PSYCHOGERIATRICS
The Official Journal of the Japanese Psychogeriatric Society

doi:10.1111/j.1479-8301.2011.00387.x PSYCHOGERIATRICS 2011; 11: 196–204

ORIGINAL ARTICLE

Specific feature of olfactory dysfunction with Alzheimer's disease inspected by the Odor Stick Identification Test

Daiki JIMBO,[1,3] Masashi INOUE,[2] Miyako TANIGUCHI[1] and Katsuya URAKAMI[1]

[1]Section of Environment and Health Science, Department of Biological Regulation, School of Health Science, Faculty of Medicine, [2]Information Media Center, Tottori University, Yonago, Tottori, and [3]Department of Anatomy, Showa University School of Medicine, Tokyo, Japan

Correspondence: Prof Katsuya Urakami MD, PhD, Section of Environment and Health Science, Department of Biological Regulation, School of Health Science, Faculty of Medicine, Tottori University 86 Nishimachi, Yonago 683-8503, Japan. Email: jinbo@society.jpn.org

Received 4 November 2010; revision received 11 May 2011; accepted 19 July 2011.

Abstract

Aim: Alzheimer's disease (AD) is one of the most significant diseases associated with ageing. As the disease progresses, symptoms including olfactory dysfunction often appear along with cognitive dysfunction. We examined olfactory and other indexes to investigate correlations between them and the validity of an olfactory test for screening for AD.

Methods: To assess whether odorant identification will be a useful diagnostic tool, we investigated the olfactory ability of Alzheimer's disease patients (ADs) using the Odor Stick Identification Test for the Japanese. As a control, we compared ADs to aged people without AD or dementia. To investigate the relationship between olfactory loss and severity of AD, we used the Mini-Mental State Examination, Alzheimer's Disease Assessment Scale, biomarkers in spinal fluid and single-photon emission computed tomography as brain imaging.

Results: In comparing the controls and ADs, we believe that there are significant differences, with ADs having particularly low activity with regard to olfactory function and some odorants. We showed that there was a definite correlation between cognitive and olfactory function. To confirm this, we sorted subjects by markers of severity scores for comparison. In all areas, the AD group had more serious olfactory dysfunction, including in the early stages of AD.

Conclusion: This study suggests that olfactory tests such as the Odor Stick Identification Test for the Japanese can be useful for assessing severity of AD, including cognitive dysfunction. Further investigations will enable us to establish an olfactory assessment method for the screening or diagnosis of AD.

Key words: *cognitive function, diagnosis, olfactory dysfunction, prevention, screening.*

INTRODUCTION

Because many diseases develop with ageing, the ageing of society has become a worldwide problem. Of these age-related diseases, dementia is one of the most serious, and its prevalence is increasing, especially for those over the age of 65 years. There are many primary diseases that present dementia as the main symptom, but Alzheimer's disease (AD) is the most common.[1] However, there are currently only a few treatment protocols for AD and dementia, including therapeutic drugs. Given this situation, early detection has become very important for timely treatment and care. Previous research has often been driven by the need to identify markers for AD,[2] and much of it has indicated that olfactory loss or dysfunction can be a symptom of AD prior to the development of neuropathology and cognitive dysfunction.[3–5] There are many common causes of olfactory dysfunction including head trauma, endocrine dysfunction, inflammatory sinusitis and other primary diseases, and with normal ageing, olfactory function often moderately worsens.[6] For example, over half of people aged 60 years and older have some problem with smell,[7] and previous studies have

suggested that problems with olfactory abilities are far more characteristic of ADs than the general population.[8] One meta-analysis of many studies on olfactory perception and AD showed that odour identification or recognition, including issues related to patient thresholds, can help predict AD symptoms.[9]

Consequently, this infers that we should more rigorously investigate the relationship between olfactory dysfunction and the risk of AD.[9] Another study suggested that participants carrying one or two copies of the e4 allele of apolipoprotein E have remarkable olfactory dysfunction compared to those without this allele. In short, there seems to be a significant correlation between AD and olfactory loss.[10]

To investigate olfactory dysfunction, we though the University of Pennsylvania Smell Identification Test and the 'Sniffin' Sticks' test might be useful.[11] However, these tests include some odorants that are not available to most Japanese people. As such, we employed the Odor Stick Identification Test for the Japanese (OSIT-J) in this study.[12–14]

MATERIALS AND METHODS

Participants

From April 2009 through August 2010, 40 non-dementia patients (12 men and 28 women) ranging in age from 30 to 87 years (mean ± SD, 69.20 ± 9.68 years) and 109 AD patients (34 men and 75 women), ranging in age from 46 to 90 years (mean ± SD, 78.48 ± 8.12 years) were set to participate in our study.

Considering the gap between the average age of the ADs and the control group, we selected age-matched subjects for analysis. Ultimately, we picked 17 non-dementia patients (6 men and 11 women), ranging in age from 65 to 87 years (mean ± SD, 75.18 ± 5.90 years) and 100 ADs (31 men and 69 women), ranging in age from 63 to 89 years (mean ± SD, 79.47 ± 5.82 years). There were no restrictions based on gender or ethnicity (Table 1). All participants underwent examination by neurologists or geriatric psychiatrists to determine study eligibility.

Clinical diagnosis

ADs were diagnosed based on the DSM-IV and the criteria of the Neurological and Communicative Disorders and Stroke–Alzheimer's Disease and Related Disorders Association.

Laboratory procedure

Odor Stick Identification Test for the Japanese (OSIT-J)

Patients' olfactory functions were evaluated by OSIT-J. This test included 12 odorants: perfume, rose, condensed milk, Japanese orange, curry, roasted garlic, fermented beans/sweaty socks, gas for a cooker, menthol, India ink, wood, and Japanese cypress. These odours were chosen from the clusters of odours familiar to Japanese people.

Each fragrance was enclosed in microcapsules made of melamine resin. These microcapsules were mixed with an odourless solid cream and then shaped to look like a lipstick. In olfactory inspection, we applied each odorant to a paraffin paper by applying the lipstick form. After application, we handed the paper to the subject, who would then sniff the paper and answer the question, 'What is this smell?' Subjects answered by selecting the answer from a set of cards, each of which listed the name of an odorant, including the correct answer. Subjects received 1 point for each question answered correctly. No points were given for incorrect answers.

Cognitive scales

Previously, the Mini-Mental State Examination (MMSE) was used for screening cognitive level,[15] but

Table 1 Number and age of participants

	Subjects (*n*)	Age (mean ± SD)	Men (*n*)	Age (mean ± SD)	Women (*n*)	Age (mean ± SD)
All subjects						
ADs	109	78.48 ± 8.12	34	78.09 ± 8.58	75	79.32 ± 7.06
Control	40	69.20 ± 9.68	12	69.92 ± 11.89	28	61.14 ± 11.81
Age-matched subjects						
ADs	100	79.47 ± 5.82	31	79.29 ± 5.78	69	79.39 ± 5.88
Control	17	75.18 ± 5.90	6	75.86 ± 7.97	11	75.27 ± 4.56

ADs, Alzheimer's disease patients.

there have been indications that the MMSE does not evaluate cognitive function to the extent necessary. Instead, we used the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) score to rigorously assess cognitive state.[16]

Cerebrospinal fluid (CSF) collection

CSF samples were collected from subject by the Department of Neurology of Shinsei Hospital (Kurayoshi, Tottori, Japan). Informed consents had been obtained from the subjects and/or their relatives before the samples were collected. The collected CSF samples were stored immediately at −80°C until use. The study was performed in accordance with the Declaration of Helsinki (2008). Amyloid beta-protein$_{1\text{-}42}$ (Aβ_{42}) and phosphor-tau (p-tau) in CSF were measured using the double-sandwich ELISA method (Wako Pure Chemical Industries, Osaka, Japan) according to the manufacturer's instructions. We also calculated derived ratios from single biomarkers, including p-tau/Aβ_{42}.

Single-photon emission computed tomography (SPECT)

Patients were injected with 600 MBq technetium-99m ethyl cysteinate dimer.[17] Then a gamma camera was rotated around the subject continuously for 16 min, and single-photon emission computed tomography (SPECT) data were arranged into 90 projections over the entire 360° spectrum. All SPECT images were obtained from Tottori Prefectural Hospital (Gotsu, Tottori, Japan).

Ethics

We provided patients and their families with detailed information regarding the methods and purpose of the study. In addition, we received informed consent from all participants. The study was approved by the Ethical Review Board of the Faculty of Medicine of Tottori University (Yonago, Tottori, Japan).

Statics

The numbers of correct answers were compared between and the control and ADs groups using non-parametric analysis (Mann–Whitney *U*-test) in Stat View ver. 4.58 (Abacus Concepts, Piscataway, NJ, USA).

ROC curve analyses and logistic regression analyses were conducted using Excel 2008 (Microsoft, Redmond, Washington, USA).

Methodology

Investigating the capability of ADs to identify smell

We used OSIT-J to investigate the ability of ADs to identify smell and compared ADs with the control group. Subjects were outpatients at Shinsei Hospital, and we employed OSIT-J to assess smell functions and other scales to assess for dementia.

RESULTS

Comparing age and OSIT-J scores between the ADs and control group

Comparing age and OSIT-J scores between the control group and ADs showed significant differences. Previous studies have found that olfactory function is closely correlated with age, but in this case, ADs showed significant olfactory dysfunction at younger ages than non-ADs (Fig. 1).[8] Therefore, we chose age-matched subjects to determine the disparity between the ADs and control subjects. We compared OSIT-J scores of ADs and control subject and found significant differences in olfactory function (Fig. 2a). ADs had particularly low activity when presented with certain smells, including India ink, rose, roasted garlic ($P < 0.01$), Japanese cypress, and wood ($P < 0.05$) (Fig. 2b,c).

Olfactory dysfunction in ADs

The notion that patients with severe AD have greater olfactory dysfunction than non-dementia individuals has attracted a great deal of attention. We showed that there is a definite correlation between ADAS-cog and OSIT-J (Fig. 3a), and through a logistic regression model, we showed that the olfactory loss is significant risk for ADs (Table 2). We sorted patients based on their scores on the p-tau/Aβ_{42}, MMSE and ADAS-cog tests and then compared OSIT-J scores for the ADs and control groups. Surprisingly, on all of the AD assessments, we found that those with severe AD group had more serious olfactory dysfunction than those with mild AD (Fig. 3b–d). On ADAS-cog, the gold standard test for measuring cognitive disorder in ADs, there were significant differences between

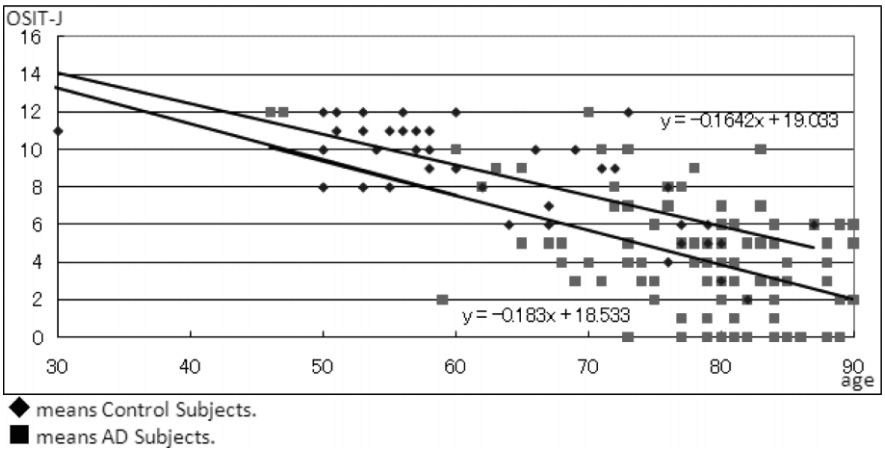


Figure 1 Relationship between age and the number of correct answers to the Odor Stick Identification Test for the Japanese.

mild ADs and the control group ($P < 0.05$) as well as between the severe ADs and the controls group ($P < 0.001$).

Hypoperfusion as a reflection of olfactory dysfunction

We used SPECT to locate hypoperfusion in the brain and then sorted subjects accordingly to compare the OSIT-J score of each group. No significant differences were found among ADs, but there was a difference between ADs and the control group (or non-definable hypoperfusion group). Differences were observed between the frontal lobe hypoperfusion group and the control group (Fig. 4a). We used the following smells to determine whether the hypoperfusion was an accurate reflection of olfactory dysfunction and found significant differences where indicated: India ink, wood, perfume, menthol, curry, gas for a cooker, rose, Japanese cypress, condensed milk, roasted garlic ($P < 0.001$), fermented beans/sweaty socks ($P < 0.01$) and Japanese orange ($P < 0.05$) (Fig. 4b,c).

Identification and specificity for checkups

Given that olfactory function varied between ADs and the control group, we investigated whether olfactory dysfunction could be used as a biomarker of AD.

In an estimated ROC, we found OSIT-J scores to be a specific marker for AD. At a cutoff of $P = 1.0$, sensitivity for identification of participants with AD was 0.75 and specificity was 0.77. At a cutoff of $P = 2.0$, sensitivity for identification of participants with AD was 0.95 and specificity was 0.30.

DISCUSSION

Although there have been many previous studies dealing with olfactory dysfunction in ADs, the tests used in these studies were not appropriate for Japanese subjects. Therefore, we employed OSIT-J to assess the ability of Japanese ADs to identify smells. Because it uses smells that are familiar to Japanese people, OSIT-J has better sensitivity for analyzing the olfactory function of Japanese ADs.

Generally, there is a marked decline in olfactory function as a result of the ageing process. Past studies have assumed there is a wholesale decline in olfactory function, and our results agree.[5] In our study, comparing age-matched ADs and a control group showed that ADs have more severe olfactory dysfunction than elderly people who do not have dementia. Additionally, we found that certain aromas are more likely to show low olfactory function in ADs, particularly India ink, rose, roasted garlic, Japanese cypress, and wood. These results are significant and rather unexpected. No significant decline in olfactory function was observed with other odorants. Given that ROC analysis showed the high sensitivity and specification of these odorants for ADs, these results are even more remarkable.

In contrast, previous studies have found that memory disorder and cognitive dysfunction affect olfactory dysfunction. In short, the development of olfactory disorders correlates closely with the progression of cognitive loss. In this study, we performed ADAS-cog, a more precise test than MMSE for evaluating cognitive function. The scores for ADAS-cog and OSIT-J are correlated. This result confirms the

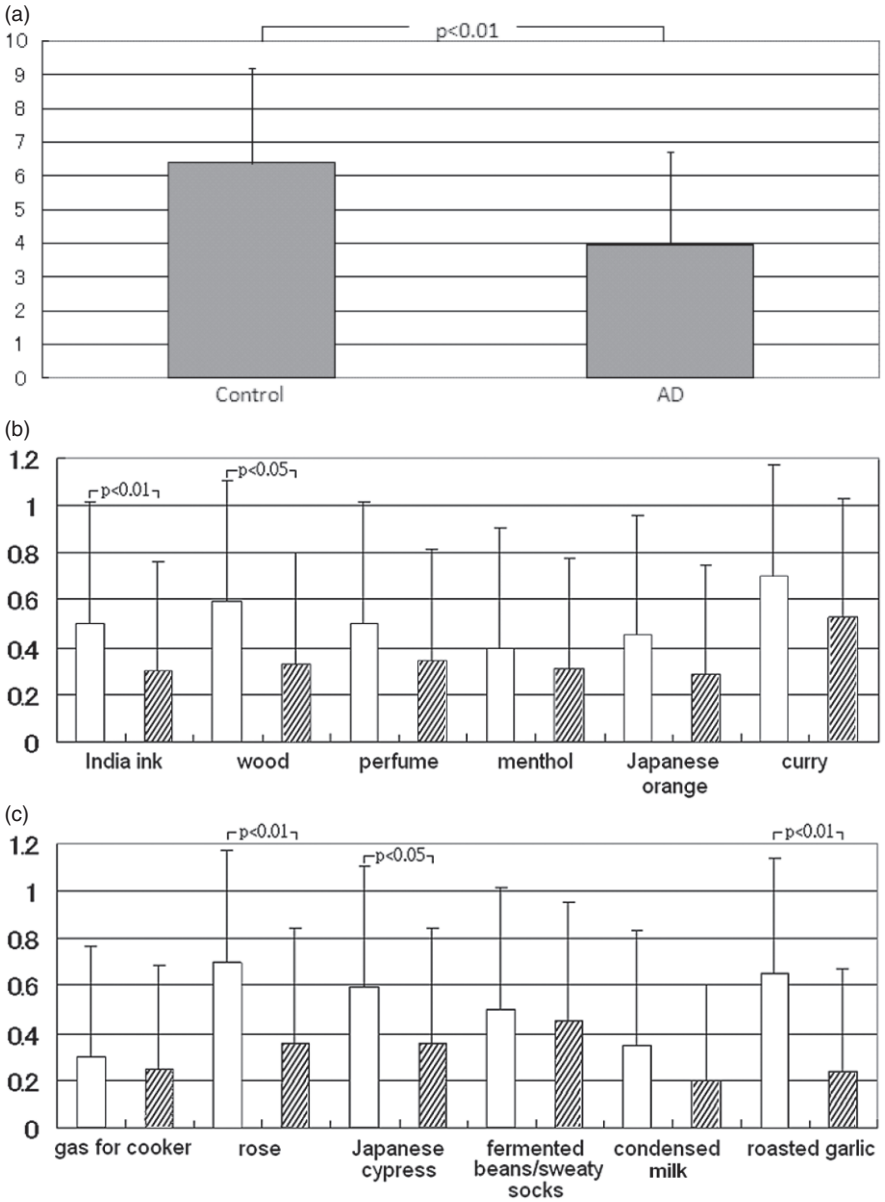
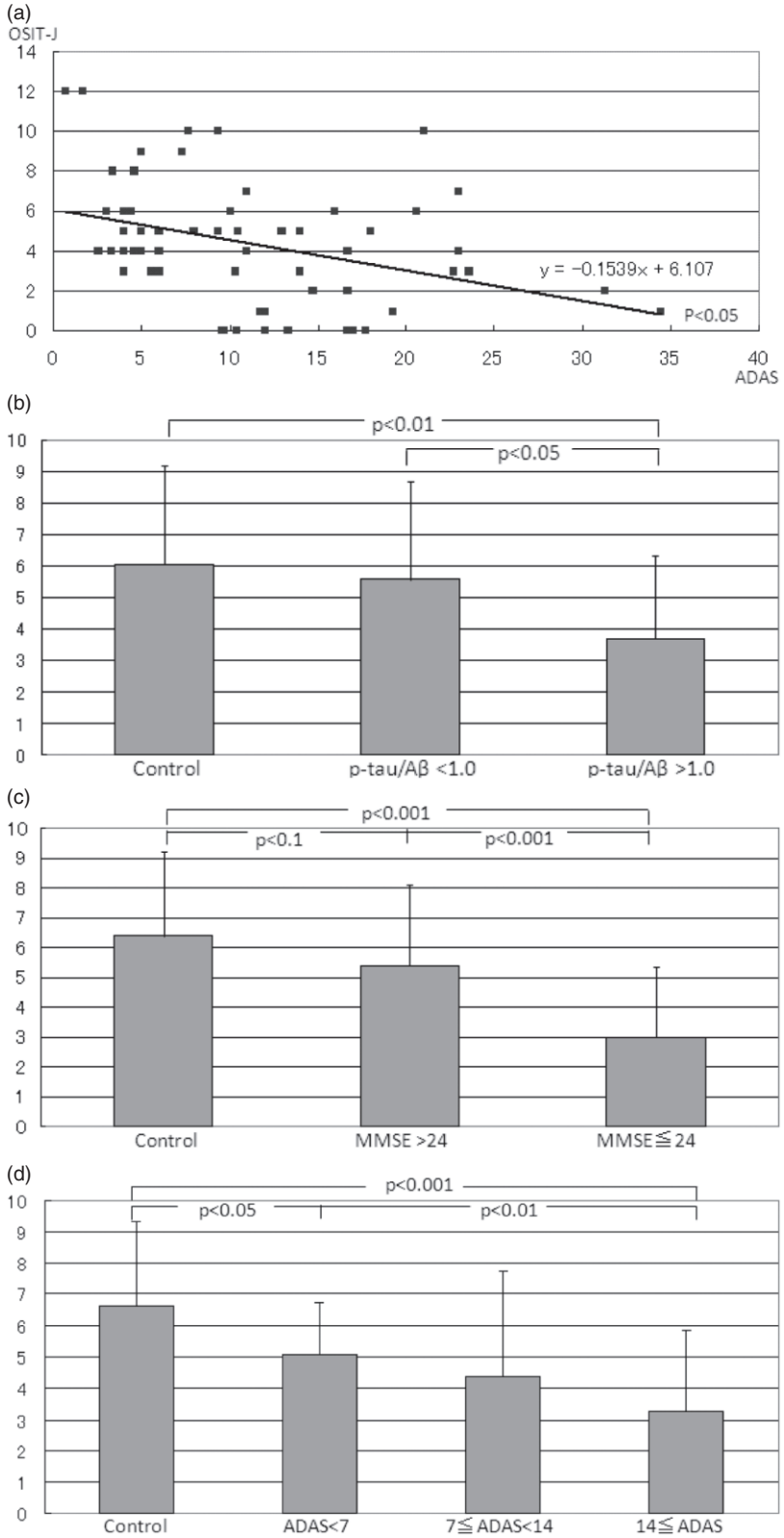


Figure 2 (a) There was a significant difference ($P < 0.01$) in total OSIT-J scores between the control group and ADs in age-matched subjects. (b) Answers for each odorant (India ink, wood, perfume, menthol, Japanese orange and curry) in control (white) and AD (striped) groups. (c) Answers for each odorant (gas for a cooker, rose, Japanese cypress, fermented beans/sweaty socks, condensed milk and roasted garlic) in control (white) and AD (striped) groups. AD, Alzheimer's disease; ADs, Alzheimer's disease patients; OSIT-J, Odor Stick Identification Test for the Japanese.

Figure 3 (a) Relationship between total ADAS-cog score and the number of correct answers to the Odor Stick Identification Test for the Japanese (OSIT-J) . There is a significant correlation between ADAS-cog and OSIT-J ($P < 0.05$, $r = -0.366$). (b) We sorted subjects based on p-tau/$A\beta_{42}$ scores and then compared each group – control ($n = 17$), p-tau/$A\beta_{42} < 1.0$ ($n = 32$), and p-tau/$A\beta_{42} \geq 1.0$ ($n = 14$) – based on OSIT-J scores. There was a significant difference between mild ADs patients and severe ADs ($P < 0.05$) and between severe ADs and the control group ($P < 0.01$). (c) We sorted based on MMSE scores and then compared each group – control ($n = 17$), MMSE ≥ 24 ($n = 39$), and MMSE ≤ 24 ($n = 59$) – based on OSIT-J scores. There was a significant difference between so mild ADs and severe ADs ($P < 0.001$) and between severe ADs and the control group ($P < 0.001$). (d) We sorted based on ADAS-cog scores and then compared each group – control ($n = 17$), ADAS-cog < 7 ($n = 21$), 7 $\leq$ ADAS-cog < 14 ($n = 25$), and 14 $\leq$ ADAS-cog ($n = 24$) – based on OSIT-J scores. There was a significant difference between mild ADs and the control group ($P < 0.05$), between mild ADs and severe ADs ($P < 0.01$), and between severe ADs and the control group ($P < 0.001$). ADAS-cog, Alzheimer's Disease Assessment Scale-cognitive subscale; ADs, Alzheimer's disease patients; MMSE, Mini-Mental State Examination; p-tau, phospho-tau.



results of past studies and reports. We sorted patients using MMSE and ADAS-cog scores to classify various stages of AD and then compared the OSIT-J score to each group. As a result, if a cognitive function disorder can be clearly noted, olfactory dysfunction can be noted, too. With ADAS-cog, a score of seven or more points generally indicates cognitive dysfunction and AD. In our study, mild ADs with a score less than seven had more significant olfactory dysfunction than those in the control group. Interestingly, the severe ADs with a score of 14 or more had severe olfactory loss, including 13 cases with anosmia.

A recent study suggested that the progression of the accumulation of neurofibrillary pathology in central olfactory regions occurs with dysosmia[18] Therefore, we sorted mild AD and other AD cases by biomarker p-tau/Aβ_{42} ratio, which reflects pathologic nerve changes, and compared the results with those of the control group. In this way we found that severe AD reflects severe olfactory dysfunction. Perhaps, the progression of AD and other nerve diseases often leads to olfactory disorders. These correlations between the levels of AD and dysosmia confirm previous research. Consequently, with these results, we can say that olfactory dysfunction sometimes reflects symptoms of AD.

Ohm and Braak said that cerebral atrophy in AD begins with atrophy of the rhinencephalon, or the olfactory brain.[19] However, the definition of rhinencephalon is low in diagnostic imaging such as SPECT, so it is impossible to examine for atrophy if the rhinencephalon cannot practically be measured. Therefore, we classified these patients into groups according to hypoperfusion location, as shown by SPECT, and then investigated whether the location related to the extent of olfactory loss. We were surprised to find there was no significant difference between the control group and ADs without hypoperfusion of the brain. In this way, we showed that patients without hypoperfusion of the brain may be free of olfactory dysfunction. Most past research has reported that olfactory dysfunction appears unconditionally in ADs, but in this study, we showed that dysosmia due to AD reflects an early encephalic pathologic change such as hypoperfusion of the brain or atrophy. However, there was no significant difference OSIT-J scores between the control group and ADs with hypoperfusion who developed dysosmia. The progress of dysosmia appears slow, particularly when the hypoperfusion is in the frontal or temporal lobe. Moreover, given the rhinencephalon's location near the frontal lobe, it is especially interesting that dysosmia in subjects with hypoperfusion near the frontal lobe tends to be worse. Incidentally, there were significant differences between ADs with hypoperfusion patients and the control group for all odorants tested. Some patients with early-stage dysosmia were unable to detect certain individual smells. Ultimately, all dysosmia patients olfactory abilities will continue to decline as hypoperfusion spreads.

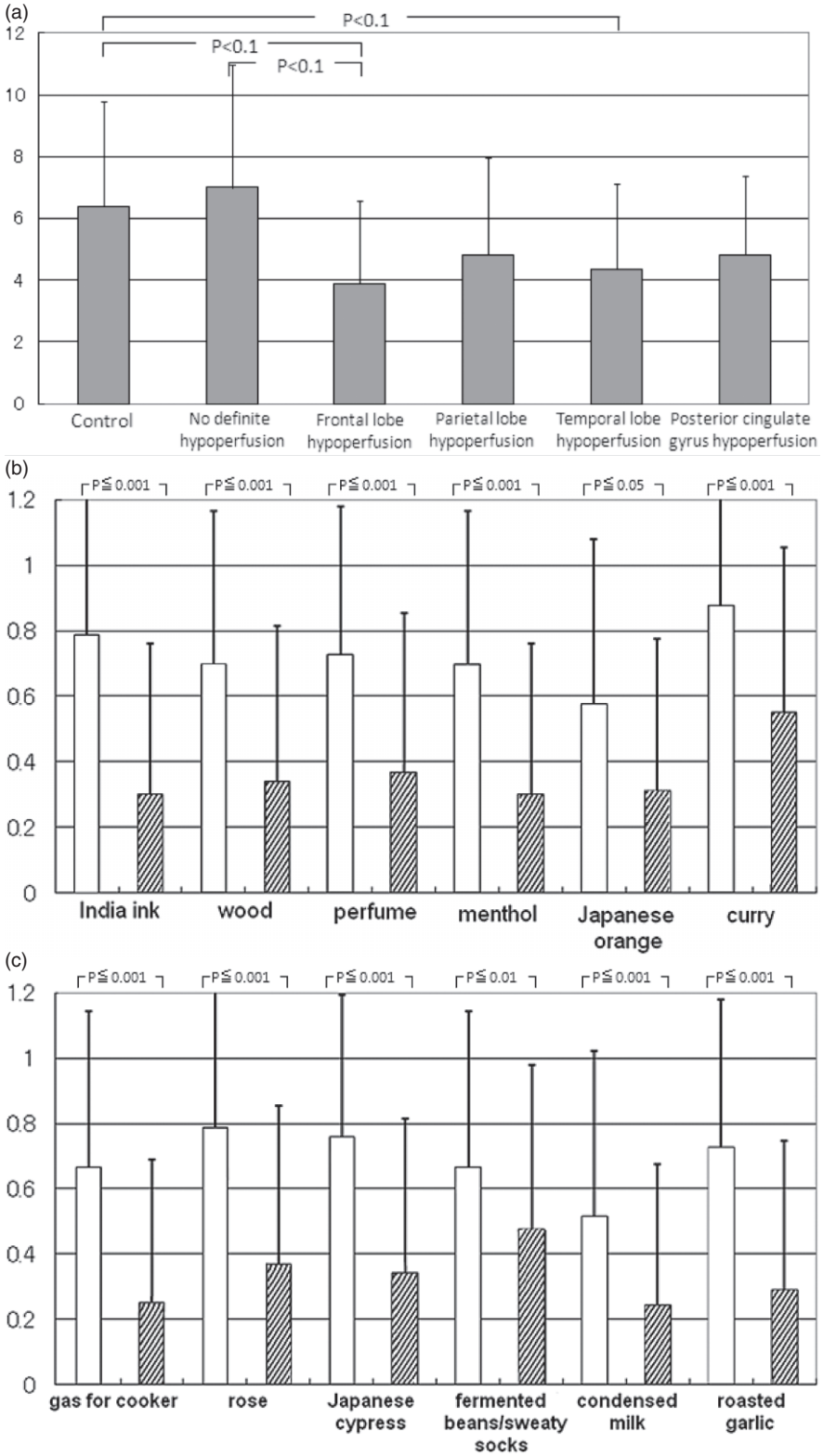
The significance of the results became clearer when comparing the control group with ADs who had hypoperfusion, under ROC analysis. At a cutoff of $P = 5.0$, sensitivity for ADs with hypoperfusion was 0.83 and specificity was 0.81 with the 10 OSIT-J odorants. We feel these scores are very promising for screening and diagnosis, as they show that patients with mild AD can have olfactory dysfunction or dysosmia and that decline in individual aroma identification is characteristic of the early stage of dysosmia. Moreover, we believe a new examination method could possibly be established for AD, including early or moderate cases, through a suitable

Table 2 OR (AD/control)

		95% CI	
	OR	Lower limit	Upper limit
Olfactory loss	4.5621	1.4548	14.3065
Age	1.1016	1.0459	1.1603

The olfactory loss group had an Odor Stick Identification Test for the Japanese score ≤ 6. ADs, Alzheimer's disease patients.

Figure 4 (a) We sorted subjects based on the appearance of hypoperfusion and then compared each group – no definite hypoperfusion ($n = 10$), frontal lobe hypoperfusion ($n = 10$), parietal lobe hypoperfusion ($n = 8$), temporal lobe hypoperfusion ($n = 12$), and posterior cingulated gyrus hypoperfusion ($n = 11$) – based on Odor Stick Identification Test for the Japanese (OSIT-J) scores. (b) Answers for each odorant (India ink, wood, perfume, menthol, Japanese orange and curry) in the control group (white) and subjects with hypoperfusion (striped). (c) Answers for each odorant (gas for a cooker, rose, Japanese cypress, fermented beans/sweaty socks, condensed milk and roasted garlic) in the control group (white) and subjects with hypoperfusion (striped).



inspection of olfactory function. Olfactometry is non-invasive and takes little time because it merely involves presenting some aromas to patients. The nature of the exam enables a wide range of health professionals, including family doctors, to use it as part of a screening battery for early AD.

At this time, OSIT-J is not suitable for clinical use. As it is not permissible to show test results to patients because of regulations protecting the marketers of the aromatic products, it can be problematic for patients who wish to see their results. It is essential that doctors be able to provide accurate and complete information, especially if the results indicate olfactory loss. Therefore, a new olfactory inspection system, one that is free of legal restrictions, must be developed for AD diagnosis.

In conclusion, this is the first study to report that Japanese ADs' ability to identify odour can be accurately estimated using the OSIT-J. Our report shows that olfactory dysfunction should be considered a marker for AD and that this type of examination can also help assess the level of AD, including cognitive dysfunction.

ACKNOWLEDGMENTS

The authors would like to thank Dr Senzou Kishida and Ms Akemi Aramatsu of Shinsei Hospital for their cooperation with this study and for advice in the writing this article, as well as Mr TL Wiltshire and Ms Nanae Itokazu for helping edit the English manuscript. I (DJ) also want to thank my family, especially Ms Yuko Jimbo, for her support in both my public and private life. Moreover, I am grateful to Dr Ryuichiro Hashimoto and Dr Seiji Shiota for their contributions to this manuscript.

REFERENCES

1 Chen JH, Lin KP, Chen YC. Risk factors for dementia. *J Formos Med Assoc* 2009; **108**: 754–764.
2 Hampel H, Frank R, Broich K *et al*. Biomarkers for Alzheimer's disease: academic, industry and regulatory perspectives. *Nat Rev Drug Discov* 2010; **9**: 560–574.
3 Doty RL. Olfactory capacities in aging and Alzheimer's disease. Psychophysical and anatomic considerations. *Ann N Y Acad Sci* 1981; **640**: 20–27.
4 Murphy C. Loss of olfactory function in dementing disease. *Physiol Behav* 1999; **66**: 177–182.
5 Eichenbaum HB, Otto TA, Wible CG *et al*. Building a model of the hippocampus in olfaction and memory. In: Davis JL, Eichenbaum HB, eds. *Olfaction: A Model System for Computational Neuroscience*. Cambridge, MA: The MIT Press, 1991; 167–210.
6 Doty RL, Kobal G. Current trends in the measurement of olfactory function. In: Doty RL ed. *Handbook of Olfaction and Gustation*. New York, NY: Marcel Dekker, 1995; 191–225.
7 Westervelt HJ, Carvalho J, Duff K. Presentation of Alzheimer's disease in patients with and without olfactory deficits. *Arch Clin Neuropsychol* 2007; **22**: 117–122.
8 Kovacs T. Mechanisms of olfactory dysfunction in aging and neurodegenerative disorders. *Ageing Res Rev* 2004; **3**: 215–232.
9 Mesholam RI, Moberg PJ, Mahr RN *et al*. Olfaction in neurodegenerative disease: a meta-analysis of olfactory functioning in Alzheimer's and Parkinson's diseases. *Arch Neurol* 1998; **55**: 84–90.
10 Gilbert PE, Murphy C. The effect of the ApoE e4 allele on recognition memory for olfactory and visual stimuli in patients with pathologically confirmed Alzheimer's disease, probable Alzheimer's disease, and healthy elderly controls. *J Clin Exp Neuropsychol* 2004; **26**: 779–794.
11 Kobal G, Hummel T, Sekinger T *et al*. 'Sniffin' sticks': screening of olfactory performance. *Rhinology* 1996; **34**: 222–226.
12 Kobayashi M. The Odor Stick Identification Test for the Japanese (OSIT-J): clinical suitability for patients suffering from olfactory disturbance. *Chem Senses* 2005; **30** (Suppl 1): i216–i217.
13 Kobayashi M, Imanishi Y, Ishikawa M *et al*. Influence of visual information and test paradigm on clinical olfactory test results. *Auris Nasus Larynx* 2008; **35**: 53–60.
14 Kobayashi M, Reiter ER, DiNardo LJ *et al*. A new clinical olfactory function test: cross-cultural influence. *Arch Otolaryngol Head Neck Surg* 2007; **133**: 331–336.
15 Tombaugh TN, McIntyre NJ. The mini-mental state examination: a comprehensive review. *J Am Geriatr Soc* 1992; **40**: 922–935.
16 Rosen WG, Mohs RC, Davis KL. A new rating scale for Alzheimer's disease. *Am J Psychiatry* 1984; **141**: 1356–1364.
17 Koslowsky IL, Brake SE, Bitner SJ. Evaluation of the stability of (99m)Tc-ECD and stabilized (99m)Tc-HMPAO stored in syringes. *J Nucl Med Technol* 2001; **29**: 197–200.
18 Wilson RS, Arnold SE, Schneider JA *et al*. The relationship between cerebral Alzheimer's disease pathology and odour identification in old age. *J Neurol Neurosurg Psychiatry* 2007; **78**: 30–35.
19 Ohm TG, Braak H. Olfactory bulb changes in Alzheimer's disease. *Acta Neuropathol* 1987; **73**: 365–369. 1991.