Odor identification as a biomarker of preclinical AD in older adults at risk

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ABSTRACT

Objective: To assess odor identification (OI) as an indicator of presymptomatic Alzheimer disease (AD) pathogenesis in cognitively normal aging individuals at increased risk of AD dementia.

Methods: In 274 members of the PREVENT-AD cohort of healthy aging persons with a parental or multiple-sibling history of AD dementia, we assessed the cross-sectional association of OI with potential indicators of presymptomatic AD. Some 101 participants donated CSF, thus enabling assessment of AD pathology with the biomarkers total tau (t-tau), phospho-tau (P_{181}-tau), and their ratios with β-amyloid ($A\beta_{1\text{-}42}$). Adjusted analyses considered age, cognition, *APOE* ε4 status, education, and sex as covariates. We measured OI using the University of Pennsylvania Smell Identification Test and cognitive performance using the Repeatable Battery for Assessment of Neuropsychological Status. Standard kits provided assays of the AD biomarkers. Analyses used robust-fit linear regression models.

Results: Reduced OI was associated with lower cognitive score and older age, as well as increased ratios of CSF t-tau and P_{181}-tau to $A\beta_{1\text{-}42}$ (all $p < 0.02$). However, the observed associations of OI with age and cognition were unapparent in adjusted models that restricted observations to CSF donors and included AD biomarkers. OI showed little association with CSF $A\beta_{1\text{-}42}$ alone except in *APOE* ε4 carriers having lowest-quartile $A\beta_{1\text{-}42}$ levels.

Conclusions: These findings from healthy high-risk older individuals suggest that OI reflects degree of preclinical AD pathology, while its relationships with age and cognition result from the association of these latter variables with such pathology. Diminished OI may be a practical and affordable biomarker of AD pathology. *Neurology*® 2017;89:327–335

GLOSSARY

Aβ = β-amyloid; **AD** = Alzheimer disease; **INTREPAD** = Impact of Naproxen Treatment on Presymptomatic Alzheimer's Disease; **OI** = odor identification; **P_{181}-tau** = phospho-tau; **PREVENT-AD** = Presymptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's Disease; **RBANS** = Repeatable Battery for Assessment of Neuropsychological Status; **t-tau** = total tau; **UPSIT** = University of Pennsylvania Smell Identification Test.

Prevention of Alzheimer disease (AD) dementia can be accomplished by retarding the progression of the disease in its presymptomatic stages, thus postponing the onset of clinical symptoms. Hence, research on the identification and development of AD preventives can be aided by quantitative measures of disease progression before symptom onset.[1] Such presymptomatic disease progress may be revealed by subtle cognitive changes, various MRI or PET imaging techniques, or AD biomarkers in the CSF. These measures are generally inconvenient, and more accessible markers of preclinical AD pathology are needed.

Because rhinencephalic brain regions are especially vulnerable to AD pathology,[2] a candidate marker for this purpose may be odor identification (OI), i.e., the ability to identify and name specific odorants.[3–10] Like cognition, OI is known to be impaired in both aging[11,12] and

Supplemental data at Neurology.org

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dementia.[7,13,14] In longitudinal studies, reduced OI performance predicts faster cognitive decline in elderly controls and persons with mild cognitive impairment or AD dementia.[5,8–10,15–17] Finally, an important study of cognitively healthy persons showed that reduced OI ability predicted postmortem AD pathology.[5]

We therefore sought to evaluate OI as a measure of presymptomatic AD pathogenesis. In a study of aging asymptomatic individuals at risk of AD dementia, we investigated the association between OI and recognized in vivo AD biomarkers such as CSF total tau (t-tau) and phospho-tau (P_{181}-tau) and their ratio with Alzheimer β-amyloid ($A\beta_{1\text{-}42}$). We hypothesized that degree of impairment in OI would predict biomarker evidence of AD neuropathology.

METHODS

The PREVENT-AD cohort. We investigated cross-sectional baseline measures from 274 participants in a cohort of cognitively unimpaired individuals assembled for Presymptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's Disease (PREVENT-AD).[1] PREVENT-AD enrollees had a parent or multiple siblings with a history of AD-like dementia. They were ≥60 years of age, except that individuals 55 to 59 years old were eligible if their current age was within 15 years of dementia onset in their youngest-affected relative. In general, persons with a first-degree family history have an elevated risk of AD.[18] We first screened their cognitive state using the Montreal Cognitive Assessment[19] and the Clinical Dementia Rating scale.[20] Individuals with questionable cognitive difficulties underwent a formal neuropsychological assessment. We analyzed data collected between September 2011 and August 2015 and archived in PREVENT-AD data release 2.0.

Nested within PREVENT-AD was a randomized trial of Impact of Naproxen Treatment on Presymptomatic Alzheimer's Disease (INTREPAD), a placebo-controlled, biomarker-endpoint prevention trial of the nonsteroidal anti-inflammatory drug naproxen in 184 participants. We obtained CSF data from a subset of 101 volunteers from that trial.

Standard protocol approvals, registrations, and patient consents. Written informed consent was obtained at each stage of the research from all participants and their collateral partners. Protocols and French and English consent forms were approved by the Institutional Review Board of the McGill University Faculty of Medicine. INTREPAD is registered with clinicatrials.gov as NCT02702817. The research was conducted in compliance with the ethics principles of the Declaration of Helsinki. For additional information, see www.prevent-alzheimer.ca.

Methods of assessment. ***Review of health and neurocognitive status.*** Participants were evaluated while accompanied by an informant. Assessments included a health history and review of systems, a standardized neurologic examination, and a cognitive examination with version A of the Repeatable Battery for Assessment of Neuropsychological Status (RBANS).[21] The RBANS is available in both English and Canadian French. It measures 5 domains of cognitive performance. Participants also underwent phlebotomy for routine laboratory tests and banking of plasma and a multimodal MRI/fMRI scan session.

CSF collection. INTREPAD volunteers' lumbar punctures were performed in the morning after an overnight fast. CSF was collected with the Sprotte 24-gauge atraumatic needle. The time of collection was recorded. All procedures followed recommendations of the BiomarkAPD project in the EU Joint Programme in Neurodegeneration.[22] Briefly, CSF was centrifuged at 3,000 RPM (2,000*g*) at room temperature to precipitate cells and other insoluble material. Within 4 hours of collection, CSF samples were frozen and stored in 0.4-mL aliquots at −80°C in 500 μL polypropylene cryotubes. The samples went through only one freeze-thaw cycle. We assayed CSF t-tau, P_{181}-tau, and $A\beta_{1\text{-}42}$ levels using the Innotest/Fujirebio (previously Innogenetics, Ghent, Belgium) ELISA kit, again following Joint Programme in Neurodegeneration–specified procedures. This technology is based on specific fluorescent antibody labeling. We used the biomarker ratios CSF t-tau/$A\beta_{1\text{-}42}$ and P_{181}-tau/$A\beta_{1\text{-}42}$ to indicate disease state.

APOE genotyping. DNA was extracted automatically from buffy coat samples with the QiaSymphony DNA mini kit (Qiagen, Toronto, ON, Canada). *APOE* genotype was determined with the PyroMark Q96 pyrosequencer (Qiagen). The DNA was amplified using reverse transcriptase–PCR, forward primers 5′-ACGGCTGTCCAAGGAGCTG-3′ (rs429358) and 5′-CTCCGCGATGCCGATGAC-3′ (rs7412), and reverse biotinylated primers 5′-CACCTCGCCGCGGTACTG-3′ (rs429358) and 5′-CCCCGGCCTGGTACACTG-3′ (rs7412). The DNA was sequenced with these primers: 5′-CGGACATGGAGGACG-3′ (rs429358) and 5′-CGATGACCTGCAGAAG-3′ (rs7412).

Odor identification. We assessed OI abilities using the 40-item University of Pennsylvania Smell Identification Test (UPSIT).[23] This test includes a simple scratch-and-sniff booklet along with multiple-choice response forms for OI. The UPSIT has been validated in people 5 to 85 years of age and shows a test-retest reliability of $r = 0.92$ to 0.95.[24,25] Its score is computed as the sum of the correct responses of a maximum possible 40. Both francophone and anglophone participants were presented with odors from the US version of the UPSIT. The francophone test used an in-house French translation. In a leave-one-out analysis, we assessed the reliability of the UPSIT in the PREVENT-AD cohort among an initial sample of 159 participants, obtaining a Cronbach α of 0.821, which suggests high internal consistency.

Data analyses. To avoid left skewness and a leptokurtic distribution, we transformed the raw OI scores to an UPSIT error score of $\log_{10}$ (41 − raw UPSIT score), as described by Moberg et al.[26] Higher transformed scores thus represented greater deficit in OI. We used a Kruskal-Wallis test to compare scores in *APOE* ε4 carriers and noncarriers. To assess the main effects on OI of various indicators of interest, we first examined bivariate relationships using simple linear regression. To examine the effect of each predictor variable in full perspective, we then constructed multivariable models, iteratively adding measures individually or in combination. Both the bivariate and multivariable analyses used robust-fit linear regression with a tuning constant of 1.205 to down-weight outliers. The latter general linear model analyses considered, in various combinations, age, sex, years of education, *APOE* ε4 carrier status, RBANS total score (global cognition), and the CSF t-tau/$A\beta_{1\text{-}42}$ ratio

Table 1 Demographics of INTREPAD participants

	INTREPAD participants with LP						INTREPAD participants no LP						Group comparison	All INTREPAD participants					
Demographics	Average	SD	Min	Median	Max	n	Average	SD	Min	Median	Max	n	*p* Value	Average	SD	Min	Median	Max	n
Age, y	62	6	55	61	84	101	64.14	5.57	55	64	84	83	0.01	63	6	55	62	84	184
Female, %	70	46				101	80	41				83	0.15	74	44				184
Education, y	15	3	10	14	29	101	15.30	3.83	7	15	29	83	0.35	15	3	7	15	29	184
APOE ε4 carrier status, %	33	47				101	36	48				81	0.66	34	48				182[a]
White, %	99	10				101	98	15				83	0.45	98	13				184
Francophone, %	87	34				101	73	44				83	0.02	81	39				184
MoCA total score	28	2	23	28	30	101	28.35	1.49	23	29	30	83	0.02	28	2	23	28	30	184
RBANS	101	11	74	101	129	98[b]	103.76	10.36	84	104	131	83	0.08	102	11	74	102	131	181[b]
UPSIT total score	35	4	21	36	40	100[b]	35.41	3.28	24	36	40	80	0.32	36	3	21	36	40	180[c]
$A\beta_{1-42}$, pg/mL	1,063	281	402	1,068	1,597	101								1,063	281	402	1,068	1,597	101
t-tau, pg/mL	273	130	90	259	851	101								273	130	90	259	851	101
P_{181}-tau, pg/mL	47	18	12	44	114	101								47	18	12	44	114	101
t-tau/$A\beta_{1-42}$	0.28	0.21	0.11	0.22	1.20	101								0.28	0.21	0.11	0.22	1.20	101
P_{181}-tau/$A\beta_{1-42}$	0.05	0.03	0.01	0.04	0.18	101								0.05	0.03	0.01	0.04	0.18	101

Abbreviations: Aβ = β-amyloid; INTREPAD = Impact of Naproxen Treatment on Presymptomatic Alzheimer's Disease; LP = lumbar puncture; Max, maximum; Min, minimum; MoCA = Montreal Cognitive Assessment; P_{181}-tau = phospho-tau; RBANS = Repeatable Battery for the Assessment of Neuropsychological Status; t-tau = total tau; UPSIT = University of Pennsylvania Smell Identification Test.

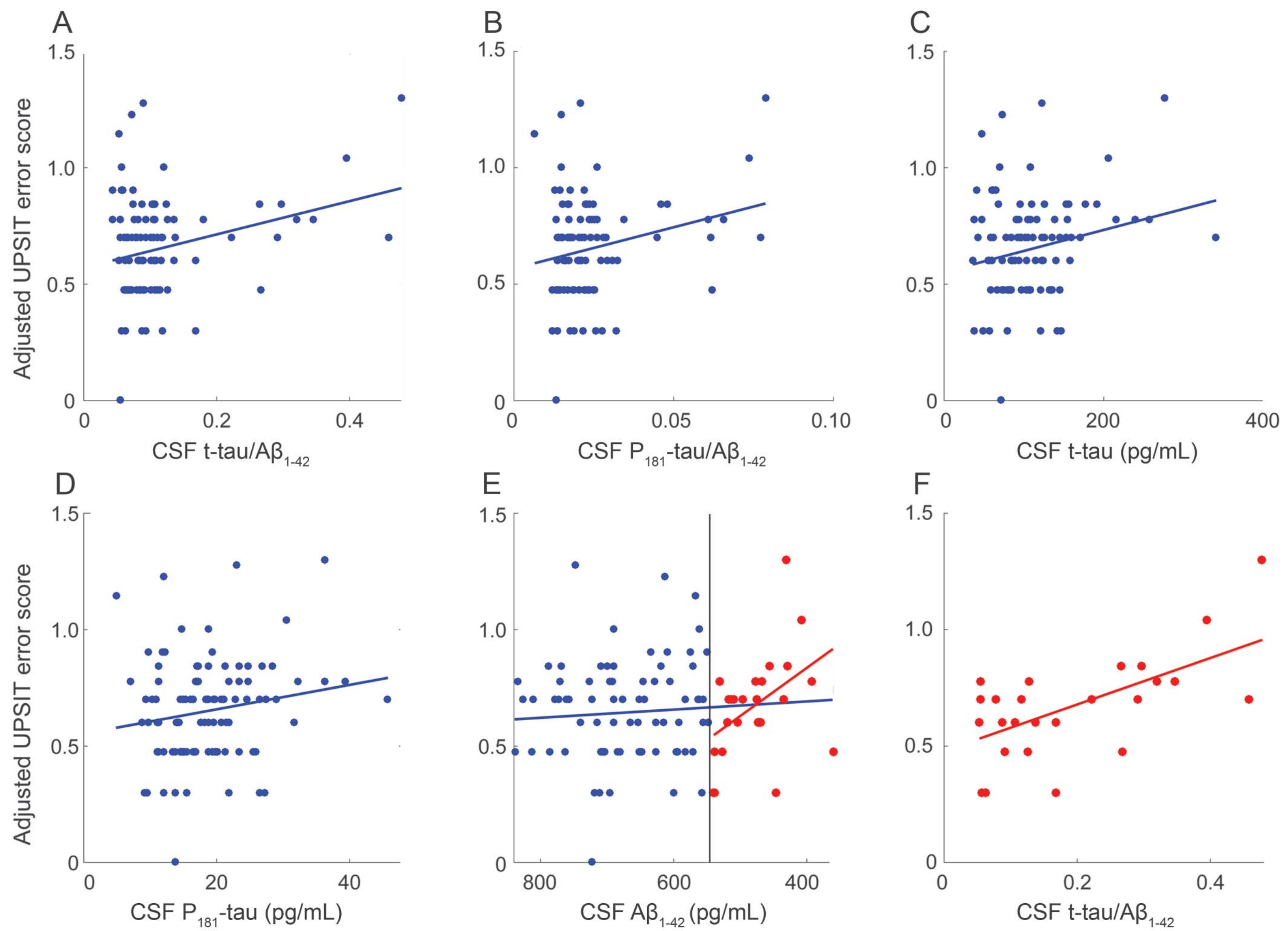
χ^2 test for categorical variables and Kruskal-Wallis test for continuous variables.

[a] Participants did not consent to genotyping.

[b] Three RBANS reports of individuals who underwent the LP were lost.

[c] One person did not consent, and some data were excluded (see Methods).

Figure 1 Robust-fit linear regression models of UPSIT error score vs CSF biomarkers of AD



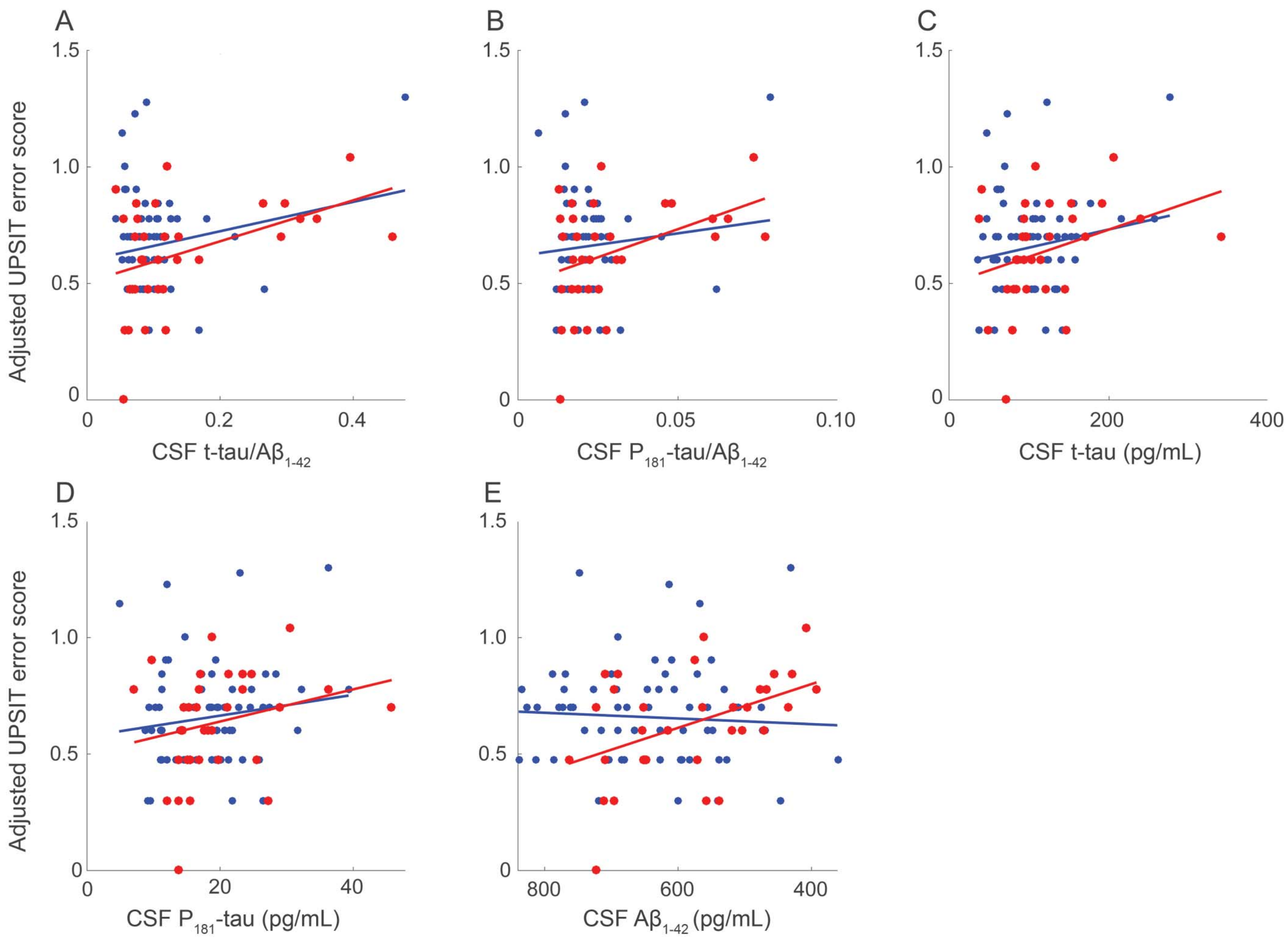
(A) CSF t-tau/$A\beta_{1\text{-}42}$ (β = 0.287, *p* = 0.00494, n = 100). (B) CSF P_{181}-tau/$A\beta_{1\text{-}42}$ (β = 1.77, *p* = 0.0165, n = 100). (C) CSF t-tau ($\beta = 3.61 \times 10^{-4}$, *p* = 0.0257, n = 100). (D) CSF P_{181}-tau ($\beta = 2.10 \times 10^{-3}$, *p* = 0.0724, n = 100). (E) CSF $A\beta_{1\text{-}42}$ ($\beta = -7.01 \times 10^{-5}$, *p* = 0.359, n = 100) and in individuals with CSF $A\beta_{1\text{-}42}$ levels below the 25th percentile (β = −0.000827, *p* = 0.0135, n = 25). (F) CSF t-tau/$A\beta_{1\text{-}42}$ in individuals with CSF $A\beta_{1\text{-}42}$ levels below the 25th percentile (β = 0.399, *p* = 0.00260, n = 25). In panel (E), blue circles represent the top 3 quartiles for Aβ concentrations, while red circles in panels (E) and (F) are data from the lowest quartile of Aβ concentrations (suggesting that they have more advanced preclinical AD). Aβ = β-amyloid; AD = Alzheimer disease; P_{181}-tau = phospho-tau; t-tau = total tau; UPSIT = University of Pennsylvania Smell Identification Test.

(sometimes substituted as specified below by other CSF indicators of AD pathogenesis). Adjusting for age, sex, years of education, and *APOE* ε4 carrier status enabled us to compare our work to other highly cited findings.[5,6] We verified the absence of collinearity in the multivariable models by investigating the variance inflation factor and tolerance. To test our primary hypothesis, we explored the relationships between OI and CSF AD biomarkers, seeking identifiable subgroups and examining interaction terms of interest.

Two sensitivity analyses assessed the effects of potential confounders on olfactory function. For both analyses, we grouped available data on brain injury, TIA, and stroke into a binary categorical variable called brain health. A second binary variable grouped nasal polyps, nasal surgeries, deviated septum, and history of a broken nose. A third such variable identified participants with a history of asthma, and a fourth identified current smokers of any substance. A final potential confounder characterized participants with any of the foregoing conditions (past or present) mentioned only at the time of olfactory testing. The first sensitivity analysis added all 5 of these potential confounders as covariates in the analytic framework of model 7. The other was a version of model 7 that excluded data from all participants with a positive rating on any of the 5 potential confounding variables.

RESULTS Of the 274 PREVENT-AD participants who met inclusion and exclusion criteria, 1 individual who consented for a lumbar puncture did not consent to the OI test. RBANS test results were lost for 4 participants. We excluded data from 8 participants who had incomplete test scores or nasal congestion on the day of testing. The analytic sample then comprised 265 PREVENT-AD participants, including 100 INTREPAD lumbar puncture volunteers who had complete CSF biomarker and OI data. The participant pool included predominantly francophone and female individuals who were well educated. As expected, their proportion of *APOE* ε4 carriers was higher than population norms (table 1). The INTREPAD participants from whom we

Figure 2 Robust-fit linear regression models of UPSIT error score vs CSF biomarkers, stratified by *APOE* ε4 status



Red circles represent *APOE* ε4 carriers. Blue circles represent *APOE* ε4 noncarriers. (A) CSF t-tau/$A\beta_{1-42}$ in carriers ($\beta = 0.352$, $p = 0.0158$, n = 33) and in noncarriers ($\beta = 0.252$, $p = 0.131$, n = 67). (B) CSF P_{181}-tau/$A\beta_{1-42}$ in carriers ($\beta = 2.416$, $p = 0.0270$, n = 33) and in noncarriers ($\beta = 0.980$, $p = 0.408$, n = 67). (C) CSF t-tau in carriers ($\beta = 4.68 \times 10^{-4}$, $p = 0.0914$, n = 33) and in noncarriers ($\beta = 0.000312$, $p = 0.144$, n = 67). (D) CSF P_{181}-tau in carriers ($\beta = 2.76 \times 10^{-3}$, $p = 0.204$, n = 33) and in noncarriers ($\beta = 0.00178$, $p = 0.218$, n = 67). (E) CSF $A\beta_{1-42}$ in carriers ($\beta = -3.76 \times 10^{-4}$, $p = 0.00841$, n = 33) and in noncarriers ($\beta = 4.890e{-}05$, $p = 0.598$, n = 67). Aβ = β-amyloid; AD = Alzheimer disease; P_{181}-tau = phospho-tau; t-tau = total tau; UPSIT = University of Pennsylvania Smell Identification Test.

collected CSF were slightly younger than other INTREPAD participants and had a higher proportion of francophone individuals and slightly lower Montreal Cognitive Assessment scores. The CSF donors appeared demographically similar to the entire PREVENT-AD group of 274 PREVENT-AD enrollees (table 1 and table e-1 at Neurology.org).

In bivariate modeling, the UPSIT error score was higher in older participants (n = 265, $\beta = 0.0134$, $p = 2.24 \times 10^{-6}$; figure e-1) and in participants with lower RBANS total score (n = 261, $\beta = -0.00666$, $p = 1.28 \times 10^{-6}$; figure e-1). However, because we were interested especially in the CSF AD biomarker data, we also evaluated models restricted to the 100 participants who had both OI and CSF data. In the reduced sample, the statistical association with older age was seen only at a trend level ($\beta = 6.79 \times 10^{-3}$, $p = 0.095$; figure e-2), but the association of reduced OI with decreased cognition remained robust ($\beta = -4.76 \times 10^{-3}$, $p = 0.011$; figure e-2). Similar unadjusted analyses showed strong association between higher UPSIT error score and increased values of CSF t-tau/$A\beta_{1-42}$ ($\beta = 0.286$, $p = 4.94 \times 10^{-3}$), P_{181}-tau/$A\beta_{1-42}$ ($\beta = 1.77$, $p = 0.0165$), and CSF t-tau levels ($\beta = 3.61 \times 10^{-4}$, $p = 0.0257$; figure 1). There was a weaker but still suggestive relationship between increased UPSIT error score and elevated CSF P_{181}-tau ($\beta = 2.10 \times 10^{-3}$, $p = 0.0724$) but no relationship with $A\beta_{1-42}$ alone ($\beta = -7.01 \times 10^{-5}$, $p = 0.359$).

We observed no group difference in OI between *APOE* ε4 carriers and noncarriers (n = 262, $p = 0.271$). A similar result was observed in the reduced sample of 100 participants with CSF ($p = 0.7129$).

Table 2 Estimated coefficients from stepwise multiple linear regression modeling to predict UPSIT error score

Predictors of UPSIT error score	Estimated coefficients (SE)						
	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7
Age, y	0.0051 (0.004)			0.0013 (0.005)	0.0031 (0.004)		0.0001 (0.005)
RBANS		−0.0037 (0.002)		−0.0034 (0.002)		−0.0028 (0.002)	−0.0028 (0.002)
t-tau/Aβ_{1-42}			0.3250 (0.107)[a]		0.3068 (0.109)[a]	0.3159 (0.106)[a]	0.3150 (0.108)[a]
Sex (female = 1)	−0.0914 (0.047)	−0.0851 (0.047)	−0.0877 (0.045)	−0.0843 (0.048)	−0.0825 (0.046)	−0.0797 (0.046)	−0.0795 (0.046)
Years of education	−0.0036 (0.007)	−0.0039 (0.007)	−0.0003 (0.007)	−0.0038 (0.007)	−0.0001 (0.007)	−0.0003 (0.007)	−0.0003 (0.007)
APOE ε4 (carrier = 1)	−0.0270 (0.046)	−0.0361 (0.045)	−0.0844 (0.045)	−0.0341 (0.046)	−0.0757 (0.047)	−0.0871 (0.045)	−0.0869 (0.047)

Abbreviations: Aβ = β-amyloid; RBANS = Repeatable Battery for the Assessment of Neuropsychological Status; SE = standard error; t-tau = total tau; UPSIT = University of Pennsylvania Smell Identification Test.
This table looks at combinations of age, RBANS, and CSF t-tau/Aβ_{1-42} as predictors of odor identification. All models are adjusted for *APOE* ε4 carrier status, sex, and education. Because of different metrics used to measure the several variables, the various coefficients are not commensurable, but the indicated *p* values show the importance of individual variables in the overall model.
[a] $p < 0.01$.

However, previously observed correlations between UPSIT error score and several CSF markers of AD pathology were now apparent only in ε4 carriers. In contrast to unstratified samples, the carriers showed an association between higher UPSIT error scores and reduced levels of Aβ_{1-42} (n = 33, $\beta = -3.76 \times 10^{-4}$, $p = 0.00841$). In keeping with previously noted findings, UPSIT error score was associated in the ε4 carriers with higher t-tau/Aβ_{1-42} ($\beta = 0.352$, $p = 0.0158$) and P_{181}-tau/Aβ_{1-42} ($\beta = 2.416$, $p = 0.0270$), but a comparable association appeared only at a trend level for t-tau ($\beta = 4.68 \times 10^{-4}$, $p = 0.0914$) and not at all for P_{181}-tau ($\beta = 2.76 \times 10^{-3}$, $p = 0.204$; figure 2). In addition, we saw that individuals with lowest CSF Aβ_{1-42} levels appeared to have a higher proportion of *APOE* ε4 carriers (supplemental materials).

The multiple linear regression models assessed the relationships between OI and age, cognition, and CSF biomarkers added sequentially in models adjusted for sex, education, and *APOE* ε4 carrier status. Multivariable models from the full PREVENT-AD dataset (without CSF variables) showed strong associations of UPSIT error score with age and diminished cognition, either alone or in combination (table e-2). Table 2 shows comparable analyses in the reduced sample, now including CSF data. Models 1, 2, and 3 in table 2 (supplemental materials) indicate no association of UPSIT error score with age, a trend with cognitive score ($p = 0.06$), but a strong association with CSF t-tau/Aβ_{1-42} ($p = 0.003$). Model 4 suggests that the trend association with cognition was unapparent after adjustment for age ($p = 0.145$). Model 5 confirms the absence (in adjusted models) of any association between OI and age, and it shows the absence of a material effect of age adjustment on the association of OI with CSF t-tau/Aβ_{1-42}. Model 6 indicates that any association of OI with cognition became unapparent after the inclusion of CSF t-tau/Aβ_{1-42} (RBANS, $p = 0.151$; CSF t-tau/Aβ_{1-42}, $p = 0.004$). Model 7, which includes all the described variables, made these findings clearer by suggesting that OI was predicted by its relationship with CSF t-tau/Aβ_{1-42} ($p = 0.005$) regardless of age, cognition, *APOE* ε4 status, sex, or education (figure e-3). This last model explained 19.7% of the variance in OI score ($F_{7,90} = 3.68$, $p < 0.00258$). Both sensitivity analysis variants of model 7 produced nearly identical results (in the first analysis, $F_{12,85} = 2.47$, $p < 0.00993$; in the second, $F_{7,83} = 2.59$, $p < 0.0236$). The latter reduced model still explained 15.9% of the variance in OI score. Other similar multiple linear regression analyses (not shown) essentially reproduced the findings of model 7, substituting t-tau or P_{181}-tau alone or the ratio of P_{181}-tau/Aβ_{1-42} as independent CSF biomarker predictors.

DISCUSSION We investigated relationships of performance in OI with global cognitive scores, established AD risk factors, and several CSF biomarkers known to predict subsequent dementia. Our main finding was that a decrease in OI was associated with increasing CSF biomarker evidence of AD pathology. This association survived adjustment not only for sex, educational attainment, *APOE* ε4 carrier status, and potential brain and olfactory health history confounders but also for age or cognitive score. The relationships of OI performance with age and cognitive ability recapitulate earlier findings.[11,12] In the present sample, however, the relationship of OI with cognitive performance appears to be spurious because it represents the conjoint (confounded) relationship of both variables with the CSF biomarkers. We suggest that the relationship between OI and age (observed in the full dataset) may similarly represent a confounded association of these 2 measures with AD pathology (hence with related impairment in OI). To the best of our knowledge, there has been no previous direct demonstration that the association of OI with cognition is driven by a confounded relationship of both variables with AD pathology.

Overall, we observed no correlation of OI with CSF $A\beta_{1-42}$ levels alone. Such a relationship was observed, however, among individuals whose CSF $A\beta_{1-42}$ levels were in the lowest quartile. This subgroup had a greater proportion of *APOE* ε4 carriers (48%), which was close to the proportion typically seen in patients with AD. Thus, impairment in OI may in fact reflect cerebral accumulation of Aβ in these sicker participants, consistent with observations with amyloid PET.[6] This notion was reinforced in our work by a statistically significant interaction between *APOE* ε4 carrier status and CSF $A\beta_{1-42}$ levels as predictors of OI performance. The same was not so for global cognition, suggesting that OI may be an earlier indicator of accumulated pathology before symptom onset. Related recent work in transgenic mice expressing human *APOE* ε4 vs ε3 demonstrated genotype-specific structural differences in midlife. These modifications appear to precede later functional differences and increasing structural abnormality in brain regions related to olfaction.[27]

More generally, OI deficit is associated with presymptomatic AD pathology.[5] Reduced structural integrity of brain regions that subserve olfaction appears especially vulnerable to such pathology. These changes include reduced entorhinal cortical thickness and hippocampus and amygdala volumes,[6,16,28] as well as fibrillar amyloid accumulation in the posterior cingulate, temporoparietal, and lingual cortical regions.[6,29] Data from $^{15}O\text{-}H_2O$-PET experiments on olfactory-evoked regional cerebral blood flow also demonstrate that patients with AD have a pattern of functional activation different from healthy controls. Specifically, regional cerebral blood flow in the right frontotemporal area shows a correlation with OI.[30] CSF clearance is reduced in AD, relating to increased amyloid accumulation. Finally, dynamic monitoring of CSF has confirmed that the fluid reaches the nasal turbinates and clears through the cribriform plate.[31]

We note that our findings appear to contradict one recent report in which OI appeared not to be related to brain accumulation of amyloid as revealed by PET.[32] As we did, the authors of that study attempted to control for known detrimental olfactory health issues. They chose to exclude participants with such issues. In addition, the earlier research was characterized by intervals ranging up to 5 years between tests of OI and PET scans, whereas our work consistently tested OI within 3 months of CSF collection. A recent study demonstrated that OI deficits are more readily detected in patients with acute brain trauma when olfactory testing is performed within days of injury.[33] Recent PET studies of olfactory sensory neurons in lesioned, aging, or AD-like animal models further support this point.[34]

Limitations of this study include its high proportion of women and participants' level of educational attainment, attributes that are common in aging volunteer cohorts. Although we attempted to control for these factors in multivariable models, participants who volunteered for lumbar punctures may be an even more select population, an important concern because these 100 participants produced our most informative findings. Because our results come from cross-sectional observations only, it remains unknown whether altered biomarker levels represent a process of ongoing change and, if so, the rate at which such change is accumulating. Longitudinal studies for this purpose are now in progress, as are studies assessing the physical accumulation of amyloid and tau with specific PET tracers. Lastly, we acknowledge that our failure to observe a relationship between OI and age in the restricted sample may result from the limited sample size of the CSF donor participant pool. Generally, age is reported to be the strongest known predictor of impaired OI[35,36] and the best known predictor of AD dementia. However, at least some of this age-related loss in olfactory function may relate to factors unrelated to AD pathogenesis (e.g., deterioration of nasal epithelium or calcification of the cribriform plate[36]).

While impaired OI may in fact help identify persons who could for various reasons eventually have cognitive impairment, we strongly urge that our present cross-sectional results not be regarded as rationale for clinical use of olfactory testing as an AD diagnostic. We suggest, however, that OI may serve for research purposes as a simple and inexpensive indicator of evolving AD pathology. Indeed, we are exploring its use as a biomarker in clinical trials among asymptomatic persons at risk of later dementia symptoms. In this and other ways, OI may add valuably to the measures available for AD prevention research.

AUTHOR CONTRIBUTIONS

Marie-Elyse Lafaille-Magnan: study concept and design, data collection (RBANS and UPSIT), statistical analysis, data interpretation, drafting/revising the manuscript, accepts responsibility for conduct of research. John Breitner: study concept and design, data interpretation, drafting/revising the manuscript. Judes Poirier: study concept and design, data interpretation, revising the manuscript. Pierre Etienne and Pedro Rosa-Neto: study concept and design, revising the manuscript. Jennifer Tremblay-Mercier and Joanne Frenette: study concept and design.

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