9%) were less common delusions. In this study, the subject of patients' PBS was mostly people closely related to the patients such as a child, sibling, grandparent, cousin, or acquaintance, even though the person had already died or left home. Although these people were not living with the patients, the patients felt as if they were present. Of the 87 patients, 51 patients (58.6%) presented more than two types of delusion.

Classification of delusions. The results of the exploratory factor analysis are shown in Table 1. The value of the Kaiser-Meyer-Olkin measure of sampling adequacy was 0.555, and Bartlett's test of sphericity reached statistical significance (χ^2 = 45.798, d.f. = 28, P = 0.018). The principal component analysis found three components with eigenvalues exceeding 1.0, explaining 22.8%, 16.0%, and 13.7% of the variance respectively. Moreover, a plain break after the third component was seen by visual inspection of the scree plot. The delusions that were loaded into Factor 1 were belief that his/her house is not his/her home, PBS, delusion of abandonment, and belief that one's spouse or others are not who they claim to be. The delusions that were loaded into Factor 2 were delusion relating to the television and delusion of persecution. The delusions that were loaded into Factor 3 were delusion of abandonment and delusional jealousy. The factor loadings of delusion of abandonment for Factor 1 and 3 were almost equivalent (0.590 and 0.579, respectively). Delusion of theft was not loaded into any of these three factors, and it was negatively loaded into Factors 1 and 2.

Study 2: Neural correlates of each classified delusions

Methods

Participants. Among the 87 patients, 25 patients underwent a ^{123}I-IMP SPECT examination. None of them had bad smoking or drinking habits nor reported complications or histories of severe heart and pulmonary diseases. Severe ischemic changes were not observed on head MR images, and motion artefacts were not observed on the head MR images and SPECT images. In Study 2, the mean age of the patients with delusions was 74.0 ± 7.2 years (range: 63–86 years). The numbers of men and women were 4 and 21, respectively. The mean years of education were 10.8 ± 2.5 (range: 8–17 years). Donepezil was prescribed to 18 patients (72.0%), and antipsychotics were prescribed to two patients (8.0%), one of whom received tiapride and the other received sulpiride. A potent vasodilator, which was nicergoline, was prescribed to four patients. The mean Mini-Mental State Examination score was 18.3 ± 4.3 (range: 8–26). The numbers of patients with CDR grades of 0.5, 1, 2, and 3 were six, ten, eight and one, respectively. The mean factor scores for each factor were 0.1449 ± 1.0141 (range: −1.53–2.32) for Factor 1, −0.2113 ± 0.9446 (range: −2.15–1.55) for Factor 2, and −0.0258 ± 0.9646 (range: −1.29–2.15) for Factor 3.

SPECT image acquisition. Patients were each administered 167-MBq ^{123}I-IMP intravenously and asked to lie supine on the scanning bed with their eyes closed in a quiet examination room while the SPECT images were acquired. The SPECT scans were performed with a four-head rotating gamma camera (Gamma View SPECT 2000H; Hitachi Medical Corporation, Tokyo, Japan) with a low-energy, medium-resolution parallel-hole collimator that allows a spatial resolution of 13 mm full width at half maximum. After each patient's head was fixed on the headrest, a laser-assisted device equipped with the gamma camera determined the orbitomeatal line.

The acquisition protocol was 20 s per step with 64 collections over 360°, and the final data set was recorded in a 64 × 64 matrix. The raw SPECT data were transferred to a nuclear medicine computer (HARP3; Hitachi Medical Corporation, Tokyo, Japan). A Butterworth filter (cut-off frequency; 0.20 cycles per pixel; order 10) pre-filtered the projection data to minimize noise, and then the data were reconstructed into transaxial sections of 4.0-mm thick slices in planes parallel to the orbitomeatal line. Chang's attenuation correction with an optimized effective attenuation coefficient of 0.08/cm was applied to the reconstructed images.

SPECT image analysis. The SPECT data were analyzed using the SPM 5 (Wellcome Department of Cognitive Neurology, London, UK) in MATLAB software (MathWorks, Natick, MA). In the pre-processing steps, each image was spatially normalized to the stereotactic 3-D space of the Montreal Neurological Institute brain and then was smoothed with an 8 mm full width at half maximum Gaussian filter to increase the signal-to-noise ratio. The relationship between each classified delusion and rCBF was examined with multiple regression models, which are covariate only design matrices, and a two sample *t*-test was employed for the delusion of theft. In the multiple regression models, the factor scores of each factor were entered into the covariate. In both statistical models, the age, the score of the Mini-Mental State Examination, and the mean of the tracer uptake in the white matter were entered into the models as nuisance covariates. Whether the other types of delusions, except delusion of theft, were present was added to the model of the two sample *t*-test. The multiple regression models identified cerebral regions that were positively or negatively correlated with factor scores. The two sample *t*-test model identified cerebral regions that were more hyperperfused or hypoperfused in the patients with the delusion of theft than the patients without the delusion of theft. Expediently, in this study, we defined the positively correlated and more hyperperfused regions as cerebral regions of hyperperfusion and the negatively correlated and more hypoperfused regions as cerebral regions of hypoperfusion. The statistical tests were performed with thresholds of uncorrected $P < 0.01$, and the normalization to the white matter was employed. We report the significant results, in which the extent threshold was more than 100 voxels, with the Montreal Neurological Institute coordinates.[39] However, descriptions of the anatomical location also relied on visual inspection of the normalized structural MR image.

Results

Factor 1, consisting of belief that his/her house is not his/her home, PBS, delusions of abandonment, and belief that one's spouse or others are not who they claim to be, was related to hypoperfusion in the right temporal pole and hyperperfusion in the bilateral medial frontal regions and the precentral gyrus (Table 2, Fig. 1). Factor 2, consisting of delusion relating to the television and delusion of persecution, was related to hypoperfusion in the bilateral precuneus and hyperperfusion in the left insula and right thalamus (Table 3, Fig. 2). Factor 3, consisting of delusion of abandonment and delusional jealousy, was related to hypoperfusion in the right inferior temporal gyrus and inferior frontal gyrus and hyperperfusion in the left middle frontal gyrus, insula, and posterior cingulate gyrus (Table 4, Fig. 3). Finally, delusion of theft was related to hypoperfusion in the bilateral thalami and left posterior cingulate gyrus, and hyperperfusion in the left inferior frontal regions and anterior cingulate gyrus was detected (Table 5, Fig. 4).

DISCUSSION

The present study classified eight delusions in 87 AD patients through a factor analysis and then investigated the relationship between each classified delusion and alterations of rCBF by using ^{123}I-IMP SPECT. When analyzing the SPECT data, we employed SPM and normalization to the white matter CBF for the statistical analyses. Furthermore, this study revealed some hypoperfused cerebral regions related to the classified delusions. Factor 1 consisted of belief that his/her house is not his/her home, PBS, delusion of abandonment, and belief that one's spouse or others are not who they claim to be. Belief that his/her house is not his/her home and belief that one's spouse or others are not who they claim to be are a form of delusional misidentification, which refers to a false belief that an identify of a place or person has been altered,[24,40] so that those two delusions have been equated.[24,40] Furthermore, PBS has been reported to be related to belief that his/her house is not his/her home and belief that one's spouse or others are not

who they claim to be.[20,24,41–43] The present study confirmed that these three types of delusions are correlated with each other and first revealed that delusion of abandonment was also related to these three delusions.

Patients with PBS in this study probably recall a person who used to live or stay with them in the home. Delusional misidentification was found to be associated with paramnesia,[40] which is a false recollection of memory that is caused by biographical memory impairment, and losing the sense of familiarity with one's environment.[44] Therefore, PBS as well as the belief that his/her house is not his/her home and the belief that one's spouse or others are not who they claim to be are probably caused by biographical memory impairment and loss of the sense of familiarity. In Study 2, these three delusions were related to hypoperfusion in the right temporal pole, indicating that dysfunction in this region causes these three delusions. Our interpretation is consistent with the findings in previous studies that delusional misidentification was associated with right cerebral hemisphere dysfunction,[23,40] that the temporal lobes are involved in both biographical memory and experiencing feelings of familiarity,[45–47] and that the right temporal pole is involved in discriminating familiar faces and scenes from unfamiliar ones on the basis of memory.[48] As for the association between the delusional misidentifications including the three delusions and delusion of abandonment, if patients have lost senses of familiarity, they ought to be uncomfortable about and alienated from their family, home, and belongings, which subsequently brings about delusion of abandonment. Hyperperfusion in the bilateral medial frontal and precentral regions, including the primary motor cortex, was associated with the delusions in Factor 1. The primary motor cortex has been reported to play an important role in action preparation, which is induced by worry and anxiety.[49] Therefore, hyperperfusion might reflect the patients' unsettled state of mind.

Delusion relating to the television and delusion of persecution were loaded into Factor 2. These related to hypoperfusion in the precuneus and hyperperfusion

Table 2 Brain regions showing significant relative hypoperfusion and hyperperfusion in relation to Factor 1

Region	Voxels	Z-score	MNI coordinates *x*	*y*	*z*
Hypoperfusion					
Right middle temporal gyrus (temporal pole)	219	3.11	64	6	−16
Hyperperfusion					
Left medial frontal gyrus (BA 6)	2988	3.21	−4	−28	68
Left precentral gyrus (BA 4)	–	3.12	−32	−30	70
Left medial frontal gyrus (BA 6)	–	3.10	−10	−12	68

BA, Brodmann area; MNI, Montreal Neurological Institute; voxels, number of voxels in each detected region.

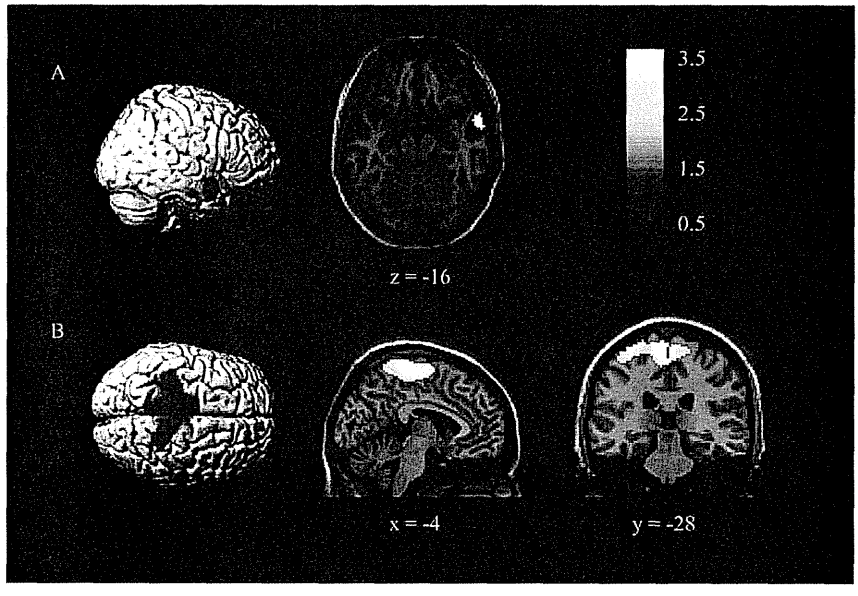

Figure 1 The results of significant (A) hypoperfusion and (B) hyperperfusion related to Factor 1 were superimposed on rendered, axial, sagittal and coronal slices of a standard brain from a single normal subject; *x*, *y* and *z* indicate the sagittal, coronal, and axial slice position in millimetres in the stereotactic space, respectively. The statistical threshold was set at $P < 0.01$ without correction for multiple comparisons. The colour bar reflects the value of the *t* statistic.

in the insula and thalamus. The relationship between the two delusions has never been reported, and thus the neural correlates have never been investigated. In previous studies, the precuneus was involved in discriminating self-relevant information from self-irrelevant information and in retrieving source memories, which are memories when and where the information was obtained.[50–52] Therefore, we thought that failing to discriminate between self-relevant and self-irrelevant information and to retrieve the information source must make the patients hypersensitive to all information. Increased activation in the insula and thalamus was reported in a functional MRI study of psychosis in schizophrenia,[53] and another functional MRI study reported that the activities of the insula are involved in suppression of negative emotions, such as fear and anger.[54] Hyperperfusion in the insula and thalamus in this study probably reflects suppression of emotional unstableness for the delusions.

Delusion of abandonment and delusional jealously were loaded into Factor 3. The relationships of the two delusions are also first reported here. The common phenomenological feature must be thoughts of abandonment. The thought of being abandoned is experienced by aged people, and it has been reported that the thought can be caused by feelings of inferiority as a result of senescence and dependence on the spouse.[55,56] AD patients feel insecurity more strongly and depend on their spouse more than aged people because of their disease; hence, the psychosocial factors are key to the two delusions. These delusions are related to hypoperfusion in the right inferior temporal gyrus and inferior frontal gyrus and hyperperfusion in the left middle frontal gyrus, insula and posterior cingulate gyrus. Previous studies revealed that the right inferolateral temporal region is involved in com-

Table 3 Brain regions showing significant relative hypoperfusion and hyperperfusion in relation to Factor 2

Region	Voxels	Z-score	MNI coordinates *x*	*y*	*z*
Hypoperfusion					
Right precuneus (BA 31)	1093	3.40	4	−74	26
Left precuneus (BA 7)	–	2.66	−14	−72	34
Hyperperfusion					
Left insula	342	2.93	−38	−12	12
Right extra-nuclear	475	2.91	26	−8	20
Right thalamus	–	2.78	24	−26	10
Right frontal sub-gyral	–	2.55	22	−18	36

BA, Brodmann area; MNI, Montreal Neurological Institute; voxels, number of voxels in each detected region.

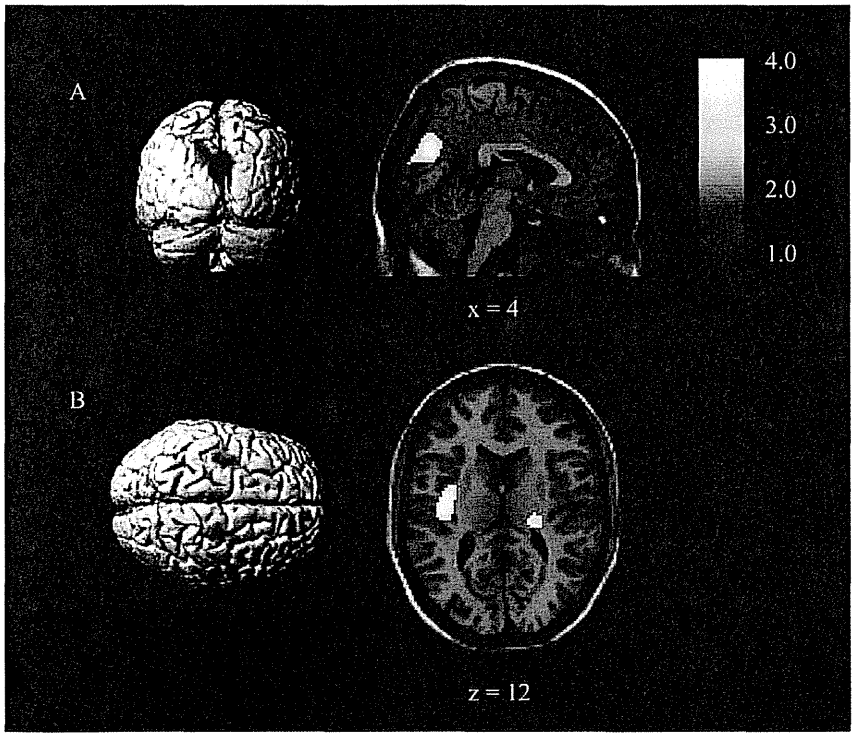

Figure 2 The results of significant (A) hypoperfusion and (B) hyperperfusion related to Factor 2 were superimposed on rendered, sagittal and axial slices of a standard brain from a single normal subject; *x* and *z* indicate the sagittal and axial slice position in millimetres in the stereotactic space, respectively. The statistical threshold was set at $P < 0.01$ without correction for multiple comparisons. The colour bar reflects the value of the *t* statistic.

prehending negative emotion exhibited by facial expression and in reading others' emotional states and feeling empathy.[57,58] Failure to comprehend others' emotional states and feel empathy strains relationships with others, which likely makes patients feel as if they have been abandoned. A previous functional MRI study found that the middle frontal gyrus, insula, and posterior cingulate gyrus, which were observed as hyperperfusion regions in our SPECT study, were activated when a participant was viewing paintings with a theme of rejection.[59] The hyperperfusion in our study must also reflect the state of mind of being abandoned.

Table 4 Brain regions showing significant relative hypoperfusion and hyperperfusion in relation to Factor 3

Region	Voxels	Z-score	MNI coordinates *x*	*y*	*z*
Hypoperfusion					
Right inferior temporal gyrus (BA 20)	1854	3.94	52	−18	−36
Right inferior frontal gyrus	133	2.78	58	42	−10
Right inferior frontal gyrus	–	2.59	60	34	−2
Right inferior frontal gyrus (BA 10)	–	2.46	54	52	2
Hyperperfusion					
Left middle frontal gyrus	161	3.33	−60	0	44
Left insula	567	3.10	−42	2	8
Left putamen (lentiform nucleus)	–	2.57	−26	−6	−6
Left posterior cingulate gyrus (BA 31)	293	3.02	−16	−44	32

BA, Brodmann area; MNI, Montreal Neurological Institute; voxels, number of voxels in each detected region.

In this study, delusion of theft was not loaded into any of the three factors, and the factor loadings for Factors 1 and 2 were negative. A previous factor analysis study of AD also found that delusion of theft differed from delusional misidentification, PBS, and delusion relating to television.[20] Another previous factor analysis study of dementia, including AD, vascular dementia, and dementia with Lewy bodies, revealed that delusion of theft was not associated with PBS, delusion of abandonment, delusion relating to the television or delusion of persecution.[19] Given the results of our study and the two previous factor analysis studies, delusion of theft does not appear to have a relationship with the other types of delusion. However, had we employed different methodologies, it is possible that such a relationship may have been found.

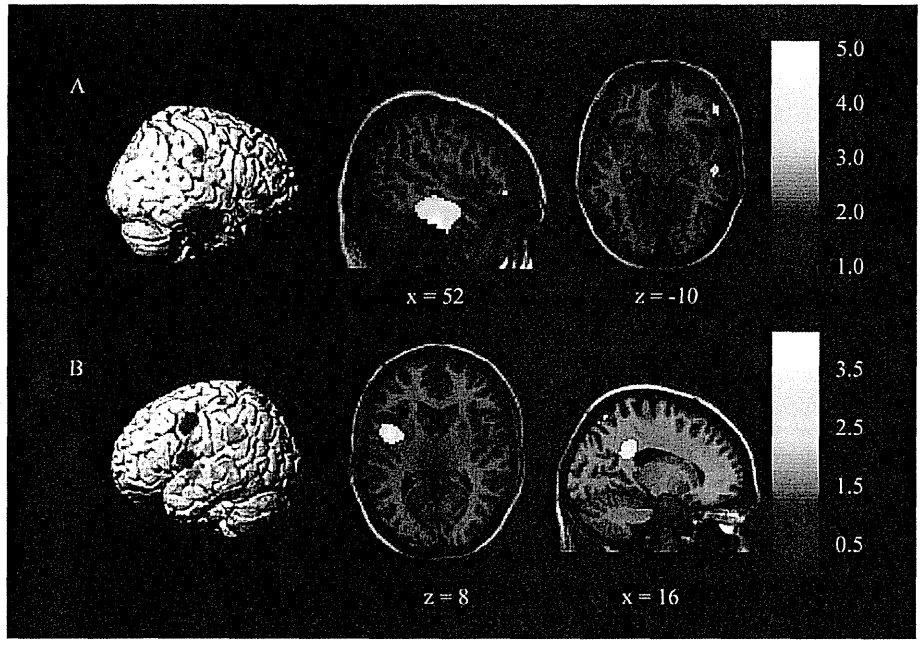

Figure 3 The results of significant (A) hypoperfusion and (B) hyperperfusion related to Factor 3 were superimposed on rendered, sagittal and axial slices of a standard brain from a single normal subject; *x* and *z* indicate the sagittal and axial slice position in millimetres in the stereotactic space, respectively. The statistical threshold was set at $P < 0.01$ without correction for multiple comparisons. The colour bar reflects the value of the *t* statistic.

The delusion of theft in this study was related to hypoperfusion in the bilateral thalami and posterior cingulate gyrus as well as to hyperperfusion in the left inferior frontal regions and anterior cingulate gyrus. The bilateral thalami and left posterior cingulate gyrus have been shown to play important roles in episodic memory in AD.[60,61] A previous study found that delusion of theft in AD was related to hypoperfusion in the right medial posterior parietal region.[22] The authors explained that the right parietal dysfunction brings attention deficit, which declines episodic memory performance, so that delusion of theft develops.[22] This indicates that the delusion of theft is associated with episodic memory impairment, which causes patients to lose their belongings and not to remember where they had left them. Increased rCBF of the left inferior frontal and anterior cingulate gyri have been related to feelings of anxiety.[62] Another study revealed that AD patients with premorbid neurotic personality tend to develop delusion of theft.[63] Hence, patients with delusion of theft are probably constantly alert and anxious that someone will steal from them, and the hyperperfusion relating to delusion of theft found in this study reflects the patients' anxiety.

Several issues in this present study should be taken considered when the findings are generalized, most importantly the small sample size, especially in Study

Table 5 Brain regions showing significant relative hypoperfusion and hyperperfusion in relation to delusion of theft

Region	Voxels	Z-score	MNI coordinates		
			x	*y*	*z*
Hypoperfusion					
Left thalamus	521	3.50	−4	−30	2
Right thalamus	–	3.06	2	−26	6
Left posterior cingulate gyrus	177	3.13	−6	−10	38
Hyperperfusion					
Left inferior frontal gyrus (BA 13)	800	3.14	−38	22	8
Left anterior cingulate gyrus	–	2.60	−20	34	20

BA, Brodmann area; MNI, Montreal Neurological Institute; voxels, number of voxels in each detected region.

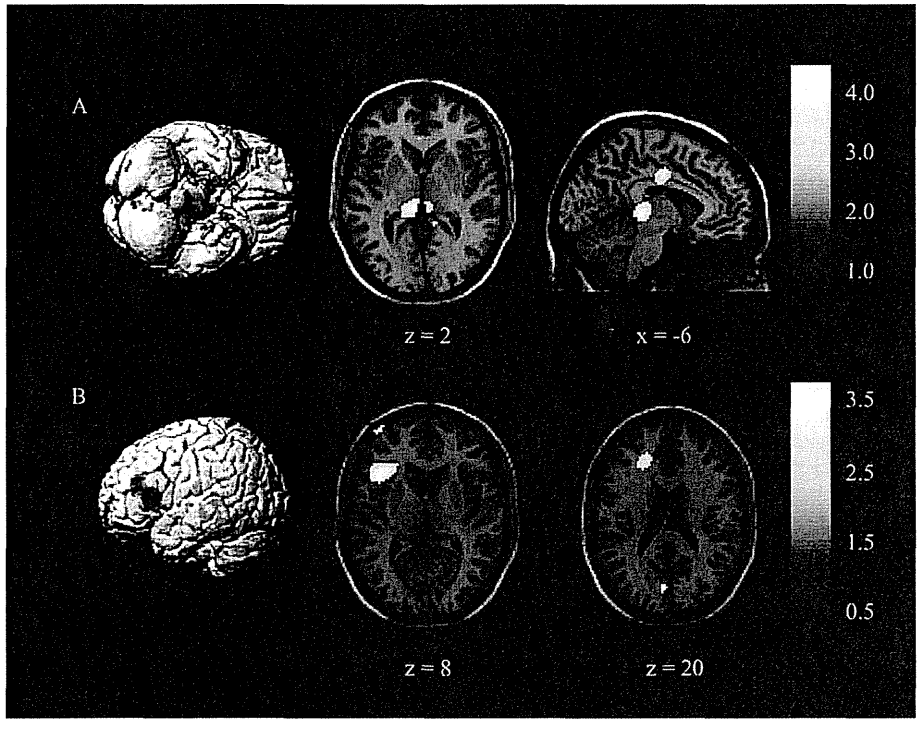

Figure 4 The results of significant (A) hypoperfusion and (B) hyperperfusion related to delusion of theft were superimposed on rendered, axial and sagittal slices of a standard brain from a single normal subject; *x* and *z* indicate the sagittal and axial slice position in millimetres in the stereotactic space, respectively. The statistical threshold was set at $P < 0.01$ without correction for multiple comparisons. The colour bar reflects the value of the *t* statistic.

2. It is difficult to administer ^{123}I-IMP SPECT examinations to patients with BPSD and cognitive impairment because they seldom keep still during the examination due to their symptoms, and sedatives cannot be given to patients before this exam. That is why the sample size for Study 2 is small. Additionally, the patients in this study could not be confirmed as having definite AD. However, most were followed up for several years, and the possibility of other disorders or diseases was ruled out. The results of this study are based on eight delusions, which were evaluated in the NPI. As such, the results might have been different if other evaluation scales, such as the Behavioural Pathology in Alzheimer's Disease Rating Scale or the Behaviour Rating Scale for Dementia, has been used.[64,65]

The present study revealed that delusions in AD are classifiable and can be correlated with rCBF in different regions of the brain. These results should help to develop more effective drugs and therapies for treating delusions in AD.

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REFERENCES

1 Mega MS, Cummings JL, Fiorello T, Gornbein J. The spectrum of behavioral changes in Alzheimer's disease. *Neurology* 1996; **46**: 130–135.
2 Robert PH, Verhey FR, Byrne EJ *et al*. Grouping for behavioral and psychological symptoms in dementia: clinical and biological aspects. Consensus paper of the European Alzheimer disease consortium. *Eur Psychiatry* 2005; **20**: 490–496.
3 Shimabukuro J, Awata S, Matsuoka H. Behavioral and psychological symptoms of dementia characteristic of mild Alzheimer patients. *Psychiatry Clin Neurosci* 2005; **59**: 274–279.
4 Hirono N, Mori E, Ishii K *et al*. Alteration of regional cerebral glucose utilization with delusions in Alzheimer's disease. *J Neuropsychiatry Clin Neurosci* 1998; **10**: 433–439.
5 Ikeda M, Shigenobu K, Fukuhara R *et al*. Delusions of Japanese patients with Alzheimer's disease. *Int J Geriatr Psychiatry* 2003; **18**: 527–532.
6 Nakano S, Yamashita F, Matsuda H, Kodama C, Yamada T. Relationship between delusions and regional cerebral blood flow in Alzheimer's disease. *Dement Geriatr Cogn Disord* 2006; **21**: 16–21.
7 Rubin EH. Delusions as part of Alzheimer's disease. *Neuropsychiatry Neuropsychol Behav Neurol* 1992; **5**: 108–113.
8 Stern Y, Mayeux R, Sano M, Hauser WA, Bush T. Predictors of disease course in patients with probable Alzheimer's disease. *Neurology* 1987; **37**: 1649–1653.
9 Allegri RF, Sarasola D, Serrano CM *et al*. Neuropsychiatric symptoms as a predictor of caregiver burden in Alzheimer's disease. *Neuropsychiatr Dis Treat* 2006; **2**: 105–110.
10 Magni E, Binetti G, Bianchetti A, Trabucchi M. Risk of mortality and institutionalization in demented patients with delusions. *J Geriatr Psychiatry Neurol* 1996; **9**: 123–126.
11 Morriss RK, Rovner BW, Folstein MF, German PS. Delusions in newly admitted residents of nursing homes. *Am J Psychiatry* 1990; **147**: 299–302.
12 Steele C, Rovner B, Chase GA, Folstein M. Psychiatric symptoms and nursing home placement of patients with Alzheimer's disease. *Am J Psychiatry* 1990; **147**: 1049–1051.
13 Liperoti R, Pedone C, Corsonello A. Antipsychotics for the treatment of behavioral and psychological symptoms of dementia (BPSD). *Curr Neuropharmacol* 2008; **6**: 117–124.
14 Starkstein SE, Vazquez S, Petracca G *et al*. A SPECT study of delusions in Alzheimer's disease. *Neurology* 1994; **44**: 2055–2059.
15 Kotrla KJ, Chacko RC, Harper RG, Jhingran S, Doody R. SPECT findings on psychosis in Alzheimer's disease. *Am J Psychiatry* 1995; **152**: 1470–1475.
16 Pontón MO, Darcourt J, Miller BL, Cummings JL, Schumann SW, Maen I. Psychometric and SPECT studies in Alzheimer's disease with and without delusions. *Neuropsychiatry Neuropsychol Behav Neurol* 1995; **8**: 264–270.
17 Staff RT, Shanks MF, Macintosh L, Pestell SJ, Gemmell HG, Venneri A. Delusions in Alzheimer's disease: SPET evidence of right hemispheric dysfunction. *Cortex* 1999; **35**: 549–560.
18 Sultzer DL, Brown CV, Mandelkern MA *et al*. Delusional thoughts and regional frontal/temporal cortex metabolism in Alzheimer's disease. *Am J Psychiatry* 2003; **160**: 341–349.
19 Ballard CG, Bannister CL, Patel A *et al*. Classification of psychotic symptoms in dementia sufferers. *Acta Psychiatr Scand* 1995; **92**: 63–68.
20 Cook SE, Miyahara S, Bacanu SA *et al*. Psychotic symptoms in Alzheimer disease: evidence for subtypes. *Am J Geriatr Psychiatry* 2003; **11**: 406–413.
21 Harwood DG, Ownby RL, Barker WW, Duara R. The behavioral pathology in Alzheimer's Disease Scale (BEHAVE-AD): factor structure among community-dwelling Alzheimer's disease patients. *Int J Geriatr Psychiatry* 1998; **13**: 793–800.
22 Fukuhara R, Ikeda M, Nebu A *et al*. Alteration of rCBF in Alzheimer's disease patients with delusions of theft. *Neuroreport* 2001; **12**: 2473–2476.
23 Forstl H, Burns A, Jacoby R, Levy R. Neuroanatomical correlates of clinical misidentification and misperception in senile dementia of the Alzheimer type. *J Clin Psychiatry* 1991; **52**: 268–271.
24 Mentis MJ, Weinstein EA, Horwitz B *et al*. Abnormal brain glucose metabolism in the delusional misidentification syndromes: a positron emission tomography study in Alzheimer disease. *Biol Psychiatry* 1995; **38**: 438–449.
25 Lipkin B. Capgras syndrome heralding the development of dementia. *Br J Psychiatry* 1988; **153**: 117–118.
26 Harvey RJ. Review: delusions in dementia. *Age Ageing* 1996; **25**: 405–408.
27 Ito H, Inoue K, Goto R *et al*. Database of normal human cerebral blood flow measured by SPECT: I. Comparison between I-123-

IMP, Tc-99m-HMPAO, and Tc-99m-ECD as referred with O-15 labeled water PET and voxel-based morphometry. *Ann Nucl Med* 2006; **20**: 131–138.
28 Kuwabara Y. [Nuclear medicine for general radiologists: clinical application of brain SPECT]. *Nippon Igaku Hoshasen Gakkai Zasshi* 2000; **60**: 671–677.
29 Matsuda H, Li YM, Higashi S *et al.* Comparative SPECT study of stroke using Tc-99m ECD, I-123 IMP, and Tc-99m HMPAO. *Clin Nucl Med* 1993; **18**: 754–758.
30 Borghammer P, Jonsdottir KY, Cumming P *et al.* Normalization in PET group comparison studies—the importance of a valid reference region. *Neuroimage* 2008; **40**: 529–540.
31 Borghammer P, Cumming P, Aanerud J, Forster S, Gjedde A. Subcortical elevation of metabolism in Parkinson's disease—a critical reappraisal in the context of global mean normalization. *Neuroimage* 2009; **47**: 1514–1521.
32 McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. 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* 1984; **34**: 939–944.
33 McKeith IG, Galasko D, Kosaka K *et al.* Consensus guidelines for the clinical and pathologic diagnosis of dementia with Lewy bodies (DLB): report of the consortium on DLB international workshop. *Neurology* 1996; **47**: 1113–1124.
34 Folstein MF, Folstein SE, McHugh PR. 'Mini-mental state'. A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res* 1975; **12**: 189–198.
35 Morris JC. The Clinical Dementia Rating (CDR): current version and scoring rules. *Neurology* 1993; **43**: 2412–2414.
36 Cummings JL, Mega M, Gray K, Rosenberg-Thompson S, Carusi DA, Gornbein J. The Neuropsychiatric Inventory: comprehensive assessment of psychopathology in dementia. *Neurology* 1994; **44**: 2308–2314.
37 Hirono N, Mori E, Ikejiri Y *et al.* [Japanese version of the Neuropsychiatric Inventory—a scoring system for neuropsychiatric disturbance in dementia patients]. *No To Shinkei* 1997; **49**: 266–271.
38 Kim JO, Mueller CW. *Factor Analysis: Statistical Methods and Practical Issues*. Newbury Park, CA: Sage Publications, 1978.
39 Mazziotta JC, Toga AW, Evans A, Fox P, Lancaster J. A probabilistic atlas of the human brain: theory and rationale for its development. The International Consortium for Brain Mapping (ICBM). *Neuroimage* 1995; **2**: 89–101.
40 Fleminger S, Burns A. The delusional misidentification syndromes in patients with and without evidence of organic cerebral disorder: a structured review of case reports. *Biol Psychiatry* 1993; **33**: 22–32.
41 Harciarek M, Kertesz A. The prevalence of misidentification syndromes in neurodegenerative diseases. *Alzheimer Dis Assoc Disord* 2008; **22**: 163–169.
42 Nagaratnam N, Irving J, Kalouche H. Misidentification in patients with dementia. *Arch Gerontol Geriatr* 2003; **37**: 195–202.
43 Hwang JP, Yang CH, Tsai SJ. Phantom boarder symptom in dementia. *Int J Geriatr Psychiatry* 2003; **18**: 417–420.
44 Feinberg TE, Shapiro RM. Misidentification-reduplication and the right hemisphere. *Neuropsychiatry Neuropsychol Behav Neurol* 1989; **2**: 39–48.
45 Kapur N, Ellison D, Smith MP, McLellan DL, Burrows EH. Focal retrograde amnesia following bilateral temporal lobe pathology. A neuropsychological and magnetic resonance study. *Brain* 1992; **115** (Pt 1): 73–85.
46 Halgren E, Babb TL, Crandall PH. Activity of human hippocampal formation and amygdala neurons during memory testing. *Electroencephalogr Clin Neurophysiol* 1978; **45**: 585–601.
47 Gloor P, Olivier A, Quesney LF, Andermann F, Horowitz S. The role of the limbic system in experiential phenomena of temporal lobe epilepsy. *Ann Neurol* 1982; **12**: 129–144.
48 Nakamura K, Kawashima R, Sato N *et al.* Functional delineation of the human occipito-temporal areas related to face and scene processing. A PET study. *Brain* 2000; **123** (Pt 9): 1903–1912.
49 Oathes DJ, Bruce JM, Nitschke JB. Worry facilitates corticospinal motor response to transcranial magnetic stimulation. *Depress Anxiety* 2008; **25**: 969–976.
50 Kircher TT, Senior C, Phillips ML *et al.* Towards a functional neuroanatomy of self processing: effects of faces and words. *Brain Res Cogn Brain Res* 2000; **10**: 133–144.
51 Lundstrom BN, Petersson KM, Andersson J, Johansson M, Fransson P, Ingvar M. Isolating the retrieval of imagined pictures during episodic memory: activation of the left precuneus and left prefrontal cortex. *Neuroimage* 2003; **20**: 1934–1943.
52 Lundstrom BN, Ingvar M, Petersson KM. The role of precuneus and left inferior frontal cortex during source memory episodic retrieval. *Neuroimage* 2005; **27**: 824–834.
53 Kumari V, Fannon D, Peters ER *et al.* Neural changes following cognitive behaviour therapy for psychosis: a longitudinal study. *Brain* 2011; **134**: 2396–2407.
54 Goldin PR, McRae K, Ramel W, Gross JJ. The neural bases of emotion regulation: reappraisal and suppression of negative emotion. *Biol Psychiatry* 2008; **63**: 577–586.
55 Takenaka S. Personality and personality disorder of senescence. *Seishin Igaku* 1987; **29**: 47–55.
56 Breitner BCC, Anderson DN. The organic and psychological antecedents of delusional jealousy in old age. *Int J Geriatr Psychiatry* 1994; **9**: 703–707.
57 Rosen HJ, Wilson MR, Schauer GF *et al.* Neuroanatomical correlates of impaired recognition of emotion in dementia. *Neuropsychologia* 2006; **44**: 365–373.
58 Schulte-Ruther M, Markowitsch HJ, Shah NJ, Fink GR, Piefke M. Gender differences in brain networks supporting empathy. *Neuroimage* 2008; **42**: 393–403.
59 Kross E, Egner T, Ochsner K, Hirsch J, Downey G. Neural dynamics of rejection sensitivity. *J Cogn Neurosci* 2007; **19**: 945–956.
60 Nestor PJ, Fryer TD, Hodges JR. Declarative memory impairments in Alzheimer's disease and semantic dementia. *Neuroimage* 2006; **30**: 1010–1020.
61 Walhovd KB, Fjell AM, Dale AM *et al.* Multi-modal imaging predicts memory performance in normal aging and cognitive decline. *Neurobiol Aging* 2010; **31**: 1107–1121.
62 Kimbrell TA, George MS, Parekh PI *et al.* Regional brain activity during transient self-induced anxiety and anger in healthy adults. *Biol Psychiatry* 1999; **46**: 454–465.
63 Murayama N, Iseki E, Endo T *et al.* Risk factors for delusion of theft in patients with Alzheimer's disease showing mild dementia in Japan. *Aging Ment Health* 2009; **13**: 563–568.
64 Reisberg B, Borenstein J, Salob SP, Ferris SH, Franssen E, Georgotas A. Behavioral symptoms in Alzheimer's disease: phenomenology and treatment. *J Clin Psychiatry* 1987; **48** (Suppl): 9–15.
65 Tariot PN, Mack JL, Patterson MB *et al.* The behavior rating scale for dementia of the consortium to establish a registry for Alzheimer's Disease. The Behavioral Pathology Committee of the Consortium to Establish a Registry for Alzheimer's Disease. *Am J Psychiatry* 1995; **152**: 1349–1357.

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Long-term multiple risk factor interventions in Japanese elderly diabetic patients: The Japanese Elderly Diabetes Intervention Trial – study design, baseline characteristics and effects of intervention

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Aim: To evaluate long-term, multiple risk factor intervention on physical, psychological and mental prognosis, and development of complications and cardiovascular disease in elderly type 2 diabetes patients.

Methods: Our randomized, controlled, multicenter, prospective intervention trial included 1173 elderly type 2 diabetes patients who were enrolled from 39 Japanese institutions and randomized to an intensive or conservative treatment group. Glycemic control, dyslipidemia, hypertension, obesity, diabetic complications and atherosclerotic disease were measured annually. Instrumental activity of daily living, cognitive impairment, depressive symptoms and diabetes burden were assessed at baseline and 3 years.

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Results: There was no significant difference in clinical or cognitive parameters at baseline between the two groups. The prevalence of low activities of daily living, depressive symptoms and cognitive impairment was 13%, 28% and 4%, respectively, and was similar in the two groups. A small, but significant difference in HbA1c between the two groups was observed at 1 year after the start of intervention (7.9% *vs* 8.1%, $P < 0.05$), although this significant difference was not observed after the second year. With the exception of coronary revascularization, there was no significant difference in fatal or non-fatal events between the two groups. Composite events were also similar in the two groups.

Conclusions: This study showed no significant differences in fatal or non-fatal events between intensive and conventional treatment. The present study might clarify whether treatment of risk factors influences function and quality of life in elderly diabetic patients.
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Keywords: diabetes mellitus, elderly, geriatric assessment, intervention, vascular complications.

Introduction

The prevalence of diabetes increases with age, with approximately 15% of elderly people in Japan having the disorder.[1] These patients often suffer from diabetic microvascular and macrovascular complications.[2] Treatment goals in this elderly diabetic population are to maintain functional abilities and quality of life, and to prevent diabetic complications. Physical functional activities[3,4] and cognitive function[5,6] are more impaired in elderly diabetic patients, with depression and low well-being being major concerns.[7,8] It is therefore important to evaluate the effects of clinical interventions on physical, psychological and mental functions, as well as on disease-related variables, such as diabetic complications, atherosclerotic disease and mortality.

The impact of intensive blood glucose, blood pressure or multiple risk factor intervention on diabetic complications in type 2 diabetes has been evaluated in the United Kingdom Prospective Diabetes Study (UKPDS),[9,10] Kumamoto Study[11] and Steno-2 Study.[12] As only a few elderly people were included in these studies, little is known on the effects of multiple risk factor intervention on diabetic complications and functional prognosis.

We therefore carried out a randomized clinical trial to evaluate the efficacy of multiple risk factor intervention on functional prognosis, and development and/or progression of diabetic complications and cardiovascular disease in elderly people with type 2 diabetes. The present study presents baseline demographic and biomedical characteristics, and describes the major outcome variables measured at baseline.

Methods

Participants

The participants recruited for the Japan Elderly Diabetes Intervention Trial (J-EDIT) were diabetic outpatients at 39 representative hospitals in Japan between March 2001 and February 2002. Written informed consent was obtained from all participants before screening, consistent with the Helsinki Declaration and the guidelines of each center's institutional ethical committee.

Initial screening tests included glycated hemoglobin A1c (HbA1c), body mass index (BMI), blood pressure, serum total cholesterol, triglycerides and high-density lipoprotein cholesterol (HDL-C). Inclusion criteria included age 65–85 years, HbA1c ≥ 7.9% or HbA1c ≥ 7.4% with at least one of following criteria: BMI ≥ 25 kg/m^2, blood pressure ≥ 130/85 mmHg, serum total cholesterol ≥ 200 mg/dL (or low-density lipoprotein cholesterol [LDL-C] ≥ 120 mg/dL in participants without coronary heart disease [CHD]) or ≥180 mg/dL (or LDL-C ≥100 mg/dL in participants with CHD), triglycerides ≥ 150 mg/dL and HDL-C < 40 mg/dL. Exclusion criteria included a recent (<6 months) myocardial infarction (MI) or stroke, acute or serious illness, aphasia and severe dementia.

Randomization and intervention

A total of 1173 diabetic outpatients were enrolled and randomly allocated to either the intensive or conventional treatment group. The randomized factors were age, sex, diabetes treatment, HbA1c, total cholesterol, triglycerides, HDL-C, blood pressure, diabetic

Table 1 Treatment goals of multiple risk factor intervention studies in patients with type 2 diabetes

	J-EDIT	UKPDS	Steno-2 Study
Mean age (years)	72	52	55
Range	(65–84)	(25–65)	(40–65)
Treatment goals			
Glucose control			
FPG (mmol/L)		<6.0	
HbA1c (%)	<6.9		<6.5
Blood pressure control (mmHg)	<130/85	<150/85	<140/85 (1993–1999) <130/80 (2000–2001)
Cholesterol (mg/dL)	<200 (<180) if one has CHD	none	<190 (1993–1999) <175 (2000–2001)
Triglycerides (mg/dL)	<150	none	<150
HDL-C (mg/dL)	>40	none	>40
Other interventions	BMI <25		Smoking cession Aspirin use

CHD, coronary heart disease; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol, J-EDIT, Japan Elderly Diabetes Intervention Trial; UKPDS, United Kingdom Prospective Diabetes Study.

microangiopathy, atherosclerotic disease, hypertension, hyperlipidemia and institutions.

The treatment goal in the intensive treatment group was HbA1c < 6.9%, BMI < 25 kg/m^2, systolic blood pressure (SBP) < 130 mmHg, diastolic blood pressure (DBP) < 85 mmHg, HDL-C > 40 mg/dL, serum triglycerides < 150 mg/dL and serum total cholesterol < 180 mg/dL (or LDL-C < 100 mg/dL if patients had CHD) or <200 mg/dL (or LDL-C < 120 mg/dL if patients did not have CHD; Table 1). If HbA1c levels did not reduce to <6.9%, oral hypoglycemic drugs (sulphonylurea, biguanides, α-glucosidase inhibitors and pioglitazone) or insulin therapy was introduced by the physician. If total cholesterol or LDL-C levels did not reach the treatment goal, the physicians were advised to use atorvastatin. Patients with a history of cerebral infarction also had antiplatelet therapy where possible.

The conventional treatment group continued their baseline treatment for diabetes, hypertension or dyslipidemia without a specific treatment goal.

Each participant had a standardized medical history and physical examination at baseline, and then annually. Baseline information included age, sex, medical history, family members with whom they lived, education, employment, height, bodyweight, waist-to-hip ratio, maximum body weight, diabetes duration, family history of diabetes and diabetes treatment. Standardized questionnaires were used to obtain self-reported data on smoking, alcohol, hypoglycemia frequency, nutritional status, dietary habits and adherence, self-efficacy, activities of daily living (ADL), physical activities, comprehensive cognitive function, and psychological status including diabetes burden and depressive symptoms.

Basic ADL was assessed by the Barthel index,[13] whereas functional disabilities were examined by the Tokyo Metropolitan Institute of Gerontology (TMIG) Index of Competence.[14] This index includes 13 items and three subscales: instrumental ADL, intellectual activity and social role. The index is well validated and is widely used to measure functional abilities in community-dwelling or institutionalized elderly subjects.[15]

Physical activities were assessed using the Baecke questionnaire.[16] The Folstein Mini-Mental State Examination (MMSE) was carried out to assess comprehensive cognitive function including orientation, memory recall and calculations.[17]

Depressive symptoms were evaluated using a short form of the Geriatric Depression Scale (15 items, GDS-15),[18] whereas diabetes-specific burden and concerns were examined using the elderly diabetes burden scale (EDBS).[19] EDBS is a short revised version of the elderly diabetes impact scale reported previously,[4] and consists of six subscales: symptom burden (4 items), social burden (5 items), diet restrictions (4 items), concern (4 items), treatment satisfaction (3 items) and burden by tablets or insulin (3 items). Each of the 23 EDBS items was rated on a four-point multiple-choice scale. The elderly diabetes burden score was calculated by reversing the scores of the treatment satisfaction subscale and summing the scores of the six subscales. EDBS has good test–retest reliability, construct validity, convergent validity and satisfactory internal consistency.

The frequency of mild or severe hypoglycemia was assessed using questionnaires (number of hypoglycemic episodes and number of comas or emergency hospital visits or admissions as a result of hypoglycemia in a year, month or week). Mild hypoglycemia episodes included the appearance of or recovery from hypoglycemic symptoms. Severe hypoglycemia episodes were defined as

coma, convulsion or incapacity of the patient sufficient to require the assistance of another person.

Nutritional intake was assessed for 1 week using the Yoshimura food frequency questionnaire[20] that estimated food and total energy intake, carbohydrate-, protein- and fat-to-energy ratios, and intake of cholesterol, salt, iron, calcium, vitamins and dietary fiber from portion sizes (relative to the standard amount) and frequency (intake number for 1 week) of 29 food groups.

Measurements

Venous blood was drawn for determination of blood glucose, HbA1c and serum concentrations of total cholesterol, HDL-C and triglycerides at baseline, and then at least twice a year. Plasma glucose was measured by the glucokinase method, and HbA1c by ion-exchange high-performance liquid chromatography. The Japan Diabetes Society (JDS) has standardized several HbA1c assays with the international standard value adjusted by the equation of HbA1c (JDS) (%) plus 0.4%. Serum insulin was measured by an enzyme immunoassay, and total cholesterol, triglycerides, HDL-C, white blood cells, red blood cells, hematocrit (Ht), blood urea nitrogen (BUN), serum creatinine, uric acid, total protein and albumin by established methods.

Blood pressure was measured with a mercury sphygmomanometer using a cuff of appropriate size. Diastolic blood pressure was determined as Korotkoff phase V. Body mass index was calculated as weight in kilograms / (height in meters)^2.

Microangiopathy (retinopathy, nephropathy and neuropathy), macroangiopathy (ischemic heart disease [IHD]), stroke and peripheral vascular disease [PVD]) were assessed at baseline, and then annually. Funduscopic examinations were carried out on dilated pupils by experienced ophthalmologists using direct ophthalmoscopy. Retinopathy status was assessed by the Japanese Diabetes Complication Study method and classified into five stages: stage 0: no retinopathy; stage 1: dot hemorrhages, hemorrhages or hard exudates; stage 2: soft exudates; stage 3: IRMA or venous deformities; stage 4: neovascularization, preretinal proliferative tissues, vitreous hemorrhages or retinal detachment. Diabetic maculopathy was assessed according to findings of hemorrhages, local edema, hard exudates and diffuse edema at macular areas. Uncorrected and corrected visual acuities, the occurrence of cataract, corneal opacity, glaucoma, age-related macular degeneration, laser photocoagulation, cataract operations and vitrectomy were assessed. Urinary albumin was measured by immunological assay. Mean urinary albumin-to-creatinine ratio (ACR; μg/mg creatinine) in two or three successive urinalyses was used to classify diabetic nephropathy as no nephropathy (ACR < 30), microalbuminuria (30 ≤ ACR < 300) or persistent proteinuria (ACR ≥ 300 or urinary protein ≥30 mg/dL). Diabetic neuropathy was defined as loss of Achilles tendon reflexes and diminished vibration sensation, and/or neuropathic symptoms including paresthesia.

Follow up

The annual examinations included bodyweight, BMI, waist-to-hip ratio, treatment of diabetes, fasting plasma glucose, serum insulin, total cholesterol, triglycerides, HDL-C, lipoprotein(a), white blood cells, red blood cells, Ht, platelet, BUN, serum creatinine, uric acid, total protein, albumin, blood pressure, visual acuity, microalbuminuria, deep tendon reflexes, neuropathic symptoms, resting electrocardiogram (ECG), chest X-ray, and the occurrence of retinopathy, nephropathy, neuropathy, IHD, stroke and PVD. HbA1c and ACR were measured biannually. Basic ADL, functional abilities, cognitive function, depressive symptoms and nutrition were assessed every other year. Use of medications, including insulin and hypoglycemic, antihypertensive, antihyperlipidemic, antiplatelet and anticoagulant drugs, was checked annually.

Data management and analyses

The main database was stored at the data management and statistical analysis center. A data sheet of each patient was mailed from the study institutions to the data management and statistical analysis center each year. The data was validated by range, combinatorial and historical checks of compatibility with previous data. A visual check of the list of abnormalities and information in the data sheets was carried out by trained staff. The study institutions were notified of unexplained abnormalities in the data that were completed or corrected before entry into the main database.

Data are presented as means ± SD or as proportions, unless otherwise specified. Data for analysis was extracted from the main database, and statistical analysis was carried out using the SAS computer programs. For univariate analysis, we used unpaired *t*-test and χ^2-test to compare baseline clinical characteristics in the two treatment groups. $P < 0.05$ was considered statistically significant.

Data security was maintained by exclusion of patient identities, password access and secure output within the data management and statistical analysis center.

End-points

Fatal and non-fatal events during follow up were certified by at least two members of the expert committee, masked to the participants' diagnosis and risk factor status. Death as a result of diabetes was defined as sudden death or death from atherosclerotic CHD (MI or heart failure as a result of ischemia) or stroke, death as

a result of renal failure, hyperglycemia or hypoglycemia. The history of macroangiopathy was obtained from medical records. Ischemic heart disease was classified as present when the patient had (i) a history of MI characterized by a typical clinical picture (chest pain, chest oppression and dyspnea), typical ECG alterations with occurrence of pathological Q waves and/or localized ST variations) and typical enzymatic changes (creatine phosphokinase); and (ii) a history of angina pectoris, positive treadmill ECG test or positive postload cardiac scintigram, confirmed by coronary angiography. Stroke was defined as clinical signs of a focal neurological deficit with rapid onset persisting ≥24 h, confirmed by either brain computed tomography or magnetic resonance imaging. No cases of asymptomatic lesions detected by brain imaging (i.e. silent infarction) were included. PVD was defined as the absence of dorsal pedal artery or posterior tibial artery pulsation and ankle–brachial index <0.8 or the presence of foot gangrene or ulcers.

All events related to diabetes were defined as any complications of cardiovascular events, fatal or non-fatal stroke, sudden death, renal death, diabetic foot complications and heart failure. All events included death unrelated to diabetes, as well as all events related to diabetes.

End-point validation

Possible clinical end-points were noted in the annual data sheets, with the diagnostic criteria for each end-point being predetermined. When an end-point was notified on a data sheet, the administrator requested full information from the data management and statistical analysis center, followed by a review by two clinical assessors of the event assignment committee. Two separate assessments for each end-point were entered on a special data sheet. If there was disagreement on the assessment, a final decision was made after discussions of the committee. The definition of the end-points is shown in the Appendix.

Statistical analysis and criteria for stopping the study

Differences in end-points (deaths or complications) between the two groups were analyzed using the log–rank test. Uni- and multivariate survival analyses were carried out using Cox proportional hazard regression models. All major analyses were according to assigned allocations (intention to treat), without exclusion of protocol deviants.

The Data and Safety Monitoring Committee examine the end-points annually and will stop the study when the difference in diabetes-related deaths or complications (disease) between the two groups becomes significant ($P < 0.001$, log–rank test).

Results

A total of 1173 outpatients with diabetes, aged over 65 years, were registered between March 2001 and February 2002. After randomization, 585 and 588 patients were allocated to intensive or conventional treatment, respectively. There were no significant differences between the two groups for age, sex, diabetes treatment, BMI, HbA1c, SBP and DBP, total cholesterol, triglycerides, HDL-C levels (Table 2), and number of risk factors (data not shown).

At baseline, the proportion of patients with a low ADL (TMIG Index of Competence ≤ 9), depressive symptoms (GDS-15 ≥ 5), or cognitive impairment (MMSE ≤ 23) were 13%, 28% and 4%, respectively. The prevalence of low ADL, depressive state and cognitive impairment was similar in the two groups (Table 2).

The dropout rate after 6 years was 8.9% (104 cases). HbA1c, total cholesterol, triglycerides, blood pressures and BMI at baseline and during follow up are shown in Table 3 and Figures 1–4. A small, but significant difference in HbA1c between the two groups was observed at 1 year after the start of intervention (7.9% *vs* 8.1%, $P < 0.05$), although this significant difference was not observed after the second year. Although SBP and DBP, total cholesterol and triglycerides levels tended to decrease by the sixth year compared with the baseline data in both groups, no significant differences in these variables were observed between the two groups during follow up (Figs 1–4). BMI and HDL-C levels did not change over the follow-up period in either group.

Table 4 shows the fatal and non-fatal events during follow up in the two groups. With the exception of coronary revascularization, there were no significant differences in fatal or non-fatal events between the groups ($P < 0.05$, log-rank test). Composite events (death as a result of diabetes, death unrelated to diabetes, coronary vascular events, stroke, total diabetes-related events and all events) were also similar in the two groups (Table 5).

Discussion

The J-EDIT study has the potential to determine whether multiple risk factor intervention prevents aggravation of complications and quality of life, and reduces mortality in elderly diabetic patients. The study has three characteristics. First, it is a large-scale study of multiple risk factor intervention in elderly diabetic patients. No or very few elderly patients were included in the UKPDS[9,10] or Steno-2 Study.[12] Second, the multiple interventions involved control of blood pressure, serum lipids, bodyweight and blood glucose. The treatment goals in the intensive treatment group were similar

Table 2 Clinical characteristics of the participants at baseline

	Conventional treatment ($n = 588$)	Intensive treatment ($n = 585$)
General characteristics		
Age (years)	71.7 ± 4.7	71.9 ± 4.6
Male (%)	46.3	46.3
Duration of diabetes (years)	18.0 ± 9.9	16.7 ± 8.5
Body mass index (kg/m^2)	24.3 ± 7.3	24.0 ± 3.9
Waist (cm)	83.6 ± 9.9	84.3 ± 10.4
Waist-to-hip ratio	0.89 ± 0.07	0.90 ± 0.07
Smoking (%) (non-/ex-smoker/current smoker)	16:31:53	15:29:56
Smoking (package × years)	848 ± 762	789 ± 601
Family history of diabetes (%)	45.8	39.7
Systolic BP (mmHg)	137 ± 17	137 ± 16
Diastolic BP (mmHg)	75 ± 10	76 ± 10
Clinical status		
Ischemic heart disease (%)	16.3	14.9
Cerebrovascular disease (%)	12.4	13.3
Retinopathy (%)		
Stage 0	53.6	51.7
Stage 1	30.5	31.4
Stage 2	7.8	9.1
Stage 3	3.3	3.4
Stage 4	4.7	4.7
Nephropathy (%) (no/microalbuminuria/persistent proteinuria)	51:30:19	53:30:17
Loss or weakness of ATR (%)	56.8	57.1
Paresthesia (%)	18.5	22.3
Laboratory data		
HbA1c (%)	8.5 ± 0.9	8.4 ± 0.8*
Fasting plasma glucose (mg/dL)	170 ± 53	168 ± 49
Fasting insulin (mIU/mL)	10.9 ± 12.0	10.3 ± 9.6
Total cholesterol (mg/dL)	202 ± 34	203 ± 34
Triglycerides (mg/dL)	131 ± 70	137 ± 110
HDL-C (mg/dL)	56 ± 18	57 ± 19
Uric acid (mg/dL)	5.1 ± 2.0	5.1 ± 1.4
Blood urea nitrogen (mg/dL)	16.9 ± 5.9	17.2 ± 6.1
Creatinine (mg/dL)	0.93 ± 1.2	0.83 ± 0.36
Treatment		
Treatment of diabetes (diet/OHA/insulin)	9.0:60.7:30.3	8.7:61.0:30.3
Sulfonylurea drugs	54.6	56.0
α-Glucosidase inhibitors (%)	30.5	28.0
Biguanides (%)	16.4	15.5
Pioglitazone (%)	4.5	5.2
Glinides (%)	2.3	2.1
Antihypertensive drugs (%)	56.4	57.4
ACE inhibitors (%)	22.9	23.3
ARB (%)	10.1	9.3
Calcium blockers (%)	42.9	41.0
β-Blockers (%)	6.2	5.7
α-Blockers (%)	6.1*	3.4
Diuretics (%)	5.1	7.5
Antihyperlipidemic drugs (%)	40.2	36.8
Statins (%)	30.3	26.5
Fibrates (%)	3.4	3.9
EPA (%)	0.7*	2.7
Nicotinates (%)	1.3	1.4
Probucol	2.2	1.6
Antiplatelet drugs (%)	25.9	27.4
Aspirin (%)	13	15
Geriatric Assessment		
Barthel index (full score: 20)	19.8 ± 0.9	19.8 ± 0.8
Prevalence of any disabilities (%)	11	14
Functional abilities (TMIG index of competence) (full score: 13)	11.6 ± 2.2	11.6 ± 2.2
Geriatric depression scale (full score: 15)	4.3 ± 3.3	4.0 ± 3.2
Depressive symptoms (%) (Geriatric depression scale ≥5)	41	36
MMSE (full score: 30)	28.0 ± 2.4	27.8 ± 3.0
Cognitive impairment (%) (MMSE ≤23)	7	6
Visual impairment (%) (≤0.1)	9	12

ARB, angiotensin II receptor blockers; ACE, angiotensin-converting enzyme; ATR, Achilles tendon reflex; BP, blood pressure; EPA, eicosapentenoic acid; HbA1c, glycated hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; MMSE, Mini-Mental State Examination; OHA, oral hypoglycaemic agents; TMIG, Tokyo Metropolitan Institute of Gerontology. *$P < 0.05$.