

Relation of Odor Identification with Alzheimer's Disease Markers in Cerebrospinal Fluid and Cognition

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Relation of Odor Identification with Alzheimer's Disease Markers in Cerebrospinal Fluid and Cognition

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Abstract.

Background: Impaired olfactory function is an early characteristic of Alzheimer's disease (AD), but it remains unclear if odor identification also relates to early markers of AD in cerebrospinal fluid (CSF).

Objective: To investigate the association between odor identification and amyloid- β 1–42 ($A\beta_{42}$) and total tau (t-tau) concentrations in CSF. In addition, to examine the relation between odor identification and cognitive function at baseline and at follow-up, and whether these associations are moderated by CSF $A\beta_{42}$ and t-tau and apolipoprotein E (APOE) genotype.

Methods: We included 160 individuals (40 with normal cognition, 45 with mild cognitive impairment (MCI), 42 with AD-type dementia, and 26 individuals with non-AD dementia) from the EDAR study. Individuals were recruited from six memory clinics across Europe. Odor identification was tested with the brief University of Pennsylvania Smell Identification Test. CSF $A\beta_{42}$ and t-tau were assessed with INNO-BIA AlzBio3 Luminex assay. Neuropsychological assessment included tests for verbal memory, verbal fluency, attention, executive function, and visuoconstruction. Follow-up was performed within 3 years after baseline.

Results: Lower odor identification scores correlated with increased CSF t-tau concentrations and with lower scores on all cognitive measures at baseline independent of diagnostic group. Lower odor identification scores predicted decline on the

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MMSE in the total group, and decline on wordlist learning and delayed recall in APOE ϵ 4 carriers and in individuals with abnormal A β ₄₂.

Conclusion: Odor identification impairment may be an indicator of neuronal injury rather than amyloid pathology.

Keywords: Alzheimer's disease, amyloid- β (1–42), cerebrospinal fluid, mild cognitive impairment, olfaction

INTRODUCTION

Impaired olfactory function is an early clinical feature of Alzheimer's disease (AD) [1, 2]. AD is characterized by amyloid plaques, neurofibrillary tangles, and neuronal loss in the brain. In early stages of the AD disease process, olfactory deficits have been correlated with neurofibrillary tangles in the central olfactory system and its olfactory projection areas [3–5]. Postmortem studies have shown that the olfactory bulb is one of the first subcortical areas in which AD-related tau neurofibrillary tangles manifest [3, 6, 7]. *In vivo*, lower cerebrospinal fluid (CSF) amyloid- β 1–42 (A β ₄₂) reflects amyloid deposition and higher CSF total tau (t-tau) reflects neuroaxonal degeneration as assessed on neuropathological examination [8], but it remains unclear if odor identification is also correlated with these markers in CSF.

Olfactory identification deficits have also been correlated with memory functioning [9, 10] and with cognitive decline or conversion to AD-type dementia [11–13]. The central olfactory system projects to several regions in the medial temporal lobe (MTL) [14], where episodic memory function is located. The association between olfaction and memory seems to be stronger in carriers of the apolipoprotein E (APOE) ϵ 4 allele [15]. However, information is still limited on the association between odor identification and performance on non-memory tests, and whether this association is also modulated by APOE genotype and AD-related CSF markers. A commonly used test to assess odor identification deficits is the University of Pennsylvania Smell Identification Test (UPSIT) [16]. The UPSIT has a high sensitivity and specificity to detect olfaction deficits, and has been able to discriminate between individuals with mild cognitive impairment (MCI), a possibly early stage of AD and AD-type dementia, and between MCI and normal cognition [11]. In this study, we examined whether odor identification, measured by a brief version of the UPSIT (i.e., the Brief Smell Identification Test (B-SIT) [17], is associated with CSF A β ₄₂ and t-tau concentrations. Second, we examined the relation between odor identification scores and cognitive function at baseline and follow-up, and whether these associations are

dependent on AD-related CSF markers and APOE genotype. We tested these associations along the clinical spectrum from normal cognition to dementia.

MATERIALS AND METHODS

Individuals

We included 160 individuals from the European study “Beta amyloid oligomers in the early diagnosis of AD and as marker for treatment response” (acronym: EDAR) based on availability of CSF, cognition scores, and scores on the B-SIT. Individuals were recruited from six memory clinics across Europe between 2008 and 2010 and represented individuals with normal cognition ($n = 40$), MCI ($n = 45$), AD-type dementia ($n = 42$), non-AD type dementia [$n = 26$, frontotemporal dementia (FTD), $n = 14$], dementia with Lewy bodies (DLB, $n = 7$), and vascular dementia (VaD, $n = 5$) and 7 individuals that were not demented but could not be classified to a diagnostic group (see below). Follow-up assessments were performed in 80 (50%) individuals within 3 years after baseline.

Individuals with normal cognition were recruited among patients attending the memory clinic ($n = 16$) or from other settings (partners from patients or via advertisements, $n = 24$). Inclusion criteria for individuals with normal cognition were: age above 40 years, no cognitive impairment on neuropsychological tests, and age and education corrected Mini-Mental State Examination score (MMSE [18]) above the 10th percentile based on local norms (unpublished data from Maastricht Aging Study). The MMSE cut points for individuals aged respectively 40–53 years, 54–63 years, 64–73 years, and age >73 years were for individuals with less than 8 y of education respectively 25, 24, 24, and 23; for individuals with 8–12 years of education respectively 27, 26, 26, and 24; and for individuals with more than 12 years of education respectively 27, 27, 26, and 25. Individuals with normal cognition from outside the memory clinic did not differ from individuals with normal cognition from the memory clinic with respect to age, educational level, MMSE score, and neuropsychological test scores. Inclusion criteria for individuals

with MCI were: memory clinic referral for the evaluation of cognitive complaints, age above 60 years, a MMSE-score above 19, a cognitive impairment on neuropsychological tests, defined as a z-score (corrected for age, gender, and education) below -1.5 on one or more neuropsychological tests (for tests see section cognition measures) according to Petersen's criteria [19] and the absence of a clinical diagnosis of dementia.

Inclusion criteria for individuals with dementia were age above 40 years and a clinical diagnosis of probable or possible AD-type dementia according to the NINCDS-ADRDA criteria [20], FTD [21], VaD according to the NINDS-AIREN criteria [22], or DLB [23]. Exclusion criteria for all individuals were contra-indications for lumbar puncture or any disorder probably related to cognitive impairment other than neurodegeneration.

Seven individuals could not be classified to a diagnostic subgroup because they had no cognitive complaints but performed poorly on one or more cognitive tests at baseline with a z-score equal to or below -2 (2 controls) or because of missing test scores (5 individuals with cognitive complaints) but they were included in the total group analyses. All individuals provided written informed consent and the medical ethics committee at each center approved the study.

Baseline and follow-up assessment

Baseline assessments included clinical history, physical examination, neuroimaging, routine laboratory tests for blood and CSF, the MMSE, and the Clinical Dementia Rating scale (CDR [24]), Functional Assessment Questionnaire (FAQ [25]), a neuropsychological examination, and an odor identification test (see below). Follow-up assessments were similar to baseline except for laboratory tests and neuroimaging and were performed once or twice within 3 years after baseline coded in 6-month intervals.

Brief smell identification test

The B-SIT is a brief 12-item version of the 40-item UPSIT [17]. In the B-SIT 12 strips are embedded with microencapsulated odorants. After scratching the strip an odorant is released and the individual has to select the identity from 4 suggested odorants. The B-SIT was administered in the individual's own language. Scores ranged from 0 (no odor correctly identified) to 12 (all odors correctly identified). Individuals were excluded if they completed less than 10

items ($n = 11$; one individual without dementia with missing cognitive tests scores, three with MCI, three with AD-type dementia; one with possible vascular dementia; and two with FTD). In individuals with 1 or 2 missing scores, a chance-level score of 0.25 was imputed for each missing item.

Cognition measures

General cognitive function was assessed with the MMSE. The neuropsychological test battery included tests for memory (wordlist learning and delayed recall of the Consortium to Establish a Registry for Alzheimer's Disease neuropsychological battery (CERAD) [26]), semantic memory and language (animal fluency), information processing speed (Trail Making Test (TMT) A), executive function (TMT B), and visuoconstruction (CERAD copy figures). Z-scores (corrected for age, gender, and education) for the CERAD wordlist learning and delayed recall, TMT A and B and copy figures were calculated according to the CERAD-Plus norms [26, 27]. For the animal fluency test, z-scores were calculated according to the norms by van der Elst et al. [28].

CSF and DNA collection, storage, and analysis

CSF was collected via a lumbar puncture in 10-mL polypropylene tubes, centrifuged at 4°C at $2000\times g$ and stored at -80°C within 1 h after collection. $\text{A}\beta_{42}$ and t-tau concentrations were measured with the INNO-BIA AlzBio3 Luminex assay (Fujirebio, formerly Innogenetics, Gent, Belgium). All CSF analyses were performed at the end of the study, in one batch, at the VU University Medical Centre (VUmc) in Amsterdam in the Netherlands. CSF concentrations below 389 (pg/ml) for $\text{A}\beta_{42}$, and above 98 (pg/ml) for t-tau were considered abnormal according to local cut off values for this assay at the VUmc [29]. Investigators that collected the clinical data were blinded to the CSF results. APOE genotype was determined by Polymerase Chain Reaction (PCR) of genomic DNA extracted from EDTA anticoagulated blood.

Statistical analyses

Data were analyzed with IBM SPSS statistics version 22. Group differences in baseline characteristics were examined with a one-way ANOVA for continuous variables and χ^2 -tests for categorical variables. The relationship between B-SIT scores and CSF

markers and between B-SIT scores and cognitive measures were analyzed by performing linear mixed with random effects for individual intercepts within center (nested design). Age, gender, and education were included as covariates when it would improve the model fit. Age was included in all analysis, and the analyses with cognitive outcomes were corrected for gender and education as well. In addition, we tested the association of a low B-SIT score (score <9 [30, 31]) and abnormal A β_{42} (score below 389) and abnormal tau (score above 98) with logistic regression corrected for age and center.

The association of the B-SIT with cognitive decline over time was examined with linear mixed models analyses of the time-by-B-SIT interaction, corrected for age, gender and education with random effects for individual intercepts within center (nested design). If required for a better fit according to likelihood ratio tests, random slopes were also included to allow for heterogeneity in individual trajectories over time. *Post hoc*, a similar linear mixed model analysis was performed of a time-by-B-SIT performance level

interaction to graphically display the association of MMSE scores over time in low and in high B-SIT performers. Current smoking (yes versus no) was not significant and therefore excluded from the models. The interaction effect of diagnosis and APOE $\epsilon 4$ status (carriers of one or two $\epsilon 4$ alleles versus no $\epsilon 4$ allele) were included separately in a second model to examine a possible moderator effect. For the cognition measures, the moderator effect of the CSF markers was also included. A *p*-value of <0.05 for two-sided tests was considered statistically significant.

RESULTS

Baseline characteristics for the total group and the subgroups are presented in Table 1. Individuals were on average 67 years old, had 11 years of education and more than half of the individuals were male (57%). Individuals with normal cognition, MCI, AD-type dementia, and non-AD dementia did not differ on age or education. Males were more common in the

Table 1
Baseline characteristics

	All (<i>n</i> = 160)	Normal cognition (<i>n</i> = 40)	MCI (<i>n</i> = 45)	AD-type dementia (<i>n</i> = 42)	Non-AD dementia (<i>n</i> = 26)
Age, mean (SD)	67.3 (9.4)	65.8 (10.6)	69.5 (9.4)	66.6 (8.4)	66.9 (9.6)
Male, <i>n</i> (%)	91 (57)	21 (53) ⁺	25 (56)	21 (50) ⁺	20 (77) ^{#§}
Education, mean <i>y</i> (SD)	11.2 (3.7)	12.1 (2.7)	10.5 (3.6)	11.3 (3.9)	11.4 (4.7)
FAQ, <i>n</i>	134	36	43	32	18
Mean (SD)	6.8 (7.8)	1.6 (3.1) ^{^§+}	5.0 (7.0) ^{#§+}	12.4 (6.7) [^]	12.4 (9.1) [^]
MMSE, <i>n</i>	156	40	45	41	23
Mean (SD)	26.3 (3.2)	28.9 (1.3) ^{^§+}	27.2 (1.8) ^{#§+}	23.2 (3.2) [^]	25.5 (3.4) [^]
CDR-SOB, <i>n</i>	138	37	45	32	19
Mean (SD)	2.3 (2.6)	0.6 (1.2) ^{^§+}	1.7 (1.2) ^{#§+}	4.3 (2.6) [^]	4.5 (3.6) [^]
APOE $\epsilon 4$ +, <i>n</i> (%)	72 (57)	11 (48)	23 (62) ⁺	26 (70) ⁺	7 (30) ^{^§}
CSF markers, <i>n</i>	132	25	39	36	25
A β_{42} (pg/ml), mean (SD)	357 (152)	418 (158) [§]	368 (127) [§]	278 (132) [^]	381 (175) [§]
Abnormal A β_{42} , <i>n</i> (%)	79 (60)	9 (38) ^{^§}	26 (67) [#]	29 (81) [^]	12 (48) [§]
t-tau (pg/ml), mean (SD)	109 (63)	78 (25) ^{^§}	111 (68) ^{#§+}	150 (73) [^]	81 (36) ^{^§}
Abnormal t-tau, <i>n</i> (%)	66 (50)	5 (21) ^{^§}	19 (49) ^{#§}	30 (83) [^]	9 (36) [§]
B-SIT, mean (SD)	6.8 (2.6)	8.7 (2.1) ^{^§+}	6.7 (2.4) ^{#§+}	5.7 (2.4) [^]	5.4 (2.1) [^]
Low B-SIT performers, <i>n</i> (%)	116 (73)	17 (43) ^{^§+}	36 (80) [#]	36 (86) [#]	24 (92) [#]
Cognition (<i>z</i> -scores), <i>n</i>	136	35	44	35	20
Wordlist learning, mean (SD)	-1.6 (1.8)	0.2 (1.0) ^{^§+}	-2.0 (1.5) ^{#§}	-2.8 (1.3) [^]	-1.7 (1.9) ^{#§}
Wordlist delayed recall, mean (SD)	-1.4 (1.7)	0.1 (0.9) ^{^§+}	-1.6 (1.5) ^{#§}	-2.7 (1.3) [^]	-1.3 (1.8) ^{#§}
Animal fluency, mean (SD)	-1.2 (1.1)	-0.3 (0.8) ^{^§+}	-1.0 (1.0) ^{#§+}	-1.5 (0.9) [^]	-1.9 (1.1) [^]
TMTA, mean (SD)	-0.9 (2.1)	0.7 (0.9) ^{^§+}	-0.9 (1.8) ^{#§}	-2.2 (2.3) [^]	-1.7 (1.9) [#]
TMTB, mean (SD)	-1.0 (2.2)	0.9 (0.7) ^{^§+}	-0.5 (1.5) ^{#§+}	-2.7 (2.1) [^]	-2.0 (2.2) [^]
Copy figures, mean (SD)	-0.9 (2.1)	0.5 (0.7) ^{^§+}	-0.9 (2.1) ^{#§}	-1.7 (2.4) [^]	-1.7 (2.0) [#]

Data are mean (SD) or valid percent. FAQ, Functional Assessment Questionnaire; MMSE, Mini-Mental State Examination; CDR-SOB, Clinical dementia Rating, Sum of Boxes; APOE, apolipoprotein E; CSF, cerebrospinal fluid; A β_{42} , amyloid- β 1-42; t-tau, total tau; B-SIT, the Brief Smell Identification Test; TMT, Trail Making Test; MCI, mild cognitive impairment; AD, Alzheimer's disease. [#]*p* < 0.05 compared to normal cognition, [^]*p* < 0.05 compared to MCI, [§]compared to AD, ⁺compared to non-AD dementia.

non-AD dementia group than in the normal cognition, MCI, and AD-type dementia group. Scores on the MMSE, FAQ, CDR memory test, cognitive tests, and CSF markers differed between groups as expected. APOE genotyping was available for 127 individuals (79%). The number of carriers of one or more APOE ε4 alleles was high in the AD-type dementia (70%) and MCI (62%) group and low in the non-AD dementia group (30%). Lower B-SIT scores were found in individuals with AD-type dementia and with non-AD dementia compared with individuals with MCI or normal cognition.

B-SIT scores and CSF markers

In the total group, lower B-SIT scores were associated with increased CSF t-tau concentrations, but not with CSF Aβ₄₂ concentrations (Fig. 1, Table 2). The association between B-SIT scores and CSF markers was independent of diagnosis, APOE ε4 status and the other CSF marker. A low B-SIT score was associated with increased likelihood for abnormal tau (OR = 2.84, *p* < 0.05) but not abnormal Aβ₄₂ (OR = 1.88, *p* = 0.12), Table 3). The association with abnormal tau was strongest in individuals with AD-type dementia with a low B-SIT score ((OR = 23.25, *p* < 0.05), Table 3).

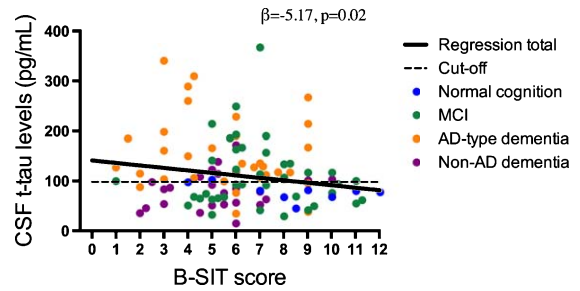


Fig. 1. Associations at baseline between B-SIT scores and CSF concentrations. The figure displays the count of individuals with normal cognition (blue), MCI (green), AD-type dementia (orange), and non-AD dementia (purple) with regression line of the total group and with a cut-off line for t-tau concentrations.

B-SIT scores and cognition

In the total group, lower B-SIT scores were associated with lower scores on the MMSE, wordlist learning, wordlist delayed recall, animal fluency, TMT A, TMT B, and copy figures (Table 2, Fig. 2). The association differed between diagnostic groups for the MMSE and TMT part A. The relation between B-SIT and MMSE scores was stronger in individuals with AD-type dementia than in individuals with MCI (difference β = 0.60, *p* < 0.01). The relation between B-SIT scores and scores on the TMT part A was

Table 2
Associations between B-SIT and CSF markers and cognition measures at baseline

	All (<i>n</i> = 160)	Normal cognition (<i>n</i> = 39)	MCI (<i>n</i> = 45)	AD-type dementia (<i>n</i> = 42)	Non-AD dementia (<i>n</i> = 26)	B-SIT* diagnosis	
						F	<i>p</i>
CSF Aβ ₄₂	6.56 (-3.45 to 16.58)	-15.69 (-40.44 to 9.06)	9.65 (-10.77 to 30.06)	-3.19 (-22.27 to 15.90)	12.59 (-13.63 to 38.80)	1.14	0.34
CSF t-tau	-5.17* (-9.36 to -0.98)	-2.32 (-12.47 to 7.84)	-2.98 (-11.36 to 5.39)	-7.77 (-15.61 to 0.07)	3.88 (-6.87 to 14.62)	0.99	0.40
MMSE	0.37*** (0.18 to 0.55)	0.00 (-0.35 to 0.35)	-0.24 (-0.53 to 0.06)	0.37* (0.06 to 0.67)	0.33 (-0.12 to 0.78)	3.20	0.03*
Wordlist learning	0.27*** (0.16 to 0.37)	0.09 (-0.12 to 0.31)	0.00 (-0.18 to 0.18)	0.15 (-0.03 to 0.32)	0.40** (0.14 to 0.67)	2.18	0.09
Wordlist delayed recall	0.27*** (0.16 to 0.36)	0.15 (-0.07 to 0.36)	0.14 (-0.03 to 0.31)	0.10 (-0.07 to 0.28)	0.33* (0.07 to 0.58)	0.71	0.55
Animal fluency	0.11** (0.04 to 0.18)	0.00 (-0.15 to 0.15)	0.00 (-0.12 to 0.12)	0.06 (-0.07 to 0.18)	0.18 (-0.01 to 0.36)	1.00	0.39
TMT A	0.28*** (0.16 to 0.40)	-0.02 (-0.28 to 0.25)	-0.02 (-0.24 to 0.20)	0.37** (0.14 to 0.59)	0.36 (-0.01 to 0.68)	2.95	0.04*
TMT B	0.26*** (0.13 to 0.38)	0.01 (-0.24 to 0.27)	-0.01 (-0.22 to 0.21)	0.27* (0.06 to 0.49)	0.02 (-0.30 to 0.35)	1.47	0.23
Copy figures	0.25*** (0.12 to 0.37)	0.10 (-0.19 to 0.40)	-0.01 (-0.25 to 0.24)	0.32** (0.08 to 0.56)	-0.11 (-0.49 to 0.26)	1.81	0.15

Numbers are beta coefficients with 95% confidence intervals between brackets. Lower scores on the B-SIT, MMSE, wordlist learning, wordlist delayed recall, animal fluency, TMT A, TMT B, and figures indicate lower performance. Decreased concentrations of CSF Aβ₄₂ and increased concentrations of CSF t-tau are abnormal. B-SIT; the Brief Smell Identification Test; CSF, cerebrospinal fluid; Aβ₄₂, amyloid-β 1-42; t-tau, total tau; MMSE, Mini-Mental State Examination; TMT, Trail Making Test; MCI, mild cognitive impairment; AD, Alzheimer's disease. Statistically significant linear mixed models were indicated with **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

Table 3
Association of low B-SIT scores with presence of abnormal A β_{42} and abnormal tau

	Abnormal A β_{42}	Abnormal t-tau
Normal cognition ($n = 25$)	0.96 (0.16 to 5.80)	2.58 (0.17 to 40.09)
MCI ($n = 39$)	0.93 (0.19 to 4.69)	2.20 (0.44 to 10.99)
AD-type dementia ($n = 36$)	0.79 (0.07 to 9.10)	23.25 (1.39 to 388.05)*
Not demented ($n = 71$) ^a	1.85 (0.69 to 4.98)	3.03 (0.98 to 9.34)
Demented ($n = 61$) ^b	1.10 (0.22 to 5.45)	1.18 (0.24 to 5.79)
All ($n = 132$)	1.88 (0.85 to 4.19)	2.84 (1.24 to 6.51)*

Numbers are odds ratios with 95% confidence intervals between brackets. Abnormal A β_{42} is defined as a score below 389; abnormal tau as a score above 98. No odds ratio could be estimated in individuals with non-AD dementia as none of the individuals had abnormal A β_{42} or tau in combination with a high B-SIT score. ^aIndividuals with normal cognition ($n = 25$), MCI ($n = 39$), and individuals without dementia who could not be classified to a diagnostic group ($n = 7$); ^bIndividuals with AD-type dementia ($n = 36$) and non-AD dementia ($n = 25$). B-SIT, the Brief Smell Identification Test. * $p < 0.05$.

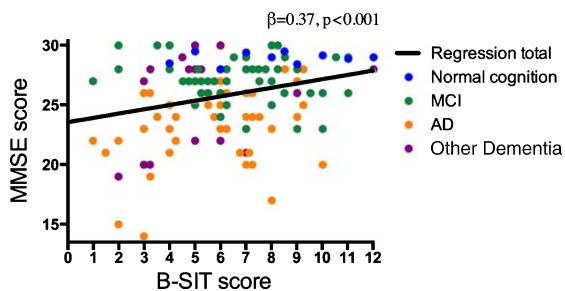


Fig. 2. Associations at baseline between B-SIT scores and MMSE scores. The figure displays the count of individuals with normal cognition (blue), MCI (green), AD-type dementia (orange), and non-AD dementia (purple) with regression line of the total group.

stronger in individuals with AD-type dementia than in individuals with MCI (difference $\beta = 0.39$, $p < 0.05$) and normal cognition (difference $\beta = 0.38$, $p < 0.05$). The association between B-SIT scores and cognition measures were independent of APOE $\epsilon 4$ carriership and independent of abnormal CSF A β_{42} and t-tau concentrations.

B-SIT scores and cognitive decline over time

Follow-up data were available for 80 individuals, including 20 individuals with normal cognition, 33 with MCI, and 23 with AD-type dementia at baseline. Another 4 individuals without dementia were included who could not be classified to a diagnostic group because of no cognitive complaints but a z-score equal to or below -2 on neuropsychological tests (1 individual) or because of missing tests scores (3 individuals). Individuals with non-AD dementia (2 individuals) were excluded in the longitudinal analyses due to the small sample size. The average follow-up interval was 1.28 (SD = 0.52) years. Individuals with follow-up data, in the total group and in

the subgroups, did not differ from individuals with only baseline data on MMSE score, age, gender, and education.

In the total group, lower baseline B-SIT scores at baseline were associated with decline on only the MMSE score at follow-up (Table 4, Fig. 3). These associations were independent of diagnostic group. The relation between B-SIT scores and decline on the wordlist learning and delayed recall at follow-up was dependent on APOE $\epsilon 4$ carriership and A β_{42} status (Supplementary Table 1). The relation between lower B-SIT scores and decline on delayed recall was stronger in APOE $\epsilon 4$ carriers ($\beta = 0.07$, $p < 0.05$) than in non-carriers ($\beta = -0.04$, $p = 0.25$). The relation between lower B-SIT scores and decline on wordlist learning and delayed recall was stronger in individuals with abnormal A β_{42} concentrations (wordlist learning: $\beta = 0.07$, $p < 0.05$; wordlist delayed recall: $\beta = 0.07$, $p < 0.05$ than in individuals with normal A β_{42} concentrations (wordlist learning: $\beta = -0.05$, $p = 0.16$; wordlist delayed recall: $\beta = -0.06$, $p = 0.09$). The associations between B-SIT scores and cognition were independent of t-tau status for all measures.

DISCUSSION

Our main results are that lower odor identification scores correlated with increased CSF t-tau concentrations and with lower scores on all cognition measures. Over time, lower identification scores at baseline were associated with a larger decline on the MMSE in the total group.

The observed relation between odor identification scores and CSF tau is in line with postmortem research showing that in early stage of AD neurofibrillary tangles are present in the olfactory bulb and the primary and secondary olfactory cortex, including

Table 4
Associations between B-SIT and cognition performance at follow-up in the total group ($N=80$)

	Time	Time*B-SIT	Time*B-SIT* diagnosis	
			F	p
MMSE	-1.56 (-2.20 to -0.92)***	0.16 (0.08 to 0.25)***	0.24	0.79
Wordlist learning	-0.19 (-0.52 to 0.15)	0.03 (-0.02 to 0.07)	1.06	0.35
Wordlist delayed recall	-0.23 (-0.53 to 0.08)	0.03 (-0.01 to 0.06)	0.65	0.52
Animal fluency	-0.09 (-0.32 to 0.15)	0.01 (-0.02 to 0.04)	0.45	0.96
TMT A	0.07 (-0.26 to 0.40)	-0.10 (-0.05 to 0.03)	0.98	0.38
TMT B	-0.31 (-0.70 to 0.08)	0.03 (-0.02 to 0.08)	0.84	0.44
Copy figures	0.01 (-0.33 to 0.36)	-0.01 (-0.05 to 0.04)	0.15	0.99

The slope for time indicates the change in cognitive score per 6-month follow-up in the total group. The time-by-B-SIT interaction indicates how the slope differs from the time slope as a function of B-SIT score. For example, the MMSE decline on average 1.56 per 6-month follow-up. For every increase in B-SIT score at baseline, the slope on the MMSE improves with 0.16. B-SIT, the Brief Smell Identification Test; MMSE, Mini-Mental State Examination; TMT, Trail Making Test. Numbers are beta coefficients with 95% confidence intervals between brackets. *** $p < 0.001$.

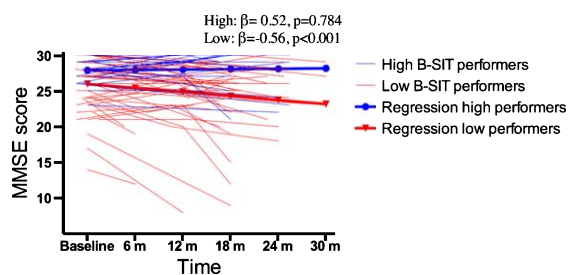


Fig. 3. MMSE scores over time in high and low B-SIT performers. The figure displays thin individual lines of high B-SIT performers (thin blue lines, i.e., B-SIT score 9 or greater) ($n=22$) and low B-SIT performers (thin red lines, i.e., B-SIT score less than 9) ($n=57$) on the MMSE score over time with solid corresponding regression lines for the two groups.

the entorhinal cortex [5] and brain areas involved in transmodal object representation such as the temporal pole. We did not find an association between odor identification and $A\beta_{42}$ concentrations. Similarly, previous research also found that olfactory identification is not strongly related to amyloid deposition measured with *in vivo* amyloid imaging [32, 33]. The associations between odor identification scores and CSF markers were independent of diagnostic group.

Lower scores on odor identification correlated with lower scores on all cognition measures at baseline. Stronger associations between odor identification and scores on the MMSE and TMT part A were found in individuals with AD-type dementia than in individuals who were not demented. For most other cognitive tests, the associations between odor identification scores and cognitive performances were stronger in individuals with dementia than in individuals without dementia although these differences were not statistically significant. This may suggest

that odor identification scores become abnormal in more advanced stages of the disease. This could also explain the stronger correlations between odor identification scores and cognition than between odor identification scores and CSF biomarkers, which are suspected to become abnormal earlier in the AD disease course [34, 35].

Longitudinally, we found that in the total group lower odor identification scores at baseline correlated with larger decline in scores on the MMSE. These findings are consistent with previous community studies showing an association between odor identification scores and decline on a general cognitive functioning measure [12, 36]. The reason that we only found an association with the MMSE may be explained by the fact that the MMSE does not have a floor effect in mild-moderate dementia and is perhaps less vulnerable to practice effects as opposed to cognitive tests. The association between odor identification scores and cognitive decline was independent of APOE $\epsilon 4$ status and CSF markers except for decline in memory scores. For these memory measures, the association with odor identification scores was stronger in APOE $\epsilon 4$ allele carriers and in individuals with abnormal $A\beta_{42}$. This is in line with previous research showing solely an association in APOE $\epsilon 4$ allele carriers between odor identification scores and long-term episodic memory decline [15]. Conceivably, as both APOE $\epsilon 4$ and amyloid pathology are strongly associated with AD, the association between odor identification and memory decline may be stronger in these individuals because of more severe underlying AD pathology.

Interestingly, the correlations of the B-SIT score with AD biomarkers tended to differ between individuals with AD-type dementia and individuals

with non-AD dementia, despite similar odor impairments. B-SIT scores in the non-AD dementia group were as low as in the AD-type dementia group; yet only one-third (36%) in the non-AD dementia group had abnormal t-tau concentrations compared with a majority (83%) in the AD-type dementia group. In addition, the relationship between B-SIT scores and CSF tau concentrations was reversed in the non-AD dementia group. This suggests that different mechanisms underlie impaired odor recognition and that this impairment is not just an effect of the dementia itself.

Arguably, other pathologies may be the cause of impaired odor identification in individuals without dementia. Alpha-synucleinopathy has been found in the olfactory bulb in an early stage of DLB [37, 38], and it has been linked to odor dysfunction. Indeed, in the non-AD dementia group, individuals with DLB ($n=7$) had the lowest B-SIT scores (DLB: mean 4.71, $SD=2.98$). B-SIT scores were also low in individuals with VaD ($n=5$, mean 6.00, $SD=2.12$) and FTD ($n=14$, mean: 5.51, $SD=2.27$), which is consistent with previous evidence suggesting impairment in odor identification in these disorders [39–41]. Yet, in these groups CSF tau concentrations are often not as high as in AD-type dementia [42]. This suggests that the underlying pathology for low B-SIT scores may depend on the clinical group in which the test is performed.

Although the memory and language component in the B-SIT is minimized due to multiple-choice options facilitating cueing, the impaired B-SIT scores may also result from AD memory dysfunction. However, after correction for semantic memory performance as assessed by verbal fluency, the relation between the B-SIT score and CSF $A\beta_{42}$ and tau remained similar ($A\beta_{42}$: $\beta=7.44$ (-2.93 to 17.81); t-tau: $\beta=-5.24$ (-9.44 to -1.03)). Previous studies showed that associations between odor recognition and neuropathology are independent of semantic memory [4]. This suggests that the effect of odor identification cannot only be explained by impairments in semantic memory.

The limitations of the study are: first, the relatively small sample size of the subgroups could have resulted in a type II error. Second, our main outcome was the association of B-SIT scores with CSF and clinical markers in the total group for two biomarkers and seven cognitive markers, which may have resulted in a type I error. However, when corrected for multiple testing according to Benjami-Hochberg, all our findings remained significant. Third, the

follow-up time was relatively short. Fourth, there is a possible selection bias due to the drop out at follow-up because of poor cognitive functioning. Individuals with follow-up data, however, did not differ on MMSE score, age, gender, or education from individuals with only baseline data, which makes selection bias less likely. Fifth, no imaging data was available for testing. Odor identification was associated with specific atrophy patterns [43–45]. Lastly, we used a brief version of the UPSIT. Previous studies have shown that the brief 12-item version has a somewhat lower reliability than the 40-item version (0.71 versus 0.95) and may have a lower predictive utility than the 40-item version for the transition from MCI to AD-type dementia [31], but the mean and frequency distribution were similar [16, 17]. Furthermore, the reliability of the brief 12-item version is still high and because the administration time is less than 5 minutes it is easier to incorporate in clinical routine.

To conclude, our study shows a relationship between lower odor identification and increased tau concentration suggesting that odor identification correlates better with measures of tau than with amyloid markers. Still, in non-AD dementias, low odor identification scores may be associated with non-tau pathologies as well. This limits the use of odor identification tests in the differential diagnosis of individuals with dementia. In addition, odor identification correlated with lower cognitive performances at baseline and with decline over time on the MMSE. Future longitudinal studies with a longer follow-up duration are recommended to further examine the prognostic value of odor identification tests in predicting AD-related pathology and cognitive decline.

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SUPPLEMENTARY MATERIAL

The supplementary material is available in the electronic version of this article: <http://dx.doi.org/10.3233/JAD-170564>.

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