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Verification of the association between cognitive decline and olfactory dysfunction using a DEmentia screening kit in subjects with Alzheimer's dementia, mild cognitive impairment, and normal cognitive function (DESK study): A multicenter, open-label, interventional study

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ABSTRACT

Background and purpose: Olfactory dysfunction may be an early symptom of degenerative neurological disorders such as mild cognitive impairment (MCI), which may progress to cognitive decline and Alzheimer's disease (AD). We investigated the relationship between cognitive decline and olfactory dysfunction in healthy controls and patients with MCI or AD using the DEmentia Screening Kit (DESK), an olfactory identification assessment tool designed for Japanese populations.

Methods: In this multicenter, open-label, interventional study conducted from 16 September 2020 to 30 April 2021, participants underwent olfactory tests using the DESK tool. This included 10 odorants at two concentrations (weak/strong) including toothpaste, butter, and India ink.

Results: Among 223 participants, 100, 61, and 62 were healthy controls, MCI patients, and AD patients (mean ages, 57.4, 72.8, and 76.3 years; total DESK olfaction scores, 18.4, 14.7, and 7.4), respectively. Significant differences in total olfaction scores were observed between groups (healthy controls vs MCI, healthy controls vs AD, and MCI vs AD). Significant between-group total score differences were shown for olfaction scores with both the 10 strong and 10 weak odorant varieties.

Conclusion: The DESK tool may discriminate between healthy individuals and those with MCI or AD, facilitating early screening for cognitive decline among Japanese patients, although the effect of age on DESK olfaction scores has not been fully explored.

1. Introduction

Among the neurocognitive disorders affecting older adults [1], it is estimated that approximately 47 million individuals have dementia worldwide, and projections estimate these numbers will increase to over 115 million by the year 2050 [2]. Alzheimer's disease (AD) accounts for approximately 60% to 80% of dementia cases [3].

Dementia places significant health and economic burdens on the families of patients and on society. Although several pharmacological therapies can ameliorate AD symptoms and slow the progression of dementia [4–6], there is currently no treatment to reverse dementia, and it remains incurable.

The randomized controlled FINGER trial showed that among adults at high risk of developing dementia, the application of a 2-year preventative multidomain intervention (i.e., diet, exercise, cognitive training, and vascular risk monitoring) improved or maintained cognitive function in comparison with only receiving general health advice [7]. The Lancet Commission on dementia prevention, intervention, and

Abbreviation: AD, Alzheimer's disease; AUC, area under the curve; DESK, DEmentia Screening Kit; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, 5th Edition; FAS, full analysis set; ICD-10, International Classification of Diseases-10; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MoCA-J, Montreal Cognitive Assessment - Japanese version; NIA-AA, National Institute on Aging and Alzheimer's Association criteria; OI, olfactory identification; PPS, per-protocol analysis set; ROC, receiver operating characteristic.

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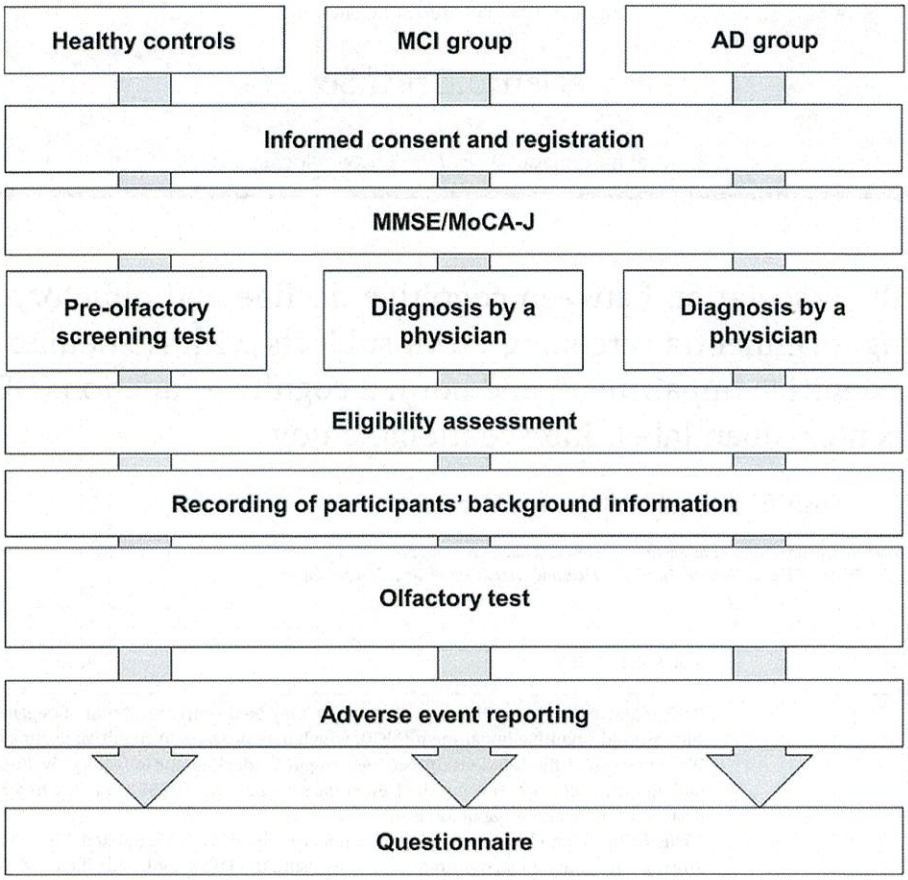

Fig. 1. Study flow diagram.
AD, Alzheimer's disease; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MoCA-J, Montreal Cognitive Assessment-Japan.

care reported that modifiable risk factors for dementia such as education in early life; hypertension, obesity, hearing impairment, traumatic brain injury, and alcohol misuse in midlife; and smoking, depression, physical inactivity, social isolation, diabetes, and air pollution in later life, can increase dementia risk [8].

Mild cognitive impairment (MCI) is a transitional state between normal aging and the development of dementia [9]. It has been estimated that between 4% and 10% of patients with MCI progress annually to AD [10]. Therefore, it is critical to identify markers for early detection of dementia symptoms and prediction of MCI or AD.

It is well known that olfactory dysfunction, MCI, and dementia are closely linked. Olfactory dysfunction has been observed to be one of the initial symptoms of AD [11,12]. Previous studies have reported olfactory dysfunction across various domains (detection, discrimination, and identification of odors) in patients with AD and that this precedes the onset of cognitive decline and is predictive of progression from normal cognitive functioning to MCI [13–18]. Of the affected olfactory domains, olfactory identification (OI) seems to be the predominant factor in AD and has been associated with rapid cognitive decline in this population [19]; thus, OI is proposed as a valuable marker of preclinical or early AD [20], and several studies have been conducted to verify this [21,22].

We hypothesized that while cognitive function cannot be evaluated by assessing OI, OI may help discriminate between normal cognitive decline, MCI, and AD. Previous studies have evaluated different olfaction screening and assessment tools for dementia, but a recent systematic review found that most tools were not designed for the examined population (those with dementia or AD) [20]. The aim of this study was to investigate the relationship between olfactory dysfunction and cognitive decline in healthy controls and patients with MCI or AD. This study used the DEmentia Screening Kit (DESK), an easy-to-apply olfaction assessment tool that was specifically designed for Japanese populations.

2. Material and methods

2.1. Study design

This study was a multicenter, open-label, interventional study that used the DESK assessment tool to evaluate the relationship between olfactory dysfunction and cognitive decline in healthy controls and patients with MCI or AD. The study was conducted at five facilities (one hospital and four clinics) in Japan from 16 September 2020 to 30 April 2021. The principal investigators at each facility were certified neurologists from the Japanese Society of Neurology who provide medical care to patients with dementia. The study was registered at the University hospital Medical Information Network (https://www.umin.ac.jp/) under the identifier number UMIN000041794.

The study protocol was approved by the Institutional Review Board of Takahashi Clinic prior to study conduct. The study sponsor, Kobayashi Pharmaceutical, contributed to the development of the study's protocol, formulation of its analysis plan, monitoring, interpretation of results, and data publication, but was not involved in data management or the conduct of statistical analyses.

The study was conducted in compliance with the ethical principles originating in the Declaration of Helsinki and Ethical Guidelines for Medical and Health Research Involving Human Subjects and was approved by the ethical review committees of each participating study site. All patients or their legal representatives (i.e., one family member, such as a parent, spouse, or a patient's child, who could represent the patient's intentions and prioritize their best interests) provided written

informed consent and subsequently registered themselves using the electronic data capture software.

2.2. Patients

The study recruited men and women aged ≥40 years and < 90 years at the time of providing informed consent, who either were healthy controls, or had MCI or AD. Healthy controls were defined as those with a Mini-Mental State Examination (MMSE) [23] score of ≥28 points. Patients with MCI were defined as those with an MMSE score of ≥24 to ≤27 points, who had previously been diagnosed with MCI and had memory impairment as per assessment by a physician specialized in dementia and based on the diagnostic criteria by Petersen et al. [24,25]. An AD diagnosis was confirmed if patients had an MMSE score of ≤23 points and had been assessed by a physician specialized in dementia using either the International Classification of Diseases-10 (ICD-10), The Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) or the National Institute on Aging and Alzheimer's Association criteria (NIA-AA).

Patients who met the criteria for AD and who had also been determined by a physician to be able to express subjective symptoms and olfaction scores appropriately were included in the study. In addition, only participants who could provide consent either firsthand or from a legally authorized representative in writing (for AD patients only) were included.

Participants were excluded from this study if they had an allergic reaction to odorants in the past; were currently pregnant or planning pregnancy; had been diagnosed with an olfactory deficit of a clear cause; had complications of epilepsy or a history thereof; had complications of depression or a history thereof; had undertaken the MMSE or Montreal Cognitive Assessment - Japanese version (MoCA-J [26]) within 1 month or less of providing informed consent; had an altered olfactory function on the day of their olfaction tests, such as those who smoked, had reduced olfactory function due to pollen allergies, rhinitis, or chronic paranasal sinusitis, had used perfumes, or consumed foods with a strong smell from the day prior to the day of their olfaction tests; were bedridden or determined to have severe AD with impaired consciousness; were current smokers; and were otherwise determined to be inappropriate for study participation by the investigator. Patient eligibility was comprehensively judged by the investigator, but there were no specific restrictions on the use of medications taken to improve cognitive function, for ethical reasons.

2.3. Procedures

The flow of study procedures is shown in Fig. 1. At enrollment, the investigator assessed the cognitive status of each participant (using the MMSE [23] or MoCA-J [26]) and an olfactory screening test was performed for healthy individuals. A physician diagnosed patients with MCI or AD based on ICD-10, DSM-5, or NIA-AA, to establish cognitive status at baseline. Background information, including demographics, medical history, comorbidities, medication use, supplement intake, and information on other interventions for dementia prevention, were collected at baseline. Healthy controls underwent a pre-olfactory test to identify olfactory blindness. Of the five sheets of paper, two with one reference odorant and three with one control odorant, healthy controls who could distinguish between the two reference odorants were judged not to be olfactory blind. The test was repeated for each of the five odorant variations as a reference odorant, and when even one of the odorants could not be discriminated, the subject was considered ineligible to participate in the study.

Healthy controls, patients with MCI, and patients with AD had their olfactory function tested by an investigator using the DESK tool developed by Kobayashi Pharmaceutical Co., Ltd. (Osaka, Japan).

2.3.1. Olfactory testing

Participants underwent olfactory tests using the DESK tool, which included 10 odorant varieties in two concentrations (weak/strong) for a total of 20 investigational substances. The 10 odorant variations were randomly divided into groups of five prior to the test. The principal investigator and sub-investigators selected one sample from each of the five different sets and then the five different odorant sets were reordered during each test. For smelling, paper cups were utilized and the two different concentrations were tested separately, with a rest period of 5 min in between each test. After smelling each odorant, the participants were asked to respond whether they could identify the odor and were provided six alternative answers to choose from (four types of scents, none of the scents among the four provided, and "I don't know"). Each correctly identified odorant was allocated 1 point, and the total number of correct answers provided the total olfaction score. The tool is used to check if the odorant are clearly identifiable.

The 10 different odorant varieties included orange, toothpaste, butter, India ink, apples, soap, coffee, *hinoki* (cypress wood), vanilla ice cream, and sweaty socks (an odorant that represents the common condition of bromodosis [foot odor]). These 10 odorants were chosen because they are odorants that the Japanese population are familiar with and frequently encounter in their daily life, they are likely to elicit similar responses in all participants, they had a highly stable composition (odorant stability tests were performed), they are not ethically inappropriate odorants (e.g., smells of decay, excreta, or stimulants), and the safety of these odorants was assured as they met the International Fragrance Association, category 11.

Data on adverse events that occurred during the olfactory test were collected from the start of the test to the end of the questionnaire. Following informed consent and prior to the olfactory test, the investigator administered questionnaires on the MMSE or MoCA-J to healthy controls, patients with MCI, and patients with AD. In addition, the questionnaires were completed by the study subjects who discontinued the study due to the occurrence of adverse events or for other reasons.

2.4. Study outcomes

The primary study outcomes were olfaction scores by cognitive status in healthy controls, patients with MCI, and patients with AD; associations between olfaction scores and cognitive assessment measures; and distinguishability of cognitive status (healthy controls, patients with MCI, and patients with AD) according to olfaction scores.

Secondary outcomes were the diagnostic performance of cognitive status in healthy controls, patients with MCI, and patients with AD, according to olfaction scores, and subgroup analysis by medical history and presence of complications.

2.5. Statistical analysis

The sample size was set based on the number of participants needed for the statistical analysis and the feasibility of recruitment at 100 healthy participants (58 men and women aged 40–59 years and 42 men and women aged 60–89 years), 61 patients with MCI (nine men and women aged 40–59 years and 52 men and women aged 60–89 years), and 62 patients with AD (three men and women aged 40–59 years and 59 men and women aged 60–89 years). The full analysis set (FAS) was defined as participants who enrolled in this study and completed the olfactory test. The per-protocol analysis set (PPS) was defined as those who were adjudicated as compliant with the protocol without protocol violations. Participants with significant protocol violations after enrollment or who were found to be ineligible after enrollment were excluded.

For analysis of the primary endpoint (olfaction scores by cognitive level), summary statistics were calculated by odorant (the 10 odorant varieties chosen) and by concentration (weak/strong). The Kruskal–Wallis test as used to compare olfaction scores of healthy controls

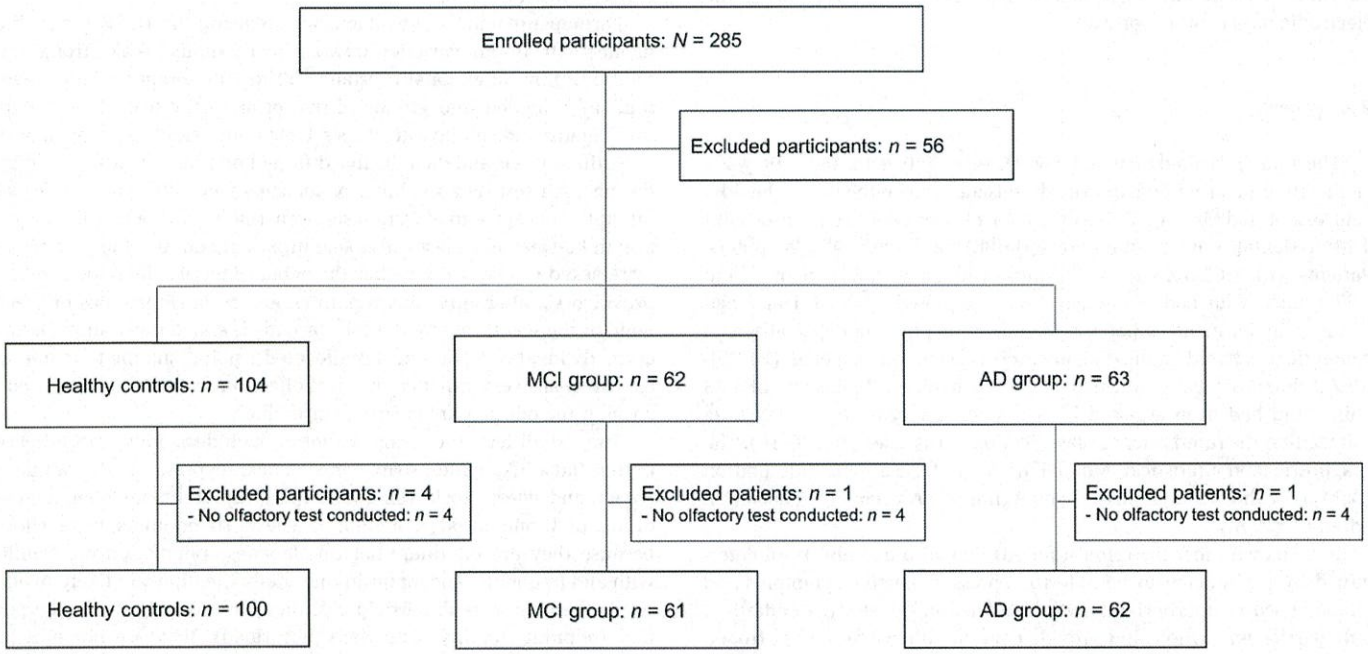

Fig. 2. Patient disposition.
AD, Alzheimer's disease; MCI, mild cognitive impairment.

with those of patients with MCI or AD (the Steel–Dwass procedure was used for multiple comparisons). Correlation coefficients (Spearman's rank-order correlations) for olfaction scores and cognitive assessment function (MMSE/MoCA-J scores) were calculated, and odorants/concentrations that corresponded with cognitive assessment function indices were chosen based on a correlation coefficient of ≥0.7.

Olfaction scores were presented by odorant (the 10 odorant varieties chosen) and concentration (weak + strong, weak/strong). For discriminating cognitive levels, receiver operating characteristic (ROC) analyses by cognitive status were then conducted for olfaction scores, and sensitivity, specificity, and area under the curve (AUC) were calculated.

For secondary outcome measures, discrimination analyses were performed using cognitive level as the objective variable and olfaction score as the explanatory variable, and the diagnostic utility of disease levels per olfaction scores (for our odorant/concentration combinations) was examined by ROC analyses. Additionally, the number of odorants (e.g., three varieties, five varieties) used for the olfaction scores was explorative changed.

For subgroup analyses, olfaction score differences were investigated by disease level as according to MMSE scores at study enrollment. Stratification by sex and age (≤65 years, >65 years) were also performed in this analysis. Multiple regression analysis was conducted to determine whether age or MMSE and MoCA-J scores had a greater influence on the olfaction score after MMSE score, MoCA-J score, age, and olfaction score were generated by standardization. For standardization, the mean was set to 0 and the standard deviation to 1. Data management and statistical analyses were performed by Mebix Inc. (Tokyo, Japan). *P*-values <0.05 were considered significant, and all tests were two-sided. Statistical analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, NC, USA).

3. Results

3.1. Patients

Of the 223 participants registered, 100 healthy controls, 61 patients with MCI, and 62 patients with AD were enrolled (Fig. 2). At baseline, the mean age of the healthy controls was lower than that of patients with MCI and AD (Table 1). The mean age of patients with MCI and AD was comparable. The proportions of patients with comorbidities and taking medication was comparable between patients with MCI and AD, but higher in these groups compared with that in healthy controls.

Table 1
Baseline patient demographic and clinical characteristics.

	Healthy controls (n = 100)	MCI group (n = 61)	AD group (n = 62)
Male, n (%)	33 (33.0)	18 (29.5)	19 (30.6)
Age (years), mean ± SD	57.4 ± 11.7	72.8 ± 10.6	76.3 ± 8.8
Age (years), median [IQR]	55 [47.5, 66.5]	76 [68.0, 80.0]	77 [73.0, 84.0]
Age (years), n (%)			
40–49, n (%)	31 (31.0)	2 (3.3)	1 (1.6)
50–59, n (%)	27 (27.0)	7 (11.5)	2 (3.2)
60–69, n (%)	26 (26.0)	8 (13.1)	9 (14.5)
70–79, n (%)	13 (13.0)	28 (45.9)	25 (40.3)
80–89, n (%)	3 (3.0)	16 (26.2)	25 (40.3)
Medical history, n (%)	6 (6.0)	4 (6.6)	6 (9.7)
Have comorbidity, n (%)	43 (43.0)	53 (86.9)	55 (88.7)
Use of medication, n (%)			
Yes	41 (41.0)	52 (85.2)	55 (88.7)
No	59 (59.0)	8 (13.1)	7 (11.3)
Unknown	0 (0.0)	1 (1.6)	0 (0.0)
Taking supplements, n (%)			
Yes	6 (6.0)	2 (3.3)	4 (6.5)
No	94 (94.0)	58 (95.1)	57 (91.9)
Unknown	0 (0.0)	1 (1.6)	1 (1.6)
Other intervention related to the prevention for dementia, n (%)			
Yes	0 (0.0)	1 (1.6)	1 (1.6)
No	100 (100.0)	59 (96.7)	61 (98.4)
Unknown	0 (0.0)	1 (1.6)	0 (0.0)
MMSE total score, mean ± SD	29.7 ± 0.6	25.7 ± 1.1	17.5 ± 4.6
MoCA-J total score	27.2 ± 2.6	21.3 ± 3.9	11.3 ± 5.5

AD, Alzheimer's disease; IQR, interquartile range; MCI, mild cognitive impairment; MMSE, Mini-Mental State Examination; MoCA-J, Montreal Cognitive Assessment-Japan, SD; standard deviation.

Table 2
Olfaction scores in each group.

Olfaction score[a]	Olfaction score, mean ± SD			P value		
	[A] Healthy controls (n = 100)	[B] MCI group (n = 61)	[C] AD group (n = 62)	[A] vs. [B]	[A] vs. [C]	[B] vs. [C]
Total (Strong + weak)[b]	18.4 ± 1.6	14.7 ± 4.5	7.4 ± 5.0	<0.0001	<0.0001	<0.0001
Strong	9.5 ± 0.8	7.8 ± 2.2	3.9 ± 2.7	<0.0001	<0.0001	<0.0001
(No. of correct responses, n [%])						
Orange	98 [98.0]	52 [85.2]	33 [53.2]	0.0029	<0.0001	0.0002
Toothpaste	98 [98.0]	43 [70.5]	20 [32.3]	<0.0001	<0.0001	<0.0001
Butter	79 [79.0]	30 [49.2]	13 [21.0]	0.0001	<0.0001	0.0013
India ink	91 [91.0]	39 [63.9]	10 [16.1]	<0.0001	<0.0001	<0.0001
Apple	97 [97.0]	48 [78.7]	30 [48.4]	0.0003	<0.0001	0.0007
Soap	99 [99.0]	52 [85.2]	32 [51.6]	0.0008	<0.0001	<0.0001
Coffee	98 [98.0]	53 [86.9]	27 [43.5]	0.0068	<0.0001	<0.0001
Hinoki	94 [94.0]	48 [78.7]	24 [38.7]	0.0051	<0.0001	<0.0001
Vanilla ice cream	99 [99.0]	54 [88.5]	31 [50.0]	0.0050	<0.0001	<0.0001
Sweaty socks	93 [93.0]	54 [88.5]	21 [33.9]	0.3913	<0.0001	<0.0001
Weak	8.9 ± 1.0	6.9 ± 2.7	3.5 ± 2.6	<0.0001	<0.0001	<0.0001
(No. of correct responses, n [%])						
Orange	99 [99.0]	48 [78.7]	36 [58.1]	<0.0001	<0.0001	0.0197
Toothpaste	93 [93.0]	40 [65.6]	21 [33.9]	<0.0001	<0.0001	0.0006
Butter	72 [72.0]	23 [37.7]	9 [14.5]	<0.0001	<0.0001	0.0040
India ink	82 [82.0]	28 [45.9]	11 [17.7]	<0.0001	<0.0001	0.0010
Apple	90 [90.0]	47 [77.0]	29 [46.8]	0.0385	<0.0001	0.0008
Soap	96 [96.0]	50 [82.0]	23 [37.1]	0.0045	<0.0001	<0.0001
Coffee	92 [92.0]	42 [68.9]	15 [24.2]	0.0003	<0.0001	<0.0001
Hinoki	77 [77.0]	43 [70.5]	25 [40.3]	0.3592	<0.0001	0.0011
Vanilla ice cream	99 [99.0]	51 [83.6]	31 [50.0]	0.0003	<0.0001	0.0001
Sweaty socks	91 [91.0]	49 [80.3]	18 [29.0]	<0.0001	<0.0001	0.0197

AD, Alzheimer's disease; MCI, mild cognitive impairment; SD, standard deviation.
[a] The olfaction score was based on the number of correct answers for a total of 20 odorant tests (10 odorants each with a strong and weak concentration).
[b] 10 different odorants, each with a strong and weak concentration.

Table 3
Distribution of olfaction scores in each group.

		Olfaction scores, n (%)										
		0	1	2	3	4	5	6	7	8	9	10
Healthy controls	Strong	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.0)	2 (2.0)	3 (3.0)	34 (34.0)	59 (59.0)
(n = 100)	Weak	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (2.0)	8 (8.0)	23 (23.0)	31 (31.0)	36 (36.0)
MCI group	Strong	1 (1.6)	0 (0.0)	0 (0.0)	2 (3.3)	3 (4.9)	4 (6.6)	3 (4.9)	7 (11.5)	15 (24.6)	12 (19.7)	14 (23.0)
(n = 61)	Weak	0 (0.0)	5 (8.2)	2 (3.3)	1 (1.6)	3 (4.9)	4 (6.6)	6 (9.8)	6 (9.8)	14 (23.0)	13 (21.3)	7 (11.5)
AD group	Strong	7 (11.3)	8 (12.9)	7 (11.3)	7 (11.3)	9 (14.5)	6 (9.7)	7 (11.3)	4 (6.5)	1 (1.6)	6 (9.7)	0 (0.0)
(n = 62)	Weak	8 (12.9)	10 (16.1)	4 (6.5)	9 (14.5)	14 (22.6)	5 (8.1)	3 (4.8)	1 (1.6)	6 (9.7)	1 (1.6)	1 (1.6)

AD, Alzheimer's disease; MCI, mild cognitive impairment.

3.2. Study outcomes

3.2.1. Olfaction scores

Mean ± standard deviation total olfaction scores in the healthy control, MCI, and AD groups were 18.4 ± 1.6, 14.7 ± 4.5, and 7.4 ± 5.0, respectively (Table 2). Statistically significant differences were observed for all between-group comparisons, healthy control vs MCI groups, healthy control vs AD groups, and MCI vs AD groups. Significant between-group total score differences were also similarly shown for olfaction scores, with both the 10 strong and 10 weak odorant varieties. Among healthy controls, the lowest olfaction score was 6, but in MCI and AD groups, the scores were widely distributed (Table 3).

The number of correct answers was the highest among healthy controls for the 10 odorants at both weak and strong concentrations, while the number of correct answers was lower for all odorants in the MCI group and was the lowest for all odorants in the AD group (Table 2, Fig. 3a and b). Significant differences were shown for all between-group comparisons, except for the strong odor of sweaty socks and the weak odor of *hinoki* in the healthy control group vs MCI group (Table 2).

3.2.2. Correlations between olfaction scores and the MMSE and MoCA-J

Correlation coefficients for the total olfaction score from the MMSE and MoCA-J were 0.74 and 0.73, respectively (Table 4), indicating a strong positive correlation. In the strong odorant concentration group, toothpaste, butter, and India ink were moderately associated with the MMSE/MoCA-J scores whereas in the weak odorant concentration group, India ink, toothpaste, and coffee were moderately associated with the MMSE/MoCA-J scores. No significant differences were noted in the olfaction scores of healthy controls by sex or age. Statistically significant differences were noted in the olfaction scores of patients with MCI and those with AD aged ≤65 years and > 65 years, with significantly lower scores among older patients in both groups (Table 5). Based on the results of multiple regression analysis, the MMSE score had a greater influence than age on the olfaction score as the absolute value of the parameter was higher for the MMSE score than age (regression coefficient [P value]; 0.60 [$P < 0.001$] and − 0.26 [$P < 0.001$], respectively) (data not shown). Similarly, MOCA-J had a greater influence than age (regression coefficient [P value]; 0.61 [$P < 0.001$] and − 0.19 [$P = 0.001$], respectively) (data not shown). Both MMSE and MoCA-J scores were found to be associated with the olfaction score (correlation coefficient [P value]; 0.73 [$P < 0.001$] and 0.72 [$P < 0.001$], respectively)

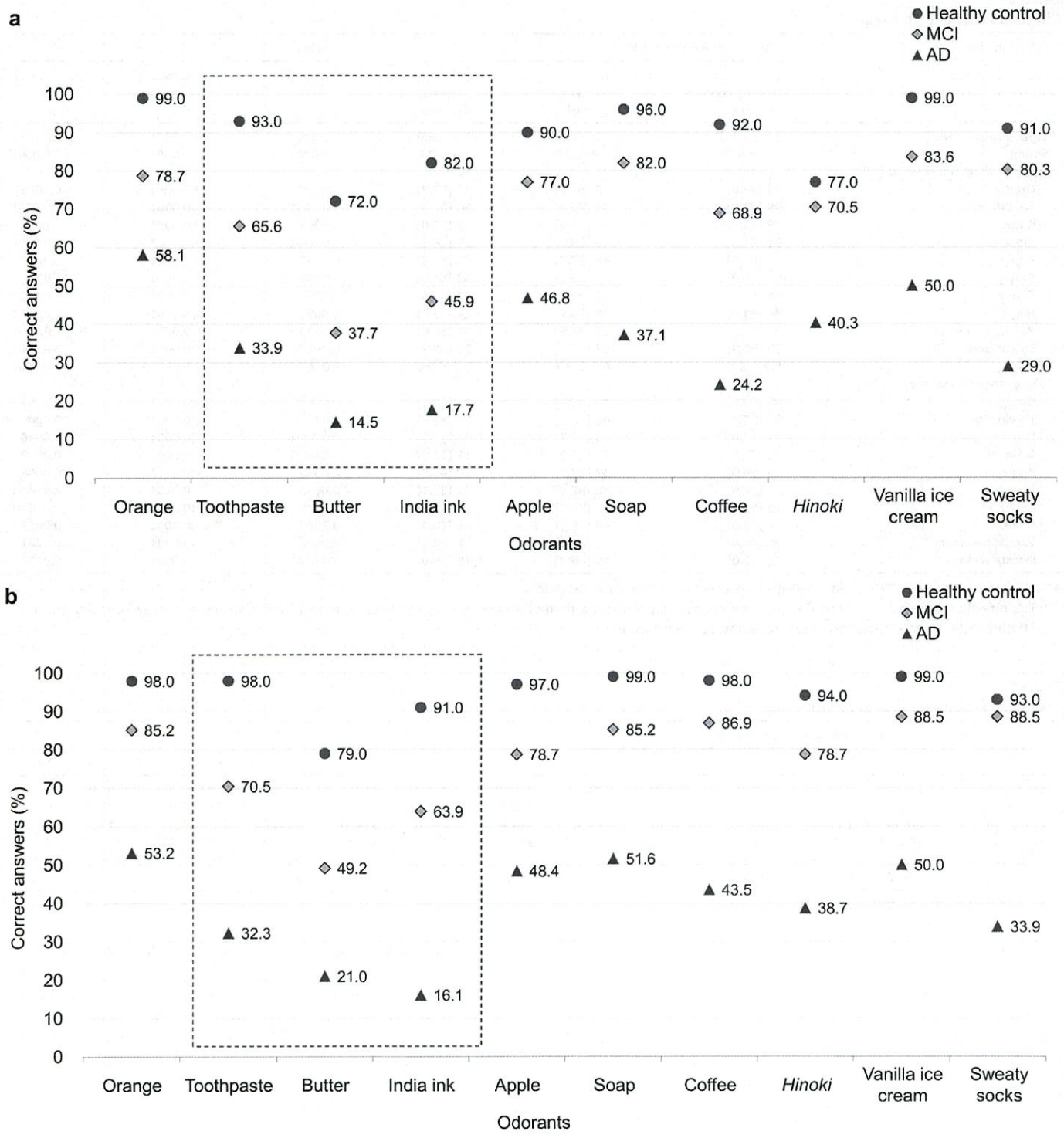

Fig. 3. Correct answer percentage for (a) each strong odorant and (b) each weak odorant.
AD, Alzheimer's disease; MCI, mild cognitive impairment.

(data not shown).

3.2.3. Investigating the diagnostic utility of olfaction scores with cognitive status (healthy controls and MCI patients)

When using a ROC analysis on the discrimination ability for the 10 odorants (both weak and strong) in healthy controls and MCI patients, we found that the discrimination ability was highest with strong odorant combinations of toothpaste, butter, and India ink. AUC increases were limited when more concentrated varieties were added on (AUC with just the three varieties = 0.766 [Fig. 4a], AUC with one more variety added = 0.771 [apple], 0.777 [soap], 0.767 [coffee], 0.767 [*hinoki*], 0.766 [vanilla ice cream], 0.754 [sweaty socks], and 0.766 [orange]) (data not shown).

3.2.4. Validating the diagnostic utility of the three odorant varieties with high discrimination ability

Toothpaste, butter, and India ink were selected for comparing the diagnostic utility between the healthy control and MCI groups, the

Table 4
Correlation coefficients between total olfaction scores and MMSE/MoCA-J.

		MMSE	MoCA-J
Total (Strong + weak)[a]		0.74	0.73
Strong	Orange	0.47	0.45
	Toothpaste	0.58	0.53
	Butter	0.51	0.48
	India ink	0.62	0.59
	Apple	0.49	0.46
	Soap	0.45	0.43
	Coffee	0.52	0.48
	Hinoki	0.50	0.49
	Vanilla ice cream	0.50	0.49
	Sweaty socks	0.52	0.53
Weak	Orange	0.44	0.42
	Toothpaste	0.52	0.57
	Butter	0.44	0.45
	India ink	0.55	0.48
	Apple	0.40	0.39
	Soap	0.51	0.46
	Coffee	0.58	0.55
	Hinoki	0.31	0.32
	Vanilla ice cream	0.49	0.51
	Sweaty socks	0.52	0.52

MMSE, Mini-Mental State Examination; MoCA-J, Montreal Cognitive Assessment-Japan.
[a] 10 different odorants, each with a strong and weak concentration.

healthy control and AD groups, and MCI and AD groups (Fig. 3a and b). Healthy controls had the highest percentages of correct answers in both the weak (93%, 72%, and 82% for toothpaste, butter, and India ink) and strong odorant concentrations (98%, 79%, and 91%, respectively). The percentages of correct answers decreased with the severity of cognitive decline, with higher percentages for MCI (weak: 65.6%, 37.7%, and 45.9%; strong: 70.5%, 49.2%, and 63.9%, respectively) than AD (weak: 33.9%, 14.5%, 17.7%; strong: 32.3%, 21.0%, and 16.1%, respectively) reported. The AUC was 0.953, 0.766, and 0.810 for healthy control vs AD groups, healthy control vs MCI groups, and MCI vs AD groups, respectively (Fig. 4b and c).

3.2.5. *Adverse events*

No adverse events were reported during this study.

4. Discussion

In this study we used the DESK tool for OI assessment to evaluate the cognitive status of three groups of patients and evaluated the diagnostic utility of the tool to discriminate between healthy controls, patients with MCI, and those with AD. Based on our findings, we consider the DESK tool to differentiate between the three cognitive function statuses reliably.

At baseline, patients with MCI and AD were older than healthy controls, with greater proportions of patients aged 70–89 years and 80–89 years. There were also greater proportions of patients in these two groups who reported the presence of comorbidities as well as lower MMSE and MoCA-J total scores.

Correct answer rates declined along with declining cognitive function for all 10 odorant varieties. We found that three odorant varieties (toothpaste, butter, and India ink) were especially useful in discriminating MCI from normal cognition and AD. Correlation coefficient scores for the 10 odorant varieties with the MMSE/MoCA-J scores were high.

Our results showed that cognitive function, as determined by the MMSE and MoCA-J scores, had a greater influence on the olfactory score than age. Therefore, the olfactory score may be considered a useful tool to predict cognitive decline.

In terms of the diagnostic utility of the DESK assessment tool used in this study, we found a high diagnostic utility with all combinations. Previous studies have demonstrated associations between cognitive function decline and olfactory function, including in Japanese and Asian populations, and indicate that impairment in olfactory discrimination can predict future cognitive decline [20,27,28]. These results suggest that the greater a patient's decline in cognitive function, the higher the possibility of reduced olfactory function occurring due to cerebral limbic system or olfactory nerve neuropathy [29,30].

Based on these results, it is possible that in some cases, the decline in olfactory function may occur either simultaneously or earlier than cognitive function decline, which is also supported by previous findings [31]. As such, while impaired olfaction is not an indicator of cognitive function per se, it may be useful as an early screening tool to identify patients with MCI or AD. Possible reasons are amyloid-β or tau protein deposition in the parahippocampal gyrus or entorhinal cortex regions, as reported previously [29,30,32–35]. In addition, dysfunction in the peripheral areas of the brain may lead to both cognitive dysfunction and olfactory dysfunction. Furthermore, even though damage to the cerebral limbic system and olfactory nerve does not immediately cause cognitive dysfunction, olfactory function is considered to become impaired first as the pathology gradually progresses. It has been reported that cognitive function starts to decline several years after the start of olfactory function impairment [36,37], hence, olfactory dysfunction may be a precursor of dementia. Therefore, identifying olfactory dysfunction early may be the first step in preventing dementia. There are reports of early detection of olfactory dysfunction and improved cognitive function with aromatherapy [38,39], suggesting that cognitive dysfunction associated with olfactory dysfunction can be addressed with treatments that approach the damaged olfactory nerve. Thus, these findings strongly suggest that there is a close relationship between olfactory function and dementia.

We surmise that once a decline in cognitive function is triggered by neuropathy, there is some interplay between areas that recognize specific odorants and the pathology because of the differences in identification ability we saw with each odorant variety. A recent study using a 3-month-old 5xFAD mouse model of AD showed that hyporeactivity to some odorants was associated with physiologically and structurally damaged areas in the peripheral olfactory system due to partial and asymmetrical accumulation of amyloid-β [40]. While there were no meaningful differences in the mean age between patients with MCI and patients with AD, there were large differences in their olfaction scores. As such, the decline in olfactory function is possibly linked to the decline in cognitive function rather than age. The main strength of this study is that DESK was specifically designed for Japanese participants as it incorporates odorants that are familiar to Japanese people and as such, should elicit similar responses across all participants.

Table 5
Sub-group analyses of olfaction scores in each group.

	Healthy controls			Mild cognitive impairment group			Alzheimer's disease group		
	n	Olfaction score[a]	*P* value	*n*	Olfaction score[a]	*P* value	*n*	Olfaction score[a]	*P* value
Male	33	18.5	0.7082	18	13.7	0.2712	19	6.8	0.5631
Female	67	18.3		43	15.1		43	7.7	
Age ≤ 65 years	74	18.5	0.0925	14	17.2	0.0143	10	11.7	0.0024
Age > 65 years	26	17.9		47	13.9		52	6.6	

[a] The olfaction score was based on the number of correct answers for a total of 20 odorant tests (10 odorants each with a strong and weak concentration).

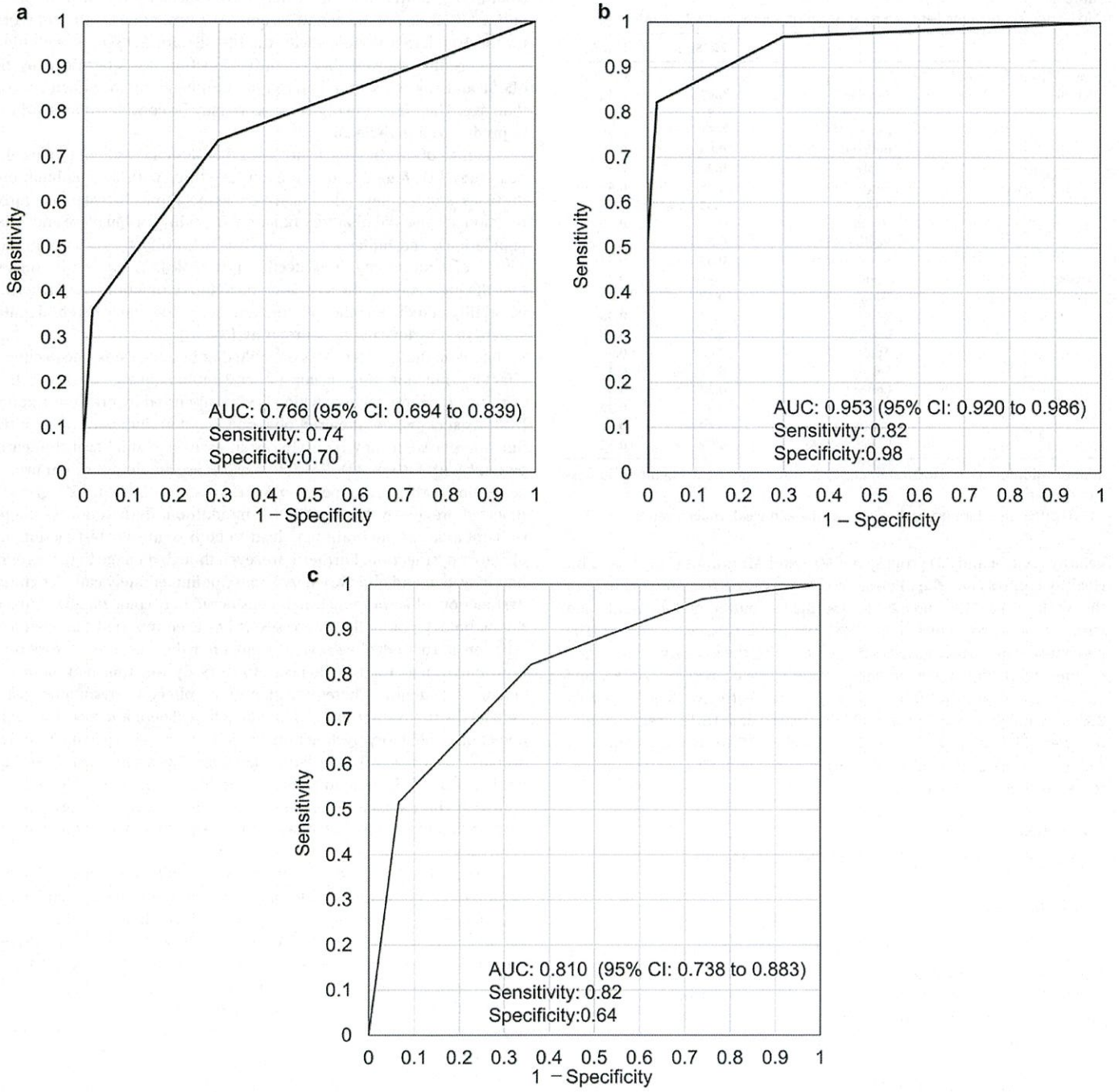

Fig. 4. ROC curves of (a) healthy controls versus the MCI group, (b) healthy controls versus the AD group, and (c) the MCI group versus the AD group. AD, Alzheimer's disease; AUC, area under the curve; CI, confidence interval; MCI, mild cognitive impairment; ROC, receiver operating characteristic.

4.1. Limitations

This study had several limitations, including the small sample size, the inclusion of participants with various causes of cognitive decline, bias resulting from the odorant test application, as well as potential biases in the responses from participants. In addition, the study participants were selected by screening with the MMSE test, but the classification of MCI was not conducted based on subdividing the results of this test. As such, it is likely that we included patients with MCI and AD, as well as other types of dementia such as vascular dementia and Lewy body dementia without odorant dysfunction. Differences in age between groups occurred, but the effect of aging on olfaction was not adjusted on analysis. As it was difficult to recruit healthy elderly subjects (≥70 years old) because of the COVID-19 pandemic, we plan to clarify the relationship between olfactory score and cognitive function in a future study in which the age factor is excluded. In the present study, the assignment to healthy control, MCI, and AD groups was only based on the MMSE and physician diagnosis using cognitive screening tools, and not based on multiple screening tests such as assessment of amyloid PET, amyloid-β in blood or cerebral fluid, or tau. Because of ethical concerns, there were no restrictions for drugs that could affect cognitive function or olfaction and no exclusion criteria related to the use of such drugs. Therefore, it is possible that some study participants who used drugs that could affect cognitive function or olfaction were included in the study. In the healthy controls, we conducted a pre-examination of olfactory function based on our assumption that healthy subjects should have good cognitive and

olfactory functions. We hypothesized that the onset of olfactory dysfunction could occur earlier than the onset of cognitive impairment, so participants with good cognitive function who had some level of olfactory dysfunction were not included in our study, which limits the generalizability of our findings. Finally, as only Japanese participants were included in this study, our findings cannot be generalized to other ethnic populations.

5. Conclusions

When using the DESK tool for OI assessment, we found significant associations between olfactory dysfunction and cognitive status among healthy controls, patients with MCI, and those with AD. The DESK tool may help discriminate between healthy individuals, those with MCI, and those with AD, allowing early screening for cognitive decline among Japanese patients at high risk of cognitive decline and dementia, although the effect of age on DESK olfaction scores has not been fully explored in this study.

Role of the funding source

This work was supported by Kobayashi Pharmaceutical, and involved in the study design, data collection, data analysis, data interpretation, and writing of the manuscript.

Author contributions

Takahiro Fukumoto: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Visualization, Writing– Original draft preparation, Writing- review & editing. Toshifumi Ezaki: Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Software, Visualization, Writing– Original draft preparation, Writing- review & editing. Katsuya Urakami: Investigation, Supervision, Writing- review & editing.

Declaration of Competing Interest

Takahiro Fukumoto is a full-time employee of Kobayashi Pharmaceutical Co., Ltd. Toshifumi Ezaki is a full-time employee of, and holds stock in, Kobayashi Pharmaceutical Co., Ltd. Katsuya Urakami has received grants from Kobayashi Pharmaceutical Co., Ltd.

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