Marie-Elyse Lafaille-Magnan

A thesis submitted to McGill University in partial fulfillment of the requirements of the degree of PhD in Neuroscience

February 2018

Supervisor: Dr. John C. S. Breitner

Title: Odor identification as a biomarker for progression of pre-symptomatic Alzheimer's disease

Integrated Program in Neuroscience

Faculty of Medicine

McGill University, Montreal

Table of contents

List of figures

List of tables

Abstract

Introduction: After several decades of research, we now understand that there is a long transition period from normal aging to dementia onset. Recently, preventive interventions have moved more upstream than ever before. This shift requires "measurement like" functional instruments or biological markers to reflect early Alzheimer neuropathological change. Previous association studies, imaging, and autopsy work had hinted that olfactory functions might be indicative of an underlying neurodegenerative disorder like AD. We sought to use odor identification as a measure of change and investigate its association with severity markers of AD in both cross-section and over time.

Method: Cognitively normal adults with a first-degree relative were given serial odor identification tests, measured using the University of Pennsylvania Smell Identification Test. Total tau (t-*tau*), phospho-*tau* (P_{181}-*tau*), β-amyloid ($A\beta_{1-42}$) were measured for those who volunteered to do lumbar punctures. We used Kruskal-Wallis and chi-square to evaluate group differences. We used robust fit linear regression methods to evaluate the effect of multiple variables entering AD risk or severity measures iteratively. We used linear mixed-effect model to assess decline in odor identification over a 2-year period. We assessed the association of repeated odor identification and AD measurements. We evaluated the effect of baseline AD severity (CSF biomarkers, hippocampal volume, global cognition) on longitudinal OI. Adjusted analyses considered age, cognition, APOE ε4 status, education, and sex as covariates. Longitudinal analyses were adjusted for treatment assignment, and time.

Results: Reduced odor identification was associated with lower cognitive score and older age, as well as increased ratios of CSF t-*tau* to $A\beta_{1-42}$. OI declined significantly over 2 years. In analyses that investigated change scores, an increase in t-*tau*/$A\beta_{1-42}$ predicted decreasing OI over two years at trend level. Higher baseline CSF t-*tau*/$A\beta_{1-42}$ predicted lower OI throughout the trial at trend level. Similarly, lower baseline OI predicted higher CSF t-*tau*/$A\beta_{1-42}$. Lower baseline immediate memory was associated with lower OI at any timepoint. Baseline global cognition and immediate memory portended declines in OI at trend level. Finally, there was no association between OI and hippocampal volumes.

Conclusion: This work contributes to the understanding of how odor identification could be a measurement for AD progression. Together, the findings from healthy high-risk older individuals suggest that odor identification reflects degree of pre-symptomatic AD pathology. Olfactory functions remain an attractive and easy way to measure early Alzheimer related changes. This method shows promising cost-effectiveness. This sensori-neural function deserves more attention, and further investigation in preventative trials.

Résumé

Introduction: Après plusieurs décennies de recherche, nous comprenons maintenant qu'il existe une longue période de transition entre le vieillissement normal et le début de la démence. Les interventions préventives sont de plus en plus en amont que jamais auparavant. Des instruments de mesure fonctionnels et des marqueurs biologiques sont nécessaires pour mesurer des changements dans la phase précoce de la maladie d'Alzheimer (MA). Des études d'association utilisant l'imagerie cérébrale et le travail d'autopsie ont suggéré que des troubles d'identification des odeurs pourraient indiquer un trouble neurodégénératif sous-jacent comme la maladie d'Alzheimer. Ces observations nous ont poussés à utiliser l'identification des odeurs comme mesure du changement et à étudier son association avec les marqueurs de sévérité de la MA dans sa phase pré-symptomatique.

Méthode : Des tests d'identification d'odeurs ont été administrés en série, en utilisant un test standardisé de l'Université de Pennsylvanie chez des adultes cognitivement normaux qui ont un parent atteint de la MA. Les niveaux de protéines *tau* totales (t-*tau*), *tau* phosphorylées (P_{181}-tau) et de β-amyloïde ($A\beta_{1-42}$) ont été mesurés dans un sous-groupe de volontaires chez qui des ponctions lombaires ont été effectuées afin de recueillir du liquide céphalo-rachidien (LCR). Les méthodes de Kruskal-Wallis et chi-carré ont été utilisées pour évaluer les différences entre les groupes de volontaires. Des méthodes de régression linéaire ont été appliquées pour évaluer l'effet de variables multiples en entrant des mesures de risque ou de sévérité de la MA de manière itérative (âge, cognition, éducation, sexe, statut *APOE* ε4, biomarqueurs du LCR). Pour évaluer le déclin de la capacité à identifier des odeurs sur une période de deux ans, un modèle linéaire à effets mixtes a été utilisé. Nous avons évalué l'association de l'identification des odeurs et des mesures de la MA répétées (biomarqueurs du LCR, volume de l'hippocampe, cognition globale) sur deux ans. Les analyses qui ont été ajustées le sont en fonction de l'âge, de la cognition, du statut *APOE* ε4, de l'éducation et du sexe Les analyses longitudinales ont été ajustées selon le temps et l'assignation à un traitement pharmacologique.

Résultats: Une capacité réduite à identifier les odeurs est associée à un score cognitif plus faible, à un âge plus avancé, ainsi qu'à des ratios élevés de t-*tau*/$A\beta_{1-42}$. La

capacité à identifier les odeurs a diminué de façon significative en deux ans. Une augmentation du ratio t-*tau*/$A\beta_{1-42}$ prédit une diminution de l'identification d'odeurs sur deux ans avec un faible niveau de confiance De même, une valeur initiale plus élevée du ratio t-*tau*/$A\beta_{1-42}$ prédit une capacité moindre à identifier les odeurs tout au long de l'étude. De même, la capacité d'identifier les odeurs plus bas au début prédit un ratio t-*tau*/$A\beta_{1-42}$ plus élevé tout au long de l'étude. Les résultats aux tests cognitifs initiaux (cognition globale ainsi que la mémoire de travail) prédisent le déclin des résultats d'identification d'odeurs. Aucune association entre les résultats d'identification d'odeurs et les volumes de l'hippocampe n'a été détectée.

Conclusion: Ce travail contribue à la compréhension de la façon dont l'identification des odeurs pourrait être une mesure de la progression de la MA. Ensemble, les résultats suggèrent que l'identification des odeurs reflète le degré de pathologie présent chez des individus sain à risque de développer la MA, considérés dans la phase pré-symptomatique. La mesure des fonctions olfactives demeurent un moyen attrayant et simple pour mesurer les changements précoces liés à la maladie d'Alzheimer. Cette méthode présente un rapport coût-efficacité prometteur et mérite son inclusion dans plus de recherches afin de mieux définir son utilisation.

Abbreviation list

5-HT serotonin
11C-PiB-PET 11C-labelled Pittsburgh compound B PET
ABCA7 ATP-binding cassette, sub-family A member 7
AC anterior commissure
AC anterior commissure
AChEI acetylcholinesterase inhibitor
ACIII or AdCy3 adenylyl cyclase 3
AD Alzheimer's Disease
ADNI Alzheimer Disease Neuroimaging Initiative
ADRDA Stroke and Alzheimer's Disease and Related Disorders Association
ANIMAL Automatic Nonlinear Image Matching and Anatomical Labeling)
AON anterior olfactory nucleus
AONc anterior olfactory nucleus (pars cortical)
AONi anterior olfactory nucleus (pars intrapeduncular)
AONpE anterior olfactory nucleus pars externa
AONr anterior olfactory nucleus (pars retrobulbar)
APA American Psychiatric Association
APOE apolipoprotein E
ApoE e4 Apolipoprotein E epsilon 4
APP Amyloid Precursor Protein
APS Alzheimer Progression Score
ATP adenosine triphosphate
Aβ beta-amyloid
Aβ1-40 beta-amyloid fragment
Aβ1-42 beta-amyloid fragment
BA Broca's area
BIN1 bridging integrator 1
BIOMARK-APD Biomarkers for Alzheimer's disease and Parkinson's disease
BOLD blood oxygenation level dependent
BSIT Brief Smell Identification Test
Ca++ Calcium
CA1 Cornu Ammonis or hippocampal area 1
CaMKII calcium and calmodulin-dependent kinase-II
cAMP cyclic adenosine monophosphate
CASS4 Cas scaffolding protein family member 4
CBF cerebral bloodflow
CD14 cluster of differentiation 14
CD2AP CD2-associated protein
CD33 or Siglec-3 sialic acid binding Ig-like lectin 3
CDK5 cyclin dependent protein kinase-5

CDR Clinical Dementia Rating Scale
CELF1 CUGBP Elav family member 1
CERAD Consortium to Establish a Registry for AD
Cl- Chloride
CLU clusterin
CN Cognitively Normal Controls
CN1 Cranial nerve 1
CNG cyclic nucleotide gated channel
COX-2 cyclooxygenase-2
CR1 complement component receptor 1
CSF cerebrospinal fluid
CTF-α C-terminal fragment α cleaved by α-secretase
CTF-β C-terminal fragment β cleaved by β-secretase
d-EPL deep EPL
DAG diacyl glycerol
DG dentate gyrus
DLB Lewy bodies dementia
DLPFC dorso-lateral pre-frontal cortex
DRB5 Human leukocyte antigen D related B5
dSA cell deep short-axon cell
DSM-V Diagnostic and Statistical Manual of Mental Disorders
EC entorhinal cortex
EEG electroencephalogram
EKG electrocardiogram
ENT ear nose throat specialist
Ent entorhinal cortex
EntRh entorhinal
EPHA1 Ephrin type-A receptor 1
EPL external plexiform layer
ERK extracellular signal related kinases
ERP event-relatd potential
ET cell external tufted cell
FAD familial autosomal dominant AD
FDG-PET Fluorodeoxyglucose PET
FERMT2 Fermitin Family Member 2
FH- no family history of AD–like dementia
FH+ family history of AD–like dementia
FL frontal lobe
fMRI functional Magnetic Resonance Imaging
FTD Fronto-temporal dementia
G-protein guanine nucleotide-binding protein
GAD glutamic acid decarboxylase
GCL granule cell layer

GDP gross domestic product
GL glomerular layer
GLM general linear model
GPCR G-protein coupled receptor
GSK-3B glycogen synthase kinase-3 beta
GWAS genome-wide association study
Gαolf G activating protein
H.M. Henry Molaison
HC or HCC hippocampal cortex
HDL High density lipoprotein
Hip hippocampus
HIV human immunodeficiency virus
HLA-DRB1 Human leukocyte antigen D related B1
HLA25 Human leukocyte antigen serotype A25
i-EPL intermediate EPL
ic internal capsule
ICC intracranial cavity
IDE insulin degrading enzyme
IFG inferior frontal gyrus
IGr indiseum griseum
IL-1β interleukin 1beta
In insula
INPP5D Phosphatidylinositol-3,4,5-trisphosphate
INTREPAD Investigations of Naproxen Treatment Effects in Pre-clinical Alzheimer's Disease
IP3 inositol triphosphate
IPL internal plexiform layer
JGcell juxtaglomerular cell
LDL Low density lipoprotein
LME linear mixed-effect
LN lenticular nucleus
LOAD Late onset AD
LV lateral ventricle
MAPK mitogen-activated protein kinases
MAPT microtubule-associated protein
MARK microtubule-affinity regulating kinase
MCI Mild Cognitive Impairment
MCL mitral cell layer
MCSA Mayo Clinic Olmsted Study of Aging
MEF2C Myocyte Enhancer Factor 2C
mGluRs metabotropic glutamate receptors
Mi mitral cell
MMSE mini-mental state examination

MND major neurocognitive disorder
MNI-ICBM152 Montreal Neurological Institute International Consortium for Brain Mapping 152
mL milliliter
MoCA Montreal Cognitive Assessment
MRI Magnetic Resonance Imaging
MS4A6A membrane-spanning 4-domains, subfamily A, member 6A
MS4A6E membrane-spanning 4-domains, subfamily A, member 4E
MTC medial temporal cortex
MTL medial temporal lobe
N.B. nota bene or to note
Na+ Sodium
NfL neurofilament light chain
Nfs neurofilaments
NFT Neurofibrillary Tangles
NGF Neuronal growth factors
NINCDS National Institute of Neurological and Communicative Disorders and
NMDA N-methyl-D-aspartate
NME8 N-terminal thioredoxin domain family memenber 8
OB olfactory bulb
OD Odor discrimination
OE olfactory epithelium
OF orbitofrontal
OFC orbitofrontal cortex
OI Odor identification
ONL olfactory nerve layer
ONs olfactory sensory neurons
OR Olfactory receptor
ORN olfactory receptor neurons
OSN Olfactory sensory neuron
OT olfactory tubercle
PC piriform cortex
PD Parkinson’s disease
PET Positron Emission Tomography
PG cell periglomerular cell
pg picogram
PHF paired helical filaments
PIB or PiB or 11C-PiB Pittsburgh compound B
PICALM phosphatidylinositol binding clathrin assembly protein
Pir piriform
Pir piriform cortex
PLC phospholipase C
POC Primary Olfactory Cortex

POC primary olfactory cortex
POC primary olfactory cortex
PP2A protein phosphatase 2
PREVENT-AD Pre-symptomatic Evaluation of Novel or Experimental Treatments for AD
PS1 Presenilin 1
PSEN1 Presenilin 1
PSEN2 Presenilin 2
PTK2B Protein tyrosine kinase 2 beta
RBANS Repeatable Battery for the Assessment of Neuropsychological Status
RBDSQ Rapid Eye Movement Disorder Screening Questionnaire
REML restricted maximum likelihood
RMS rostral migratory stream
ROI Region of Interest
s-EPL superficial EPL
sAPPα soluble extracellular fragment cleaved by α-secretase
sAPPβ soluble extracellular fragment cleaved by β-secretase
SD standard deviation
SEN or Sen Odor sensation
SLC2A4A solute carrier 2 facilitated glucose transporter, member 4
Sn Sniff press
SNP single nucleotide polymorphism
SORL1 sortilin related receptor 1
SPECT single-photon emission computed tomography
sSA cell superficial short-axon cell
SToP-AD study of Alzheimer's disease in its pre-symptomatic stages
Sup Occ gyrus superior occipital gyrus
SUVR standardized uptake value ratio
SVZ subventicular zone
SVZ subventricular zone
T1w T1 weighted image
TCd tail of caudate nucleus
TE/TR echo time/ repetition time
TH tyrosine hydroxylase
TL temporal lobe
TLV temporal lateral ventricle
TNFα Tumor necrosis factor alpha
TSR trim scoring regression
UPSIT University of Pennsylvania Smell Identification Test
USD United States Dollar
VGAT vesicular GABA transporter
VGLUT vesicular glutamate transporter
Viz. videlicet or namely or that is to say

ZCWPW1 Zinc finger CW-type and PWWP domain containing 1

Acknowledgements

I would like to thank first and foremost the PREVENT-AD participants who gave their consent and participated in the olfactory identification study. I would like to thank all the SToP-AD nurses, research assistant, physicians, students, and affiliated members.

I appreciate the interest Dr. John C. S. Breitner has shown towards my project and opportunities I have had during his tutelage. I am grateful for the time he has taken to teach me his craft. Thank you for poking at the holes in my logic. I am grateful you constantly challenged me and made me defend my arguments relentlessly.

I would like to thank Dr. Marilyn Jones-Gotman and Dr. Andrea Leblanc for their valuable advice throughout my graduate studies, both members of my PhD committee.

I would like to also thank Dr. Judes Poirier, Dr. Pedro Rosa-Neto, Dr. Véronique Bohbot, Dr. Katarina Dedovic, Dr. Tobias Luck, Dr. Vinod Venugopalan, Dr. Pierre Etienne, Dr. Yasser Iturria-Medina, Dr. Jeannie-Marie Leoutsakos, Dr. Gabriel Devenyi, Dr. Mallar Chakravaty, Dr. Marina Tedeshi-Dauar, Dr. Kyoko Konishi, Dr. Louisa Dhamani, Dr. Natasha Rajah, Dr. Blandine Courcot, Karina Borja, Dr. Renee Gordon, Dr. David Maillet, Dr. David Fontaine, Dr. Villeneuve for your questions, your advice, your training and your guidance throughout these years as a graduate student.

I am grateful for the many times Thomas Beaudry, Dr. Tobias Luck, Dr. Vinod Venugopalan, Melissa Savard, Miranda Tuwaig, Angela Tam, Cécile Madjar, Jennifer Tremblay-Mercier, Melissa Appleby, Laura Mahar, Galina Pogossova, Isabelle Vallée-Beaupré, Fabiola Ferdinand, Marianne Dufour, Christina Kazazian, Pierre-François Meyer, Alexa Pichette Binette, Dr. Cynthia Picard, Justin Miron, Nathalie Nilsson, Yasser Iturria-Medina, Karen Wong, Renuka Giles have listened and helped me over the past couple years.

Thank you, David Maillet, for introducing me to Matlab. Thank you, John Breitner, Mélissa Savard, Cécile Madjar, Blandine Courcot, Yasser Iturria-Medina, Gabriel

Devenyi, Jeannie-Marie Leoutsakos, Pierre-François Meyer, Thomas Beaudry, for making me push through my boundaries and learn proper analytical and programming skills to exploit modern analytical methods.

Thomas Beaudry, thank you for rescuing me with your great shell scripting skills.

I appreciate the excellent lecturers I was lucky to have during my PhD: Claire Infante-Rivard, Brenda Milner, Claudio Cuello, Michael Petrides, Edith Hamel.

I would like to thank Yvonne Yau, Pascal Kropft, Dorelle Hinton, Chiu-An Lo, Ayokunle Olanrewaja, Anthony Gifuni and all other members of the McGill Peer Writing Groups I was involved with.

I am glad for the people I met in class and collaborating labs. Special thanks also go to my friends, cousins, and aunts and uncles; I would particularly like to thank the following: Sara Boussekri, Katarina Dedovic, Shaun Pancham, Angela Tam, Soraya Esmail, Jacques Magnan, Marc-André Lafaille Magnan, Marc-André Argentino, Valérie Sekla, Valérie Nantel, Miranda Tuwaig, Mélissa Savard, Daphnée Lalonde, Nolan Mills, Sarah Legault, Rosanne DeWolfe Sonja Damika, Will Sanderson and Sabrina March. I am immensely grateful for your friendship, support, and laughs.

To Tom, you have been supportive of my goals. You have helped me get through it all. I love you immensely. I am lucky to call you my husband. No words will ever describe how grateful I am for all of the opportunities you have given me.

To my brother, brother-in-law, and Liba, thank you for all the babysitting you have done and for acting as a personal assistant.

To my mom and dad, thank you for raising me and your lifelong love care and support.

To my mom, thank you for traveling to Washington and babysit while Tom and I attended our conference.

To Victoria-Elizabeth, I am so proud to show you what I was able to accomplish during your early years.

Finally, I would like to thank my family for their encouragement and, especially, my husband for his love and support, which made this thesis possible. I dedicate this thesis to him and Victoria-Elizabeth. Thanks for enduring me, when there was a cloud over my head.

In the words of Arnold Schwarzenegger: “I could not have done it alone”.

Funding

I would like to thank Dr. John Breitner, StoP-AD centre, and the Lazlo & Etelka Kollar Fellowship for their financial support along with travel support from IPN and the Alzheimer Association International conference.

This work was supported by McGill University, the Douglas Mental Health University Institute Research Centre, Canada Foundation for Innovation, Fonds de recherche Santé Québec, Pharmascience Inc. and Pfizer.

Contributions of authors

Contribution to the overall thesis:

Ethics and funding: Marie-Elyse Lafaille-Magnan, Dr. John Breitner, Jennifer Tremblay-Mercier and Joanne Frenette

Study design: Marie-Elyse Lafaille-Magnan, Dr. John Breitner

Consent and nursing: Joanne Frenette, Ginette Mérand, Isabelle Vallée-Beaupré, Fabiola Ferdinand, Rana El Khoury, Valérie, Gerda Duclerc

Data collection by student and research assistant: Marie-Elyse Lafaille-Magnan, Karen Wan, Galina Pogossova, Laura Mahar, Melissa Appleby, Tanya Lee, Renuka Giles, Vinod Venugopalan

Data analysis: Marie-Elyse Lafaille-Magnan, Mélissa Savard, Jeannie Leoutsakos

Preparation of the thesis: Marie-Elyse Lafaille-Magnan and Dr. John Breitner

Proofreading and draft editing: Marie-Elyse Lafaille-Magnan, Dr. John Breitner, Thomas Beaudry, Angela Tam, Renuka Giles, Miranda Tuwaig, Marina Tedeschi Dauar, Justin Miron, Christina Kazazian, Dorelle Hinton, Judith Sabetti, PWG members

Chapter 1:

Marie-Elyse Lafaille-Magnan: Literature review, writing of text, cartoon

John Breitner: Editorial comments

We obtained the rights to reprint previously published figures in this thesis and not for commercial purposes (credit attributed for each individual figure in chapter)

Chapter 2:

Marie-Elyse Lafaille-Magnan: Literature review and writing of text

John Breitner: Editorial comments

Jeannie-Marie Leoutsakos: Power analysis table and description of her analysis
We obtained the rights to reprint previously published figures in this thesis and not for commercial purposes (credit attributed for each individual figure in chapter)

Chapter 3:

Marie-Elyse Lafaille-Magnan: Design of study, Data collection, Analysis, Writing of Manuscript
John Breitner: Design of study, Writing of Manuscript, Editorial comments
Pedro Rosa-Neto: Design of study, Data collection, Editorial comments
Judes Poirier: Design of study
Pierre Etienne: Design of study, Data collection
Joanne Frenette: Design of study, Data collection
Jennifer Tremblay-Mercier: Coordinator of Study
Prevent-AD group: Data collection

Chapter 4:

Marie-Elyse Lafaille-Magnan: Design of study, randomization of drug assignment, data collection, Mean and linear equating for RBANS adjustment, X^2, Kruskal-Wallis, linear regressions, LME, Writing of manuscript, figure
John Breitner: Design of study, Editorial comments
Pedro Rosa-Neto: Design of study, Data collection
Louis Collins: Design of study, MRI analysis
Vladimir Fonov: Design of study, MRI analysis, and writing of MRI processing method
Mallar Chakravarty: Proofread imaging content (MRI method and discussion)
Jeannie-Marie Leoutsakos: general statistics advice and design of equating method
Judes Poirier: Design of study
Prevent-AD group: Data collection

Anne Labonté: amyloid$_{1-42}$, tau, p-*tau*

Chapter 5: Discussion

Marie-Elyse Lafaille-Magnan: Writing of text, and figures

John Breitner: Editorial comments

Appendix:

Marie-Elyse Lafaille-Magnan: Amendment to protocol, writing of consent form, Ethics application

Preface to the thesis

There are difficulties in relying on only cognitive outcomes to detect the progress of AD in the healthy elderly (chapter 1). Nonetheless, we are resolved to explore cognitive tests as correlative measures alongside biological markers, imaging assays, and sensory-neural functions of disease progression. Olfactory dysfunction is an important marker of neurological diseases.[1-4] Early protein accumulation, atrophy, and synaptic loss in areas of the brain involved in odor identification processing may be detected in analyses using MRI, CSF proteins, and cognitive and olfactory performance alteration (Chapters 1 & 2).

We investigated whether olfactory identification relates to a probable ongoing disease state. Because structures involved in processing odors are affected by AD neuropathology (Chapter 2), we first examined odor identification in relation to cross-sectional AD biomarker data (brain imaging, CSF proteins, and cognitive testing; Chapter 3).[5] Secondly, we examined whether poor performance on olfactory identification tests predicted brain changes related to the progression of AD. These studies examined the earliest (pre-clinical) stages of AD development by performing these experiments in cognitively normal participants with a first-degree relative affected by Alzheimer dementia (Chapter 4).

Our overarching hypothesis is that ongoing change, such as a reduction of neurogenesis, synaptic dysfunction, synaptic loss, increased cell death, atrophy, and inflammation in the olfactory bulb and primary olfactory cortex, lead to olfactory disruption. We therefore predicted that odor identification could monitor brain health and reflect functional brain changes over time (Chapter 4). We expected that the relationship of olfactory identification to multiple MRI and biochemical measurements could advance our understanding of age-related and AD neuropathology-related changes (Chapter 2). This thesis explored if olfactory identification testing could be used as a biomarker for

AD (chapter 3 & 4). Thus, it expands current understanding of the relation between olfactory identification, cognition, and *in vivo* neuropathology of AD (Chapters 3 & 4).

This thesis is presented in the manuscript format for Doctoral thesis as described by the Department of Graduate and Postdoctoral Studies. Chapter 1 is an introduction and review of literature relevant to this thesis. Chapter 2 is a review of the anatomy and physiology of the brain's olfactory system, originating with olfactory receptors themselves. Chapter 3 was published in *Neurology*.[5] Chapter 4 is a more detailed version of a second manuscript that we intend shortly to submit to *Neurology*. Chapter 5 is a summary and discussion of findings from Chapter 3 and Chapter 4 and a consideration of future directions. Also presented, as appendices are a series of published abstracts on related topics. All this work was prepared with mentorship and guidance from my supervisor, Dr. John Breitner. Most of the work represents collaborative efforts with the PREVENT-AD Research Group.

Original contributions to knowledge

In the first of this thesis's 'three publishable manuscripts,' the review (Chapter 2), we describe odor identification and present anatomical and functional data that explain why odor identification is expected to decline during AD progression. This manuscript explains the multiple mechanisms that make olfactory functions vulnerable to AD. It suggests the potential use of odor identification in prevention and treatment trials, among which it has been used little if at all.

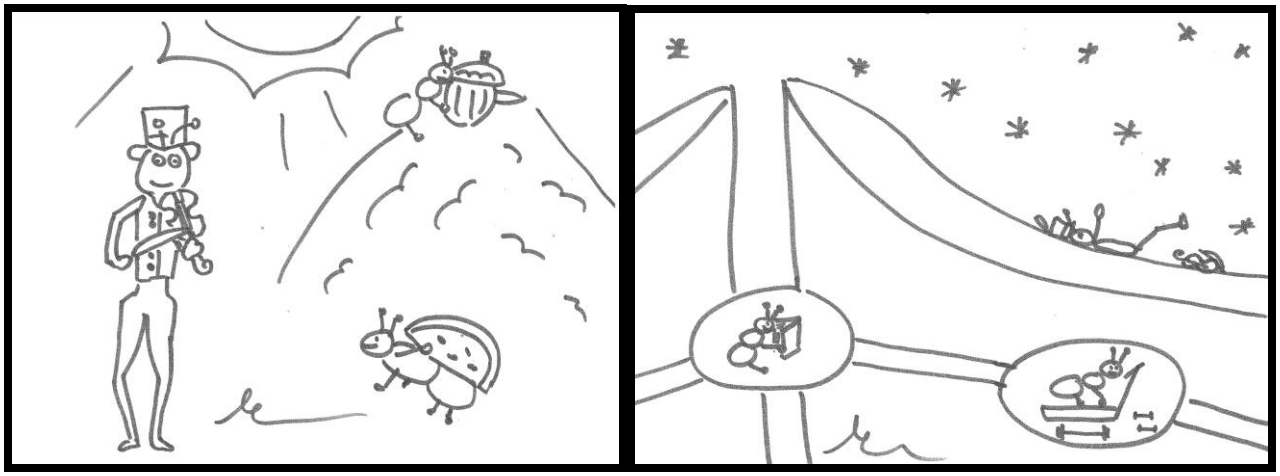
The second manuscript is an original scientific paper published in *Neurology* (the principal journal of the American Academy of Neurology; impact factor 8.32 in 2016) with public access.[5] As of 2 February 2018 this manuscript had been downloaded 1571 times. The work received overwhelming attention and currently has an Altmetric score of 268, placing it in the 99th percentile of 9,000,597 research outputs tracked by Almetric. This manuscript places #52 out of the 10,000+ outputs from *Neurology,* and has been already commented and cited by other scholars. The publication presented for the first time a description of transparent and robust associations between odor identification and cerebrospinal fluid (CSF) biomarkers measures related to AD neurodegeneration. Methods were rigorous and controlled extensively for covariates. It noted that AD-related CSF biomarkers predict variance in odor identification in cognitively normal individuals at risk for AD dementia. These novel findings were also described after stratification of the study population by presence of the strong AD genetic risk factor, *APOE* ε4. Although such stratification revealed no ε4 carrier effect on OI in the entire dataset (about half of which lacked CSF), we did find that association between OI and CSF biomarkers of AD were attributable principally to ε4 carriers.

Finally, the third manuscript, in preparation for submission to *Neurology*, uses longitudinal data with repeated measures to replicate the main findings that odor identification is related to CSF biomarkers of AD. It further expands on its predecessor

by investigating specific domains of cognition and hippocampal volume for their possible association with OI. A key finding of this manuscript is that there is a readily detectable decline in OI performance over a relatively brief 2-year interval of observations. In addition to reinforcing our previous findings, this last manuscript also shows that OI (which is relatively easily measured) can predict functional decline, which is a different question of whether AD biomarkers predict OI. Altogether, these manuscripts suggest that OI reflects an AD-related process and may be useful as a tool for recruiting participants for AD prevention trials, or for monitoring the progression of their disease before such progression is evident from crude symptoms.

CHAPTER 1: Alzheimer's disease and the need for pre-symptomatic biomarkers

La cigale et la fourmi - Jean de Lafontaine



"By failing to prepare,

you are preparing to fail"

Benjamin Franklin

1. Alzheimer's disease and the need for pre-symptomatic biomarkers: a literature review

1.1 What is Alzheimer's disease?

1.1.1 Alzheimer dementia

Alzheimer's disease (AD) is a neurodegenerative disorder of the brain that has specific pathological signature. However, the disease does not imply the existence of dementia. Instead, it is widely recognized that AD evolves over a period of several decades from clinically imperceptible early changes in brain through milder symptomatic states, *viz*. Mild Cognitive Impairment (MCI), and, in most instances, eventually to the characteristic dementia, which was used in the past as a synonym for the disease. To help avoid confusion between the underlying disease and its familiar dementia, the latter is best referred as AD dementia or "Alzheimer's dementia"). AD dementia is the most common form of dementia in the elderly. Studies over the prior decades showed that the emergence of cognitive impairment is provoked by synaptic dysfunction, widespread neurodegeneration and pathological protein accumulation (i.e. *tau* neurofibrillary tangles and neuritic plaques that are loaded with aggregated amyloid-β. This chapter will review these characteristic brain changes.

Clinically, late onset AD (LOAD, which constitutes at least 99% of all AD) appears initially with gradual memory impairments specifically, affecting semantic memory abilities[6], although there is increasing recognition also of executive dysfunction (much more difficult to measure) as an early symptom.[7-10] Language dysfunction commonly compounds patients' memory difficulties. Thus, patients may have early difficulty in naming animate objects (i.e., animals and people).[11,12] Later, more intense difficulties with other cognitive functions including executive and visuospatial skills and attention begin to impair functional abilities owing to poor judgment, loss of ability to reason or plan, and a tendency to get lost. Commonly, these more extreme symptoms

alert family and caregiver and motivate them to seek medical attention. At this point the diagnosis of AD is often made with cognitive test batteries that assess the presence of characteristic neuro-psychological features and based on reliance of neurological examination and imaging.[13-15] Ultimately, there are devastating losses in language (aphasia, both oral and written, receptive and expressive), coordination of movements or ideas (apraxia) or recognition of familiar persons or objects (agnosia).[16]

Presence of neuropsychiatric symptoms (NPS), such as agitation, apathy or aggressiveness, are often apparent before the advent of dementia, e.g., in MCI patients.[17] Interestingly, NPS are increasingly recognized as risk factors for conversion from MCI to AD dementia,[18] probably because they are common in AD and may be less characteristic of MCI. In any event, they can substantially exaggerate difficulties with care. Thus, presence of delusions, agitation, and depression render management more difficult. Additionally, patients with AD suffer from insomnia and/or sleep disturbances.[19] As part of their difficulties with praxis, dementia patients may be unable to use particular limbs because they cannot coordinate the needed information between brain and muscle.[16] Similarly, executive dysfunction in AD patients can lead to disorganized planning, problems initiating, or controlling of movements,[20] which may explain the increased risk of falls among patients with dementia.[21] Ultimately, this disease destroys all abilities to function independently so that persons affected by later-stage AD dementia become heavily dependent on caregivers or nursing assistants. The end comes commonly as a result of inability to coordinate swallowing movements resulting in aspiration of foodstuffs, causing pneumonia or other upper respiratory problems.[22]

Alzheimer's disease is a grave public health concern. *The World Alzheimer Report 2015: The Global Impact of Dementia* reveals that every 3 seconds someone develops dementia.[23] That report projects that 74.7 million people will have dementia in 2030, and 131.5 million worldwide by 2050.[23] The global cost of treating AD in 2015 was estimated at $818 billion USD, which represents 1.09% of the global GDP.[23] Alzheimer's disease presents an excessive and increasing toll on society. Thus, AD prevention is an urgent priority. To understand the current state of this objective, we will

review what has been known about AD (the disease, apart from its symptoms) from its first description to our current understanding, known causes, risk and protective factors, as well as the nature of the transition period before dementia onset.

1.1.2 First descriptions of Alzheimer's disease

Alzheimer disease (AD) was named after Alois Alzheimer, a psychiatrist who first described the famous case of Auguste Dieter in a paper presented on November 4th 1906. Frau Dieter was hospitalized at the age of 47, presenting with delusions of morbid jealousy along with other neuropsychiatric symptoms like hallucinations.[24] Alzheimer's observations revealed a marked aphasia, and cognitive decline thereafter was severe and rapid, causing total loss of daily functional abilities.[25,26] When Frau Dieter died at 51, Alzheimer examined her brain postmortem and described extensive atrophy. Microscopic examination revealed abundant extracellular plaques as well as numerous intra-neuronal filamentous tangles.[25-27] Perusini subsequently examined the histopathology of 4 cases, including Frau Dieter's, and illustrated these characteristic lesions.[26,28]

1.1.3 Histological Hallmarks of Alzheimer's disease

Further histologic examination, including histochemistry, of the brains of patients dying with AD led to more complete descriptions of the disease as being characterized by (1) the deposition of amyloid plaques in the extracellular space, and (2) by the intra-neuronal neurofibrillary tangles in the brain.[29-31] For a century, these characteristic plaques and tangles were studied through post-mortem staining of brain slices. Over the past 30 years, however, biochemical methods have been used to isolate the components of these lesions from tissue and cerebrospinal fluid (CSF), while *in vivo* imaging techniques have enabled visualization and quantitation of AD neuropathology. With these newer methods it is no longer necessary to await death (and post-mortem examination of brain) to achieve a confident diagnosis of AD. Importantly, in this time it has also become possible to demonstrate and measure Alzheimer neuropathology

before clinical symptoms emerge.[32,33] Quantification of misfolded proteins from PET imaging and CSF collection methods have been validated by studying their concordance with autopsy examination of AD and control brains.[34-37] These more recent methods can also be used to help track the disease *in vivo*. After case ascertainment at autopsy, CSF $A\beta_{42}$ was identified as the most sensitive single biomarker for AD diagnosis vs. controls.[38] However, the ratio of CSF total-*tau* to $A\beta_{42}$ had a high sensitivity in predicting conversion from MCI (by that stage, advanced amyloid changes in CSF are typically well established) to dementia.[38] Finally, in addition to plaques and tangles, AD neuropathological features include synaptic and neuronal loss.[39-41] The spreading of these pathological features occurs progressively as the severity of symptoms increases. In theory, these observations suggest that if we can identify someone on a disease trajectory early enough, we may attempt to modify their disease course, mitigate symptom appearance or, at the very least, plan for the future.

1.1.3.1 Amyloid pathology

Amyloid plaques, first described as “senile plaques”, consist mainly of aggregated amyloid-β peptide, surrounded by dystrophic neurites.[42] The protein fragments come from proteolytic cleavage of the Amyloid Precursor Protein encoded by the *APP* gene on the long arm of Chromosome 21). Two alternate cleavage pathways were discovered by which the precursor can be broken down (Fig. 1-1). In the non-amyloidogenic cleavage pathway, the so-called α-secretase enzyme cleaves near the middle of the Aβ region to produce a soluble extracellular fragment (sAPPα) and a C-terminal fragment-α CTF-α.[43] In the alternate pathway, β-secretase, a subunit of the BACE protease complex, cleaves the precursor and generates sAPP-β and the C-terminal fragment beta CTF-β.[44] Subsequently, the so-called ɣ-secretase domain including protein presenilins 1 and 2 (encoded by *PS/1* on Chromosome 14 and *PS/2* on Chromosome 1) cleaves CTF-β in its intra-membrane region into Aβ fragments that are typically 48-52 amino acids long along with soluble CTF- ɣ. The longer Aβ monomers are quickly cleaved by several alternate pathways to the more stable species

known as $A\beta_{1-40}$ and $A\beta_{1-42}$ or (less commonly) $A\beta_{1-38}$. $A\beta_{1-40}$ and, especially, $A\beta_{1-42}$ are hydrophobic and lipophilic; when not bound with cell or organelle membranes they have a tendency to aggregate into oligomers and higher-order insoluble polymeric cross-β fibrils and accumulate in the extracellular space.[45,46] Ultimately, these fibrils precipitate into what we call “plaques” in the extracellular space rather than being excreted directly via the CSF.

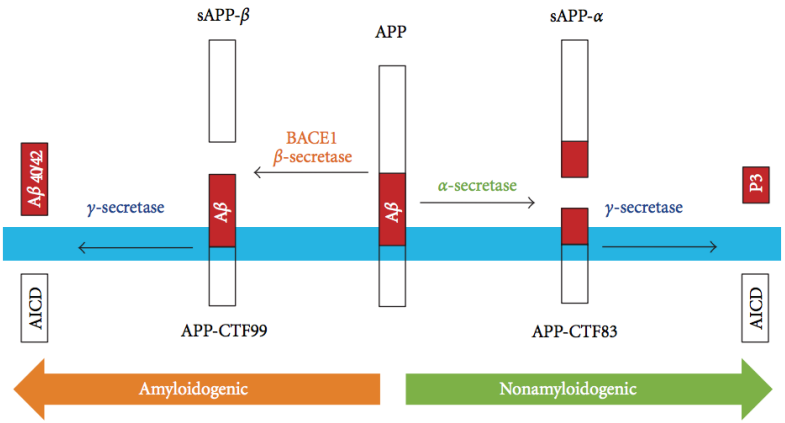


FIGURE 1: A diagram of amyloid precursor protein (APP) processing pathways. The transmembrane protein APP (membrane indicated in blue) can be processed by two pathways, the nonamyloidogenic α-secretase pathway and the amyloidogenic β-secretase pathway. In the nonamyloidogenic pathway, α-secretase cleaves in the middle of the β-amyloid (Aβ) region (red) to release the soluble APP-fragment sAPP-α. The APP C-terminal fragment 83 (APP-CTF83) is then cleaved by γ-secretase to release the APP intracellular domain (AICD) and P3 fragment. In the amyloidogenic pathway, β-secretase cleaves APP to produce the soluble fragment sAPP-β. APP-CTF99 is then cleaved by γ-secretase to produce Aβ_{40}, Aβ_{42} and AICD.

Figure 1-1 Two alternate cleavage pathways of APP

In the non-amyloidogenic cleavage pathway, α-secretase cleaves the Aβ region of the APP to produce a soluble extracellular fragment (sAPPα) and a C-terminal fragment-α CTF-α.[43] In the amyloidogenic pathway, β-secretase, a subunit of the BACE protease, cleaves APP and generates sAPPβ and the C-terminal fragment beta CTF-β.[44] Subsequently, the ɣ-secretase domain of presenilins 1 and 2 cleaves CTF-β in its intra-membrane region into Aβ monomers of 36 to 42 amino acids long and soluble CTF- ɣ. This figure was published in Hui Wang et al., 2012 and reproduced here by permission under the Creative Commons Attribution License.[47]

The amyloid hypothesis of AD pathogenesis suggests that Aβ oligomers disrupt synapses, leading in turn to neuronal dysfunction, synaptic loss, and neuronal death causing AD dementia,[48-52] although this idea remains criticized due to inconclusive results from amyloid-targeted treatment trials.[53] There are endogenous metabolisms and clearance mechanism for Aβ species. The Aβ monomers can be broken down by the insulin-degrading enzyme (IDE), neprilysin, and endothelin-converting enzyme.[54] Astrocytes and microglia can also clear Aβ peptides.[55] CSF has the ability to clear soluble APP metabolites from the brain and carry them to the lymphatic system.[56] While the normal function of APP remains unclear, it is argued that APP may act in cellular signaling cascades, as both a growth factor and receptor[57-59] and/or to modulate bcl-2, an anti-apoptotic protein.[60]

Through extensive post-mortem histological staining of brains at various Alzheimer neuropathological stages, Thal and his colleagues established a temporal and spatial pattern of diffuse and dense amyloid accumulation that typifies AD progression.[61] The neuropathology is categorized into 6 phases: in phase 0 there is no amyloid; in phase 1 there is accumulation in the isocortex; in phase 2, there is accumulation in the limbic cortex; in phase 3, there is amyloid in the basal ganglia; in phase 4, there are deposits in the basal forebrain and midbrain; and finally, in phase 5, the amyloid accumulation reaches the pons/medulla oblongata and cerebellum.[61,62] The Consortium to Establish a Registry for AD (CERAD) introduced an alternate way to study amyloid patterns using three stages (A-C) that qualify the extent of dense amyloid plaque deposits.[29] The CERAD score reflects the abundance/frequency of observing plaques in three brain regions (frontal, temporal, and parietal). The Thal stages and the CERAD score are complementary and are both currently used to characterize AD pathology post-mortem.[62]

1.1.3.2 Tau pathology

Tau proteins are microtubule-associated protein (MAPT) that are integral to the neuronal cytoskeleton. *Tau* has a many phosphorylation sites of which usually a few are phosphorylated. In AD, however, there is increased phosphorylation. Normally, *tau* assembles and supports microtubules by interacting with tubulin in axons.[63] *Tau* plays a dynamic role in migration, axon guidance, and intracellular transport, specifically, axonal transport.[64]

Kinases are enzymes that catalyze protein phosphorylation. Kinases can potentially lead to increased phosphorylation of *tau*, hyperphosphorylation, through upregulation of their activity (Fig. 1-2).[65-69] These kinases include glycogen synthase kinase-3 beta (GSK-3B),[70] cyclin dependent protein kinase-5 (CDK5),[71-73] microtubule-affinity regulating kinase (MARK),[74] calcium and calmodulin-dependent kinase-II (CaMKII),[75] as well as mitogen-activated protein kinases (MAPK)[76] and extracellular signal related kinases (ERK) 1 and 2.[77] Phosphatases are enzymes that catalyze protein dephosphorylation. Downregulation of phosphatases such as protein phosphatase 2 (PP2A) can also lead to increased *tau* phosphorylation.[78,79] Several conditions (i.e. pesticides, aluminum, hypoxia) are thought to activate kinase and inhibit phosphatase activity.[80] Hyperphosphorylation enables *tau* to polymerize into paired helical filaments (PHF; Fig. 1-2). These filaments in turn complex into neurofilaments (Nfs) and make up neurofibrillary tangles (NFTs; Fig. 1-2). Increased phosphorylated *tau* (P-*tau*) levels, beyond those of a healthy brain, accumulate as NFTs inside neuronal cells (Fig. 1-2).[81,82] Therefore, elevated P-*tau* levels can disrupt the structural cell integrity and lead to axonal degeneration and cell death.[82-84]

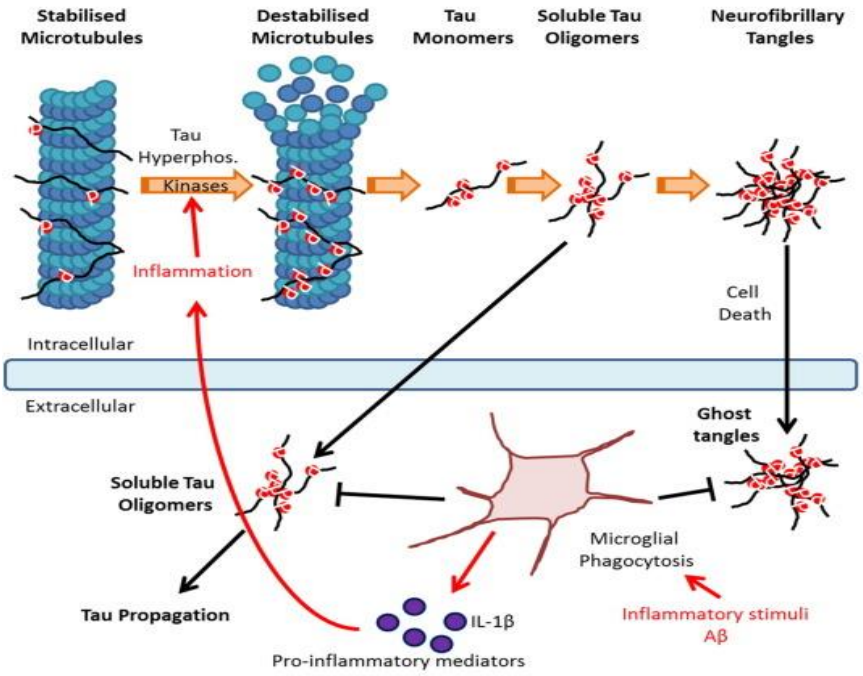


Figure 1-2 Formation of neurofibrillary tangles

Progression of tau pathology: Under physiological conditions tau regulates microtubule stabilisation. In tauopathies, tau hyperphosphorylation triggers a loss in microtubule affinity. Soluble tau aggregates into pathological soluble tau oligomers, ultimately forming pathological insoluble neurofibrillary tangles (NFT). Tau oligomers are secreted into the extracellular compartment contributing to the propagation of tau pathology into neighbouring neurons. Inflammatory stimuli, such as Aβ, stimulate microglial production of pro-inflammatory mediators such as IL-1β leading to the up-regulation of kinases involved in tau phosphorylation and exacerbation of the pathology. However, inflammation can have beneficial effects on tau pathology by inducing microglial phagocytosis of extracellular tau species. Image adapted from National Institute of Ageing. These caption and figure were published in Barron, 2017 and reproduced here by permission under the Creative Commons Attribution License.[85]

Similar to the Thal stages, the Braak stages (I-VI) describe the temporal and topographic maps of NFT accumulation (Fig. 1-3). Through extensive post-mortem

histological staining of brains at various AD dementia stages, Braak found that tangles typically appear in the entorhinal cortex (EC), and spread to the hippocampus, thalamus, and basal magnocellular complex, culminating in the neocortex as the disease progresses.[30,86] The appearance of cognitive symptoms usually indicates that NFTs have spread to the subcortical structures involved in memory processes.[87] The NFT density in the entorhinal cortex correlates with MCI status.[88,89] In fact, the presence of NFTs is more homogeneous in patients than amyloid plaques and correlates more strongly with cognitive decline.[86,90] Whereas, amyloid accumulates in cognitively normal individuals and reaches a plateau as dementia emerges,[85,91-94] tau pathology appears to accrue into much more advanced disease stages. Importantly for this thesis, Christen-Zaech (2003) identified neuropil threads and NFTs in the olfactory bulb and tract in early AD-related neurodegeneration.[95] The NFT protein accumulation that is typical of AD eventuates in progressive cell death, neuronal loss, and atrophy.[96,97]

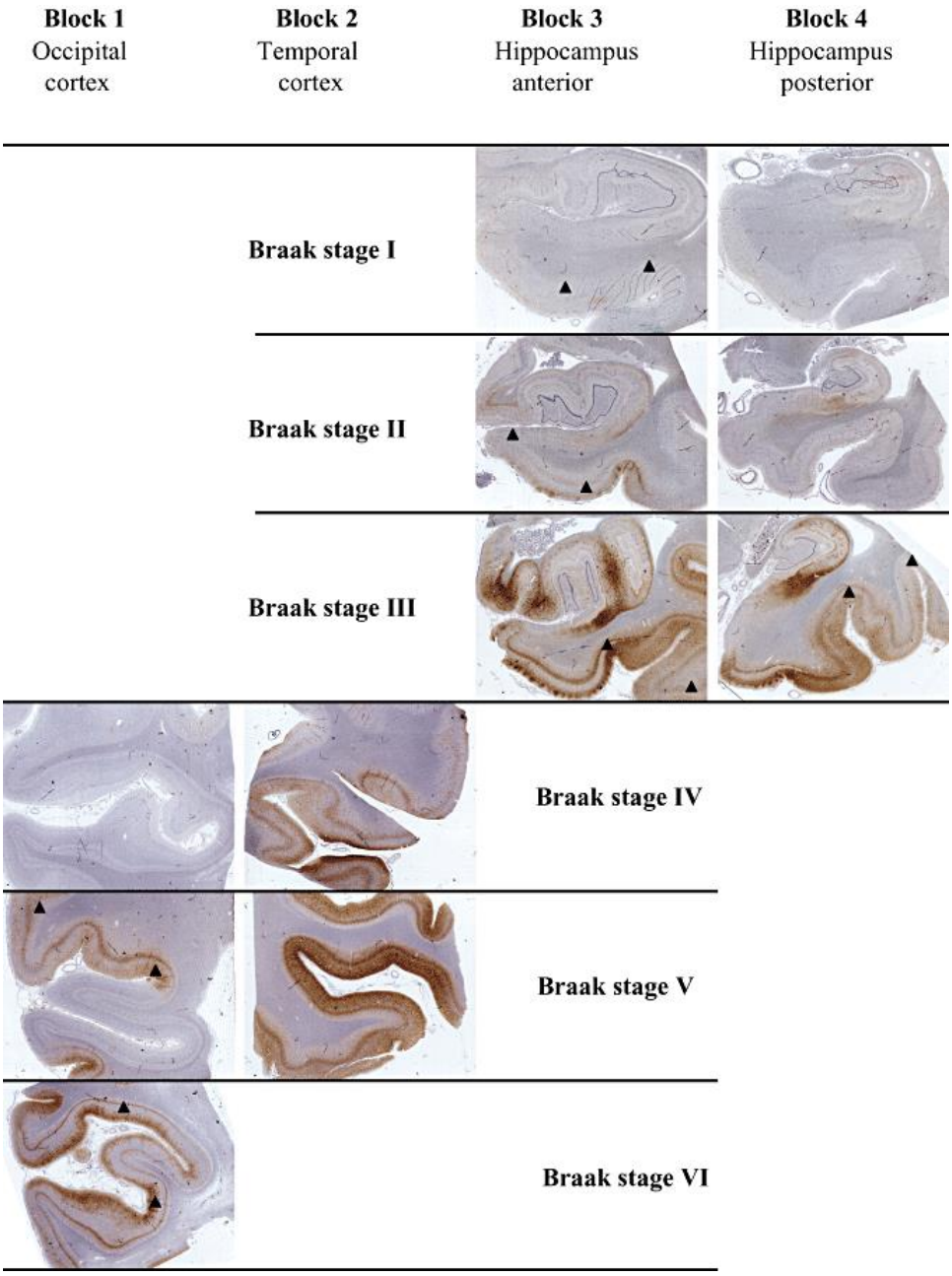


Figure 1-3 Braak Stages

Staging of Neurofibrillary Pathology in Alzheimer's Disease: A Study of the BrainNet Europe Consortium

Scanned immunohistochemically stained sections applying AT8 antibody. Section from: Block 1 – occipital cortex including calcarine fissure; Block 2 – temporal cortex including

middle temporal gyrus and at least a part of superior temporal gyrus; Block 3 – anterior hippocampus at the level of uncus; and Block 4 – posterior hippocampus at the level of lateral geniculate nucleus. The regions are given from left to right in the suggested order of assessment. The arrowheads indicate borders for the relevant neuroanatomical regions for each given stage: Braak I – transentorhinal region; Braak II – entorhinal region; Braak III – temporo- occipital gyrus; Braak IV – temporal cortex; Braak V – peristriatal cortex; and Braak VI striatal cortex.

This figure was published by Alafuzoff et al 2008 in **Brain Pathology** Volume 18, Issue 4, pages 484-496, 27 MAR 2008 DOI: 10.1111/j.1750-3639.2008.00147.x http://onlinelibrary.wiley.com/doi/10.1111/j.1750-3639.2008.00147.x/full#f6 and reproduced here by permission under the Creative Commons Deed, Attribution 2.5.[98]

1.1.3.3 Neuronal loss

A gradual loss of synapses parallels the clinical manifestations of the disease. The pattern of progressive synaptic loss may explain why episodic memory is among the first areas of cognition affected by AD.[99-102] Synaptic loss, especially in the frontal cortex, occurs prior to neuronal loss,[103,104] thereby possibly explaining why synaptic density explains cognitive impairment better than other neuropathological markers.[103,105] In fact, neurons can sustain pruning and damage for years,[106] which may explain why functional changes can sometimes be detected before neuronal atrophy is evident. Neuronal loss, when it occurs, takes place in a non-homogeneous pattern. This lack of homogeneity might explain incongruent structural brain imaging findings in early stages of AD. Nonetheless, the entorhinal cortex (EC) in the medial temporal cortex (MTC) emerges as one of the first affected areas; where up to a 32% of neurons are lost.[96] EC neurons are the primary cortical afferents to the hippocampal cortex (HC). Thus, these neurons play an important role in episodic memory.[107-109] Loss of entorhinal neurons leads to HC deafferentation in AD.[110] Furthermore, innervation of the superior temporal cortex by cholinergic neurons in the basal forebrain (nucleus basalis of Meynert) is disrupted due to neuronal loss in the latter area, leading to cholinergic dysfunction and impaired learning and memory.[111-116] In addition, the limbic cortices, the posterior cingulate gyrus, and the superior parietal lobule, also suffer neuronal loss in AD.[41,117] Moreover, there is also loss of the noradrenergic neurons of the locus coeruleus[118,119] and the serotonergic neurons of the dorsal raphe nucleus[120]. which may explain non-cognitive symptoms.[121]

1.1.4 Etiology

What could cause amyloid plaques, NFTs, synaptic dysfunction and subsequent neuronal loss? Although we have learned a lot from a century of studies, we still don't understand the Alzheimer disease process. While we know that AD is associated with

age and a number of risk factors, there is a form of AD that develops in young to middle age adults and is caused by specific genetic mutations. This leads to a separation of etiology, simple vs complex. We will briefly review the known genetic causes and influences, as well as the environmental factors that affect the two AD dementia forms characterized by the above-described neuropathology.

1.1.4.1 Brief history of familial Alzheimer's disease and late-onset Alzheimer's disease

In the beginning of the 1900s, Auguste Dieter's case stood out because Alzheimer's observations were made in an individual who was much younger than the typical "senile dementia" patient of that time. In those years, "senile dementia" was typically considered to be a normal consequence of aging. Therefore, Emil Kraepelin, the Director of the Tubingen Institute where Alzheimer practiced, named this condition "Alzheimer's Pre-senile Dementia" in the 8th edition of his *Handbook of Psychiatry*.[122] After describing a patient aged 60 with histopathology similar to Auguste D., Alzheimer retracted his characterization of "Alzheimer's pre-senile dementia" stating that young and old have dementia alike.[26,123,124] Subsequently, various investigators re-discovered that the brains of dementia patients, whether young or old, often appeared similar.[125-129] Although the debate continued as to whether there were two distinct conditions, both senile and pre-senile dementia were later established to have the same chemical compositions in their characteristic hallmarks, Amyloid-Beta (Aβ) peptides[130] and hyperphosphorylated *tau* protein.[131] These analyses confirmed why the brains of younger adults and elderly appeared to be similar, despite clear evidence for distinct causes of these two disease forms.[132] As such, we will segregate the two forms in the material that follows.

1.1.4.2 Familial Alzheimer's disease

We now know that Auguste Dieter, had a *PS/1* mutation. Thus, she had familial AD (FAD), the rare early-onset genetic form of the illness.[133-135] In this genetic form, symptom onset occurs typically between 40 to 60 years of age (varying somewhat,

depending on which mutation is present in which vulnerable gene). There are three specific genes in which mutations can cause autosomal dominant patterns of segregation of FAD. These are: *APP* on chromosome 21,[44,136-138] presenilin-1 (*PS/1*) on chromosome 14[139] and presenilin-2 (*PS/2*) on chromosome 1.[140-145] There are 32 known *APP*, 185 *PS/1* and 13 *PS/2* mutations.[146] All these different mutations have 100% penetrance in presence of a single copy. These mutations commonly increase presence of APP fragments.[147] In a related set of circumstances, Down Syndrome patients have extra copies of the (normal) *APP* gene due to their extra chromosome 21. These individuals inevitably develop an Alzheimer-like pathology and an early onset dementia.[148-150] These genetic studies demonstrate that, if a gene is mutated or has an extra copy at any one of these three loci, the processing of the amyloid precursor protein is affected and Aβ peptides are increased. These observations led to initial enthusiasm for the "amyloid cascade" hypothesis of AD which, despite its detractors, has remained a favorite way to conceptualize the pathological process of AD.[48,151] Looking at the penetrance in and the Alzheimer-like phenotype of individuals with Down Syndrome, we understand why delving more deeply in this simpler form appealed to scientists. Beyond these genetic causes we know very little, and the etiology of AD dementia remains a puzzle in 99% of cases.[152]

1.1.4.3 Late onset Alzheimer's disease

Late-onset Alzheimer's disease (LOAD), is the most common form of AD.[153] It appears typically after 65 years of age and cannot be traced to a single genetic mutation. Age remains the principal risk factor for LOAD, as the disease's incidence increases steadily with age.[154,155] In a recent editorial, Breitner has argued that, provided persons live to around age 100, developing AD dementia is more the rule than the exception.[156] At any rate, with lifespans now approaching or exceeding 90 years, the lifetime incidence of dementia approximates 50%. We still don't know the proportion of individuals at this age who have developed AD pathology without dementia.

LOAD is a complex disorder with a multifactorial etiology that remains poorly understood. In recent years, evidence has accrued in that FAD is characterized by the over-production of the neurotoxic $A\beta_{1-42}$ peptide, whereas LOAD patients appear to have a normal rate of Aβ production but suffer from a reduced clearance of the $A\beta_{1-42}$ peptide in particular.[157-159] In fact, in addition to CSF dynamics, it is now evident that Aβ clearance is impaired in individuals with an *APOE* ε4 allele.[160]

1.1.4.3.1 Genetics

Twin studies have proven useful to illustrate the "heritability" of AD, i.e., the proportion of the total population risk in predisposition that can be ascribed specifically to familial predisposition.[161] The twin approach imputes genetic causality according to the difference in concordance between monozygotic (MZ) and dizygotic (DZ) twin pairs (assuming that environmental influences are shared equally between the two types of pairs). Despite the absence of a culpable fully penetrant gene (or genes), LOAD appears to be highly heritable. The Swedish HARMONY study found concordance of 59% for AD dementia[162], and several estimates have placed the heritability of this illness at or near 70%.[163-165] In earlier work, Breitner et al. investigated 79 persons with typical features of AD dementia whose families included a history of AD-like dementia in at least one first-degree relative. Compared with relatives of patients with other forms of dementia, the 379 AD first-degree relatives were found to be at some three-fold increased risk of developing dementia themselves.[166]

Genetic variants may influence the underlying brain architecture and subsequent development of neurodegeneration and limit an individual's functional plasticity. Some twenty-one genetic loci have been associated with LOAD through meta-analysis of genome wide association studies GWAS.[167] These susceptibility gene variants have small effect sizes in comparison to the *APOE* ε4 allele. They include: BIN1, CLU, ABCA7, CR1, PICALM, MS4A6A, CD33, MS4A6E, CD2AP, EPHA1, HLA25, DRB5/HLA-DRB1, PTK2B, SORL1, SLC2A4A, INPP5D, MEF2C, NME8, ZCWPW1,

FERMT2, CELF1 and CASS4.[167,168] It is striking that so many of these genes encode proteins with prominent roles in immune and cellular transport mechanisms, suggesting these mechanisms may play important roles in AD pathogenesis. It bears mention that the polymorphic *APOE* gene itself, by far the most important susceptibility locus for LOAD,[168,169] encodes a protein whose prominent function is in cholesterol transport.[170,171] The linkage of this genetic system (and the *APOE* ε4 allele in particular) to AD risk has a P-value of 10^-47.[167]

Cholesterol, itself, plays an important role in cellular lipid bilayer fluidity, as well as cellular signaling and trafficking; it is a component of myelin sheaths that serve to efficiently conduct signals across axons efficiently.[170,172] Cholesterol is also a precursor for important micronutrients and steroid hormones such as vitamin D and sex hormones. Thus, *APOE* modulation has the potential to influence neuronal functions, neuronal survival, synaptic plasticity, dendritic remodeling, lipoprotein metabolism and signaling cascades.[173-175]

The three important *APOE* alleles, ε2, ε3, and ε4 differ by two amino acids at position 112 and 158.[171,176] The arginine residue at position 112 and 158 of the ε4 variant affects the conformation of the gene's protein product and impairs its functionality.[177] In contrast to ε4, the ε2 allele protects against cognitive disorder.[178] ε2 carriers have higher plasma levels of apoE protein, and increased cholesterol and lipid transportation compared to ε4 carriers, while, ε3 is associated with intermediate apolipoprotein plasma levels.[179]

Interestingly, the ε4 allele is uncommon in centenarians.[180] The *APOE* ε4 allele frequency (p) is typically around 13% in Caucasian normal controls (this varies with ethnic group), but this allele frequency was enriched to p = 36.7% in typical AD dementia Caucasian patient populations.[181] Because humans are diploid, inheriting one allele from each parent, the corresponding frequency of persons who bear at least one

ε4 allele is given by Hardy-Weinberg equilibrium (p2 + 2pq) as 25.7 % in typical Caucasian normal controls, but the proportion of ε4 carriers rises above 50% in AD dementia patients.[140,181] Even in healthy aging, ε4 carriers suffer from memory, learning, attention, and olfactory deficits,[182-184] suggesting the protein functions of this allele on human brain function. Although *APOE* ε4 is not a determinant of AD pathogenesis, substantial evidence suggests that this genetic variant exerts an influence on the progression of AD. Thus, *APOE* ε4 carriers have an increased abundance of amyloid plaques compared to non-carriers measured both *in vivo* and *post-mortem*.[185-187] Furthermore, *APOE* ε4 is associated with cholinergic deficits and with an earlier disease onset.[188,189]

Likewise, functional brain imaging suggests differences between carriers and non-carriers for both task-related activity and resting network connectivity.[190,191] The ε4 carriers are also burdened by structural abnormalities, and it is argued that this gene polymorphism predisposes the brain to inflammation and neurodegeneration even during development.[192] The effect of the *APOE* ε4 allele is so strong, that it alters the outcome of preventative interventions such as physical activity, cholesterol lowering, and functional diets.[193] Accordingly, ε4 carriers have a poor recovery after brain injury or neurodegeneration.[194-197]

1.1.4.3.2 Interaction of genetic and non-genetic risk factors

A large twin study of 11,884 monozygotic and dizygotic twin pairs, among which 392 were affected by AD dementia in one or both members, demonstrated that LOAD heritability did not vary substantially between men and women. However, the same study suggested existence of *non-shared* (i.e., adult) environmental susceptibility factors.[198] Recent work suggests an interaction between genetic (e.g., *APOE* ε4) and non-genetic risks. Interestingly, blood pressure in pre-hypertensive individuals interacts with the *APOE* ε4 allele to predict diminished cognitive performance in healthy middle-

aged adults.[199] Furthermore, diabetes and *APOE* ε4 act together to increase the risk of dementia.[200] Moreover, possession of a ε4 allele markedly increases the risk of dementia associated with depressive symptoms.[201] These findings suggest that *APOE* ε4 carriers might in some instances mitigate their risk for AD symptoms by paying close attention to environmental factors such as health-conscious behavior, early treatment of risk-modifying conditions, or changing habits such as nicotine or alcohol dependence.

1.1.4.3.3 Non-genetic risk factors

Even in *APOE* ε4 non-carriers, AD is a multifactorial pathology influenced by a balance between risk and protective factors, one of them being vascular in nature. Epidemiological studies identified that mid-life individuals with vascular diseases and elevated cholesterol levels are more susceptible to AD.[202-206] Low density lipoprotein (LDL) levels, known as the “bad cholesterol”, are higher in AD patients compared to controls.[207] In fact statins, cholesterol-lowering drugs, may be associated with reduced risk of AD and reduce Aβ levels in the brain *in vivo*.[202,203,208-211] Additionally, hypertension in midlife predicts subsequent dementia[212,213] and cerebral blood flow decreases with the degree of AD advancement.[214] Finally, insulin resistance and hyperinsulinemia, also risk factors for cardiovascular disease, increase the risk for developing Alzheimer’s disease.[215-219] Thus, there appears to be an important cardiovascular risk component to the development of clinical symptoms. It is not surprising that oxidative stress, cholesterol, oxidized cholesterol, and metabolic syndrome are associated with increased risk for AD.[153,204,220] Furthermore, modern computational modeling suggests that a vascular vulnerability most probably triggers LOAD.[221] This is meaningful as the co-occurrence of AD pathology and vascular disease is has high as 84%.[222]

Several AD risk factors have been identified in other domains. These include psychiatric health, which include depression and personality disorders[223] and head trauma.[224,225]

Finally, it is important to highlight that there are some factors that may be protective and render individuals more resilient to AD. For example, cognitive reserve, a concept of resilience to sustain brain damage often associated with education and occupation, appears to delay dementia onset.[226-228] Other suggested protective factors include literacy, years of education, physical and mental activity, and a Mediterranean diet.[229-231] *The Lancet* International Commission on Dementia Prevention, Intervention and Care, conducted a large meta-analysis that evaluated modifiable health and lifestyle factors as an approach to prevention of dementia.[232] These include attending school at least until age 15, physical activity, and social engagement.[232] They also include avoiding or stabilizing hypertension, obesity, hearing loss, depression, diabetes, and smoking.[232]

Interestingly, some of these risk factors for dementia also influence the sense of smell, olfaction, a possible indicator of pre-symptomatic AD. The last will be discussed in chapter 5, but we shall first briefly review what is known about early AD pathogenesis and markers that may characterize this process.

1.1.5 Chronic disease

Along with his important synthesis of “Alzheimer’s disease” as an entity that included both early- and late-onset variants (implying that so-called “senile dementia” was a disease not unlike “Alzheimer’s pre-senile dementia), Katzman (1976)[127] pointed to a body of evidence that “dementia of the Alzheimer type” (Katzman’s preferred term) was in fact a chronic illness with a lengthy period of transition between normal cognitive decline and dementia. Several attempts were then made to describe the spectrum of individual cognitive deficits in aging and their relationship to early or prodromal AD dementia.[233] Some of the terms suggested to describe intermediate states now appear to have been misleading in their lack of acknowledgement that milder difficulties might signal later development of more serious difficulty. These terms include “benign senescent forgetfulness”,[234] “age-associated memory impairment”,[235] “aging-associated

cognitive decline",[236] "age-related cognitive decline",[237] and "cognitive impairment, no dementia".[238] In 1991, Flicker proposed the term "mild cognitive impairment",[239] and Petersen (1999) adopted this expression to describe a long period of decline that precedes the onset of dementia, emphasizing that many persons meeting criteria for this condition (especially those with an APOE ε4 allele) were destined later to develop AD dementia.[240] "Petersen MCI" specifically denoted a degree of impairment measurable with psychometric instruments that exceeded those of "normal" aging persons, even though these difficulties did not affect usual daily living. In fact by the time most patients are diagnosed with MCI, they already have AD neuropathology in stages three and four of the six Braak stages (NFT in the entorhinal and transentorhinal; Braak, 1991; Markesbury, 2010).[86,241] MCI is often divided into amnestic and non-amnestic types (aMCI and naMCI),[15] depending on whether memory failure is the predominant feature.

1.1.6 Treatments

Currently, no treatment is effective at targeting the underlying pathology of AD and its related clinical syndromes. Intervention trials in people with dementia have widely failed.[242] Therefore, effective therapies are needed to postpone or prevent the onset of cognitive impairment so individuals can live successfully into old age. For some years, trials have been attempted in patients with mild cognitive impairment (MCI), with the intention of delaying their "conversion" to dementia.[243] Since trials in both MCI and AD have failed, the field is moving more "upstream" and considering the prevention of AD. In fact, it is now commonly thought that brain deterioration may be beyond repair by the time dementia, or probably even MCI, are evident. By contrast, disease-modifying prevention strategies might delay the onset of symptoms and thereby reduce prevalence.[244] More recently, the approach is to develop pre-symptomatic secondary preventive interventions to mitigate change and intervene in individuals that would otherwise likely have clinical symptoms in a few years (e.g. cognitively normal individuals at high risk of developing Alzheimer's dementia).[245] To this end,

characterization and the measurement of progress in pre-symptomatic AD are needed.

1.1.7 Biomarkers: How can we track pre-symptomatic Alzheimer's disease progression?

Though not curable, AD dementia may be preventable if we can gain a better understanding of its pre-symptomatic stages. Several investigators are now looking retrospectively of prospectively in younger and healthier individuals to assess when the initial brain changes can be quantified. For example, Bateman has observed hippocampal volume reductions 15 years before estimated onset of dementia in pre-symptomatic individuals with FAD-causing mutations in the DIAN study.[246] This seminal study demonstrated dynamic changes in several biomarkers such as cognition, cerebrospinal fluid (CSF) Aβ and *tau* levels, elevated amyloid-PET (Positron Emission Tomography) measurement, reduced FDG-PET (fluorodeoxyglucose, a measure of glucose metabolism) as well as hippocampal volume before their expected age of dementia onset. As we continue to characterize risk factors and biomarker trajectories in the preclinical stage, there is a lengthy AD development phase in which biomarkers start to change before MCI onset.[94]

The sequence of events in pre-clinical late onset AD is less clear, but cerebrospinal fluid (CSF) accumulation of *tau* and decline in $A\beta_{1-42}$ (which indicates accumulation in brain), both well-known markers of AD, appear to signal the presence of pre-clinical AD in cognitively normal individuals.[32] Supporting this finding, longitudinal data from the BIOCARD study illustrated that baseline CSF *tau* or $A\beta_{1-42}$ levels predicted subsequent development of MCI over five years, as did the rate of change in the ratio of these two markers.[247] Observations of amyloid accumulation by PET with ^{11}C-labelled Pittsburgh compound B (^{11}C-PiB) estimated that sporadic AD dementia occurred only after 17 or more years of progressive fibrillar Aβ deposition based on a decade of data acquisition when the Standardized Uptake Value Ratio (SUVR, a measure of A deposition) was $\geq$ 1.5.[248] Taken together, these findings suggest that AD pathogenesis remains

unapparent for many years while massive damage accumulates. Prevention appears to be key.

To achieve prevention and possibly offset the disease, there must be measures to detect individuals at elevated risk of AD. Additionally, these individuals should be counseled and educated about known risk and preventative lifestyle factors, similar to interventions that have reduced the toll of HIV or cancer. Alternatively, one may administer medicines that slow progression of the disease process, thus deferring symptom onset. Demonstration of efficacy in either of these strategies requires methods that can track change in disease status over time. How can we measure AD progression?

It is difficult to conduct prevention trials because one cannot rely on dementia as their outcome since the preceding stages could last a decades or longer before diagnosis. In fact, the conversion rate is 1-2% in normal adult, whereas MCI patients develop AD at a rate of 10-15% year.[100,249] These projects can be too expensive to be sustainable and may lead to emergent adverse events (i.e. falls, strokes…) that may not be caused by interventions but would make continuing trials unethical. Thus, prevention trials enrich their participants for risk factors of AD if they want to measure change within relatively short periods of time in the years before dementia. Cummings suggested to use an age cutoff, inclusion of individuals with genetic risk factors or other known risk factors, or to use identify individuals by determining biomarker changes.[250] This thesis focuses on a population enriched through a family history of AD–like dementia (FH+) and willingness to participate in a prevention trial. FH+ increases an individual's age-specific risk of AD by a factor of 2-3 using guidelines like those from to the Cache County Study.[251,252]

Prevention requires mitigation of pre-symptomatic brain changes. To show such mitigation, one must measure the degree of accumulated damage and its change over time in individuals with pre-symptomatic AD,[253-255] although disease progression is likely to vary across individuals. To deal with this heterogeneity, one may measure multiple

disease markers. Well studied in this regard are structural magnetic resonance imaging (MRI) changes in vulnerable brain regions and various aspects of cognition. These markers change as people age, but their currently known relationship with subsequent AD dementia is insufficiently specific to allow their use as measures of disease progression. Potentially more specific markers include CSF $A\beta_{1-42}$ and *tau*, (presumed to be more direct indicators of Alzheimer neuropathology), amyloid PET, FDG-PET. However, the methods to measure these markers are inconvenient, invasive, or costly, and they are not widely available or culturally accepted.[256] Criteria are constantly changing to meet technological progress, as is our understanding of the progression from normal to demented. Acceptable quality-adjusted life-years (QALY) should be considered even in research to identify individuals at risk of progressing through the AD continuum. More convenient and accessible indicators of asymptomatic AD pathology are needed. Auditory processing, eye neuropathology, motor processing, and olfactory functions are being explored as less invasive functional reflections of AD risk, accumulated damage, and AD progression.[257,258]

Unfortunately, commonly used biomarkers lack practicality beyond research and clinical trial settings. Therefore, the need to recognize individuals with declining cognitive issues beyond normal aging still remains. This issue is more relevant now than ever as we are attempting to successfully prevent or delay the disease onset rather than “empty the water out of a sinking ship.”

In summary, these studies have moved the field forward by demonstrating that there are measurable dynamic changes that remain unapparent in both FAD and LOAD. The ability to detect AD in pre-symptomatic stages opens a critical window for the evaluation of preventative interventions before massive damage accumulates. This dissertation appraises odor identification as a marker of presymptomatic AD.

1.2. Odor identification: a predictor of cognitive decline

1.2.1. Odor identification, Aging, and Alzheimer’s disease

As with cognition, olfactory abilities decline with age.[259-264] Older adults demonstrate retrieval impairment for specific information (analogies, verbal label, vocabulary, information), although familiarity (semantic knowledge) is less affected, demonstrating decline in cognitive abilities. Episodic memory problems are one of the first clinical signs that manifest years before a person can be diagnosed as suffering from AD.[265-267] Like cognition, olfaction is impaired in dementia (reviewed by Sun, 2012 and Rahayel, 2012).[1,268] Olfactory dysfunction corresponds to the early AD pathology observed in the primary olfactory cortex, located across the frontal and temporal lobe, as well as in key areas responsible for memory.

In 1974, Waldton observed that olfaction declined over 5 longitudinal visits with 6-month intervals, in demented women and healthy controls; this study suggested that olfactory impairment increases with progressive dementia.[269] Two decades later, Mesholam published a meta-analysis of 43 studies on olfaction in patients with AD; in contrast to controls, AD cases have deficits in odor detection thresholds, identification, and recognition (a familiarity task).[270] Several more recent studies indicate that odor identification (OI) skills are correlated with poor cognitive performance in patients with AD dementia patients compared to elderly controls.[1,271-274] Furthermore, among patients with AD dementia, reduced OI performance predicts more rapid cognitive decline.[275] Failure to identify odors has been suggested to be a sensitive indicator of AD progression.[276] In fact, odor identification appears to be an important part of the clinical picture as 90% of AD patients show impairment.[277] We will review how difficulties in identifying and naming odors are associated with declines in cognitive performance and the emergence of AD-type dementia.

1.2.2. Longitudinal studies

Since olfactory dysfunction manifests before cognitive decline,[278] smell identification deficits have been used to predict subsequent development of MCI [271,278-280] or AD dementia.[279-282] These studies come together and motivate the evaluation of

odor identification as a preclinical marker of odor identification in further details. They also suggest odor identification may be useful as a functional measure of cognitive decline and ongoing degeneration affecting the olfactory circuitry.

1.2.2.1 Odor identification predicts later cognitive decline and AD dementia

We will review key longitudinal studies that demonstrate that odor identification in normal individuals can predict later symptoms related to dementia progression. In a first large population study from the Betula Project, the baseline odor identification score and subjective olfactory complaints, in addition to age and MMSE, emerged as independent predictors of dementia conversion over 10 years in a logistic regression of 1529 cognitively normal 66.9 years old individuals.[281]

Separate projects looking at shorter follow-up periods have reported similar findings. A prospective study of 1,920 cognitively normal individuals showed that lower smell identification test scores at baseline were associated with greater 5-year incidence of cognitive impairment (OR 6.62, 95% CI: 4.36–10.05).[283] After adjusting for an exhaustive list of covariates, difficulties in odor identification remained predictive of cognitive impairments (OR 3.72, 95% CI: 2.31-5.99).[283] In 589 older adults without cognitive impairment or dementia from the Rush Memory and Aging Project, odor identification predicted the emergence of an MCI diagnosis over 5 years of follow-up in a proportional hazards model adjusted for age, sex, and education (relative risk 1.15; 95% CI, 1.07-1.23).[271] When investigating change over time in a subset of these individuals with the use of a linear mixed-effects model, lower odor identification was associated with faster decline in episodic memory (estimate=0.014, SE=0.004, $p<0.001$) after adjusting for age, sex, education, ε4, and baseline episodic memory.[278]

These findings were independently replicated over a shorter time period and with a larger sample. Among 1430 cognitively normal individuals from the Mayo Clinic Study of Aging (MCSA), 250 were diagnosed with MCI over a follow-up of 3.5 years and 64

progressed to dementia.[279] In this longitudinal study, olfactory identification test scores improved the MCI progression prediction model concordance (HR 1.10 [95% CI, 1.04-1.16], P<0.001).[279] The worst olfactory identification was associated with an amnestic MCI, aMCI, and AD dementia diagnosis.[279] Through a linear mixed effects model, Roberts et al. observed that lower cross-sectional odor identification test scores can predict a decline in memory, executive function, language, and global cognition.[279] These longitudinal studies inform us that olfactory identification can add to predictive models of AD progression even very early in the pre-symptomatic phase. A possible application is to use this information in a clinical trial design, in order to enrich a population with increased risk of cognitive decline, and to schedule further testing of clinical symptoms or neuropathology.

1.2.2.2 Reproducibility of odor identification as a predictor of later decline in diverse populations

It is a concern that very few studies go out of their way to investigate odor identification or other olfactory tasks in populations of different ethnic backgrounds. Luckily, there are some findings across various cultures and multi-ethnic cohorts that corroborate the association between odor identification and cognitive decline. There are studies on odor identification, aging and dementia conducted across the world and many cultures have developed or adapted OI tests.[284-287] Here we briefly review some exemplary studies. In one multi-ethnic study conducted in a large urban community (n=1037) on the East Coast of the United States with almost a 1:1:1 ratio of Whites, African-Americans, and Hispanics, impairment in OI was predictive of cognitive decline in cognitively normal individuals.[282] This study reported no APOE ε4 effect on the odor identification test.

In a biracial cohort of 2428 black and white Americans from the North and South US who participated in the Health, Aging, and Body Composition (Health ABC) study, poor OI at baseline was associated with an increased risk of dementia over 12 years.[280]

Unfortunately, this study stratified black and white participants instead of creating a dummy variable for ethnicity to maximize their sample size in their risk analysis. As observed in a multi-ethnic study of New Yorkers, this large study did not find an interaction with APOE ε4 between OI and dementia status.[280,282]

Likewise, the National Social Life, Health, and Aging Project found a simple baseline identification test of 5 odors (peppermint, fish, orange, rose, and leather) using picture or word multiple-choice options was predictive of AD dementia in 2677 normal elderly participants.[288] Individuals with 3 or fewer correctly identified odors had higher odds of developing dementia (odds ratio =2.14, 95% CI: 1.32-3.43).[288] Race was a covariate in their analysis as the cohort was 81% white, 10% black, 7% nonblack Hispanic, and 3% other races.[288] Adams and colleagues reported a 47% sensitivity and a 79% specificity to predict conversion 5 years before cognitive impairment.[288] Although, they found that 91% of those who did poorly on their odor identification did not develop dementia within the 5-year follow-up.[288] This observation suggests that repeated measures may be important to assess a decline in odor identification performance and to take one measure of OI lightly.

Across the pond, in Indonesia, a study of 109 participants on average 64 years old with low education levels (40% had less than 6 years of education), revealed that OI testing enhanced the detection of aMCI among normal individuals. Combining odor identification assessment to a multi-domain evaluation like pupillary response to tropicamide, an anti-cholinergic drug, increased the specificity of the test to 91% and the positive predictive value to 87% to diagnose aMCI.[289] The combine use of olfactory deficit and pupillary response was superior to other combinations of APOE ε4 status, BDNF plasma level, olfactory deficit and pupillary response. These authors also replicated their results with an external sample. Although most studies represent highly educated European descendants, these few examples suggest that odor identification deficits and cognitive decline are related cross-culturally, in various ethnicities and among less educated populations. The use of odor identification as a biomarker may

therefore be advantageous in research or clinical settings since olfaction does not appear to be affected by education as most neuropsychological tests. The replication of the association between odor identification and cognitive decline appear to be generalizable.

1.3 Rationale for thesis

The increased focus on the preventative intervention in the pre-symptomatic phase of AD underlines the importance of sensitive and accurate biomarkers. Thus, this work will present a novel marker that can be used to monitor the disease progression, predict symptoms, and may represent an anatomical and functional underlying process. This measurement, odor identification, appears to demonstrate some degree of synchrony or concordance in appearance with other known biomarkers during the MCI and AD stages. In a healthy population of 471 elderly subjects without clinical manifestation of a cognitive impairment, low scores in the brief smell identification test (BSIT) was associated with a higher probability of developing decline in episodic memory, MCI, and AD-type pathology at autopsy.[278] These findings support the idea that pathology present in the olfactory network is associated with higher-order processing of odors in normal individuals. Prior to this thesis, Wilson and colleagues published the strongest report linking OI to AD by observing an association between odor identification scores and NFTs and plaques *post-mortem*.[278,290] In order for the field to move forward, it remained important to reproduce these findings *in vivo*, which was the goal of this thesis (chapter 3). An additional and important goal is to demonstrate how olfactory identification may be representative of disease progression and if we can observe declining function that parallels disease hallmarks in cognitive individuals at risk for Alzheimer's disease (chapter 2, 3 & chapter 4). An obvious application of such markers is validating the testing of preventive interventions among asymptomatic individuals at risk of AD dementia. We assessed odor identification in the PREVENT-AD cohort and used data acquired during a 2-year period.

1.4 Objectives

Our objectives are spread across 3 manuscripts, a review, a cross-sectional data paper, and a longitudinal data paper:

1.4.1 Manuscript 1

To assess the potential of odor identification as a marker of presymptomatic change related to AD in preventative trials.

For this:

1. We review literature on olfactory system, its vulnerability, and evidence of AD neuropathology across several structures (chapter 2);
2. We review previous use in treatment trials of MCI and AD (chapter 2);
3. We review how odor identification adds to composite measures of AD (chapter 2);
4. We introduce the contribution of odor identification to a composite measure recently developed using data collected for this dissertation, the Alzheimer Progression Score (chapter 2);

1.4.2 Manuscript 2

To investigate the relationship between OI and measures of AD severity, in a cohort of individuals at risk for AD dementia.

For this, we evaluated the relationship between:

1. OI performance and AD risk or protective factors such as age, sex, and education (chapter 3);
2. OI performance and genetic risk associated with the polymorphic *APOE* locus

(chapter 3);

3. OI performance and total or subscale cognitive test scores (chapter 3);
4. OI performance and CSF markers of AD progression such as Aβ42, total-*tau*, phospho-*tau*, and the ratios of total *tau*/Aβ42 and phospho-*tau*/Aβ42 (chapter 3);

1.4.3 Manuscript 3

To evaluate olfaction identification longitudinal performance in cognitively normal adults at risk in the INTREPAD trial. For this, we investigated serial odor identification and multi-modal measurements.

Specifically, we assess:

1. OI performance decline over a 2-year period and the effect of recognized covariates (chapter 4).
2. Baseline and Longitudinal CSF markers of AD progression such as *tau*/Aβ42 and phospho-*tau*/Aβ42 effect on repeated measures of OI performance (chapter 4);
3. Baseline and Longitudinal hippocampal volume effect on repeated measures of OI performance (chapter 4).
4. Baseline and Longitudinal total or domain specific cognitive scores on repeated measures of OI performance (chapter 4);
5. Baseline OI performance effect on repeated measures of AD progression, hippocampal volume, global and domain specific cognition (chapter 4).

1.5 References

1 Rahayel, S., Frasnelli, J. & Joubert, S. The effect of Alzheimer's disease and Parkinson's disease on olfaction: a meta-analysis. *Behavioural brain research* **231**, 60-74, doi:10.1016/j.bbr.2012.02.047 (2012).

2 Doty, R. L. Olfactory dysfunction in Parkinson disease. *Nature Reviews Neurology* **8**, 329-339 (2012).

3 Rey, N. L., Wesson, D. W. & Brundin, P. The olfactory bulb as the entry site for prion-like propagation in neurodegenerative diseases. *Neurobiology of disease*, doi:10.1016/j.nbd.2016.12.013 (2016).

4 Roalf, D. R. *et al.* A quantitative meta-analysis of olfactory dysfunction in mild cognitive impairment. *Journal of neurology, neurosurgery, and psychiatry* **88**, 226-232, doi:10.1136/jnnp-2016-314638 (2017).

5 Lafaille-Magnan, M.-E. *et al.* Odor identification as a biomarker of preclinical AD in older adults at risk. *Neurology* **89**, 327-335 (2017).

6 Ferris, S. H. & Farlow, M. Language impairment in Alzheimer's disease and benefits of acetylcholinesterase inhibitors. *Clin Interv Aging* **8**, 1007-1014, doi:10.2147/CIA.S39959 (2013).

7 Perry, R. J. & Hodges, J. R. Attention and executive deficits in Alzheimer's disease. *Brain : a journal of neurology* **122**, 383-404 (1999).

8 Bäckman, L., Jones, S., Berger, A.-K., Laukka, E. J. & Small, B. J. Cognitive impairment in preclinical Alzheimer's disease: a meta-analysis. *Neuropsychology* **19**, 520 (2005).

9 Tabert, M. H. *et al.* Neuropsychological prediction of conversion to Alzheimer disease in patients with mild cognitive impairment. *Archives of general psychiatry* **63**, 916-924 (2006).

10 Johnson, D. K., Storandt, M., Morris, J. C. & Galvin, J. E. Longitudinal study of the transition from healthy aging to Alzheimer disease. *Archives of neurology* **66**, 1254-1259 (2009).

11 Mazzoni, M., Moretti, P., Lucchini, C., Vista, M. & Muratorio, A. Category-specific semantic disorders in Alzheimer's disease. *Rivista di neurologia* **61**, 77-85 (1991).

12 Silveri, M. C., Daniele, A., Giustolisi, L. & Gainotti, G. Dissociation between knowledge of living and nonliving things in dementia of the Alzheimer type. *Neurology* **41**, 545-545 (1991).

13 Bennett, D. A. Mild cognitive impairment. *Clinics in geriatric medicine* **20**, 15-25, doi:10.1016/j.cger.2003.10.001 (2004).

14 Folstein, M. F., Folstein, S. E. & McHugh, P. R. Mini-Mental state. A practical method for grading the cognitive state of patients for the clinician. *Journal of psychiatric research* **12**, 189-198 (1975).

15 McKhann, G. M. *et al.* The diagnosis of dementia due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **7**, 263-269, doi:10.1016/j.jalz.2011.03.005 (2011).

16 Foundas, A. L. Apraxia: neural mechanisms and functional recovery. *Handb Clin Neurol* **110**, 335-345, doi:10.1016/B978-0-444-52901-5.00028-9 (2013).

17 Lyketsos, C. G. *et al.* Neuropsychiatric symptoms in Alzheimer’s disease. *Alzheimer's & dementia: the journal of the Alzheimer's Association* **7**, 532-539 (2011).

18 Rosenberg, P. B. *et al.* Neuropsychiatric symptoms in MCI subtypes: the importance of executive dysfunction. *International journal of geriatric psychiatry* **26**, 364-372, doi:10.1002/gps.2535 (2011).

19 Cipriani, G., Lucetti, C., Danti, S. & Nuti, A. Sleep disturbances and dementia. *Psychogeriatrics* **15**, 65-74, doi:10.1111/psyg.12069 (2015).

20 Hausdorff, J. M. & Buchman, A. S. (Oxford University Press US, 2013).

21 Sheridan, P. L. & Hausdorff, J. M. The role of higher-level cognitive function in gait: executive dysfunction contributes to fall risk in Alzheimer's disease. *Dementia and geriatric cognitive disorders* **24**, 125-137, doi:10.1159/000105126 (2007).

22 Scheltens, P. *et al.* Alzheimer's disease. *Lancet*, doi:10.1016/S0140-6736(15)01124-1 (2016).

23 Prince, M. J. *World Alzheimer Report 2015: the global impact of dementia: an analysis of prevalence, incidence, cost and trends*. (Alzheimer's Disease International, 2015).

24 Cipriani, G., Vedovello, M., Nuti, A. & di Fiorino, A. Dangerous passion: Othello syndrome and dementia. *Psychiatry and clinical neurosciences* **66**, 467-473 (2012).

25 Alzheimer, A. Uber eine eigenartige Erkrankung der Hirnrinde. *Allgem.Zeit.Psychiat.Psych-Gerich.Med.* **64**, 146-148 (1907).

26 Maurer, K., Volk, S. & Gerbaldo, H. Auguste D and Alzheimer's disease. *The Lancet* **349**, 1546-1549 (1997).

27 Alzheimer, A., Stelzmann, R. A., Schnitzlein, H. N. & Murtagh, F. R. An English translation of Alzheimer's 1907 paper, "Uber eine eigenartige Erkankung der Hirnrinde". *Clinical anatomy* **8**, 429-431, doi:10.1002/ca.980080612 (1995).

28 Perusini, G. Über klinisch und histologisch eigenartige psychische Erkrankungen des späteren Lebensalters. *Histologische und Histopathologische Arbeiten. Jena: Verlag G Fischer*, 297-351 (1909).

29 Mirra, S. S. *et al.* The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part III. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* **41**, 479-486 (1991).

30 Braak, H. & Braak, E. Staging of Alzheimer's disease-related neurofibrillary changes. *Neurobiology of aging* **16**, 271-278 (1995).

31 Newell, K. L., Hyman, B. T., Growdon, J. H. & Hedley-Whyte, E. T. Application of the National Institute on Aging (NIA)-Reagan Institute criteria for the neuropathological diagnosis of Alzheimer disease. *Journal of neuropathology and experimental neurology* **58**, 1147-1155 (1999).

32 Dubois, B. *et al.* Preclinical Alzheimer's disease: Definition, natural history, and diagnostic criteria. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **12**, 292-323, doi:10.1016/j.jalz.2016.02.002 (2016).

33 Mattsson, N. *et al.* Revolutionizing Alzheimer's disease and clinical trials through biomarkers. *Alzheimer's & Dementia: Diagnosis, Assessment & Disease Monitoring* **1**, 412-419 (2015).

34 McGeer, P. L. *et al.* Positron emission tomography in patients with clinically diagnosed Alzheimer's disease. *CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne* **134**, 597-607 (1986).

35 Ikonomovic, M. D. *et al.* Post-mortem correlates of in vivo PiB-PET amyloid imaging in a typical case of Alzheimer's disease. *Brain : a journal of neurology* **131**, 1630-1645, doi:10.1093/brain/awn016 (2008).

36 Engelborghs, S. *et al.* Diagnostic performance of a CSF-biomarker panel in autopsy-confirmed dementia. *Neurobiology of aging* **29**, 1143-1159, doi:10.1016/j.neurobiolaging.2007.02.016 (2008).

37 Struyfs, H., Molinuevo, J. L., Martin, J. J., De Deyn, P. P. & Engelborghs, S. Validation of the AD-CSF-index in autopsy-confirmed Alzheimer's disease patients and healthy controls. *Journal of Alzheimer's disease : JAD* **41**, 903-909, doi:10.3233/JAD-131085 (2014).

38 Shaw, L. M. *et al.* Cerebrospinal fluid biomarker signature in Alzheimer's disease neuroimaging initiative subjects. *Annals of neurology* **65**, 403-413 (2009).

39 Spillane, J. *et al.* Accelerated ageing or selective neuronal loss as an important cause of dementia? *The Lancet* **313**, 11-14 (1979).

40 Bowen, D. M. in *Aging of the Brain and Dementia* (ed L.et al Amaducci) 127-138 (Raven Press, 1980).

41 Brun, A. & Englund, E. Regional pattern of degeneration in Alzheimer's disease: neuronal loss and histopathological grading. *Histopathology* **5**, 549-564 (1981).

42 Glenner, G. G. & Wong, C. W. Alzheimer's disease: initial report of the purification and characterization of a novel cerebrovascular amyloid protein. *Biochemical and biophysical research communications* **120**, 885-890 (1984).

43 Haass, C. Take five--BACE and the gamma-secretase quartet conduct Alzheimer's amyloid beta-peptide generation. *EMBO J* **23**, 483-488, doi:10.1038/sj.emboj.7600061 [doi];7600061 [pii] (2004).

44 Kang, J. *et al.* The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* **325**, 733-736, doi:10.1038/325733a0 (1987).

45 Hamilton, J. A. *et al.* Alzheimer's disease amyloid beta and prion protein amyloidogenic peptides promote macrophage survival, DNA synthesis and enhanced proliferative response to CSF-1 (M-CSF). *Brain research* **940**, 49-54 (2002).

46 Cappai, R. & White, A. R. Amyloid β. *The international journal of biochemistry & cell biology* **31**, 885-889 (1999).

47 Wang, H., Megill, A., He, K., Kirkwood, A. & Lee, H. K. Consequences of inhibiting amyloid precursor protein processing enzymes on synaptic function and plasticity. *Neural Plast* **2012**, 272374, doi:10.1155/2012/272374 (2012).

48 Hardy, J. & Higgins, G. Alzheimer's disease: the amyloid cascade hypothesis. *Science* **256**, 184-185, doi:10.1126/science.1566067 (1992).

49 Hardy, J. & Selkoe, D. J. The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics. *Science* **297**, 353-356 (2002).

50 Selkoe, D. J. Toward a comprehensive theory for Alzheimer's disease. Hypothesis: Alzheimer's disease is caused by the cerebral accumulation and cytotoxicity of amyloid beta-protein. *Ann N Y Acad Sci.* **924**, 17-25. (2000).

51 Selkoe, D. J. & Hardy, J. The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med* **8**, 595-608, doi:10.15252/emmm.201606210 (2016).

52 Tanzi, R. & Bertam, L. Twenty Years of the Alzheimer's Disease Amyloid Hypothesis A Genetic Perspective. **120**, 11 (2005).

53 Hardy, J. Alzheimer's disease: the amyloid cascade hypothesis: an update and reappraisal. *Journal of Alzheimer's Disease* **9**, 151-153 (2006).

54 Jha, N. K. *et al.* Impact of insulin degrading enzyme and neprilysin in Alzheimer's disease biology: Characterization of putative cognates for therapeutic applications. *Journal of Alzheimer's disease* **48**, 891-917 (2015).

55 Ries, M. & Sastre, M. Mechanisms of Aβ clearance and degradation by glial cells. *Frontiers in aging neuroscience* **8**, 160 (2016).

56 Tarasoff-Conway, J. M. *et al.* Clearance systems in the brain [mdash] implications for Alzheimer disease. *Nature Reviews Neurology* **11**, 457-470 (2015).

57 Meziane, H. *et al.* Memory-enhancing effects of secreted forms of the beta-amyloid precursor protein in normal and amnestic mice. *Proceedings of the National Academy of Sciences of the United States of America* **95**, 12683-12688 (1998).

58 Roch, J. M. *et al.* Increase of synaptic density and memory retention by a peptide representing the trophic domain of the amyloid beta/A4 protein precursor. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 7450-7454 (1994).

59 Rossjohn, J. *et al.* Crystal structure of the N-terminal, growth factor-like domain of Alzheimer amyloid precursor protein. *Nat. Struct. Biol* **6**, 327-331, doi:10.1038/7562 [doi] (1999).

60 Paradis, E., Douillard, H., Koutroumanis, M., Goodyer, C. & LeBlanc, A. Amyloid β peptide of Alzheimer's disease downregulates Bcl-2 and upregulates Bax expression in human neurons. *Journal of Neuroscience* **16**, 7533-7539 (1996).

61 Thal, D. R., Rüb, U., Orantes, M. & Braak, H. Phases of Aβ-deposition in the human brain and its relevance for the development of AD. *Neurology* **58**, 1791-1800 (2002).

62 Hyman, B. T. *et al.* National Institute on Aging–Alzheimer's Association guidelines for the neuropathologic assessment of Alzheimer's disease. *Alzheimer's & dementia: the journal of the Alzheimer's Association* **8**, 1-13 (2012).

63 Iqbal, K., Liu, F., Gong, C.-X. & Grundke-Iqbal, I. Tau in Alzheimer disease and related tauopathies. *Current Alzheimer Research* **7**, 656-664 (2010).

64 Conde, C. & Cáceres, A. Microtubule assembly, organization and dynamics in axons and dendrites. *Nature Reviews Neuroscience* **10**, 319 (2009).

65 Iqbal, K. *et al.* Tau pathology in Alzheimer disease and other tauopathies. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* **1739**, 198-210 (2005).

66 Pei, J. J. *et al.* Okadaic-acid-induced inhibition of protein phosphatase 2A produces activation of mitogen-activated protein kinases ERK1/2, MEK1/2, and p70 S6, similar to that in Alzheimer's disease. *The American journal of pathology* **163**, 845-858, doi:10.1016/S0002-9440(10)63445-1 (2003).

67 Hoover, B. R. *et al.* Tau mislocalization to dendritic spines mediates synaptic dysfunction independently of neurodegeneration. *Neuron* **68**, 1067-1081 (2010).

68 Zempel, H., Thies, E., Mandelkow, E. & Mandelkow, E.-M. Aβ oligomers cause localized Ca2+ elevation, missorting of endogenous Tau into dendrites, Tau phosphorylation, and destruction of microtubules and spines. *Journal of Neuroscience* **30**, 11938-11950 (2010).

69 Morris, M., Maeda, S., Vossel, K. & Mucke, L. The many faces of tau. *Neuron* **70**, 410-426 (2011).

70 Llorens-Marítin, M., Jurado, J., Hernández, F. & Ávila, J. GSK-3β, a pivotal kinase in Alzheimer disease. *Frontiers in molecular neuroscience* **7**, 46 (2014).

71 Lee, M.-S. & Tsai, L.-H. Cdk5: one of the links between senile plaques and neurofibrillary tangles? *Journal of Alzheimer's Disease* **5**, 127-137 (2003).

72 Monaco 3rd, E. & Vallano, M. L. Role of protein kinases in neurodegenerative disease: cyclin-dependent kinases in Alzheimer's disease. *Frontiers in bioscience : a journal and virtual library* **10**, 143-159 (2005).

73 Lim, A. C. & Qi, R. Z. Cyclin-dependent kinases in neural development and degeneration. *Journal of Alzheimer's Disease* **5**, 329-335 (2003).

74 Chin, J. Y. *et al.* Microtubule-affinity regulating kinase (MARK) is tightly associated with neurofibrillary tangles in Alzheimer brain: a fluorescence resonance energy transfer study. *Journal of Neuropathology & Experimental Neurology* **59**, 966-971 (2000).

75 Wei, Y. *et al.* Ribosylation triggering Alzheimer's disease- like Tau hyperphosphorylation via activation of CaMKII. *Aging cell* **14**, 754-763 (2015).

76 Amadoro, G. *et al.* NMDA receptor mediates tau-induced neurotoxicity by calpain and ERK/MAPK activation. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 2892-2897 (2006).

77 Fang, H., Zhang, L.-F., Meng, F.-T., Du, X. & Zhou, J.-N. Acute hypoxia promote the phosphorylation of tau via ERK pathway. *Neuroscience letters* **474**, 173-177 (2010).

78 Goedert, M. *et al.* Reduced binding of protein phosphatase 2A to tau protein with frontotemporal dementia and parkinsonism linked to chromosome 17 mutations. *Journal of neurochemistry* **75**, 2155-2162 (2000).

79 Sontag, E., Nunbhakdi-Craig, V., Lee, G., Bloom, G. S. & Mumby, M. C. Regulation of the phosphorylation state and microtubule-binding activity of Tau by protein phosphatase 2A. *Neuron* **17**, 1201-1207 (1996).

80 Ma, R.-h. *et al.* Role of microtubule-associated protein tau phosphorylation in Alzheimer's disease. *Journal of Huazhong University of Science and Technology [Medical Sciences]* **37**, 307-312 (2017).

81 Khatoon, S., Grundke-Iqbal, I. & Iqbal, K. Brain levels of microtubule-associated protein tau are elevated in Alzheimer's disease: a radioimmuno-slot-blot assay for nanograms of the protein. *Journal of neurochemistry* **59**, 750-753 (1992).

82 Alonso, A. D., Grundke-Iqbal, I., Barra, H. S. & Iqbal, K. Abnormal phosphorylation of tau and the mechanism of Alzheimer neurofibrillary degeneration: sequestration of microtubule-associated proteins 1 and 2 and the disassembly of microtubules by the abnormal tau. *Proc. Natl. Acad. Sci. U. S. A* **94**, 298-303 (1997).

83 Lindwall, G. & Cole, R. D. Phosphorylation affects the ability of tau protein to promote microtubule assembly. *The Journal of biological chemistry* **259**, 5301-5305 (1984).

84 Alonso, A. C., Zaidi, T., Grundke-Iqbal, I. & Iqbal, K. Role of abnormally phosphorylated tau in the breakdown of microtubules in Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America* **91**, 5562-5566 (1994).

85 Barron, M., Gartlon, J., Dawson, L. A., Atkinson, P. J. & Pardon, M.-C. A state of delirium: Deciphering the effect of inflammation on tau pathology in Alzheimer's disease. *Experimental gerontology* **94**, 103-107, doi:https://doi.org/10.1016/j.exger.2016.12.006 (2017).

86 Braak, H. & Braak, E. Neuropathological stageing of Alzheimer-related changes. *Acta neuropathologica* **82**, 239-259 (1991).

87 Van Petten, C. Relationship between hippocampal volume and memory ability in healthy individuals across the lifespan: review and meta-analysis. *Neuropsychologia* **42**, 1394-1413 (2004).

88 Hof, P., Glannakopoulos, P. & Bouras, C. The neuropathological changes associated with normal brain aging. *Histology and histopathology* **11**, 1075-1088 (1996).

89 Almkvist, O. Neuropsychological features of early Alzheimer's disease: preclinical and clinical stages. *Acta neurologica Scandinavica* **94**, 63-71 (1996).

90 Sämgård, K. *et al.* Cerebrospinal fluid total tau as a marker of Alzheimer's disease intensity. *International journal of geriatric psychiatry* **25**, 403-410 (2010).

91 Fagan, A. M. *et al.* Cerebrospinal fluid tau/β-amyloid42 ratio as a prediction of cognitive decline in nondemented older adults. *Archives of neurology* **64**, 343-349 (2007).

92 Stomrud, E., Hansson, O., Blennow, K., Minthon, L. & Londos, E. Cerebrospinal fluid biomarkers predict decline in subjective cognitive function over 3 years in healthy elderly. *Dementia and geriatric cognitive disorders* **24**, 118-124, doi:10.1159/000105017 (2007).

93 Buchhave, P. *et al.* Cerebrospinal fluid levels ofβ-amyloid 1-42, but not of tau, are fully changed already 5 to 10 years before the onset of Alzheimer dementia. *Archives of general psychiatry* **69**, 98-106 (2012).

94 Jack, C. R., Jr. & Holtzman, D. M. Biomarker modeling of Alzheimer's disease. *Neuron* **80**, 1347-1358, doi:S0896-6273(13)01133-1 [pii];10.1016/j.neuron.2013.12.003 [doi] (2013).

95 ChristenZaech, S. *et al.* Early olfactory involvement in Alzheimer's disease. *Canadian Journal of Neurological Sciences* **30**, 20-25 (2003).

96 Gómez-Isla, T. *et al.* Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer’s disease. *The Journal of Neuroscience* **16**, 4491-4500 (1996).

97 Kordower, J. H. *et al.* Loss and atrophy of layer II entorhinal cortex neurons in elderly people with mild cognitive impairment. *Annals of neurology* **49**, 202-213 (2001).

98 Alafuzoff, I. *et al.* Staging of neurofibrillary pathology in Alzheimer's disease: a study of the BrainNet Europe Consortium. *Brain pathology* **18**, 484-496 (2008).

99 Jacobs, D. M. *et al.* Neuropsychological detection and characterization of preclinical Alzheimer's disease. *Neurology* **45**, 957-962 (1995).

100 Tierney, M. *et al.* Prediction of probable Alzheimer's disease in memory-impaired patients A prospective longitudinal study. *Neurology* **46**, 661-665 (1996).

101 Aggarwal, N., Wilson, R., Beck, T., Bienias, J. & Bennett, D. Mild cognitive impairment in different functional domains and incident Alzheimer's disease. *Journal of Neurology, Neurosurgery & Psychiatry* **76**, 1479-1484 (2005).

102 Blacker, D. *et al.* Neuropsychological measures in normal individuals that predict subsequent cognitive decline. *Archives of neurology* **64**, 862-871 (2007).

103 Terry, R. D. *et al.* Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Annals of neurology* **30**, 572-580 (1991).

104 DeKosky, S. T. & Scheff, S. W. Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. *Annals of neurology* **27**, 457-464 (1990).

105 Terry, R. D. Cell death or synaptic loss in Alzheimer disease. *Journal of Neuropathology & Experimental Neurology* **59**, 1118-1119 (2000).

106 Coleman, P., Federoff, H. & Kurlan, R. A focus on the synapse for neuroprotection in Alzheimer diseas and other dementias. *Neurology*, 1155-1162 (2004).

107 Masliah, E. *et al.* Synaptic and neuritic alterations during the progression of Alzheimer's disease. *Neurosci. Lett* **174**, 67-72 (1994).

108 Izquierdo, I. *et al.* Memory processing by the limbic system: role of specific neurotransmitter systems. *Behavioural brain research* **58**, 91-98 (1993).

109 Witter, M. P. Organization of the entorhinal—hippocampal system: A review of current anatomical data. *Hippocampus* **3**, 33-44 (1993).

110 Sze, C. I. *et al.* Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. *J. Neuropathol. Exp. Neurol* **56**, 933-944 (1997).

111 Sims, N. R. *et al.* Presynaptic cholinergic dysfunction in patients with dementia. *J. Neurochem* **40**, 503-509 (1983).

112 Bartus, R. T., Dean, R. L., 3rd, Beer, B. & Lippa, A. S. The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217**, 408-414 (1982).

113 Etienne, P., Robitaille, Y., Gauthier, S. & Nair, N. P. Nucleus basalis neuronal loss and neuritic plaques in advanced Alzheimer's disease. *Canadian journal of physiology and pharmacology* **64**, 318-324 (1986).

114 Whitehouse, P. J., Price, D. L., Clark, A. W., Coyle, J. T. & DeLong, M. R. Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. *Annals of neurology* **10**, 122-126 (1981).

115 Fibiger, H. C. Cholinergic mechanisms in learning, memory and dementia: a review of recent evidence. *Trends in neurosciences* **14**, 220-223 (1991).

116 Whitehouse, P. J. *et al.* Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* **215**, 1237-1239 (1982).

117 Thompson, P. M. *et al.* Dynamics of gray matter loss in Alzheimer's disease. *Journal of Neuroscience* **23**, 994-1005 (2003).

118 Bondareff, W., Mountjoy, C. & Roth, M. Loss of neurons of origin of the adrenergic projection to cerebral cortex (nucleus locus ceruleus) in senile dementia. *Neurology* **32**, 164-168 (1982).

119 Mann, D., Lincoln, J., Yates, P., Stamp, J. & Toper, S. Changes in the monoamine containing neurones of the human CNS in senile dementia. *The British Journal of Psychiatry* **136**, 533-541 (1980).

120 Mann, D. & Yates, P. Serotonin nerve cells in Alzheimer's disease. *Journal of neurology, neurosurgery, and psychiatry* **46**, 96 (1983).

121 Palmer, A. M. & DeKosky, S. T. Monoamine neurons in aging and Alzheimer's disease. *Journal of Neural Transmission* **91**, 135-159 (1993).

122 Kraepelin, E. *Senile and pre-senile dementias*. (1910).

123 Alzheimer, A. Über eigenartige Krankheitsfälle des späteren Alters. *Zeitschrift für die gesamte Neurologie und Psychiatrie* **4**, 356 (1911).

124 Derouesné, C. Alzheimer and Alzheimer's disease: the present enlighted by the past. An historical approach. *Psychologie & neuropsychiatrie du vieillissement* **6**, 115-128 (2008).

125 Newton, R. D. The identity of Alzheimer's disease and senile dementia and their relationship to senility. *Journal of Mental Science* **94**, 225-249 (1948).

126 Lauter, H. & Mayer, J. E. in *Senile Dementia* (eds C. Müller & L. Ciompi) 13-27 (Huber, 1968).

127 Katzman, R. Editorial: The prevalence and malignancy of Alzheimer disease. A major killer. *Archives of neurology* **33**, 217-218 (1976).

128 Blessed, G., Tomlinson, B. E. & Roth, M. The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. *British Journal of Psychiatry* **114**, 797-811 (1968).

129 Terry, R. D. *Aging, Senile Dementia and Alzheimer's Disease*. Vol. (Aging, Vol. 7) 11-14 (Raven Press, 1978).

130 Glenner, G. G., Wong, C. W., Quaranta, V. & Eanes, E. D. The amyloid deposits in Alzheimer's disease: their nature and pathogenesis. *Applied Pathology* **2**, 357-369 (1984).

131 Grundke-Iqbal, I. *et al.* Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. *The Journal of biological chemistry* **261**, 6084-6089 (1986).

132 Cruchaga, C. *et al.* Rare variants in APP, PSEN1 and PSEN2 increase risk for AD in late-onset Alzheimer's disease families. *PloS one* **7**, e31039 (2012).

133 Bekris, L. M., Yu, C.-E., Bird, T. D. & Tsuang, D. W. Review article: genetics of Alzheimer disease. *Journal of geriatric psychiatry and neurology* **23**, 213-227 (2010).

134 Müller, U., Winter, P. & Graeber, M. B. A presenilin 1 mutation in the first case of Alzheimer's disease. *The Lancet Neurology* **12**, 129-130 (2013).

135 Rocchi, A., Pellegrini, S., Siciliano, G. & Murri, L. Causative and susceptibility genes for Alzheimer's disease: a review. *Brain research bulletin* **61**, 1-24 (2003).

136 Chartier-Harlin, M. C. *et al.* Early-onset Alzheimer's disease caused by mutations at codon 717 of the B-amyloid precursor protein gene. *Nature* **353**, 844-846 (1991).

137 Goate, A. *et al.* Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. *Nature* **349**, 704-706, doi:10.1038/349704a0 [doi] (1991).

138 Goate, A. Segregation of a missense mutation in the amyloid β-protein precursor gene with familial Alzheimer's disease. *Journal of Alzheimer's Disease* **9**, 341-347 (2006).

139 Sherrington, R. *et al.* Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **375**, 754-760, doi:10.1038/375754a0 (1995).

140 Corder, E. *et al.* Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. *Science* **261**, 921-923, doi:10.1126/science.8346443 (1993).

141 Levy-Lahad, E. *et al.* Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* **269**, 973-977 (1995).

142 Hendriks, L. *et al.* Presenile dementia and cerebral haemorrhage linked to a mutation at codon 692 of the beta-amyloid precursor protein gene. *Nature genetics* **1**, 218-221, doi:10.1038/ng0692-218 (1992).

143 Selkoe, D. J. Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* **81**, 741-766 (2001).

144 Mullan, M. *et al.* A pathogenic mutation for probable Alzheimer's disease in the APP gene at the N-terminus of beta-amyloid. *Nature genetics* **1**, 345-347, doi:10.1038/ng0892-345 (1992).

145 Haass, C. & De Strooper, B. The presenilins in Alzheimer's disease--proteolysis holds the key. *Science* **286**, 916-919 (1999).

146 Cruts, M., Theuns, J. & Van Broeckhoven, C. Locus-specific mutation databases for neurodegenerative brain diseases. *Human mutation* **33**, 1340-1344, doi:10.1002/humu.22117 (2012).

147 Cacace, R., Sleegers, K. & Van Broeckhoven, C. Molecular genetics of early-onset Alzheimer's disease revisited. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **12**, 733-748, doi:10.1016/j.jalz.2016.01.012 (2016).

148 Glenner, G. G. & Wong, C. W. Alzheimer's disease and Down's syndrome: sharing of a unique cerebrovascular amyloid fibril protein. *Biochemical and biophysical research communications* **122**, 1131-1135 (1984).

149 Sanchez, M. M. *et al.* Neurobiological elements of cognitive dysfunction in down syndrome: exploring the role of APP. *Biological psychiatry* **71**, 403-409 (2012).

150 Teller, J. K. *et al.* Presence of soluble amyloid β–peptide precedes amyloid plaque formation in Down's syndrome. *Nature medicine* **2**, 93-95 (1996).

151 Hardy, J. Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci* **20**, 154-159, doi:S0166223696010302 [pii] (1997).

152 Bateman, R. J. *et al.* Autosomal-dominant Alzheimer's disease: a review and proposal for the prevention of Alzheimer's disease. *Alzheimer's research & therapy* **3**, 1 (2011).

153 Frisardi, V. *et al.* Metabolic-cognitive syndrome: a cross-talk between metabolic syndrome and Alzheimer's disease. *Ageing research reviews* **9**, 399-417 (2010).

154 Association, A. s. 2017 Alzheimer's disease facts and figures. *Alzheimer's & Dementia* **13**, 325-373 (2017).

155 Hux, M. J. *et al.* Relation between severity of Alzheimer's disease and costs of caring. *Canadian Medical Association Journal* **159**, 457-465 (1998).

156 Breitner, J. C. What should we do if we were wrong and Alzheimer was right? *International psychogeriatrics / IPA* **26**, 3-6, doi:10.1017/S1041610213001749 (2014).

157 Mawuenyega, K. G. *et al.* Decreased clearance of CNS β-amyloid in Alzheimer's disease. *Science* **330**, 1774-1774 (2010).

158 Iturria-Medina, Y., Sotero, R. C., Toussaint, P. J., Evans, A. C. & Alzheimer's Disease Neuroimaging, I. Epidemic spreading model to characterize misfolded proteins propagation in aging and associated neurodegenerative disorders. *PLoS Comput Biol* **10**, e1003956, doi:10.1371/journal.pcbi.1003956 (2014).

159 Suzuki, Y. *et al.* Reduced CSF Water Influx in Alzheimer's Disease Supporting the beta-Amyloid Clearance Hypothesis. *PloS one* **10**, e0123708, doi:10.1371/journal.pone.0123708 (2015).

160 Castellano, J. M. *et al.* Human apoE isoforms differentially regulate brain amyloid-β peptide clearance. *Science translational medicine* **3**, 89ra57-89ra57 (2011).

161 Breitner, J. C. S. *et al.* The use of twin cohorts for research in Alzheimer's disease. *Neurology* **43**, 261-267 (1993).

162 Gatz, M. *et al.* Complete ascertainment of dementia in the Swedish Twin Registry: the HARMONY study. *Neurobiology of aging* **26**, 439-447, doi:10.1016/j.neurobiolaging.2004.04.004 (2005).

163 Green, R. C. *et al.* Risk of dementia among white and African American relatives of patients with Alzheimer disease. *JAMA : the journal of the American Medical Association* **287**, 329-336 (2002).

164 Loy, C. T., Schofield, P. R., Turner, A. M. & Kwok, J. B. Genetics of dementia. *The Lancet* **383**, 828-840 (2014).

165 Avramopoulos, D. Genetics of Alzheimer's disease: recent advances. *Genome Medicine* **1**, 34 (2009).

166 Breitner, J. C. S., Murphy, E. A., Silverman, J. M., Mohs, R. C. & Davis, K. L. Age-dependent expression of familial risk in Alzheimer's disease. *American journal of epidemiology* **128**, 536-548 (1988).

167 Lambert, J. C. *et al.* Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nature genetics* **45**, 1452-1458, doi:10.1038/ng.2802 (2013).

168 Poirier, J. *et al.* Apolipoprotein E polymorphism and Alzheimer's disease. *The Lancet* **342**, 697-699 (1993).

169 Strittmatter, W. J. *et al.* Apolipoprotein E: high-avidity binding to B-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 1977-1981 (1993).

170 Poirier, J. Apolipoprotein E and cholesterol metabolism in the pathogenesis and treatment of Alzheimer's disease. *Trends in Molecular Medicine* **9**, 94-101, doi:10.1016/S1471-4914(03)00007-8 (2003).

171 Poirier, J. Apolipoprotein E, cholesterol transport and synthesis in sporadic Alzheimer's disease. *Neurobiol. Aging* **26**, 355-361, doi:S0197-4580(04)00293-3 [pii];10.1016/j.neurobiolaging.2004.09.003 [doi] (2005).

172 Beffert, U. & Poirier, J. ApoE associated with lipid has a reduced capacity to inhibit beta-amyloid fibril formation. *Neuroreport* **9**, 3321-3323 (1998).

173 Poirier, J. Apolipoprotein E in animal models of CNS injury and in Alzheimer's disease. *Trends in Neurosciences* **17**, 525-530 (1994).

174 Masliah, E., Mallory, M., Alford, M., Ge, N. & Mucke, L. *Abnormal synaptic regeneration in hAPP695 transgenic and APOE knockout mice*. (1995).

175 Krzywkowski, P. *et al.* Cholinergic systems and long-term potentiation in memory-impaired apolipoprotein E-deficient mice. *Neuroscience* **92**, 1273-1286 (1999).

176 Hanlon, C. S. & Rubinsztein, D. C. Arginine residues at codons 112 and 158 in the apolipoprotein E gene correspond to the ancestral state in humans. *Atherosclerosis* **112**, 85-90 (1995).

177 Hatters, D. M., Peters-Libeu, C. A. & Weisgraber, K. H. Apolipoprotein E structure: insights into function. *Trends Biochem Sci* **31**, 445-454, doi:10.1016/j.tibs.2006.06.008 (2006).

178 Corder, E. H. *et al.* Protective effect of apolipoprotein E type 2 allele for late onset Alzheimer disease. *Nature genetics* **7**, 180-184, doi:10.1038/ng0694-180 (1994).

179 Riddell, D. R. *et al.* Impact of apolipoprotein E (ApoE) polymorphism on brain ApoE levels. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **28**, 11445-11453, doi:10.1523/JNEUROSCI.1972-08.2008 (2008).

180 Riedel, B. C., Thompson, P. M. & Brinton, R. D. Age, APOE and sex: Triad of risk of Alzheimer's disease. *J Steroid Biochem Mol Biol* **160**, 134-147, doi:10.1016/j.jsbmb.2016.03.012 (2016).

181 Farrer, L. A. *et al.* Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. *Journal of the American Medical Association* **278**, 1349-1356 (1997).

182 Graves, A. B. *et al.* Impaired olfaction as a marker for cognitive decline Interaction with apolipoprotein E ε4 status. *Neurology* **53**, 1480-1480 (1999).

183 Mann, D. Alzheimer's disease and Down's syndrome. *Histopathology* **13**, 125-137 (1988).

184 Helkala, E.-L. *et al.* The association of apolipoprotein E polymorphism with memory: a population based study. *Neuroscience letters* **191**, 141-144 (1995).

185 Schmechel, D. *et al.* Increased amyloid beta-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. *Proceedings of the National Academy of Sciences* **90**, 9649-9653 (1993).

186 Rebeck, G. W., Reiter, J. S., Strickland, D. K. & Hyman, B. T. Apolipoprotein E in sporadic Alzheimer's disease: allelic variation and receptor interactions. *Neuron* **11**, 575-580 (1993).

187 Mann, D. *et al.* Preferential deposition of amyloid β protein (Aβ) in the form Aβ40 in Alzheimer's disease is associated with a gene dosage effect of the apolipoprotein E E4 allele. *Neuroscience letters* **221**, 81-84 (1997).

188 Lai, M. K. *et al.* Selective effects of the APOE epsilon4 allele on presynaptic cholinergic markers in the neocortex of Alzheimer's disease. *Neurobiology of disease* **22**, 555-561, doi:10.1016/j.nbd.2005.12.016 (2006).

189 Allen, S. *et al.* Reduced cholinergic function in normal and Alzheimer's disease brain is associated with apolipoprotein E4 genotype. *Neuroscience letters* **239**, 33-36 (1997).

190 Filippini, N. *et al.* Distinct patterns of brain activity in young carriers of the APOE-epsilon4 allele. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 7209-7214, doi:10.1073/pnas.0811879106 (2009).

191 Fleisher, A. S. *et al.* Resting-state BOLD networks versus task-associated functional MRI for distinguishing Alzheimer's disease risk groups. *NeuroImage* **47**, 1678-1690, doi:10.1016/j.neuroimage.2009.06.021 (2009).

192 Calderon-Garciduenas, L. *et al.* White matter hyperintensities, systemic inflammation, brain growth, and cognitive functions in children exposed to air

pollution. *Journal of Alzheimer's disease : JAD* **31**, 183-191, doi:10.3233/JAD-2012-120610 (2012).

193 Schipper, H. M. Apolipoprotein E: implications for AD neurobiology, epidemiology and risk assessment. *Neurobiology of aging* **32**, 778-790 (2011).

194 Arendt, T. Plastic neuronal remodeling is impaired in patients with Alzheimer's disease carrying apolipoprotein E4 allele. *Journal of Neuroscience* **17**, 516-529 (1997).

195 Niskakangas, T. *et al.* Association of apolipoprotein E polymorphism with outcome after aneurysmal subarachnoid hemorrhage: a preliminary study. *Stroke* **32**, 1181-1184 (2001).

196 Crawford, F. *et al.* APOE genotype influences acquisition and recall following traumatic brain injury. *Neurology* **58**, 1115-1118 (2002).

197 Ariza, M. *et al.* Influence of APOE polymorphism on cognitive and behavioural outcome in moderate and severe traumatic brain injury. *Journal of Neurology, Neurosurgery & Psychiatry* **77**, 1191-1193 (2006).

198 Gatz, M. *et al.* Role of genes and environments for explaining Alzheimer disease. *Archives of general psychiatry* **63**, 168-174 (2006).

199 Oberlin, L. E. *et al.* Blood pressure interacts with APOE epsilon4 to predict memory performance in a midlife sample. *Neuropsychology* **29**, 693-702, doi:10.1037/neu0000177 (2015).

200 Irie, F. *et al.* Enhanced risk for Alzheimer disease in persons with type 2 diabetes and APOE ε4: the Cardiovascular Health Study Cognition Study. *Archives of neurology* **65**, 89-93 (2008).

201 Irie, F. *et al.* Apolipoprotein E ε4 allele genotype and the effect of depressive symptoms on the risk of dementia in men: the Honolulu-Asia Aging Study. *Archives of general psychiatry* **65**, 906-912 (2008).

202 Shobab, L. A., Hsiung, G.-Y. R. & Feldman, H. H. Cholesterol in Alzheimer's disease. *The Lancet Neurology* **4**, 841-852 (2005).

203 Hirsch-Reinshagen, V., Burgess, B. L. & Wellington, C. L. Why lipids are important for Alzheimer disease? *Molecular and cellular biochemistry* **326**, 121-129 (2009).

204 Czech, C. *et al.* Apolipoprotein E-4 gene dose in clinically diagnosed Alzheimer's disease: prevalence, plasma cholesterol levels and cerebrovascular change. *European archives of psychiatry and clinical neuroscience* **243**, 291-292 (1994).

205 Notkola, I. L. *et al.* Serum total cholesterol, apolipoprotein E epsilon 4 allele, and Alzheimer's disease. *Neuroepidemiology* **17**, 14-20 (1998).

206 De la Torre, J. Alzheimer disease as a vascular disorder: nosological evidence. *Stroke* **33**, 1152-1162 (2002).

207 Kuo, Y.-M. *et al.* Elevated low-density lipoprotein in Alzheimer's disease correlates with brain Aβ 1–42 levels. *Biochemical and biophysical research communications* **252**, 711-715 (1998).

208 Fassbender, K. *et al.* Simvastatin strongly reduces levels of Alzheimer's disease beta -amyloid peptides Abeta 42 and Abeta 40 in vitro and in vivo. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 5856-5861, doi:10.1073/pnas.081620098 (2001).

209 Wolozin, B. Cholesterol and Alzheimer's disease. *Biochemical Society transactions* **30**, 525-529 (2002).

210 Wolozin, B. A fluid connection: cholesterol and Aβ. *Proceedings of the National Academy of Sciences* **98**, 5371-5373 (2001).

211 Kandiah, N., Tan, K., Lim, C. C. & Venketasubramanian, N. Hyperglycemic choreoathetosis: role of the putamen in pathogenesis. *Mov Disord* **24**, 915-919, doi:10.1002/mds.22277 (2009).

212 Skoog, I. *et al.* 15-year longitudinal study of blood pressure and dementia. *Lancet* **347**, 1141-1145 (1996).

213 Launer, L. J. *et al.* Midlife blood pressure and dementia: the Honolulu–Asia aging study☆. *Neurobiology of aging* **21**, 49-55 (2000).

214 Wang, Z. *et al.* Arterial spin labeled MRI in prodromal Alzheimer's disease: a multi-site study. *NeuroImage: Clinical* **2**, 630-636 (2013).

215 Leibson, C. L. *et al.* Risk of dementia among persons with diabetes mellitus: a population-based cohort study. *American journal of epidemiology* **145**, 301-308 (1997).

216 Luchsinger, J. A. *et al.* Relation of diabetes to mild cognitive impairment. *Archives of neurology* **64**, 570-575, doi:64/4/570 [pii]
10.1001/archneur.64.4.570 [doi] (2007).

217 Luchsinger, J. A. Adiposity, hyperinsulinemia, diabetes and Alzheimer's disease: an epidemiological perspective. *European journal of pharmacology* **585**, 119-129 (2008).

218 Craft, S. Insulin resistance syndrome and Alzheimer's disease: age-and obesity-related effects on memory, amyloid, and inflammation. *Neurobiology of aging* **26**, 65-69 (2005).

219 Sims-Robinson, C., Kim, B., Rosko, A. & Feldman, E. L. How does diabetes accelerate Alzheimer disease pathology? *Nature Reviews Neurology* **6**, 551 (2010).

220 Gamba, P. *et al.* Oxidized cholesterol as the driving force behind the development of Alzheimer's disease. *Front Aging Neurosci* **7**, 119, doi:10.3389/fnagi.2015.00119 (2015).

221 Iturria-Medina, Y. *et al.* Multifactorial causal model of brain (dis)organization and therapeutic intervention: Application to Alzheimer's disease. *NeuroImage* **152**, 60-77, doi:10.1016/j.neuroimage.2017.02.058 (2017).

222 Attems, J. & Jellinger, K. A. The overlap between vascular disease and Alzheimer’s disease-lessons from pathology. *BMC medicine* **12**, 206 (2014).

223 Kokmen, E. *et al.* Clinical risk factors for Alzheimer's disease: a population-based case-control study. *Neurology* **41**, 1393-1397 (1991).

224 Mortimer, J. A., French, L. R., Hutton, J. T. & Schuman, L. M. Head injury as a risk factor for Alzheimer's disease. *Neurology* **35**, 264-267 (1985).

225 Mortimer, J. A. *et al.* Head trauma as a risk factor for Alzheimer's disease: a collaborative re-analysis of case-control studies. EURODEM Risk Factors Research Group. *International journal of epidemiology* **20 Suppl 2**, S28-35 (1991).

226 Stern, Y. The concept of cognitive reserve: a catalyst for research. *Journal of clinical and experimental neuropsychology* **25**, 589-593, doi:10.1076/jcen.25.5.589.14571 (2003).

227 Wang, H.-X., Xu, W. & Pei, J.-J. Leisure activities, cognition and dementia. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease* **1822**, 482-491 (2012).

228 Wang, H.-X. *et al.* Late life leisure activities and risk of cognitive decline. *Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences* **68**, 205-213 (2012).

229 Kalaria, R. N. *et al.* Alzheimer's disease and vascular dementia in developing countries: prevalence, management, and risk factors. *The Lancet Neurology* **7**, 812-826, doi:10.1016/s1474-4422(08)70169-8 (2008).

230 Norton, S., Matthews, F. E., Barnes, D. E., Yaffe, K. & Brayne, C. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. *The Lancet Neurology* **13**, 788-794, doi:10.1016/s1474-4422(14)70136-x (2014).

231 Bilbul, M. & Schipper, H. M. Risk profiles of Alzheimer disease. *Canadian Journal of Neurological Sciences* **38**, 580-592 (2011).

232 Livingston, G. *et al.* Dementia prevention, intervention, and care. *The Lancet*, doi:10.1016/s0140-6736(17)31363-6 (2017).

233 Petersen, R. C., Doody, R. & Kurz, A. Current concepts in mild cognitive impairment. *Archives of ...* **1**, doi:10.1001/archneur.58.12.1985 (2001).

234 Kral, V. A. Senescent forgetfulness: benign and malignant. *Canadian Medical Association Journal* **86**, 257-260 (1962).

235 Crook, T. *et al.* Age-associated memory impairment: proposed diagnostic criteria and measures of clinical change - Report of a National Institute of Mental Health Work Group. *Developmental Neuropsychology* **2**, 261-276 (1986).

236 Levy, R. Aging-associated cognitive decline. *International Psychogeriatrics* **6**, 63-68 (1994).

237 Association, A. P. *DSM-IV® Sourcebook*. Vol. 1 (American Psychiatric Pub, 1994).

238 Graham, J. E. *et al.* Prevalence and severity of cognitive impairment with and without dementia in an elderly population. *Lancet* **349**, 1793-1796 (1997).

239 Flicker, C., Ferris, S. H. & Reisberg, B. Mild cognitive impairment in the elderly predictors of dementia. *Neurology* **41**, 1006-1006 (1991).

240 Petersen, R. C. Mild Cognitive Impairment. *Archives of neurology* **56**, 303-303, doi:10.1001/archneur.56.3.303 (1999).

241 Markesbery, W. R. Neuropathologic alterations in mild cognitive impairment: a review. *Journal of Alzheimer's Disease* **19**, 221-228 (2010).

242 Sperling, R. A. *et al.* Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **7**, 280-292, doi:10.1016/j.jalz.2011.03.003 (2011).

243 Fink, H. A., Jutkowitz, E., McCarten, J. & et al. Pharmacologic interventions to prevent cognitive decline, mild cognitive impairment, and clinical alzheimer-type dementia: A systematic review. *Annals of internal medicine* **168**, 39-51, doi:10.7326/M17-1529 (2018).

244 Brookmeyer, R., Johnson, E., Ziegler-Graham, K. & Arrighi, H. M. Forecasting the global burden of Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **3**, 186-191, doi:10.1016/j.jalz.2007.04.381 (2007).

245 Sperling, R., Mormino, E. & Johnson, K. The evolution of preclinical Alzheimer's disease: implications for prevention trials. *Neuron* **84**, 608-622, doi:10.1016/j.neuron.2014.10.038 (2014).

246 Bateman , R. J. *et al.* Clinical and Biomarker Changes in Dominantly Inherited Alzheimer's Disease. *New Engl J Med* **367**, 795-804, doi:doi:10.1056/NEJMoa1202753 (2012).

247 Moghekar, A. *et al.* CSF biomarker changes precede symptom onset of mild cognitive impairment. *Neurology* **81**, 1753-1758, doi:10.1212/01.wnl.0000435558.98447.17 (2013).

248 Villemagne, V. L. *et al.* Amyloid beta deposition, neurodegeneration, and cognitive decline in sporadic Alzheimer's disease: a prospective cohort study. *Lancet neurology* **12**, 357-367, doi:10.1016/S1474-4422(13)70044-9 (2013).

249 Petersen, R. C. *et al.* Mild cognitive impairment: clinical characterization and outcome. *Archives of neurology* **56**, 303-308 (1999).

250 Cummings, J. L., Doody, R. & Clark, C. Disease-modifying therapies for Alzheimer disease Challenges to early intervention. *Neurology* **69**, 1622-1634 (2007).

251 Breitner, J. C. *et al.* APOE-epsilon4 count predicts age when prevalence of AD increases, then declines: the Cache County Study. *Neurology* **53**, 321-331 (1999).

252 Huang, W., Qiu, C., von Strauss, E., Winblad, B. & Fratiglioni, L. APOE genotype, family history of dementia, and Alzheimer disease risk: a 6-year follow-up study. *Archives of neurology* **61**, 1930-1934 (2004).

253 Breitner, J., Poirier, J., Etienne, P., Leoutsakos, J. & Group, P.-A. R. Rationale and structure for a new center for Studies on Prevention of Alzheimer's Disease (StoP-AD). *J Prev Alzheimers Dis* **3**, 236-242 (2016).

254 Fleisher, A. S. *et al.* Florbetapir PET analysis of amyloid-β deposition in the presenilin 1 E280A autosomal dominant Alzheimer's disease kindred: a cross-sectional study. *The Lancet Neurology* **11**, 1057-1065 (2012).

255 Takeda, A. *et al.* A systematic review of the clinical effectiveness of donepezil, rivastigmine and galantamine on cognition, quality of life and adverse events in Alzheimer's disease. *International journal of geriatric psychiatry* **21**, 17-28 (2006).

256 Menéndez-González, M. Routine lumbar puncture for the early diagnosis of Alzheimer's disease. Is it safe? *Frontiers in Aging Neuroscience* **6**, 65 (2014).

257 Tuwaig, M. *et al.* Deficit in Central Auditory Processing as a Biomarker of Pre-Clinical Alzheimer's Disease. *Journal of Alzheimer's Disease*, 1-12 (2017).

258 Albers, M. W. *et al.* At the interface of sensory and motor dysfunctions and Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **11**, 70-98, doi:10.1016/j.jalz.2014.04.514 (2015).

259 Larsson, M. & Bäckman, L. Semantic activation and episodic odor recognition in young and older adults. *Psychology and aging* **8**, 582 (1993).

260 Ship, J. A., Pearson, J. D., Cruise, L. J., Brant, L. J. & Metter, E. J. Longitudinal changes in smell identification. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* **51**, M86-M91 (1996).

261 Lehrner, J. P., Glück, J. & Laska, M. Odor identification, consistency of label use, olfactory threshold and their relationships to odor memory over the human lifespan. *Chemical Senses* **24**, 337-346 (1999).

262 Hummel, T., Heilmann, S. & Murphy, C. Age-related changes of chemosensory functions. *Olfaction, taste, and cognition*, 441-456 (2002).

263 Nordin, S. *et al.* Olfactory event-related potentials in young and elderly adults: evaluation of tracking task versus eyes open/closed recording. *Chemical senses* **24**, 459-464 (1999).

264 Wehling, E. I., Wollschlaeger, D., Nordin, S. & Lundervold, A. J. Longitudinal changes in odor identification performance and neuropsychological measures in aging individuals. *Neuropsychology* **30**, 87 (2016).

265 Grober, E. *et al.* Memory impairment, executive dysfunction, and intellectual decline in preclinical Alzheimer's disease. *Journal of the International Neuropsychological Society* **14**, 266-278 (2008).

266 Grober, E., Lipton, R. B., Hall, C. & Crystal, H. Memory impairment on free and cued selective reminding predicts dementia. *Neurology* **54**, 827-832 (2000).

267 Bäckman, L., Small, B. J. & Fratiglioni, L. Stability of the preclinical episodic memory deficit in Alzheimer's disease. *Brain : a journal of neurology* **124**, 96-102 (2001).

268 Sun, G. H., Raji, C. A., Maceachern, M. P. & Burke, J. F. Olfactory identification testing as a predictor of the development of Alzheimer's dementia: a systematic review. *The Laryngoscope* **122**, 1455-1462, doi:10.1002/lary.23365 (2012).

269 Waldton, S. Clinical observations of impaired cranial nerve function in senile dementia. *Acta psychiatrica Scandinavica* **50**, 539-547 (1974).

270 Mesholam, R. I., Moberg, P. J., Mahr, R. N. & Doty, R. L. Olfaction in neurodegenerative disease: a meta-analysis of olfactory functioning in Alzheimer's and Parkinson's diseases. *Archives of neurology* **55**, 84-90 (1998).

271 Wilson, R. S. *et al.* Olfactory identification and incidence of mild cognitive impairment in older age. *Archives of general psychiatry* **64**, 802-808 (2007).

272 Djordjevic, J., Jones-Gotman, M., De Sousa, K. & Chertkow, H. Olfaction in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiology of aging* **29**, 693-706 (2008).

273 Kjelvik, G., Sando, S. B., Aasly, J., Engedal, K. A. & White, L. R. Use of the Brief Smell Identification Test for olfactory deficit in a Norwegian population with Alzheimer's disease. *International journal of geriatric psychiatry* **22**, 1020-1024 (2007).

274 Makowska, I., Kloszewska, I., Grabowska, A., Szatkowska, I. & Rymarczyk, K. Olfactory deficits in normal aging and Alzheimer's disease in the polish elderly population. *Archives of Clinical Neuropsychology* **26**, 270-279 (2011).

275 Velayudhan, L., Pritchard, M., Powell, J. F., Proitsi, P. & Lovestone, S. Smell identification function as a severity and progression marker in Alzheimer's disease. *International psychogeriatrics* **25**, 1157-1166 (2013).

276 Tabert, M. H. *et al.* A 10- item smell identification scale related to risk for Alzheimer's disease. *Annals of neurology* **58**, 155-160 (2005).

277 Devanand, D. *et al.* Olfactory identification deficits and MCI in a multi-ethnic elderly community sample. *Neurobiology of aging* **31**, 1593-1600 (2010).

278 Wilson, R. S. *et al.* Olfactory impairment in presymptomatic Alzheimer's disease. *Annals of the New York Academy of Sciences* **1170**, 730-735, doi:10.1111/j.1749-6632.2009.04013.x (2009).

279 Roberts, R. O. *et al.* Association Between Olfactory Dysfunction and Amnestic Mild Cognitive Impairment and Alzheimer Disease Dementia. *JAMA Neurol*, 1-9, doi:10.1001/jamaneurol.2015.2952 (2015).

280 Yaffe, K. *et al.* Olfaction and risk of dementia in a biracial cohort of older adults. *Neurology* **88**, 456-462 (2017).

281 Stanciu, I. *et al.* Olfactory impairment and subjective olfactory complaints independently predict conversion to dementia: a longitudinal, population-based study. *Journal of the International Neuropsychological Society* **20**, 209-217 (2014).

282 Devanand, D. *et al.* Olfactory deficits predict cognitive decline and Alzheimer dementia in an urban community. *Neurology* **84**, 182-189 (2015).

283 Schubert, C. R. *et al.* Olfaction and the 5- Year Incidence of Cognitive Impairment in an Epidemiological Study of Older Adults. *Journal of the American Geriatrics Society* **56**, 1517-1521 (2008).

284 Ogihara, H., Kobayashi, M., Nishida, K., Kitano, M. & Takeuchi, K. Applicability of the cross-culturally modified University of Pennsylvania Smell Identification Test in a Japanese population. *American journal of rhinology & allergy* **25**, 404-410 (2011).

285 Fornazieri, M. A. *et al.* A new cultural adaptation of the University of Pennsylvania Smell Identification Test. *Clinics* **68**, 65-68 (2013).

286 Picillo, M. *et al.* Validation of an Italian version of the 40- item University of Pennsylvania Smell Identification Test that is physician administered: Our experience on one hundred and thirty- eight healthy subjects. *Clinical Otolaryngology* **39**, 53-57 (2014).

287 Taherkhani, S. *et al.* Iran Smell Identification Test (Iran-SIT): a Modified Version of the University of Pennsylvania Smell Identification Test (UPSIT) for Iranian Population. *Chemosensory perception* **8**, 183-191 (2015).

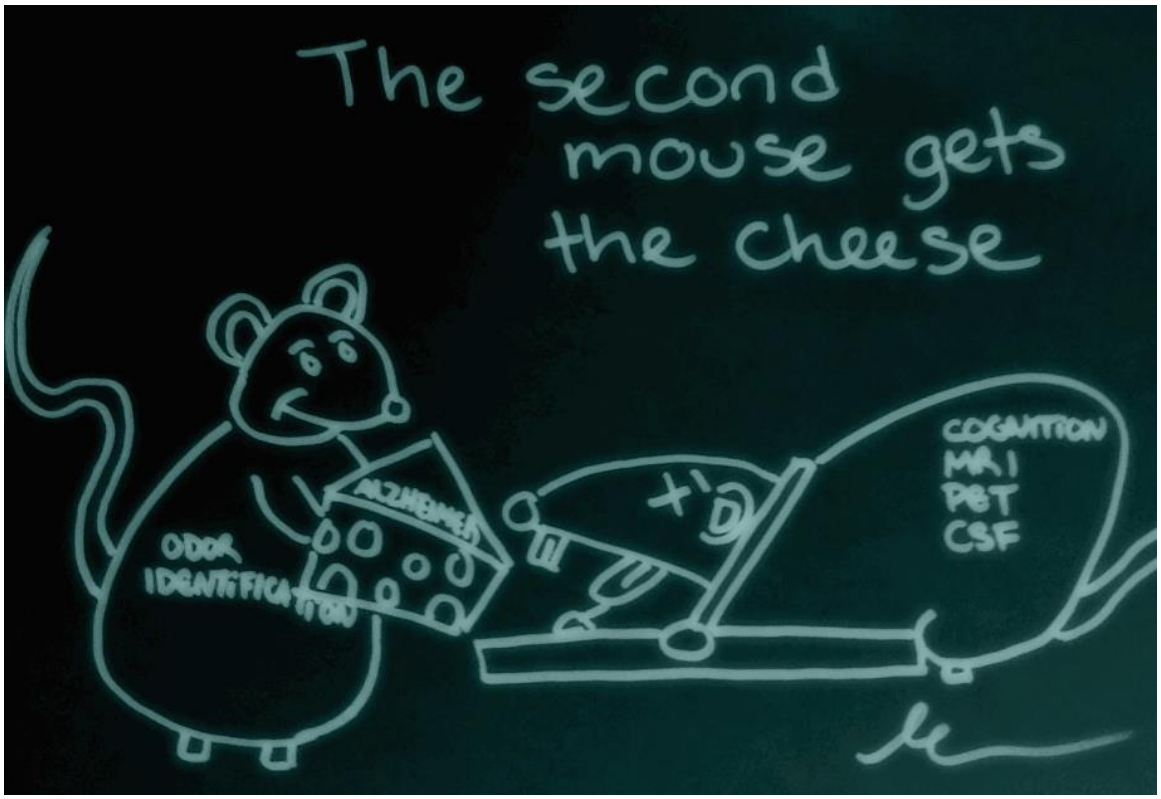
288 Adams, D. R. *et al.* Olfactory Dysfunction Predicts Subsequent Dementia in Older U.S. Adults. *Journal of the American Geriatrics Society*, doi:10.1111/jgs.15048 (2017).

289 Turana, Y. *et al.* Enhancing diagnostic accuracy of aMCI in the elderly: combination of olfactory test, pupillary response test, BDNF plasma level, and APOE genotype. *International Journal of Alzheimer's Disease* **2014** (2014).

290 Wilson, R. S., Arnold, S. E., Schneider, J. A., Tang, Y. & Bennett, D. A. The relationship between cerebral Alzheimer's disease pathology and odour identification in old age. *Journal of neurology, neurosurgery, and psychiatry* **78**, 30-35, doi:10.1136/jnnp.2006.099721 (2007).

CHAPTER 2:

Olfaction: "The Holy Grail" of Alzheimer's disease biomarkers or the second mouse gets the cheese



2. Olfactory identification as a practical marker of neurodegeneration in Alzheimer's disease

2.1 Chapter 2 preamble

There are no available disease modifiers for AD dementia. We need to understand the pre-symptomatic phase of AD (*i.e*., before symptoms are manifested) and how Alzheimer neuropathology leads to AD dementia. One method to track transition from normal to mild cognitive impairment (MCI) might be odor identification (OI). Despite numerous studies on variability in the probability of conversion to MCI or AD, few have incorporated OI into research on pre-symptomatic AD including prevention trials. This review focuses on OI as a sensory-neural task that may reflect change in regions known to be vulnerable to AD. We explain the functional anatomy of the olfactory system, showing how several key structures are vulnerable in this way. To support our arguments, we will discuss imaging studies of olfactory tasks in healthy aging and AD dementia, as well as, evidence for the association of OI performance with Alzheimer brain changes. Our intention is to publish this work as a review to establish a rationale for use of odor identification as an early disease marker.

2.2 What is olfaction and what are olfactory network structures?

The sense of smell, olfaction, is the perception of odorant molecules. The ability to smell and discriminate odors serves many purposes like detecting hazards, eating, interacting with others, and mating. It may be important for survival of the individual and the species. Generally, the term 'olfaction' refers to a few functions of the sense of smell, namely detection, discrimination and identification. Detection becomes possible at some odor threshold at which an odorant is reliably perceived. Discrimination is the ability to distinguish two or more odors. This task requires acuity in odor detection as well as working memory that can distinguish different odors. In general, odor identification refers to the identification and the naming of an odor. In research settings,

odor identification builds on detection and discrimination. It does require identifying an odor and assigning a verbal label or visual representation as distinct from other options. Typically, tests of smell identification force the participant to name an odorant through a multiple-choice paradigm.

Although olfaction appears to activate many areas of the brain, the term 'olfactory system' typically describes the olfactory epithelium, the olfactory nerve, the olfactory bulb and the primary olfactory cortex. Odor perception then recruits several brain regions including: the frontal, temporal, and parietal cortex, as well as the limbic system and cerebellum.[1-10] For an overview, please refer to the schematic illustration of the olfactory system (Fig. 2-1).[11] You can refer to figure 2-7 to visualize what regional task activation (Fig. 2-7).[2]

Olfactory disorders may include partial or complete loss of olfactory functions (hyposmia or miscrosmia and anosmia), exaggerated odor acuity (hyperosmia), distorted smells (dysosmia), and perception in absence of stimulus (phantosmia).[12,13] Odor threshold, discrimination, and identification impairments have been described across different stages of AD.[14-17]

Several studies have found that odor identification deficits are increased among AD and MCI patients as compared with normal individuals.[14,17] In addition, OI impairment increases in proportion to severity of AD dementia.[18] A study conducted at McGill University demonstrated differences between normal controls and AD patients in odor detection, discrimination and identification.[15] This research suggested that OI might be used as a surrogate for olfactory discrimination.[15] A more recent meta-analysis also suggested that deficits in odor recognition and identification may be more prominent in AD then Parkinson's disease.[16] In general, examination of OI performance in neurodegenerative illnesses has become popular. Therefore, we focus this review on this OI function. We include a review of the pathways by which olfactory information is processed. We also discuss the particular characteristics of the olfactory system

including extensive and complex rhinencephalic brain regions that make them vulnerable to early AD pathology (see Fig 2-1, Fig 2-10).[19] We then present data suggesting that decline in OI may be a marker of ongoing AD-related brain changes. Finally, we suggest that OI abilities may be used in pre-symptomatic research and AD and trials.

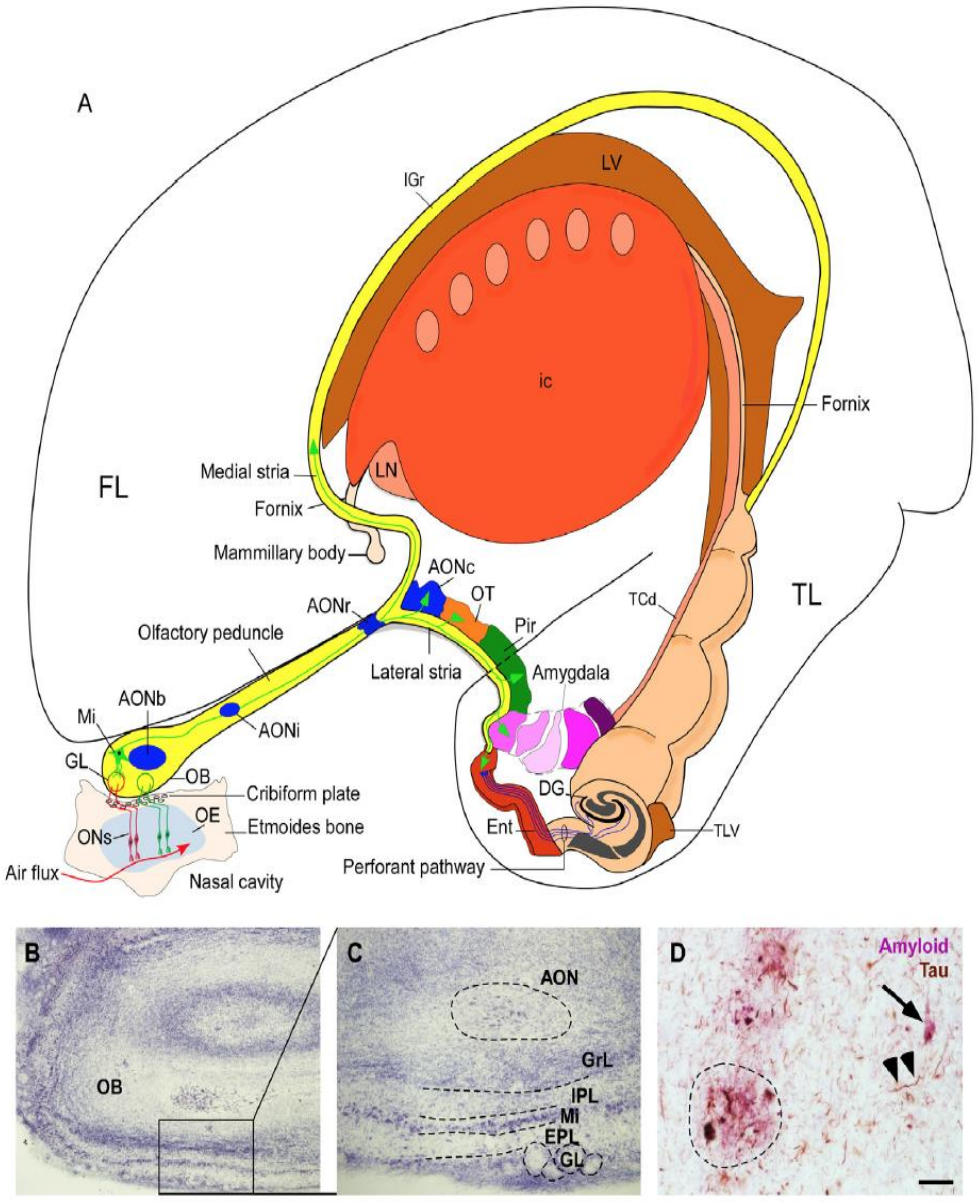


Figure 2-1 Representation of olfactory system and evidence for presence of AD pathological markers

Schematic representation of the human olfactory system. A; anterolateral view of the principal olfactory areas in the human brain. The olfactory sensory neurons detect odors after inhalation of air in the nasal cavity. This primary information is processed in the

olfactory bulb and the mitral and tufted cells send their axons to the olfactory cortex (green projections). Anterior olfactory nucleus is distributed in different parts during the course of the olfactory bulb, olfactory peduncle and olfactory cortex and constitutes the first relay of the olfactory information. In the frontal lobe is located the olfactory tubercle which constitutes part of the ventral striatum and contains the Calleja's islands (with unknown function; not represented). The principal olfactory area is the piriform cortex. It is divided into two parts (dash line) regarding the anterior location in the frontal lobe and the posterior part in the temporal lobe. Inside the temporal lobe resides the amygdala, which is divided into different nuclei. The cortical and medial nuclei are primary involved in processing olfactory information. The last olfactory inputs from the olfactory bulb reach the rostral part of the entorhinal cortex, which in turn forms the main connection with the limbic system via the perforant pathway (blue lines). Please, note the hippocampus has been separated laterally from the amygdala and the relationships with the entorhinal cortex are topologically modified for clarity. B and C; Nissl stain of the olfactory bulb with a high magnification image detailing different olfactory bulb layers. D; double immunohistochemistry of amyloid-beta (purple) and Tau protein (brown) in the olfactory cortex showing typical senile plaque with central accumulation of amyloid-beta surrounded by Tau (circle). A detail of neuron with amyloid-beta affectation (arrow) and dystrophic neurite filled by Tau (arrowheads). Scale bar=B, 400 μm; C, 200 μm and D, 80 μm. Abbreviations: AONb, anterior olfactory nucleus (pars bulbaris); AONc anterior olfactory nucleus (pars corticalis), AONi, anterior olfactory nucleus (pars intrapeduncularis); AONr, anterior olfactory nucleus (*pars retrobulbaris*); DG, dentate gyrus; Ent, entorhinal cortex; FL, frontal lobe; GL, glomerular layer; ic, internal capsule; IGr, indiseum griseum; LN, lenticular nucleus; LV, lateral ventricle; Mi, mitral cell; OB, olfactory bulb; OE, olfactory epithelium; ONs, olfactory sensory neurons; OT, olfactory tubercle; Pir, piriform cortex; TCd, tail of caudate nucleus; TL, temporal lobe; TLV, temporal lateral ventricle (figure and caption from Daniel Saiz-Sanchez's dissertation and reprinted in Saiz-Sanchez, 2016, and reproduced here by permission from authors Daniel Saiz-Sanchez and Alino Jose Martinez Marcos).[11,20]

2.2 Anatomy

2.2.1 Olfactory epithelium

Physiologically, the olfactory network begins in the nose where molecules from the air dissolve in the mucus that covers the olfactory epithelium (Fig 2-2). The odorant molecules activate chemoreceptors on the cilia of olfactory sensory neurons (OSN). These are known as olfactory receptors (OR) (Fig. 2-2 & 2-3).[21] The cilia contains dendrites of olfactory receptor neurons (ORN) alongside glia-like supporting cells, basal cells, and bowman glands. Odorants reach the OR through the orthonasal olfactory system via the nose or through the retronasal olfactory system via the throat.[22] The oro-nasal cavity is an open-air area, where neurons are directly exposed to the environment. The mucus contains several immune system molecules and it may serve as a physical barrier for neurons.[23] We discuss the significance of the exposure to the environment and the implication of immune response later in this review (2.3.1).

Virtually nothing compares to the variety of olfactory receptors in the human genome present in the olfactory epithelium. In 1991, Richard Axel and Linda Buck discovered the olfactory receptor (OR) genes expressed on the apical part of OSN.[24] Each neuron expresses one receptor type coded by one gene.[24] There are 861 genes of which 401 produce functional OR proteins.[25] The remaining 460 OR genes are pseudogenes with DNA sequences closely related to OR genes; together this genetic receptor family represents approximately 3% of the human genome.[25-31] In contrast, there are only 42 genes for taste.[32] Although not much is known about olfactory pseudogenes, they were previously considered to be non-functional, and now appear to code for RNA, and may thus have important roles in physiological and pathological processes.[33]

To further complicate matters, several odorant molecules can activate a single OR with varying magnitude.[34,35] Once an odorant molecule binds to an OR, this triggers

a G-protein-coupled receptor (GPCR) signalling cascade that leads to an action potential.[36] The way OR activation and consequent signaling cascade lead to an action potential is illustrated in Figure 2-3.

Axonal projections of olfactory sensory neurons (OSN) form large bundles that make up the olfactory nerve. These fibers enter the cranial cavity through perforations in the cribiform plate of the ethmoid bone, i.e., the roof of the upper nasal cavity, and project to the olfactory bulb (OB), which lies within the anterior cranial fossa on the ventral surface of the brain (Fig 2-2).[37] The neurons that make up this first cranial nerve (CN1) transduce the signal and project it to synapses in bulb where it is processed for subsequent projection to posterior and deeper areas of the brain.

The purpose of olfaction appears closely linked to survival (danger avoidance), quality of life, eating, mating, and navigating.[38-47] Therefore, it is not surprising that OSN can regenerate continuously from basal stem cells,[48-50] although the potential for such self-renewal power diminishes with age.[51] The potential of stem cells to replenish the OSN population in the periphery is interesting as is indicates a remedial method by which olfactory abilities can be preserved after exposure to insults or damage (see 2.3.1 & 2.3.2).

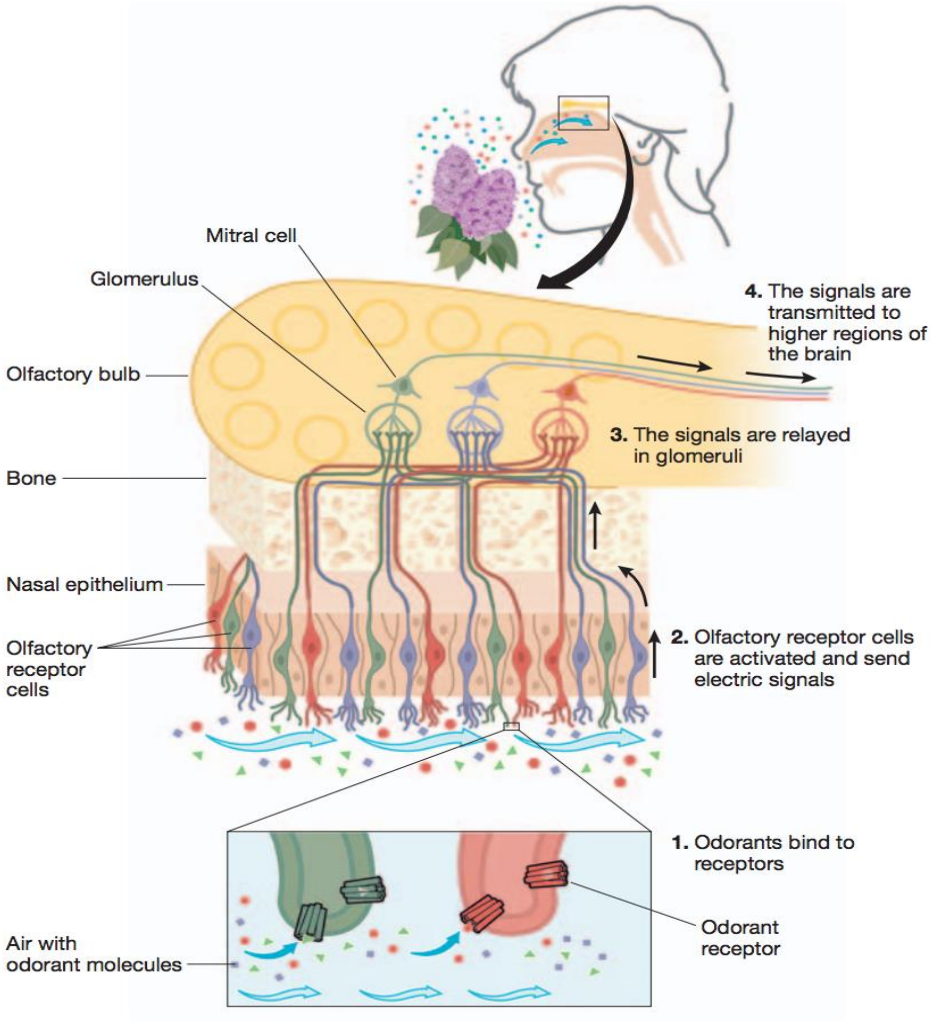


Figure 2-2 The human olfactory system.

Credit: Karolinska Institutet and Nobel Foundation, Stockholm, Sweden.
This figure was published in Press Release: The 2004 Nobel Prize in Physiology or Medicine to Richard Axel and Linda B. Buck". *Nobelprize.org.* Nobel Media AB 2014. Accessed;19 Jan 2018.
http://www.nobelprize.org/nobel_prizes/medicine/laureates/2004/press.html

Odorant → Olfactory receptor → G-protein activation → G-alpha olf (Gαolf) dissociates from the olfactory receptor → ↑Adenylyl cyclase 3 → converts ATP to cyclic AMP (cAMP) → opens cyclic nucleotide gated channels → Ca^{++} influx and Na^{+} influx → Cl^{-} efflux→ depolarization → action potential.

Figure 2-3 Common olfactory receptor signal transduction cascade

When an odorant binds to an olfactory receptor, there is a conformation change that triggers G activating protein (Gαolf) to dissociate from the OR receptor and activate adenylyl cyclase3 (AdCy3 or ACIII).[53-56] AdCy3 converts ATP into the second messenger cAMP (cyclic adenosine monophosphate), which then binds and activates a cyclic nucleotide gated channel (CNG).[57-59] The activated CNG channel opens and leads to a cellular influx of sodium and calcium. Subsequently, calcium-activated chloride channels open, enabling Cl- to efflux from the neuron.[60,61] This influx and efflux of ions into the pre-synaptic terminal depolarizes the neuron and eventually generates an action potential.[61-64] The action potential ends in glutamate release at the synaptic terminal onto glomeruli cells of the olfactory bulb.[65-68]

N.B. Some ORs activate an alternate cascade through phospholipase C, which increases downstream second messengers diacyl glycerol (DAG) and inositol triphosphate (IP_3).[69-71]

2.2.2 Olfactory bulb

The olfactory bulbs are symmetrical ovoid macrostructures that are the rostral and expanded part of the olfactory stalk (peduncle or tracts) at the base of the frontal lobe (Fig. 2-1, Fig. 2-4). The OSNs project to the mitral or tufted cells and terminate into glomeruli within one ipsilateral olfactory bulb.[72] The projections are ipsilateral (i.e. same side), but there is an eventual crossover of information (Fig 2-5).[73,74] The olfactory bulb serves as a relay, and each glomerulus receives the afferent fibers from OSN of only one OR receptor-type.[75] There are 6 layers in the olfactory bulb: olfactory nerve layer, the glomerular layer, the external plexiform layer, the mitral cell layer, the internal plexiform layer, and the granule cell layer (Fig 2-1).[13,76] The glomerular layer forms a map where signals are integrated in a topographic manner. There are medial and lateral almost twin maps of glomeruli.[77] Each odor has a different pattern of glomerular activation.[78,79]

This network has many important intra-regional neuronal connections that work together to reduce signal to noise, discriminate odors and relay the signals to downstream processing areas like the piriform cortex. There is a concerted signaling of the glomeruli, periglomerular, mitral, tufted, short-axon, and granule cells that enhances the signal-to-noise ratio of the activity patterns.[76,80-86] The reduction of background noise facilitates the brain's perception of a distinct odor image or fingerprint. Table 2-1 highlights the function and characteristics of the different neuronal cell type found in the olfactory bulb.

In addition to intra-bulb modulation, this complex neuronal network receives input from several brain areas. There are noradrenergic fibers that originate from the locus coeruleus (LC).[87] There is serotonergic modulation (5-HT) from the raphe nuclei.[88] Lastly, there are cholinergic afferents from the horizontal limb of the diagonal band of Broca that appear to be important for encoding and recall.[89-92] The inter-regional connections may serve as a feedforward inhibition.[93] In summary, there is an incredible

amount of information processed in periphery before the identification and naming of an odor can occur.

Unlike other sensory networks (e.g. auditory, proprioceptive), there is also a constant renewal of the neuronal cell population that migrates from the temporal lobe to the olfactory bulbs, even in adulthood. Adult brain neurogenesis occurs in the subventricular zone (SVZ) and the subgranular zone of the hippocampus.[94,95] SVZ neural stem cells give rise to neuroblasts that in turn migrate through the rostral migratory stream (RMS) to the olfactory bulb where these cells will differentiate into glia and neurons.[96] The migration is facilitated by blood vessels parallel to the RMS.[97-100] The newborn neurons differentiate into granule cells or periglomerular cells.[99] Noteworthy, neuronal maintenance requires neuronal activity.[101]

Trophic factors involved in neurogenesis are proteins capable of stimulating cellular growth, proliferation and differentiation. These neurotrophins include Neuronal Growth Factor (NGF) like Brain Derived Neuron Factor (BDNF),[102] which prevents degeneration of cholinergic neurons and maintains their anatomical phenotype.[103,104] This self-renewal process affects cells in both the hippocampus and the olfactory bulb. The neurogenesis mechanism could in part explain how olfactory functions may relate to learning and memory.[105,106]

We can appreciate the importance of maintaining the integrity of the olfactory network inasmuch as this system can yield both peripheral and central self-renewal of its neuronal population. Thus, the olfactory network may be so critical for life that it was “designed” with two points of repair. Alternatively, it could also point to how vulnerable the infrastructure is to damage. By studying the function of the olfactory system through tasks like odor identification, we may be able to measure neurogenesis. This makes the sense of smell appealing as a way to track the neural integrity of the network, even though less than 1% of the OB cell population turns over through the lifespan and the renewal process remains controversial in humans.[107,108]

Layer	Glomerular Layer			External plexiform layer and mitral cell layer			Granule cell layer	
Cell Type	Periglomerular cell (PG cell)	Superficial short-axon cell (sSA cell)	External tufted cell (ET cell)	Tufted cell	Mitral cell	Interneurons	Granule cell	Deep short-axon cell
Transmitter	GABA, dopamine (in the TH+ subtype)	GABA, dopamine	Glutamate, GABA (in VGLUT3+ subtype), CCK, vasopressin	Glutamate	Glutamate	Almost all of EPL interneurons are GABAergic	GABA	GABA
Function	Inhibition within the glomerulus	Inhibition across glomeruli	Excitation within the glomerulus, Connecting circuits associated with the glomeruli of the same ORN	Output within OB and output out of OB to anterior part of olfactory cortex, Higher sentitivity to the odor stimuli, odor evoke spiked activity during early phase respiratory cycle	Output within OB and output out of OB to the entire olfactory cortex, Lower sensitivity to the odor stimuli, odor evoked spike activity during late phase of respiratory cycle		Modulators of principal neurons to influence pattern separation/ discrimination (Gheusi, 1999; Sahay, 2011)	Tufted cell inhibition (Burton, 2017)

Table 2-1 Summary of neural cell types in the olfactory bulb and their function

Modified from Nagayama, 2014 and additional information from (Gheusi, 1999, Sahya, 2011, Burton, 2017).[76,109-111]

CCK = Cholecystokinin EPL = External plexiform layer ET = external tufted cell ORN = Olfactory receptor neuron PG=Periglomerular cell sSA =superficial short axon cell OB=olfactory bulb GABA = TH+ = positive for tyrosine hydroxylase VGLUT3+ = positive for glutamate vesicular transporter type 3

2.2.3 Primary Olfactory Cortex

After processing in the olfactory bulb, the signal travels through the olfactory tracts to the next level of information processing. Mitral and tufted cell axons form the olfactory tracts (stalk of the olfactory bulb or peduncle), which project to the pyramidal neurons of secondary olfactory structures.[112] These structures are on the surface of the basal forebrain and mesial temporal lobe. Information from the olfactory bulb is sent to the dispersed area called the primary olfactory cortex (POC). The primary olfactory cortex (POC) structures are: the anterior olfactory nucleus (AON); piriform cortex in the ventromedial temporal lobe in proximity of the optic chiasm; anteromedial entorhinal cortex; portions of the amygdala (Fig. 2-6).[113] There are reciprocal afferents from these olfactory areas that project to the OB.[11] The tracts run inferiorly to the frontal lobe and reach the anterior perforated substance, where the tracts divide at the olfactory trigone into medial and lateral striae. The anterior commissure AC connects the primary olfactory cortices of opposite hemispheres and enables a contralateral exchange of information (Fig. 2-4).[114,115] Notable, OB projections also reach the lateral olfactory tract nucleus, the olfactory tubercle, the peri-amygdaloid cortex, the anterior cortical nucleus of the amygdala, the ventral tenia tecta, and the entorhinal cortex, and the hypothalamus (Fig. 2-4 & 2-5).[73,116-119]

The piriform cortex overlies the lateral olfactory stria, and is located in between the insula and the rostral pole of the temporal lobe and the anterior and lateral amygdala.[120] The piriform cortex discriminates between similar odors and is able to complete patterns when a molecular component of the odor image is missing.[121] In fact, the right piriform cortex activity seems to be especially important for odor naming. A unirhinal task, *viz*. use of one nostril at a time, activates the right hemisphere and does not activate the left hemisphere when the left nostril is blocked; in contrast, when the right nostril is blocked, the left and right piriform become active.[3]

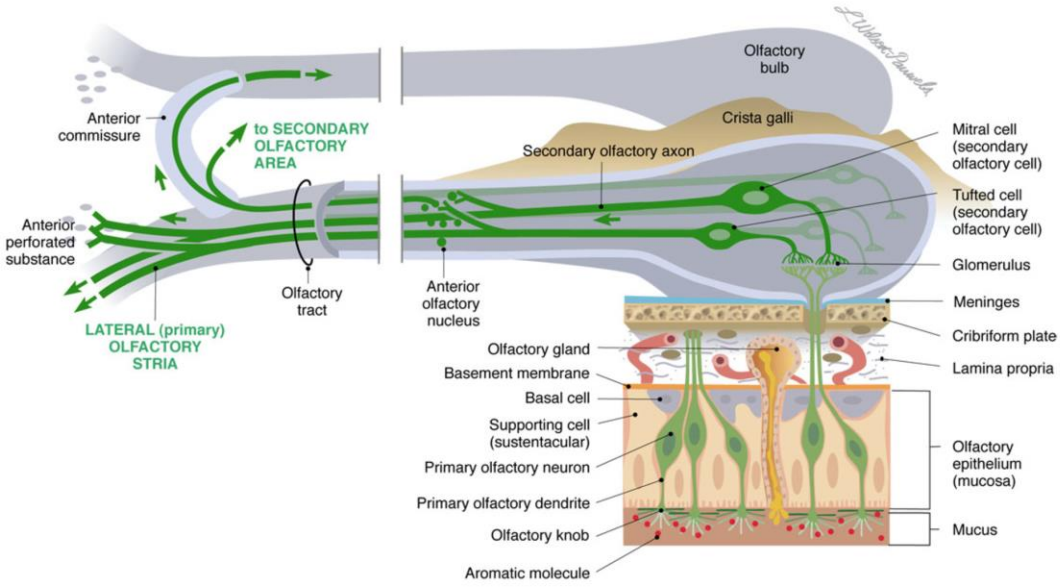


Figure I–2 Olfactory epilthelium, bulb, and tracts (structures are enlarged for clarity).

From Cranial Nerves 3rd Ed. ©2010 Wilson-Pauwels, Stewart, Akesson, Spacey, PMPH-USA

Figure 2-4 Contralateral exchange of information occurs at the anterior commissure. Projections from olfactory sensory neurons are ipsilateral. The olfactory tracts are symmetrical and parallel. This was published in Wilson-Pauwels, Sewart, Akesson, Spacey (2010) Cranial Nerves 3rd Edition reproduced here by permission.[122]

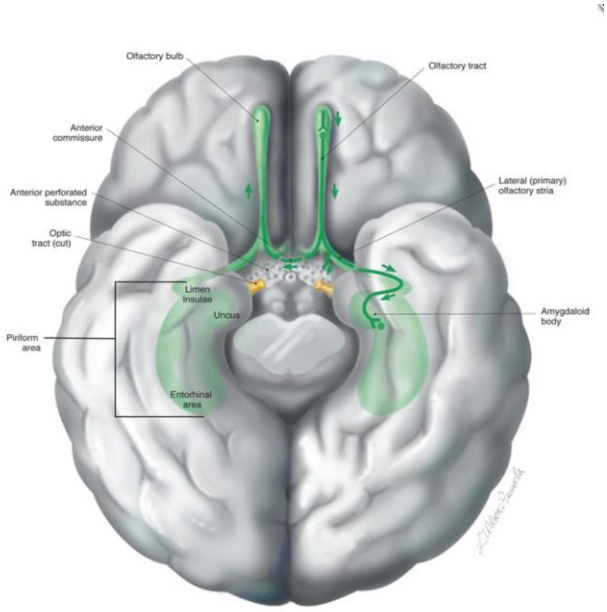


Figure I–5 Olfactory areas (inferior view).

A)

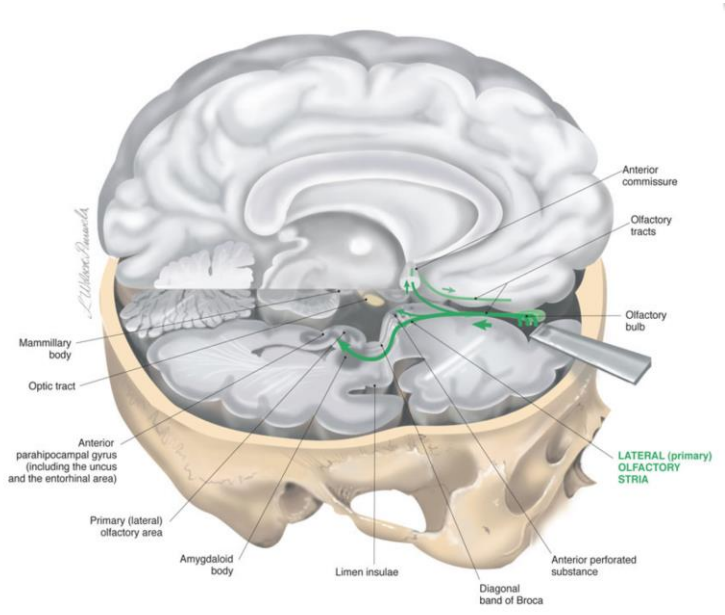


Figure I–4 Overview of the olfactory nerve.

B)

Figure 2-5 Anatomical olfactory system from different views

This was published in Wilson-Pauwels, Sewart, Akesson, Spacey (2010) Cranial Nerves 3rd Edition reproduced here by permission.[122]

2.2.4 Secondary higher olfactory areas

There are many so-called secondary olfactory areas to which the primary olfactory cortex (POC) neurons project directly or, in the case of the orbitofrontal cortex (OFC), indirectly. The projection areas include: the dorsomedial nucleus of the thalamus, the caudal OFC (via thalamic relay), the amygdala, the entorhinal cortex, the agranular (rostral) insula, the hippocampus, the basal ganglia (in the ventral striatum comprising the nucleus accumbens, olfactory tubercle; in the dorsal striatum comprising ventral putamen, caudate, and the.[73,119,123-130] Imaging studies show that odors engage several higher order areas like the amygdala, piriform cortex, orbitofrontal cortex (OFC), medial thalamus, the insula, and the cingulate cortices (Fig. 2-7).[1-3,5-8,10,131-133]

As mentioned above, the odor signal travels to more central areas including the amygdala, hippocampus, orbitofrontal cortex, insula, and the thalamic dorsomedial nucleus.[134] Unlike other senses, the olfactory network connects without an obligatory relay step (in the thalamus) to structures that support emotion and memory functions.[135] However, there is also an indirect circuit that sends odorant information from the primary olfactory cortex to the mediodorsal thalamic nucleus, and this may be important for modulation of attention and behavior by the cortico-thalamo-cortical network, *viz* the salience network SN.[127,136-140] More work is required to clarify the importance of this circuit and thalamic contribution to odor processing.

Olfactory activation occurs in a variety of areas more central than those of the olfactory bulb and primary olfactory cortex. The Papez circuit, part of the limbic system, starts and ends in the hippocampus[141] This circuit is important for the regulation of hormones, olfaction, memory, and emotion.[142] It has been shown that olfactory stimuli form long lasting memories by tapping into the Papez cicuit (amygdaloid and hippocampal systems).[143,144]

The entorhinal cortex is implicated in odor memory, emotion and autonomic responses as it transmits information from the neocortex to the hippocampus.[145,146] The entorhinal cortex transmits input, such as sensory information, and projects directly to hippocampal regions that are part of the perforant pathway;[147] thus, these neuronal connections are important for new episodic memory encoding and perhaps even smell identification.[148,149] The hippocampal cortex is thought to feed back this information to the medial entorhinal cortex.[150] Olfactory function may reflect abnormalities in either the Papez circuit or the perforant pathway as they emerge with AD severity. For instance, many individuals with learning and recalling impairment diagnosed with MCI or AD dementia suffer from neuropsychiatric symptoms and sleep disturbance.[151-153]

H.M., a patient with bilateral medial temporal resection, demonstrated that lesions in more central areas lead to loss in discrimination of odor, while conserving the ability to detect odors.[148] Higher order tasks like odor recognition depend on recall of a previously encountered odor without context. Similarly, odor identification requires several levels of sensory processing,[154] including detection, discrimination, recognition, as well as retrieval of a verbal label.[155] The need for this intense cognitive processing likely renders tests of olfactory identification more sensitive to malfunction than an odor detection test alone.[155] In other words, good sensory function is not sufficient to name odors.[156] Even in normal individuals, naming odors spontaneously (without cueing in a multiple-choice paradigm) is extremely challenging with a success rate of 22 to 57% using 7 to 80 common odorants (e.g. chocolate).[157-159] A multiple-choice format for odor naming reduces cognitive demands and yields improved performance.[157,160-162] In normal settings, we constantly smell a mixture of odors and integrate multiple senses together; for example, we pair odors with objects or tastes.[163] A functional MRI study supports this idea by demonstrating that easily named odors activate a higher-order processing brain region, the inferior frontal gyrus (IFG), while, un-nameable odors activated the piriform cortex (PC).[164] The IFG is important to access a lexicon and provide a name.[165] When contrasting odor detection and identification tasks activaty, the inferior frontal gyrus and Broca’s area were more recruited during the odor identification

task.[2] Several imaging studies have illustrated that odor identification involves the IFG, the fusiform gyrus, and the temporal regions.[166-168] Odor identification appears to require an extensive amount of processing in periphery, central areas of the brain as well as, structures important for attention and language. Figure 2-7 illustrates different levels of projections within the olfactory network, while figure 2-8 is a top-down mindmap of the olfactory network. This suggests that odor identification is a complex task that can potentially tap into the broad brain functional integrity. Several concerted mechanism of neurodegenerative disorders like AD have the potential to impact odor identification from various vantage points. As pathology spreads, olfactory functions have the potential to decline and reflect progressive brain damage. In the next section, we will review how AD pathology is found across the olfactory system and mechanisms that may influence its performance.

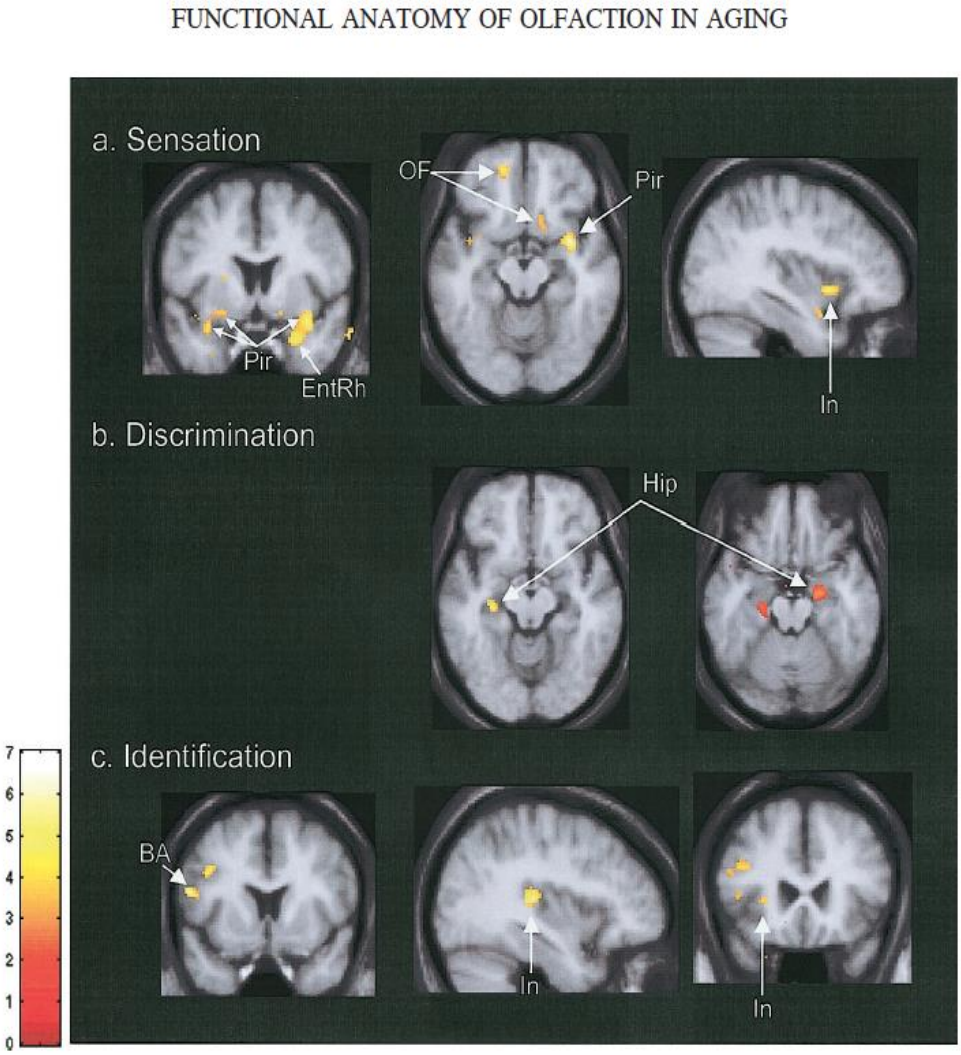


Figure 2-6 – Task activation

This figure illustrates task activation during a PET with labels for oxygen. Tasks activation were contrasted to observes regions activated by higher-order processing.

Odor sensation (Figure 6a). SEN (odor sensing activation minus the sniff button press; Sen - Sn) produced activation bilaterally in the medial temporal area, involving both frontotemporal junctions (piriform cortex). Left frontal piriform cortex was also present. A subpeak in the right piriform area included the uncus, which corresponded to the anterior entorhinal area. Medial temporal/piriform activity was most extensive on the

right. The medial portion of right orbital cortex (deep in the olfactory sulcus) was activated, as was a more rostral orbital area on the right. Activation in the left anterior insula was also apparent.

Odor discrimination (Figure 6b). OD (Od-Sen, odor discrimination activation minus odor sensation, reflecting activity above and beyond sensory stimulation) did not produce greater activation in piriform or orbitofrontal areas. Instead, the most prominent activation was in the left hippocampus. Activation in the left inferior temporal gyrus and in Broca' s area was also present. Given the left hippocampal activation, activation was also sought in the contralateral (right) hippocampus by using a volume of interest defi ned on MRI. This analysis showed a peak ($p = .01$) at the hippocampal head of 63 voxels (corrected cluster statistic $p<.05$ at height threshold $p<05$, uncorrected).

Odor identification (Figure 6c). As in discrimination, OI (Oi –Sen, odor identification minus odor sensation activation) did not reveal added activity in piriform or orbitofrontal olfactory areas. The most significant foci in OI were instead in Brodmann' s area (BA) 44 (Broca' s area) and in the left inferior frontal gyrus more generally. A region in the posterior insula was also prominent, while a smaller activated cluster appeared in the left superior temporal gyrus, slightly rostral to traditional Wernicke' s area. Finally, there was a small, subthreshold area (< 20 voxels) in the left anterior insula, proximate to the left insula location that was activated in SEN. Using corrected cluster statistics ($p <05$, height threshold $p <.05$, uncorrected) from a large volume of interest traced on MRI of the anterior insula, this area was significant (69 voxels).

Figure 2, p.487 from Kareken, D. A., Mosnik, D. M., Doty, R. L., Dzemidzic, M., & Hutchins, G. D. (2003).[2] Functional anatomy of human odor sensation, discrimination, and identification in health and aging. *Neuropsychology*, *17*(3), 482-495, 2003, APA, reprinted with permission.

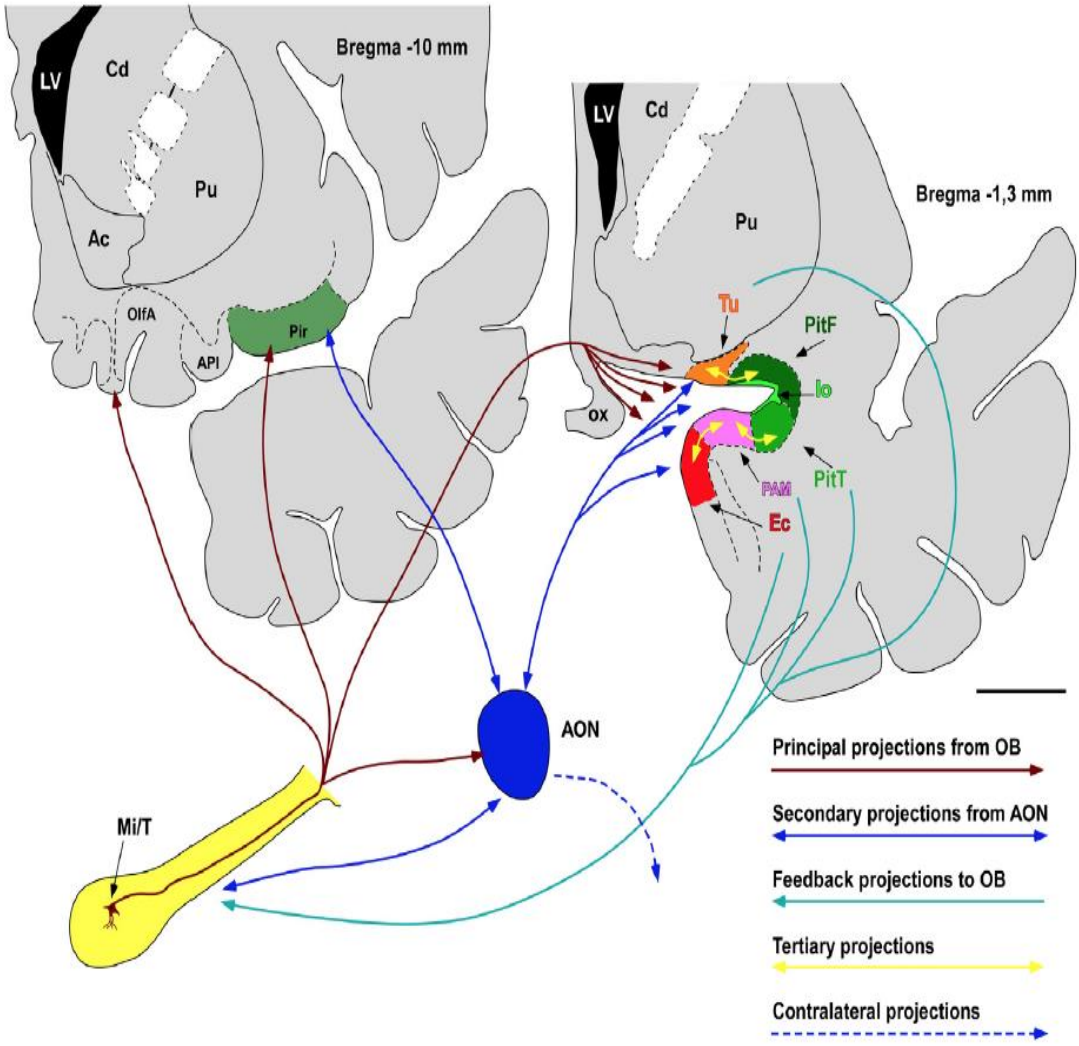


Figure 2-7 Olfactory sytem with secondary and tertiary projections.

The olfactory system as potential via to spreading pathology. According with Braak stages key olfactory regions such as the olfactory bulb, anterior olfactory nucleus and entorhinal cortex are deeply affected by NFTs since the beginning of Alzheimer's disease (Braak and Braak, 1991).[169] The progression of both Aβ and Tau could take advantage from the olfactory connections. This figure aims to represent most of the olfactory pathways that can be involved in the spreading of the neuropathological proteins. First, the olfactory bulb sends direct projections to all olfactory areas (brown arrows). Secondary projections from the anterior olfactory nucleus (blue arrows) reach and receive reciprocal projections from all the olfactory areas. In addition, it is the

principal olfactory structure sending contralateral projections. Anterior olfactory nucleus highlights as an important area that can send and receive neuropathological proteins from all areas of the olfactory system not only ipsilaterally, but contra-laterally. Each olfactory area from the cortex sends back projections to the olfactory bulb. These projections could be involved in the very early presence of NFTs in the olfactory bulb described by Braak and Braak (1991).[169] Finally, microcircuitry may act as spreading via between adjacent olfactory areas. Interneurons could be determinant regarding these last connections.

Abbreviations: Ac, Nucleus Accumbens; AON, anterior olfactory nucleus; API, area piriformis insulae; Cd, caudate nucleus; lo, lateral olfactory tract; LV, lateral ventricle; Mi, mitral cell; OB, olfactory bulb; OlfA, olfactory area; Ox, optic chiasm; PAM, periamygdaloid cortex; Pir, piriform cortex; PirF, frontal piriform cortex; PirT, temporal piriform cortex; Pu, putamen; T, tuft cell; Tu, olfactory tubercle.

This figure and caption are from Daniel Saiz-Sanchez's dissertation and reprinted in Saiz-Sanchez, 2016, and reproduced here by permission from authors Daniel Saiz-Sanchez and Alino Jose Martinez Marcos).[11,20]

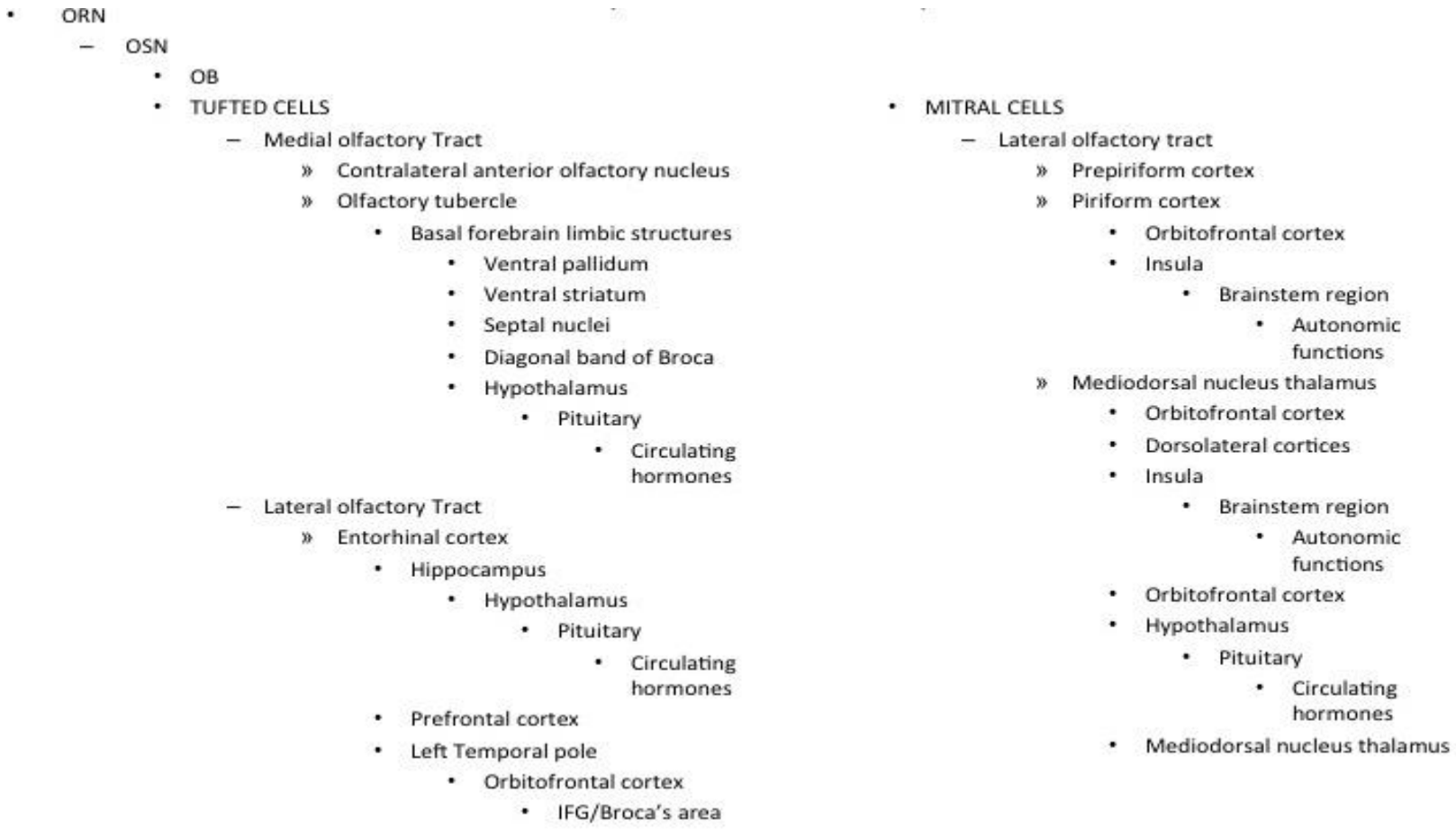


Figure 2-8 Olfactory network mindmap. Summary of olfactory pathways. Information from Price 1990; Carmichael, 1994; Doty, 2011; Doty, 2012; Lötsch, 2012; Olofsson, 2015 was used to develop this map. Autonomic functions include heart rate, digestion, respiratory rate, pupillary response, urination and sexual arousal.[73,124,165,170-172]

2.3 Pathology

2.3.1 Peripheral vulnerability to pathology

The olfactory network is vulnerable to neurodegeneration through various physical, inflammatory and toxicological mechanisms. The nasal cavity is a gateway to the brain, making the nasal epithelium susceptible to toxic molecules or viruses that can contact OSN. This vulnerability may be pertinent for AD, as several studies have pointed to pollution as a potential contributor to both peripheral and central nervous system damage.[173-175]

Autopsy studies of Mexico city residents have documented an increased cyclooxygenase-2 (COX-2) expression and $A\beta_{1-42}$ accumulation in the olfactory bulb.[176,177] In studies of olfactory bulbs from children, these workers documented increased levels of COX-2, interleukin 1beta (IL-1β) and CD14.[178] Children exposed to pollution have white matter hyperintensities and changes in brain development.[179] Calderon et al. have corroborated their findings in autopsies of dogs by observing both inflammation and AD-like pathology.[180,181] Others have demonstrated in rodents how six months of diesel exhaust exposure increased $A\beta_{42}$ levels in the frontal lobe, *tau* levels in the frontal and temporal lobe, and TNFα almost everywhere in the brain.[182] In a similar way, inflammation in the olfactory epithelium leads to inflammation in central areas of the brain in animals.[89] Rombeaux et al. correlated a decline in OB volume with increasing degree of nasal inflammation (2008).[183] In addition, inflammation inhibits OSN cell renewal.[184] Older adults suffer from reduced epithelium area, which is replaced by respiratory epithelium,[185] and neuronal cell loss.[186] These peripheral phenomena are important as they may modulate an ongoing pathology in deep brain areas. As postulated by Hebb, repeated simultaneous activity associates cells or systems together and one cell can modulate the other.[187] A complete loss of smell lasting more than 2 years is associated with lower grey matter volume in the medial prefrontal cortex, anterior cingulate and middle cingulate cortex, dorso-lateral prefrontal cortex, cerebellum, superior occipital gyrus, orbitofrontal cortex, supramarginal gyrus,

precuneus, hippocampus, and parahippocampal.[188] This volume reduction is an important finding that emphasizes the importance of this sensory function.

2.3.2 Conduction interference

The olfactory sensory neurons' axons are thin and go through small cranial foramina.[189] The OSNs are particularly vulnerable to pressure and sheering forces during head trauma that can therefore lead to loss of conduction.[37,190,191] Obstruction from sinusitis, chronic nasal inflammation, deviated septum, and polyps can also cause olfactory loss due to conduction loss.[192] It is even argued that nasal obstruction could lead to disrupted clearance of CSF, Aβ oligomers and other extracellular excreted product accumulation, and even hydrodynamic change and increased intracranial pressure.[37] There is a glymphatic clearance pathway that drains CSF containing metabolites through the medial temporal lobe, the lateral olfactory stria, the olfactory trigone, the tracts, the bulbs, the cribriform plate, and seeps into the nasal submucosa, and flows into the cervical lymphatics.[37,193] Until recently this pathway was theoretical in humans. Dynamic monitoring of CSF has confirmed that *tau* tracer labeled metabolites clear through the cribiform plate and reach the nasal turbinates.[194] In fact, CSF dynamics are reduced and amyloid accumulates instead of being cleared in late onset AD.[37,195-198] Studies by de Leon and colleagues on nasal CSF drainage in AD observed a reduction in lateral ventricle clearance associated with increased PiB-labeled amyloid accumulation.[194] This recent study supports earlier post-mortem olfactory epithelium biopsy staining that revealed the presence of *tau* and amyloid outside the cranial cavity.[192] Additionally, this older histological analysis revealed OSN losses in patients with Alzheimer's disease.[192] Taken together, it appears important for our health to prevent and treat conduction interference before it leads to complications like CSF clearance disruption, peripheral neuronal loss, and central volume loss. The olfactory epithelium, mucus composition, and general olfactory functions may indicate exposure or foreshadow more central pathological protein accumulation.

2.3.3 AD neuropathology from periphery to central areas of the olfactory system

In describing the brains of people aged 70 years and older, Liss and Gomez identified that OR cells, the olfactory bulbs, and olfactory tracts showed generalized atrophy, neuronal loss, astrogliosis, and *corpora amylacea*, a type of glycoprotein inclusion that accumulates to a greater extent in AD.[199,200] Terminology has changed over the years, but these brains exhibited a pattern of “senile dementia”, most of which is in fact late onset AD. The fact that the studied brains were AD-like could explain the absence of a correlation between age and the severity of neurodegeration (sampling bias). Interestingly, Liss and Gomez (1958) concluded that degeneration of the bulb was secondary to degeneration of the OSN in the epithelium.[199] Thus, their results and interpretation supports the loss-of-conduction theory.[191]

Furthermore, tangles in the olfactory bulb have been observed in 86% of non-demented adults with an average of 75 years of age. Additionally, a third of these adults had plaques.[201] NFT observations in the olfactory bulbs in these normal individuals may have been related to early AD changes, and perhaps the individuals were already in transition.[201]

Subsequently, autopsy studies in older adults and in Alzheimer disease patients confirmed cell loss in the olfactory bulb as well as the anterior olfactory nucleus.[202,203] In the same way, amyloid plaques and tangles were observed in the olfactory bulbs.[202,204,205] In addition to olfactory bulb volume loss, the neuronal integrity of the olfactory system is affected.[204] For example, there is evidence for axonal loss in the olfactory bulb.[206] Davies et al. reported that AD patients had fewer myelinated axons in the olfactory tract than controls.[206]

In fact, Mann suggested that the olfactory bulbs and tracts might be where AD pathology initiates, inasmuch as the plaques and tangles are both present in these structures (contrasts to the presence of tangles in the hippocampus but plaques in the

amygdala).[207] Simultaneous co-localization is not the case for the Braak and Thal maps of *tau* and amyloid. Mann et al explain that pathological agents could enter the brain through the olfactory network and induce downstream pathology supporting the above-mentioned gateway and lack of barrier.[207] Moreover, autopsy work from Christen-Zaech et al. supports the occurrence of early changes in the olfactory bulb and tract related to AD neuropathology.[208] In 19 cases with severe, 14 cases with moderate, and 58 cases with discrete AD-type cortical changes, 84% of patients had pathology in their olfactory bulb or tract. Severity and frequency of the peripheral pathology of the olfactory system correlated with more central AD-related pathology.

Seminal works described the presence of both plaques and NFTs in the POC. For instance, amyloid plaques have been observed in the anterior olfactory nucleus,[202] while there have been several descriptions of tangle accumulation in the anterior olfactory nucleus, originating in the entorhinal and transentorhinal.[145,169,209,210] The entorhinal cortex and the periamygdaloid nucleus are damaged by NFTs in AD patients, while other sensory areas (i.e. auditory or visual cortex) appear to be spared.[211,212]

Importantly, NFTs seem to appear in the olfactory system as soon as they are present in the entorhinal cortex, thus indicating that the olfactory system is involved very early in the degeneration process.[208] Again in a post-mortem study, *tau* pathology in the olfactory bulb increased with AD severity.[211,213] Although, Reyes and colleagues found that the primary olfactory cortex contained the most pathological markers of AD, they also found the presence of NFT and plaques in the dorsomedial nucleus of the thalamus and the bulb and tract.[211]

In fact, Alzheimer neuropathology is believed to originate in the entorhinal and transentorhinal regions of the brain, with initial neurofibrillary tangle accumulation in these areas, corresponding to Braak stages 1 and 2.[145,169,210] Tangles were confirmed in the entorhinal cortex and amygdala of AD patients,[202,209] and plaques appeared across the entorhinal cortex.[209] Although AD pathological markers have been identified in the

locus coeruleus or olfactory bulb before the entorhinal cortex (EC) is even affected, the entorhinal cortex remains consistently cited as the first area in which there is neuronal loss related to AD neuropathology.[214] The introduction and acceptance of a transition period leading to AD pathogenesis is a fairly modern idea; thus, many studies that reported AD-like pathology prior to cognitive decline might have been describing individuals with pre-symptomatic AD. AD-related damage likely interferes with projections from the olfactory bulbs to the temporal lobes and bidirectional projections between the entorhinal cortex and hippocampus. The EC is the major source of afferent input to the hippocampus;[118] furthermore, entorhinal degeneration leads to a decrease in density of hippocampal synapses.[215] Braak suggested that AD-related lesions could lead to conduction loss and an inability to transduce information to the hippocampus for memory encoding or retrieval.[216] In summary, AD neuropathology, including amyloid, *tau* and neuronal loss, can be observed across the olfactory peripheral and central key structures (Fig. 2-9). These observations motivate the study of olfaction in Alzheimer's disease.

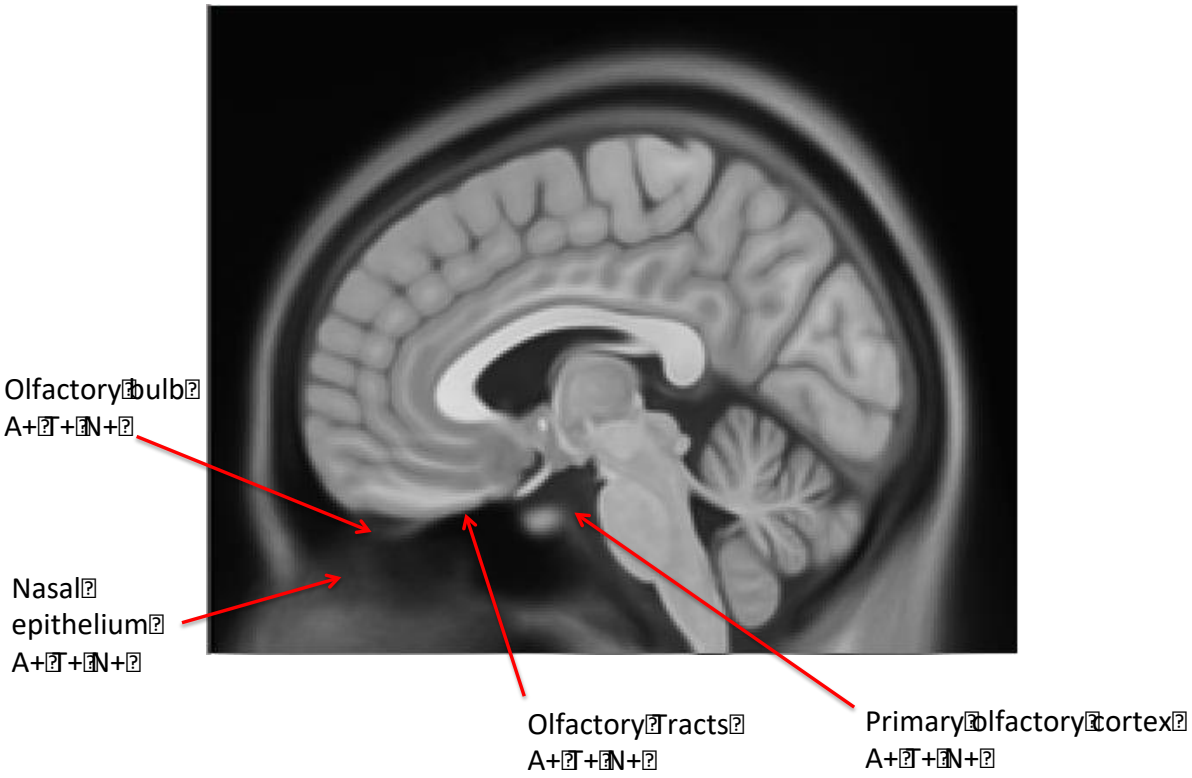


Figure 2-9 Presence across the olfactory system of amyloid, tau, and neurodegeneration

Sagittal view of the ICBM152 human anatomical template. Important structures of the olfactory system (labeled using the A/T/N framework from Jack, 2017)[217] have been shown to be vulnerable to amyloid and tau pathology, as well as neurodegeneration. Note how the vulnerable olfactory system spans the frontal lobe and reaches deep structures, perhaps accounting for the prominent sensori-neural deficits that occur with AD progression. (A+ = presence of amyloid; T+ = presence of tauopathy; N=presence of neurodegenation)

2.3.4 Possible impact of pathology on olfactory function

Progressive neurodegeneration may interfere with central processing of sensory input like odor identification. Olfactory identification and naming deficits could stem from accumulated deterioration of the signal over multiple steps of information processing.[165] Picture identification does not differentiate normal controls from MCI individuals, while odor identification is worse in the cognitively impaired than the normal controls in the same populations.[15] Both tasks require naming capabilities, but odor identification appears notably to distinguish a normal individual from those further along the AD continuum. Olfactory areas may be vulnerable to AD neuropathology early on, whereas, the visual cortex and associated areas are affected later during the disease process.[145]

As stated above, the loss or dysfunction of neurons in the entorhinal cortex could explain cognitive impairments and decline in olfactory identification, which require the EC neurons to project and transmit signals to the HC. Smell identification requires intact detection and discrimination, which occurs upstream. AD neuropathology at the level of the EC may interfere with the recognition and identification function necessary to name odors correctly. In a similar way, hippocampal neuronal loss or dysfunction, as in Alzheimer neuropathology, could contribute to olfactory identification deficits.

2.3.5 Odor identification and neuroimaging

2.3.5.1 Atrophy

Several functional and imaging studies support the concept of conduction loss. The following studies expand on existing autopsy work by providing anatomical and functional evidence for the association of OI with AD related changes. The identification and naming of odors may be a potential measure of evolving pathology in regions vulnerable to AD neuropathology across both pre-clinical and clinical stages of AD.[15,18,218-221] Lower smell identification test scores are associated with increased atrophy, i.e. reduced entorhinal cortical thickness, reduced amygdala volume.[220-222]

Likewise, piriform cortex and hippocampal volumes were atrophied in MCI and AD patients compared to normal controls.[223] Lower performance in olfactory tasks has been repeatedly associated with lower hippocampal volumes.[12,224-226] Inclusion of olfactory tests with psychometric tests and brain imaging data (MRI volume) improves the sensitivity of the progression prediction from MCI to AD dementia by reflecting functional olfactory circuits affected in early AD.[222] In this study, those with odor identification deficits were at a 4-5 fold increased risk of converting.[222] A recent study of blast victims suggested that OI deficits are more readily detected in patients with acute brain trauma when olfactory testing is performed within days of injury.[227] Recent PET studies with a tracer that monitored newborn olfactory sensory neurons in lesioned, aging, or AD-like animal models further support this point.[228] Although, these last two studies were not performed in patients with AD, they demonstrated that odor identification as a functional task is altered with brain damage and recovery. In addition, data suggest that structural damage or loss of integrity can affect function. In a study investigating atrophy and blood oxygen level dependent (BOLD) activation pattern during an olfactory task, greater atrophy in the POC was associated with a decline in olfactory fMRI activity.[223] This study illustrates ways structures may affect functions. Thus, we can suggest that as key regions of interest decline in volume with the advancement of AD, olfactory tasks like odor identification should reflect this detrimental change.

2.3.5.2 Functional imaging

In addition to structural neuroimaging, we can use several different methods to investigate brain function in relation to olfaction. Studies using olfactory event related potentials (ERPs), for one, suggested a decline in activity in individuals at risk for early AD.[229] Data from ^{15}O-H2O-PET olfactory-evoked regional cerebral blood flow (rCBF) illustrate that AD patients have a different pattern of functional activation than healthy controls. In fact, in this study, there was decreased engagement of the frontotemporal

junction in the AD patients group. Additionally, this study demonstrated a linear relationship for odor identification performance and increased regional blood flow in the right piriform cortex, which they suggested could be due to age-associated reduced function in the left temporal area.[1] Förster and colleagues conducted similar work with a different PET tracer. They investigated FDG-PET and found that AD patients had hypometabolism during odor identification, discrimination or threshold.[230] In these patients, odor identification increased glucose reuptake, a measure of functional activity, in the right superior parietal lobule, fusiform gyrus, inferior frontal gyrus, and precuneus. It is relevant that odor discrimination activated the left postcentral cortex, while simple detection activated the right thalamus and cerebellum. These findings may have been expected because odor identification is known to be a higher order process that builds on these lower order tasks. PET imaging and fMRI imaging have revealed blunted activation in the piriform cortex activation in AD patients.[1,231-233] The reduced activation represents a weaker blood flow signal in the piriform cortex in AD than in controls, thus demonstrating a reduced energy requirement perhaps due to regional loss of function. Vasavada and colleagues investigated central and peripheral involvement of the olfactory system using an fMRI tasks and concluded that olfactory problems of those on an AD trajectory related to central rather than peripheral damage.[234] These various functional imaging methods demonstrate how odor identification could represent change brain activity in regions important for higher-order processing of senses, language processing, and attention. OI could serve as a reflection of anatomical integrity and functional activity affected by AD neuropathology.

2.3.5.3 Amyloid imaging

In an attempt to study the relationship of fibrillar amyloid deposition and odor identification, two groups have shown that poor odor identification is weakly associated with pathological accumulation of amyloid plaques in the posterior cingulate, temporo-parietal, and lingual cortical regions.[220,235] A separate amyloid PET study observed that amyloid accumulation was not correlated to odor identification.[236] We suggest that the

methodology of the last study was less than ideal, as it tested olfaction at an average of 2 years after a PET scan had taken place. Additionally, the delay ranged in some instances up to five years following the scan. Among cognitively normal individuals from the Mayo Clinic Study on Aging (MCSA), loss of odor identification was associated with a tendency toward reduced cortical thickness in entorhinal, inferior temporal, middle temporal and fusiform cortices (n=826), and in hippocampal volume (n=821). All these are known regions of AD neurodegeneration.[237] Within this large study, 305 had amyloid PET and 18F-FDG PET data. There was no association of an abnormal glucose metabolism imaging marker with odor identification. However, this study observed associations between glucose metabolism in regions of interest representing primary and secondary olfactory areas with the odor identification test.[238] Those with odor identification loss, *viz.* anosmia, were at an increased risk of abnormal amyloid accumulation, a finding that strengthens the link between odor identification and AD pathology.[237] Lastly, individuals with both abnormal amyloid accumulation and neurodegeneration had increased odds of lower smell test scores and odor identification loss (OR 3.84; 95%CI: 1.14-12.97; p=0.03). This large study reproduced the Growdon et al. observations in cognitively normal individuals and expanded it further by characterizing odor identification with multi-modal imaging and applied the new Jack et al A/T/N framework.[217] Overall, most of the above evidence for an association between OI and amyloid accumulation is somewhat inconsistent. It might be insightful to explore continuous measures of tracer uptake instead of threshold cut-off values or uptake patterns over time.[239] Nonetheless, there appear to be sufficient autopsy, structural and functional imaging data associating olfactory structures with AD neuropathology to support further explorion of odor identification as a marker of early AD progression.

2.3.6 Studies of olfactory function and AD pathological markers, or the proof of the pudding is in the eating

A decade ago, a breakthrough study examined the difficulty in identifying odors in 77 autopsied cases from the Rush Memory and Aging Project.[218] Using two separate

methods to quantify AD pathology, *viz*. silver staining and immunochemistry, they showed that reduced odor identification an average 2.2 years before death was associated with a composite measure of plaques and tangles. This association survived adjustments for age, sex, education, cognitive function, and even diagnosis. Moreover, it was not the demented cases that drove the association.[218] Neuritic plaques were associated with deficits in odor identification, although diffuse plaques were not.[240] This study demonstrated that poorer odor identification was strongly associated with increased *tau*-immunoreactive neurofribrillary tangles in the CA1 and subiculum areas of the hippocampus and the entorhinal cortex (both $p<0.001$) after adjustments. Wilson et al. also observed an association of reduced odor identification and increased tangles in the inferior-temporal cortex ($p=0.012$). Trends were found for increased tangles in the anterior cingulate cortex and primary-visual cortex $p=0.114$; $p=0.064$. After revisiting these data, Wilson et al. found that odor identification was associated with increased accumulation of AD neuropathology in 34 individuals who died without cognitive impairment.[219] This association survived adjustment for age, *APOE* ε4 allele carrier, sex, education, and cognitive function.

Recently, Lafaille-Magnan and colleagues corroborated some of these seminal findings *in vivo*. In 100 cognitively normal adults at increased risk for AD from the PREVENT-AD study, reduced odor identification was associated with AD-related changes in the CSF, specifically with increased t-*tau*/$A\beta_{1-42}$ and P_{181}-*tau*/$A\beta_{1-42}$ ($p<0.002$).[241] However, there was no apparent association with CSF $A\beta_{42}$ except in *APOE* ε4 allele carriers or in those with lower-quartile $A\beta_{42}$ levels.[241] The relationship of the tau/Aβ biomarkers and reduced odor identification survived adjustment for sex, education, age, possession of an ε4 allele and global cognition.[241]

Reijs and colleagues corroborated the *in vivo* association of OI and CSF *tau,* described by Lafaille-Magnan and colleagues, in 160 individuals with average age of 67 years and 11 years of education (40 normals, 45 MCI, 42 AD-dementia, 26 non-AD-dementia) Their work from the EDAR study found no association between OI and CSF

$A\beta_{42}$..[242] These findings were independent of diagnosis or ε4 status. The results are interesting inasmuch as OI was assessed with a shorter test, the Brief Smell Identification Test (BSIT). This instrument is thought to be less reliable, but appeared to be sufficient to reflect ongoing neurodegeneration. Unfortunately, Reijs et al. did not report examination the CSF tau/$A\beta_{42}$ ratio, a more specific marker for AD diagnosis than either marker alone.[243] In this same study, authors found that lower OI was associated with lower performance on all cognitive measures, but baseline OI was only predictive of MMSE decline in 80 individuals over 1.28 years of follow-up. Interestingly, OI predicted decline on word list learning and delayed recall in ε4 carriers and in individuals with abnormal $A\beta_{42}$. This suggests that in individuals at increased risk of dementia or with dementia already, OI can serve as a marker of memory decline.

2.4 Use of odor identification in clinical trials

Autopsy work has observed that tau, amyloid, and neurodegeneration are found across the olfactory network. Odor identification impairment is associated with atrophy and changes in brain activity. The considerations of OI as a marker in AD pathogenesis is relatively novel; yet there are reports of trials in which OI was examined as a secondary marker. While examples are few, we can cite them to further exemplify the use of odor identification as an outcome measure for preventive or treatment trials.

2.4.1 MCI trials

Pelton and colleagues have demonstrated that olfactory identification measured with the University of Pennsylvania Smell Identification Test, UPSIT, could be a valuable predictive secondary measure in a clinical trial of 22 individuals with both depression and mild cognitive impairment.[244] Participants were treated with sertraline or an alternative antidepressant and they were randomized to donepezil, a cholinesterase inhibitor, or placebo.[244] The olfactory performance was a helpful predictor of treatment effects. Baseline odor naming ability (UPSIT score) was worse in those who improved on episodic verbal memory performance (8 to 20 weeks) in the donepezil ($r=-0.56$;

p=0.07) but not the placebo (r=−0.20; p=0.6) arm.[244] Among the donepezil and antidepressant group, based on a median split stratification of the UPSIT score, the individuals with low UPSIT scores had a 44% improvement vs an 11% improvement on the Selective Reminding Test in those with higher UPSIT scores.[244] This study suggests that individuals with lower OI could benefit especially from a treatment intervention, perhaps because their cholinergic system would have been more affected. In 1976, Davies and colleagues reported the loss of choline acetylase and acetylcholinesterase activity in AD patients.[245] After which, Whitehouse and colleagues demonstrated that there was a significant cholinergic neuronal loss in demented patients.[246] These findings led to the cholinergic hypothesis wherein loss of cholinergic input to the hippocampus and cerebral cortex leads to the cognitive decline observed in AD patients.[247,248] This randomized controlled trial supports the potential of odor identification in prevention trials in MCI patients, i.e., before the onset of AD dementia.

2.4.2 AD dementia trials

Even in mild to moderate AD dementia patients, open label trial data demonstrated that one can distinguish donepezil responders using the UPSIT.[249] Velayudhan et al. followed acute 3-month change in 25 patients with AD. An improvement in olfactory identification was observed in those who improved on the Clinical Interview Based Impression of Change plus caregiver scale (CIBIC-plus).[249] This finding suggests that odor identification may provide useful monitoring of cholinesterase inhibitor efficacy. Another study by the same group demonstrated that the UPSIT could be used as a severity marker in 57 AD patients and 24 elderly controls.[18] These patients were still able to complete the smell identification test despite their MMSE test scores ranging from 15 to 25. The ability to name an odor was reduced in AD patients who demonstrated more rapid decline, as compared with non-rapid decliners over a 3-month period.[18] These data add to idea that odor identification might serve as an corroborative outcome measure for cholinergic drug intervention.

2.5 How can we use odor identification in clinical trials?

Beyond response to donepezil, use of odor identification as a clinical endpoint is a relatively new field. The idea is that individuals with pathological AD as their leading cause of cognitive dysfunction should also have impairment in olfactory identification. Alternately stated, we may be able to use tests of OI to follow evolving AD pathology. The above findings from the autopsy study of Wilson et al. (2007) demonstrate that odor identification makes an independent contribution apart from other cognitive measures.[218] Devanand et al. have demonstrated that odor identification strongly predicts conversion from MCI to AD dementia when combined with age, MMSE, episodic memory, and a functional activities questionnaire.[222] Several other important works across various populations observed improved prediction of cognitive decline when baseline odor identification was considered in the model.[250-252] In fact, a larger study of 757 New Yorkers with up to 4-year follow-up demonstrated that the ability to name odors was superior to episodic memory in identifing the 101 participants who subsequently transitioned to AD dementia.[250]

Recently, Leoutsakos et al. included OI scores, a measure collected for this work, in the development of an Alzheimer Progression Score (APS).[253] This score is based on an item response theory latent-variable analysis and summarizes multi-modal information to assess AD progression in a clinical trial of AD prevention.[253] Authors used a combination of odor identification, cognitive score and imaging to demonstrate how investigators can take advantage of multiple biomarkers available such as in the PREVENT-AD study. The APS methodology has been previously applied to predict subsequent "conversion" to MCI or AD dementia.[253] The concerted use of neurodegenerative disease biomarkers may enable to follow diseases on a continuum like AD. This methodology could be applied using different instruments to other diseases like Parkinson’s disease, also associated with olfactory dysfunction.

In an unpublished work, Leoutsakos fit random intercept models to the APS and each of its constituents in the PREVENT-AD cohort. The investigator used those parameter estimates to run a Monte Carlo simulation to calculate the power to detect a treatment effect in a randomized clinical trial lasting two years with annual visits and a sample size of 200. Table 2-2 shows the power to detect effects equivalent to a 40% and 60% reduction in rate of decline in a putative treatment arm. The APS, the index for progression outperformed all of its constituents. Since CSF data was not available in non-trial participants, it was not possible to determine how the addition of such variables would affect power. Given how much it increased the predictive utility in the BIOCARD study, we expect that it would similarly improve the power in PREVENT-AD. This exercise highlights the importance of relying on several markers to measure AD related change. This power calculation shows how odor identification may not be powerful enough to accomplish treatment efficacy evaluation alone, but appears superior to immediate memory, hippocampal or ventricle volumes, and anterior cingulate blood flow.

Table 1. Power To Detect A Treatment Effect For APS and its Constituents			
	Fitted Rate of Change (SE)	Treatment Effect	
Measure		40%	60%
APS	**.16 (.02)**	**.68**	**.95**
RBANS Attention	-1.46 (.51)	.14	.22
RBANS Immediate Memory	-.76 (.49)	.10	.12
UPSIT Total	-.48 (.14)	.17	.32
fMRI Behavioral Task	-1.31 (.52)	.18	.34
Entorhinal Density	-.003 (.001)	.37	.72
Lingual Density	-.004 (.0004)	.47	.84
Putamen Density	-.003 (.0004)	.39	.74
Right Superior Parietal Thickness	-.02 (.003)	.36	.71
Right Dorsal Frontal Thickness	-.02 (.003)	.32	.62
Right Rostral Anterior Cingulate Cerebral Blood Flow	-.87 (.54)	.12	.19
Hippocampal Volume	-25.59 (2.83)	.07	.07
Lateral Ventricle Volume	807.39 (48.03)	.08	.10

Table 2-2 Power To Detect A Treatment Effect For the Alzheimer Progression Score developed with a selection of PREVENT-AD measures and its Constituents

2.6 Conclusion

Work conducted in MCI with depressive symptoms and AD patients suggest the potential use of odor identification to evaluate changes related to the disease or the treatment assignment.[244,249] We acknowledge that AD presents heterogeneously and many patients have mixed dementia. If donepezil treats "true blue" AD going back to the original cholinergic hypothesis, it is interesting to reflect on this finding. Will other drug treatments be able to show an effect on odor identification? Clinical trials are moving upstream of cognitive decline. It would be very interesting to include olfactory testing in randomized control trials of preventative interventions to further explore its potential.

The value of altered odor identification in people at risk of Alzheimer dementia is under-appreciated. Taken together, these findings suggest the importance in studying olfactory function early on in the AD disease continuum, to be used as an early indicator of pathway integrity. Although decades of autopsy studies suggest an association between the olfactory bulb, tract, entorhinal cortex, and even hippocampus and the Alzheimer degeneration process, odor identification testing remains absent from most studies assessing the clinical picture of AD dementia. Odor identification requires peripheral and central neuronal integrity. Recent relationships between odor function with *tau* and amyloid accumulation have begun to associate decline and abnormal change to an early AD progression.[220,237,241,242] This task appears well suited to evaluate the conservation, recovery, and deterioration of the olfactory network.

There are very few studies that exploit longitudinal odor identification data.[254-256] To our knowledge, there is an absence of literature that investigates odor identification and the Alzheimer neuropathology parallel processes. OI screening for predisposition to AD is noninvasive and can be used in remote

regions, and may improve diagnosis or evaluation of treatment efficacy in combination with other classical biomarkers.[257] The smell identification test is not only cost effective and easy, but also practical.

2.7 References

1 Kareken, D. A. *et al.* Olfactory-evoked regional cerebral blood flow in Alzheimer's disease. *Neuropsychology* **15**, 18 (2001).

2 Kareken, D. A., Mosnik, D. M., Doty, R. L., Dzemidzic, M. & Hutchins, G. D. Functional anatomy of human odor sensation, discrimination, and identification in health and aging. *Neuropsychology* **17**, 482 (2003).

3 Zatorre, R. J., Jones-Gotman, M., Evans, A. C. & Meyer, E. Functional localization and lateralization of human olfactory cortex. *Nature* **360**, 339 (1992).

4 Zatorre, R. J., Jones-Gotman, M. & Rouby, C. Neural mechanisms involved in odor pleasantness and intensity judgments. *Neuroreport* **11**, 2711-2716 (2000).

5 Zatorre, R. J. & Jones-Gotman, M. in *Brain mapping: The systems* 403-424 (Elsevier, 2000).

6 Sobel, N. *et al.* A method for functional magnetic resonance imaging of olfaction. *Journal of neuroscience methods* **78**, 115-123 (1997).

7 Sobel, N. *et al.* Sniffing and smelling: separate subsystems in the human olfactory cortex. *Nature* **392**, 282 (1998).

8 Sobel, N. *et al.* Odorant-induced and sniff-induced activation in the cerebellum of the human. *Journal of Neuroscience* **18**, 8990-9001 (1998).

9 Savic, I., Gulyas, B., Larsson, M. & Roland, P. Olfactory functions are mediated by parallel and hierarchical processing. *Neuron* **26**, 735-745 (2000).

10 Yousem, D. M. *et al.* Functional MR imaging during odor stimulation: preliminary data. *Radiology* **204**, 833-838 (1997).

11 Saiz-Sanchez, D., Flores-Cuadrado, A., Ubeda-Banon, I., de la Rosa-Prieto, C. & Martinez-Marcos, A. Interneurons in the human olfactory system in Alzheimer's disease. *Experimental neurology* **276**, 13-21, doi:10.1016/j.expneurol.2015.11.009 (2016).

12 Murphy, C., Jernigan, T. L. & Fennema-Notestine, C. Left hippocampal volume loss in Alzheimer's disease is reflected in performance on odor identification: a

structural MRI study. *Journal of the International Neuropsychological Society* **9**, 459-471 (2003).

13 Doty, R. L. in *Seminars in neurology*. 074-081 (© Thieme Medical Publishers).

14 Mesholam, R. I., Moberg, P. J., Mahr, R. N. & Doty, R. L. Olfaction in neurodegenerative disease: a meta-analysis of olfactory functioning in Alzheimer's and Parkinson's diseases. *Archives of neurology* **55**, 84-90 (1998).

15 Djordjevic, J., Jones-Gotman, M., De Sousa, K. & Chertkow, H. Olfaction in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiology of aging* **29**, 693-706 (2008).

16 Rahayel, S., Frasnelli, J. & Joubert, S. The effect of Alzheimer's disease and Parkinson's disease on olfaction: a meta-analysis. *Behavioural brain research* **231**, 60-74, doi:10.1016/j.bbr.2012.02.047 (2012).

17 Roalf, D. R. *et al.* A quantitative meta-analysis of olfactory dysfunction in mild cognitive impairment. *Journal of neurology, neurosurgery, and psychiatry* **88**, 226-232, doi:10.1136/jnnp-2016-314638 (2017).

18 Velayudhan, L., Pritchard, M., Powell, J. F., Proitsi, P. & Lovestone, S. Smell identification function as a severity and progression marker in Alzheimer's disease. *International psychogeriatrics* **25**, 1157-1166 (2013).

19 Nicola-Antoniu, I. in *The Clinical Spectrum of Alzheimer's Disease -The Charge Toward Comprehensive Diagnostic and Therapeutic Strategies* (ed Suzanne De La Monte) Ch. 07 (InTech, 2011).

20 Saiz-Sanchez, D. *Neuroanatomical changes in the olfactory system in Alzheimer's disease* PhD thesis, Universidad de Castilla-La Mancha, (2012).

21 Sela, L. & Sobel, N. Human olfaction: a constant state of change-blindness. *Experimental brain research. Experimentelle Hirnforschung. Experimentation cerebrale* **205**, 13-29, doi:10.1007/s00221-010-2348-6 (2010).

22 Shepherd, G. M. The human sense of smell: are we better than we think? *PLoS Biol* **2**, E146, doi:10.1371/journal.pbio.0020146 (2004).

23 Getchell, M. L. & Getchell, T. V. Immunohistochemical localization of components of the immune barrier in the olfactory mucosae of salamanders and rats. *The Anatomical Record* **231**, 358-374 (1991).

24 Buck, L. & Axel, R. A novel multigene family may encode odorant receptors: a molecular basis for odor recognition. *Cell* **65**, 175-187 (1991).

25 (HGNC)www.genenames.org., H. G. N. C. <https://www.genenames.org/cgi-bin/genefamilies/set/141>

26 Glusman, G., Yanai, I., Rubin, I. & Lancet, D. The complete human olfactory subgenome. *Genome research* **11**, 685-702, doi:10.1101/gr.171001 (2001).

27 Nei, M., Niimura, Y. & Nozawa, M. The evolution of animal chemosensory receptor gene repertoires: roles of chance and necessity. *Nat Rev Genet* **9**, 951-963, doi:10.1038/nrg2480 (2008).

28 Crasto, C., Marenco, L., Miller, P. & Shepherd, G. Olfactory Receptor Database: a metadata-driven automated population from sources of gene and protein sequences. *Nucleic acids research* **30**, 354-360 (2002).

29 Zhang, X. & Firestein, S. The olfactory receptor gene superfamily of the mouse. *Nature neuroscience* **5**, 124 (2002).

30 Alioto, T. S. & Ngai, J. The odorant receptor repertoire of teleost fish. *BMC genomics* **6**, 173 (2005).

31 Zhang, X. *et al.* Characterizing the expression of the human olfactory receptor gene family using a novel DNA microarray. *Genome biology* **8**, R86 (2007).

32 (HGNC)www.genenames.org., H. G. N. C. <http://www.genenames.org/cgi-bin/genefamilies/set/289> (

33 Shi, X., Nie, F., Wang, Z. & Sun, M. Pseudogene-expressed RNAs: a new frontier in cancers. *Tumor Biology* **37**, 1471-1478 (2016).

34 Malnic, B., Hirono, J., Sato, T. & Buck, L. B. Combinatorial receptor codes for odors. *Cell* **96**, 713-723 (1999).

35 Saito, H., Chi, Q., Zhuang, H., Matsunami, H. & Mainland, J. D. Odor coding by a Mammalian receptor repertoire. *Sci. Signal.* **2**, ra9-ra9 (2009).

36 Sela, L. & Sobel, N. Human olfaction: a constant state of change-blindness. *Experimental brain research* **205**, 13-29 (2010).

37 Ethell, D. W. Disruption of cerebrospinal fluid flow through the olfactory system may contribute to Alzheimer's disease pathogenesis. *Journal of Alzheimer's Disease* **41**, 1021-1030 (2014).

38 Asahina, K., Pavlenkovich, V. & Vosshall, L. B. The survival advantage of olfaction in a competitive environment. *Current Biology* **18**, 1153-1155 (2008).

39 Reinhard, J., Srinivasan, M. V. & Zhang, S. Olfaction: scent-triggered navigation in honeybees. *Nature* **427**, 411 (2004).

40 Wallraff, H. G. Avian olfactory navigation: its empirical foundation and conceptual state. *Animal Behaviour* **67**, 189-204 (2004).

41 Murphy, M. R. & Schneider, G. E. Olfactory bulb removal eliminates mating behavior in the male golden hamster. *Science* **167**, 302-304 (1970).

42 Heimer, L. & Larsson, K. Mating behavior of male rats after olfactory bulb lesions. *Physiology & Behavior* **2**, 207-209 (1967).

43 Yamazaki, K. *et al.* Control of mating preferences in mice by genes in the major histocompatibility complex. *Journal of Experimental Medicine* **144**, 1324-1335 (1976).

44 Hummel, T. & Nordin, S. Olfactory disorders and their consequences for quality of life. *Acta oto-laryngologica* **125**, 116-121 (2005).

45 Miwa, T. *et al.* Impact of olfactory impairment on quality of life and disability. *Archives of Otolaryngology–Head & Neck Surgery* **127**, 497-503 (2001).

46 Croy, I., Nordin, S. & Hummel, T. Olfactory disorders and quality of life—an updated review. *Chemical Senses* **39**, 185-194 (2014).

47 Conover, M. R. *Predator-prey dynamics: the role of olfaction*. (CRC Press, 2007).

48 Moulton, D. G. Dynamics of cell populations in the olfactory epithelium. *Annals of the New York Academy of Sciences* **237**, 52-61 (1974).

49 Graziadei, P. & Graziadei, G. M. Neurogenesis and neuron regeneration in the olfactory system of mammals. I. Morphological aspects of differentiation and structural organization of the olfactory sensory neurons. *Journal of neurocytology* **8**, 1-18 (1979).

50 Graziadei, G. M. & Graziadei, P. P. C. Neurogenesis and neuron regeneration in the olfactory system of mammals. II. Degeneration and reconstitution of the olfactory sensory neurons after axotomy. *Journal of neurocytology* **8**, 197-213 (1979).

51 Enwere, E. *et al.* Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. *Journal of Neuroscience* **24**, 8354-8365 (2004).

52 Röhl, I. A. *The 2004 Nobel Prize in Physiology or Medicine to Richard Axel and Linda B. Buck.* , <http://www.nobelprize.org/nobel_prizes/medicine/laureates/2004/press.html >

53 Jones, D. T. & Reed, R. R. Golf: an olfactory neuron specific-G protein involved in odorant signal transduction. *Science* **244**, 790-795 (1989).

54 Jones, D., Barbosa, E. & Reed, R. in *Cold Spring Harbor symposia on quantitative biology.* 349-353 (Cold Spring Harbor Laboratory Press).

55 Belluscio, L., Gold, G. H., Nemes, A. & Axel, R. Mice deficient in Golf are anosmic. *Neuron* **20**, 69-81 (1998).

56 Wong, S. T. *et al.* Disruption of the type III adenylyl cyclase gene leads to peripheral and behavioral anosmia in transgenic mice. *Neuron* **27**, 487-497 (2000).

57 Ronnett, G. V., Cho, H., Hester, L. D., Wood, S. F. & Snyder, S. H. Odorants differentially enhance phosphoinositide turnover and adenylyl cyclase in olfactory receptor neuronal cultures. *Journal of Neuroscience* **13**, 1751-1758 (1993).

58 Buck, L. B. Information coding in the vertebrate olfactory system. *Annual review of neuroscience* **19**, 517-544 (1996).

59 Brunet, L. J., Gold, G. H. & Ngai, J. General anosmia caused by a targeted disruption of the mouse olfactory cyclic nucleotide–gated cation channel. *Neuron* **17**, 681-693 (1996).

60 Stephan, A. B. *et al.* ANO2 is the cilial calcium-activated chloride channel that may mediate olfactory amplification. *Proceedings of the National Academy of Sciences* **106**, 11776-11781 (2009).

61 Zufall, F., Leinders-Zufall, T. & Greer, C. A. Amplification of odor-induced Ca2+ transients by store-operated Ca2+ release and its role in olfactory signal transduction. *Journal of neurophysiology* **83**, 501-512 (2000).

62 Firestein, S., Darrow, B. & Shepherd, G. M. Activation of the sensory current in salamander olfactory receptor neurons depends on a G protein-mediated cAMP second messenger system. *Neuron* **6**, 825-835 (1991).

63 Breer, H., Raming, K. & Krieger, J. Signal recognition and transduction in olfactory neurons. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research* **1224**, 277-287 (1994).

64 Breer, H. in *Seminars in cell biology*. 25-32 (Elsevier).

65 Berkowicz, D. A., Trombley, P. Q. & Shepherd, G. M. Evidence for glutamate as the olfactory receptor cell neurotransmitter. *Journal of neurophysiology* **71**, 2557-2561 (1994).

66 Su, C.-Y., Menuz, K. & Carlson, J. R. Olfactory perception: receptors, cells, and circuits. *Cell* **139**, 45-59 (2009).

67 DeMaria, S. & Ngai, J. The cell biology of smell. *The Journal of cell biology* **191**, 443-452 (2010).

68 Mombaerts, P. Axonal wiring in the mouse olfactory system. *Annu. Rev. Cell Dev. Biol.* **22**, 713-737 (2006).

69 Boekhoff, I., Tareilus, E., Strotmann, J. & Breer, H. Rapid activation of alternative second messenger pathways in olfactory cilia from rats by different odorants. *The EMBO journal* **9**, 2453-2458 (1990).

70 Boekhoff, I., Strotmann, J., Raming, K., Tareilus, E. & Breer, H. Odorant-sensitive phospholipase C in insect antennae. *Cellular signalling* **2**, 49-56 (1990).

71 Breer, H. & Boekhoff, I. Odorants of the same odor class activate different second messenger pathways. *Chemical Senses* **16**, 19-29 (1991).

72 Bozza, T., Feinstein, P., Zheng, C. & Mombaerts, P. Odorant receptor expression defines functional units in the mouse olfactory system. *Journal of Neuroscience* **22**, 3033-3043 (2002).

73 Carmichael, S. T., Clugnet, M. C. & Price, J. L. Central olfactory connections in the macaque monkey. *Journal of Comparative Neurology* **346**, 403-434 (1994).

74 Mori, K., Nagao, H. & Yoshihara, Y. The olfactory bulb: coding and processing of odor molecule information. *Science* **286**, 711-715 (1999).

75 Mori, K., Takahashi, Y. K., Igarashi, K. M. & Yamaguchi, M. Maps of odorant molecular features in the mammalian olfactory bulb. *Physiological reviews* **86**, 409-433 (2006).

76 Nagayama, S., Homma, R. & Imamura, F. Neuronal organization of olfactory bulb circuits. *Frontiers in neural circuits* **8** (2014).

77 Mori, K. & Sakano, H. How is the olfactory map formed and interpreted in the mammalian brain? *Annual review of neuroscience* **34**, 467-499 (2011).

78 Mori, K. & Yoshihara, Y. Molecular recognition and olfactory processing in the mammalian olfactory system. *Progress in neurobiology* **45**, 585-619 (1995).

79 Royet, J., Sicard, G., Souchier, C. & Jourdan, F. Specificity of spatial patterns of glomerular activation in the mouse olfactory bulb: computer-assisted image analysis of 2-deoxyglucose autoradiograms. *Brain research* **417**, 1-11 (1987).

80 Aungst, J. *et al.* Centre–surround inhibition among olfactory bulb glomeruli. *Nature* **426**, 623 (2003).

81 Rall, W., Shepherd, G., Reese, T. & Brightman, M. Dendrodendritic synaptic pathway for inhibition in the olfactory bulb. *Experimental neurology* **14**, 44-56 (1966).

82 Willhite, D. C. *et al.* Viral tracing identifies distributed columnar organization in the olfactory bulb. *Proceedings of the National Academy of Sciences* **103**, 12592-12597 (2006).

83 Maresh, A., Gil, D. R., Whitman, M. C. & Greer, C. A. Principles of glomerular organization in the human olfactory bulb–implications for odor processing. *PloS one* **3**, e2640 (2008).

84 Migliore, M. & Shepherd, G. M. Dendritic action potentials connect distributed dendrodendritic microcircuits. *Journal of computational neuroscience* **24**, 207-221 (2008).

85 Fukunaga, I., Berning, M., Kollo, M., Schmaltz, A. & Schaefer, A. T. Two distinct channels of olfactory bulb output. *Neuron* **75**, 320-329 (2012).

86 Shepherd, G. M., Chen, W. R., Willhite, D., Migliore, M. & Greer, C. A. The olfactory granule cell: from classical enigma to central role in olfactory processing. *Brain research reviews* **55**, 373-382 (2007).

87 Jahr, C. & Nicoll, R. Noradrenergic modulation of dendrodendritic inhibition in the olfactory bulb. *Nature* **297**, 227 (1982).

88 McLean, J. H. & Shipley, M. T. Serotonergic afferents to the rat olfactory bulb: I. Origins and laminar specificity of serotonergic inputs in the adult rat. *Journal of Neuroscience* **7**, 3016-3028 (1987).

89 Doty, R. L. Olfactory dysfunction in neurodegenerative diseases: is there a common pathological substrate? *The Lancet Neurology* **16**, 478-488, doi:10.1016/s1474-4422(17)30123-0 (2017).

90 Schliebs, R. & Arendt, T. The cholinergic system in aging and neuronal degeneration. *Behavioural brain research* **221**, 555-563 (2011).

91 Devore, S. Rapid control of olfaction. *Nature neuroscience* **19**, 181 (2016).

92 de Almeida, R. F. *et al.* Olfactory bulbectomy in mice triggers transient and long-lasting behavioral impairments and biochemical hippocampal disturbances. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **76**, 1-11 (2017).

93 Bazhenov, M. & Stopfer, M. Forward and back: motifs of inhibition in olfactory processing. *Neuron* **67**, 357-358 (2010).

94 Alvarez-Buylla, A. & Lim, D. A. For the long run: maintaining germinal niches in the adult brain. *Neuron* **41**, 683-686 (2004).

95 Ming, G.-l. & Song, H. Adult neurogenesis in the mammalian central nervous system. *Annu. Rev. Neurosci.* **28**, 223-250 (2005).

96 Pignatelli, A., Belluzzi, O. & Menini, A. The neurobiology of olfaction. (2010).

97 Shen, Q. *et al.* Adult SVZ stem cells lie in a vascular niche: a quantitative analysis of niche cell-cell interactions. *Cell stem cell* **3**, 289-300 (2008).

98 Whitman, M. C., Fan, W., Rela, L., Rodriguez- Gil, D. J. & Greer, C. A. Blood vessels form a migratory scaffold in the rostral migratory stream. *Journal of Comparative Neurology* **516**, 94-104 (2009).

99 Whitman, M. C. & Greer, C. A. Adult neurogenesis and the olfactory system. *Progress in neurobiology* **89**, 162-175 (2009).

100 Martoncikova, M. *et al.* Astrocytic and vascular scaffolding for neuroblast migration in the rostral migratory stream. *Current neurovascular research* **11**, 321-329 (2014).

101 Sultan, S. *et al.* Learning-dependent neurogenesis in the olfactory bulb determines long-term olfactory memory. *The FASEB Journal* **24**, 2355-2363 (2010).

102 Pezet, S. & McMahon, S. B. Neurotrophins: mediators and modulators of pain. *Annu. Rev. Neurosci.* **29**, 507-538 (2006).

103 Williams, L. J. & Hazer, J. T. Antecedents and consequences of satisfaction and commitment in turnover models: A reanalysis using latent variable structural equation methods. *Journal of applied psychology* **71**, 219 (1986).

104 Rylett, R., Goddard, S., Schmidt, B. & Williams, L. Acetylcholine synthesis and release following continuous intracerebral administration of NGF in adult and aged Fischer-344 rats. *Journal of Neuroscience* **13**, 3956-3963 (1993).

105 Koehl, M. & Abrous, D. N. A new chapter in the field of memory: adult hippocampal neurogenesis. *European Journal of Neuroscience* **33**, 1101-1114 (2011).

106 Lledo, P.-M., Alonso, M. & Grubb, M. S. Adult neurogenesis and functional plasticity in neuronal circuits. *Nature Reviews Neuroscience* **7**, 179 (2006).

107 Bergmann, O., Spalding, K. L. & Frisén, J. Adult neurogenesis in humans. *Cold Spring Harbor perspectives in biology* **7**, a018994 (2015).

108 Bergmann, O. *et al.* The age of olfactory bulb neurons in humans. *Neuron* **74**, 634-639 (2012).

109 Burton, S. D., LaRocca, G., Liu, A., Cheetham, C. E. & Urban, N. N. Olfactory Bulb Deep Short-Axon Cells Mediate Widespread Inhibition of Tufted Cell Apical Dendrites. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **37**, 1117-1138, doi:10.1523/JNEUROSCI.2880-16.2016 (2017).

110 Sahay, A. *et al.* Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature* **472**, 466 (2011).

111 Gheusi, G. *et al.* Importance of newly generated neurons in the adult olfactory bulb for odor discrimination. *Proceedings of the National Academy of Sciences* **97**, 1823-1828 (2000).

112 Nagayama, S. *et al.* Differential axonal projection of mitral and tufted cells in the mouse main olfactory system. *Frontiers in neural circuits* **4**, 120 (2010).

113 Sankaran, S., Khot, L. R. & Panigrahi, S. Biology and applications of olfactory sensing system: A review. *Sensors and Actuators B: Chemical* **171**, 1-17 (2012).

114 Price, J. L. An autoradiographic study of complementary laminar patterns of termination of afferent fibers to the olfactory cortex. *Journal of Comparative Neurology* **150**, 87-108 (1973).

115 Haberly, L. B. & Price, J. L. Association and commissural fiber systems of the olfactory cortex of the rat. I. Systems originating in the piriform cortex and adjacent areas. *Journal of Comparative Neurology* **178**, 711-740 (1978).

116 Allison, A. The secondary olfactory areas in the human brain. *Journal of anatomy* **88**, 481 (1954).

117 Amaral, D., Insausti, R. & Cowan, W. The entorhinal cortex of the monkey: I. Cytoarchitectonic organization. *Journal of Comparative Neurology* **264**, 326-355 (1987).

118 Insausti, R., Amaral, D. & Cowan, W. The entorhinal cortex of the monkey: II. Cortical afferents. *Journal of Comparative Neurology* **264**, 356-395 (1987).

119 Carmichael, S. & Price, J. Architectonic subdivision of the orbital and medial prefrontal cortex in the macaque monkey. *Journal of Comparative Neurology* **346**, 366-402 (1994).

120 Howard, J. D., Plailly, J., Grueschow, M., Haynes, J. D. & Gottfried, J. A. Odor quality coding and categorization in human posterior piriform cortex. *Nature neuroscience* **12**, 932-938, doi:10.1038/nn.2324 (2009).

121 Wilson, D. A. & Stevenson, R. J. *Learning to smell: olfactory perception from neurobiology to behavior*. (JHU Press, 2006).

122 Wilson-Pauwels, L. *Cranial nerves: function and dysfunction*. (PMPH-USA, 2010).

123 Price, J. L., Slotnick, B. M. & Revial, M. F. Olfactory projections to the hypothalamus. *The Journal of comparative neurology* **306**, 447-461, doi:10.1002/cne.903060309 (1991).

124 Price, J. L. in *The human nervous system* 979-998 (Elsevier, 1990).

125 Benjamin, R. M. & Jackson, J. C. Unit discharges in the mediodorsal nucleus of the squirrel monkey evoked by electrical stimulation of the olfactory bulb. *Brain research* **75**, 181-191 (1974).

126 Krettek, J. & Price, J. Amygdaloid projections to subcortical structures within the basal forebrain and brainstem in the rat and cat. *Journal of Comparative Neurology* **178**, 225-253 (1978).

127 Powell, T., Cowan, W. & Raisman, G. The central olfactory connexions. *Journal of Anatomy* **99**, 791 (1965).

128 Price, J. L. & Slotnick, B. M. Dual olfactory representation in the rat thalamus: an anatomical and electrophysiological study. *Journal of Comparative Neurology* **215**, 63-77 (1983).

129 Scott, J. W. & Leonard, C. M. The olfactory connections of the lateral hypothalamus in the rat, mouse and hamster. *Journal of Comparative Neurology* **141**, 331-344 (1971).

130 Johnson, D. M., Illig, K. R., Behan, M. & Haberly, L. B. New features of connectivity in piriform cortex visualized by intracellular injection of pyramidal cells suggest that "primary" olfactory cortex functions like "association" cortex in other sensory systems. *Journal of Neuroscience* **20**, 6974-6982 (2000).

131 Hanson, A. J. *et al.* Effect of apolipoprotein E genotype and diet on apolipoprotein E lipidation and amyloid peptides: randomized clinical trial. *JAMA Neurol* **70**, 972-980, doi:10.1001/jamaneurol.2013.396 (2013).

132 Sobel, N. *et al.* Time course of odorant-induced activation in the human primary olfactory cortex. *Journal of neurophysiology* **83**, 537-551 (2000).

133 Savic, I. & Gulyas, B. PET shows that odors are processed both ipsilaterally and contralaterally to the stimulated nostril. *Neuroreport* **11**, 2861-2866 (2000).

134 Hummel, T. *et al.* Position paper on olfactory dysfunction. *Rhinology* (2017).

135 Brodal, A. The olfactory pathways. *Neurological anatomy*, 640-697 (1981).

136 Courtiol, E. & Wilson, D. A. The olfactory thalamus: unanswered questions about the role of the mediodorsal thalamic nucleus in olfaction. *Front Neural Circuits* **9**, 49, doi:10.3389/fncir.2015.00049 (2015).

137 Peters, S. K., Dunlop, K. & Downar, J. Cortico-Striatal-Thalamic Loop Circuits of the Salience Network: A Central Pathway in Psychiatric Disease and Treatment. *Front Syst Neurosci* **10**, 104, doi:10.3389/fnsys.2016.00104 (2016).

138 Tham, W. W., Stevenson, R. J. & Miller, L. A. The role of the mediodorsal thalamic nucleus in human olfaction. *Neurocase* **17**, 148-159 (2011).

139 Keller, A. Attention and olfactory consciousness. *Frontiers in Psychology* **2**, 380 (2011).

140 Tham, W. W., Stevenson, R. J. & Miller, L. A. The functional role of the medio dorsal thalamic nucleus in olfaction. *Brain research reviews* **62**, 109-126 (2009).

141 Papez, J. W. A proposed mechanism of emotion. *Archives of Neurology & Psychiatry* **38**, 725-743 (1937).

142 Rajmohan, V. & Mohandas, E. The limbic system. *Indian journal of psychiatry* **49**, 132 (2007).

143 Willander, J. & Larsson, M. Smell your way back to childhood: Autobiographical odor memory. *Psychonomic bulletin & review* **13**, 240-244 (2006).

144 Willander, J. & Larsson, M. Olfaction and emotion: The case of autobiographical memory. *Memory & cognition* **35**, 1659-1663 (2007).

145 Braak, H. & Braak, E. Evolution of the neuropathology of Alzheimer's disease. *Acta neurologica Scandinavica* **94**, 3-12 (1996).

146 Coutureau, E. & Di Scala, G. Entorhinal cortex and cognition. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **33**, 753-761 (2009).

147 Witter, M. P. The perforant path: projections from the entorhinal cortex to the dentate gyrus. *Progress in brain research* **163**, 43-61 (2007).

148 Eichenbaum, H., Morton, T. H., Potter, H. & Corkin, S. Selective olfactory deficits in case HM. *Brain : a journal of neurology* **106**, 459-472 (1983).

149 Masliah, E. *et al.* Synaptic and neuritic alterations during the progression of Alzheimer's disease. *Neuroscience letters* **174**, 67-72 (1994).

150 Witter, M. P., Doan, T. P., Jacobsen, B., Nilssen, E. S. & Ohara, S. Architecture of the entorhinal cortex a review of entorhinal anatomy in rodents with some comparative notes. *Frontiers in systems neuroscience* **11**, 46 (2017).

151 Lyketsos, C. G. *et al.* Neuropsychiatric symptoms in Alzheimer's disease. *Alzheimer's & dementia: the journal of the Alzheimer's Association* **7**, 532-539 (2011).

152 Rosenberg, P. B., Nowrangi, M. A. & Lyketsos, C. G. Neuropsychiatric symptoms in Alzheimer's disease: What might be associated brain circuits? *Mol Aspects Med* **43-44**, 25-37, doi:10.1016/j.mam.2015.05.005 (2015).

153 Cipriani, G., Lucetti, C., Danti, S. & Nuti, A. Sleep disturbances and dementia. *Psychogeriatrics* **15**, 65-74, doi:10.1111/psyg.12069 (2015).

154 Crowder, R. G., Schab, F., Schab, F. & Crowder, R. Odor recognition memory. *Memory for odors. Mahwah, NJ: Lawrence Erlbaum Associates, Inc., Publishers*, 9-20 (1995).

155 Morgan, C. D., Nordin, S. & Murphy, C. Odor identification as an early marker for Alzheimer's disease: impact of lexical functioning and detection sensitivity. *Journal of clinical and experimental neuropsychology* **17**, 793-803 (1995).

156 Murphy, C. & Cain, W. S. Odor identification: The blind are better. *Physiology & Behavior* **37**, 177-180 (1986).

157 Cain, W. S. To know with the nose: keys to odor identification. *Science* **203**, 467-470 (1979).

158 Chobor, K. L. in *Science of olfaction* 355-377 (Springer, 1992).

159 Schab, F. R. Odor memory: taking stock. *Psychological bulletin* **109**, 242 (1991).

160 Doty, R. L., Shaman, P. & Dann, M. Development of the University of Pennsylvania Smell Identification Test: a standardized microencapsulated test of olfactory function. *Physiology & behavior* **32**, 489-502 (1984).

161 De Wijk, R. A. & Cain, W. S. Odor quality: discrimination versus free and cued identification. *Perception & psychophysics* **56**, 12-18 (1994).

162 <larsson1999.pdf>.

163 Yeshurun, Y. & Sobel, N. An odor is not worth a thousand words: from multidimensional odors to unidimensional odor objects. *Annual review of psychology* **61**, 219-241 (2010).

164 Zelano, C., Montag, J., Khan, R. & Sobel, N. A specialized odor memory buffer in primary olfactory cortex. *PloS one* **4**, e4965 (2009).

165 Olofsson, J. K. & Gottfried, J. A. The muted sense: neurocognitive limitations of olfactory language. *Trends in cognitive sciences* **19**, 314-321 (2015).

166 Levy, L. M., Henkin, R. I., Hutter, A., Lin, C. S. & Schellinger, D. Mapping brain activation to odorants in patients with smell loss by functional MRI. *Journal of computer assisted tomography* **22**, 96-103 (1998).

167 Savic, I. & Berglund, H. Passive perception of odors and semantic circuits. *Hum Brain Mapp* **21**, 271-278, doi:10.1002/hbm.20009 (2004).

168 Ciumas, C., Lindström, P., Aoun, B. & Savic, I. Imaging of odor perception delineates functional disintegration of the limbic circuits in mesial temporal lobe epilepsy. *NeuroImage* **39**, 578-592 (2008).

169 Braak, H. & Braak, E. Neuropathological stageing of Alzheimer-related changes. *Acta neuropathologica* **82**, 239-259, doi:10.1007/BF00308809 (1991).

170 Doty, R. L., Hawkes, C. H. & Berendse, H. W. Olfactory dysfunction in Parkinson's disease and related disorders. *Non-dopamine Lesions in Parkinson's Disease*, 65-91 (2011).

171 Doty, R. L. Olfactory dysfunction in Parkinson disease. *Nature Reviews Neurology* **8**, 329-339 (2012).

172 Lötsch, J., Geisslinger, G. & Hummel, T. Sniffing out pharmacology: interactions of drugs with human olfaction. *Trends in pharmacological sciences* **33**, 193-199 (2012).

173 Ajmani, G. S., Suh, H. H. & Pinto, J. M. Effects of Ambient Air Pollution Exposure on Olfaction: A Review. *Environ Health Perspect* **124**, 1683-1693, doi:10.1289/EHP136 (2016).

174 Ajmani, G. S. *et al.* Fine particulate matter exposure and olfactory dysfunction among urban-dwelling older US adults. *Environmental research* **151**, 797-803 (2016).

175 Calderon-Garciduenas, L. *et al.* Decreases in Short Term Memory, IQ, and Altered Brain Metabolic Ratios in Urban Apolipoprotein epsilon4 Children Exposed to Air Pollution. *Journal of Alzheimer's disease : JAD* **45**, 757-770, doi:10.3233/JAD-142685 (2015).

176 Calderon-Garciduenas, L. *et al.* Brain inflammation and Alzheimer's-like pathology in individuals exposed to severe air pollution. *Toxicologic pathology* **32**, 650-658, doi:10.1080/01926230490520232 (2004).

177 Calderon-Garciduenas, L. *et al.* Urban air pollution: influences on olfactory function and pathology in exposed children and young adults. *Exp Toxicol Pathol* **62**, 91-102, doi:10.1016/j.etp.2009.02.117 (2010).

178 Calderon-Garciduenas, L. *et al.* Systemic inflammation, endothelial dysfunction, and activation in clinically healthy children exposed to air pollutants. *Inhalation toxicology* **20**, 499-506 (2008).

179 Calderon-Garciduenas, L. *et al.* Exposure to severe urban air pollution influences cognitive outcomes, brain volume and systemic inflammation in clinically healthy children. *Brain and cognition* **77**, 345-355, doi:10.1016/j.bandc.2011.09.006 (2011).

180 Calderon-Garciduenas, L. *et al.* DNA damage in nasal and brain tissues of canines exposed to air pollutants is associated with evidence of chronic brain inflammation and neurodegeneration. *Toxicologic pathology* **31**, 524-538, doi:10.1080/01926230390226645 (2003).

181 Calderon-Garciduenas, L. *et al.* Air pollution, cognitive deficits and brain abnormalities: a pilot study with children and dogs. *Brain and cognition* **68**, 117-127, doi:10.1016/j.bandc.2008.04.008 (2008).

182 Levesque, S., Surace, M. J., McDonald, J. & Block, M. L. Air pollution & the brain: subchronic diesel exhaust exposure causes neuroinflammation and elevates early markers of neurodegenerative disease. *Journal of neuroinflammation* **8**, 105 (2011).

183 Rombaux, P., Potier, H., Bertrand, B., Duprez, T. & Hummel, T. Olfactory bulb volume in patients with sinonasal disease. *American journal of rhinology* **22**, 598-601 (2008).

184 Turner, J. H., Liang, K. L., May, L. & Lane, A. P. Tumor necrosis factor alpha inhibits olfactory regeneration in a transgenic model of chronic rhinosinusitis–associated olfactory loss. *American journal of rhinology & allergy* **24**, 336 (2010).

185 Paik, S. I., Lehman, M. N., Seiden, A. M., Duncan, H. J. & Smith, D. V. Human olfactory biopsy: the influence of age and receptor distribution. *Archives of Otolaryngology–Head & Neck Surgery* **118**, 731-738 (1992).

186 Chen, Y., Getchell, T. V., Sparks, D. L. & Getchell, M. L. Patterns of adrenergic and peptidergic innervation in human olfactory mucosa: Age- related trends. *Journal of Comparative Neurology* **334**, 104-116 (1993).

187 Hebb, D. O. *The organization of behavior: A neuropsychological theory*. (Psychology Press, 2005).

188 Bitter, T. *et al.* Anosmia leads to a loss of gray matter in cortical brain areas. *Chem Senses* **35**, 407-415, doi:10.1093/chemse/bjq028 (2010).

189 Morrison, E. E. & Costanzo, R. M. Morphology of the human olfactory epithelium. *Journal of Comparative Neurology* **297**, 1-13 (1990).

190 Kern, R., Quinn, B., Rosseau, G. & Farbman, A. Post- traumatic olfactory dysfunction. *The Laryngoscope* **110**, 2106-2109 (2000).

191 Seiden, A. M. & Duncan, H. J. The diagnosis of a conductive olfactory loss. *The Laryngoscope* **111**, 9-14 (2001).

192 Talamo, B. R. *et al.* Pathological changes in olfactory neurons in patients with Alzheimer's disease. *Nature* **337**, 736-739 (1989).

193 Johnston, M., Zakharov, A., Papaiconomou, C., Salmasi, G. & Armstrong, D. Evidence of connections between cerebrospinal fluid and nasal lymphatic vessels in humans, non-human primates and other mammalian species. *Cerebrospinal Fluid Res* **1**, 2, doi:10.1186/1743-8454-1-2 (2004).

194 de Leon, M. J. *et al.* CSF clearance in Alzheimer Disease measured with dynamic PET. *J Nucl Med*, doi:10.2967/jnumed.116.187211 (2017).

195 Mawuenyega, K. G. *et al.* Decreased clearance of CNS β-amyloid in Alzheimer's disease. *Science* **330**, 1774-1774 (2010).

196 Castellano, J. M. *et al.* Human apoE isoforms differentially regulate brain amyloid-β peptide clearance. *Science translational medicine* **3**, 89ra57-89ra57 (2011).

197 Iturria-Medina, Y., Sotero, R. C., Toussaint, P. J., Evans, A. C. & Alzheimer's Disease Neuroimaging, I. Epidemic spreading model to characterize misfolded proteins propagation in aging and associated neurodegenerative disorders. *PLoS Comput Biol* **10**, e1003956, doi:10.1371/journal.pcbi.1003956 (2014).

198 Suzuki, Y. *et al.* Reduced CSF Water Influx in Alzheimer's Disease Supporting the beta-Amyloid Clearance Hypothesis. *PloS one* **10**, e0123708, doi:10.1371/journal.pone.0123708 (2015).

199 Liss, L. & Gomez, F. The nature of senile changes of the human olfactory bulb and tract. *Arch Otolaryngol* **67**, 167-171 (1958).

200 Song, W. *et al.* Astroglial heme oxygenase-1 and the origin of corpora amylacea in aging and degenerating neural tissues. *Experimental neurology* **254**, 78-89 (2014).

201 Kovacs, T., Cairns, N. & Lantos, P. beta-amyloid deposition and neurofibrillary tangle formation in the olfactory bulb in ageing and Alzheimer's disease. *Neuropathology and applied neurobiology* **25**, 481-491 (1999).

202 Ohm, T. & Braak, H. Olfactory bulb changes in Alzheimer's disease. *Acta neuropathologica* **73**, 365-369 (1987).

203 Bhatnagar, K. P., Kennedy, R. C., Baron, G. & Greenberg, R. A. Number of mitral cells and the bulb volume in the aging human olfactory bulb: a quantitative morphological study. *The Anatomical record* **218**, 73-87 (1987).

204 Thomann, P. A. *et al*. Reduced olfactory bulb and tract volume in early Alzheimer's disease—a MRI study. *Neurobiology of aging* **30**, 838-841 (2009).

205 Esiri, M. M. & Wilcock, G. K. The olfactory bulbs in Alzheimer's disease. *Journal of Neurology, Neurosurgery & Psychiatry* **47**, 56-60 (1984).

206 Davies, D., Brooks, J. & Lewis, D. Axonal loss from the olfactory tracts in Alzheimer's disease. *Neurobiology of aging* **14**, 353-357 (1993).

207 Mann, D. M., Tucker, C. M. & Yates, P. O. Alzheimer's disease: an olfactory connection? *Mechanisms of ageing and development* **42**, 1-15 (1988).

208 ChristenZaech, S. *et al*. Early olfactory involvement in Alzheimer's disease. *Canadian Journal of Neurological Sciences* **30**, 20-25 (2003).

209 Price, J. L., Davis, P. B., Morris, J. C. & White, D. L. The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiology of aging* **12**, 295-312 (1991).

210 Braak, H. & Braak, E. Staging of Alzheimer-related cortical destruction. *International psychogeriatrics / IPA* **9 Suppl 1**, 257-261; discussion 269-272 (1997).

211 Reyes, P. F., Deems, D. A. & Suarez, M. G. Olfactory-related changes in Alzheimer's disease: a quantitative neuropathologic study. *Brain research bulletin* **32**, 1-5 (1993).

212 Van Hoesen, G. W. & Solodkin, A. Cellular and systems neuroanatomical changes in Alzheimer's disease. *Annals of the New York Academy of Sciences* **747**, 12-35 (1994).

213 Attems, J., Lintner, F. & Jellinger, K. Olfactory involvement in aging and Alzheimer's disease: an autopsy study. *Journal of Alzheimer's disease* **7**, 149-157 (2005).

214 Gómez-Isla, T. *et al.* Profound loss of layer II entorhinal cortex neurons occurs in very mild Alzheimer's disease. *The Journal of Neuroscience* **16**, 4491-4500 (1996).

215 Sze, C. I. *et al.* Loss of the presynaptic vesicle protein synaptophysin in hippocampus correlates with cognitive decline in Alzheimer disease. *J. Neuropathol. Exp. Neurol* **56**, 933-944 (1997).

216 Braak, H. & Braak, E. Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* **82**, 239-259 (1991).

217 Jack Jr, C. R. *et al.* Age-specific and sex-specific prevalence of cerebral β-amyloidosis, tauopathy, and neurodegeneration in cognitively unimpaired individuals aged 50–95 years: a cross-sectional study. *The Lancet Neurology* **16**, 435-444 (2017).

218 Wilson, R. S., Arnold, S. E., Schneider, J. A., Tang, Y. & Bennett, D. A. The relationship between cerebral Alzheimer's disease pathology and odour identification in old age. *Journal of neurology, neurosurgery, and psychiatry* **78**, 30-35, doi:10.1136/jnnp.2006.099721 (2007).

219 Wilson, R. S. *et al.* Olfactory impairment in presymptomatic Alzheimer's disease. *Annals of the New York Academy of Sciences* **1170**, 730-735, doi:10.1111/j.1749-6632.2009.04013.x (2009).

220 Growdon, M. E. *et al.* Odor identification and Alzheimer disease biomarkers in clinically normal elderly. *Neurology* **84**, 2153-2160, doi:10.1212/WNL.0000000000001614 (2015).

221 Hagemeier, J. *et al.* Odor identification deficit in mild cognitive impairment and Alzheimer's disease is associated with hippocampal and deep gray matter atrophy. *Psychiatry Research: Neuroimaging* **255**, 87-93 (2016).

222 Devanand, D. P. *et al.* Combining early markers strongly predicts conversion from mild cognitive impairment to Alzheimer's disease. *Biol Psychiatry* **64**, 871-879 (2008).

223 Vasavada, M. M. *et al.* Olfactory cortex degeneration in Alzheimer's disease and mild cognitive impairment. *Journal of Alzheimer's Disease* **45**, 947-958 (2015).

224 Devanand, D. *et al.* Olfactory identification deficits and MCI in a multi-ethnic elderly community sample. *Neurobiology of aging* **31**, 1593-1600 (2010).

225 Kjelvik, G. *et al.* The brain structural and cognitive basis of odor identification deficits in mild cognitive impairment and Alzheimer's disease. *BMC neurology* **14**, 168 (2014).

226 Marigliano, V. *et al.* Olfactory deficit and hippocampal volume loss for early diagnosis of Alzheimer disease: a pilot study. *Alzheimer Disease & Associated Disorders* **28**, 194-197 (2014).

227 Xydakis, M. S. *et al.* Olfactory impairment and traumatic brain injury in blast-injured combat troops A cohort study. *Neurology* **84**, 1559-1567 (2015).

228 <mahlknecht2015.pdf>.

229 Morgan, C. D. & Murphy, C. Individuals at risk for Alzheimer's disease show differential patterns of ERP brain activation during odor identification. *Behavioral and Brain Functions* **8**, 37 (2012).

230 Forster, S. *et al.* Functional representation of olfactory impairment in early Alzheimer's disease. *Journal of Alzheimer's disease : JAD* **22**, 581-591, doi:10.3233/JAD-2010-091549 (2010).

231 Wang, J. *et al.* Olfactory deficit detected by fMRI in early Alzheimer's disease. *Brain research* **1357**, 184-194 (2010).

232 Li, W., Howard, J. D. & Gottfried, J. A. Disruption of odour quality coding in piriform cortex mediates olfactory deficits in Alzheimer's disease. *Brain : a journal of neurology* **133**, 2714-2726 (2010).

233 Li, W. *et al.* Right orbitofrontal cortex mediates conscious olfactory perception. *Psychological science* **21**, 1454-1463 (2010).

234 Vasavada, M. M. *et al.* Central Olfactory Dysfunction in Alzheimer's Disease and Mild Cognitive Impairment: A Functional MRI Study. *Journal of Alzheimer's disease : JAD* **59**, 359-368, doi:10.3233/JAD-170310 (2017).

235 Bahar-Fuchs, A. *et al.* Olfactory deficits and amyloid-beta burden in Alzheimer's disease, mild cognitive impairment, and healthy aging: a PiB PET study. *Journal of Alzheimer's disease : JAD* **22**, 1081-1087, doi:10.3233/JAD-2010-100696 (2010).

236 Dhilla Albers, A. *et al.* Episodic memory of odors stratifies Alzheimer biomarkers in normal elderly. *Annals of neurology* **80**, 846-857, doi:10.1002/ana.24792 (2016).

237 Vassilaki, M. *et al.* Neuroimaging biomarkers and impaired olfaction in cognitively normal individuals. *Annals of neurology* **81**, 871-882, doi:10.1002/ana.24960 (2017).

238 Roberts, R. O. *et al.* Association Between Olfactory Dysfunction and Amnestic Mild Cognitive Impairment and Alzheimer Disease Dementia. *JAMA Neurol*, 1-9, doi:10.1001/jamaneurol.2015.2952 (2015).

239 Villeneuve, S. *et al.* Existing Pittsburgh Compound-B positron emission tomography thresholds are too high: statistical and pathological evaluation. *Brain : a journal of neurology* **138**, 2020-2033, doi:10.1093/brain/awv112 (2015).

240 <wilson2007 (1).pdf>.

241 Lafaille-Magnan, M.-E. *et al.* Odor identification as a biomarker of preclinical AD in older adults at risk. *Neurology* **89**, 327-335 (2017).

242 Reijs, B. L. R. *et al.* Relation of Odor Identification with Alzheimer's Disease Markers in Cerebrospinal Fluid and Cognition. *Journal of Alzheimer's disease : JAD* **60**, 1025-1034, doi:10.3233/JAD-170564 (2017).

243 Blennow, K. A Review of Fluid Biomarkers for Alzheimer's Disease: Moving from CSF to Blood. *Neurol Ther* **6**, 15-24, doi:10.1007/s40120-017-0073-9 (2017).

244 Pelton, G. H., Soleimani, L., Roose, S. P., Tabert, M. H. & Devanand, D. P. Olfactory Deficits Predict Cognitive Improvement on Donepezil in Patients With Depression and Cognitive Impairment: A Randomized Controlled Pilot Study. *Alzheimer disease and associated disorders* **30**, 67-69, doi:10.1097/WAD.0000000000000107 (2016).

245 Davies, P. & Maloney, A. J. Selective loss of central cholinergic neurons in Alzheimer's disease. *Lancet* **2**, 1403 (1976).

246 Whitehouse, P. J. *et al.* Alzheimer's disease and senile dementia: loss of neurons in the basal forebrain. *Science* **215**, 1237-1239 (1982).

247 Bartus, R. T., Dean, R. L., III, Beer, B. & Lippa, A. S. The cholinergic hypothesis of geriatric memory dysfunction. *Science* **217**, 408-414 (1982).

248 Rogers, S. L. & Friedhoff, L. T. The efficacy and safety of donepezil in patients with Alzheimer's disease: results of a US Multicentre, Randomized, Double-Blind, Placebo-Controlled Trial. The Donepezil Study Group. *Dementia* **7**, 293-303 (1996).

249 Velayudhan, L. & Lovestone, S. Smell identification test as a treatment response marker in patients with Alzheimer disease receiving donepezil. *Journal of clinical psychopharmacology* **29**, 387-390, doi:10.1097/JCP.0b013e3181aba5a5 (2009).

250 Devanand, D. *et al.* Olfactory deficits predict cognitive decline and Alzheimer dementia in an urban community. *Neurology* **84**, 182-189 (2015).

251 Turana, Y. *et al.* Enhancing diagnostic accuracy of aMCI in the elderly: combination of olfactory test, pupillary response test, BDNF plasma level, and APOE genotype. *International Journal of Alzheimer's Disease* **2014** (2014).

252 Stanciu, I. *et al.* Olfactory impairment and subjective olfactory complaints independently predict conversion to dementia: a longitudinal, population-based study. *Journal of the International Neuropsychological Society* **20**, 209-217 (2014).

253 Leoutsakos, J., Gross, A., Jones, R., Albert, M. & Breitner, J. Alzheimer progression score: development of a biomarker summary outcome for AD prevention trials. *J Prev Alz Dis* **3**, 229-235 (2016).

254 Wilson, R. S. *et al.* Olfactory Impairment in Presymptomatic Alzheimer's Disease. *Annals of the New York Academy of Sciences* **1170**, 730-735, doi:10.1111/j.1749-6632.2009.04013.x (2009).

255 Josefsson, M., Larsson, M., Nordin, S., Adolfsson, R. & Olofsson, J. APOE-ε4 effects on longitudinal decline in olfactory and non-olfactory cognitive abilities in middle-aged and old adults. *Scientific Reports* **7** (2017).

256 Roberts, R. O. *et al.* Association Between Olfactory Dysfunction and Amnestic Mild Cognitive Impairment and Alzheimer Disease Dementia. *JAMA Neurol* **73**, 93-101, doi:10.1001/jamaneurol.2015.2952 (2016).

257 Wesson, D. W., Wilson, D. A. & Nixon, R. A. Should olfactory dysfunction be used as a biomarker of Alzheimer's disease? *Expert review of neurotherapeutics* **10**, 633-635 (2010).

Chapter 3: Odor identification as a biomarker of preclinical AD in older adults at risk

.

“They took all the trees
And put them in a tree museum
And they charged all the people
A dollar and a half to see 'em
Don't it always seem to go
That you don't know what you've got
'Till it's gone
They paved paradise
And they put up a parking lot”
Joni Mitchell –Big Yellow Taxi

3.1 Pre-amble

The development and identification of new methods to monitor Alzheimer's disease-related brain changes are essential to improve the understanding of the underlying pathophysiology, discovery of new targets and assessment of new therapeutics. Ideally, we need sensitive measures that correlate with the neural changes associated with the pre-symptomatic stage of AD.

A challenge in identifying early markers is the lack of prospective evidence. Traditional measures include cognitive batteries (CDR, MMSE, MoCA…), CSF biomarker levels, Magnetic resonance imaging (MRI), and Positron Emission Tomography (PET) neuroimaging. Interesting, subtle olfactory changes are potentially useful to supplement these classical tools. Important areas of the olfactory network exhibit initial AD-related neuropathological changes (Chapter 2).[1-4] These pathophysiological changes in olfactory structures can precede cognitive symptoms by a decade.[5] Therefore, olfactory deficits could be a sign of what is occurring inside the brain during the pre-symptomatic phase of AD.[6] Smell loss could be attributed to biochemical alterations in the olfactory network.

Losing the sense of smell is characteristic of both normal aging and neurodegenerative diseases, including AD.[7-12] Although found in both, the loss of olfactory ability is greater in neurodegenerative diseases. There is demonstrable deterioration in odor identification (OI), the ability to identify and name specific odorants, as well as, pathological and morphological changes support the accuracy of this sensory dysfunction in pre-symptomatic AD.[13,14] In fact, decline in odor naming abilities in cognitively normal older adults, and MCI could be used to predict cognitive decline, and MCI to AD conversion (Chapter 1).[15-17]

In this chapter, we investigate whether odor identification performance in normal individuals at risk of AD is different in older individuals, APOE ε4 carriers, and those with lower cognitive performance. In addition, we attempt to reproduce findings of post-mortem association between odor identification and AD histopathological staining.[6,18] To expand on previous works, we study classical AD markers found in the cerebrospinal fluid of 101 volunteer and we further stratify the sample to characterize ε4 carriers and non-carriers. After inspection we chose the 25th percentile to dichotomize participants as having high or low CSF Aβ concentrations. Recently, CSF biomarker levels have been classified into stages of Alzheimer's disease progression.[19] Normal individuals in Stage 0 have high $A\beta_{1-42}$ and low t-*tau*, whereas individuals in the Stage 1-3 have low CSF $A\beta_{1-42}$. The chosen cut-off would suggest that 75% of the population was considered normal and 25% were considered to be further along the way (Stage 1-2 but not 3, as it is associated with cognitive impairment). This is analogous to other investigation of odor identification and CSF biomarkers or PET imaging markers of AD progression where there is a separate analysis that focuses on abnormal $A\beta_{1-42}$ levels.[13,14]

3.1.1 References

1 Price, J. L., Davis, P. B., Morris, J. C. & White, D. L. The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiology of aging* **12**, 295-312 (1991).

2 Braak, H. & Braak, E. Neuropathological stageing of Alzheimer-related changes. *Acta neuropathologica* **82**, 239-259 (1991).

3 Braak, H. & Braak, E. Staging of Alzheimer's disease-related neurofibrillary changes. *Neurobiology of aging* **16**, 271-278 (1995).

4 Ferreyra-Moyano, H. The olfactory system and Alzheimer's disease. *International Journal of Neuroscience* **49**, 157-197 (1989).

5 Albert, M. S. *et al.* The diagnosis of mild cognitive impairment due to Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia :*

the journal of the Alzheimer's Association **7**, 270-279, doi:10.1016/j.jalz.2011.03.008 (2011).

6 Wilson, R. S. *et al.* Olfactory impairment in presymptomatic Alzheimer's disease. *Annals of the New York Academy of Sciences* **1170**, 730-735, doi:10.1111/j.1749-6632.2009.04013.x (2009).

7 Ship, J. A., Pearson, J. D., Cruise, L. J., Brant, L. J. & Metter, E. J. Longitudinal changes in smell identification. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* **51**, M86-M91 (1996).

8 Mesholam, R. I., Moberg, P. J., Mahr, R. N. & Doty, R. L. Olfaction in neurodegenerative disease: a meta-analysis of olfactory functioning in Alzheimer's and Parkinson's diseases. *Archives of neurology* **55**, 84-90 (1998).

9 Doty, R. L. Olfactory dysfunction in Parkinson disease. *Nature Reviews Neurology* **8**, 329-339 (2012).

10 Rahayel, S., Frasnelli, J. & Joubert, S. The effect of Alzheimer's disease and Parkinson's disease on olfaction: a meta-analysis. *Behavioural brain research* **231**, 60-74, doi:10.1016/j.bbr.2012.02.047 (2012).

11 Roalf, D. R. *et al.* A quantitative meta-analysis of olfactory dysfunction in mild cognitive impairment. *Journal of neurology, neurosurgery, and psychiatry* **88**, 226-232, doi:10.1136/jnnp-2016-314638 (2017).

12 Wehling, E. I., Lundervold, A. J., Nordin, S. & Wollschlaeger, D. Longitudinal Changes in Familiarity, Free and Cued Odor Identification, and Edibility Judgments for Odors in Aging Individuals. *Chem Senses* **41**, 155-161, doi:10.1093/chemse/bjv066 (2016).

13 Growdon, M. E. *et al.* Odor identification and Alzheimer disease biomarkers in clinically normal elderly. *Neurology* **84**, 2153-2160, doi:10.1212/WNL.0000000000001614 (2015).

14 Vassilaki, M. *et al.* Neuroimaging biomarkers and impaired olfaction in cognitively normal individuals. *Annals of neurology* **81**, 871-882, doi:10.1002/ana.24960 (2017).

15 Devanand, D. *et al.* Olfactory identification deficits and MCI in a multi-ethnic elderly community sample. *Neurobiology of aging* **31**, 1593-1600 (2010).

16 Devanand, D. *et al.* Olfactory deficits predict cognitive decline and Alzheimer dementia in an urban community. *Neurology* **84**, 182-189 (2015).

17 Roberts, R. O. *et al.* Association Between Olfactory Dysfunction and Amnestic Mild Cognitive Impairment and Alzheimer Disease Dementia. *JAMA Neurol*, 1-9, doi:10.1001/jamaneurol.2015.2952 (2015).

18 Wilson, R. S., Arnold, S. E., Schneider, J. A., Tang, Y. & Bennett, D. A. The relationship between cerebral Alzheimer's disease pathology and odour identification in old age. *Journal of neurology, neurosurgery, and psychiatry* **78**, 30-35, doi:10.1136/jnnp.2006.099721 (2007).

19 Sperling, R. A. *et al.* Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's Association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimer's & dementia : the journal of the Alzheimer's Association* **7**, 280-292, doi:10.1016/j.jalz.2011.03.003 (2011).

3.2 Title and authors

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

Marie-Elyse Lafaille-Magnan, BSc, MSc, Judes Poirier, PhD, CQ, Pierre Etienne, MD Jennifer Tremblay-Mercier, MSc, Joanne Frenette, BSN, MN, Pedro Rosa-Neto, MD, PhD, John C.S. Breitner, MD, MPH, For the PREVENT-AD Research Group

Correspondence to M.E. Lafaille-Magnan:
Marie-Elyse.Lafaille-Magnan@mail.mcgill.ca

From the Centre for Studies on Prevention of AD (M.-E.L.-M., J.P., P.E., J.T.-M., J.F., P.R.-N., J.C.S.B.) and McGill Centre for Studies in Aging (P.R.-N.), Douglas Mental Health University Institute, McGill University, Faculty of Medicine, Montreal, Quebec, Canada. Coinvestigators are listed at Neurology.org. Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article. The Article Processing charge was funded by McGill University.

3.3 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 ($A\beta_{1-42}$). 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 $A\beta_{1-42}$ (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 $A\beta_{1-42}$ alone except in APOE ε4 carriers having lowest-quartile $A\beta_{1-42}$ 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;89:1–9

3.4 Glossary

Aβ β-amyloid; AD Alzheimer disease; INTREPAD Impact of Naproxen Treatment on PresymptomaticAlzheimer'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.

3.5 Background

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 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 ($A\beta_{1-42}$). We hypothesized that degree of impairment in OI would predict biomarker evidence of AD neuropathology.

3.6 Methods

3.6.1 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] PREVENTAD 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.

3.6.2 Methods of assessment.

3.6.2.1 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.

3.6.2.2 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 (2,000g) 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 2808C in 500mL polypropylene cryotubes. The samples went through only one freeze-thaw cycle. We assayed CSF t-*tau*, P_{181}-*tau*, and $A\beta_{1-42}$ 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*/ $A\beta_{1-42}$ and P_{181}-*tau* / $A\beta_{1-42}$ to indicate disease state.

3.6.2.3 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).

3.6.2.4 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.

3.6.3 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*/ $A\beta_{1-42}$ ratio (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.

Characteristics of the seven multivariable models presented in Table 2:

Model 1 UPSIT error score ~ 1+ *APOE* 4 carrier status + sex + education + age

Model 2 UPSIT error score ~ 1+ *APOE* 4 carrier status + sex + education+ RBANS

Model 3 UPSIT error score ~ 1+ *APOE* 4 carrier status + sex + education + CSF total-*tau*/$A\beta_{1-42}$

Model 4 UPSIT error score ~ 1+ *APOE* 4 carrier status + sex + education + age + RBANS

Model 5 UPSIT error score ~ 1+ *APOE* 4 carrier status + sex + education + age + CSF total-*tau*/$A\beta_{1-42}$

Model 6 UPSIT error score ~ 1+ *APOE* 4 carrier status + sex + education + RBANS + CSF total-*tau*/$A\beta_{1-42}$

Model 7 UPSIT error score ~ 1+ *APOE* 4 carrier status + sex + education + age + RBANS + CSF total-*tau*/$A\beta_{1-42}$

We considered the need for adjusting by education, sex, and *APOE* 4 carrier status. Inclusion of these variables did not change the iterative modeling output, however, we kept these known determinants in to be comparable to studies of Alzheimer's disease.

3.7 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 3-1). The INTREPAD participants from whom we 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 3-1 and Table 3-2).

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]
$A\beta_{1\text{-}42}$, 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/$A\beta_{1\text{-}42}$	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/$A\beta_{1\text{-}42}$	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).

Table 3-1 Demographics of participants

PREVENT-AD cohort participants				
Demographics	**Average**	**S.D.**	**range (min median max)**	**n**
Age (years)	63.41	5.43	55 - 62 - 84	274
Sex (% Female)	73	44		274
Education (years)	15.15	3.47	7 - 15 - 29	274
E4 carrier status (%)	33	47		268
Caucasian (%)	98	13		274
Francophone (%)	81	39		274
MoCA total score	28.09	1.52	23 - 28 - 30	274
RBANS	101.92	11.16	73 - 101 - 140	270*
UPSIT total score	35.41	3.65	13 - 36 - 40	265**
$A\beta_{1\text{-}42}$ (pg/mL)	1062.91	280.65	402.35 - 1068.4 - 1596.9	101
t-*tau* (pg/mL)	273.09	129.97	90 - 259.06 - 851	101
P_{181}-*tau* (pg/mL)	46.83	18	12. 1 - 43.9 - 114.4	101
t-*tau* / $A\beta_{1\text{-}42}$	0.28	0.21	0.11 - 0.22 - 1.20	101
P_{181}-*tau*/ $A\beta_{1\text{-}42}$	0.05	0.03	0.01 - 0.04 - 0.18	101

*Four RBANS reports of individuals who underwent the lumbar puncture were lost.

**One person refused the olfactory testing and was not included in the reported analyses. Additionally, there were 8 incomplete tests or completed when congested not included in any analysis.

Table 3-2. Demographics of PREVENT-AD cohort participants

MoCA = Montreal Cognitive Assessment; RBANS = Repeatable Battery for the Assessment of Neuropsychological Status; UPSIT = University of Pennsylvania Smell Identification Test.

In bivariate modeling, the UPSIT error score was higher in older participants (n = 265, $\beta = 0.0134$, $p= 2.24 \times 10^{-6}$; Fig. 3-1) and in participants with lower RBANS total score (n = 261, $\beta = -0.00666$, $p= 1.28 \times 10^{-6}$; Fig. 3-2). 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; Fig. 3-2), but the association of reduced OI with decreased cognition remained robust ($\beta = -4.76 \times 10^{-3}$, p= 0.011; Fig. 3-2). 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; Fig. 3-3). 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 ($\beta = 27.01 \times 10^{-5}$, p= 0.359).

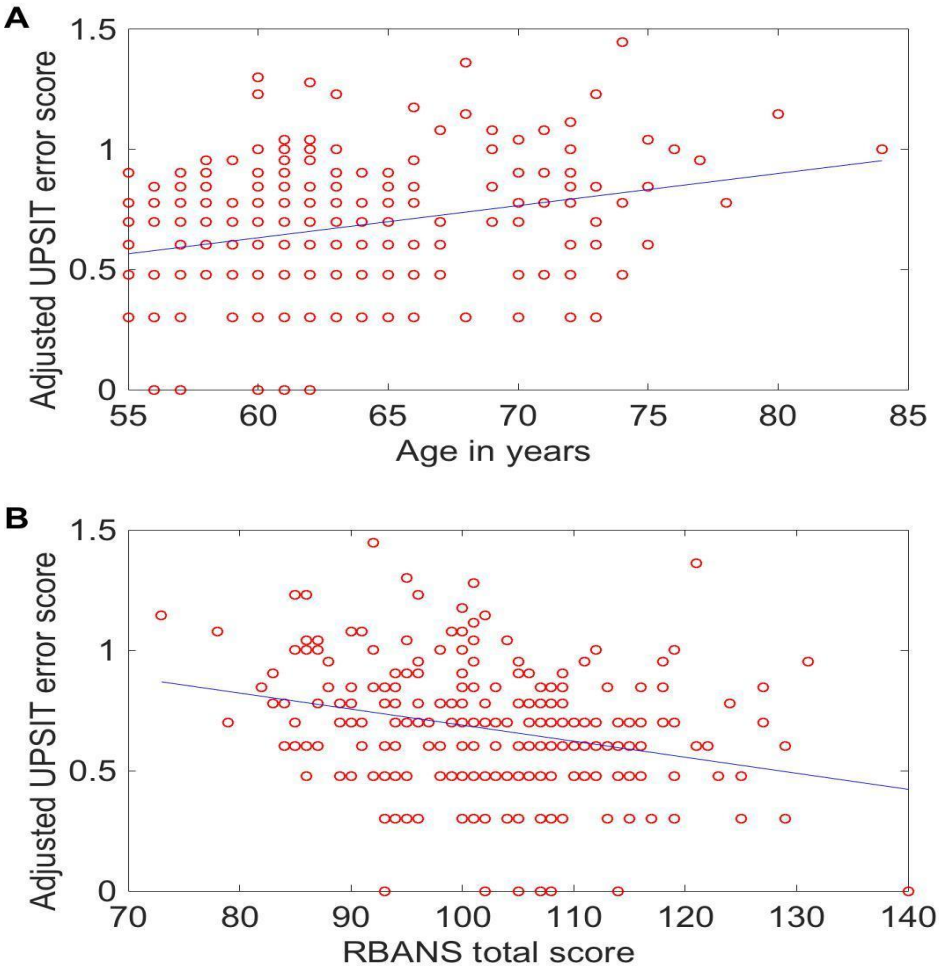


Figure 3-1. Robust-fit linear regression models of UPSIT error score vs. age and cognition.

A) UPSIT error score vs. age ($\beta = 0.0134$, $p = 2.24 \times 10^{-6}$, n=265),

B) UPSIT error score vs. RBANS total score ($\beta = -0.00666$, $p = 1.28 \times 10^{-6}$, n=261)

RBANS=Repeatable Battery for the Assessment of Neuropsychological Status; UPSIT=University of Pennsylvania Smell Identification Test

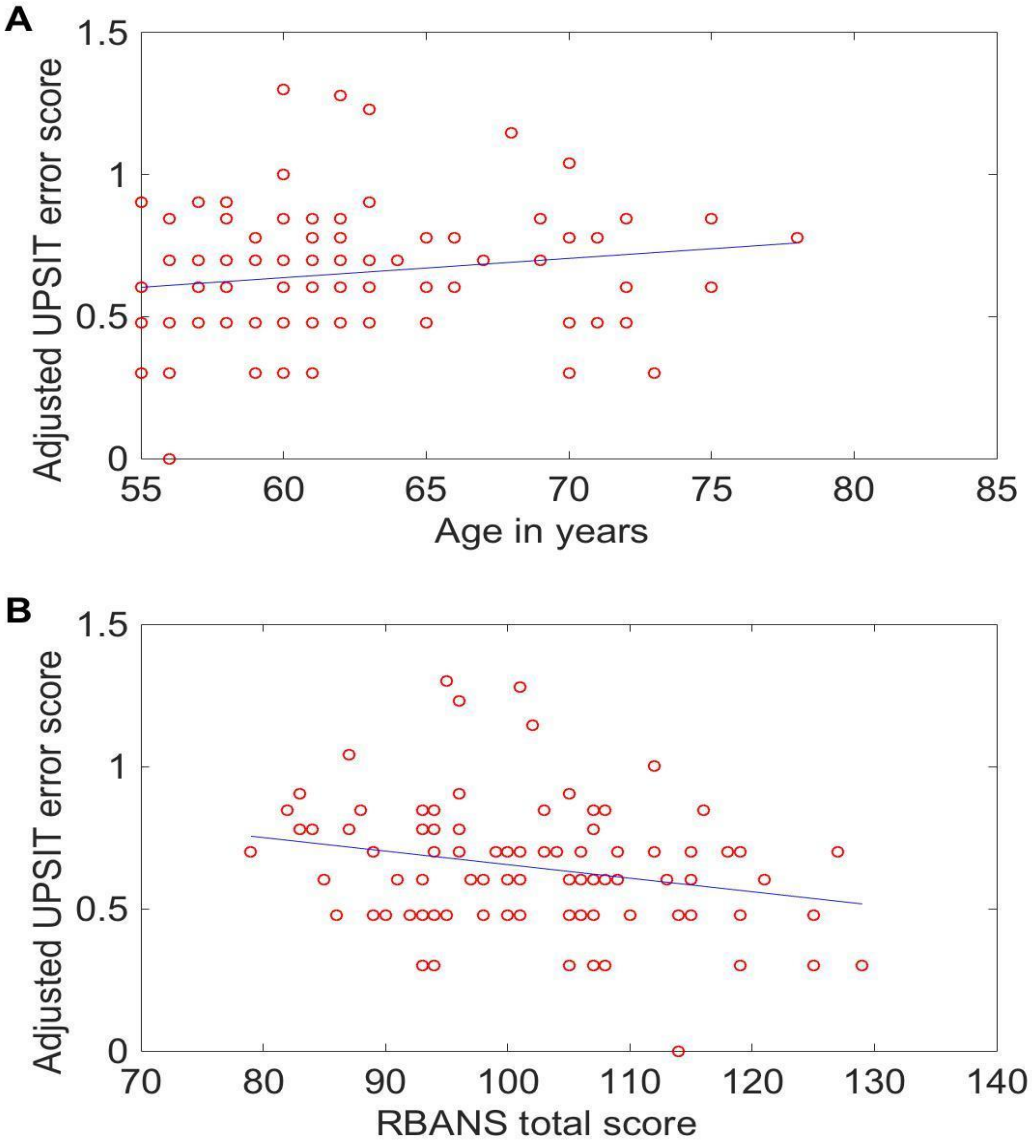


Figure 3-2. Robust-fit linear regression models of UPSIT error score vs. age and cognition in those with both CSF and odor identification data.

A) UPSIT error score vs. age ($\beta=6.79x10^{-3}$, p=0.095, n=100),

B) UPSIT error score vs. RBANS total score ($\beta=-4.76\ x10^{-3}$, p=0.011, n=100)

RBANS=Repeatable Battery for the Assessment of Neuropsychological Status; UPSIT=University of Pennsylvania Smell Identification Test

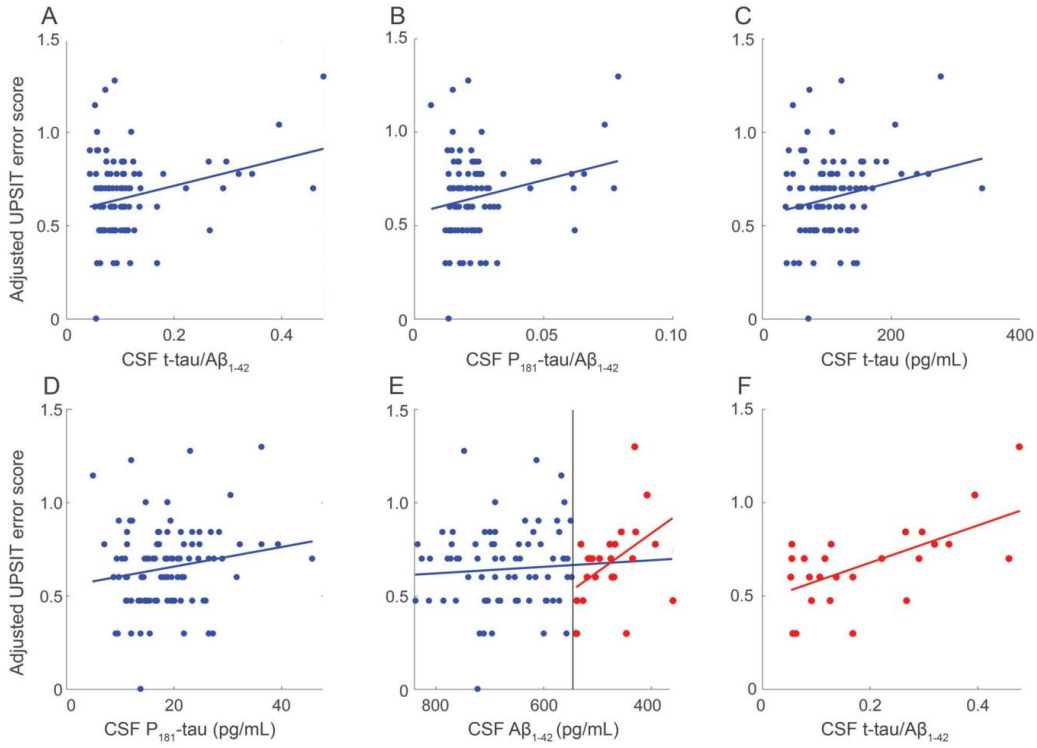


Figure 3-3 Robust-fit linear regression models of UPSIT error score vs CSF biomarkers of AD

(A) CSF t-*tau*/Aβ1-42 (β = 0.287, *p* = 0.00494, n = 100). (B) CSF P181-*tau*/Aβ1-42 (β = 1.77, *p* = 0.0165, n = 100). (C) CSF t-*tau* (β = 3.61 ×10^{-4}, *p* = 0.0257, n = 100). (D) CSF P181-*tau* (β = 2.10 ×10^{-3}, *p* = 0.0724, n = 100). (E) CSF Aβ1-42 (β = −7.01 ×10^{-5}, *p* = 0.359, n = 100) and in individuals with CSF Aβ1-42 levels below the 25th percentile (β = −0.000827, *p* = 0.0135, n = 25). (F) CSF t-*tau*/Aβ1-42 in individuals with CSF Aβ1-42 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; P181-*tau* = phospho-*tau*; t-*tau* = total *tau*; UPSIT = University of Pennsylvania Smell Identification Test.

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). 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 $A\beta_{1-42}$ (n=33, $\beta = 23.76 \times 10^{-4}$, p= 0.00841). In keeping with previously noted findings, UPSIT error score was associated in the ε4 carriers with higher t-*tau*/ $A\beta_{1-42}$ (β = 0.352, p= 0.0158) and P_{181}-*tau* / $A\beta_{1-42}$ (β = 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; Fig. 3-4). In addition, we saw that individuals with lowest CSF $A\beta_{1-42}$ levels appeared to have a higher proportion of APOE ε4 carriers.

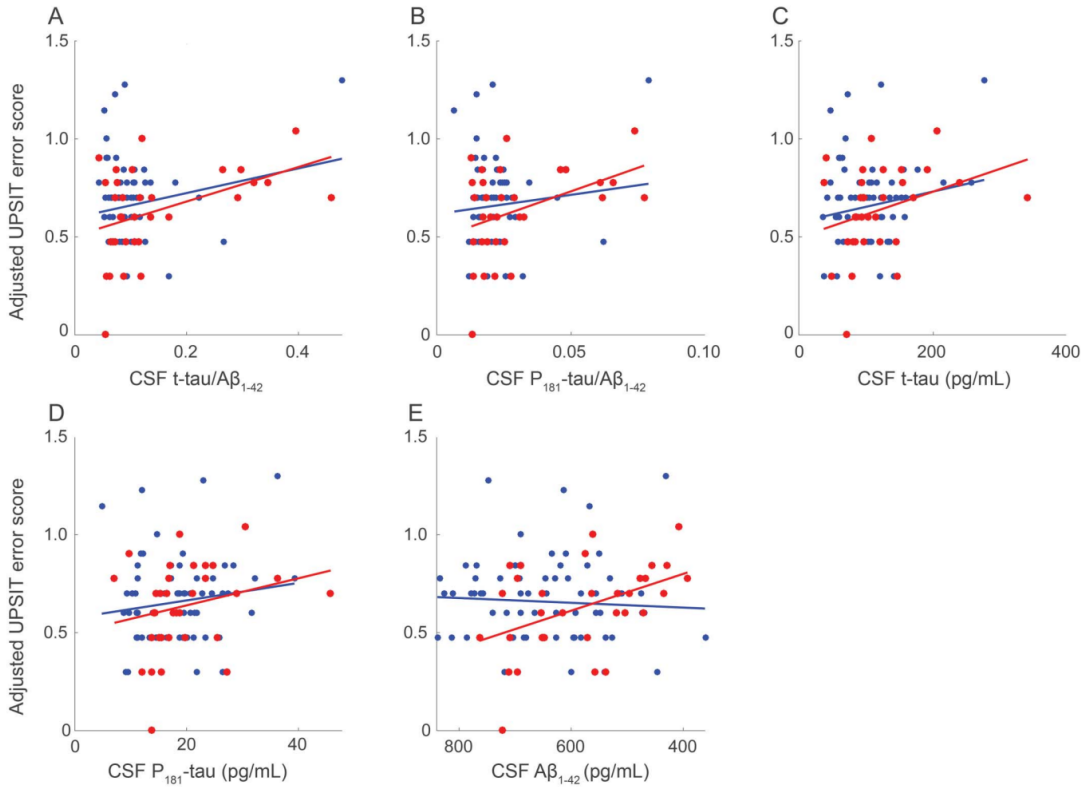


Figure 3-4 Robust-fit linear regression models of UPSIT error score vs CSF biomarkers, stratified by *APOE* ε4 status

Red circles represent *APOE* ε4 carriers. Blue circles represent *APOE* ε4 noncarriers. (A) CSF t-*tau*/Aβ1-42 in carriers (β = 0.352, *p*= 0.0158, n = 33) and in noncarriers (β = 0.252, *p* = 0.131, n = 67). (B) CSF P181-*tau*/Aβ1-42 in carriers (β = 2.416, *p* = 0.0270, n = 33) and in noncarriers (β = 0.980, *p* = 0.408, n = 67). (C) CSF t-*tau* in carriers (β = 4.68 $\times 10^{-4}$, *p* = 0.0914, n = 33) and in noncarriers (β = 0.000312, *p* = 0.144, n = 67). (D) CSF P181-*tau* in carriers (β = 2.76 $\times 10^{-3}$, *p* = 0.204, n = 33) and in noncarriers (β = 0.00178, *p* = 0.218, n = 67). (E) CSF Aβ1-42 in carriers (β = -3.76×10^{-4}, *p* = 0.00841, n = 33) and in noncarriers (β = 4.890e−05, *p* = 0.598, n = 67). Aβ = β-amyloid; AD = Alzheimer disease; P181-*tau* = phospho-*tau*; t-*tau* = total *tau*; UPSIT = University of Pennsylvania Smell Identification Test.

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 3-3). Table 3.4 shows comparable analyses in the reduced sample, now including CSF data. Models 1, 2, and 3 in table 3.4 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*/$A\beta_{1-42}$ ($p= 0.003$). 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*/$A\beta_{1-42}$. Model 6 indicates that any association of OI with cognition became unapparent after the inclusion of CSF t-*tau*/$A\beta_{1-42}$ (RBANS, $p= 0.151$; CSF t-*tau*/ $A\beta_{1-42}$, $p= 0.004$). 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*/$A\beta_{1-42}$ ($p= 0.005$) regardless of age, cognition, APOE ε4 status, sex, or education (Fig. 3-5). 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*/$A\beta_{1-42}$ as independent CSF biomarker predictors.

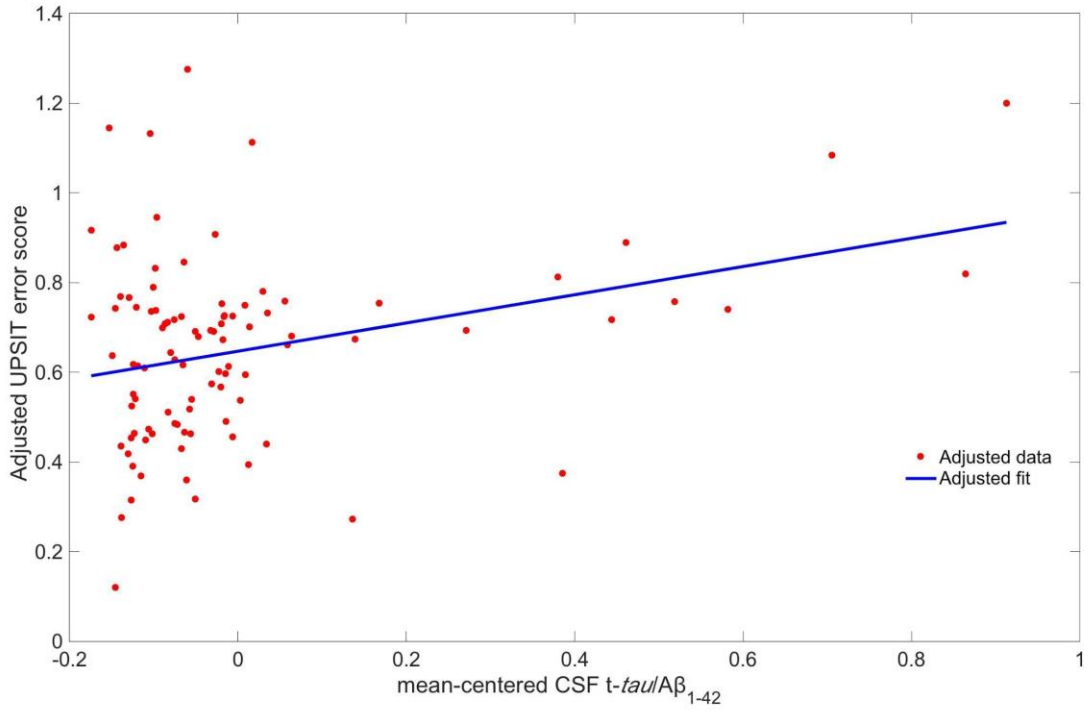


Figure 3-5. Model 7: UPSIT error score vs CSF total-*tau*/Aβ$_{1-42}$ adjusted for age, cognition, *APOE* ε4 status, sex, education

This is a graph of model 7 from Table 2. It shows an increase in the UPSIT error score with an increase in mean-centered CSF total-*tau*/β$_{1-42}$ after adjusting for age, RBANS total score, *APOE* ε4 status, sex, and education. Worse odor identification ability is correlated with higher levels of AD biomarkers.

RBANS=Repeatable Battery for the Assessment of Neuropsychological Status; UPSIT=University of Pennsylvania Smell Identification Test

	Estimated coefficients [s.e.]		
Predictors of OI	**Model 1**	**Model 2**	**Model 4**
n	262	258	258
Age in years	0.0119 [0.003] ***		0.0094 [0.003] **
RBANS		-0.0052 [0.002] ***	-0.0033 [0.002] *
t-*tau*/Aβ1-42			
Sex (Female=1)	-0.1162 [0.034] ***	-0.1037 [0.035] **	-0.1024 [0.035] **
Education in years	-0.0062 [0.004]	-0.0033 [0.005]	-0.0038 [0.005]
APOE ε4 (Carrier=1)	-0.0424 [0.032]	-0.0379 [0.032]	-0.0380 [0.032]

Table 3-3. **Estimated coefficients from step-wise multiple linear regression modeling to predict UPSIT error score**.

This table looks at combinations of age, RBANS, and CSF total-*tau*/$A\beta_{1\text{-}42}$ as predictors of odor identification. All Models are adjusted for *APOE* ε4 carrier status, sex, and education. We explored the multivariable corresponding to Model 1, 2, 4 in table 3-4 using the full data set. 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. We found that there are strong associations of greater UPSIT error score with older age (=0.0119, p=3.55 $x10^{-5}$) and lower RBANS (= -0.00515, p=9.41 $x10^{-3}$). Model 4 suggested that age and cognition were independent predictors of OI but the association of odor identification with cognition was weakened after adjustment for age (Age, =0.00941, p=0.002; RBANS =-0.00328, p=0.0397). The coefficients are labeled with asterisks according to the size of their p-value (*p<0.05, **p<0.01, ***p<0.001).

RBANS=Repeatable Battery for the Assessment of Neuropsychological Status; UPSIT=University of Pennsylvania Smell Identification Test

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/A$\beta_{1\text{-}42}$			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/A$\beta_{1\text{-}42}$ 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] $p < 0.01$.

Table 3-4 Estimated coefficients from stepwise multiple linear regression modeling to predict UPSIT error score

To evaluate the odor identification-CSF marker relationship in individuals likely to have more advanced pathology, we conducted sub-analyses among individuals with CSF A_{1-42} concentrations below the 25th percentile (864.621 pg/mL) (Fig. 3-3 E & F, red circles). These individuals showed correlations between UPSIT error score and CSF $A\beta_{1-42}$ levels (β=-8.27 x 10^{-4}; p=0.0135; n=25), t-*tau*/$A\beta_{1-42}$ (β= 0.399; p=0.00260), and P_{181}-*tau*/$A\beta_{1-42}$ (β= 0.301; p = 0.0109) (the latter not shown). As these CSF ratios are driven by elevated *tau* or decreasing amyloid levels, this analysis reproduces the relationships observed across the entire dataset for the ratio and odor identification in individuals with low CSF $A\beta_{1-42}$. We explored the relationship of the UPSIT error score and CSF *tau* above 335.1243 pg/mL, CSF P_{181}-*tau* above 55.3472 pg/mL, CSF t-*tau*/$A\beta_{1-42}$ above 0.2867, CSF P_{181}-*tau*/$A\beta_{1-42}$ above 0.0498 in individuals in these various CSF upper quartile levels. Similarly, among persons above the 75th percentile for CSF markers of neurodegeneration, UPSIT error score was related to CSF t-*tau* (β= 8.16 x 10^{-4}; p = 0.0151; n=25), t-*tau*/$A\beta_{1-42}$ (β= 0.313, p = 0.0358), and P_{181}-*tau*/$A\beta_{1-42}$ (β= 2.6386, p = 0.0314; n=25), but not P_{181}-*tau* (β= 1.76 x 10^{-3}; p = 0.587). Whereas individuals with CSF $A\beta_{1-42}$ levels below the 25th percentile included a high proportion of *APOE* ε4 carriers (48%) the proportion of 4 carriers was 28% for individuals in the upper three quartiles of CSF $A\beta_{1-42}$ concentration.

There was no evident relationship of odor identification and CSF $A\beta_{1-42}$ alone. However, after inspection of the curve, the data suggest the possibility of such a relationship among people with low CSF $A\beta_{1-42}$ level. Therefore, we explored interaction

models looking at individuals more likely to have advanced pathology. Exploratory analyses suggested a possible interaction among persons with CSF $A\beta_{1-42}$ concentrations below the 25th percentile (864.621 pg/mL) and a trend among persons with CSF t-*tau* levels above the 75th percentile (335.124 pg/mL) (Fig. 3-6 A & C; B & D).

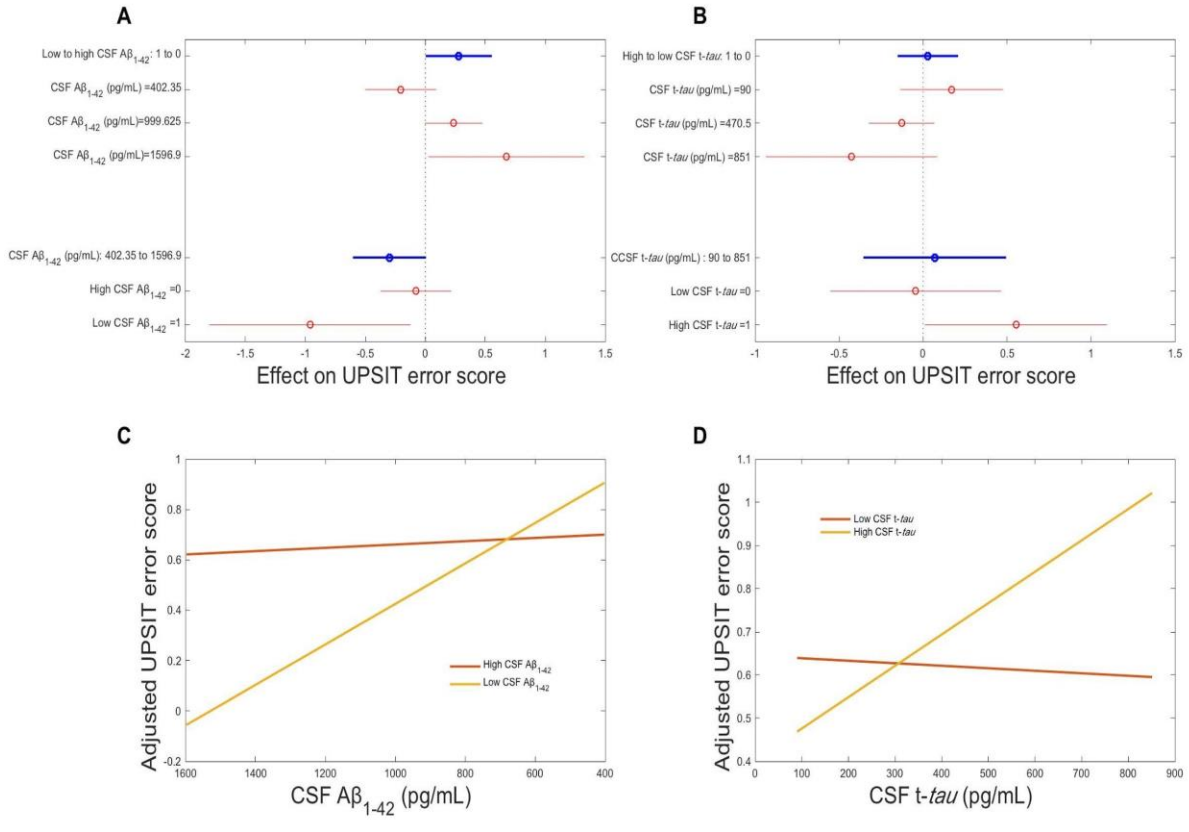


Figure 3-6. Advanced stage progression and CSF biomarker level interaction model for UPSIT error score

A & B) plot of interaction effect on UPSIT error score, with a horizontal bar showing the confidence interval for the estimated effect

A & B) blue circles indicate main effects and red circles indicate effect for set variables

C) response curve as a function of CSF $A\beta_{1-42}$ level, with *ad progression* fixed at 1 for closer to ad or 0 for individuals further away

D) response curve as a function of CSF t-*tau* level, with *ad progression* fixed at 1 for closer to ad or 0 for individuals further away

A & C) Interaction of CSF $A\beta_{1-42}$ level predicts UPSIT error score (model, F=1.89, n=100, df=96, R^2=0.0558, p=0.136; high low CSF $A\beta_{1-42}$, β =0.503, p=0.0859; CSF $A\beta_{1-}$

$_{42}$ level, β=-6.565e-05, p=0.599; high-low CSF $Aβ_{1-42}$ interaction with CSF $Aβ_{1-42}$ level, β=-0.000739, p=0.0516).

B & D) Interaction of CSF t-*tau* level predicts UPSIT error score (model, F=2.93, n=100, df=96, R^2=0.0839, p=0.0374; high low CSF t-*tau*, β=-0.241, p=0.214, CSF t-*tau* level, β =-5.80e-05, p=0.864; high-low CSF t-*tau* interaction with CSF t-*tau* level, β=0.000784, p=0.114).

Ultimately, we clarified the differences between the ε4 carriers and non-carriers by exploring the interaction between *APOE* ε4 carrier status and amyloid levels to predict odor identification. There was a statistical interaction of *APOE* ε4 carrier state and CSF $A\beta_{1-42}$ levels that predicted the UPSIT error score (=-4.28 $x10^{-4}$, p=0.0104, n=100) (Figure 3-7 A & C). This suggests that odor identification was substantially worse in those with lower CSF $A\beta_{1-42}$ concentrations and an *APOE* ε4 allele. There was no interaction for any other CSF marker. We attempted but failed to reproduce the ε4 carrier and CSF $A\beta_{1-42}$ interaction effect with RBANS total index score (Figure 3-7 B & D). This could be because odor identification deficit precedes cognitive loss.

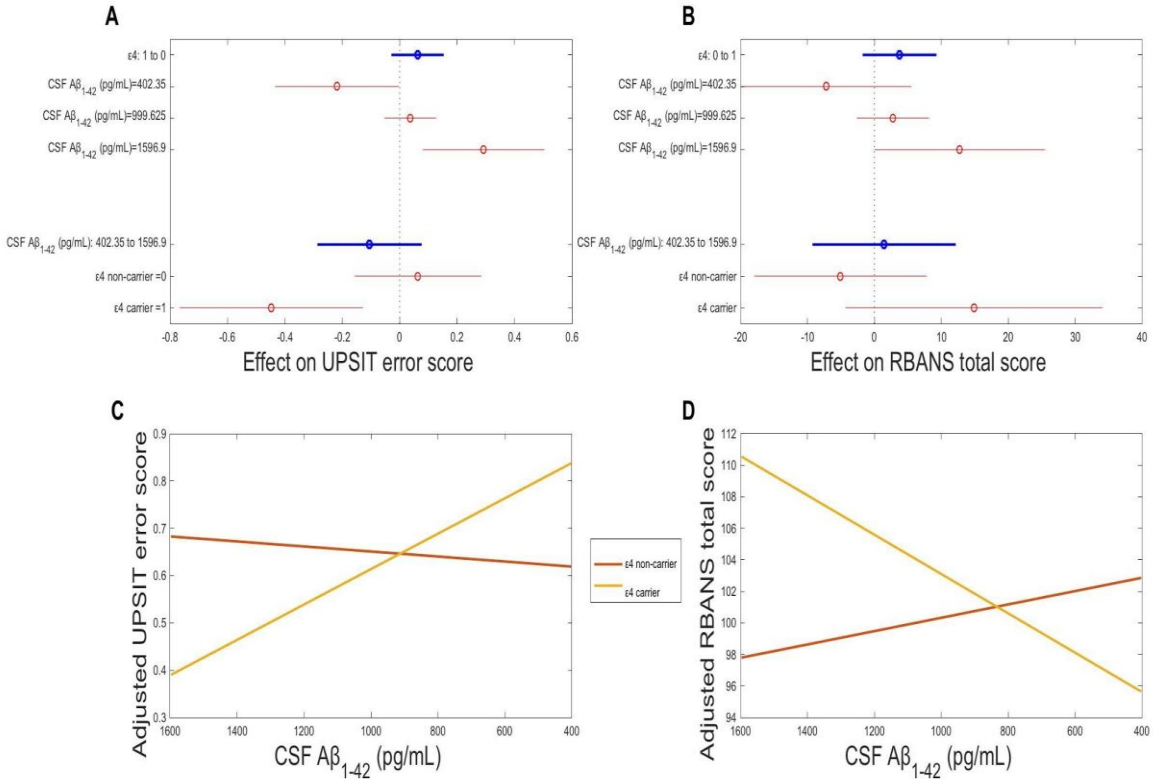


Figure 3-7. *APOE* 4 carrier status and CSF Aβ1-42 level interaction model for UPSIT error score and RBANS prediction

A) plot of interaction effect on UPSIT error score, with a horizontal bar showing the confidence interval for the estimated effect

B) plot of interaction effect on RBANS total index score, with a horizontal bar showing the confidence interval for the estimated effect

A & B) blue circles indicate main effects and red circles indicate effect for set variables

C) response curve as a function of CSF Aβ1-42 level, with *APOE* ε4 carrier fixed at 1 for carriers or 0 for non-carriers

D) response curve as a function of CSF Aβ1-42 level, with *APOE* ε4 carrier fixed at 1 for carriers or 0 for non-carriers

A & C) Interaction of CSF Aβ1-42 level and *APOE* ε4 carrier status predicts UPSIT error score (model, F=2.88, n=100, df=96, R^2=0.205, p=0.04; ε4 carrier status, β =-0.0641, p=0.167; CSF Aβ1-42 level, β =-5.314e-05, p=0.570; ε4 carrier status interaction with CSF Aβ1-42 level, β =-0.000428, p=0.0104).

B & D) Interaction of CSF $A\beta_{1-42}$ level and *APOE* ε4 carrier status predicts RBANS total index score (model, F=1.34, n=98, df=94, R^2=0.0411, p=0.266; ε4 carrier status, β =3.8284, p=0.17276; CSF $A\beta_{1-42}$ level, β =-0.00423, p=0.438; ε4 carrier status interaction with CSF $A\beta_{1-42}$ level, β =0.0167,p=0.0900).

3.8 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-H2O-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.

3.9 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.

3.10 Acknowledgement

The authors thank David Fontaine, PhD, who oversaw cognitive testing at enrollment, scored the RBANS cognitive evaluations, and administered additional cognitive batteries when indicated. Anne Labonté, BSc, conducted the CSF biomarker analyses. Cécile Madjar, MSc, directed data management, and Doris Dea and Louise Théroux did the genotyping. Cynthia Picard, PhD, helped write the descriptions of laboratory methods. Tanya Lee, Melissa Appleby, Laura Mahar, Galina Pogossova, Renuka Giles, and Karen Wan collected cognitive and olfaction data. All are with the Centre for Studies on Prevention of AD, Douglas Mental Health University Institute. Thomas Beaudry, BSc (McGill Centre for Studies in Aging, Douglas Mental Health University Institute; Perform Centre, Concordia University), helped edit the manuscript. Tharick Ali Pascoal, MD, Marina Tedeshi Dauar, MD, and Laksanun Cheewakriengkrai, MD (McGill Centre for Studies in Aging and Centre for Studies on Prevention of AD, Douglas Mental Health University Institute), collected the CSF. Katarina Dedovic, PhD (Canadian Institutes for Health Research, Ottawa), provided valuable advice, as did Miranda

Tuwaig, MSc, Angela Tam, MSc, Christina Kazazian, Pierre-Francois Meyer, MSc, and Melissa Savard, MSc (all at the Centre for Studies on Prevention of AD, Douglas Mental Health University Institute). Special thanks go to Marianne Dufour, administrative assistant, and to Ginette Mayrand, Joanne Frenette, MSc, Isabelle Vallée, Rana El-Khoury, and Fabiola Ferdinand, all nurses who met with participants, as well as the entire PREVENT-AD Research Group (https://preventad.loris.ca/acknowledgements/acknowledgements.php?date5201 7-1-26). The authors acknowledge the generosity and commitment of all research participant volunteers for this work.

3.11 Study funding

This study was funded by an unrestricted gift from Pfizer Canada, by generous support from McGill University, and by infrastructure support from the Canada Fund for Innovation. Support for genetic and laboratory work was provided by the Fonds de Récherche du Québec–Santé and by the Levesque Foundation. The Douglas Mental Health University Institute Foundation provided additional support.

3.12 Disclosure

M.-E. Lafaille-Magnan has received research support from the Lazlo & Etelka Kollar fellowship fund. M.-E. Lafaille-Magnan is the Brain Reach High School program coordinator, for which she has received an award from the McGill Integrated Program in Neuroscience. M.-E. Lafaille-Magnan receives honoraria from serving as a board member of the clinical excellence committee on social services with a focus on youth, mental health, and dependence for the Institut National d’excellence en santé et en services sociaux.

J. Poirier receives royalties from a publication he has cowritten titled Alzheimer's Disease: A Guide, published in 2011 in French, English, German, Portuguese, and Chinese.

P. Etienne, J. Tremblay-Mercier, and J. Frenette report no disclosures relevant to the manuscript.

P. Rosa-Neto receives a salary award from le Fonds de recherché du Québec–Santé (Chercheurs-boursiers–Junior 2). He receives research support from the Canadian Institutes of Health Research, Canadian Consortium on Neurodegeneration in Aging, Weston Brain Institute, and Enigma radiopharmaceuticals. Dr. Rosa-Neto is a principal investigator in industry-sponsored clinical trials for TauRx, Jensen, and Eli Lilly. He received honoraria from serving on Eli Lilly's scientific advisory board. He serves as a consultant for Kalgene and Enigma radiopharmaceuticals. He does not hold stock in or receive royalties from any pharma or biotech company.

J. Breitner has received research support from Pfizer Canada. He also received research support from McGill University, the Douglas Hospital Foundation, le Fonds de recherché du Québec–Santé, Canadian Consortium on Neurodegeneration in Aging, Weston Brain Institute, and the government of Canada (Canadian Institutes for Health Research, Tier 1 Canada Research Chair, and the Canadian Fund for Innovation). Go to Neurology.org for full disclosures.

Received January 26, 2017. Accepted in final form April 28, 2017.

3.13 References

1. Breitner JCS, Poirier J, Etienne PE, Leoutsakos JM; PREVENT-AD Research Group. Rationale and structure for a new center for Studies on Prevention of Alzheimer's Disease (StoP-AD). J Prev Alzheimers Dis 2016;3:236– 242.

2. Daulatzai MA. Olfactory dysfunction: its early temporal relationship and neural correlates in the pathogenesis of Alzheimer's disease. J Neural Transm (Vienna) 2015; 122: 1475–1497.

3. Tabert MH, Liu X, Doty RL, et al. A 10-item smell identification scale related to risk for Alzheimer's disease. Ann Neurol 2005;58:155–160.

4. Djordjevic J, Jones-Gotman M, De Sousa K, Chertkow H. Olfaction in patients with mild cognitive impairment and Alzheimer's disease. Neurobiol Aging 2008;29:693–706.

5. Wilson RS, Arnold SE, Schneider JA, Boyle PA, Buchman AS, Bennett DA. Olfactory impairment in presymptomatic Alzheimer's disease. Ann NY Acad Sci 2009;1170: 730–735.

6. Growdon ME, Schultz AP, Dagley AS, et al. Odor identification and Alzheimer disease biomarkers in clinically normal elderly. Neurology 2015;84:2153–2160.

7. Velayudhan L, Pritchard M, Powell JF, Proitsi P, Lovestone S. Smell identification function as a severity and progression marker in Alzheimer's disease. Int Psychogeriatr 2013;25: 1157–1166.

8. Yaffe K, Freimer D, Chen H, et al. Olfaction and risk of dementia in a biracial cohort of older adults. Neurology 2017;88:456–462.

9. Roberts RO, Christianson TJH, Kremers WK, et al. Association between olfactory dysfunction and amnestic mild cognitive impairment and Alzheimer disease dementia. JAMA Neurol 2016;73:93–101.

10. Devanand DP, Lee S, Manly J, et al. Olfactory deficits predict cognitive decline and Alzheimer dementia in an urban community. Neurology 2015;84:182–189.

11. Ship J, Pearson J, Cruise L, Brant L, Metter EJ. Longitudinal changes in smell

identification. J Gerontol A Biol Med Sci 1996;51:86–91.

12. Wehling EI, Lundervold AJ, Nordin S, Wollschlaeger D. Longitudinal changes in familiarity, free and cued odor identification, and edibility judgments for odors in aging individuals. Chem Senses 2016;41:155–161.

13. Sun GH, Raji CA, MacEachern MP, Burke JF. Olfactory identification testing as a predictor of the development of Alzheimer's dementia: a systematic review. Laryngoscope 2012;122:1455–1462.

14. Rahayel S, Frasnelli J, Joubert S. The effect of Alzheimer's disease and Parkinson's disease on olfaction: a meta-analysis. Behav Brain Res 2012;231:60–74.

15. Wilson RS, Arnold SE, Schneider JA, Tang Y, Bennett DA. The relationship between cerebral Alzheimer's disease pathology and odour identification in old age. J Neurol Neurosurg Psychiatry 2007;78:30–35.

16. Devanand DP, Liu X, Tabert MH, et al. Combining early markers strongly predicts conversion from mild cognitive impairment to Alzheimer's disease. Biol Psychiatry 2008; 64:871–879.

17. Stanciu I, Larsson M, Nordin S, Adolfsson R, Nilsson LG, Olofsson JK. Olfactory impairment and subjective olfactory complaints independently predict conversion to dementia: a longitudinal, population-based study. J Int Neuropsychol Soc 2014;20:209–217.

18. Lampert EJ, Roy Choudhury K, Hostage CA, Petrella JR, Doraiswamy PM. Prevalence of Alzheimer's pathologic endophenotypes in asymptomatic and mildly impaired first-degree relatives. PLoS One 2013;8:e60747.

19. Nasreddine Z, Phillips N, Bédirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. J Am Geriatr Soc 2005;53: 695–699.

20. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL. A new clinical state for the staging of dementia. Br J Psychiatry 1982;140:566–572.

21. Randolph C, Tierney MC, Mohr E, Chase TN. The Repeatable Battery for the

Assessment of Neuropsychological Status (RBANS): preliminary clinical validity. J Clin Exp Neuropsychol 1998;20:310–319.

22. Lelental N, Brandner S, Kofanova O, et al. Comparison of different matrices as potential quality control samples for neurochemical dementia diagnostics. J Alzheimers Dis 2016;52:51–64.

23. Doty RL, Shaman P, Dann M. Development of the University of Pennsylvania Smell Identification Test: a standardized microencapsulated test of olfactory function. Physiol Behav 1984;32:489–502.

24. Doty RL, Newhouse MG. Azzalina JD. Internal consistency and short-term test-retest reliability of the University of Pennsylvania Smell Identification Test. Chem Senses 1985;10:297–300.

25. Doty RL, Frye RE, Agrawal U. Internal consistency reliability of the fractionated and whole University of Pennsylvania Smell Identification Test. Percept Psychophys 1989;45:381–384.

26. Moberg PJ, Doty RL, Turetsky BI, et al. Olfactory identification in elderly schizophrenia and Alzheimer's disease. Neurobiol Aging 1997;18:163–167.

27. Peng KY, Mathews PM, Levy E, Wilson DA. Apolipoprotein E4 causes early olfactory network abnormalities and short-term olfactory memory impairments. Neuroscience 2017;343:364–371.

28. Hagemeier J, Woodward MR, Rafique UA, et al. Odor identification deficit in mild cognitive impairment and Alzheimer's disease is associated with hippocampal and deep gray matter atrophy. Psychiatry Res 2016;255:87–93.

29. Bahar-Fuchs A, Chételat G, Villemagne VL, et al. Olfactory deficits and amyloid-b burden in Alzheimer's disease, mild cognitive impairment, and healthy aging: a PiB PET Study. J Alzheimers Dis 2010;22:1081–1087.

30. Kareken DA, Mosnik D, Doty RL, et al. Olfactory-evoked regional cerebral blood flow in Alzheimer's disease. Neuropsychology 2001;15:18–29.

31. Leon De MJ, Li Y, Okamura N, et al. CSF clearance in Alzheimer disease measured

with dynamic PET. J Nucl Med Epub 2017 Mar 16.

32. Albers AD, Asafu-Adjei J, Delaney MK, et al. Episodic memory of odors stratifies Alzheimer biomarkers in normal elderly. Ann Neurol 2016;80:846–857.

33. Xydakis MS, Mulligan LP, Smith AB, Olsen CH, Lyon DM, Belluscio L. Olfactory impairment and traumatic brain injury in blast-injured combat troops: a cohort study. Neurology 2015;84:1559–1567.

34. Van de Bittner GC, Riley MM, Cao L, et al. Nasal neuron PET imaging quantifies neuron generation and degeneration. J Clin Invest 2017;127:681–694.

35. Doty RL. The olfactory system and its disorders. Semin Neurol 2009;29:74–81.

36. Doty RL, Kamath V. The influences of age on olfaction: a review. Front Psychol 2014;5:1–20.

Chapter 4

"It is wiser to find out than suppose."
- *More Maxims of Mark*, Johnson, 1927

4.1 Chapter 4 preamble

Having demonstrated a cross-sectional association between odor identification (OI) and early Alzheimer pathology (reflected by abnormal CSF amyloid and *tau* concentrations), we sought to measure whether this association is apparent also in longitudinal data. Because our earlier study (Chapter 3) could not discern whether a deficit in OI would predict subsequent volumetric or CSF biomarker change, or further cognitive decline (general or domain-specific), we address these topics here.

Earlier studies had demonstrated that OI deficit could predict the progress of AD pathology as well as onset or progression of MCI (reviewed in Chapter 1). We reproduce previous findings and expand on our previous work by exploring domain-specific associations. We now test the hypothesis that OI itself declines as a function of age, time, and accumulation of AD neuropathology. Beyond this, we investigate the hypothesis that baseline OI can serve as a surrogate marker and predict subsequent cognitive decline or accumulation of AD pathology, as indicated by the above metrics. The data for these investigations come from a two-year randomized controlled trial with a highly structured and vigorously monitored protocol for data collection over four serial time points.

4.2 Title: Longitudinal measures of odor identification as an index of preclinical AD progression

Marie-Elyse Lafaille-Magnan, BSc, MSc, Pedro Rosa-Neto, MD, PhD, Jeannie-Marie Leoutsakos, PhD, Mélissa Savard, MSc, Anne Labonté, BSc, Vladimir S. Fonov, PhD, Louis Collins, Judes Poirier, PhD, CQ, John C.S. Breitner, MD, MPH, For the PREVENT-AD Research Group

From the Centre for Studies on Prevention of AD (M.-E.L.-M., M.S., A.L., J.P., P.R.-N., J.C.S.B.), the McGill Centre for Studies in Aging (P.R.-N., M.S.), the Montreal Neurological Institute (V.F., L.C.) McGill University, Faculty of Medicine, Montreal, Quebec, Canada, John Hopkins University, Bloomberg School of Public Health, Baltimore, USA (J.-M.L).

4.3 Abstract

Objective:

To assess longitudinal associations of odor identification (OI) with pre-symptomatic Alzheimer's disease (AD).

Methods: In 160 members of the PREVENT-AD cohort of healthy aging persons with a parental or multiple-sibling history of AD dementia, we tested odor identification (OI) using the University of Pennsylvania Smell Identification Test (UPSIT) annually. Also assessed were the cerebrospinal fluid (CSF) biomarkers of AD t-*tau*/A$\beta_{1\text{-}42}$ and P_{181}-*tau*/A$\beta_{1\text{-}42}$, hippocampal volume, and global and domain-specific measures of cognitive deficit on the Repeatable Battery for Assessment of Neuropsychological Status (RBANS). We tested: 1) whether our prior observations of association between OI, CSF biomarkers, and cognitive performance were reproducible; 2) whether OI performance declined over a two-year interval; 3) whether change-over-time was predicted by changes in CSF AD biomarkers, hippocampal volume or global or domain-specific cognitive performance; 4) whether baseline AD biomarker measures predicted OI decline; 5) whether baseline OI predicted trajectory of the AD biomarkers. Multivariable linear regression included, when appropriate, linear mixed effects models controlling for repeated measures. Covariates included age at baseline, *APOE* ε4 status, education, and sex.

Results: Lower repeated measures of OI were associated with higher corresponding levels of CSF t-*tau*/A$\beta_{1\text{-}42}$ (β=-3.03, CI: -5.43 — -0.61, P<0.01; number of observations = 318). OI declined significantly over 2 years. Age, but not *APOE* ε4 carrier status, education or sex, predicted this decline. Higher baseline CSF t-*tau*/A$\beta_{1\text{-}42}$ and P_{181}-*tau*/A$\beta_{1\text{-}42}$ predicted lower OI throughout the two-year interval (β=-3.51, CI: -6.93 – -0.08, P=0.04; β=-21.68, CI: -46.19 – +2.82, P=0.08, n for both = 350). Similarly, lower baseline OI predicted higher CSF t-*tau*/A$\beta_{1\text{-}42}$ and P_{181}-*tau*/A$\beta_{1\text{-}42}$ at all timepoints (β=-0.016, CI: -0.029 – -0.004; P=0.009; β=-0.002, CI: -0.004 – -0.0004, P=0.02, n = 325).

Notably, CSF biomarkers did not decline significantly over the interval, but lower repeated measures of OI were associated with lower corresponding immediate memory scores (β=0.02, CI: 0.004 – 0.040, P<0.02, but adjustment for multiple cognitive domain predictors yielded P_{FDR}=0.1, n=596). Lower baseline immediate memory was associated with lower OI at any timepoint (β=0.06, SE=0.02, CI: 0.02 – 0.11, P=0.01, P_{FDR} =0.05 n=587). Baseline global cognition and immediate memory portended declines in OI at trend level (β=0.05, SE=0.03, CI: -0.02 – 0.02, P=0.06; β=-0.01, SE=0.01; CI: -0.03 – 0.0004, P=0.06, n=587). Finally, there was no association between OI and hippocampal volumes.

Conclusions: Lower OI appears to reflect evolving Alzheimer disease, as revealed by AD biomarkers and declining cognitive performance. OI may be a practical measure for prevention trials and other studies of preclinical AD.

4.4 Introduction

Alzheimer disease (AD) neuropathology begins decades before onset of symptoms.[1] Hence, the pre-symptomatic stage of AD offers an opportunity for interventions that may alter the trajectory of disease. To assess the effects of such intervention, one needs measures of brain function or anatomy. Because rhinencephalic areas are among the first sites to show AD-related brain change, we chose to study olfactory function as an indicator of early AD progression.[2,3]

In older individuals with and without dementia, reduced odor identification (OI) can predict autopsy findings of increased AD plaques and neurofibrillary tangles in the olfactory bulb, entorhinal cortex, and CA1 regions of the hippocampus.[4] Later observations in 34 cognitively normal individuals demonstrated that lower OI was associated with a worse composite measure of AD neuropathology in general.[5] Similarly, others have shown that, regardless of clinical diagnosis, lower OI performance is associated with reduced hippocampal volume.[6-10]

We recently reported that reduced OI was associated in cross-section with increased cerebro-spinal fluid (CSF) t-*tau*/Aβ_{1-42} in 100 cognitively normal adults at increased risk for AD.[11] These findings persisted after sensitivity analyses accounted for several potential confounders.[11] Subsequently, Reijs and colleagues reported a similar association between OI and CSF t-*tau* in 160 individuals (40 normals, 45 with MCI, 42 with AD-dementia, 26 non-AD-dementia).[12]

Because several investigators have found that baseline deficit in OI precedes cognitive impairment,[5,13-19] we examined longitudinal OI performance as an indicator of evolving AD neuropathology. If odor identification does indeed indicate an early AD process, we reasoned that declining OI would parallel decline in cognition and in hippocampal volume, and a corresponding increase in CSF t-*tau*/Aβ_{1-42}, or P_{181}-*tau*/Aβ_{1-42}. We tested this idea a subset of individuals from the PREVENT-AD cohort who had repeated observations, including serial donations of CSF for an AD prevention trial.[20]

4.5 Methods

4.5.1 Participants

We studied members of the PREVENT-AD Cohort of cognitively and physically healthy volunteers with a parental or multiple-sibling family history of AD.[20] Nested within PREVENT-AD was a two-year pharmaco-prevention trial (INTREPAD, NCT02702817). We analyzed data collected between September 2011 and June 2017 (PREVENT-AD data release 4.0, published 30 June, 2017). The trial's modified intent-to-treat (mITT) analysis pool included 160 participants who had remained on-protocol for at least 90 days (i.e., had at least one follow-up assessment) and were examined thereafter at 12 and 24 months following randomization. Of these, 93 individuals agreed to undergo serial lumbar punctures (LPs) for CSF biomarker studies. The McGill University Faculty of Medicine Institutional Review Board approved all protocols, and participants and their collateral study partners provided written informed consent. All research complied with ethical principles of the Declaration of Helsinki.

4.5.2 Methods of assessment

Detailed inclusion and exclusion criteria as well as data collection procedures for the trial have been described.[20] Screening procedures included brief magnetic resonance (MR) imaging to exclude obvious structural brain disease. Absence of a diagnosable cognitive syndrome was established by screening with the Montreal Cognitive Assessment and the brief cognitive questionnaire from the Clinical Dementia Rating scale.[21,22] Individuals with questionable cognitive difficulties underwent a formal neuropsychological assessment. In addition to items described below, baseline, 3-, 12-, and 24-month data collection procedures included a medical review of systems, structured neurological evaluation, and phlebotomy for routine laboratory studies.

4.5.3 Cerebrospinalspinal fluid collection and measurements

LPs were performed following an overnight fast using the Sprotte 24-gauge atraumatic needle. Completion rates for the LPs were high: 72 volunteers (75%) underwent all four procedures, and 79 (85%) completed at least three. We used standard procedures of the BIOMARK-APD consortium to measure CSF concentrations of the AD biomarkers total-*tau* (t-*tau*) and P_{181}-*tau* (P-*tau*), amyloid-beta$_{1-42}$ ($A\beta_{1-42}$) using the Innotest "ALZbio3" ELISA kit (Fujirebio, Ghent, Belgium).[23] To evaluate pre-symptomatic AD progression, we measured CSF t-*tau,* P_{181}-*tau,* $A\beta_{1-42,}$ and the ratios of t-*tau* and P_{181}-*tau* with the latter.[24]

4.5.4 *APOE* genotyping

The *APOE* genotype was determined using the PyroMark Q96 pyrosequencer (Qiagen, Toronto, ON, Canada), as previously described.[11]

4.5.5 Odor Identification

Smell identification abilities were tested using the University of Pennsylvania Smell Identification Test (UPSIT) in a well-ventilated room. This test uses a "scratch-and-sniff" stimulus of 40 items (4 randomized booklets of 10 odorants each). The UPSIT score then represents the number of correct choices, so that higher scores indicate better performance. The trained technician who administered the test did not allow participants to skip items. Score analyses excluded data from days when participants complained of nasal congestion. For francophone participants, we used an in-house translation of odor labels.[11] For the nine instances in which test results included one or two missing responses, we extrapolated scores proportionally to values predicted from 40 items.

4.5.6 Cognition

We evaluated cognitive performance using French and English versions of the Repeatable Battery for Assessment of Neuropsychologic Status (RBANS).[25] This RBANS draws on 12 tests to yield 5 cognitive domain scores (Table 4-1).[25] A global cognition score then summarizes all these domain scores. Because we wished to assess cognitive performance irrespective of age, we interpreted all test results using the single set of scoring norms, for persons aged 60-69.

Cognitive domain scores	**Component Tests**
Immediate Memory score	List Learning Story Memory
Visuospatial/Constructional score	Figure Copy Line Orientation
Language score	Picture Naming Semantic Fluency
Attention score	Digit Span Coding
Delayed Memory score	List Recall List Recognition Story Recall Figure Recall

Table 4-1 RBANS Cognitive domain scores and tests

RBANS = Repeatable Battery for Assessment of Neuropsychologic Status

Equivalency of the four alternate forms for the English language RBANS has been verified by the battery's author, whose recommended procedure is to add four points to the raw score of the semantic fluency test in form C only. Because participants were selected for their cognitive health and physical fitness, we assumed no or little change after 3 months. To insure equivalency for the French language version, adjustment factors to derive equivalent scores for all four forms (using A as a template) were drawn from linear mixed-effects models with random intercepts, two time points and dummy variables for alternate forms. We used 133 form A administered at baseline and the three alternate forms (60 for form B, 44 for form C, and 29 for form D) administered three months later. This equivalency procedure is similar to mean

equating (equating B to A, then C to A, and then D to A). Owing to our small sample size, we tested the robustness of our coefficients using a bootstrapping approach.

The models were run 5000 times, while resampling from the sample data on each iteration. This was done at the individual level including the baseline and 3-month visit for each individual in the model. The mean estimates resulting from these models were used as adjustment factors for the French RBANS forms. This equating was applied to the 5 cognitive domains' scores. The total score was then calculated using the look up tables with the 5 adjusted domain scores.

We then ran similar models on the adjusted data to verify the removal of a form effect. In addition, we verified form equivalence through linear equating.

4.5.7 Magnetic Resonance Imaging (MRI)

Participants were scanned on a Siemens TIM Trio 3 Tesla MRI system (Siemens Healthcare, Erlangen, Germany) using the Siemens standard 12-channel head coil at the Cerebral Imaging Centre of the Douglas Mental Health University Institute. Scanning sequences included T1-weighted (TE/TR = 2.3 / 2.98 ms) images with 1x1x1mm resolution. Scans were preprocessed using the following steps to obtain the volume ratio of total hippocampi to intracranial cavity, thusly:

1. All scans were corrected for geometric distortions using information from the closest available lego phantom scan.[26]
2. Data were processed using a longitudinal pipeline,[27] with the following options (intracranial cavity volume):
    a. Distortion corrected scans were de-noised using non-local patch based de-noising with parameters: beta=0.7, block=1, b_space=2, neighborhood=1, search=5, m_min=0.95, v_min=0.5.[28] The level of noise was estimated as per Coupe, 2009.[29]
    b. Non-uniformity correction using N3 with parameters optimized for 3T scans.

   c. Tissue classification and lobe segmentation was done using “hybrid method”.
3. Spatial registration was done using ANIMAL (Automatic Nonlinear Image Matching and Anatomical Labeling) technique and deep structures atlas defined in MNI-ICBM152 2009c template space.[30-33]
4. Additional processing was performed to calculate the right and left hippocampal volumes. When multiple timepoints were available for a given subject, non-linear registration and patch-based multi-atlas label fusion,[34,35] were applied to the subject-specific non-linear average template. The obtained volumes were then non-linearly warped to each time point. The detailed algorithm to create the template is described in Aubert-Broche, 2013.[27] This process creates an average volume and non-linear transformations mapping it to each of the timepoints.
   a. Hippocampus and amygdala segmentation library based on 80 manual segmentations of ICBM subjects was used.[36]
5. Left and right hippocampal volumes were summed and divided by the intracranial cavity volume, a volume that includes whole brain volume and ventricular space.

4.5.8 Statistical Analyses

We inspected spaghetti plots of repeated measures. No markers appeared to have non-linear effects (Fig. 4-1). 1) We attempted to reproduce our previous associations of OI - CSF and OI – global cognition in repeated-measures using linear mixed effects models to control for lack of independence. To expand on our previous work, we investigated the total hippocampal volume adjusted for intracranial space and we did a *post-hoc* analysis of RBANS domain-specific cognitive performance. 2) We assessed whether OI declined over time by performing a linear mixed effects analysis of the relationship between olfactory identification and time. We reproduced previous OI – age associations, as well as, other risk and protective factors for AD 3) Using change scores (data at 24 months – data at baseline), we employed a robust fit linear regression method to assess if changes in markers of AD progression explained changes in OI at 24 months. 4) We evaluated whether baseline markers of AD predicted repeated measures of OI or its decline. 5) Finally, in an attempt to use OI as a predictor, we

evaluated whether baseline OI predicted trajectory in AD markers. This was a similar logistic regression model looking at OI as a predictor and conversion as the outcome.

Unless stated otherwise, we tested these predictions with mixed-effect linear regression. When doing linear mixed effects modeling, we used the fitlme function with restricted maximum likelihood as implemented in Matlab (Matlab (R2016a); Mathworks inc.; Natick, Massachusetts). The time intervals were expressed in years with a maximum of 4 time points (Year 0 = baseline visit, Year 0.25 = 3 month visit, Year 1 = 12 month visit, Year 2 = 24 month visit). Models evaluating repeated measures considered intercepts as random effects and included time in the model. We adjusted for age at baseline, sex, educational attainment, and *APOE* ε4 carrier status as traditional covariates for studies on aging, cognition and Alzheimer's disease. Also included were treatment assignment and the interaction between time and treatment assignment as these were nuisance variables. In order to have an intercept that was within the range of the raw data, age at baseline, education, and predictors were mean-centered to baseline values.

For our first aim, we used time-varying measures of OI. Therefore, no term for time-by-OI interaction was necessary. These models evaluated whether OI and markers of progression were associated even across time. OI measures were mean-centered to baseline values, and their coefficients indicate annual change during the trial follow-up period.

For models investigating change at 24 months, we included baseline values of the dependent variable, and these were mean-centered.

In a separate set of models, to test whether baseline AD progression markers showed an effect on OI decline, each model included an interaction between the baseline marker values and time. In a final set of models, we repeated this method but explored the contra-positive condition; this approach assessed whether OI predicted cognitive decline and AD-related pathological severity measures. We conducted *post-hoc* analyses looking at domain-specific cognitive performance. We controlled for

multiple hypothesis testing using the Benjamini and Hochberg False Discover Rate (FDR) method, and generated corrected P-values labeled in this text as P_{FDR}.[37] This method controls for false positive observations, but inevitably increases the chance of missing a true association.

Plotting a histogram of the residuals assessed our assumptions. In the case of a non-normal distribution, we log-transformed the dependent variable. This did not change the results; therefore, we used the raw data. Potential confounding by drug-(naproxen)-related adverse events was examined in separate models. To do this we included longitudinal hemoglobin and hematocrit levels in blood, which were altered as a result of drug exposure in a safety analysis (Breitner, in preparation). These were mean-centered, and there was no observed effect.

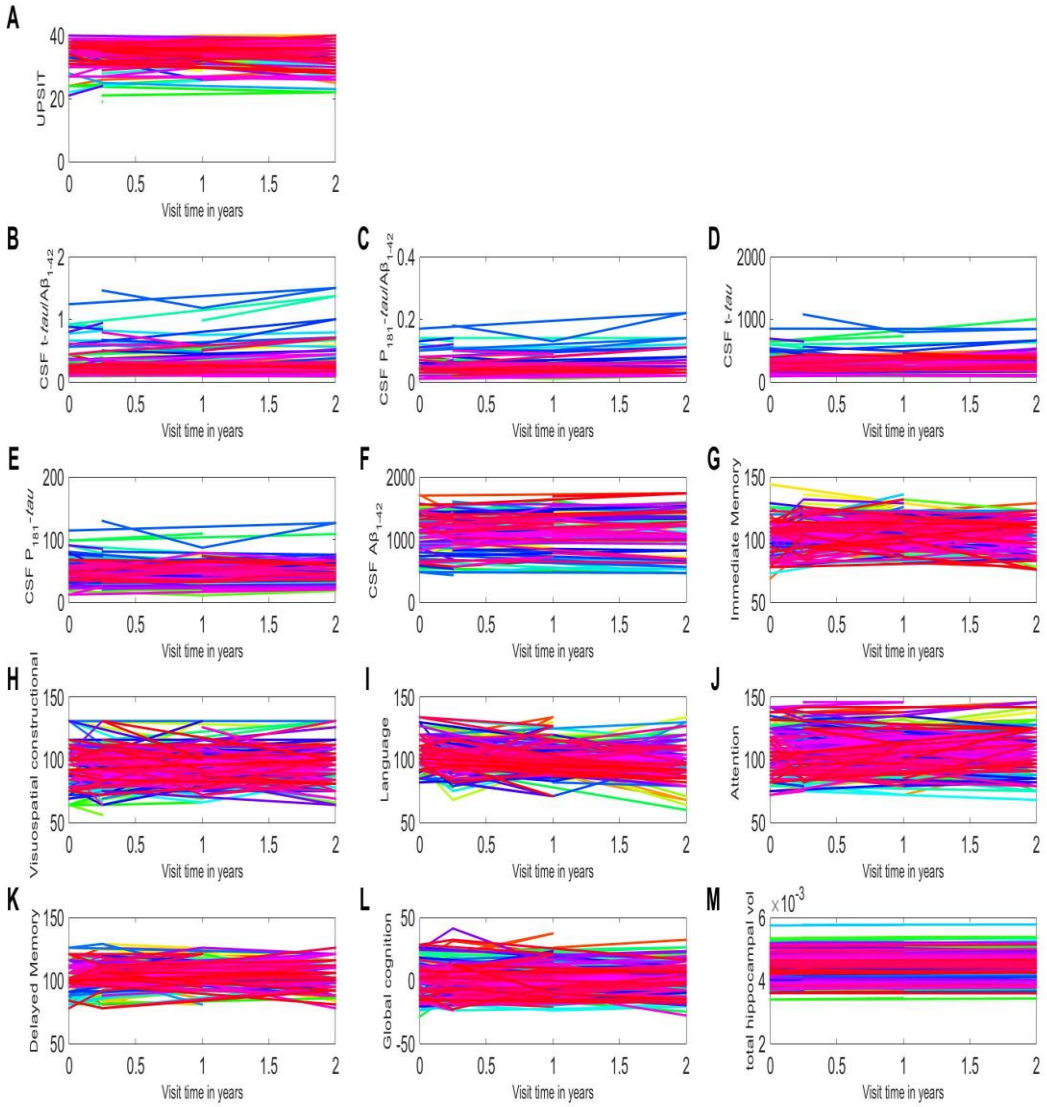


Figure 4-1 Spaghetti plots of repeated measures over a 2-year period.
A) UPSIT B) CSF t-*tau*/Aβ1-42 C) CSF P181-*tau*/Aβ1-42 D) CSF CSF t-*tau* (pg/mL) E) CSF P181-*tau* (pg/mL) E) CSF Aβ1-42 pg/mL G) Immediate Memory H) Visuospatial Constructional I) Language J) Attention K) Delayed Memory L) Global Cognition M) Hippocampal Volume adjusted for intracranial cavity

4.6 Results

4.6.1 Sample demographics

There were 160 cognitively normal INTREPAD trial participants in the mITT pool. Of these, >90% completed the protocol. Baseline demographic characteristics of the participants are summarized in Table 4-2. Participants were mostly female and well educated. The mean age was 63 years at baseline (760 months). There was notable enrichment in persons with at least one *APOE* ε4 allele, most likely as a result of the family history inclusion requirement. Among the 93 individuals who volunteered for LPs, 75% donated all four CSF samples. At baseline, participants had an average UPSIT score of 35.62 ± s.d. 3.2. By the end of the protocol their average was down by 1 point (34.55 ± 3.3). Some variables appeared to be different in those who volunteered for lumbar punctures (Table 4-2). Demographics for each follow up visit are provided in Table 4-3.

	Participants with LP				*Participants no LP*				Group Comparison	*All INTREPAD*			
Demographics	**Average**	**S.D.**	**min median max**	**n**	**Average**	**S.D.**	**min median max**	**n**	**p-value**	**Average**	**S.D.**	**min median max**	**n**
On Treatment (%)	55	50		93	54	50		67	<0.01*	55	50		160
Age (years)	761	68	663 - 734 - 999	93	772	67	662 - 765 - 1011	67	<0.01*	761	68	662 - 740 - 1011	160
Sex (% Female)	74	44		93	82	39		67	<0.01*	74	44		160
Education (years)	15	3	10 – 15 - 27	93	15	4	7 – 15 - 29	67	0.11	15	3	7 – 15 - 29	160
E4 carrier status (%)	34	48		93	33	47		67	0.45	34	48		160
Caucasian (%)	100	13		93	99	12		67	0.84	100	13		160
Francophone (%)	83	38		93	78	42		67	0.01*	83	38		160
MoCA total score	28	2	23 - 28 - 30	93	28	1	23 - 29 - 30	67	0.0398*	28	2	23 - 28 - 30	160
RBANS	102	11	74 - 101 - 129	90	104	11	84 - 104 - 131	67	0.23	102	11	74 - 103 - 131	157
RBANS immediate memory	103	11	78 - 106 - 144	90	103	12	69 - 106 - 129	67	0.54	103	11	69 - 106 - 144	157
RBANS visuospatial	95	14	64 - 96 - 131	90	96	15	64 - 96 - 131	67	0.88	95	14	64 - 96 - 131	157
RBANS language	104	11	82 - 101 - 134	90	105	9	92 - 104 - 130	67	0.77	104	11	82 - 101 - 134	157
RBANS attention	106	16	75 - 106 - 142	90	108	16	72 - 106 - 142	67	0.04*	106	16	72 - 106 - 142	157
RBANS delayed memory	102	9	78 - 102 - 126	90	103	9	84 - 102 - 126	67	0.31	102	9	78 - 102 - 126	157
UPSIT total score	36	3	21 - 36 - 40	92	35	3	24 - 36 - 40	64	0.02*	36	3	21 - 36 - 40	157
t-*tau* (pg/mL)	48	18	90 - 260 - 851	93						278	133	90 - 260 - 851	93
P_{181}-*tau* (pg/mL)	1133	279	12 - 46 - 114	93						48	18	12 - 46 - 114	93
$A\beta_{1-42}$ (pg/mL)	278	133	479 - 1188 - 1712	93						1133	279	479 - 1188 - 1712	93
t-*tau* / $A\beta_{1-42}$	0.27	0.19	0.09 - 0.22 - 1.24	93						0.27	0.19	0.09 - 0.22 - 1.24	93
P_{181}-*tau*/ $A\beta_{1-42}$	0.05	0.03	0.01 - 0.04 - 0.17	93						0.05	0.03	0.01 - 0.04 - 0.17	93
HCC/ICC	0.0045	0.0004	0.0034 - 0.0045 - 0.0053	88	0.0045	0.0004	0.0036 - 0.0045 - 0.0057	67	0.30	0.0045	0.0004	0.0034 - 0.0045 - 0.0057	155
Time between LP and UPSIT, m	-0.71	0.7	-3.3 - -0.4 - 0	93						-0.71	0.7	-3.3 - -0.4 - 0	93

Table 4-2 Baseline Demographics

HCC/ICC= Hippocampal cortex over intracranial cavity; m=month; MoCA= Montreal Cognitive Assessment; RBANS = Repeatable Battery for the Assessment of Neuropsychological Status; UPSIT= University of Pennsylvania Smell Identification Test; y=year

Visit	36 month follow-up visit						120 month follow-up visit						240 month follow-up visit					
Demographics	mean	sd	min	median	max	n	mean	sd	min	median	max	n	mean	sd	min	median	max	n
Treatment	55%	50%				160	54%	50%				152	53%	50%				147
Age, m	764.57	68.19	664.8	745.2	1013.7	159	768.98	62.17	665.8	753	955.8	151	781.84	65.51	685.3	765	1036	147
Female, %	74%	44%				160	74%	44%				152	75%	44%				147
Education, y	15.17	3.42	7	15	29	160	15.14	3.46	7	15	29	152	15.13	3.48	7	15	29	147
APOE ε4 carrier status, %	34%	48%				160	35%	48%				152	35%	48%				147
White, %	100%					160	100%					152	100%					147
Francophone, %	83%	38%	0	1	1	160	82%	38%	0	1	1	152	82%	39%				147
MoCA	28.04	1.56	23	28	30	160	28.04	1.57	23	28	30	152	28.03	1.55	23	28	30	147
UPSIT	34.91	3.44	19	36	39	154	34.77	2.91	24	35	40	146	34.55	3.3	22	35	40	142
t-*tau*/$A\beta_{42}$	0.3	0.23	0.09	0.21	1.46	83	0.28	0.19	0.09	0.21	1.18	79	0.3	0.26	0.08	0.215	1.5	72
P_{181}-*tau*/$A\beta_{42}$	0.05	0.03	0.02	0.04	0.18	83	0.05	0.02	0.01	0.04	0.13	79	0.05	0.03	0.02	0.04	0.22	72
t-*tau* (pg/mL)	291.57	143.25	99.00	274.00	1077.43	83	285.17	129.02	94.53	253.70	793.18	79	295.77	166.95	82.50	253.65	1001.49	72
P_{181}-*tau* (pg/mL)	48.21	17.58	19.10	45.46	129.71	83	47.61	16.93	10.90	47.16	109.50	79	47.12	18.47	18.10	42.80	126.47	72
$A\beta_{42}$ (pg/mL)	1127.61	306.58	431.29	1174.83	1605.20	83	1138.59	299.07	479.09	1175.85	1722.80	79	1121.59	303.06	459.88	1110.18	1739.60	72
Total RBANS score	101.89	11.45	79	101	144	159	103.11	10.79	79	102	140	152	103.05	10.84	75	102	135	147
Immediate memory	106.95	10.82	81	109	136	159	106.49	11.41	81	109	136	152	103.76	11.37	76	106	129	147
Visuconstructional	92.09	13.46	56	92	131	159	93.84	12.74	66	96	131	152	96.65	12.8	64	100	131	147
Language	98.56	9.75	68	98	127	159	98.86	10.82	71	98	134	152	99.73	11.6	60	98	134	147
Attention	105.96	15.21	75	106	146	159	107.28	14.82	72	106	146	152	106.88	15.86	68	106	146	147
Delayed memory	104.64	9.14	78	102	129	159	106.18	8.25	81	106	126	152	105.56	8.64	78	106	126	147
HCC/ICC	0.0046	0.0006	0.0022	0.0046	0.0062	113	0.0046	0.0006	0.0031	0.0046	0.0062	112	0.0045	0.0006	0.0027	0.0045	0.0059	108
Time between LP and UPSIT, m	-0.69	0.76	-4.1	-0.4	0.3	83	-0.52	0.78	-5.5	-0.3	1.2	79	-0.33	0.38	-1.7	-0.2	0.5	72

Table 4-3 Follow-up Demographics

HCC/ICC= Hippocampal cortex over intracranial cavity; m=month; MoCA= Montreal Cognitive Assessment; RBANS = Repeatable Battery for the Assessment of Neuropsychological Status; UPSIT= University of Pennsylvania Smell Identification Test; y=year

4.6.2 OI association with markers of AD progression

We constructed a mixed-effects model to test reproducibility in a larger sample of our previously observed association of OI with CSF markers and cognitive scores. The model used time-varying global cognition and CSF indicators of AD pathology, t-*tau*/$A\beta_{1-42}$ and P_{181}-*tau*/$A\beta_{1-42}$. Results showed that lower repeated measures of OI were associated with higher CSF t-*tau*/$A\beta_{1-42}$ but not P_{181}-*tau*/$A\beta_{1-42}$ (β=-3.03, CI:-5.43 - -0.61, P<0.01, n=318; β=-4.37, CI: -21.94 – 13.2, CI: 13.20 – - 0.37, P=0.62, n=318; Table 4-4).[11,38] However, an early observation of association between baseline OI and hippocampal volume could not be reproduced once we adjusted results appropriately for total intracranial volume (β=-150.19, SE=592.18, CI: -1313.3 - 1012.9, P=0.80, n=568; Table 4-4).[38]

Our previous work suggested an association between OI and global cognition in a large dataset (n=258), but once again we failed to observe this association in longitudinal data (β= 0.0014, SE=0.01, CI:-0.02293 - 0.025648, P=0.91, n=583; Table 4-4). In exploratory analyses of cognitive subscores, however, we saw an association between OI and poorer immediate memory. The estimated P-value for this association (0.02) was reduced to trend-level after adjustment for testing of multiple cognitive domains (P_{FDR}=0.095). These results did not change substantially (P>0.1) after we removed several superfluous covariates from the models, leaving terms for age, year of observation, and time-varying markers of AD progression and cognitive decline.

A

Models	t-*tau*/$A\beta_{42}$			P_{181}-*tau*/$A\beta_{42}$		
Predictors	β	SE	P	β	SE	P
Predictors of longitudinal UPSIT	-3.03	1.23	0.01	-4.37	8.93	0.62
Year	-0.34	0.19	0.08	-0.37	0.19	0.06
Age	-0.01	0.00	0.02	-0.01	0.00	0.01

B

Models	Total RBANS Score			Adjusted hippocampal volume		
Predictors	β	SE	P	β	SE	P
Predictors of longitudinal UPSIT	0.00	0.01	0.91	-150.19	592.18	0.80
Year	-0.17	0.15	0.25	-0.41	0.14	0.00
Age	-0.02	0.00	<0.01	-0.02	0.00	<0.01

C

Models	Immediate memory				Delayed memory			
Predictors	β	SE	P	PFDR	β	SE	P	PFDR
Predictors of longitudinal UPSIT	0.02	0.01	0.02	0.10	0.00	0.01	0.78	0.78
Year	-0.13	0.15	0.37	0.37	-0.17	0.15	0.25	0.37
Age	-0.01	0.00	0.00	<0.01	-0.02	0.00	0.00	<0.01

D

Models	Visuoconstructional				Language				Attention			
Predictors	β	SE	P	PFDR	β	SE	P	PFDR	β	SE	P	PFDR
Predictors of longitudinal UPSIT	-0.01	0.01	0.29	0.49	0.01	0.01	0.53	0.66	-0.01	0.01	0.18	0.45
Year	-0.15	0.15	0.32	0.37	-0.17	0.15	0.25	0.37	-0.17	0.15	0.26	0.37
Age	-0.02	0.00	0.00	<0.01	-0.02	0.00	0.00	<0.01	-0.02	0.00	0.00	<0.01

Table 4-4 Linear mixed-effects models of AD progression markers as predictors of UPSIT performance over a 2-year period Table 4-4 provides results from linear-mixed effects models. Estimated coefficients (β) with standard error (SE) are shown for each test with corresponding p-values. All models were adjusted for treatment, treatment by time interaction, year, sex, education, *APOE* ε4 carrier status. A & B) presents 4 different models (one for each predictor being tested for its association with OI) C & D) presents 5 different models (one for each cognitive domain being tested for its association with OI). Models were adjusted for multiple comparisons using false discovery rate and new p-values are labeled as P_{FDR}. UPSIT= University of Pennsylvania Smell Identification Test

4.6.3 Longitudinal change in odor identification

The data showed a 2% decline in (unadjusted) UPSIT performance over the two years of observation. We examined this further using linear mixed-effects models that again showed OI decline over time (year, β=-0.3774, SE=0.1351, CI:-0.64 – -0.11, P<0.01; Fig 4-2; Table 4-3). Both time in years and age were important predictors of UPSIT performance during the 2-year observation period (age in months, β=-0.0152, SE=0.0034, CI: -0.02 – -0.01, p<0.001). Consistent with this observation, Model 2 reproduced associations with time and age but not *APOE* ε4 carrier status, education or sex after adjustment for treatment assignment and treatment by time interaction (Table 4-5).

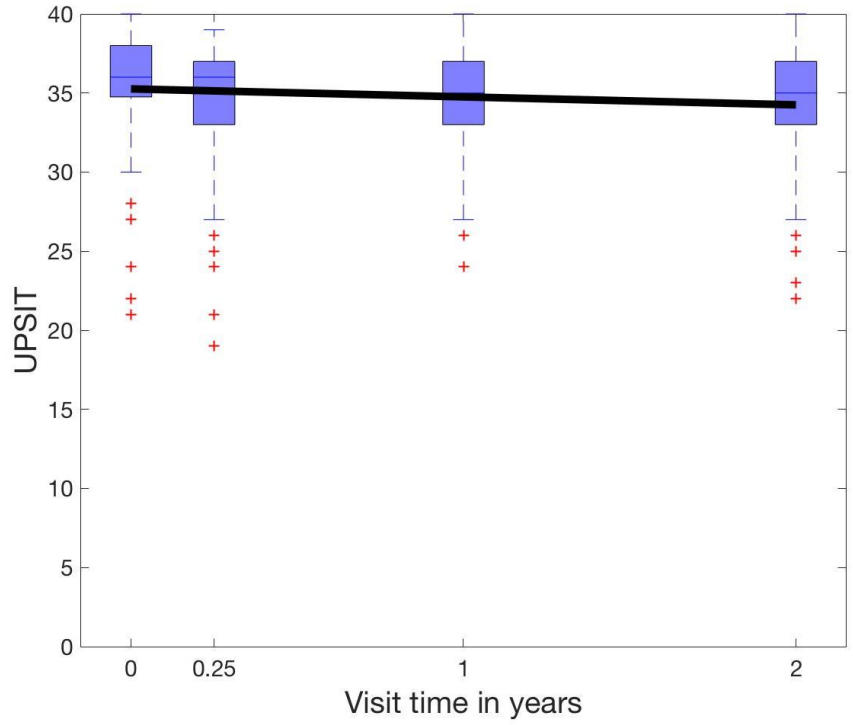


Figure 4-2 Boxplot of OI with fitted curve of change during the 2 year follow period

UPSIT= University of Pennsylvania Smell Identification Test

Model	Independent variable	Estimate	SE	tstat	p-value	CI	
Model 1 n=594	Intercept	35.2956	0.3541	99.6689	0	34.6001	35.991
	year	-0.3774	0.1351	-2.7942	0.0054	-0.6427	-0.1121
	Age at baseline	-0.0152	0.0034	-4.4386	1.08E-05	-0.022	-0.0085
Model 2 n=591	Intercept	34.9215	0.5575	62.6368	4.14E-263	33.8265	36.0164
	year	-0.3778	0.1351	-2.797	0.0053	-0.6431	-0.1125
	Age at baseline	-0.0151	0.0035	-4.3648	1.50E-05	-0.0219	-0.0083
	Female	0.5146	0.5152	0.9989	0.3183	-0.4972	1.5264
	Education, y	0.0507	0.0659	0.7689	0.4422	-0.0787	0.18
	E4 carrier	-0.0384	0.4801	-0.0799	0.9364	-0.9812	0.9045

Table 4-5 Linear mixed-effects models of demographic and time as predictors of UPSIT performance over a 2-year period

Table 4-5 provides results from linear-mixed effects models. Estimated coefficients (β) with standard error (SE) are shown for each test with corresponding p-values. Model 1 was adjusted for treatment and time interaction and it looks at year and age at baseline as predictors of the UPSIT performance. Model 2 was adjusted for treatment and time interaction and looks at year, sex, education, *APOE* ε4 carrier status as predictors of the UPSIT performance. The time in year was predictive of the UPSIT score during a 2-year period. Age at baseline was also an important predictor of the UPSIT score during a 2-year period.

UPSIT= University of Pennsylvania Smell Identification Test; y=year

4.6.4 Assessment of 24-month change

In robust-fit linear regression models, we examined change in the OI – AD biomarker association after 24 months. An increase in t-*tau*/$A\beta_{1-42}$ accompanied decreases in OI over 2 years, but only at a trend-level (β=-4.75, SE=2.84, CI: -0.84 – 1.66, P=0.1, n=66; Table 4-6). We investigated *post-hoc* the specific cognitive domain associations with OI. A suggestive association between OI change and immediate memory change did not survive adjustment for multiple comparisons (β=0.03, SE=0.02, CI: 0.002 – 0.07, P=0.04, P_{FDR}=0.2, n=135). All models were adjusted for treatment, treatment-by-time interaction, year, sex, education, *APOE* ε4 carrier status, and baseline UPSIT performance. We then investigated models of 24-months change in AD progression markers or related cognitive decline adjusted for time in years and baseline UPSIT performance (Fig. 4-3). This modification suggested a stronger relationship between OI and t-*tau*/$A\beta_{1-42}$, but no improvement in the association of OI with immediate memory (β=-4.29, SE=2.39, CI: -1.41 – -0.38, P=0.08, n=66; β=0.03, SE=0.02, CI: 0.00002 – 0.07, P=0.05, n=135).

A

Models	t-*tau*/$A\beta_{42}$ change			P_{181}-*tau*/$A\beta_{42}$ change		
Predictors	β	SE	P	β	SE	P
Predictors of UPSIT change	-4.75	2.84	0.10	-19.02	21.92	0.39
Age	0.00	0.00	0.90	0.00	0.00	0.98
Baseline UPSIT	-0.31	0.10	<0.01	-0.29	0.10	<0.01

B

Models	Total RBANS score change			Adjusted hippocampal volume change		
Predictors	β	SE	P	β	SE	P
Predictors of UPSIT change	0.00	0.02	0.92	223.43	5364.06	0.97
Age	0.00	0.00	0.42	0.00	0.00	0.42
Baseline UPSIT	-0.27	0.07	<0.01	-0.31	0.07	<0.01

C

Models	Immediate memory change				Delayed memory change			
Predictors	β	SE	P	PFDR	β	SE	P	PFDR
Predictors of UPSIT change	0.04	0.02	0.04	0.20	-0.01	0.02	0.70	0.97
Age	0.00	0.00	0.28	0.48	0.00	0.00	0.41	0.48
Baseline UPSIT	-0.27	0.07	<0.01	<0.01	-0.27	0.08	<0.01	<0.01

D

Models	Visuoconstructional change				Language change				Attention change			
Predictors	β	SE	P	PFDR	β	SE	P	PFDR	β	SE	P	PFDR
Predictors of UPSIT change	0.00	0.01	0.97	0.97	0.00	0.02	0.92	0.97	-0.02	0.02	0.40	0.97
Age	0.00	0.00	0.42	0.48	0.00	0.00	0.43	0.48	0.00	0.00	0.48	0.48
Baseline UPSIT	-0.27	0.07	<0.01	<0.01	-0.27	0.07	<0.01	<0.01	-0.29	0.07	<0.01	<0.01

Table 4-6 Robust fit linear models of AD progression marker 24 month change as predictors of UPSIT 24 month change in performance Table 4-6 provides results from robust fit models. Estimated coefficients (β) with standard error (SE) are shown for each test with corresponding p-values. Change at 24 months was calculated by subtracting baseline results from 24-month visit results. All models were adjusted for treatment, year, sex, education, *APOE* ε4 carrier status, and baseline UPSIT performance. A & B) presents 4 different models (one for each predictor being tested for its association with OI) C & D) presents 5 different models (one for each cognitive domain being tested for its association with OI). Models were adjusted for multiple comparisons using false discovery rate and new p-values are labeled as P_{FDR}. UPSIT= University of Pennsylvania Smell Identification Test

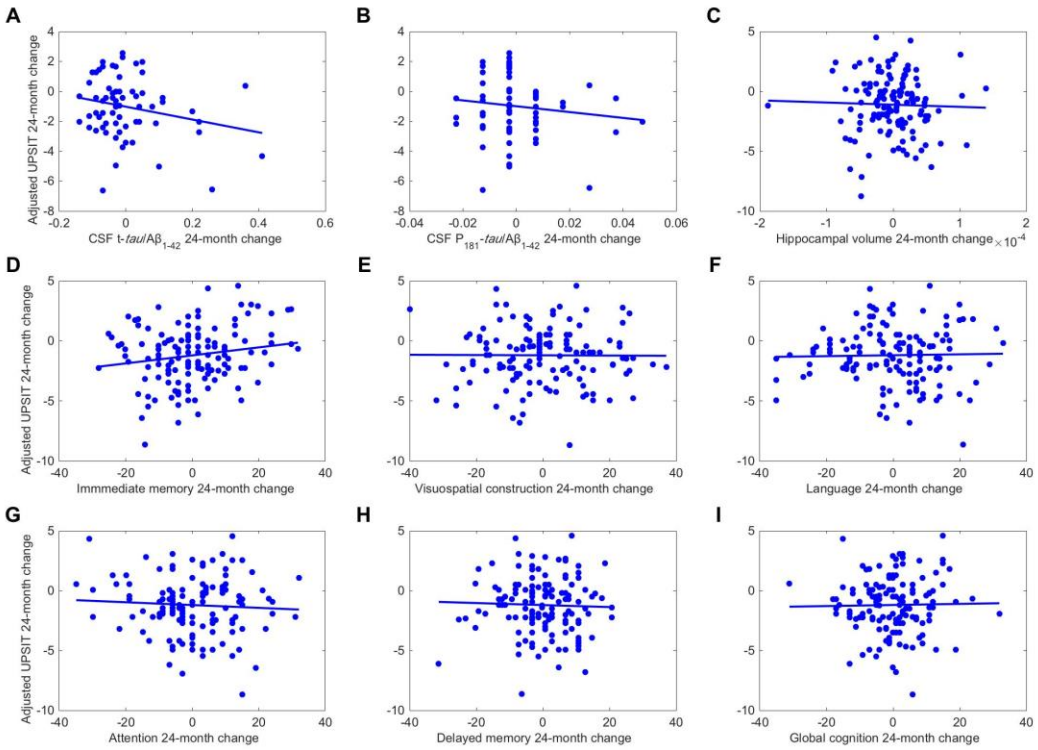


Figure 4-3 Robust fit linear models of AD progression marker 24-month change as predictors of UPSIT 24-month change in performance

Figure 4-3 provides results from robust fit models. Change at 24 months was calculated by subtracting baseline results from 24-month visit results. All models were adjusted for time in years and baseline UPSIT performance. This figure presents the nine different models from table 4-6 (one for each predictor being tested for its association with OI change after 24 months)

A) CSF t-*tau*/$A\beta_{1-42}$ B) CSF P_{181}-*tau*/$A\beta_{1-}$ C) Hippocampal Volume adjusted for intracranial cavity D) Immediate Memory E) Visuospatial Constructional F) Language G) Attention H) Delayed Memory I) Global Cognition ; UPSIT= University of Pennsylvania Smell Identification Test

4.6.5 Baseline cognition, CSF biomarkers, and HCC/ICC volume effect as predictors of longitudinal odor identification

Higher baseline CSF t-*tau*/$A\beta_{1-42}$ and P_{181}-*tau*/$A\beta_{1-42}$ was predictive of OI at any time point but not to its decline (β=*-3.51*, SE=1.*74*, CI: -6.93 – -0.08, P=0.04, n=340; β=-21.68, SE=12.46, CI: -46.191 – 2.82, P=0.08, n=350, Table 4-7). Lower baseline immediate memory was weakly associated with lower OI at any timepoint (β=0.06, SE=0.02, CI: 0.02 – 0.11, P=0.01, n=587; Table 4-7). This association still remained after multiple comparisons correction (P_{FDR} =0.05; Table 4-7). Baseline global cognition and immediate memory predicted declines in OI at trend level (β=0.05, SE=0.03, CI: -0.02 – 0.02, P=0.06; β=-0.01, SE=0.01; CI: -0.03 – 0.0004, P=0.06, n=587; Table 4-7).

A

Models	t-*tau*/$A\beta_{42}$			P_{181}-*tau*/$A\beta_{42}$		
Predictors	β	SE	P	β	SE	P
Baseline marker	-3.51	1.74	0.04	-21.68	12.46	0.08
Year	-0.31	0.18	0.10	-0.31	0.18	0.10
Baseline marker * time	-0.55	0.72	0.44	-4.85	5.00	0.33
Age	-0.01	0.00	0.02	-0.01	0.00	0.02

B

Models	Total RBANS Score			Adjusted hippocampal Volume		
Predictors	β	SE	P	β	SE	P
Baseline marker	0.05	0.03	0.06	-170.64	622.42	0.78
Year	-0.16	0.15	0.28	-0.41	0.14	0.00
Baseline marker * time	0.00	0.01	0.99	-74.42	223.77	0.74
Age	-0.01	0.00	<0.01	-0.02	0.00	<0.01

C

Models	Immediate memory				Delayed memory			
Predictors	β	SE	P	PFDR	β	SE	P	PFDR
Predictors of longitudinal UPSIT	0.06	0.02	0.01	0.05	0.03	0.03	0.34	0.57
Year	-0.17	0.15	0.26	0.29	-0.16	0.15	0.28	0.29
Baseline marker * time	-0.01	0.01	0.06	0.30	0.01	0.01	0.22	0.55
Age	-0.01	0.00	<0.01	<0.01	-0.01	0.00	<0.01	<0.01

D

Models	Visuoconstructional				Language				Attention			
Predictors	β	SE	P	PFDR	β	SE	P	PFDR	β	SE	P	PFDR
Predictors of longitudinal UPSIT	0.01	0.02	0.47	0.58	0.04	0.02	0.09	0.23	0.00	0.02	0.98	0.98
Year	-0.16	0.15	0.28	0.29	-0.16	0.15	0.29	0.29	-0.16	0.15	0.28	0.29
Baseline marker * time	0.00	0.01	0.58	0.73	0.01	0.01	0.36	0.60	0.00	0.01	0.89	0.89
Age	-0.01	0.00	<0.01	<0.01	-0.01	0.00	<0.01	<0.01	-0.02	0.00	<0.01	<0.01

Table 4-7 Linear mixed-effects models of baseline AD progression marker measurement as predictors of UPSIT performance over a 2-year period Table 4-7 provides results from linear-mixed effects models. Estimated coefficients (β) with standard error (SE) are shown for each test with corresponding p-values. All models were adjusted for treatment, treatment by time interaction, year, sex, education, *APOE* ε4 carrier status. A & B) presents 4 different models looking at baseline AD marker as predictors and the effect of baseline AD marker measures by time interaction (one for each predictor being tested for its association with OI) C & D) presents 5 different models looking at baseline cognitive domain result as a predictor and the effect of the cognitive domain by time interaction (each cognitive domain being tested for its association with OI). Models were adjusted for multiple comparisons using false discovery rate and new p-values are labeled as P_{FDR}. UPSIT= University of Pennsylvania Smell Identification Test

4.6.6 Baseline odor identification effect on longitudinal cognition, CSF biomarkers, and HCC/ICC volume

Table 4-8 shows mixed-effects models that evaluate the ways baseline odor identification predicted specific CSF biomarkers, global cognition, and hippocampal volumes, and again, probed for domain specific cognition. Models were adjusted for age, sex, education, and *APOE* ε4 status, treatment, treatment-by-time, and time-squared. Lower baseline OI predicted higher CSF t-*tau*/$A\beta_{1-42}$ and P_{181}-*tau*/$A\beta_{1-42}$ at any time point (β=-0.016, SE=0.01, CI: -0.029 – -0.004; P=0.009; β=-0.002, SE=0.00, CI: -0.004 – -0.0004, P=0.02, n=325; Table 4-8). Lower baseline OI predicted lower declines in immediate memory but it did not survive multiple comparisons (β=0.48, SE=0.22, CI: 0.05 – 0.91, P=0.03, P_{FDR}=0.15, n=322; Table 4-8). In these models, we did not observe any decline in cognition or increase t-*tau*/$A\beta_{1-42}$ and P_{181}-*tau*/$A\beta_{1-42}$ or reduction in hippocampal volume with time.

A

Models	t-*tau*/$A\beta_{42}$			P_{181}-*tau*/$A\beta_{42}$		
Predictors	β	SE	P	β	SE	P
Baseline UPSIT	-0.02	0.01	0.01	0.00	0.00	0.02
Year	0.01	0.01	0.13	0.00	0.00	0.76
Baseline UPSIT * time	0.00	0.00	0.72	0.00	0.00	0.38
Age	0.00	0.00	0.07	0.00	0.00	0.02

B

Models	Total RBANS Score			Adjusted hippocampal volume		
Predictors	β	SE	P	β	SE	P
Baseline UPSIT	0.34	0.28	0.23	0.00	0.00	0.55
Year	0.83	0.76	0.27	0.00	0.00	0.57
Baseline UPSIT * time	-0.14	0.16	0.38	0.00	0.00	0.42
Age	-0.07	0.01	<0.01	0.00	0.00	<0.01

C

Models	Immediate memory				Delayed memory			
Predictors	β	SE	P	PFDR	β	SE	P	PFDR
Baseline UPSIT	0.31	0.29	0.29	0.48	0.14	0.24	0.54	0.54
Year	-1.86	1.04	0.08	0.33	0.88	0.86	0.30	0.38
Baseline UPSIT * time	0.48	0.22	0.03	0.15	0.05	0.18	0.80	0.80
Age	-0.03	0.01	0.03	0.03	-0.04	0.01	<0.01	<0.01

D

Models	Visuoconstructional				Language				Attention			
Predictors	β	SE	P	PFDR	β	SE	P	PFDR	β	SE	P	PFDR
Baseline UPSIT	0.40	0.34	0.25	0.48	0.46	0.28	0.11	0.48	-0.30	0.45	0.51	0.54
Year	1.64	1.24	0.19	0.33	0.83	1.14	0.47	0.47	1.36	1.06	0.20	0.33
Baseline UPSIT * time	-0.34	0.26	0.19	0.32	-0.26	0.24	0.28	0.35	-0.30	0.22	0.19	0.32
Age	-0.07	0.01	<0.01	<0.01	-0.04	0.01	<0.01	<0.01	-0.08	0.02	<0.01	<0.01

Table 4-8 Linear mixed-effects models of baseline UPST measurement as predictors of AD progression marker over a 2-year period Table 4-8 provides results from linear-mixed effects models. Estimated coefficients (β) with standard error (SE) are shown for each test with corresponding p-values. All models were adjusted for treatment, treatment by time interaction, year, sex, education, *APOE* ε4 carrier status. A & B) presents 4 different models looking at baseline UPSIT scores as predictors and the effect of baseline UPSIT scores by time interaction (one for AD progression marker outcome being tested for its association with OI) C & D) presents 5 different models looking at baseline UPSIT result as a predictor and the effect of the UPSIT score by time interaction (each cognitive domain outcome being tested for its association with OI). Models were adjusted for multiple comparisons using false discovery rate and new p-values are labeled as P_{FDR}. UPSIT= University of Pennsylvania Smell Identification Test

4.7 Discussion

We made repeated measures of CSF AD biomarkers, cognition, and the University of Pennsylvania odor identification test (UPSIT) in individuals at risk of AD. In general, the results corroborated a previously observed association of OI with CSF biomarkers of AD, and possibly with related deficits in cognitive abilities.[11,38,39] OI performance itself declined over a 2-year interval. The relation between *decline* in OI and several key indicators of ongoing disease progression suggests that previously observed cross-sectional associations may reflect an underlying longitudinal process. Furthermore, these findings in non-symptomatic individuals suggest that OI changes predate declines in other modalities that may accompany progression of symptomatic disease. Our main findings support previous work and encourage continuous investigation of olfactory functions in early AD research.[4,5,16,40,41]

We also explored whether OI was associated with reduction in hippocampal grey matter volume, adjusting for total intra-cranial volume,[47] frequently used as a volumetric measure of AD-related atrophy.[6,8-10] However, we found no meaningful association between these measures using an image-processing pipeline designed to improve volumetric accuracy compared with voxel based morphometry. Specifically, we used a T1 template that represents an aging population of normal adult controls, individuals with MCI, and patients with AD dementia, while others may have used a common T1 template representing a normal young adult population.[48,49]

4.7.1 Strengths

Important earlier studies illustrated that OI performance is compromised in individuals with AD pathology *post mortem*.[4,5] Olfactory mucosal biopsy, autopsy, and imaging work have pointed to mechanisms that could explain associations between OI and AD progression – in the present instance, a change in CSF biochemistry and cognitive decline.[7,40-43] Our study points to smell identification as an indicator of accrued damage.

We characterized the relationship between smell identification and the performance of several cognitive domains. While odor identification has been thought of mostly in terms of language and memory abilities, we found that lower OI was associated principally with reduced immediate memory performance.[44] Baseline global and immediate memory scores portended decline in OI. Cognition did not decline detectably over the study period, but OI demonstrated a significant decline, a finding that could suggest odor identification is an earlier detectable marker of pathology.

Our findings were generally consistent with previous, well-designed studies. Thus, others showed in 589 older adults without cognitive impairment or dementia from the Rush Memory and Aging Project that OI predicted the emergence of an MCI diagnosis over 5 years of follow-up (hazard ratio 1.15; 95% CI, 1.07-1.23).[13] In that study, baseline OI predicted a more rapid rate of decline in episodic and semantic memory, and in perceptual speed using adjusted mixed-effects models.[13] In a previous iteration of that analysis based on 3 years of data, Wilson et al failed to detect an association of OI with a decline in semantic memory.[45] Similarly, a linear mixed-effects model in 1430 cognitively normal adults demonstrated that lower baseline OI test scores predict declines in memory, executive function, language, and global cognition. Some 250 of these latter participants developed MCI, and 64 developed dementia, over 3.5 years of

follow-up. As expected, the worst olfactory identification performance was associated with amnestic MCI (aMCI) and dementia.[16] Adams and colleagues reported that a simple test for OI displays a 47% sensitivity and 79% specificity of predicting dementia five years before detection of cognitive impairment in 2677 participants.[19] However, they found that 91% of those who did poorly on OI did *not* develop dementia within five years of follow-up.[19] Based on these observations, one might expect that a longer follow-up period may reveal that reduced baseline OI should predict worse cognitive function outcomes. Our findings are similar to those from a linear mixed-effects analysis in which longitudinal OI was associated with more rapid decline in episodic memory (estimate=0.014, SE=0.004, p<0.001) after adjustment for age, sex, education, ε4, and baseline episodic memory.[46] Together, these associations suggest that OI may predict later cognitive decline even in normal individuals at risk of AD.

4.7.7 Limitations

Our results relating OI and hippocampal volume also contrast with preliminary data reported earlier (Spearman rho=0.243 P=0.014). Those results described a stronger association with the right hippocampal volume (rho= 0.254 P=0.010) than left (rho=0.196 P=0.047) in 103 PREVENT-AD participants.[38] It is possible that our grouping of left and right hippocampal volume may have obscured a lateralized finding.[50] With more data, we plan to revisit these analyses and investigate left and right hippocampal formations independently, as well as left and right CA1 and subiculum hippocampal subfields, and entorhinal cortices.

We had prior bases for expecting many of our hypotheses to be sustained,[4-6,8,11,12,16,38,40,41] and therefore we did not adjust results from different domains (*viz.* CSF biomarkers, imaging volumetrics, global cognition) for multiple

comparisons. The same logic did not apply, in our opinion, to our post-hoc sub-analyses of multiple domain-specific aspects of cognition.

We noticed the UPSIT instrument fluctuated up in some and down in others. The messy appearance could be due to practice effect in some. Although, we had randomized the booklet presentation to avoid such issue, the practice effect through repeated exposure and familiarity could have weakened our longitudinal observations.[51] A longer study period may clarify these relationships and we may observe different trajectories in those who learned the task versus those who displayed immediate and steady decline.[52] Other limitations apply to the generalizability of our observations. The participants were mostly French Canadian women with a high level of educational attainment and a first degree relative affected by Alzheimer's disease.

4.8 Conclusion

Our findings suggest the potential utility of OI as a quantitative measure during a longer transition period from normal to MCI and the exploration of individual modality trajectories through the use of parallel processing analysis. Currently, preventative trials have moved "upstream" to consider changes in the pre-symptomatic phases of AD pathogenesis, but they still rely on "classical" markers such as cognitive abilities or CSF biomarkers. Based on data presented here, we suggest that OI as a quasi-continuous measure may add to the repertoire of useful disease markers for this purpose.

4.9 Author contributions

Marie-Elyse Lafaille-Magnan: study concept and design, data collection (RBANS and UPSIT and imaging), 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.
Pedro Rosa-Neto: study concept and design, revising the manuscript.
Jeannie-Marie S. Leoutsakos: study analysis design (RBANS adjustment and general statistical advice)
Judes Poirier: study concept and design, data interpretation, revising the manuscript.
Louis Collins and Vladimir Fonov: study concept and design, imaging pipeline design and imaging analysis.

4.10 Acknowledgement

The authors thank David Fontaine, PhD, who oversaw cognitive testing at enrollment, scored the RBANS cognitive evaluations, and administered additional cognitive batteries when indicated. Anne Labonté, BSc, conducted the CSF biomarker analyses. Tanya Lee, Melissa Appleby, Laura Mahar, Galina Pogossova, Renuka Giles, and Karen Wan collected cognitive and olfaction data. Cécile Madjar, MSc, directed data management, and Doris Dea and Louise Théroux did the genotyping. All are with the Centre for Studies on Prevention of AD, Douglas Mental Health University Institute. Thomas Beaudry, BSc (Perform Centre, Concordia University), helped edit the manuscript. Tharick Ali Pascoal, MD, Marina Tedeshi Dauar, MD, and Laksanun Cheewakriengkrai, MD (McGill Centre for Studies in Aging and Centre for Studies on Prevention of AD, Douglas

Mental Health University Institute), collected the CSF. Melissa Savard, MSc (McGill Centre For Studies in Aging) and Yasser Iturria-Medina (Montreal Neurological Institute) provided valuables advice and help. Special thanks go to Marianne Dufour, administrative assistant, and to Ginette Mayrand, Joanne Frenette, MSc, Isabelle Vallée, Rana El-Khoury, and Fabiola Ferdinand, all nurses who met with participants, as well as the entire PREVENT-AD Research Group (https://preventad.loris.ca/acknowledgements/acknowledgements.php?date=2018-02-10). The authors acknowledge the generosity and commitment of all research participant volunteers for this work.

4.11 Study funding

This study was funded by an unrestricted gift from Pfizer Canada, by generous support from McGill University, and by infrastructure support from the Canada Fund for Innovation. Support for genetic and laboratory work was provided by the Fonds de Récherche du Québec–Santé and by the Levesque Foundation. The Douglas Mental Health University Institute Foundation provided additional support.

4.12 Disclosure

M.-E. Lafaille-Magnan has received research support from the Lazlo & Etelka Kollar fellowship fund. M.-E. Lafaille-Magnan receives honoraria from serving as a board member of the clinical excellence committee on social services with a focus on youth, mental health, and dependence for the Institut National d'excellence en santé et en services sociaux. M-E Lafaille-Magnan has health and biotech stocks (AGN, APH, GILD, GOOG, GOOGL, ILMN).

J. Poirier receives royalties from a publication he has cowritten titled *Alzheimer's*

Disease: A Guide, published in 2011 in French, English, German, Portuguese, and Chinese. J Poirier receives royalties from a book titled *Jeune et Centenaire*, published in 2017 in French.

P. Rosa-Neto receives a salary award from le Fonds de recherché du Québec–Santé (Chercheurs-boursiers–Junior 2). He receives research support from the Canadian Institutes of Health Research, Canadian Consortium on Neurodegeneration in Aging, Weston Brain Institute, and Enigma radiopharmaceuticals. Dr. Rosa-Neto is a principal investigator in industry-sponsored clinical trials for TauRx, Jensen, and Eli Lilly. He received honoraria from serving on Eli Lilly's scientific advisory board. He serves as a consultant for Kalgene and Enigma radiopharmaceuticals. He does not hold stock in or receive royalties from any pharma or biotech company.

J. Breitner has received research support from Pfizer Canada. He also received research support from McGill University, the Douglas Hospital Foundation, le Fonds de recherché du Québec–Santé, Canadian Consortium on Neurodegeneration in Aging, Weston Brain Institute, and the government of Canada (Canadian Institutes for Health Research, Tier 1 Canada Research Chair, and the Canadian Fund for Innovation). Go to Neurology.org for full disclosures.

4.13 References

1 Katzman, R. Editorial: The prevalence and malignancy of Alzheimer disease. A major killer. *Archives of neurology* **33**, 217-218 (1976).

2 Daulatzai, M. A. Olfactory dysfunction: its early temporal relationship and neural correlates in the pathogenesis of Alzheimer's disease. *J Neural Transm (Vienna)* **122**, 1475-1497, doi:10.1007/s00702-015-1404-6 (2015).

3 Braak, H. & Braak, E. Evolution of the neuropathology of Alzheimer's disease. *Acta psychiatrica Scandinavica* **Suppl. 165**, 3-12 (1996).

4 Wilson, R. S., Arnold, S. E., Schneider, J. A., Tang, Y. & Bennett, D. A. The relationship between cerebral Alzheimer's disease pathology and odour identification in old age. *Journal of neurology, neurosurgery, and psychiatry* **78**, 30-35, doi:10.1136/jnnp.2006.099721 (2007).

5 Wilson, R. S. *et al.* Olfactory impairment in presymptomatic Alzheimer's disease. *Annals of the New York Academy of Sciences* **1170**, 730-735, doi:10.1111/j.1749-6632.2009.04013.x (2009).

6 Devanand, D. *et al.* Olfactory identification deficits and MCI in a multi-ethnic elderly community sample. *Neurobiology of aging* **31**, 1593-1600 (2010).

7 Growdon, M. E. *et al.* Odor identification and Alzheimer disease biomarkers in clinically normal elderly. *Neurology* **84**, 2153-2160, doi:10.1212/WNL.0000000000001614 (2015).

8 Murphy, C., Jernigan, T. L. & Fennema-Notestine, C. Left hippocampal volume loss in Alzheimer's disease is reflected in performance on odor identification: a structural MRI study. *Journal of the International Neuropsychological Society* **9**, 459-471 (2003).

9 Kjelvik, G. *et al.* The brain structural and cognitive basis of odor identification deficits in mild cognitive impairment and Alzheimer's disease. *BMC neurology* **14**, 168 (2014).

10 Marigliano, V. *et al.* Olfactory deficit and hippocampal volume loss for early diagnosis of Alzheimer disease: a pilot study. *Alzheimer Disease & Associated Disorders* **28**, 194-197 (2014).

11 Lafaille-Magnan, M.-E. *et al.* Odor identification as a biomarker of preclinical AD in older adults at risk. *Neurology* **89**, 327-335 (2017).

12 Reijs, B. L. R. *et al.* Relation of Odor Identification with Alzheimer's Disease Markers in Cerebrospinal Fluid and Cognition. *Journal of Alzheimer's disease : JAD* **60**, 1025-1034, doi:10.3233/JAD-170564 (2017).

13 Wilson, R. S. *et al.* Olfactory identification and incidence of mild cognitive impairment in older age. *Archives of general psychiatry* **64**, 802-808 (2007).

14 Stanciu, I. *et al.* Olfactory impairment and subjective olfactory complaints independently predict conversion to dementia: a longitudinal, population-based study. *Journal of the International Neuropsychological Society* **20**, 209-217 (2014).

15 Devanand, D. *et al.* Olfactory deficits predict cognitive decline and Alzheimer dementia in an urban community. *Neurology* **84**, 182-189 (2015).

16 Roberts, R. O. *et al.* Association Between Olfactory Dysfunction and Amnestic Mild Cognitive Impairment and Alzheimer Disease Dementia. *JAMA Neurol*, 1-9, doi:10.1001/jamaneurol.2015.2952 (2015).

17 Yaffe, K. *et al.* Olfaction and risk of dementia in a biracial cohort of older adults. *Neurology* **88**, 456-462 (2017).

18 Josefsson, M., Larsson, M., Nordin, S., Adolfsson, R. & Olofsson, J. APOE-ε4 effects on longitudinal decline in olfactory and non-olfactory cognitive abilities in middle-aged and old adults. *Scientific Reports* **7** (2017).

19 Adams, D. R. *et al.* Olfactory Dysfunction Predicts Subsequent Dementia in Older U.S. Adults. *Journal of the American Geriatrics Society*, doi:10.1111/jgs.15048 (2017).

20 Breitner, J. C. S. How can we really improve screening methods for AD prevention trials? *Alzheimer's & Dementia: Translational Research & Clinical Interventions* **2**, 45-47, doi:10.1016/j.trci.2015.12.004 (2016).

21 Nasreddine, Z. S. *et al.* The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *Journal of the American Geriatrics Society* **53**, 695-699, doi:10.1111/j.1532-5415.2005.53221.x (2005).

22 Hughes, C. P., Berg, L., Danzinger, W. L., Coben, L. A. & Martin, R. L. A new clinical scale for the staging of dementia. *British Journal of Psychiatry* **140**, 556-572 (1982).

23 Lelental, N. *et al.* Comparison of Different Matrices as Potential Quality Control Samples for Neurochemical Dementia Diagnostics. *Journal of Alzheimer's disease : JAD* **52**, 51-64, doi:10.3233/JAD-150883 (2016).

24 Blennow, K. A Review of Fluid Biomarkers for Alzheimer's Disease: Moving from CSF to Blood. *Neurol Ther* **6**, 15-24, doi:10.1007/s40120-017-0073-9 (2017).

25 Randolph, C., Tierney, M. C., Mohr, E. & Chase, T. N. The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS): preliminary clinical validity. *Journal of clinical and experimental neuropsychology* **20**, 310-319 (1998).

26 Fonov, V. S. *et al.* in *Medical Imaging and Augmented Reality: 5th International Workshop, MIAR 2010, Beijing, China, September 19-20, 2010. Proceedings* (eds Hongen Liao *et al.*) 324-333 (Springer Berlin Heidelberg, 2010).

27 Aubert-Broche, B. *et al.* A new method for structural volume analysis of longitudinal brain MRI data and its application in studying the growth trajectories of anatomical brain structures in childhood. *NeuroImage* **82**, 393-402, doi:10.1016/j.neuroimage.2013.05.065 (2013).

28 Coupe, P. *et al.* An Optimized Blockwise Nonlocal Means Denoising Filter for 3-D Magnetic Resonance Images. *IEEE Transactions on Medical Imaging* **27**, 425-441, doi:10.1109/TMI.2007.906087 (2008).

29 Coupé, P. *et al.* in *Medical Image Computing and Computer-Assisted Intervention – MICCAI 2009: 12th International Conference, London, UK, September 20-24, 2009, Proceedings, Part II* (eds Guang-Zhong Yang *et al.*) 601-608 (Springer Berlin Heidelberg, 2009).

30 Collins, D. L. & Evans, A. C. Animal: Validation and Applications of Nonlinear Registration-Based Segmentation. *International Journal of Pattern Recognition and Artificial Intelligence* **11**, 1271-1294, doi:10.1142/S0218001497000597 (1997).

31 Collins, D. L., Barré, W. F. C. & Evans, A. C. in *Annual symposium on information processing in medical imaging.* (eds A. Kuba, M. Samal, & A. Todd-Pokropek) 210–223 (Springer).

32 Fonov, V. *et al.* Unbiased average age-appropriate atlases for pediatric studies. *NeuroImage* **54**, 313-327, doi:10.1016/j.neuroimage.2010.07.033 (2011).

33 Fonov, V. S., Evans, A. C., McKinstry, R. C., Almli, C. & Collins, D. Unbiased nonlinear average age-appropriate brain templates from birth to adulthood. *NeuroImage* **47**, S102 (2009).

34 Coupe, P. *et al.* Patch-based segmentation using expert priors: application to hippocampus and ventricle segmentation. *NeuroImage* **54**, 940-954, doi:10.1016/j.neuroimage.2010.09.018 (2011).

35 Weier, K., Fonov, V., Lavoie, K., Doyon, J. & Collins, D. L. Rapid automatic segmentation of the human cerebellum and its lobules (RASCAL)—Implementation and application of the patch- based label- fusion technique with a template library to segment the human cerebellum. *Human brain mapping* **35**, 5026-5039 (2014).

36 Collins, D. L. & Pruessner, J. C. Towards accurate, automatic segmentation of the hippocampus and amygdala from MRI by augmenting ANIMAL with a template library and label fusion. *NeuroImage* **52**, 1355-1366, doi:10.1016/j.neuroimage.2010.04.193 (2010).

37 Benjamini, Y. & Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the royal statistical society. Series B (Methodological)*, 289-300 (1995).

38 Lafaille-Magnan, M.-E. *et al.* in *2014 Alzheimer's Imaging Consortium* (Poster presentation : Presentation number: IC-P-149, 2014).

39 Lafaille-Magnan, M.-E., Fontaine, D. & Breitner, J. C. S. Olfactory identification is associated with verbal intrusion and primacy effects in cognitively normal adults at risk of Alzheimer's dementia. *Alzheimer's & Dementia* **11**, P823-P824, doi:10.1016/j.jalz.2015.06.1825 (2015).

40 Vassilaki, M. *et al.* Neuroimaging biomarkers and impaired olfaction in cognitively normal individuals. *Annals of neurology* **81**, 871-882, doi:10.1002/ana.24960 (2017).

41 Ayala- Grosso, C. A. *et al.* Amyloid- Aβ Peptide in Olfactory Mucosa and Mesenchymal Stromal Cells of Mild Cognitive Impairment and Alzheimer's Disease Patients. *Brain Pathology* **25**, 136-145 (2015).

42 de Leon, M. J. *et al.* CSF clearance in Alzheimer Disease measured with dynamic PET. *J Nucl Med*, doi:10.2967/jnumed.116.187211 (2017).

43 Kareken, D. A. *et al.* Olfactory-evoked regional cerebral blood flow in Alzheimer's disease. *Neuropsychology* **15**, 18 (2001).

44 Djordjevic, J., Jones-Gotman, M., De Sousa, K. & Chertkow, H. Olfaction in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiology of aging* **29**, 693-706 (2008).

45 Wilson, R. S., Arnold, S. E., Tang, Y. & Bennett, D. A. Odor identification and decline in different cognitive domains in old age. *Neuroepidemiology* **26**, 61-67 (2006).

46 Wilson, R. S. *et al.* Olfactory Impairment in Presymptomatic Alzheimer's Disease. *Annals of the New York Academy of Sciences* **1170**, 730-735, doi:10.1111/j.1749-6632.2009.04013.x (2009).

47 Whitwell, J. L., Crum, W. R., Watt, H. C. & Fox, N. C. Normalization of cerebral volumes by use of intracranial volume: implications for longitudinal quantitative MR imaging. *American Journal of Neuroradiology* **22**, 1483-1489 (2001).

48 Mazziotta, J. C., Toga, A. W., Evans, A., Fox, P. & Lancaster, J. A probabilistic atlas of the human brain: theory and rationale for its development. *NeuroImage* **2**, 89-101 (1995).

49 Mazziotta, J. *et al.* A probabilistic atlas and reference system for the human brain: International Consortium for Brain Mapping (ICBM). *Philosophical Transactions of the Royal Society B: Biological Sciences* **356**, 1293-1322 (2001).

50 Tardif, C. L. *et al.* Investigation of the confounding effects of vasculature and metabolism on computational anatomy studies. *NeuroImage* **149**, 233-243 (2017).

51 Engen, T. Effect of practice and instruction on olfactory thresholds. *Perceptual and motor skills* **10**, 195-198 (1960).

52 Villeneuve, S. Potential Utility of Practice Effects in Preventive Trials. *The journal of prevention of Alzheimer's disease* **4**, 140-142 (2017).

Chapter 5:

Discussion

Old ways won't open new doors

"The secret of change is to focus all of your energy, not on fighting the old, but on building the new." – Socrates

There is increasing interest in identifying earlier AD-related change, as well as expectations for better psychological and psychiatric measures for pre-symptomatc AD. This doctoral study focused on the use of odor identification (OI) to follow the disease trajectory in a sample of individuals at elevated risk for AD, due to a parent or multiple siblings with AD dementia diagnosis. Building on recent evidence that OI impairment appears to be a marker for AD,[1-4] we investigated the association between OI and age, among other key variables including CSF biomarkers, cognitive performance, structural imaging, the APOE ε4 genotype, sex, and education, taking both risk and protective factors for AD into account. Findings suggested that OI performance was related to early changes in CSF biomarkers, which may reflect misfolded protein aggregation or early synaptic dysfunction and neurodegeneration.

5.1 Originality of the research

The main contribution of the present research was to identify a relationship between OI and neurodegenerative changes, independent of normal aging (chapters, 2, 3, and 4). Chapter 1 reviewed Alzheimer disease pathology and the need to monitor early change, whereas Chapter 2 reviewed the anatomy and function of the olfactory system, identifying only a very few studies linking histopathology work with olfactory functional testing. Chapter 2 also identified studies where OI was used as a secondary outcome measure in trials and highlighted its potential use in preventive intervention trials. Chapter 3 was the first published study on CSF AD-related biomarker levels in individuals at risk for AD, and Chapter 4 investigated the association between concurrent OI performance and CSF *tau* and amyloid levels as well as hippocampal volume *in vivo,* at baseline and over time. It also assessed whether OI declined during a 2-year period. We assessed this decline by introducing an odor identification test to a randomized controlled trial of healthy individuals at risk for AD (Chapter 4), which provided us with new perspectives on the disease continuum. Finally, this current

chapter reviews studies that may explain how olfactory tasks may reflect AD neuropathology.

Notably, this doctoral thesis contributed to the development of an Alzheimer Progression Score that exploited latent class/item response theory for the first time, and was used as the primary outcome measure for the INTREPAD prevention trial (described in Chapter 2).[5] Overall, this thesis appears to be the first investigation to introduce OI as a quasi-continuous outcome in relation to several different CSF measures of AD progression in at-risk individuals.

5.2. Overview of main findings

5.2.1 Cross-sectional results

Prior to this work, Wilson and colleagues had paved the way towards our research by investigating *ante mortem* odor identification performance in relation to pathological staining at autopsy.[6,7] Our findings corroborate and extend their work using a systematic approach in vivo. Our initial cross-sectional study examined the ways in which odor identification was associated with *tau*, and P-*tau*, and amyloid, as well as their ratio, in the CSF. We thus investigated whether healthy adults at risk of AD make more errors on a smell identification test if they have indication of more severe AD pathology. As expected, we observed that older individuals had lower odor identification performance. Individuals with lower cognitive performance, measured more traditionally, were also worse at identifying and naming odors in a multiple-choice paradigm. In addition, we examined how odor identification was associated with risk and protective factors of AD in order to better understand the factors associated with reduced a reduced ability to name odors. Finally, we investigated the effect of the APOE ε4 status on odor identification but found no group differences between carriers and non-carriers.

Specifically, we observed that odor identification was associated with neurodegeneration by observing an association with t-*tau*, P_{181}-*tau*, and their ratio with

$A\beta_{1-42}$. By contrast, we saw no association between odor identification and amyloid in the CSF. Historically, cognitive task performances tend to correlate better with t-*tau*, a measure of neurodegeneration. Surprisingly, however, when characterizing the association between smell identification and amyloid in individuals having the lowest $A\beta_{1-42}$ levels, we did find worse performance on the smell identification test with declining CSF $A\beta_{1-42}$, *viz*. a measure of increasing brain amyloid accumulation. We used a cut-off for amyloid to evaluate the individuals that could have more advanced pathology. This enabled us to evaluate abnormal amyloid levels as per Growdon, 2015 and Vassiliki, 2017.[2,4] After stratifying the population by the *APOE* ε4 gene risk factor, we also observed that associations between odor identification and AD pathological hallmarks were present only in 33 ε4 carriers (out of 100 total). Both these observations appear to suggest that the associations observed represent a nexus between OI and Alzheimer's disease specifically. While this thesis work was being conducted, several studies appeared investigating OI and AD-related atrophy using structural brain imaging and a few reported on PET amyloid accumulation (discussed in Chapter 2, 3, and 4).

5.2.2 Longitudinal results

Our first association study indicated that odor identification was associated with AD biomarkers, cognition, and aging, but it did not enable us to evaluate whether repeated measures in AD biomarkers or cognitive decline affect OI over time (Chapter 4). Our second study examined the way in which odor identification declined over 2 years in individuals enrolled in the INTREPAD clinical trial. We looked specifically at associations with: t-*tau*/$A\beta_{1-42}$, P_{181}-*tau*/$A\beta_{1-42}$ ratios in the CSF as a predictor or continuous measure of change. We also explored whether odor identification was associated with hippocampi volume adjusted for the intra-cranial space. Finally, we included non-genetic and genetic risk and protective factors of AD in our models to better understand the factors associated with a decline in odor identification in individuals at risk of AD. Notably, only age appeared to predict lower OI over 2 years of observations.

As per Wilson, Roberts and colleagues, we also investigated the relation of odor identification and sub-domains of cognition.[6-9] We found that lower OI was associated with lower immediate memory over a two-year span. While OI is thought to be driven mostly by language and memory processes, it is interesting to note that in our experience the latter appeared more strongly related to OI in aging individuals at high risk for AD.

5.3 Implication

To assess the biological implications of our findings, we considered OI as a biomarker of AD using Bradford-Hill's criteria. We also reviewed molecular and genetic mechanisms that may increase the olfactory network's vulnerability. The latter review includes investigations of olfactory receptor genes and AD, genome wide association studies of olfactory abilities in aging individuals. We also compared our results with other first-degree relative studies.

5.3.1 Biological plausibility and Bradford-Hill criteria

In 1965, Sir Austin Bradford-Hill proposed a list of "common sense" criteria to gauge whether an association is likely causal. The criteria may be summarized as: **strength**, **consistency**, **specificity**, **temporal sequence**, **dose-response**, **experimental evidence**, **biological plausibility**, **coherence**, and **analogy.**[10] The principle that OI is an indicator of AD progression fulfills most of these criteria. First, several meta-analyses have shown that the effect size **(strength)** of association between OI and AD dementia or MCI, is large.[1,3,11] Our work corroborated an observed association of OI with AD pathology through investigation of CSF biomarkers *in vivo* (**consistency),** extending it to pre-symptomatic individuals with 2 years of observations.[6,7,12-14] AD progression causes several symptoms from neuropsychiatric symptoms to cognitive impairment.[15] In fact, despite a strong association, no study has

claimed an obligatory loss of smell as a result of AD. Anosmia or declines in odor identification doesn't appear to be specific to AD progression (**specificity**). Olfactory function declines in both AD and PD, although, a meta-analysis suggests that higher-order tasks like OI are more prominent in AD.[1] Odor detection appears to have a larger effect in PD than in AD patients.[1] In fact, NFTs initially accumulate in the entorhinal and hippocampus of AD brains, whereas Lewy bodies have a different distribution pattern. Despite the lack of specificity, we established that this test could be useful in a composite measure to track progression in Chapter 2. OI has also been used to diagnose Parkinson's disease (PD) in similar ways. In PD patients, OI provides valuable information to discriminate patients who eventually develop cognitive decline 2-years later. In this PD specific model, age, UPSIT, Rapid Eye Movement Disorder Screening Questionnaire (RBDSQ), CSF $A\beta_{1-42}$, and the caudate dopamine uptake transporter DAT contributed to the prediction of cognitive decline.[16] A higher-order task like OI and the right combination of accompanying measures may be instructive in pre-symptomatic AD to track change.

Chapter 1 supports the idea that OI and AD pathology have a relationship with time. In fact, we reviewed several key studies illustrating that OI performance could predict the transition to MCI. Chapter 1 also reviewed the effect of non-genetic factors on AD risk and progression. We have learned that cognitive impairment appears after decades of damage accrual related to AD progression (Chapter1). There are empirical evidence for the association between the latter risk factors and olfactory decline. OI decline occurs after exposure to risk factors such as those in figure 5-1. If Alzheimer's disease pathology causes OI decline, we would expect that exposure to risk factors for AD would be associated with its decline. In our longitudinal study (Chapter 4), the presence of elevated CSF biomarker ratio at baseline predicted a lower repeated measured in OI (**Temporal sequence**).

In the latter chapter, we attempted to understand further temporality and found that OI related to CSF t-*tau*/$A\beta_{1-42}$ and immediate memory during a 2-year period. Elevated degree of pathology or increased memory decline was associated with

reduced OI in both our cross-sectional and longitudinal study (Chapter 3 & 4). Our results demonstrate a gradient effect of AD pathology on OI performance. In fact, Velayudhan and colleagues demonstrated a similar **dose-response** relationship in AD patients. They found that AD dementia severity was inversely related to odor naming performance.[17]

AD patients respond to Donepezil better than other forms of dementia. We posit that AD is a cause of olfactory deficit. OI is associated to a response or predicts response to Donepezil in AD and MCI.[18,19] A novel PET imaging technique that uses GV1-57 to quantify the olfactory sensory neuron (OSN) population during normal development, removal of the olfactory bulb, recovery, aging, and AD, highlights how olfactory loss can be reversible.[20] Cell renewal in pre-clinical animal supports positive observations of Donepezil on OI in individuals with AD (**experimental evidence)**.[18-20]

In our review of the anatomy and pathology (Chapter 2), we established **biological plausibility** for the association of odor identification deficit and AD neuropathology, even early in the AD pathological cascade. The section below on neurogenesis and genetics (5.3.2) in particular builds on our previous arguments and presents additional underlying mechanistic plausibility for our associations.

Animal studies demonstrate **coherence** with the implication of olfactory dysfunction in AD. In mice models of AD, an over-expression of human APP, in comparison to a control mice model, leads to decreased olfactory functions.[21,22]

Analogously, researchers are investigating associations between the integrity of the eye with presence of AD biomarkers in order to take advantage of an alternate and less invasive window into the brain in the early stages of AD.[23] Others have turned to central auditory processing (CAP) as an on-going sensori-neural function that could reflect AD-related change.[24] In summary, olfactory loss appears causally associated with AD progression more then chance alone and fullfills 8 out of the 9 Bradford-Hill criteria. OI may be a marker of neurodegeneration like AD.

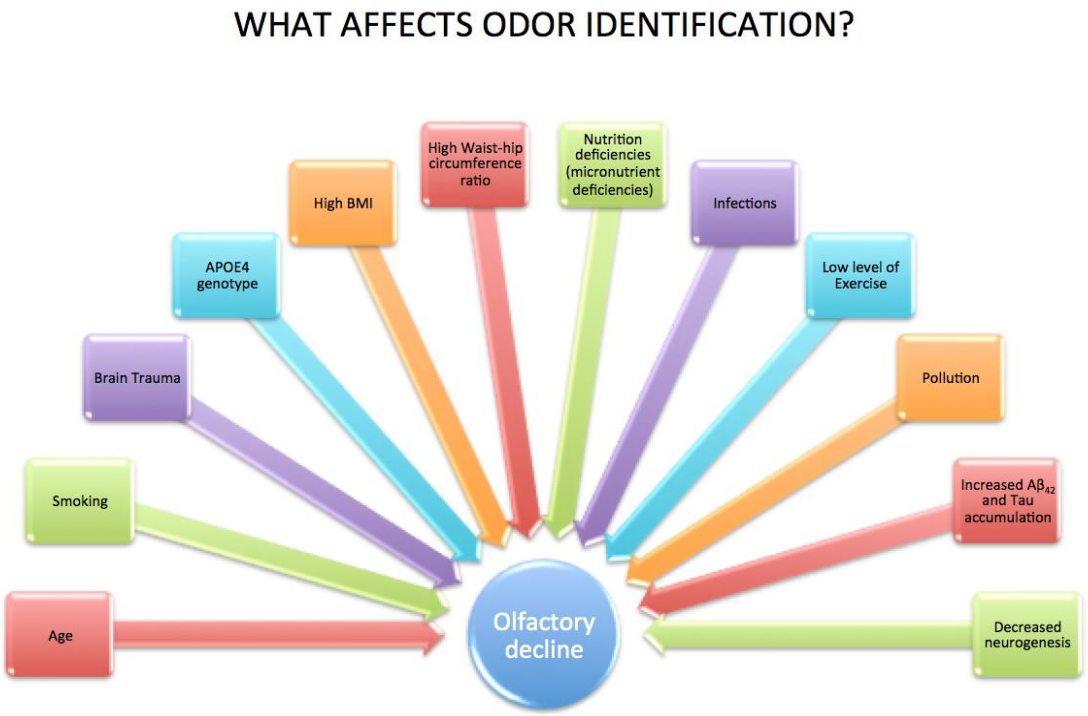


Figure 5-1 Lifestyle and environment factors for Alzheimer disease that also affect olfactory decline This figure is an overview of known environmental, lifestyle, and genetic factors that have the potential to modulate OI. The brain consumes 20% of the body's oxygen at rest.[25] Various health parameters impact olfactory dysfunction and increase the risk for AD: age, smoking, hypertension, high cholesterol levels, diabetes, obesity, sleep apnea, vascular diseases, and all cause mortality.[26-39] These variables may reflect reduced brain oxygenation. In addition, lesions from head trauma or strokes and their recovery are associated with declined olfactory function.[40-42]

5.3.2 Neurogenesis, Degeneration and Genetics

In addition to the arguments for causality (5.3.1), we highlight molecular and genetic mechanism that may affect olfactory functions and relate to subsquent AD dementia. The olfactory system has the remarkable ability to maintain and renew cell populations in the nasal epithelium and olfactory bulb (OB). Stem cells migrate from the temporal lobe to the olfactory bulbs through the rostral migratory stream (RMS), although the self-renewal power slows down as we age.[43,44] AD damages areas that support neurogenesis (Chapter 1 & 2). During regeneration, neuroblasts first make it through the tangles and plaques of a demented brain. Expectedly, as the pathology accumulates, the brain environment becomes increasingly harsh, and neurogenesis detrimentally changes with more advanced Braak stages.[45] As the OB are far from the subventricular zone, we could posit that olfactory dysfunction may reflect the inability to maintain the cell population even before cognitive symptoms of AD appear. In addition, the renewal mechanism itself makes the olfactory circuitry vulnerable to cell cycling errors or alteration in asymmetric cell division. Stem cells have a limited lifespan and suffer from cellular senescence.[46] Individuals with abnormal neuronal apoptosis from causes like acute brain trauma or a neurodegenerative disorder can lead to a higher demand for cell renewal. These sicker individuals could in theory run out of cell cycles in contrast to normal individuals with a lower cell turn-over rate. Stated differently, the stem cells have the potential to renew or maintain the OB population but chronic damage could lead to premature aging when cells reach the end of their life cycle. Declining ability to resupply and repair would then lead to a loss of integrity. Notably, the OB cell renewal reflects the upstream process that repopulates hippocampal neurons, a key structure for memory (Chapter 2). This process makes the olfactory system quite interesting to study in the context of AD. In fact, decreased plasticity and trophic maintenance of the cholinergic neurons may play a role in AD pathogenesis.[47,48] If OI deficits reflects a loss of cholinergic neuron that occurs early in AD progression (Chapter 1), OI could prove useful in trials of Donepezil (Chapter 2). In addition to the ability of the olfactory network to sustain adult cell renewal, its olfactory receptors (OR)

have a complex genetics, *viz.* 861 genes with 401 functional OR.[46,49-51]

Recently, studies have begun examining the genetic predisposition or alterations that contribute to AD and its progression. Data mining work has discovered associations between an AD-related phenotype and OR genes.[52] In ADNI, the Alzheimer's Disease Neuroimaging Initiative, grey matter density, the brain's neuronal cell bodies, was associated to 10 single nucleotide polymorphisms (SNPs) across different diagnostic groups (204 HC, 354 MCI, and 175 AD). Five of the SNPs from the olfactory pathways, reached a genome-wide significance level (*OR8K1, OR8K3, OR8K5, OR5R1, OP8U1*).[52]

Likewise, a high OR copy number was associated with a younger age at onset of AD.[53] This high copy number was located in the 14q.11.2 chromosomal segment and contained *OR4M1*, *OR4N2*, *OR4K2*, *OR4K5*, and *OR4K1.*[53] The authors suggested that olfaction might be a potential modifier for the age of onset.[53] Thus, OR gene variants may predispose the brain to earlier AD onset. High copy numbers can be due to a replication error that can occur during the development or adult cell renewal process. Both Shaw, Zieselman, and colleagues have investigated Alzheimer neuropathology and found a link with olfactory genetics; however, they were unable to address whether this genetic predisposition leads to olfactory impairment.[52,53] In Chapter 1, we reported a way in which AD pathology appears to be present across the olfactory anatomical circuitry. These genetics studies appear to show that OR genes may predispose the brain to AD.

Genetic studies have investigated the correlation of OI among older adults and its association to genetic polymorphisms in genome-wide meta-analysis association studies (GWAS). The first of these studies was in 1065 participants from the European American individuals from Communities (ARIC) study, the Health, Aging, and Body Composition (Health ABC) study, the Religious Orders Study and the Rush Memory and Aging Project (ROS/MAP). This GWAS suggested that 2 SNPs with cis (on the same

chromosome) on the microtubule-associated protein *tau* (MAPT) expression are susceptibility loci for OI impairment.[54] Furthermore, the CDK5 signaling pathway (involved in *tau* phosphorylation) was identified as the most likely genetically associated pathway with OI.[54] In a follow-up study of 6582 European Americans and 1979 African Americans, 37 SNPs achieved genome-wide significance in the African American sample only.[55] The function of the novel loci associated with OI in older adults include: neuron generation, neuron/neurite development, neurogenesis, neurotrophin signaling, cell projection, neuron differentiation, BAD phosphorylation, apoptosis, immunity, and cellular morphogenesis.[55]

In preliminary works, when investigating a subset of the PREVENT-AD individuals, the investigators found associations between OI and candidate neurodegeneration-associated polymorphisms *MAPT* and *CDK5RAP2* (Appendix E).[56] Thirty-one SNPs related to smell identification were significant after adjustments for age, gender, and APOE ε4 carrier status with a genome-wide significance threshold of $5*10^{-8}$ (Appendix E).[56] There were four SNPs on chromosome 3 and 14 that were strongly associated with OI ($p<5*10^{-12}$, Appendix E).[56] The population included in this work was mostly French Canadians descending from the French founding population and benefits from a signal/noise background. The SNPs identified were more prevalent in this population as compared to their expected allele frequency.

As for AD, hereditability appears to also play a great role. Studies of olfaction in first-degree relatives of AD patients found that these individuals have a worse sense of smell compared with family history negative controls.[57-59] In 28 family history positive relatives of AD dementia cases, the OI performance was 31.93, while it was 35.43 for family history negative controls on a 40-item scale.[58] In multiplex families from the Bryan Alzheimer Disease Research Centre (ADRC), with mutigenerational evidence of dementia, olfactory impairment in family history positive individuals appeared independent from an ε4 effect.[57] Another study found that OI was reduced in individuals at a higher risk for AD and that there were no differences in OI among ε4 carriers or

non-carriers.[59] We reproduced the latter observation and found that there were no obvious group differences in OI across ε4 carriers or non-carriers. Interestingly in Hendley et al.'s work, an ε4 carrier status by family history interaction explained worse OI performance, which meant that ε4 family history positive individuals did the worst and family history negative non-carriers performed the best.[59] In our work investigating 262 first-degree relatives of AD patients, we found no difference in odor identification in *APOE* ε4 carriers, but there was an interaction for lower OI in individuals with low levels of CSF amyloid and a concurrent ε4 carrier status (Chapter 3).[60] However, the relationship between odor identification and *APOE* remains unclear. The ε4 effect may differ throughout the progression of AD and observations may depend on the concomitant risk factors like family history. Together these family history studies highlighted the association of odor identification and cognitive decline related to AD. Furthermore, the population we tested had relatively low genetic mixing and a similar environmental background (Appendix E). Their family history and the increased prevalence of rare genes may hide an expected ε4 effect (Chapter 3 & 4). Further research investigating odor identification and genetic risks factors in family history positive and negative is warranted.

5.4 Limitations

5.4.1 Generalizability of findings

In this work, we report data from healthy adults. These individuals were predominately highly educated French women. In addition, our participants were healthier than the general public. Also, we might have suffered from self-selective attrition, and the most cognitively impaired subjects may have voluntarily dropped out of the study, or not undergone lumbar punctures. As successful agers may be more likely to continue follow-up visits, we could also suffer from an ascertainment bias. We had no external group without family history to compare our results with.

These studies we conducted in Montreal, Quebec, Canada, and we can assume a high proportion of individuals are from the founding population (approximately 85%). The French Canadian population is exceptionally interesting to study. Before the Quiet Revolution in the 1960s, this population, which most of our participants belong too, was governed by the Roman Catholic Church and were instructed to marry and have large families. Since the initial French settlements.[61-63] 8500 “survivors” of a long boat journey colonized Quebec in the 17th-18th century.[63] Surprisingly, less than 10 percent of these immigrants were female. This natural selection environment led to low genetic mixing and rare gene variant enrichment. Genealogical reconstructions indicate that fewer than 250 settlers introduced 21 diseases to Quebec.[63] From this founding population, some 34 founders contributed to more than one disease.[63] We may have benefited from this population with relatively low genetic background noise and increased prevalence of rare alleles. This peculiar genetic population might play a role in capturing early change in individuals at risk. Certainly, the large constitution of French Quebecers is a limiting factor that could make it difficult to reproduce our findings in non-founding populations.

In addition to the low genetic noise in the French Quebec population, the Quiet Revolution introduced the creation of Health and Education ministries to govern the standard medical and social care.[61] As a result, the variability in the health monitoring or general health may be narrower than studies done outside the Quebec population. In addition to the free and mandatory education, many study participants are bilingual. Bilingualism reduces the vulnerability to cognitive decline and it is associated with a more favorable CSF biomarker profile.[64,65] We have not explored this protective factor in our analyses, although recruiting more bilinguals than the general population could contribute to a selection bias.

We think that it is very important to replicate our findings in individuals from other founding populations such as the Northern European population, and also in more general populations that have a better representation of minorities and other cultural background. Also, the choice of the population used in the current thesis does not

permit us to assess odor identification progressively across mild cognitively impaired individuals or demented AD patients. A longer follow-up period is needed to evaluate a causal relationship if and when individuals begin to show cognitive symptoms.

5.4.2 Measurement and methodological limitations

There are potential limitations of using longitudinal data including practice effects. As suggested by Engen, participants may have gained experience and became accustomed to the experimental condition and learned what to pay attention to.[66] Longer studies where the practice effect disappears may be more insightful and clarify the observations from Chapter 4.

Although the UPSIT is correlated with odor detection ($r=-0.794$, $p<0.001$), our work did not include odor discrimination or detection information; therefore, we are uncertain as to what is attributable to the other aspects of olfaction.[67] Prior to our work, a picture odor identification test that removed lexical demands stood out from other studies of OI.[68,69] Schubert and colleagues reported that poor performers on this picture smell identification test were at greater risk to develop cognitive impairment (OR 6.62, 95% CI: 4.36–10.05).[69] This study used visual cues, which is a paradigm shift away from lexical cues that could have altered the multi-sensory processing of the individual. In fact, visual-olfactory congruence can speed up the odor detection process as observed in “the nose smells what the eyes sees” experiment.[70] In contrast, visual incongruence can interfere with sensory processing. In a classical example, oenology students were duped by the visual cue and confused white wines colored red with red wines.[71] Beyond a possible interaction between visual and odor cues, the OI task from Schubert and colleagues, appears to be a discrimination task, *viz.* evaluation of similarities or differences, as there were eight pictures that represent odors being tested and 12 distractors on a picture board to aid in identification.[69] Participants were free to vocalize or point to what the odor-object represented.[69] Surprisingly others have shown that a composite of olfactory function that included odor detection, discrimination, and

identification was predictive of subsequent cognitive decline, as was, discrimination alone, but not OI alone.[72] In contrast, a study suggested that poor odor discrimination is partly due to a higher odor detection threshold in MCI but not AD patients,[73] while between-group differences in OI (controls vs MCI, controls vs AD) remained after controlling for both detection and discrimination.[73] Despite discrimination having a lower hierarchical processing than OI, it may be quite interesting to investigate its value in pre-symptomatic AD. Odor discrimination may be indicative of an important ongoing process related to Alzheimer neuropathology, especially since imaging has shown that such a task elicits hippocampal activity.[74] Hippocampal activation during discrimination supports the pursuit to evaluate and compare odor discrimination and identification in pre-symptomatic AD.[74]

Another potential limitation is that we used the 40-item test instead of the shorter and more commonly used 10-12 item versions, so we may be using a more sensitive test with a broader range of scores that can reflect subtler change. This raises the issue that shorter and more economical tests may be less sensitive. It would be interesting to evaluate if we would have found the same association with a smaller spread of odors, like those used in the BSIT as opposed to the UPSIT. Would we have come to the same conclusions using alternate tests?

Olfaction tests are not always administered during a neurological examination unless there is a complaint. Even then physicians may use different kits or tests when they conduct their examination. It would be very helpful to suggest to doctors which patients should get an olfactory test as a routine and which test or odorants should be used in a way that it is practical and not too expensive. Future exploration of our results will aim to evaluate specific odor changes (item-level). An odor identification test is not a substitution for a neuropsychological test battery. For now, we are humble and refrain to recommend any specific odor or odor test for current clinical diagnostic of pre-symptomatic Alzheimer's disease. We also warrant that physicians should not expect accurate self-reporting of olfactory deficits or loss.[85,86] If a patient complains of olfactory

problems, they should refer them to otorhinolaryngology specialists for an objective evaluation with a test like the UPSIT or Sniffin' Sticks and an appropriate intervention if necessary. We do urge others to include more sensory testing in studies to expand on our work in clinical trials and longitudinal studies.

There are no treatments to reverse olfactory decline in Alzheimer disease. A mindfull practice of smelling a variety of odors several times a day has been demonstrated favorable for PD patients.[80,81] Perfumers appear to have enhanced thickness of the orbitofrontal cortex and piriform gyrus compared to non-experts.[83] Olfactory training could potentially prevent olfactory loss in older people.[82] Studies of smell experts comprising of wines experts or perfume creators, may point to a new intervention method, or the use of less appreciated approach such as multi-sensory ecosystem/Snoezelen. Recently, The Lancet Commission on Dementia Prevention, Intervention, and Care has made recommendations to reduce incidence of dementia. The commission suggested active treatment of hypertension in 45-65 year olds.[84] They recommended social engagement, exercise, reducing smoking, management of diabetes, depression, and obesity. Most relevant to this thesis, the commission recommended addressing hearing loss.[84] Perhaps with enough research, recommendations for olfactory loss will follow.

5.5 Future direction

Jack and colleagues developed a hypothetical framework of biomarker change and detection threshold.[87] Based on results from Chapter 4 and the above section on neurogenesis (5.3.2.), I argue that odor identification changes should appear first (Fig. 5-2). In the future, we will further explore this with big data algorithms and study its association with multiple imaging modalities over time to better understand odor identification dysfunction. Further analyses using multi-modal imaging will enable us to disentangle what processes are simultaneously involved and which make individuals at risk of AD more vulnerable to decline. This should enlighten us as to where odor identification performance fits on the AD continuum.

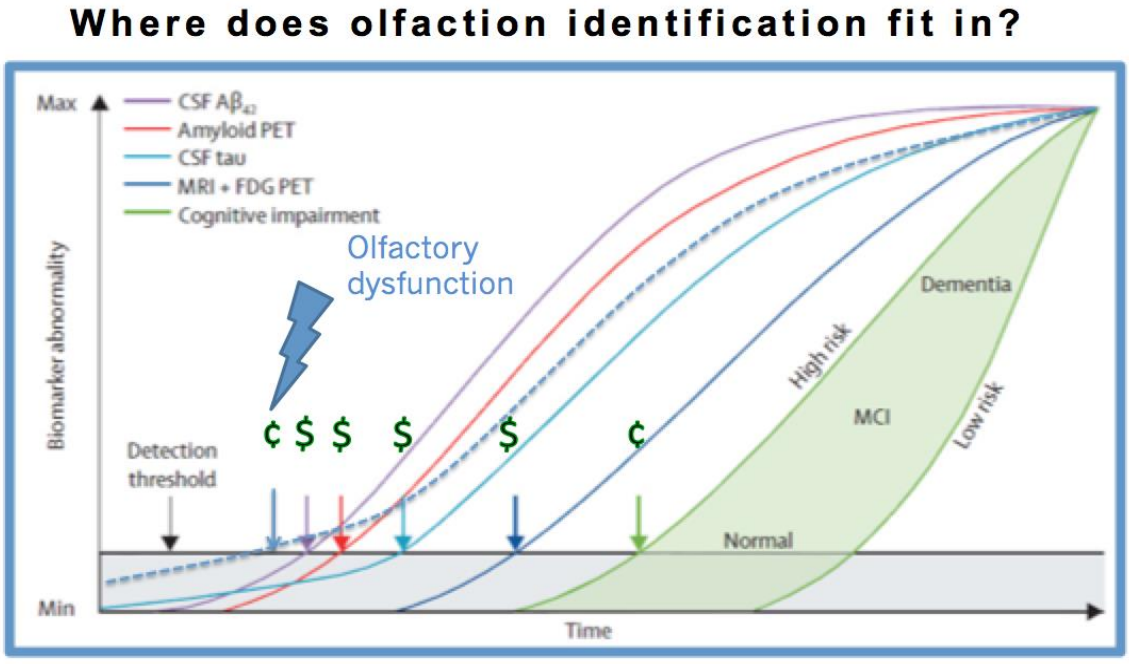


Figure 5-2 Hypothetical model of AD biomarker detection.

This figure includes olfactory dysfunction. Modified from Jack, 2013[87]. Dollar symbols indicate costly measures and cent symbols highlight economical measures.

To paraphrase Hachinski on CBF: “Our ignorance far exceeds our knowledge about [olfactory identification] in [Alzheimer’s disease]”.[88] As mentioned above, there are many non-genetic factors, besides age, education, and sex that can affect olfactory function. Some of these include cardio-vascular risk factors, sleep apnea, nutrition, air pollution and smoke exposure. In fact, bariatric surgery in morbidly obese patients, has shown improved chemosensory function, which includes olfactory functions.[89] This example illustrates how powerful lifestyle can affect OI and how it could be an interesting avenue into prevention. We are interested to investigate the association between blood pressure, cerebral blood flow, the control of their blood sugar level (HBA1C), and cholesterol level in the PREVENT-AD population. To date, we have preliminary results that we will expand upon with 5 years of data acquisition (Appendix B). Furthermore, we are curious to evaluate if OI or olfactory function reflects accumulated damage of pollution as in the works of Calderon-Garciduenas.[90] We are interested in exploring the impact of pollution on upper respiratory function, cardiovascular function, neurodegeneration, mortality and whether OI can mirror general brain health irrespective of the co-morbidity. Recently, Canadian data suggest that pollution exposure may increase the risk for AD.[91] Such findings were repeatedly observed in Mexico, after matching for socio-economic factors to the best of their ability.[92,93] These studies have great importance for public health initiatives and national transport and environmental policy making. We applied for ethics and collected information in order to conduct work on pollution exposure and odor identification in the PREVENT-AD cohort.

Going back the OI’s lack of specificity (5.3.1), there may be a way to circumvent this problem and improve the study paradigm. Data suggests that homozygotes are more vulnerable to a decline in function.[94] Sophisticated OI test variations may be more sensitive to an ε4 effect. Olfactory testing after intra-nasal atropine, an anti-cholinergic drug, is an alternative test paradigm that can inform us on the cholinergic vulnerability of participants, and has detected a difference in ε4 carriers and non-carriers.[95,96] This is important as AD dementia cases have an increased prevalence of carriers compared to the normal population. This smell test is analogous to pupil dilatation with tropicamide

and tests the integrity of the cholinergic system.[95-97] This “olfactory stress test” is reminiscent of the original cognitive “stress test” and its effect on dichotic listening task results in young individuals who behaved as if they had cognitive decline once scopolamine blocked cholinergic neurons.[98] The olfactory stress test appeared to show differences in ε4 carriers and non-carriers that have suggested that non-carriers can sustain some cholinergic inhibition or loss but carriers are not as resilient. This new testing paradigm could be interesting to further investigate the association between odor identification and the cholinergic system. Notably, most AD dementia treatment drugs inhibit acetylcholine esterase, a major enzyme involved in the breakdown of acetylcholine, the neurotransmitter of cholinergic neurons (see Chapter 2).

Noteworthy, we did not pay much attention to trigeminal function or its possible contribution. It is important to note that the trigeminal nerve contributes to olfaction.[75,76] For instance, olfactory loss is associated with trigeminal impairment.[77,78] Most odors also stimulate the trigeminal nerve.[79] This cranial nerve receives nociceptive input and there are several areas of the brain, such as the piriform cortex, insula, middle frontal gyrus, and the orbiotofrontal cortex, that are both activated by the olfactory and trigeminal nerve.[75] In the future, we could expand our work to assess the effect of nociception on OI performance.

In Appendix B, we provide a preliminary study of how cardiovascular health and risk factors associate with OI performance. Later, in appendix C, we provide a preliminary study of how odor identification is associated with hippocampal volume. In Appendix D, we provide a preliminary study of how odor identification performance is associated with verbal intrusions and primacy during list learning and story memory. Finally, in appendix E, we provide a preliminary study of how odor identification is associated with a variant of MAPT and CDK5rap2, as well as several rare variants obtained by GWAS in a mostly homogenous genetic and cultural population with a first-degree relative affected by AD-type dementia. We will expand this work using 5 years of longitudinal cohort data and external datasets.

5.6 Summary

This thesis intended to empirically demonstrate that declines in odor identification occur before the emergence of cognitive impairment. Figure 5-3, below, illustrates how we envision the AD-related neurodegeneration process occurs and affects olfactory functions.

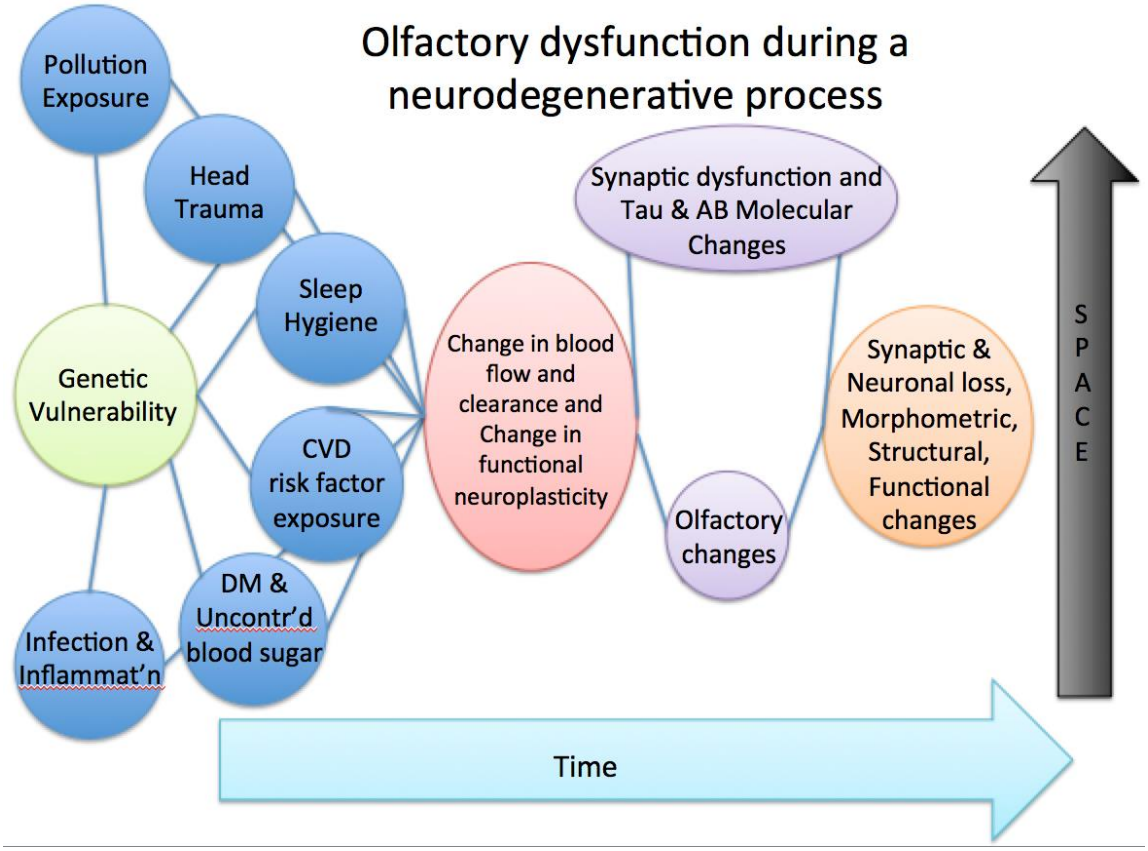


Figure 5-3 Olfactory dysfunction during a neurodegenerative process

Although the results presented in this thesis are novel, they build on previous literature and other modalities to demonstrate an encouraging prospect for early detection in a population at increased risk of AD. We observed decline in olfaction that were related to AD neuropathology as measured by CSF t-*tau*/$A\beta_{1-42}$, and P_{181}-*tau*/$A\beta_{1-42}$, immediate memory decline, and aging. Our work supports the potential use of odor identification to evaluate changes related to AD.

Presently, clinical trials are moving upstream of cognitive decline, and including broader olfactory testing could inform us on sensori-neural mechanisms. This method shows promising cost-effectiveness. In the future, the combination of the UPSIT with other markers of AD may help to decide if a person should have further tests done (expensive or invasive). Odor identification testing may have a low cost per quality-adjusted life-year (QALY) if it proves to lead to better health monitoring. Overall, this PhD advanced the field of OI and AD with regards to modeling risk and progression in otherwise healthy individuals.

5.7 References

1 Rahayel, S., Frasnelli, J. & Joubert, S. The effect of Alzheimer's disease and Parkinson's disease on olfaction: a meta-analysis. *Behavioural brain research* **231**, 60-74, doi:10.1016/j.bbr.2012.02.047 (2012).

2 Growdon, M. E. *et al.* Odor identification and Alzheimer disease biomarkers in clinically normal elderly. *Neurology* **84**, 2153-2160, doi:10.1212/WNL.0000000000001614 (2015).

3 Roalf, D. R. *et al.* A quantitative meta-analysis of olfactory dysfunction in mild cognitive impairment. *Journal of neurology, neurosurgery, and psychiatry* **88**, 226-232, doi:10.1136/jnnp-2016-314638 (2017).

4 Vassilaki, M. *et al.* Neuroimaging biomarkers and impaired olfaction in cognitively normal individuals. *Annals of neurology* **81**, 871-882, doi:10.1002/ana.24960 (2017).

5 Leoutsakos, J., Gross, A., Jones, R., Albert, M. & Breitner, J. Alzheimer progression score: development of a biomarker summary outcome for AD prevention trials. *J Prev Alz Dis* **3**, 229-235 (2016).

6 Wilson, R. S., Arnold, S. E., Schneider, J. A., Tang, Y. & Bennett, D. A. The relationship between cerebral Alzheimer's disease pathology and odour identification in old age. *Journal of neurology, neurosurgery, and psychiatry* **78**, 30-35, doi:10.1136/jnnp.2006.099721 (2007).

7 Wilson, R. S. *et al.* Olfactory impairment in presymptomatic Alzheimer's disease. *Annals of the New York Academy of Sciences* **1170**, 730-735, doi:10.1111/j.1749-6632.2009.04013.x (2009).

8 Roberts, R. O. *et al.* Association Between Olfactory Dysfunction and Amnestic Mild Cognitive Impairment and Alzheimer Disease Dementia. *JAMA Neurol*, 1-9, doi:10.1001/jamaneurol.2015.2952 (2015).

9 Wilson, R. S., Arnold, S. E., Tang, Y. & Bennett, D. A. Odor identification and decline in different cognitive domains in old age. *Neuroepidemiology* **26**, 61-67 (2006).

10 Bradford, A. Association or Causation? *Proc R Soc Med.* **58**, 295-300 (1965).

11 Mesholam, R. I., Moberg, P. J., Mahr, R. N. & Doty, R. L. Olfaction in neurodegenerative disease: a meta-analysis of olfactory functioning in Alzheimer's and Parkinson's diseases. *Archives of neurology* **55**, 84-90 (1998).

12 Ayala- Grosso, C. A. *et al.* Amyloid- Aβ Peptide in Olfactory Mucosa and Mesenchymal Stromal Cells of Mild Cognitive Impairment and Alzheimer's Disease Patients. *Brain Pathology* **25**, 136-145 (2015).

13 Lafaille-Magnan, M.-E. *et al.* Odor identification as a biomarker of preclinical AD in older adults at risk. *Neurology* **89**, 327-335 (2017).

14 Reijs, B. L. R. *et al.* Relation of Odor Identification with Alzheimer's Disease Markers in Cerebrospinal Fluid and Cognition. *Journal of Alzheimer's disease : JAD* **60**, 1025-1034, doi:10.3233/JAD-170564 (2017).

15 Scheltens, P. *et al.* Alzheimer's disease. *Lancet*, doi:10.1016/S0140-6736(15)01124-1 (2016).

16 Schrag, A., Siddiqui, U. F., Anastasiou, Z., Weintraub, D. & Schott, J. M. Clinical variables and biomarkers in prediction of cognitive impairment in patients with newly diagnosed Parkinson's disease: a cohort study. *The Lancet Neurology* **16**, 66-75 (2017).

17 Velayudhan, L., Pritchard, M., Powell, J. F., Proitsi, P. & Lovestone, S. Smell identification function as a severity and progression marker in Alzheimer's disease. *International psychogeriatrics* **25**, 1157-1166 (2013).

18 Pelton, G. H., Soleimani, L., Roose, S. P., Tabert, M. H. & Devanand, D. P. Olfactory Deficits Predict Cognitive Improvement on Donepezil in Patients With Depression and Cognitive Impairment: A Randomized Controlled Pilot Study. *Alzheimer disease and associated disorders* **30**, 67-69, doi:10.1097/WAD.0000000000000107 (2016).

19 Velayudhan, L. & Lovestone, S. Smell identification test as a treatment response marker in patients with Alzheimer disease receiving donepezil. *Journal of clinical psychopharmacology* **29**, 387-390, doi:10.1097/JCP.0b013e3181aba5a5 (2009).

20 Van de Bittner, G. C. *et al.* Nasal neuron PET imaging quantifies neuron generation and degeneration. *J Clin Invest* **127**, 681-694, doi:10.1172/JCI89162 (2017).

21 Macknin, J. B., Higuchi, M., Lee, V. M.-Y., Trojanowski, J. Q. & Doty, R. L. Olfactory dysfunction occurs in transgenic mice overexpressing human τ protein. *Brain research* **1000**, 174-178 (2004).

22 Wesson, D. W., Levy, E., Nixon, R. A. & Wilson, D. A. Olfactory dysfunction correlates with amyloid-beta burden in an Alzheimer's disease mouse model. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **30**, 505-514, doi:10.1523/JNEUROSCI.4622-09.2010 (2010).

23 Shah, T. M., Gupta, S. M., Chatterjee, P., Campbell, M. & Martins, R. N. Beta-amyloid sequelae in the eye: a critical review on its diagnostic significance and clinical relevance in Alzheimer's disease. *Mol Psychiatry* **22**, 353-363, doi:10.1038/mp.2016.251 (2017).

24 Tuwaig, M. *et al.* Deficit in Central Auditory Processing as a Biomarker of Pre-Clinical Alzheimer's Disease. *Journal of Alzheimer's Disease*, 1-12 (2017).

25 Raichle, M. E. *et al.* A default mode of brain function. *Proc Natl Acad Sci U S A* **98**, 676-682, doi:10.1073/pnas.98.2.676 (2001).

26 Ajmani, G. S., Suh, H. H., Wroblewski, K. E. & Pinto, J. M. Smoking and olfactory dysfunction: A systematic literature review and meta-analysis. *The Laryngoscope* **127**, 1753-1761, doi:10.1002/lary.26558 (2017).

27 Daulatzai, M. A. Olfactory dysfunction: its early temporal relationship and neural correlates in the pathogenesis of Alzheimer's disease. *Journal of neural transmission* **122**, 1475-1497 (2015).

28 Daulatzai, M. A. Dysfunctional Sensory Modalities, Locus Coeruleus, and Basal Forebrain: Early Determinants that Promote Neuropathogenesis of Cognitive and Memory Decline and Alzheimer's Disease. *Neurotox Res* **30**, 295-337, doi:10.1007/s12640-016-9643-3 (2016).

29 Fischer, M. E. *et al.* The association of taste with change in adiposity-related health measures. *Journal of the Academy of Nutrition and Dietetics* **114**, 1195-1202 (2014).

30 Ekstrom, I. *et al.* Smell Loss Predicts Mortality Risk Regardless of Dementia Conversion. *Journal of the American Geriatrics Society*, doi:10.1111/jgs.14770 (2017).

31 Schubert, C. R. *et al.* Sensory Impairments and Risk of Mortality in Older Adults. *The journals of gerontology. Series A, Biological sciences and medical sciences* **72**, 710-715, doi:10.1093/gerona/glw036 (2017).

32 Pinto, J. M. *et al.* Global Sensory Impairment Predicts Morbidity and Mortality in Older U.S. Adults. *Journal of the American Geriatrics Society*, doi:10.1111/jgs.15031 (2017).

33 Devanand, D. P. *et al.* Olfactory identification deficits and increased mortality in the community. *Annals of neurology* **78**, 401-411 (2015).

34 Wilson, R. S., Yu, L. & Bennett, D. A. Odor identification and mortality in old age. *Chemical senses* **36**, 63-67 (2010).

35 Fischer, M. E. *et al.* Age-Related Sensory Impairments and Risk of Cognitive Impairment. *Journal of the American Geriatrics Society* **64**, 1981-1987, doi:10.1111/jgs.14308 (2016).

36 Schubert, C. R. *et al.* Inflammatory and vascular markers and olfactory impairment in older adults. *Age and ageing* **44**, 878-882 (2015).

37 Doty, R. L. The olfactory vector hypothesis of neurodegenerative disease: is it viable? *Annals of neurology* **63**, 7-15 (2008).

38 Doty, R. L. in *Seminars in neurology*. 074-081 (© Thieme Medical Publishers).

39 Doty, R. L. Olfactory dysfunction in Parkinson disease. *Nature Reviews Neurology* **8**, 329-339 (2012).

40 Frasnelli, J. *et al.* Olfactory function in acute traumatic brain injury. *Clinical neurology and neurosurgery* **140**, 68-72 (2016).

41 Alosco, M. L. *et al.* Olfactory Function and Associated Clinical Correlates in Former National Football League Players. *Journal of neurotrauma* **34**, 772-780, doi:10.1089/neu.2016.4536 (2017).

42 Wehling, E. *et al.* Olfactory dysfunction in chronic stroke patients. *BMC neurology* **15**, 199 (2015).

43 Curtis, M. A. *et al.* Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. *science* **315**, 1243-1249 (2007).

44 Enwere, E. *et al.* Aging results in reduced epidermal growth factor receptor signaling, diminished olfactory neurogenesis, and deficits in fine olfactory discrimination. *Journal of Neuroscience* **24**, 8354-8365 (2004).

45 Perry, E. K. *et al.* Neurogenic abnormalities in Alzheimer's disease differ between stages of neurogenesis and are partly related to cholinergic pathology. *Neurobiology of disease* **47**, 155-162 (2012).

46 Fuchs, E. The tortoise and the hair: slow-cycling cells in the stem cell race. *Cell* **137**, 811-819, doi:10.1016/j.cell.2009.05.002 (2009).

47 Counts, S. E. & Mufson, E. J. The role of nerve growth factor receptors in cholinergic basal forebrain degeneration in prodromal Alzheimer disease. *Journal of Neuropathology & Experimental Neurology* **64**, 263-272 (2005).

48 Mufson, E. J. *et al.* Loss of nucleus basalis neurons containing trkA immunoreactivity in individuals with mild cognitive impairment and early Alzheimer's disease. *The Journal of comparative neurology* **427**, 19-30 (2000).

49 Eriksson, P. S. *et al.* Neurogenesis in the adult human hippocampus. *Nature medicine* **4** (1998).

50 Curtis, M. A. *et al.* Human neuroblasts migrate to the olfactory bulb via a lateral ventricular extension. *Science* **315**, 1243-1249, doi:10.1126/science.1136281 (2007).

51 (HGNC)www.genenames.org., H. G. N. C. <https://www.genenames.org/cgi-bin/genefamilies/set/141> (

52 Zieselman, A. L. *et al.* Computational genetics analysis of grey matter density in Alzheimer's disease. *BioData mining* **7**, 17 (2014).

53 Shaw, C. *et al.* Olfactory copy number association with age at onset of Alzheimer disease. *Neurology* **76**, 1302-1309 (2011).

54 Dong, J. *et al.* Genome-wide meta-analysis on the sense of smell among US older adults. *Medicine* **94** (2015).

55 Dong, J. *et al.* Genome-Wide Association Analysis of the Sense of Smell in US Older Adults: Identification of Novel Risk Loci in African-Americans and European-Americans. *Molecular neurobiology*, 1-12 (2016).

56 Lafaille-Magnan, M.-E. *et al.* CANDIDATE GENE STUDIES AND GWAS SUGGEST SUBSTANTIAL GENETIC INFLUENCE ON DEFICITS IN OLFACTORY IDENTIFICATION AMONG PERSONS AT RISK OF AD. *Alzheimer's & Dementia* **13**, P1289-P1290 (2017).

57 Schiffman, S., Graham, B., Sattely-Miller, E., Zervakis, J. & Welsh-Bohmer, K. Taste, smell and neuropsychological performance of individuals at familial risk for Alzheimer's disease. *Neurobiology of aging* **23**, 397-404 (2002).

58 Serby, M. *et al.* Olfactory identification deficits in relatives of Alzheimer's disease patients. *Biological psychiatry* **39**, 375-377 (1996).

59 Handley, O. J., Morrison, C. M., Miles, C. & Bayer, A. J. ApoE gene and familial risk of Alzheimer's disease as predictors of odour identification in older adults. *Neurobiology of aging* **27**, 1425-1430, doi:10.1016/j.neurobiolaging.2005.08.001 (2006).

60 Lafaille-Magnan, M.-E. *et al.* Odor identification as a biomarker of preclinical AD in older adults at risk. *Neurology*, doi:10.1212/wnl.0000000000004159 (2017).

61 Bernier, L., Bouchard, M. & Lévesque, B. Attending to the general interest: New mechanisms for mediating between the individual, collective and general interest in Quebec. *Annals of public and cooperative economics* **74**, 321-348 (2003).

62 Laberge, A. M. *et al.* Population history and its impact on medical genetics in Quebec. *Clinical genetics* **68**, 287-301 (2005).

63 Scriver, C. R. Human genetics: lessons from Quebec populations. *Annual review of genomics and human genetics* **2**, 69-101 (2001).

64 Li, L. *et al.* How bilingualism protects the brain from aging: Insights from bimodal bilinguals. *Hum Brain Mapp* **38**, 4109-4124, doi:10.1002/hbm.23652 (2017).

65 Estanga, A. *et al.* Beneficial effect of bilingualism on Alzheimer's disease CSF biomarkers and cognition. *Neurobiology of aging* **50**, 144-151, doi:10.1016/j.neurobiolaging.2016.10.013 (2017).

66 Engen, T. Effect of practice and instruction on olfactory thresholds. *Perceptual and motor skills* **10**, 195-198 (1960).

67 Doty, R. L., Shaman, P. & Dann, M. Development of the University of Pennsylvania Smell Identification Test: a standardized microencapsulated test of olfactory function. *Physiol Behav* **32**, 489-502 (1984).

68 Morgan, C. D., Nordin, S. & Murphy, C. Odor identification as an early marker for Alzheimer's disease: impact of lexical functioning and detection sensitivity. *Journal of clinical and experimental neuropsychology* **17**, 793-803 (1995).

69 Schubert, C. R. *et al.* Olfaction and the 5- Year Incidence of Cognitive Impairment in an Epidemiological Study of Older Adults. *Journal of the American Geriatrics Society* **56**, 1517-1521 (2008).

70 Gottfried, J. A. & Dolan, R. J. The nose smells what the eye sees: crossmodal visual facilitation of human olfactory perception. *Neuron* **39**, 375-386 (2003).

71 Morrot, G., Brochet, F. & Dubourdieu, D. The color of odors. *Brain and language* **79**, 309-320 (2001).

72 Sohrabi, H. *et al.* Olfactory discrimination predicts cognitive decline among community-dwelling older adults. *Translational Psychiatry* **2**, e118 (2012).

73 Djordjevic, J., Jones-Gotman, M., De Sousa, K. & Chertkow, H. Olfaction in patients with mild cognitive impairment and Alzheimer's disease. *Neurobiology of aging* **29**, 693-706 (2008).

74 Kareken, D. A., Mosnik, D. M., Doty, R. L., Dzemidzic, M. & Hutchins, G. D. Functional anatomy of human odor sensation, discrimination, and identification in health and aging. *Neuropsychology* **17**, 482 (2003).

75 Albrecht, J. *et al.* The neuronal correlates of intranasal trigeminal function—an ALE meta-analysis of human functional brain imaging data. *Brain research reviews* **62**, 183-196 (2010).

76 Pinto, J. M. Olfaction. *Proceedings of the American Thoracic Society* **8**, 46-52, doi:10.1513/pats.201005-035RN (2011).

77 Frasnelli, J., Schuster, B. & Hummel, T. Interactions between olfaction and the trigeminal system: what can be learned from olfactory loss. *Cerebral Cortex* **17**, 2268-2275 (2006).

78 Tremblay, C., Martel, P. D. & Frasnelli, J. Trigeminal system in Parkinson's disease: A potential avenue to detect Parkinson-specific olfactory dysfunction. *Parkinsonism & Related Disorders* (2017).

79 Brand, G. Olfactory/trigeminal interactions in nasal chemoreception. *Neurosci Biobehav Rev* **30**, 908-917, doi:10.1016/j.neubiorev.2006.01.002 (2006).

80 Knudsen, K., Flensborg Damholdt, M., Mouridsen, K. & Borghammer, P. Olfactory function in Parkinson's Disease–effects of training. *Acta neurologica Scandinavica* **132**, 395-400 (2015).

81 Haehner, A. *et al.* Olfactory training in patients with Parkinson's disease. *PloS one* **8**, e61680, doi:10.1371/journal.pone.0061680 (2013).

82 Schriever, V. A., Lehmann, S., Prange, J. & Hummel, T. Preventing olfactory deterioration: olfactory training may be of help in older people. *Journal of the American Geriatrics Society* **62**, 384-386 (2014).

83 Delon-Martin, C., Plailly, J., Fonlupt, P., Veyrac, A. & Royet, J.-P. Perfumers' expertise induces structural reorganization in olfactory brain regions. *Neuroimage* **68**, 55-62 (2013).

84 Livingston, G. *et al.* Dementia prevention, intervention, and care. *The Lancet*, doi:10.1016/s0140-6736(17)31363-6 (2017).

85 White, T. L., Sadikot, A. F. & Djordjevic, J. Metacognitive knowledge of olfactory dysfunction in Parkinson's disease. *Brain and cognition* **104**, 1-6 (2016).

86 Walliczek-Dworschak, U. & Hummel, T. The Human Sense of Olfaction. *Facial Plastic Surgery* **33**, 396-404 (2017).

87 Jack, C. R., Jr. & Holtzman, D. M. Biomarker modeling of Alzheimer's disease. *Neuron* **80**, 1347-1358, doi:S0896-6273(13)01133-1 [pii];10.1016/j.neuron.2013.12.003 [doi] (2013).

88 Hachinski, V. C. *et al.* Cerebral blood flow in dementia. *Archives of neurology* **32**, 632-637 (1975).

89 Holinski, F., Menenakos, C., Haber, G., Olze, H. & Ordemann, J. Olfactory and Gustatory Function After Bariatric Surgery. *Obes Surg* **25**, 2314-2320, doi:10.1007/s11695-015-1683-x (2015).

90 Calderon-Garciduenas, L., Torres-Jardon, R., Kulesza, R. J., Park, S. B. & D'Angiulli, A. Air pollution and detrimental effects on children's brain. The need for a multidisciplinary approach to the issue complexity and challenges. *Frontiers in human neuroscience* **8**, 613, doi:10.3389/fnhum.2014.00613 (2014).

91 Chen, H. *et al.* Living near major roads and the incidence of dementia, Parkinson's disease, and multiple sclerosis: a population-based cohort study. *The Lancet* **389**, 718-726, doi:10.1016/s0140-6736(16)32399-6 (2017).

92 Calderon-Garciduenas, L. *et al.* Neuroinflammation, hyperphosphorylated tau, diffuse amyloid plaques, and down-regulation of the cellular prion protein in air pollution exposed children and young adults. *Journal of Alzheimer's disease : JAD* **28**, 93-107, doi:10.3233/JAD-2011-110722 (2012).

93 Calderon-Garciduenas, L. *et al.* White matter hyperintensities, systemic inflammation, brain growth, and cognitive functions in children exposed to air pollution. *Journal of Alzheimer's disease : JAD* **31**, 183-191, doi:10.3233/JAD-2012-120610 (2012).

94 Josefsson, M., Larsson, M., Nordin, S., Adolfsson, R. & Olofsson, J. APOE-ε4 effects on longitudinal decline in olfactory and non-olfactory cognitive abilities in middle-aged and old adults. *Scientific Reports* **7** (2017).

95 Schofield, P. W., Ebrahimi, H., Jones, A. L., Bateman, G. A. & Murray, S. R. An olfactory ‘stress test’may detect preclinical Alzheimer’s disease. *BMC neurology* **12**, 24 (2012).

96 Schofield, P. W., Finnie, S. & Yong, Y. M. The role of olfactory challenge tests in incipient dementia and clinical trial design. *Current neurology and neuroscience reports* **14**, 479 (2014).

97 Doty, R. L. Olfactory dysfunction in neurodegenerative diseases: is there a common pathological substrate? *The Lancet Neurology* **16**, 478-488, doi:10.1016/s1474-4422(17)30123-0 (2017).

98 Drachman, D. A., Noffsinger, D., Sahakian, B. J., Kurdziel, S. & Fleming, P. Aging, memory, and the cholinergic system: a study of dichotic listening. *Neurobiology of aging* **1**, 39-43 (1980).

Appendix A - Approved Ethics, Study Renewal, and Consent Form for Studies on Olfaction in the PREVENT-AD cohort

Protocol, Version 1.8
Amendment April 6, 2017

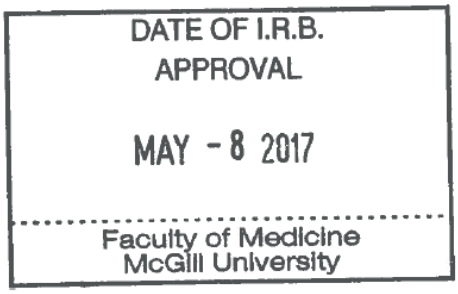
PRE-SYMPTOMATIC EVALUATION OF EXPERIMENTAL OR NOVEL TREATMENTS FOR ALZHEIMER'S DISEASE (PREVENT-AD)

- The core PREVENT-AD cohort -

PRINCIPAL INVESTIGATOR: John C. S. Breitner, MD, MPH
Director, Center for Studies on Prevention of Alzheimer's Disease (StoP-AD)
Douglas Mental Health University Institute – Research Centre Professor, Department of Psychiatry, McGill University Faculty of Medicine

OTHER INVESTIGATORS:
Veronique Bohbot, PhD
Pierre Etienne, MD
Joanne Frenette, RN
Serge Gauthier, MD
Marie-Elyse Lafaille-Magnan (PhD Candidate)
Vasavan Nair, MD
Sylvia Villeneuve, PhD
Judes Poirier, PhD
M. Natasha Rajah, PhD
Jens Preussner, PhD
Pedro Rosa-Neto, MD

Research funded by McGill University, by an unrestricted research grant from Pfizer Canada, and by the Douglas Mental Health University Institute – Research Centre, and by an Alzheimer's Association Research Grant



MCGILL IRB APPROVAL – AUGUST 29TH, 2016

McGill Faculty of Medicine

Institutional Review Board
- CONTINUING REVIEW FORM -

The completed form is to be submitted electronically to submit2irb.med@mcgill.ca. The continuing review form must be received at least **one (1) month** before the expiration of the last ethics approval. If you require additional information, please visit the IRB website at: http://www.mcgill.ca/medresearch/ethics/ or by calling 514-398-3124.

Principal Investigator	John C.S. Breitner, MD, MPH
Faculty and Department	Medicine, Psychiatry
Study Coordinator, if applicable	Marie-Elyse Lafaille-Magnan interim (Jennifer Tremblay-Mercier on Mat leave)
Address:	6875, boulevard LaSalle (Perry Pavilion E-2209), Montreal, Qc
E-mail	marie-elyse.lafaille-magnan@mail.mcgill.ca Telephone: 514-761-6131 #3959
Study Title	Pre-symptomatic evaluation of emerging novel treatments for prevention of Alzheimer's Disease
Grant title, if different from study title.	
IRB Study Number	A05-B16-11B Date of last approval 09/05/2016
Has there been a change or addition to the financial support for this study?	(●) YES ( ) NO
If yes, please specify the changes/additions.	Alzheimer's Association Research Grant

Status of the Protocol

- [x] Active enrolment — When did this study begin? 08/29/2011
- [ ] Recruitment complete
- [ ] Recruitment on hold
- [ ] Data analysis
- [ ] Secondary Analysis only
- [ ] Inactive/dormant**

RECEIVED
McGill University
APR 7 - 2017
FACULTY OF MEDECINE
IRB

**If the study is inactive/ dormant (i.e., there are no participants enrolled in the study and no study activity is occurring), please specify the reason:

If the study is is actively enrolling participants, or if enrolment is complete, please answer the following questions:

Study sample size:	500
Total number enrolled in the study:	435

Number of participants that have completed this study: 0

Total number of participants withdrawn: 79

Projected date of completion of study enrolment:

Projected date of study completion:

Please provide a brief description of what has occurred since the IRB's last ethics approval.

We continued enrollment. Participants can give consent to participate in sub-study on PET imaging. We applied for ethics to have a sub-study on stem cells using participants urine samples. We applied to have ethics to do more olfactory tests.

Has the study revealed any new findings or knowledge relevant to the potential benefits and/or study risks that may influence participants' willingness to continue in the study?
- ○ YES
- ◉ NO
- ○ N/A

Has this new information been communicated to participants?
- ○ YES
- ○ NO
- ◉ N/A

If applicable, please describe the findings.

Has an amendment(s) to the protocol been submitted to the IRB in the past year?
- ◉ YES
- ○ NO

What is the version date of the most recent IRB- approved protocol? Version v1.16, August 8th 2016

Has the consent form(s) been revised in the past year?
- ◉ YES
- ○ NO
- ○ N/A

Have consent form modifications been reported to the IRB?
- ◉ YES
- ○ NO
- ○ N/A

Version date/s of the most recently approved consent form(s): Version 1.2 12 avril 2016

Have any adverse events occurred since the last approval?
- ○ YES
- ◉ NO
- ○ N/A

If yes, how many at McGill sites? 24

How many at all sites?

DATE OF I.R.B. APPROVAL
MAY - 8 2017
Faculty of Medicine
McGill University

Have the adverse events been reported to the IRB? If no, submit all adverse events with this form.
- ◉ YES
- ○ NO
- ○ N/A

Have there been any publications?
- ◉ YES
- ○ NO

If yes, append list:

SIGNATURES

Principal Investigator — Date: 6 April 2017

IRB Chair: Roberta Palmour — Date: May 8, 2017

PARTICIPANT INFORMED CONSENT FORM

PRE-SYMPTOMATIC EVALUATION OF EXPERIMENTAL OR NOVEL TREATMENTS FOR ALZHEIMER'S DISEASE (PREVENT-AD)

OLFACTION STUDIES IN RESEARCH ON PREVENTION OF AD

PRINCIPAL INVESTIGATOR: John C. S. Breitner, MD, MPH
Director, Center for Studies on Prevention of Alzheimer's Disease (StoP-AD)
Douglas Mental Health University Institute
Professor, Department of Psychiatry, McGill University Faculty of Medicine

OTHER INVESTIGATORS: Marie-Elyse Lafaille-Magnan, PhD Candidate- Project Leader

Research funded by McGill University, by an unrestricted research grant from Pfizer Canada, and by the Douglas Mental Health University Institute – Research Centre

……..

Introduction / purpose of the study. You have agreed to participate in a program of research called Pre-symptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's Disease (PREVENT-AD). This research group will help us learn about memory and brain changes in healthy people who are 60 or older or age 55 or older if current age is within 15 years of affected relative's estimated age at onset of AD dementia. Among a number of studies in this group of volunteers, we are doing a research sub-study called "Olfaction Studies in Research on Prevention of AD." We are asking you now to join this olfaction sub-study of PREVENT-AD using the 40-item forced-choice University of Pennsylvania Smell Identification Test (UPSIT) and additional olfaction tests. Olfaction is the sense of smell. The purpose of the study is to learn how people's olfaction is affected by their brain health and memory status. This research will take place at McGill University and the Douglas Mental Health University Institute ("the Douglas") in Montreal.

As people age, they often have brain changes that can be seen long before the appearance of symptoms of forgetfulness or similar problems. The PREVENT-AD program seeks to learn whether these same brain changes may cause problems with olfaction. If so, clinicians and scientists might use olfactory abilities to reveal these brain changes even though people have not yet developed symptoms. We wish to test this idea by learning whether olfaction changes as people age, and we also want to learn whether some treatments can prevent or reverse these olfactory difficulties suggesting prevention or reversal of underlying brain changes. For these reasons, we wish to study olfaction in people as they age, and to examine olfaction in people

Appendix B - Olfactory identification correlates with cerebral blood flow in cognitively normal adults at risk of Alzheimer's dementia

Marie-Elyse Lafaille-Magnan1 , Cécile Madjar2 , Rick Hoge3 , John C.S. Breitner4,5 , the PREVENT-AD Research Group, 1 Douglas Mental Health Research Institute affiliated with McGill University, Montreal, QC, Canada; 2 Douglas Mental Health University Institute, Verdun, QC,
Canada; 3 Montreal Neurological Institute, Montreal, QC, Canada; 4 Douglas Hospital Research Centre, Montreal, QC, Canada; 5 McGill University Faculty of Medicine, Montreal, QC, Canada. Contact e-mail: Marie-Elyse.Lafaille-Magnan@mail.mcgill.ca

Background

Cerebral perfusion decreases in normal individual at risk of Alzheimer's disease who eventually progress to AD (Johnson, 2000). Similarly perfusion is known to be reduced in AD and, to a lesser extent, in MCI. This phenomenon is most evident in precuneus and bilateral parietal cortices as regions of interest (ROI) (Binnewijzend, 2013). Such hypoperfusion may reflect reduced metabolic demand in regions undergoing neuronal loss. Neurodegeneration may also be revealed by deficits in olfactory identification, especially in AD where olfactory loss correlates with neuropathology and severity of clinical symptoms. If olfactory impairments reflect reduced neural mass and AD pathology, they should be related to cerebral blood-flow (CBF). Hence, we hypothesized that olfactory identification would be associated with CBF in a cohort at risk of pre-symptomatic AD.

Methods

Cognitively normal subjects with a parental history of AD were scanned using MPRAGE T1 and pCASL sequences. Their odor identification performance was assessed with the 40-item University of Pennsylvania Smell Identification Test. Neurolens software was used for ASL analysis. After pre-processing (motion-correction, subtraction of tagged images from the control images and spatial smoothing of 6 mm, a GLM was fitted to the flow series and CBF was computed. Individual grey matter (GM) probability masks were created from the T1 scan using CIVET automatic segmentation algorithms. Anterior and

posterior cingulate cortices (ACC, PCC) and precuneus were defined as ROIs using the AAL template and multiplied by the GM mask. Resulting ROI masks were registered to the ASL space and used for average CBF extraction. Due to noise, we were unable to investigate hippocampal, and entorhinal perfusion.

Results

In age adjusted robust-fit regression analyses, olfactory identification correlated with global average CBF in GM (fig.1a), precuneus (fig.1b) and PCC (fig.1c), but not in ACC (fig.1d).

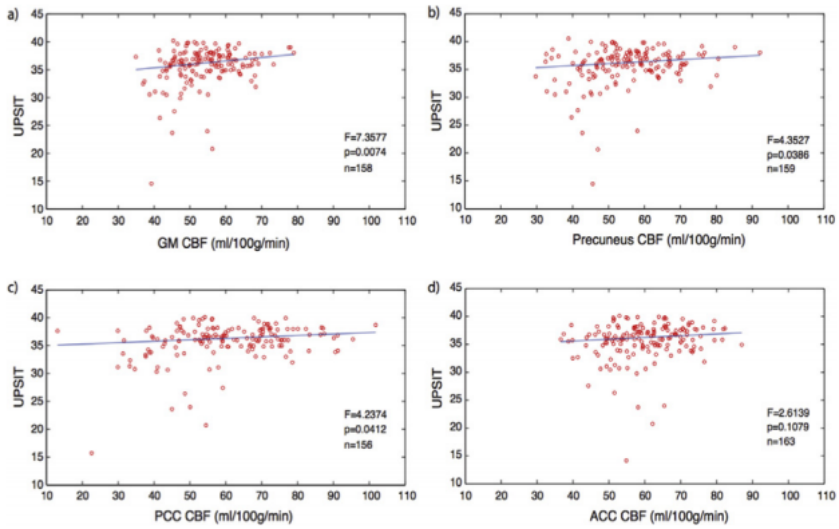


Figure 1. Scatterplot graphs of partial regression analysis for smell identification and perfusion for GM, precuneus, PCC, ACC.
Scatterplot graphs show partial robust fit regression plots showing CBF association with olfactory identification, after removing the effect of age: a) overall GM CBF is positively associated to UPSIT scores (F=8.74, p= 2.07e-05, r2=0.144 n=158); b) precuneus CBF is positively associated to UPSIT(F=12, p=1.47e-05 r2=0.133, n=159); c) PCC CBF is positively associated to UPSIT (F=11.8, p= 1.71e-05, r2=0.134, n=156); d) ACC CBF is not associated to UPSIT as correlation is driven by age (F=l 1.3, p=2.5e-05, r2=0.124, n=163).

Olfactory circuitry parallels major arteries in the medial brain and may be associated with cardiovascular health, as we also found a correlation between olfaction and systolic blood pressure (fig.2).

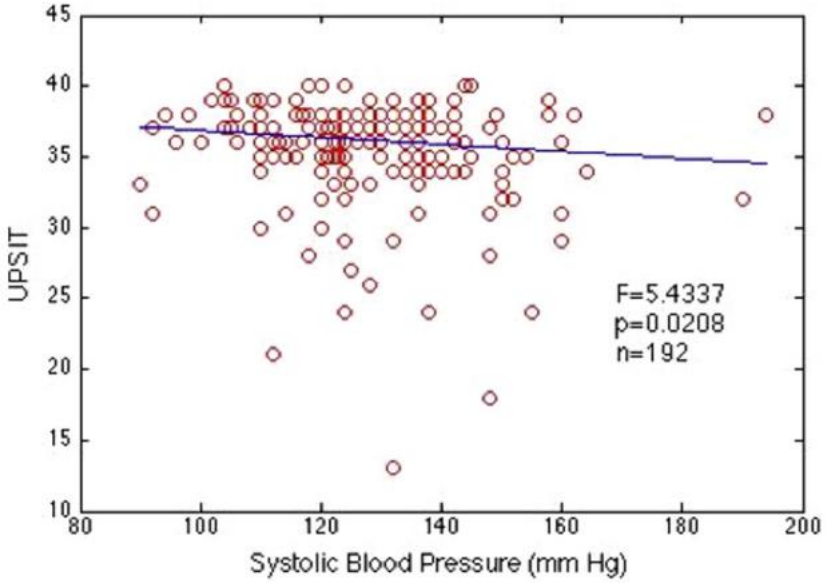


Fig.2. Scatter plot graph of correlation between smell identification and systolic blood pressure.
Scatterplot graph shows an inverse correlation between olfactory identification and systolic blood pressure (F=26.8, p = 5.7e-07, r2=0.1124, n=192).

Conclusions

Impaired olfactory identification is associated with reduced CBF in the overall GM and in key ROIs vulnerable to early changes in AD (Iturria-Medina, 2014). Olfactory identification deficits therefore hold interest as potential markers of pre-symptomatic AD.

Appendix C - Olfactory identification is associated with verbal intrusion and primacy effects in cognitively normal adults at risk of Alzheimer's dementia

Marie-Elyse Lafaille-Magnan1, David Fontaine2 , John C.S. Breitner3,4 , PREVENT-AD Research Group1 Douglas Mental Health Research Institute affiliated with McGill University, Montreal, QC, Canada; 2 Centre for Studies on Prevention of Alzheimer's Disease (StoP-AD Centre), Douglas Mental Health Institute, Montreal, QC, Canada; 3 Douglas Hospital Research Centre, Montreal, QC, Canada; 4 McGill University Faculty of Medicine, Montreal, QC, Canada. Contact e-mail: Marie-Elyse. Lafaille-Magnan@mail.mcgill.ca

Alzheimer disease (AD) is characterized by memory loss and cognitive decline associated with other symptoms such as olfactory impairments. Olfactory identification is associated with global cognition and memory, as measured by the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) in a population of cognitively normal individuals at risk of AD (Lafaille-Magnan, 2014). To further investigate these findings, we studied serial position pattern performance (primacy, middle, recency) and total verbal intrusions in tasks used to make up immediate and delayed memory scores (list and story learning and recall). Primacy effect alteration occurs in the early stages of AD and may relate to rapid forgetting and problems with consolidation (Burkart, 1998 ;Bruno, 2013). AD patients also display more intrusions during cognitive tasks due to interference sensitivity and decreased attention (Helkala, 1989; Macoir, 2002).

Methods

We report cross-sectional findings from a cohort of cognitively normal volunteers aged >55. Participants underwent cognitive evaluation using RBANS, without age-adjustment norms. Concurrently, they were given the University of Pennsylvania Smell Identification Test (UPSIT). The serial position pattern performance score was established by subdividing the first attempt at list learning task and first attempt at the story memory task into 3 parts: primacy, middle, recency. Total verbal intrusions is a summation of

irrelevant responses given in the list learning or story memory tasks as well as during the delayed recall task.

Results

Results: We found that UPSIT scores positively correlated with primacy but not with middle or recency effects and were inversely correlated with verbal intrusions (Table 1).

Table 1
Bivariate correlation of olfaction identification performance and RBANS item order and verbal intrusions

Correlation	Spearman's rho	p-value	n
UPSIT and RBANS list learning primacy	0.204	0.005	186
UPSIT and RBANS story memory primacy	0.149	0.042	186
UPSIT and RBANS list learning middle	0.075	0.308	186
UPSIT and RBANS story memory middle	0.114	0.122	186
UPSIT and RBANS list learning recency	0.005	0.941	186
UPSIT and RBANS story memory recency	-0.029	0.692	186
UPSIT and RBANS verbal memory total intrusions	-0.174	0.017	186

Conclusions

In cognitively normal older adults at increased risk for AD, olfactory identification decline was associated with primacy effect and total verbal intrusions. The perforant pathway, a memory pathway involving several anatomical structures of the limbic system, overlaps with the primary olfactory cortex (POC) and hippocampus. We suggest that deficits in higher level sensory processing may be indicative of pathology and neurodegeneration in normal aging but, more importantly, early stages of AD pathology. Ongoing pathological changes in the POC and hippocampus could be an underlying biological

mechanism that explains the correlation of olfactory function with early cognitive symptoms.

Appendix D - ASSOCIATION OF OLFACTORY PERFORMANCE AND HIPPOCAMPAL VOLUME IN PERSONS AT RISK OF ALZHEIMER'S DEMENTIA

Marie-Elyse Lafaille-Magnan[1], Louis Collins[2], Vladimir Fonov[2], David Fontaine[3], Pierre Etienne[4], Judes Poirier[1], John C.S. Breitner[5],

1-McGill University, Verdun, Quebec, Canada;

2-Montreal Neurological Institute, Montreal, Quebec, Canada;

3-Douglas Institute, Verdun, Quebec, Canada;

4-Douglas Hospital, Montreal, Quebec, Canada;

5-Douglas Mental Health University Institute, Montreal, Quebec, Canada.

Contact e-mail: marie-elyse.lafaille-magnan@mail.mcgill.ca

Background: Olfactory deficits are clearly documented in Alzheimer's dementia and may also to be a feature of pre-symptomatic and prodromal Alzheimer's disease (AD). Olfaction is dependent on the cholinergic network and structures implicated in early stages of the disease. However the rate of evolution in dysfunction, and the predictive value of olfactory deficit remain unknown. Because AD has a lengthy pre-symptomatic phase; it is not unreasonable to look "upstream" to characterize these changes. Methods: Participants were 211 cognitively normal (clinical dementia rating [CDR].0, Montreal Cognitive Assessment [MOCA]>24) volunteers aged at least 60 years with a family history of AD in a first-degree relative. Persons aged 55-59 were also admitted if their relative had dementia onset within 15 years of their own age. Participants were evaluated cognitively using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) without age adjusted norms. We investigated participants' sense of smell using the University of Pennsylvania Smell Identification Test (UPSIT). 26 participants were enrolled in a randomized trial and presently have 3-month cognitive follow-up data available. Results: Participants were 72.5% women; 32% were APOE ε4 carriers. Mean age was 64.5 6 s.d. 7.5 yrs, mean education was 15.3 6 s.d. 3.5 years; mean MOCA score at eligibility was 28.1 6 s.d. 1.6. In a subset of 89 participants, we found that the UPSIT was significantly associated at baseline with the RBANS immediate memory, delayed memory, and total index scores (Table 1). In

an overlapping subset of 103 participants with MRI scans, we found that the UPSIT was significantly associated with hippocampal volumes bilaterally (Table 1). In the subset of participants with 3-month RBANS follow-up scores, we found that low UPSIT at baseline predicted a decline in attention sub-score (r.-0.429 sig.0.037 df.22) after controlling for age, and gender. Conclusions: Observations to date suggests that people with good higher-order processing of odors have better cognitive scores and increased hippocampal volumes. Our data substantiate other reports of olfactory identification abnormalities in pre-clinical Alzheimer's disease and suggest the potential utility of olfactory testing as a ''biomarker'' to track progress of pre-symptomatic AD.

Table 1
Bivariate correlation of olfaction identification performance and RBANS and hippocampal volume

Correlations	Spearman's rho	p-value	N
UPSIT and RBANS immediate memory index score	0.337	.001	89
UPSIT and RBANS delayed memory index score	0.217	.042	89
UPSIT and RBANS total scale index score	0.244	.021	89
UPSIT and Right hippocampal volume	0.254	.010	103
UPSIT and Left hippocampal volume	0.196	.047	103
UPSIT and Total hippocampal volume	0.243	.014	103

Appendix E - Candidate Gene Studies and GWAS Suggest Substantial Genetic Influence on Deficits in Olfactory Identification Among Persons at Risk of AD

Marie-Elyse Lafaille-Magnan, MSc[1]; Cynthia Picard, PhD[2];Justin Miron, B.Sc.[2];Judes Poirier, PhD[3];John C S Breitner, MD, MPH[4] and the PREVENT-AD Research Group, (1)Douglas Mental Health Research Institute affiliated with McGill University, Montreal, QC, Canada, (2)Douglas Mental Health University Institute Research Centre, Montréal, QC, Canada, (3)Centre for the Studies on the Prevention of Alzheimer's disease, Douglas Mental Health University Institute, Montréal, QC, Canada, (4)McGill University Faculty of Medicine, Montreal, QC, Canada

Background:The olfactory network is vulnerable to AD pathology. We recently reported that deficits in olfactory identification (OI) are associated with CSF AD biomarkers. Past genome-wide association studies (GWAS) suggested that MAPT (encodes microtubule-associated protein tau) is a susceptibility locus for OI impairment (Dong, 2015). The CDK5 signalling pathway (involves tau phosphorylation) was also strongly associated with OI. Because genetic influences on OI may illuminate underlying structures' susceptibility to neurodegeneration, we investigated association of polymorphisms with OI. Using a candidate gene approach in individuals at risk of AD, we examined association of OI with neurodegeneration-associated SNPs rs199443 in MAPT and rs10984186 in CDK5RAP2, focusing on OI - SNP associations reported by Dong (2015, 2016) and several other AD-related SNPs (Lambert, 2013). Pyrosequencing techniques and a 2.5M-SNP GeneChip provided data for these analyses and an exploratory GWAS for SNPs associated with OI. Methods: We studied the family-history-positive PREVENT-AD cohort of cognitively normal individuals aged 55-83 using the 40-item University of Pennsylvania Smell Identification Test (UPSIT). DNA was extracted from blood. Automated genotyping relied on the Illumina Infinium Omni 2.5M-8 GeneChip. We used Matlab to evaluate the differences in OI across polymorphisms and in linear

regression for allele-dosage studies. We identified SNPs associated with OI using PLINK and Haploview. Results: Genome-wide data were available for 136 participants. After quality-control 2.28 million SNPs were available for analysis. We found a modest association of OI impairment with rs10984186 in CDK5RAP2 (Kruskal-Wallis, $p<0.05$) and a trend for rs199443 in MAPT ($p=0.07$; Figs 1 & 2). Allelic dose response analysis further clarified these correlations (CDK5RAP2 $\beta=0.0521$, $p=0.07$; MAPT $\beta=0.701$, $p=0.0238$). Several SNPs identified by Dong (2015, 2016) and Lambert (2013) showed no significant association. The GWAS identified 26 SNPs with genome-wide significance ($P < 5 \times 10-8$; Fig 3), of which 22 survived adjustment for age, gender, and APOE $\varepsilon 4$ status (Fig 4). Conclusions: In a sample at risk of AD we found limited association of OI impairment with polymorphisms in MAPT and CDK5RAP2. A GWAS identified several new SNPs related to OI, including three on chromosome 14 and one on chromosome 3 with p-values <10-12.

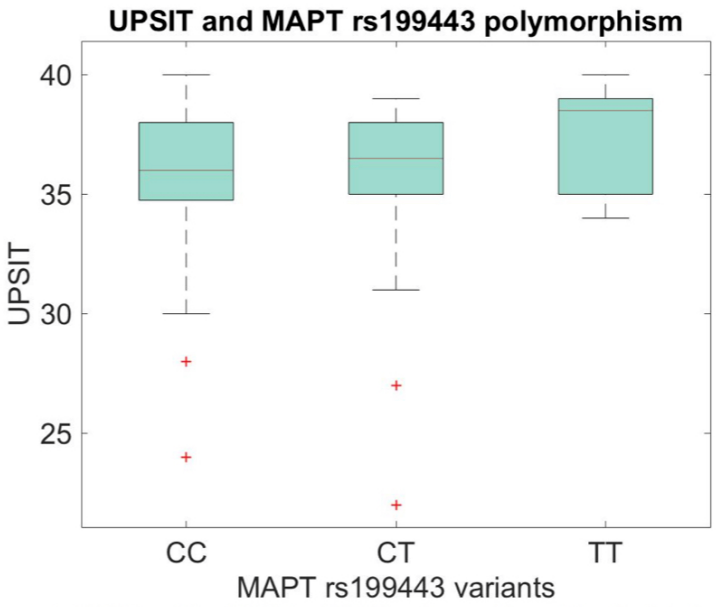


Figure 1. UPSIT and the MAPT rs199443 polymorphisms. The C allele is the risk allele is associated with increased *MAPT* expression level in the frontal cortex [2](Dong, 2015). Presence of the C allele is associated with lower OI in older adults at risk of AD. Participants included were 81 CC, 44 CT, 10 TT. A Kruskal-Wallis revealed a trend for group differences in OI ($X^2(2)=5.29$, $p=0.071$, $df=2$, $n=135$). Linear regression suggested that a minor ("protective") allele dose response ($F(1,133)=3.7683$, $p=0.0543$, $R^2=0.0508$). The dosage ranges from 0 to 2, where 0 doesn't have a copy of the minor allele (T allele) and 2 as 2 copies of the minor allele.

UPSIT = University of Pennsylvania Smell Identification Test

MAPT = microtubule-associated protein tau.

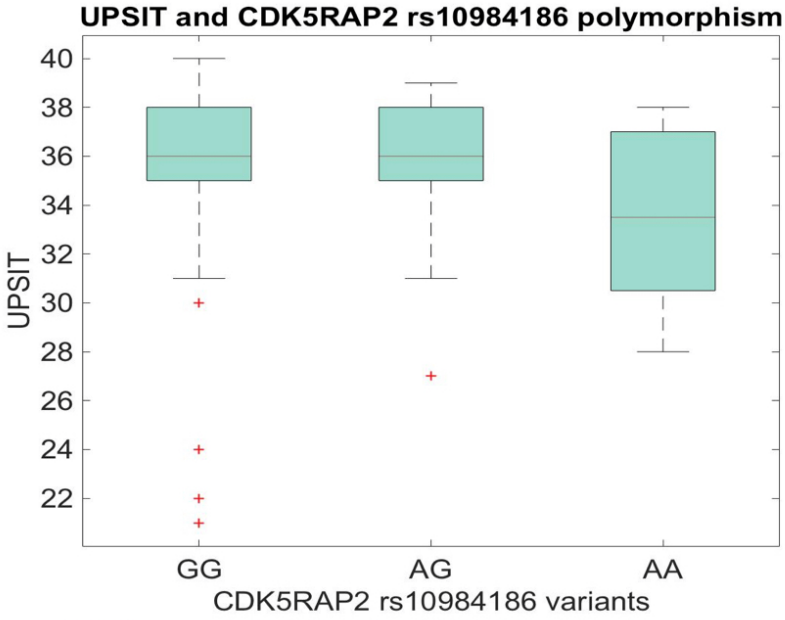


Figure 2. UPSIT and the CDK5RAP2 rs10984186 polymorphisms.
This genotyping was pyrosequenced separately from the gene chip, thus we have data on 141 individuals. CDK5 regulatory subunit-associated protein 2 (CDK5RAP2) binds to the CDK5 complex, which is involved in Tau phosphorylation. Participants included were 77 GG, 53 AG, 12 AA. A Kruskal-Wallis revealed there were significant group differences in OI ($X^2(2)= 6.13$, p=0.0466, df=2, n=141). Linear regression suggested that the minor allele dose response predicts lower OI (F(2,139)=3.61, p=0.0297, R^2=0.0494). The dosage ranges from 0 to 2, where 0 doesn't have a copy of the minor allele (A allele) and 2 as 2 copies of the minor allele.

UPSIT = University of Pennsylvania Smell Identification Test

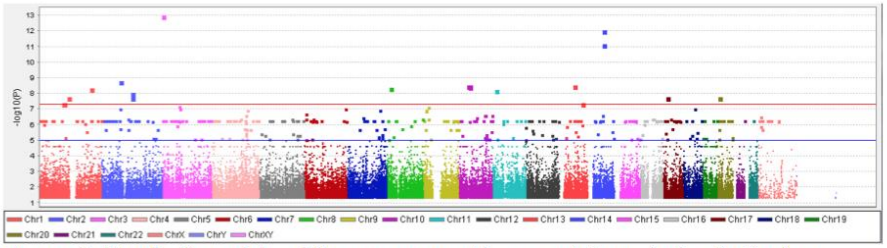


Figure 3. Manhattan plots of the genome-wide association study of olfactory identification.

The blue line indicates the genome-wide suggestive threshold ($1*10^{-5}$), The red line indicates the genome-wide significance threshold ($5*10^{-8}$). There were 26 SNPs that reached the genome-wide significance threshold. Note that 4 SNPs on chromosome 3 and 14 had a $P<5*10^{-12}$.

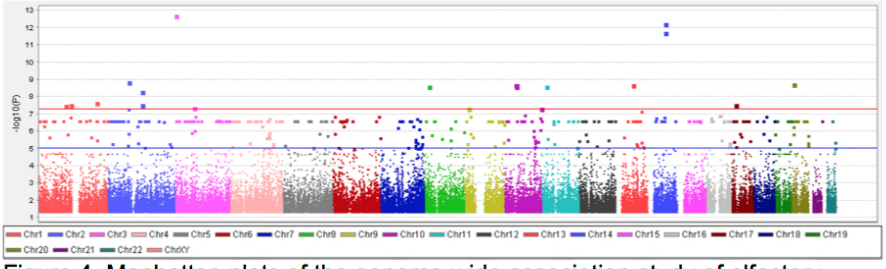


Figure 4. Manhattan plots of the genome-wide association study of olfactory identification after adjusting for age, gender, and APOE 4.

The blue line indicates the genome-wide suggestive threshold ($1*10^{-5}$), The red line indicates the genome-wide significance threshold ($5*10^{-8}$). We found that 22 SNPs survived adjustment for age, gender, and APOE 4 carrier status and identified 9 new SPNs for a total of 31 SPNs. Note that 4 SNPs on chromosome 3 and 14 conserved a $P<5*10^{-12}$ after adjusting for covariates.

Appendix F - Related contributions to the thesis, the SToP-AD centre work, the Douglas, the field, the program

- Identified marker to add in PREVENT-AD cohort and prevention trials.
- Contributed to the PREVENT-AD cohort protocol and Naproxen trial protocol by including olfaction identification in the study design.
- Contributed to randomization of NAPROXEN in the INTREPAD trial using www.randomization.com .
- Recruited participants through advertising at retirement homes and condominium across the Island of Montreal and in public and private clinics, stores, and advertisement boards.
- Recruited participants at events held at the Douglas Hall.
- Wrote consent form and made amendments to the PREVENT-AD and Naproxen trial protocols to include olfaction sub-study.
- Contributed to the translation of web material, consent forms, protocols.
- Came up with slogan for the STOP-AD Centre (STOP-AD - it’s a family affairs!).
- Randomized order of UPSIT booklets for different visits using www.randomization.com.
- Tested UPSIT.
- Tested RBANS.
- Scanned MRI sessions.
- I developed an excel sheet to compute fMRI data for the contextual memory task.
- I extracted task data from 500+ MRI visits.
- From these, I extracted outcome measures and event onsets of fMRI behavioral from eprime files output files.
- I developed a pipeline to analyze fMRI data using SPM8.

- I updated the pipeline from SPM8 to SPM12b.
- Suggested key ways to improve data in the Loris database: inclusion of RBANS raw score, addition of some MOCA sub scores, option to download data cross-sectionally and longitudinally, download with a time stamp, search box for all tools and definitions, brought up incongruence of education years across different tool.
- Analyzed UPSIT results used in grant application and PREVENT-AD online material.
- Analyzed RBANS results used in grant application and PREVENT-AD online material.
- Analyzed fMRI used in grant application and PREVENT-AD online material.
- Appeared in TVA news story on Alzheimer's research in the Fall 2012.
- Accompanied a participant to take-part of a public interview with Dr. Breitner part of the mini-series on mental health in the Fall 2012.
- Worked at the Bal des Lumières, a mental health fundraiser.
- Presented 1 poster on thesis results at AAIC 2013 in Boston, USA.
- Published abstract in Alzheimer's and Dementia, August 2013.
- Gave talk to PREVENT-AD collaborators at the MNI in August 2013.
- Invited speaker at the MCSA and presented to Dr. Pedro Rosa-Neto's laboratory and visiting speaker, Dr. David Bennett in September 2013.
- Invited speaker at the IPN retreat 2013 in Westmount in September 2013.
- Wrote in the PREVENT-AD newsletter.
- Extra in Jean-Luc Mongrain documentary on criminal claiming to be mentally unfit in the Fall 2013.
- Prepared data for presentations on NSAIDS in AD prevention presented by Dr. Breitner in Bonn, Germany; on latent variable model presented by Dr. Breitner in the USA; and on updates of latent variable presented by Dr.

Breitner at CITAD in California, USA.

- Helped out to set up, and clean up a gala for our study participants Fall 2013.
- Presented a poster at the Sensorimotor Integration Conference and co-author of another poster at the Sensorimotor Integration Conference.
- Judes Poirier appeared on TVA television and mentioned my project in Spring 2014.
- Giving a talk at the Douglas Student Day in June 2014.
- Presenting a poster on thesis results at AAIC 2014 in Copenhagen, Denmark.
- Co-author of a poster on DTI in MCI and AD from ADNI at AAIC 2014 in Copenhagen, Denmark.
- Presenting a poster at the imaging pre-conference in Copenhagen, Denmark.
- Abstracts published in Alzheimer's and Dementia, August 2014.
- Knowledge Translation of memory research with Bohbot laboratory for kids and teenagers at summer camp (part of a play that on memory and different function of anatomical structures)
- Mentored students on ethics, the study, and their projects and, on graduate application.
- Took part of a documentary segment for Banc Public on Alzheimer's disease aired on Tele-Quebec.
- Presented 3 posters and one oral presentation on thesis work, co-authored oral presentation and was the modulator for a session at AAIC 2015 in Washington, USA.
- Invited speaker at the IPN retreat 2015 in Westmount in September 2015.
- Poster was selected for the PhD competition in Washington, USA and additionally was featured on the Alzheimer's Society Canada newsletter.

- Presented memory tasks at the Douglas booth for the Verdun triathlon, August 2016.
- Served as the Brain Reach English High School coordinator 2016-2017.
- Presented 1 poster at CTAD 2016 in San Diego, USA.
- Selected out of 300 candidates to serve a 4-year mandate as a citizen consultant on a government panel on mental health, dependence, youth and their families.
- Gave talk to PREVENT-AD collaborators at the Douglas in February 2017.
- Created dummy variables and calculated adjustments factors for the different French RBANS versions. Adjusted versions and checked version equivalence via alternate adjustment methods. Subsequently, all French RBANS version B, C, D were computed with these adjustment factors in the INTREPAD trial, Probucol trial, PREVENT-AD cohort.
- Applied and obtained ethics to collect postal codes and more information on pollution exposure and smoking history Spring 2017.
- Applied and obtained odor identification, genetic, pathology, psychological tests, medical information from the RUSH University Chicago USA, Spring 2017.
- Gave talk to visiting Dutch faculty and students Spring 2017.
- Presented 1 poster at AAIC 2017 in London, UK.
- Was featured on NBC Today show, TVA news, Nathalie Normandeau radio-show, CBC radio-show, in several newspapers, online newspapers such as The Huffington Post, magazines such as Readers Digest, all McGill social media, WebMD, and blogs for work published in Neurology.
- Prepared Kiosk at gala for PREVENT-AD participants.