Spatial Extent of Amyloid-β Levels and Associations with Alzheimer's Disease Biomarkers

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Abstract: English

Alzheimer's Disease (AD) is a progressive neurodegenerative disease characterized by the accumulation of abnormal proteins, neurodegeneration, and cognitive decline. Amyloid-beta (Aβ) and tau are the main pathological hallmarks of AD, and they start to accumulate up to 20 years before the onset of clinical symptoms. While tau deposition may initiate independently and even before Aβ accumulation, it is hypothesized that Aβ pathology is required for tau to propagate outside of the medial temporal lobe and start the pathological cascade leading to AD dementia and is therefore an ideal target for clinical trials. Despite this hypothesis, most clinical trials that targeted Aβ have failed to slow down cognitive impairments. One possible explanation for these failures is that Aβ is targeted too late in the disease process, and that if we want to stop the disease, we should stop Aβ before its triggers tau accumulation. Measuring pathological levels of Aβ in-vivo, however, remains difficult and lacks consensus.

Aβ positivity is usually defined using a global composite score that represents the mean of several cortical brain regions. Given increasing evidence suggesting that subthreshold Aβ accumulation in older adults is biologically relevant, this thesis aims to investigate the associations between the spatial extent of Aβ deposition and various AD markers in (1) the PREVENT-AD cohort with cognitively unimpaired older adults with a family history of AD dementia and (2) validate the main analyses in the Alzheimer's Disease Neuroimaging Initiative (ADNI) cohort. Our main objectives were to test if we could use Aβ regional positivity to classify individuals as having early Aβ accumulation and if these individuals already have tau pathology and related cognitive decline. We used Gaussian-mixture models to create region-specific thresholds of Aβ positivity in seven regions identified previously to be sensitive to early Aβ accumulation.

Individuals who were Aβ-positive in all regions were classified as the "Widespread Aβ group"; those who were positive in one or more regions were included in the "Regional Aβ group", while the rest were considered the "Negative Aβ group". We compared the demographic and genetic characteristics, subjective memory complaints, tau-PET binding, longitudinal cognitive performance, and longitudinal Aβ accumulation (only in ADNI) of the three groups.

Our results showed that the Regional Aβ groups had higher numbers of APOE ε4 carriers than the Negative Aβ groups, but more restricted tau-PET binding compared with the Widespread Aβ group, and they did not show cognitive decline over time or complain about their cognition. A gradient was also found in both cohorts for CSF Aβ42 levels. The Widespread group had lower values (representing higher brain Aβ) compared to the Regional groups, which also had lower values than the Negative groups. Interestingly, the Regional groups did not have more CSF p-tau or total tau than the Negative groups. Longitudinal Aβ was only available in ADNI, and the results suggest that both Widespread and Regional Aβ groups accumulated Aβ faster than the Negative group. These results suggest that individuals with regional Aβ binding have started accumulating brain Aβ but that they do not yet have significant tau or related cognitive decline. They might be the best targets for amyloid therapies.

Le Résumé: Français

La maladie d'Alzheimer (MA) est une maladie neurodégénérative progressive caractérisée par l'accumulation de protéines anormales, la neurodégénérescence et le déclin cognitif. Le bêta-amyloïde (Aβ) et le tau sont les principales caractéristiques pathologiques de la MA et ils commencent à s'accumuler jusqu'à 20 ans avant l'apparition des symptômes cliniques. Alors que le dépôt de tau peut s'initier indépendamment et même avant l'accumulation d'Aβ, il est supposé que la pathologie Aβ est nécessaire pour que tau se propage à l'extérieur du lobe temporal médian et démarre la cascade pathologique menant à la démence MA, ce qui en fait une cible idéale pour les essais cliniques. Malgré cette hypothèse, la plupart des essais cliniques ciblant l'Aβ n'ont pas réussi à ralentir les troubles cognitifs. Une explication possible de ces échecs est que Aβ est ciblé trop tard dans le processus de la maladie, que si nous voulons arrêter la maladie, nous devons arrêter Aβ avant qu'il ne déclenche l'accumulation de tau. La mesure des taux pathologiques d'Aβ in-vivo reste cependant difficile et ne fait pas consensus.

La positivité Aβ est généralement définie à l'aide d'un score composite global qui représente la moyenne des régions cérébrales corticales serval. Compte tenu des preuves croissantes suggérant que l'accumulation d'Aβ sous le seuil chez les personnes âgées est biologiquement pertinente, cette thèse vise à étudier les associations entre l'étendue spatiale du dépôt d'Aβ et divers marqueurs de la MA (1) PREVENT-AD avec des personnes âgées non altérées cognitivement ayant des antécédents familiaux de La démence MA et (2) valider les principales analyses de la cohorte de l'Initiative de Neuro-imagerie de la Maladie d'Alzheimer (ADNI). Nos principaux objectifs étaient de tester si nous pouvions utiliser la positivité régionale Aβ pour classer les individus comme ayant une accumulation précoce d'Aβ et si ces individus avaient déjà

une pathologie tau et un déclin cognitif associé. Nous avons utilisé des modèles de mélange gaussien pour créer des seuils de positivité Aβ spécifiques à chaque région dans sept régions identifiées précédemment comme étant sensibles à l'accumulation précoce d'Aβ. Les personnes Aβ-positives dans toutes les régions ont été classées dans le « groupe Aβ répandu » ; ceux qui étaient positifs dans une ou plusieurs régions ont été inclus dans le « groupe Aβ régional », tandis que les autres ont été considérés comme « groupe Aβ négatif ». Nous avons comparé les caractéristiques démographiques et génétiques, les plaintes de mémoire subjectives, la liaison tau-PET, les performances cognitives longitudinales et l'accumulation longitudinale d'Aβ (uniquement dans ADNI) des trois groupes.

Nos résultats ont montré que les groupes Aβ régionaux avaient des transporteurs APOE ε4 plus élevés que les groupes Aβ négatifs, une liaison tau-PET plus restreinte par rapport aux groupes Aβ-négatifs et ils n'ont pas montré de déclin cognitif au fil du temps ou se sont plaints de leur cognition. Un gradient a également été trouvé dans les deux cohortes pour les niveaux de CSF $A\beta_{42}$. Les groupes avaient des valeurs inférieures (représentant un Aβ cérébral plus élevé) par rapport aux groupes régionaux, qui avaient également des valeurs inférieures à celles des groupes Négatifs. Il est intéressant de noter que les groupes régionaux n'avaient pas plus de pTau ou de tau total du CSF que les groupes négatifs. L'Aβ longitudinal n'était disponible que dans ADNI et les résultats suggèrent que les groupes Aβ étendus et régionaux ont accumulé Aβ plus rapidement que le groupe Négatif. Ces résultats suggèrent que les individus ayant une liaison régionale à l'Aβ ont commencé à accumuler de l'Aβ cérébral mais qu'ils n'ont pas encore de tau significatif ou de déclin cognitif associé. Ils pourraient être les meilleures cibles pour les thérapies amyloïdes.

List of Abbreviations

Aβ: amyloid-beta

AD: Alzheimer's disease

ADNI: Alzheimer's Disease Neuroimaging Initiative

***APOE*:** Apolipoprotein E

CSF: Cerebrospinal fluid

FTP: [^{18}F] Flortaucipir

GMM: Gaussian mixture modelling

MRI: Magnetic resonance imaging

NAV: [^{18}F]NAV4694

p-tau: phosphorylated tau

PET: Positron emission tomography

PREVENT-AD: PResymptomatic EValuation of Experimental and Novel Treatment of Alzheimer's disease

RBANS: Repeatable Battery for the Assessment of Neuropsychological Status

ROI: Region of interest

SCD: Subjective Cognitive Decline

SUVR: Standardized uptake value ratio

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Acknowledgments and Contributions of Co-Authors

Hazal Ozlen: study concept and design, analysis and interpretation of data, drafting, and revising the manuscript.

Alexa Pichet-Binette: study concept and design, analysis and interpretation of data, revision of the manuscript.

Theresa Köbe: data analysis, revision of the manuscript.

Pierre François Meyer: data analysis, revision of the manuscript.

John Breitner: data acquisition, protocol concept and design.

Judes Poirier: data acquisition, protocol concept and design, revision of the manuscript.

Sylvia Villeneuve: data acquisition, study concept and design, interpretation of data, revision of the manuscript.

Data used in the preparation of this article were obtained from the Pre-symptomatic Evaluation of Novel or Experimental Treatments for Alzheimer's Disease (PREVENT-AD) program data release 5.0 (November 30, 2017). PREVENT-AD was launched in 2011 as a $13.5 million, 7-year public-private partnership using funds provided by McGill University, the Fonds de Recherche du Québec – Santé (FRQ-S), an unrestricted research grant from Pfizer Canada, the Levesque Foundation, the Douglas Hospital Research Centre and Foundation, the Government of Canada, and the Canada Fund for Innovation. Private sector contributions are facilitated by the Development Office of the McGill University Faculty of Medicine and by the Douglas Hospital Research Centre Foundation (http://www.douglas.qc.ca/). The primary goal of PREVENT-AD is to test whether the serial determination of multi-modal biomarkers of Alzheimer's disease may be measured and used in pre-symptomatic persons at high risk of subsequent AD dementia to trace the progression of the disease process and to measure effects of any potentially preventive treatment interventions. This work is intended to provide preliminary data regarding the probable efficacy and safety of potential new treatments for the prevention of AD dementia. The Founders of the program were John C. S. Breitner, MD, MPH, Judes Poirier, Ph.D. and Pierre Etienne, MD, Douglas Hospital Research Centre and Faculty of Medicine, McGill University, Montréal, QC, Canada. Program Current Director is Judes Poirier, Ph.D., the Co-Director is Sylvia Villeneuve, Ph.D. and the Study Coordinator is Jennifer Tremblay-Mercier, MSc. PREVENT-AD is the result of the efforts of many other co-investigators from a range of academic institutions and private corporations, as well as an extraordinarily dedicated and talented clinical and technical assistance staff, students, and post-doctoral fellows. Subjects are recruited from the greater Montréal area

and more distant locations in Québec. For up-to-date information, see https://douglas.research.mcgill.ca/stop-ad-centre.

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CHAPTER I:

INTRODUCTION AND LITERATURE REVIEW

Alzheimer's disease (AD) is currently the most frequent cause of dementia in Western societies and severely diminishes a person's quality of life. Approximately 500,000 Canadians are living with some form of cognitive impairment or dementia, an estimated 60%-80% of whom have Alzheimer's [1-3].

The consequences of AD place a high clinical, social, and economic burden on our society, especially as the ageing population continues to grow. Thus, the early identification and management of Alzheimer's disease is a critical priority for public health, but remains challenging.

Pathological Hallmarks of Alzheimer's Disease

AD is a progressive neurodegenerative condition that starts several decades prior to the appearance of the clinical symptoms. The disease pathology starts with the accumulation of extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tau tangles, which are the pathological hallmarks of AD. Therefore, the presence of these two proteins is required for the clinical diagnosis of AD.

Aβ

The Aβ plaques result from the extracellular accumulation and deposition of the abnormal processing of Aβ precursor protein (APP), and imbalance in the production and clearance of the pathways [4]. When APP is activated, it is cut by other proteins into parts that stay inside and outside of the cell [5]. One of the parts from this cutting sometimes produces beta- Aβ. These plaques are

built up in the space between nerve cells. The accumulation triggers the disruption of communication between cells and increases inflammation by activating the immune cells for clearance [6].

Aβ plaques mainly accumulate in the isocortex. According to the staging proposed by Braak and Braak (1991), Aβ accumulation follows three stages. The first stage starts with Aβ accumulation in basal frontal and temporal lobes, followed by neocortices and hippocampus in Stage 2. Finally, the accumulation reaches primary cortices, subcortical nuclei, and cerebellum in the last stage. To have a more precise understanding of Aβ accumulation, Thal et al. (2002) suggested five phases system for Aβ deposition starting with the neocortex in the first phase. Then, the deposition continues to allocortex in the second phase, and subcortical nuclei, including the striatum, in phase 3. Then, Aβ spreads to the brainstem in phase 4 and finally reaches the cerebellum in phase 5.

Tau

The other hallmark protein of AD is tau tangles, which build up inside the cells as the neurodegenerative process of AD advances [4,7]. In the human brain, tau protein exists to stabilize the microtubules to aid intra-cellular transport. However, tau proteins in AD are hyperphosphorylated and associated with neuronal death and disintegration [8,9]. In 1991, Braak et al. explained the distribution pattern of tau in six stages: starting from the trans-entorhinal region in the first 2 stages (I-II),then expanding to entorhinal and limbic areas (stage III-IV), eventually reaching the isocortex in the last stages (V-VI) [10-12].

Traditionally, the spreading of these pathologies was captured through neuropathological methods. However, recent developments of Aβ and tau imaging have allowed us to capture the in

vivo visualization of the distributions [13]. The primary purpose of biomarkers in AD are to accurately diagnose the disease at a very early stage, distinguish AD from other dementia disorders and clarify those at risk of developing AD. In addition, in vivo techniques have created the greatest potential for understanding the disease progression and development of AD pathogenesis.

The first signs of AD can be detected in the CSF, a valuable tool to detect biomarkers, which mirrors the biochemical changes happening in the brain. A variety of CSF biomarkers are relevant to AD and neurodegeneration, such as CSF $A\beta_{1-42}$, phosphorylated tau (P-tau), and total tau (T-tau). As the disease progression continues, decreased CSF $A\beta_{1-42}$ levels reflect the accumulation of Aβ in the brain[14] and one of the earliest detectable changes in AD pathology [15]. Following Aβ, both CSF P-tau and T-tau levels increase in AD compared to healthy controls [16,17]. Even though CSF biomarkers have proven to distinguish AD from healthy controls with high specificity, the distinction between AD and other dementia types is hard to detect solely with CSF measurements[18].

Another promising neuroimaging tool, positron emission tomography (PET), captures the signals of radioactive tracers to detect whether and where these proteins are present in the brain [19]. The tracers show different rates of uptake depending on the areas, and higher absorption indicates higher pathology [20]. Visualization and quantification of the tracer uptake are essential for learning the evolution of AD. This new diagnostic biomarker has helped physicians differentiate AD from other dementia types and examine unclear clinical presentations [21,22]. Furthermore, the accuracy of diagnoses has improved significantly since the introduction of PET imaging. Grundman et al. showed that physicians changed the diagnosis in 54.6% of patients after PET scan results, and their confidence in the diagnosis improved 21.6% on average[23]. Previous studies also showed a high

correlation between in vivo PET and post-mortem results directly linking the tracer uptake in the AD patients to the amount of Aβ plaques [24,25].

Preclinical AD

Advancements in new in-vivo techniques provided the first evidence that AD pathology starts to accumulate decades before the onset of clinical symptoms, and allowed us to characterize where and when the pathology starts to accumulate. [26-28]. Therefore, instead of categorizing the stages of AD as preclinical and symptomatic stages (mild cognitive impairment (MCI) and dementia) [29], National Institute on Aging and Alzheimer's Association (NIA-AA) 2018 guidelines proposed a new definition of AD not solely based on clinical symptoms but also including biomarkers [30]. Advances with the biomarkers have provoked a shift in AD definition into a continuum rather than discrete clinical stages[30,31].

The new diagnostic framework also introduced the ATN classification system which differentiates the biomarkers into three binary groups [32]. The proposed ATN classification aimed to captured the earliest changes in AD and to improve our knowledge of preclinical phases. The first component of the scheme is "A", which represents the biomarker of Aβ. Aβ is captured at the earliest stages of AD and identified by cortical Aβ-PET binding or low CSF $A\beta_{42}$. Furthermore, "T" represents the tau pathology biomarkers captured either by tau-PET scans or CSF p-tau values. Finally, "N" categorization represents the neurodegeneration or neural injury expressed through FDG PET, CSF t-tau or structural MRI [33].

Previous longitudinal studies have shown evidence for gradual Aβ accumulation over time, even in individuals with low Aβ, and detected an increase in regional Aβ deposition 17 years before Aβ positivity [34]. The association between tau and Aβ has also been explored in the

preclinical phase, and tau accumulation was strongly correlated with the rate of Aβ accumulation, more than the Aβ levels [35]. There has also been some evidence of tau accumulation in Aβ -negative individuals[36]. Moreover, even though cognition is generally unaffected at the preclinical phase of AD, several studies provided evidence that subtle changes of cognition in preclinical AD are predictors of future cognitive decline [37,38]. In addition, previous studies showed similar results with other AD biomarkers such as atrophy patterns and similar structural MRI changes in the preclinical AD phase [39], elevated inflammation [40] and loss of hippocampal volume [41].

Improvements in defining the preclinical AD concept has allowed us to study the earliest signs of AD and advance our understanding of the relationship between the biomarkers. However, the time-dependent ordering of the biomarkers is still unclear. Classically, researchers proposed the Amyloid Cascade Theory which hypothesizes that in addition to being the first pathological event of the disease progression, Aβ is also believed to exert neurodegenerative processes by mediating the tau accumulation, with the combined effect of the pathologies leading to cognitive decline and neurodegeneration [42]. Even though the two pathologies follow different spreading patterns, many studies provided evidence for the interaction, such as the mediating effect of Aβ on tau pathogenesis [43] and enhanced tau aggregation in mice [44]. Considering these interactions, it is crucial to capture the earliest accumulation of Aβ to understand how the disease unfolds.

Thresholds and Subthreshold Aβ

In Aβ-PET imaging, to eliminate the noise capturing of the signals and ascertain what proportion of cognitively healthy individuals will progress to the clinical state of AD, dichotomizing the Aβ-PET values is commonly used in many studies [45-48]. Previous studies showed evidence for non-specific binding of the Aβ-PET tracers, such as non-specific white matter

binding, inaccurate labelling of gray matter and CSF, and sometimes subject motion [49,50]. Classification of Aβ (+) or Aβ (-) simplifies the data and increases sensitivity by eliminating potential false positives.

The main goal of the thresholds is to detect participants with "meaningful" accumulation of pathology. The summary score of Aβ-PET is commonly used as a mean for cortical Aβ deposition and calculated by AD regions of interest from previous publications. A summary score is derived by an average of the four large regions of interest: frontal (inferior frontal gyrus, middle frontal gyrus, orbitofrontal cortex, superior frontal gyrus, frontal pole), cingulate (anterior cingulate, posterior cingulate, isthmus cingulate), parietal (precuneus, inferior parietal cortex, superior parietal cortex, supramarginal gyrus), and lateral temporal (middle temporal, superior temporal gyri) [51-54].

Furthermore, looking at the correlation between neuropathology and PET imaging, lower Aβ-PET thresholds in early Aβ accumulation regions such as precuneus, anterior cingulate cortex, or orbitofrontal cortex [55-57] revealed 83% sensitivity to detect positivity while higher thresholds only provided 62% sensitivity [57]. Thus, the use of dichotomized thresholds places participants with low quantities of Aβ deposition into Aβ (-) groups before reaching AD-comparable levels.

Thresholds are not unified – each tracer has different cut-off values. Furthermore, the thresholds derived from each sample are dependent on the distribution of Aβ values within the participants. A case study by Sokjova et al. (2011) demonstrated elevated Aβ PET signals regionally presenting limited agreement with The Consortium to Establish a Registry for Alzheimer's Disease (CERAD) neuropathologic protocol and provide more supporting evidence for the existence of subthreshold, regional, levels of Aβ [55]. Other AD-related biomarkers also

showed biological relevance for subthreshold levels of Aβ, such as whiter matter hyperintensities [58] and lower $A\beta_{42}$ levels [59]. In addition, subthreshold Aβ accumulation is predictive of future decline in episodic memory [60]. Given these examples, identifying early Aβ accumulation is crucial to intervene at the right stage of the disease progression.

Key Studies on Earliest Signals and Staging of Aβ

Originally staged at death [61], PET studies allowed finer-grained models that could track the progression of AD longitudinally, particularly in the preclinical phase of the disease, instead of only at death. Aβ accumulates simultaneously in multiple regions in the brain and progresses slowly. In 2015, Villeneuve et al. examined the spatial pattern of early Aβ-PET accumulation. They identified the anterior cingulate cortex and medial frontal regions as the earliest Aβ regions and proposed a spreading model from medial to lateral frontal and parietal regions, later involving the lateral temporal lobe [57].

In 2017, Grothe et al. introduced a new staging method that divides the Aβ accumulation into four distinctive stages. They included 667 participants from the ADNI cohort: including cognitively unimpaired, mild cognitive impairment and AD dementia. These stages were created based on the ranking of the frequency of Aβ deposition, and individuals needed to be positive according to a cut-off of regional $SUVR_{Cer} = 0.92$ in at least 50% of the regions included to be "positive" in the stage. According to the 4-stage model, the spreading of Aβ starts from temporobasal and frontomedial areas. The following stages show the progression from the neocortex towards primary sensory-motor areas, medial temporal lobe, and striatum in line with the previous studies [62]. However, compared to the previous studies, temporal regions were more involved in the early stages, and 98% of the sample fit the model.

A different approach from Palmqvist et al. (2017) identified default mode network (DMN) regions, including cingulate, orbitofrontal, precuneus and insula, as early Aβ accumulation regions. Furthermore, they also showed that early patterns of Aβ accumulation were apparent even in participants with normal Aβ-PET/ CSF $A\beta_{42}$ and those with abnormal CSF $A\beta_{42}$ values [26].

Following Palmqvist's work, Mattson et al. modified a four-level staging system with longitudinal [^{18}F] AV-45 PET and CSF data from cognitively unimpaired, MCI and AD dementia. The staging system is similar to Braak & Braak's (1991) study; stage 0 is mainly cognitively unimpaired, while moving to other stages, more diversity along the continuum of symptoms is present. The earliest Aβ deposition was captured in the precuneus, posterior cingulate, and medial orbitofrontal regions. Although each stage had different characteristics and showed differences in the accumulation rate between the stages, intermediate levels displayed the most change over time [63].

In 2020, a new multi-tracer approach was proposed by Collij et al. for staging the cortical Aβ deposition. Their goal was to adopt a model that can be generalized from single tracer models to multi-tracer models and created the model using six cohorts with four different Aβ-PET tracers. The model includes 5-stages and can classify 99% of the sample. Participants were abnormal at their baseline visit, primarily at cingulate regions, followed by orbitofrontal, precuneus, and finally with temporal and parietal regions [64].

Overall, across different methodologies, staging models highlight medial regions, especially precuneus, cingulate and frontal regions that detect the earliest Aβ-PET signals. Even though the tracer differences cause slight variances, previous studies generally agree with the stages of Aβ spreading.

Rationale of the Thesis

Multiple staging techniques generally agree on the early Aβ accumulation regions and how the spreading of Aβ works in the cortex during the AD spectrum, although different tracers cause some variability. However, most of these staging techniques use the AD spectrum, including MCI and AD dementia patients who likely have much more Aβ accumulation compared to the individual at risk of AD. In addition, while the biological and clinical characteristics of the presymptomatic individuals are still unknown, most of the staging models categorized cognitively unimpaired individuals as single-stage or as Stage 0. Moreover, the association between early Aβ accumulation and other AD biomarkers (e.g., APOE e4, tau-PET, CSF) remains unclear.

With the growing interest in preclinical AD and early intervention, my thesis project will look at the spatial distribution of levels in solely cognitively unimpaired participants. This is because we expect that, within pre-symptomatic individuals, there is already significant pathological accumulation, which might or might not be detected with current thresholds. In addition, we decided to use region-specific thresholds from our sample to support a more fine-grained staging method and higher sensitivity. We used Gaussian-Mixture models to fit two normal distributions for "low" and "high" Aβ levels in each region and defined regional threshold corresponding to 90% probability of belonging to the low Aβ distribution. Participants who had higher levels of Aβ than the regional threshold were classified as Aβ positive for the region, while those with lower values were classified as Aβ negative.

Thereafter, based on Villeneuve (2015), we established a set of seven early regions for Aβ accumulation. Participants with no positive region were deemed negative, the participants with between 1 and 6 regions were deemed "regional" and participants with all 7 regions positive were

deemed “widespread”. This is done with the assumption that if participants do not present significant Aβ in any of these early regions, we do not expect them to be at risk of developing AD while presenting Aβ in all regions would indicate a widespread pattern of Aβ, indicative of the disease. We were particularly interested in capturing the stage between these two for our analyses, to determine whether this “regionality” was indicative of a group on the pathway to AD.

By combining these two methods, the study will be conducted on cognitively normal individuals from the Presymptomatic Evaluation of Experimental or Novel Treatments for Alzheimer’s Disease (PREVENT-AD) cohort, which consists of cognitively unimpaired older adults with a family history of AD dementia, and in the Alzheimer’s Disease Neuroimaging Initiative (ADNI), a multi-centric US-Canada cohort. Specifically, in two large and well-characterized cohorts of cognitively unimpaired older adults, the thesis aims to provide a better understanding of regional Aβ levels and advance the risk profiling of the individuals throughout the AD continuum.

Hypothesis

Our hypothesis is that the Regional Aβ group, defined by early Aβ regions will have biological and clinical relevance for AD pathology. Additionally, we expected to see differences between the Regional Aβ group and the other two groups regarding other AD biomarkers.

Objective #1: Define the three groups of Aβ-PET accumulation in pre-symptomatic individuals, and assess their biological (e.g., APOE e4, CSF) and clinical characteristics (e.g., subjective cognitive decline)

Hypothesis #1: The three Aβ groups will differ, and the Regional Aβ group will show biological and clinical relevance to AD. The Regional Aβ groups will have lower CSF $A\beta_{1-42}$ levels compare to the Negative Aβ group, showing signs of incipient cerebral accumulation of Aβ. Furthermore, compared to the Negative Aβ group, Regional Aβ groups will have higher percentages of *APOE* ε4 carriers, which places them at increased risk for developing the disease.

Objective #2: Assess the relationship between Aβ PET signal and AD-related biomarkers (i.e., tau PET signal and cognitive performance) across the three groups

Hypothesis #2: Elevated tau PET signal and worse cognitive performance will be found in the Widespread and Regional Aβ groups compared to the Negative Aβ group.

Objective #3: Replicate objectives 1 and 2 in a larger cohort with longitudinal Aβ-PET scans.

Hypothesis #3: The main findings of aims 1 and 2 will be replicated. We expect to see the biological and clinical relevance of the Regional Aβ group in ADNI. The Widespread Aβ group will also show elevated tau-PET signals, a higher number *APOE* ε4 carriers and worse cognitive performance than the other two groups. Furthermore, Regional and Widespread groups will accumulate Aβ at a faster rate compared to the Negative Aβ group.

CHAPTER II: MANUSCRIPT

Spatial Extent of Amyloid-β Levels and Associations with Alzheimer's Disease Biomarkers

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[#] Data used in the preparation for this article were obtained from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database (adni.loni.usc.edu). As such, the investigators within the ADNI contributed to the design and implementation of ADNI and/or provided data but did not participate in the analysis or writing of this report. A complete listing of ADNI investigators can be found at:
http://adni.loni.usc.edu/wp-content/uploads/how_to_apply/ADNI_Acknowledgement_List.pdf
†Data used in the preparation of this article were obtained from the Pre-symptomatic Evaluation of Novel or Experimental Treatments for Alzheimer's Disease (PREVENT-AD) program (https://douglas.research.mcgill.ca/stop-ad-centre), data release 5.0 (November 30, 2017). A complete listing of PREVENT-AD Research Group can be found in the PREVENT-AD database: https://preventad.loris.ca/acknowledgements/acknowledgements.php?date=[2021-04-28].

Abstract:

Objective: To investigate the biological and clinical correlates of regional and widespread Aβ deposition levels in two cohorts of cognitively unimpaired older adults.

Methods: We included 529 cognitively unimpaired older adults from the PREVENT-AD (mean age 63±5 years; n=129) and the ADNI (mean age 74±6 years; n=400) cohorts who underwent Aβ- and tau-PET scans. We used Gaussian-mixture models to identify region-specific thresholds of Aβ positivity in seven brain regions prone to early Aβ accumulation. Individuals were classified as having "widespread" Aβ deposition if they were positive in all seven regions, "regional" Aβ deposition if they were positive in one to six regions, or Aβ negative if negative in all regions. We compared the demographic and genetic characteristics, subjective memory complaints, tau-PET binding, and cognitive performance of the three groups.

Results: In both cohorts, most participants with regional Aβ-PET binding did not meet the cohort-specific criteria for Aβ-positivity (79% for PREVENT-AD, 57% for ADNI). Regional Aβ groups had normal baseline cognition and relatively normal tau-PET binding, but a greater proportion of *APOE* ε4 carriers, decreased CSF $A\beta_{1-42}$ levels, and faster longitudinal Aβ-PET binding accumulation (only available in ADNI) when compared with the Negative Aβ groups. Widespread Aβ groups had lower baseline cognitive performance (PREVENT-AD only), faster cognitive decline and more tau binding than the other groups.

Conclusions: Individuals with regional Aβ deposition might be the best candidate for preventive trials since they do not yet have widespread tau and cognitive decline. Widespread levels of Aβ seem to be needed for tau spreading.

Introduction

Amyloid-β (Aβ) and tau are the main pathological hallmarks of Alzheimer's disease (AD). The deposition of these pathological proteins is a continuous process that starts decades before the onset of AD symptoms [12,65]. While tau deposition may initiate prior to Aβ accumulation[66], it is widely held that Aβ pathology is required to start the pathological cascade leading to AD dementia, making it an ideal target for clinical trials [67,68,69,70]. Several clinical trials have now successfully reduced brain Aβ without slowing down AD clinical progression[71,72]. While the role of Aβ in the pathological cascade of AD has been questioned based on large-scale clinical trial failures[73], one could argue that Aβ needs to be targeted before the spread of tau pathology[74]. The appropriate timing likely corresponds to a stage where there is a limited amount and spreading of Aβ pathology, hence making it challenging to identify[75,76].

Accumulation of Aβ starts in a few distinct brain regions almost simultaneously, which makes it possible to characterize early regional Aβ deposition *in vivo* using positron emission tomography (PET) imaging, before it rapidly evolves to widespread distribution[26,77,78]. In general, most studies have used global brain load to classify individuals with intermediate or high levels of Aβ[62,77,79]. We took advantage of the spatial distribution of Aβ deposition to identify cognitively unimpaired individuals with regional Aβ tracer uptake with the objective of identifying early Aβ deposition. We sought to investigate the associations between the extent of Aβ deposition with various AD markers in two cohorts of cognitively unimpaired older adults, including one with a family history of AD dementia.

Methods

a. Participants and Study Design

PREVENT-AD

One hundred twenty-nine participants were recruited from the Presymptomatic Evaluation of Experimental or Novel Treatments for Alzheimer's Disease (PREVENT-AD) cohort. The PREVENT-AD cohort is an ongoing longitudinal observational study launched in 2011 comprising a total sample size of 385 individuals[80]. Only the subsample of PREVENT-AD participants who underwent Aβ and tau PET imaging were included in the current study. Enrollment criteria included: (1) having a parent or multiple siblings with a history of AD; (2) age >60 years or age between 55 and 59 years if the onset of symptomatic dementia of their youngest affected relative was within 15 years of their age; (3) no major neurological diseases; and (4) no evidence of cognitive impairment at enrollment[81]. All participants enrolled were cognitively unimpaired when they performed their PET scan.

ADNI

Four hundred participants were included from the Alzheimer's Disease Neuroimaging Initiative (ADNI; adni.loni.usc. edu) database (adni.loni.usc.edu). The ADNI was launched in 2003 as a public-private partnership, led by Principal Investigator Michael W. Weiner, MD. The primary goal of ADNI has been to test whether serial magnetic resonance imaging (MRI), positron emission tomography (PET), other biological markers, and clinical and neuropsychological assessment can be combined to measure the progression of mild cognitive impairment (MCI) and early Alzheimer's disease (AD). The current study included individuals from the ADNI-2

extension. Participants were cognitively unimpaired at the time of the PET scan and they all had at least one Aβ Florbetapir (AV45) scan.

Standard protocol approvals, registrations, and patient consents. All PREVENT-AD participants were fully briefed and gave their explicit consent for participation using procedures and consent forms approved by the Institutional Review Board of the McGill University Faculty of Medicine. Data collection and sharing in ADNI were approved by the Institutional Review Board of each participating institution and written informed consent was obtained from all participants.

b. Neuropsychological Evaluation

As part of the PREVENT-AD battery, all participants underwent annual cognitive testing using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS)[82]. Objective cognitive performance was assessed using the total score and the five RBANS composite domain scores derived from twelve tasks: Immediate Memory, Attention, Visuospatial Construction, Language and Delayed Memory. Longitudinal cognitive assessment (annually) was available for all subjects, with a median follow-up time of 7 [interquartile range (IQR): 2, 8] years.

All participants from ADNI received detailed cognitive assessments approximately every year from the ADNI neuropsychological battery[83]. The four composite scores reflect memory, executive functions, language, and visuospatial functioning, and assessment procedures have been previously described[83,84]. Longitudinal cognitive assessment was available for 393 (98%) subjects, with a median follow-up time of 6 [interquartile range (IQR): 1, 14] years.

c. Subjective Cognitive Decline (SCD)

Both in PREVENT-AD and ADNI, participants were classified as having SCD if they answered "yes" to the question "Do you think your memory is becoming worse?". Contrariwise, participants

were classified as not-SCD if they responded “no”. There were 75 participants (58%) who had SCD in PREVENT-AD and 189 participants (47%) from ADNI.

d. APOE genotyping

Genomic DNA was extracted from whole blood, and apolipoprotein E (*APOE*) genotype was determined [85]. The same procedure was done for both PREVENT-AD and ADNI participants at their baseline visit. Participants were classified as *APOE* ε4 carriers (*i.e.*, those who had at least one ε4 allele) or noncarriers.

e. Cerebrospinal fluid analysis

In PREVENT-AD, a subsample of 77 participants underwent a lumbar puncture up to 2 years before their PET scans as previously described (mean difference to PET scan 10.42 ± 8.38 months)[80,86]. In brief, cerebrospinal fluid samples (CSF) were collected in the morning after an overnight fast and stored in cryovial tubes at −80°C. CSF $A\beta_{1\text{-}42}$, p-tau (phosphorylated at threonine 181) and total tau levels were assayed in duplicate with the INNOTEST ELISA (Fujirebio, Ghent, Belgium).

In ADNI, lumbar punctures were performed on a subsample of 276 participants as described in the ADNI procedures manual (http://www.adni-info.org/). All samples were collected in the morning before breakfast and after an overnight fast of a minimum of 6 hours. CSF samples were frozen within 1 hour after collection and were shipped overnight frozen on dry ice to the Penn AD Biomarker Fluid Bank Laboratory. Aliquots of 500 μL were stored in polypropylene tubes at −80°C. CSF $A\beta_{1\text{-}42}$ and p-tau (phosphorylated at threonine 181) were measured using Elecsys immunoassays[87]. We selected CSF data that was acquired within two years of the PET scan (mean time from PET scan 0.40 ± 0.75 months).

f. Image Acquisition

PET imaging in PREVENT-AD was performed using [18F]NAV4694 (NAV; Navidea Biopharmaceuticals, Dublin, OH) for Aβ and Flortaucipir (FTP) (Eli Lilly&Co, Indianapolis, IN) for tau deposition. Aβ scans were performed 40 to 70 minutes after injection (≈6mCi) and tau scans 80 to 100 minutes after tracer injection (≈10 mCi). Most scans were operated on 2 consecutive days, and all were acquired no more than 9 months apart (median delay 1 [interquartile range (IQR): 1, 246] day). Imaging was performed at the McConnell Brain Imaging Centre at the Montreal Neurological Institute (Montreal, Canada) between February 2017 and May 2019. T1-weighted structural MRI scans had been acquired ≈1 year before the PET scans (median delay 9.06 [interquartile range (IQR): 0.03,34] months) on a 3T Siemens Trio scanner (Siemens, Munich, Germany) at the Brain Imaging Centre of the Douglas Mental Health University Institute (Montreal, Canada) with the following parameters: repetition time of 2300 milliseconds, echo time of 2.98 milliseconds, 176 slices, and 1-mm slice thickness[80].

Acquisition of the multicentric MRI and PET imaging data in ADNI has been reported previously and is described in detail at adni.loni.usc.edu/methods/. Briefly, Florbetapir-PET scans ([18F]AV45, Aβ) were acquired during a 50 to 70 minute interval following a 370 MBq bolus injection (≈10 mCi) and FTP scans were acquired during a 75 to 105 minute interval following a 370 MBq bolus injection (≈10 mCi). Tau-PET scans were acquired starting from 2016; most scans were acquired 5 years after the Aβ-PET scan (median delay 5 [interquartile range (IQR): 0, 8] years). T1-weighted structural MRI data were acquired on 3T scanning platforms using sagittal 3D magnetization-prepared rapid gradient-echo sequences. The T1 sequence was the same as for the PREVENT-AD cohort.

g. Image Processing and Classical Threshold Definition

T1-weighted MRI processing for PREVENT-AD was performed using the software FreeSurfer, version 5.3, and automatic parcellation according to the Desikan-Killiany atlas[88]. PET images were processed with a standard, in-house pipeline (available on Github: https://github.com/villeneuvelab/vlpp). Briefly, the 4D PET images were realigned, averaged, and registered to the corresponding T1-weighted MRI. Thereafter, the images were masked to exclude CSF signal and smoothed with 6 mm^3 Gaussian kernel. Standardized uptake value ratios (SUVRs) were computed by dividing the tracer uptake in each voxel by the uptake in the whole cerebellum gray matter for NAV scans[57] and the inferior cerebellum gray matter for FTP scans[89].

PET images for ADNI went through standardized preprocessing steps in order to increase data uniformity across the multicentric data acquisition[25]. Briefly, Florbetapir-PET scans (5 minutes x 4 frames) were co-registered, averaged, reoriented into a standardized image and voxel size and smoothed to produce a uniform resolution. FTP scans were acquired from a 30-minute dynamic scan (5 minutes x 6 frames) and co-registered and resliced to the structural MRI closest in time to the FTP-PET. The cerebellum gray matter was used as the reference region for Florbetapir scans and the inferior cerebellum gray matter was used for FTP scans. The Aβ and tau data were downloaded from the ADNI database. We used the 2019-12-04 version for Aβ and the 2020-02-04 version for tau.

In PREVENT-AD, to quantify the global Aβ burden, 20 regions of interest were included to calculate the average SUVR from precuneus, posterior cingulate, parietal lobe, frontal lobe, and lateral temporal regions[90]. The threshold for NAV positivity was 1.37, as previously reported[90]. In ADNI, the summary Aβ score for Florbetapir was created by the average of the volume-weighted

uptake from lateral and medial frontal, anterior and posterior cingulate, lateral parietal and lateral temporal regions. Aβ positivity was determined using a threshold of 1.1, as in previous publications[91,92].

h. Regional Thresholds of Aβ Positivity

Aβ PET values were extracted across seven bilateral regions which were hypothesized to be sensitive to early Aβ accumulation: medial orbitofrontal, rostral anterior cingulate, posterior cingulate, superior frontal, inferior parietal, precuneus, rostral middle temporal[57]. Tracer uptake in five (precuneus, medial orbitofrontal, posterior cingulate, rostral anterior cingulate, rostral middle temporal) of these regions has also been found to be elevated in Aβ-negative individuals who subsequently had significant evidence of Aβ deposition[76].

Tau-PET scans were available for 129 PREVENT-AD participants and 176 ADNI participants. Tau-PET values were extracted across six bilateral regions that represent early tau-PET deposition: entorhinal cortex, amygdala, fusiform, parahippocampal, inferior temporal, and middle temporal cortex[90]. The hippocampus was not included given the off-target binding spillover from the choroid plexus[93,94].

A Gaussian mixture modelling approach (GMM) was used to quantify region-specific Aβ thresholds in the 7 bilateral regions hypothesized to be sensitive to early Aβ accumulation. Typically, Aβ follows a bimodal distribution and thus we fitted two Gaussian distributions as commonly used to categorize Aβ positivity[57,62,95]. The two distributions acquired from GMM assigned each participant a probability of belonging to either the lower or higher distributions. We set a cut-off at the 90th percentile of the lower distribution. Those who had higher SUVR values than the regional cut-off was classified as "positive" for that specific region. According to the

region-specific positivity, individuals who were Aβ-positive in all 7 regions were classified as the "Widespread Aβ group"; those who were positive in at most 6 regions were included in the "Regional Aβ group"; those who were negative in all the regions were considered as the "Negative Aβ group".

As expected, the SUVR regional distribution of the data in both cohorts differed because of tracer differences. For the NAV-PET tracer (PREVENT-AD), GMM analyses provided a clear distinction between individuals with and without tracer binding using thresholds for each region which corresponds to a 90% probability of belonging to the low Aβ distribution (Figure e-1). The distinction between regional positive and negative binding was less evident with Florbetapir (ADNI), and therefore complicates their probability of belonging to the low distribution compared to PREVENT-AD. As shown in Figure e-1, participants from ADNI followed a more continuous distribution without a distinctive cut-off between lower and higher distributions. This interfered with using the same cut-off criteria as used in the PREVENT-AD cohort and might partly be due to the different properties of the tracers. Therefore, in ADNI, prior to defining the groups we used a 50% probability of belonging to the low-Aβ distribution as cut-off criteria solely depending on the distribution of regional SUVRs, also in line with the previous studies[51,53,96]. We acknowledge the fact that these two methods of defining thresholds are not identical, which nevertheless represents the challenge of working with multiple datasets.

i. Statistical Analysis

We compared demographics, baseline cognition and tau-PET of the Aβ groups by using analysis of covariance tests and chi-squared tests for normally distributed continuous variables and categorical variables, respectively. Tukey HSD post hoc test and Bonferroni correction were

applied to examine differences between the three Aβ groups. Regarding cognitive decline, we conducted linear mixed-effects models to test for the main effect of Aβ groups. Participants who had at least 2 assessments were included in the analysis. Models included random slope and intercept, where the time by subject interaction determined change in the cognitive scores, along with age, sex, and education as covariates. The analyses were anchored at the participant's baseline visit.

Longitudinal Aβ data was only available for the ADNI cohort. In a prospective analysis, we compared the 3 Aβ groups on their rate of Aβ accumulation (up to 4 years follow-up with a median follow-up time of 3 [interquartile range (IQR): 1, 4] years). Participants who had at least 2 measures were included in a linear mixed-effects model with a random slope and intercept where the time by subject interaction determined change in the Aβ burden accumulation, along with age, sex, and education as covariates.

All statistical analyses were conducted using RStudio, version 1.2.5001. The cutoff and mixtools packages (github.com/choisy/cutoff) were used for GMM and lme4 for mixed-effects models. The criterion for statistical significance was $\alpha \leq 0.05$ after correction for multiple comparisons by Tukey's test.

Results

a. Defining Amyloid Groups

In each cohort, GMMs were fit to the 7 bilateral early Aβ regions (Figure 1). Region-specific thresholds for both cohorts are presented in detail in Table e-1. In the PREVENT-AD cohort, 81 participants (62%) were in the Negative Aβ group, 28 participants were in the Regional Aβ group (22%) and 20 exceeded the positivity thresholds in all regions and were placed in the Widespread

Aβ group (16%). Applying the thresholds to the ADNI cohort resulted in 202 (50.5%) in the Negative Aβ group, 108 (27%) individuals in the Regional Aβ group and 90 (22.5%) individuals in the Widespread Aβ group.

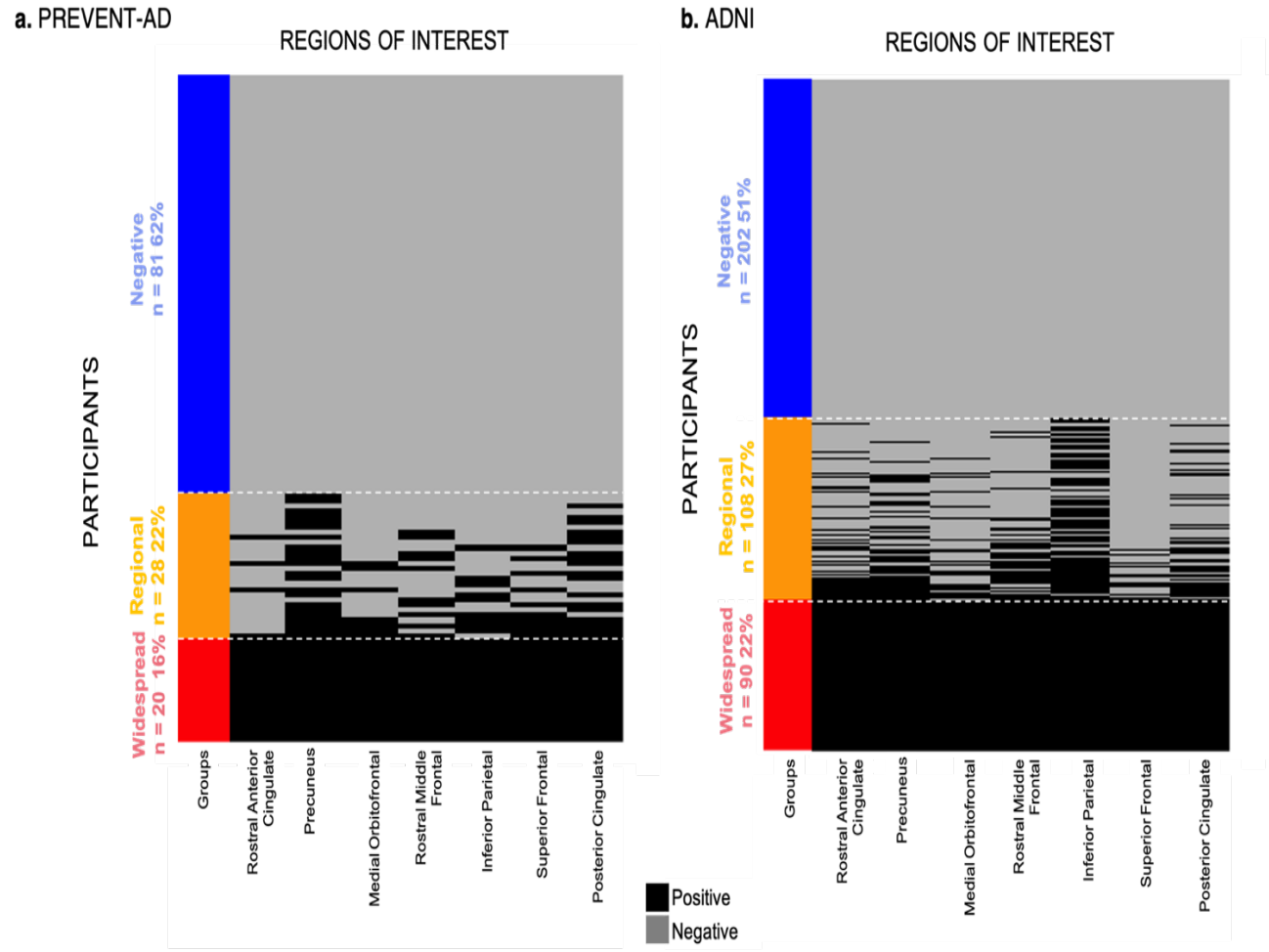

Figure 1. Defining the Aβ Groups.
Individuals were separated into three groups based on their Aβ status in seven cortical regions: rostral anterior cingulate, precuneus, medial orbitofrontal, rostral middle frontal, inferior parietal, superior frontal, and posterior cingulate. According to the region-specific positivity, individuals who were Aβ-positive in all 7 regions were classified as the "Widespread Aβ group"; those who were positive in at most 6 regions were included in the "Regional Aβ group", while those negative in all the regions were considered as the "Negative Aβ group".

Using a more standard binary classifier of positive/negative based on a global Aβ score in the PREVENT-AD cohort (SUVR 1.37; Centiloid 26.7)[90], 26 participants (20%) would have been classified as Aβ positive. This included all 20 participants in the Widespread group and 6 out of the 28 participants from the Regional group (21%). For ADNI, 137 (34%) participants would have been classified as positive based on a similar classical "global Aβ score" cut-off (SUVR 1.1; Centiloid 18.5)[60,91]. Specifically, all of the Widespread group in ADNI would have been classified as positive, all of the Negative as negative, and the Regional group would have been split almost in half between positive (43%) and negative (57%).

The results presented below, comparing the groups on biological and clinical markers, consist of all the Regional participants. The analyses were repeated when including only the Regional participants that would have been classified as Aβ-negative based on the global Aβ scores (n=22 for the PREVENT-AD and n=61 for ADNI). The results were unchanged for both PREVENT-AD and ADNI (see Table e-2, Figure e-2 & Figure e-3).

b. Biological Markers of Interest

Between the Aβ groups, there were no differences in education [$F(2, 126) = 2.89$, $p = 0.058$] or sex [X^2 (2, $N = 129$) = 1.81, $p = 0.4$] in PREVENT-AD; however, compared with the participants in the Negative Aβ group, participants in the Widespread Aβ group were older [$F(2, 126) = 3.95$, $p < 0.05$]. In ADNI, there were no differences in education between the Aβ groups [$F(2, 380) = 2.35$, $p = 0.1$]. However, the Widespread Aβ group were older [$F(1, 292) = 5.94$, $p < 0.05$] and had a higher percentage of females [X^2 (1, $N = 292$) = 4.39, $p < 0.05$] compared to the Negative Aβ group. Participants were also older in the Widespread Aβ group compared to the Regional Aβ group [$F(1, 198) = 5.94$, $p < 0.01$]. While participants in the Widespread Aβ group (Prevent-AD:

35%; ADNI: 66%) had higher proportions of SCD than those in the Negative (Prevent-AD: 25%; ADNI: 50%) and Regional (Prevent-AD: 19%; ADNI: 51%) Aβ groups in both cohorts, only the participants in the Widespread Aβ group in ADNI had significantly higher proportions of SCD compare to the Negative Aβ group [X^2 (1, N = 179) = 6.9, $p < 0.01$].

The Widespread and Regional Aβ groups had larger proportions of *APOE* ε4 carriers than the Negative Aβ groups in both the PREVENT-AD (Widespread *vs.* Negative X^2 (1, N = 101) = 8.54, $p < 0.01$; Regional *vs.* Negative X^2 (1, N = 109) = 10.8, $p < 0.01$) and ADNI (Widespread *vs.* Negative X^2 (1, N = 292) = 29.11, $p < 0.01$; Regional *vs.* Negative X^2 (1, N = 310) = 5.24, $p < 0.05$) cohorts (Table 1). In ADNI, the Widespread Aβ group also had a larger proportion of *APOE* ε4 carriers compare to the Regional Aβ group [X^2 (1, N = 198) = 6.98, $p < 0.01$].

CSF biomarker measures were available for 77 PREVENT-AD participants and for 276 ADNI participants. In PREVENT-AD, when compared with the Negative group, both the Regional (F (2, 71) = 24.01, p<0.001) and Widespread groups (F (2, 71) = 24.01, p<0.001) had lower CSF $A\beta_{1-42}$ levels. The Widespread group also had lower CSF $A\beta_{1-42}$ levels than the Regional group (F (2, 71) = 24.01, p<0.001). In ADNI, Regional Aβ (F (2, 275) = 71.76, p < 0.001) and Widespread groups (F (2, 275) = 71.76, p < 0.001) had lower $A\beta_{1-42}$ levels when compared to the Negative Aβ group. The Widespread group also had lower CSF $A\beta_{1-42}$ levels than the Regional group (F (2, 275) = 71.76, p < 0.001).

In PREVENT-AD, CSF p-tau and total tau levels were higher in the Widespread group compared to the Negative group (p-tau: [F (2,76) = 4.70, p<0.05]; total tau: [F (2,76) = 5.09, p<0.05]). In ADNI, CSF p-tau levels were higher in the Widespread Aβ group compared to the Regional Aβ (F (2, 274) = 26.77, p < 0.001) and Negative Aβ groups (F (2, 274) = 26.77, p < 0.001).

Table 1. Biological and Clinical Characteristics of Aβ groups.

	PREVENT-AD Aβ Groups				ADNI Aβ Groups			
	Negative (n = 81)	Regional (n = 28)	Widespread (n = 20)	*p < 0.05*	Negative (n = 202)	Regional (n =108)	Widespread (n =90)	*p < 0.05*
Age	**63 (0.51)**	63 (0.87)	**66 (1.03)**	*b*	**73 (0.41)**	**73 (0.57)**	**76 (0.62)**	*b,c*
Education	16 (0.36)	15 (0.61)	14 (0.72)		17 (0.19)	17 (0.26)	16 (0.28)	
Sex, female (%)	60 (74%)	23 (82%)	13 (65%)		**94 (47%)**	61 (57%)	**55 (61%)**	*b*
APOE ε4 carriership (%)	**22 (27%)**	**18 (64%)**	**13 (65%)**	*a, b*	**38 (19%)**	**34 (31%)**	**45 (50%)**	*a,b,c*
Subjective Cognitive Decline (%)	17 (25%)	5 (19%)	6 (35%)		**117 (50%)**	42 (51%)	**55 (66%)**	*b*
CSF $A\beta_{1-42}$*	**1265 (37.78)**	**1043 (60.09)**	**718 (71.53)**	*a,b,c*	**1448 (30.13)**	**1158 (40.07)**	**802 (45.69)**	*a,b,c*
CSF pTau*	**46 (3.14)**	55 (4.89)	**67 (6.15)**	*b*	**19 (0.72)**	**22 (0.96)**	**29 (1.10)**	*b,c*
CSF Total Tau*	**264 (26.74)**	363 (41.60)	**435 (52.34)**	*b*	**219 (7.33)**	**239 (9.75)**	**294 (11.12)**	*b,c*

The values are reported as Mean (SD) except for Sex, *APOE* ε4, and Subjective Cognitive Decline which are reported as Number of participants (% of group). BOLD text represents the groups between which there were significant differences: a = $p<0.05$ between Negative Aβ and Regional Aβ groups; b = $p<0.05$ between Negative Aβ and Widespread Aβ groups; c = $p<0.05$ between Regional Aβ and Widespread Aβ groups. *In PREVENT-AD, CSF samples were available for 46 Negative, 19 Regional, and 12 Widespread; in ADNI, CSF samples were available for 138 Negative, 78 Regional and 60 Widespread. *APOE* ε4: Apolipoproteinε4; Aβ: beta-amyloid; CSF: Cerebrospinal fluid.

In PREVENT-AD, the Widespread Aβ group had elevated Tau-PET signal when compared with Negative and Regional Aβ groups across the five regions investigated (Entorhinal, Amygdala, Fusiform, Inferior Temporal, and Parahippocampal) (Table 2). The Regional Aβ group had elevated tau-PET binding only in the Entorhinal cortex ($F (2, 128) = 19.21$, $p<0.05$) and Middle Temporal gyrus ($F (2.128) = 14.06$, $p<0.05$) when compared with the Negative Aβ group. In ADNI, the Widespread Aβ group had elevated Tau-PET signal compared with Negative and Regional Aβ groups across all regions investigated (Table 2) (Figure 2).

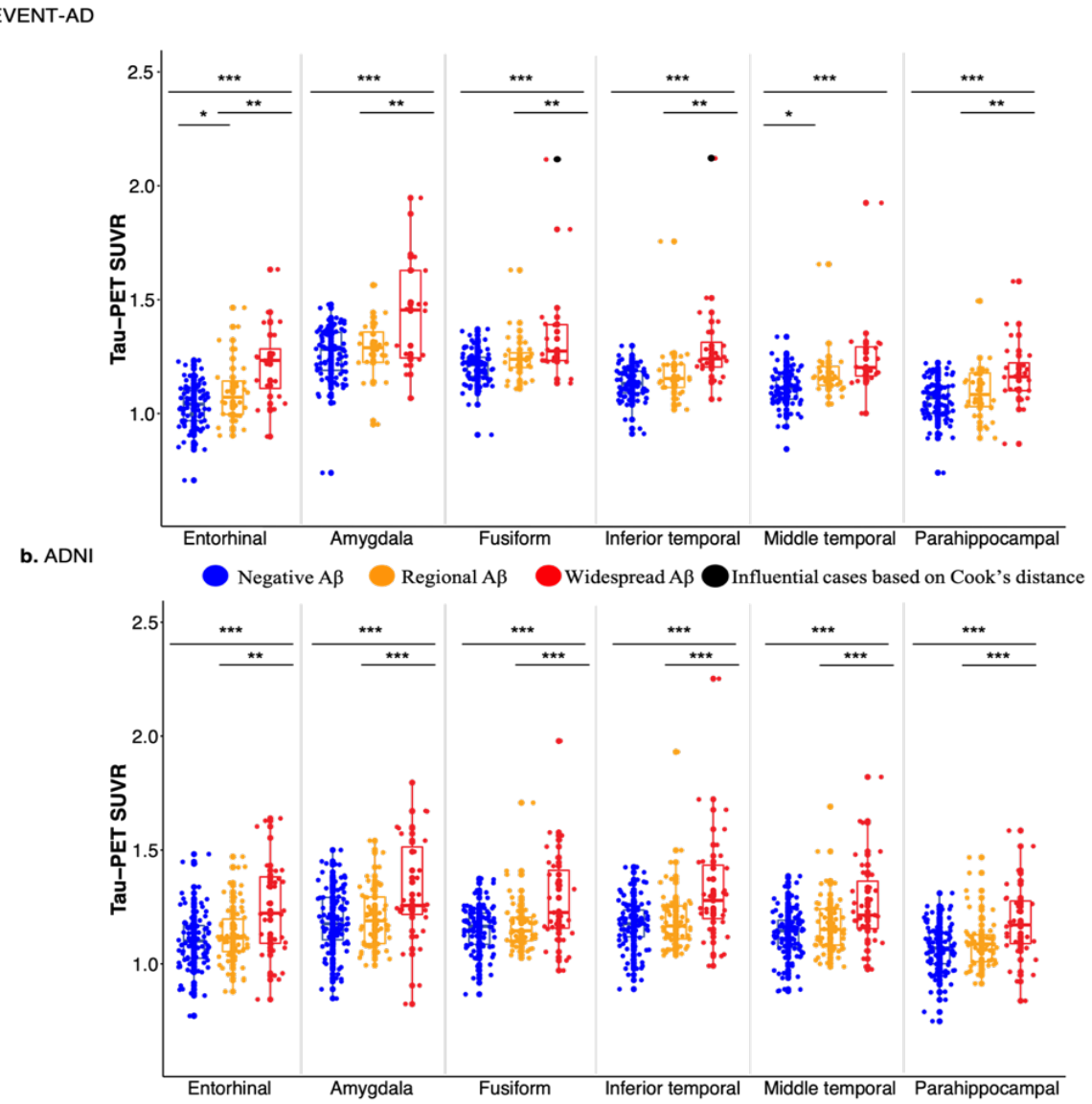

Figure 2. Tau-PET Uptake Across the 3 Aβ groups in PREVENT-AD and ADNI.
Six regions were chosen to represent areas of early tau-PET accumulation[31]. Tau-PET scans were available for 129 PREVENT-AD participants and 176 ADNI participants. **(A)** In PREVENT-AD, the Widespread Aβ group had elevated Tau-PET signal when compared with Negative and Regional Aβ groups across six regions. The Regional Aβ group had elevated tau-PET binding only in the Entorhinal cortex and Middle Temporal gyrus when compared with the Negative Aβ group. **(B)** In ADNI, the Widespread Aβ group had elevated Tau-PET signal compared with Negative and Regional Aβ groups across all regions. One PREVENT-AD Regional participant and one PREVENT-AD Widespread participant were considered influential cases based on their Cook's distance. Removing these participants did not influence the results. Analyses were corrected for age and sex. * $p<0.05$; ** $p<0.01$; ***$p<0.001$. *SUVR*: standardized uptake value ratio.

Table 2. Tau-PET Uptake in Early Tau Regions

A. PREVENT-AD	Negative Aβ (n = 81)	Regional Aβ (n = 28)	Widespread Aβ (n = 20)	P<0.05
Entorhinal	**1.04 (0.01)**	**1.10 (0.02)**	**1.22 (0.03)**	*a,b,c*
Amygdala	**1.26 (0.02)**	**1.28 (0.03)**	**1.44 (0.03)**	*b,c*
Fusiform	**1.20 (0.01)**	**1.25 (0.02)**	**1.35 (0.03)**	*b,c*
Inferior Temporal	**1.13 (0.01)**	**1.18 (0.02)**	**1.30 (0.03)**	*b,c*
Middle Temporal	**1.11 (0.01)**	**1.18 (0.02)**	**1.25 (0.02)**	*a,b*
Parahippocampal	**1.06 (0.01)**	**1.10 (0.02)**	**1.18 (0.02)**	*b,c*
B. ADNI	Negative Aβ (n = 91)	Regional Aβ (n = 54)	Widespread Aβ (n = 31)	
Entorhinal	**1.10 (0.01)**	**1.13 (0.02)**	**1.24 (0.03)**	*b,c*
Amygdala	**1.19 (0.02)**	**1.20 (0.02)**	**1.32 (0.03)**	*b,c*
Fusiform	**1.16 (0.01)**	**1.17 (0.02)**	**1.29 (0.02)**	*b,c*
Inferior Temporal	**1.18 (0.02)**	**1.20 (0.02)**	**1.33 (0.03)**	*b,c*
Middle Temporal	**1.13 (0.01)**	**1.17 (0.02)**	**1.27 (0.02)**	*b,c*
Parahippocampal	**1.07 (0.01)**	**1.09 (0.02)**	**1.18 (0.02)**	*b,c*

Using ANCOVA and multiple comparisons corrected for age and sex, we test whether Tau-PET uptake in early tau regions significantly differed between the Aβ groups in the **(A)** PREVENT-AD cohort and **(B)** ADNI cohort. For post-hoc analysis, Bonferroni correction was applied when comparing the pair of group means. BOLD text represents the significant between-group differences. a = $p<0.05$ between Negative Aβ and Regional Aβ Groups; b = $p<0.05$ between Negative Aβ and Widespread Aβ Groups; c = $p<0.05$ between Regional Aβ and Widespread Aβ Groups.

c. Cross-sectional and Longitudinal Cognition

We examined 129 participants with a cognitive assessment follow-up period of up to 8 years from the PREVENT-AD cohort (median follow-up time of 7 [interquartile range (IQR): 2, 8] years) and 393 participants with a follow-up period of up to 14 years from the ADNI cohort (median follow-up time of 6 [interquartile range (IQR): 1, 14] years).

In PREVENT-AD, when compared with the Negative Aβ group, the Widespread Aβ group performed worse in the Delayed Memory cognitive domain [$F(2, 112) = 3.923$, $p < 0.05$] at their baseline visit. There were no differences in baseline cognitive performance between any other

groups (Table 3). In ADNI, there were no differences in baseline cognitive performance between any of the groups (Table 3).

Table 3. Baseline Cognition

A. PREVENT-AD	Negative Aβ (n = 81)	Regional Aβ (n = 28)	Widespread Aβ (n = 20)	P<0.05
Immediate Memory Score	103.15 (1.26)	104.82 (2.14)	104.05 (2.54)	
Delayed Memory Score	**104.28 (1.02)**	100.71 (1.73)	**97.20 (2.05)**	*b*
Attention Score	106.96 (1.67)	106.00 (2.84)	109.65 (3.36)	
Language Score	103.21 (1.02)	105.46 (1.73)	103.65 (2.05)	
Visuospatial Score	95.24 (1.45)	94.21 (2.48)	92.45 (2.93)	
Total Index Score	102.98 (1.06)	102.61 (1.81)	101.30 (2.15)	
B. ADNI	Negative Aβ (n = 202)	Regional Aβ (n =108)	Widespread Aβ (n =90)	
Memory Score	1.08 (0.04)	1.11 (0.05)	0.95 (0.06)	
Executive Function Score	0.95 (0.06)	0.84 (0.08)	0.62 (0.08)	
Language Score	0.91 (0.05)	0.85 (0.07)	0.70 (0.07)	
Visuospatial Score	0.24 (0.04)	0.22 (0.06)	0.28 (0.06)	

Cognitive test scores were compared at the baseline visit corrected for age and sex; test scores are reported as Mean (SD). (**A)** As part of the PREVENT-AD battery, all participants undergo annual cognitive testing using the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). (**B)** In ADNI, participants received detailed cognitive assessments from which composite scores are derived. All the composite scores have a mean of 0 and a standard deviation of 1. BOLD text represents the significant between-group differences: a = $p<0.05$ between Negative Aβ and Regional Aβ groups; b = $p<0.05$ between Negative Aβ and Widespread Aβ groups; c = $p<0.05$ between Regional Aβ and Widespread Aβ groups.

In PREVENT-AD, the Widespread Aβ group experienced greater cognitive decline compared with the Negative Aβ group on the Total (β [SE], -0.11 [0.03]; $p < 0.001$), Immediate Memory (β [SE], -0.13 [0.05]; $p < 0.01$), and Delayed Memory (β [SE], -0.10 [0.04]; $p < 0.05$) index scores (Figure 3). The Widespread group also declined faster than the Regional group on the Total (β [SE], -0.08 [0.04]; $p < 0.05$), Immediate Memory (β [SE], -0.11 [0.05]; $p < 0.05$), and Delayed Memory (β [SE], -0.13 [0.05]; $p < 0.01$) index scores.

In ADNI, the Widespread Aβ group had faster cognitive decline compared with the Negative Aβ group on Memory (β [SE], -0.10 [0.006]; $p < 0.001$), Executive Function (β [SE], -0.08 [0.008]; $p < 0.001$), Language (β [SE], -0.08 [0.008]; $p < 0.001$), and Visuospatial (β [SE], -0.07 [0.009]; $p < 0.001$) scores. The Widespread group also declined faster than the Regional group on Memory (β [SE], -0.07 [0.007]; $p < 0.001$), Executive Function (β [SE], -0.06 [0.01]; $p < 0.001$), Language (β [SE], -0.06 [0.01]; $p < 0.001$), and Visuospatial (β [SE], -0.06 [0.01]; $p < 0.001$) assessments. Although the Regional Aβ group did not have worse cognitive performance at baseline than the Negative Aβ group, they experienced greater cognitive decline over the 14 year-follow up period compared to the Negative Aβ group in Memory (β [SE], -0.03 [0.006]; $p < 0.001$), Executive Function (β [SE], -0.03 [0.008]; $p < 0.001$), and Language (β [SE], -0.02 [0.008]; $p < 0.05$), but not Visuospatial (β [SE], -0.01 [0.009]; $p = 0.27$) scores.

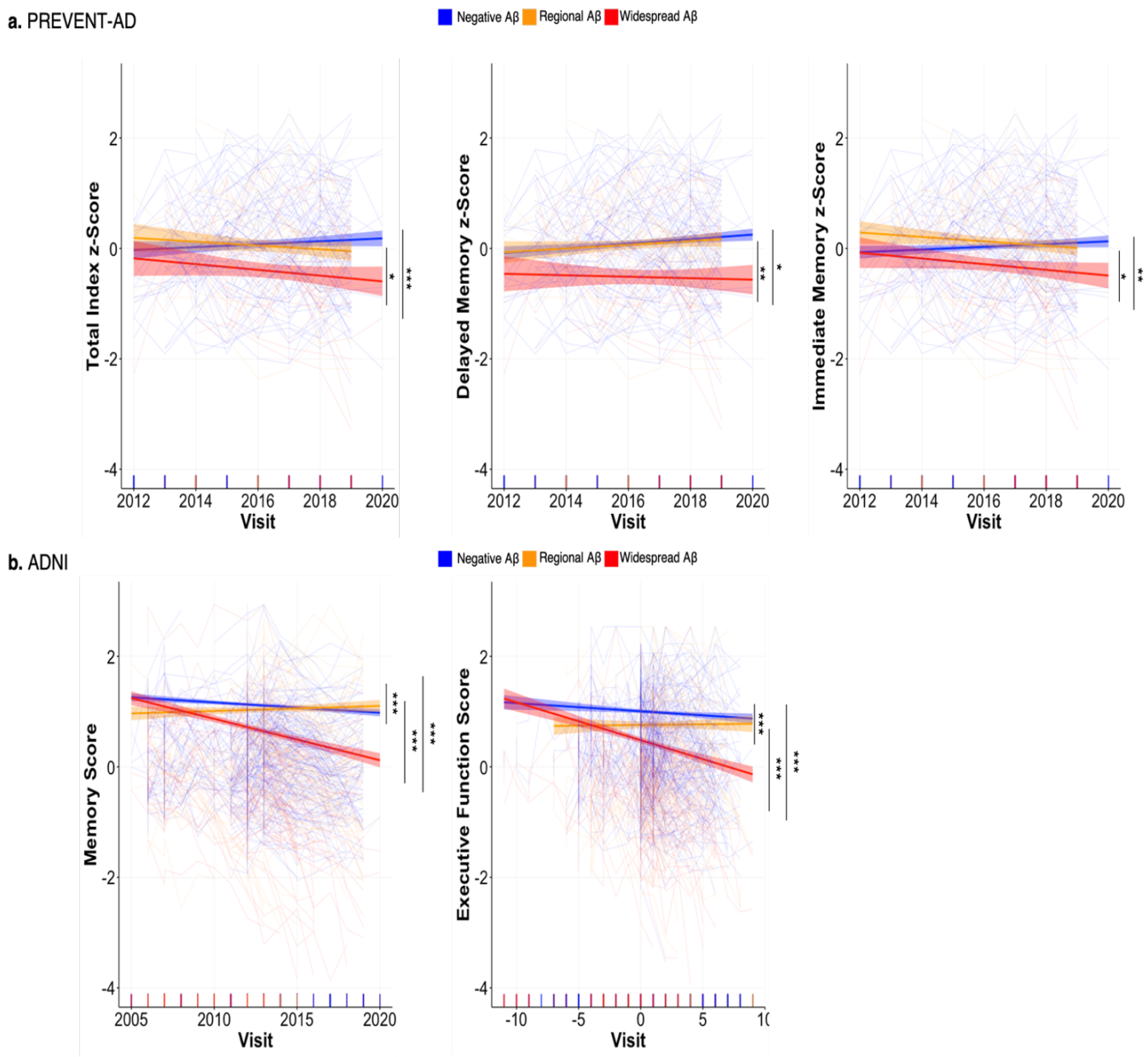

Figure 3. Change in Cognition Over Time Between the three Aβ Groups.
Linear mixed-effect models were used to assess the effects of Aβ status on longitudinal cognition, corrected for sex and education. Only significant cognitive domains are shown in the figure and the analyses were anchored at the participants' baseline visit date. **(A)** For PREVENT-AD, cognitive test scores of "Total Score", "Immediate Memory" and "Delayed Memory" on the RBANS. **(B)** In ADNI, cognitive test scores of "Memory" and "Executive Function" over time in the three different groups. The Widespread Aβ group showed a greater decline in their cognition scores when compared with the two other groups in both cohorts. * $p<0.05$; ** $p<0.01$; ***$p<0.001$.

d. Longitudinal Aβ Trajectories

To examine further whether the rates of Aβ accumulation differed depending on the Aβ groups, we conducted a series of linear mixed-effects models in the ADNI cohort using the 311 individuals with at least 2 Aβ-PET scans. All groups showed Aβ accumulation rates significantly different from zero over up to 4 years, with a median follow-up time of 3 [interquartile range (IQR): 1, 4] years (Figure 4). The rate of Aβ accumulation, however, differed between the groups (Table 4). The Regional Aβ group showed faster Aβ accumulation over time than the Negative Aβ group both in the total Aβ score (β [SE], 0.04 [0.01]; $p < 0.001$) and in the 7 early regions of interest (Table 4). The Widespread group also showed faster Aβ accumulation than the Negative Aβ group in the total Aβ score (β [SE], 0.05 [0.01]; $p < 0.001$), and in the 7 early regions of interest (Table 4). Interestingly, no difference was found between the Regional and Widespread Aβ group regarding Aβ accumulation over time both in the total Aβ score (β [SE], -0.0002 [0.01]; $p = 0.98$) and in the 7 early regions of interest (Table 4).

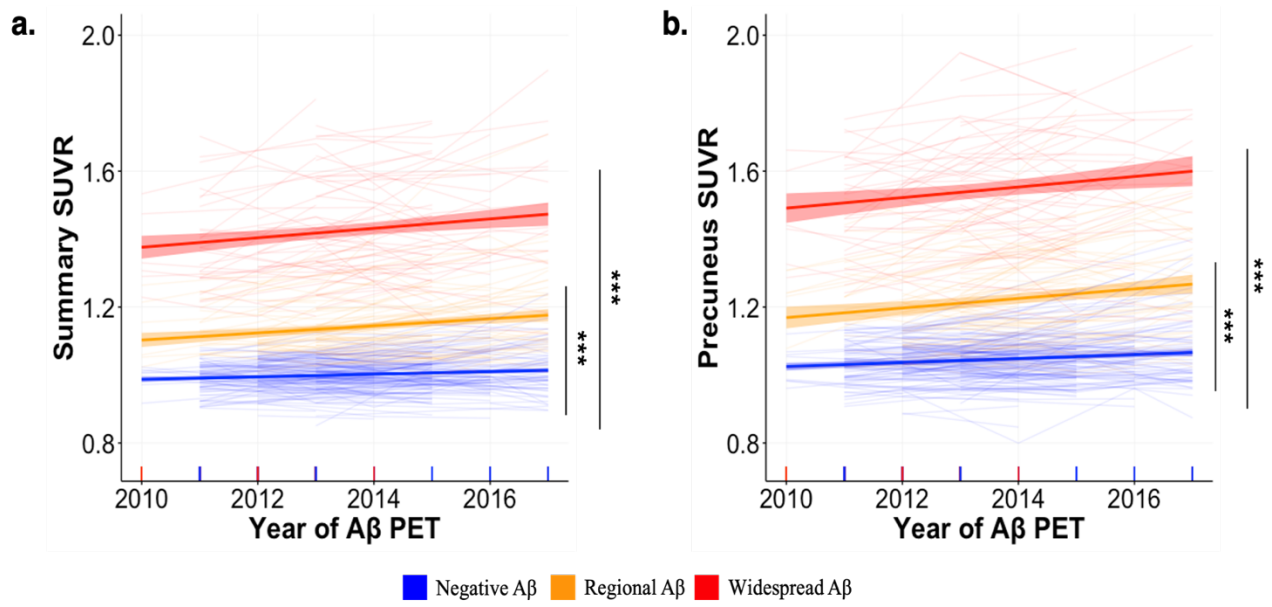

Figure 4. Change in Aβ Uptake Over Time Between the three Aβ Groups in ADNI.
Linear mixed-effect models investigating the effect of the groups on Aβ accumulation rate over time in ADNI cohort corrected for age, sex, and education. Plotted is the association between Aβ groups based on **(A)** total Aβ summary score and **(B)** Precuneus SUVR score over the years from their first scan. While both the Regional and Widespread Aβ group accumulated Aβ at a faster rate compared to the Negative Aβ group; the two groups did not differ in their rate of accumulation. * $p<0.05$; ** $p<0.01$; ***$p<0.001$; etc. *SUVR*: standardized uptake value ratio.

Table 4. Aβ Accumulation Rate in ADNI

Variable	Global SUVR	Rostral Anterior Cingulate	Precuneus	Medial Orbitofrontal	Rostral Middle Frontal	Inferior Parietal	Superior Frontal	Posterior Cingulate
Time	0.017 (0.006), **0.004**	0.012 (0.006), 0.064	0.024 (0.006), **<0.001**	0.016 (0.006), **0.008**	0.009 (0.006), 0113	0.024 (0.006), **<0.001**	0.005 (0.006), 0.472	0.037 (0.006), **<0.001**
Age	0.003 (0.004), 0.431	0.0005 (0.005), 0.918	0.002 (0.005), 0.590	0.006 (0.005), 0.233	0.007 (0.004), 0.106	0.003 (0.004), 0.480	0.007 (0.004), 0.107	0.003 (0.004), 0.520
Sex	0.010 (0.048), 0.039	0.056 (0.058), 0.331	0.034 (0.054), 0.532	-0.002 (0.057), 0.979	0.170 (0.053), **0.0012**	0.127 (0.052), **0.017**	0.055 (0.051), 0.280	0.055 (0.052), 0.293
Negative: Regional	0.047 (0.010), **<0.001**	0.049 (0.011), **<0.001**	0.052 (0.010), **<0.001**	0.056 (0.011), **<0.001**	0.042 (0.010), **<0.001**	0.030 (0.010), **0.004**	0.044 (0.011), **<0.001**	0.050 (0.011), **<0.001**
Negative: Widespread	0.047 (0.012), **<0.001**	0.028 (0.013), **0.032**	0.042 (0.012), **<0.001**	0.044 (0.012), **<0.001**	0.047 (0.012), **<0.001**	0.040 (0.012), **0.001**	0.058 (0.013), **<0.001**	0.028 (0.013), **0.030**
Regional: Widespread	-0.001 (0.013), 0.995	-0.020 (0.015), 0.168	-0.010 (0.013), 0.449	-0.014 (0.014), 0.322	0.004 (0.013), 0.762	0.009 (0.013), 0.484	0.014 (0.014), 0.329	-0.022 (0.014), 0.135

Using linear mixed-effects models, we tested whether Aβ groups were associated with a change in accumulation rate throughout the whole brain (global) and in different regions of interest corrected for baseline age, sex and time. Unstandardized estimates (Est.), standard errors (SE), and p-values are presented. The Negative group was assigned as a reference group while comparing Negative: Regional and Negative: Widespread; Regional group was set as reference group when comparing Regional: Widespread. Models employed *SUVR*: standardized uptake value ratio.

Discussion

We investigated the biological and clinical relevance of Aβ spatial extent severity on AD biomarkers in two independent cohorts of cognitively normal older adults. We classified participants as having widespread, regional or no Aβ tracer binding to quantify Aβ spatial extent severity. We focused on seven regions hypothesized to be early Aβ accumulating regions[57,76] and classified participants into Widespread (7 regions with significant Aβ-PET binding), Regional (1-6 regions with Aβ-PET binding) and Negative groups. Our results suggest that individuals with Regional Aβ-PET binding have a higher proportion of *APOE* ε4 carriers when compared to individuals with no Aβ-PET binding in both cohorts. This proportion of *APOE* ε4 carriers reached 64% in the PREVENT-AD cohort, a cohort of cognitively normal individuals with a first-degree family history of AD dementia and therefore with a higher risk to develop the disease themselves. In both cohorts, we found a grading effect of (quasi-continuous) $A\beta_{1-42}$ levels such that participants in the Widespread and Regional groups had decreased CSF $A\beta_{1-42}$ levels when compared to the Negative group, and the Widespread group had decreased CSF $A\beta_{1-42}$ levels compared to the Regional group. Furthermore, in ADNI, the Regional group had a faster rate of Aβ-PET accumulation when compared to the Negative group (information that was not available in the PREVENT-AD cohort). Of interest, however, the Regional groups showed no or very little tau-PET binding and no baseline cognitive impairment in both cohorts. While these findings suggest that the regional Aβ PET-binding signal is biologically relevant, they also suggest that widespread Aβ is necessary to detect tau-PET signals outside of the entorhinal cortex and cognitive impairment.

Continuous variables are often dichotomized in the clinic to provide a straightforward diagnosis or identify patients that would benefit from treatment[97]. They are also used in research to simplify

the interpretation of results and provide empirical evidence for clinical practice. The most common approach to analyzing Aβ-PET is to classify individuals into two groups, Aβ-negative and Aβ-positive. This approach is not always optimal to detect individuals with early Aβ levels, mainly if Aβ has accumulated regionally but is not yet globally widespread[57,60]. There is a growing body of literature documenting the earliest topographical distribution of Aβ-PET binding in individuals with and without cognitive impairment[62,77,98]. We took advantage of this literature to identify seven regions with early detectable Aβ-PET binding, and instead of classifying our participants on a "global" Aβ index, we dichotomized Aβ positivity/negativity within each of these regions and counted the number of regions with positive Aβ-PET binding. In PREVENT-AD, most of the participants with regional binding (79%) would have been classified as negative using a "global" Aβ index. This number was slightly lower in ADNI (57%), which can probably be explained by the fact that their global threshold is slightly lower than what was used in the PREVENT-AD (centiloid 26.7 vs 18.5).

Anti-Aβ therapeutic trials have failed to improve or slow down cognitive symptoms[71,99,100]. The association of cognition and neuronal loss is stronger with the tau-PET signal compared to Aβ[101]. The failure of these trials could be partly due to the inclusion of individuals who already have elevated tau-PET signals. Our results showed that cognitively normal individuals with widespread Aβ have detectable tau-PET signals in several temporal brain regions. Interestingly, elevated tau PET-binding was absent (ADNI) or restricted to the entorhinal and the middle temporal cortices (PREVENT-AD) in individuals showing regional Aβ-PET. Binding in the entorhinal cortex is common with advanced age[102]. The CSF data corroborate these findings in both cohorts, with CSF p-tau and total-tau levels being higher in the Widespread group compared to the two other groups,

with an absence of significant differences between the Regional and the Negative groups. Previous studies have shown that when Aβ pathology reaches "widespread" spatial distribution, tau-PET uptake increases faster compared to the individuals with lower Aβ[103], and the rate of tau-PET change is associated with cognitive decline[35]. In line with these results, baseline cognitive impairment was only found in the Widespread groups, and cognitive decline was also mainly restricted to the Widespread groups. The ADNI regional group also showed cognitive decline when compared to the Negative group after a decade of follow-up, by which time most Regional individuals probably developed Widespread Aβ binding[76]. Increased tau pathology might, therefore, be too advanced to stop the cognitive decline or disease progression after the removal of the Aβ plaques from the brain in individuals that have already reached the Aβ positivity thresholds. Our results suggest that anti-Aβ trials should be performed in individuals with regional binding at the latest since even individuals classified as being Negative showed Aβ-PET binding accumulation over time.

Furthermore, our findings highlight the biological relevance of the Regional Aβ group. Our result showed that the Regional Aβ groups had intermediate CSF $A\beta_{1-42}$ levels between the Widespread (lower $A\beta_{1-42}$) and Negative (higher $A\beta_{1-42}$) Aβ groups, showing signs of incipient cerebral accumulation of Aβ[15]. Even though Aβ increases with older age, Regional Aβ group participants were in the same age range as the Negative Aβ group, which was younger than the Widespread Aβ group in both cohorts. Furthermore, the Regional Aβ ADNI group accumulated Aβ faster than the Negative group. Another crucial difference between groups was marked by *APOE* ε4 carrier status; compared to the Negative Aβ group, both Regional and Widespread Aβ groups had higher percentages of *APOE* ε4 carriers, which places them at increased risk for developing the disease[104].

There is an increasing interest in the biological relevance of regional Aβ and the assessment of regional patterns[26,77]. Recent studies have shown decreased CSF $A\beta_{1-42}$ levels in participants with regional Aβ[62,64], as well as higher proportions of *APOE* ε4 carriers, compared to Aβ negative participants[105]. Even in individuals categorized as Aβ negative, subthreshold Aβ predicted a slight memory decline[106] and the development of tau pathology over five years[37]. *APOE* ε4 carriership has also been associated with increased Aβ load compared to noncarriers across all clinical diagnostic groups[107]. Taken together, our findings highlight the biological relevance of the Regional Aβ group, for which tau and cognitive impairments are still minimal. Therefore, most individuals with regional Aβ binding are at the earliest stage of the AD continuum and only a few years away from when cognitive decline is about to start.

There are several limitations to take into account. An important factor that may have impacted the current study results is the Aβ groups' disproportion due to the small sample size of the Widespread and Regional groups in PREVENT-AD. More than 63% of the cohort were in the Negative Aβ group, which led to the Regional and Widespread groups consisting of less than 30 individuals each. To address this limitation and to have a bigger sample size for the Aβ groups, the ADNI cohort with 400 participants was included in the study. ADNI, however, used the Florbetapir tracer, for which it might be more difficult to establish clear dichotomized values given the high variability related to white matter signal[108]. In addition, previous studies also reported that this tracer had shown a low correlation between tracer-specific regional rankings compared to four other tracers[53,64]. Despite the differences in the study designs, it is nevertheless important to mention that most of the results in both cohorts were comparable.

In conclusion, assessing the spatial Aβ burden could be a powerful way to identify the best candidate for preventive clinical trials. Assessing the presence of Aβ-PET binding in early accumulating regions can help identify individuals with biologically relevant signals that would have been classified as being negative using more established whole brain thresholds for Aβ positivity. While these individuals accumulate Aβ over time, they do not yet have significant tau or cognitive decline, making them a better target for anti-Aβ, and probably tau, therapies than individuals with widespread Aβ.

CHAPTER III:

DISCUSSION AND FUTURE DIRECTIONS

Our study is an investigation of the importance of the spatial extent of Aβ accumulation in early regions and the associations of Aβ deposition levels with various AD markers in two cohorts of cognitively unimpaired individuals. We characterized different levels of early Aβ accumulation in three groups, compared their characteristics, and determined how they were associated with different AD markers.

The results indicated that while most of these individuals would have been classified as having no Aβ using conventional classification, individuals with regional Aβ PET-binding had a greater proportion of *APOE* ε4 carriers, decreased CSF $A\beta_{1-42}$ levels, and faster longitudinal Aβ-PET binding accumulation when compared with individuals without regional Aβ. However, their cognition was preserved compared with individuals with widespread Aβ, and they did not show tau-PET signal outside of the entorhinal cortex. These results suggest that regional Aβ deposition is associated with alterations of key AD markers in individuals that do not yet have tau or cognitive impairments. In the study, we decided to use CSF and *APOE* ε4 proportions as proxies to evaluate and display characteristics of a group in the preclinical stage of the disease and at greater risk of becoming Aβ+. CSF Aβ starts to become abnormal earlier than PET Aβ, thus seeing the gradation in the CSF levels being mirrored in the three Aβ-PET groups reinforces an intermediate group with regional PET. The Regional group individuals present early signs of amyloidosis and show greater Aβ accumulation over time suggests that they are becoming "Aβ -positive" in the typical way that we classify individuals. The standard way that Aβ-PET is analyzed currently, we would miss the

individuals in the intermediate group.

Our results underscore how Aβ in a specific spatial distribution may be associated with specific clinical characteristics and how Aβ in these regions is indicative of accelerated accumulation of Aβ over time. This is likely important for diagnostics purposes, as new quantitative guidelines could focus on the accumulation in these regions rather than a global Aβ SUVR index. In addition, such an intermediate stage might be important to consider in future prevention trials aiming at identifying at-risk individuals that have not yet have extensive tau and related cognitive decline. More and more trials on Aβ removal focus on the early stages of the disease and the Regional Aβ group which indeed does have a milder level of amyloidosis would fit these criteria. And because the regional group is showing characteristics of the sign of early amyloidosis (as reflected in lower CSF and a greater proportion of APOE carriers), it might be an important group to target, especially in terms of future clinical trials.

Clinical Trials

An intermediate stage proposes a new screening for at-risk individuals and provides a new inclusion criterion for the clinical trials. Positive Aβ in vivo measurements (either CSF $A\beta_{42}$ values or Aβ PET scans) are generally an inclusion criterion for the clinical trials. This inclusion criterion aims to reduce the high-screen failure rate considering only a subsample of the individuals develop AD. However, late initiation of the treatment might alter the effects of the treatment. Instead, intervention during the preclinical phase of AD might allow altering of the disease progression.

The failure of anti-Aβ drug trials has raised questions about the hypothesis of the causative role of Aβ in the AD continuum. Removal of senile plaques does not affect cognitive decline[109-111]. With the causative role of Aβ in question, other hypotheses are gaining interest. One hypothesis

states that Aβ might require reaching a certain threshold to cause harm and activate other cascades of events in the disease progression[112]. Intervention prior to this threshold or lowering the amount of Aβ below the threshold could benefit and stop the disease progression. Another hypothesis suggests a trigger effect of Aβ, putting Aβ at the beginning of the temporal order of biomarkers and mediating the neurodegeneration[112]. Unlike the Aβ threshold theory, a trigger point is lower than the threshold and removing the senile plaques will not make a difference once the trigger point is passed[112]. Despite the differences, both hypotheses emphasize the importance of preclinical AD and the time window for the intervention in the disease course.

As shown in our study, the established cut-offs are too high for interventions. The presence of tau pathology in the Regional Aβ group, which consists of individuals with subthreshold Aβ, reveals that the interaction of Aβ and other AD biomarkers commences before meeting the criteria for the cut-off points. In an anti-Aβ drug trial scenario, the inclusion of the participants with widespread Aβ spatial distribution might affect the analysis because of the higher tau in their brains. Even with the removal of Aβ, these participants will have tau in their brains. Therefore, the therapeutic interventions should include individuals who are at risk but without any widespread Aβ accumulation.

Early Intervention

Conceptual differences between "clinical" and "biological" stages cause discrepancies in the research. While clinically early stages are used mainly for treatment and interventions, research showed that early phases of biological manifestations appear decades before[26,27]. One of the benefits of intervening at the preclinical AD stage is to step in early on "biological" stages before the destruction of the synapses and neuronal death. A therapeutic approach at the late stage of AD,

when disease severity is high, will not be as beneficial and will not change the course of the disease[113,114]. Furthermore, early intervention will lead to higher treatment efficiency and less economic burden than intervention later in the disease progression[115,116]. In addition, with the advancement of the disease, the cost of treatment increases every year. The delay placement in long-term care benefits estimated cost savings of $1 billion per year in the medical care system[115]. The currently available treatments do not provide a cure for AD; however, implementing these treatments in the early stages of AD delays the placement in long-term care[117,118]. Our results, which propose an intermediate stage to identify those participants, is beneficiary considering the clinical trial populations, selection of participants, and duration of the therapy.

Future Directions

Future research would be useful to extend the current findings by creating better-proportioned Aβ groups and longitudinal assessments for Aβ progression. Ideally, these findings should be replicated in a study with longer follow-up for both amyloid and tau PET scans. In addition, future studies with multiple tracers should extend the explanations of thresholds and adapt to the centiloid method to standardize the cut-off points.

Conclusion

In conclusion, the present study's data were consistent with the previous literature suggesting that regional Aβ categorization, identified by early Aβ regions, is biologically relevant and can be detected before using the established thresholds for Aβ positivity. Thus, identifying early Aβ accumulation may help us identify the at-risk population better, reduce the sample sizes for drug trials, help researchers monitor the disease progression early on, facilitate the development of effective prevention and early intervention strategies to alleviate Alzheimer's Disease

factors. Furthermore, the present study has enhanced our understanding of the relationship between regional Aβ and various AD markers. We hope that the current research will stimulate further investigation of this vital area.

SUPPLEMENTARY

Table e-1. Regional Specific Thresholds.

Cohort	Rostral Anterior Cingulate	Precuneus	Medial Orbitofrontal	Rostral Middle Frontal	Inferior Parietal	Superior Frontal	Posterior Cingulate
PREVENT-AD	1.57	1.50	1.33	1.30	1.44	1.28	1.78
ADNI	1.19	1.18	1.12	1.12	1.09	1.15	1.25

GMM analyses provided thresholds for each region which correspond to a 90% probability of belonging to the low Aβ distribution in PREVENT-AD and a 50% probability of belonging to the low Aβ distribution in the ADNI cohort.

Table e-2. Sample Demographics

	PreventAD Aβ Groups (excluding Regional Aβ+ individuals)				ADNI Aβ Groups (excluding Regional Aβ+ individuals)			
	Negative (n = 81)	Regional (n =22)	Widespread (n =20)	*p < 0.05*	Negative (n = 202)	Regional (n =61)	Widespread (n =90)	*p < 0.05*
Age	**63 (0.51)**	64 (1.00)	**66 (1.03)**	*b*	**73 (0.41)**	**71 (0.77)**	**76 (0.62)**	*b,c*
Education	16 (0.36)	15 (0.70)	14 (0.72)		17 (0.19)	17 (0.26)	16 (0.28)	
Sex, female (%)	60 (74%)	17 (77%)	13 (65%)		**94 (47%)**	26 (46%)	**55 (61%)**	*b*
APOE ε4 carriership (%)	**22 (27%)**	**14 (64%)**	**13 (65%)**	*a, b*	**38 (19%)**	**16 (27%)**	**45 (50%)**	*b,c*
Subjective Cognitive Decline (%)	17 (25%)	4 (19%)	6 (38%)		**117 (50%)**	29 (53%)	**55 (66%)**	*b*
CSF $A\beta_{1-42}$*	**1265 (37.78)**	**1058 (72.38)**	**718 (71.53)**	*a,b,c*	**1448 (30.13)**	**1236 (49.65)**	**802 (45.69)**	*a,b,c*
CSF pTau*	**46 (3.14)**	53 (5.86)	**67 (6.15)**	*b*	**19 (0.72)**	**20 (1.19)**	**29 (1.10)**	*b,c*
CSF Total Tau*	**264 (26.74)**	367 (50.82)	**435 (52.34)**	*b*	**219 (7.33)**	**220 (12.10)**	**294 (11.12)**	*b,c*
Memory Score	-	-	-		1.08 (0.04)	1.15 (0.07)	0.95 (0.06)	
Executive Function Score	-	-	-		0.95 (0.06)	0.98 (0.10)	0.62 (0.08)	
Immediate Memory Score	103.15 (1.26)	105.81 (2.39)	104.05 (2.54)		-	-	-	
Delayed Memory Score	**104.28 (1.02)**	100.64 (1.94)	**97.20 (2.05)**	b	-	-	-	
Attention Score	106.96 (1.67)	102.86 (2.05)	109.65 (3.36)		-	-	-	

The original analyses were replicated in both cohorts excluding the Aβ+ participants in Regional Aβ Groups. The values are reported as Mean (SD) except for Sex, *APOE* ε4, and Subjective Cognitive Decline which are reported as the Number of participants (% of the group). Cognitive test scores were compared at the baseline visit corrected for age and sex; test scores are reported as Mean (SD). BOLD text represents the groups between which there were significant differences: a = $p<0.05$ between Negative Aβ and Regional Aβ groups; b = $p<0.05$ between Negative Aβ and Widespread Aβ groups; c = $p<0.05$ between Regional Aβ and Widespread Aβ groups.

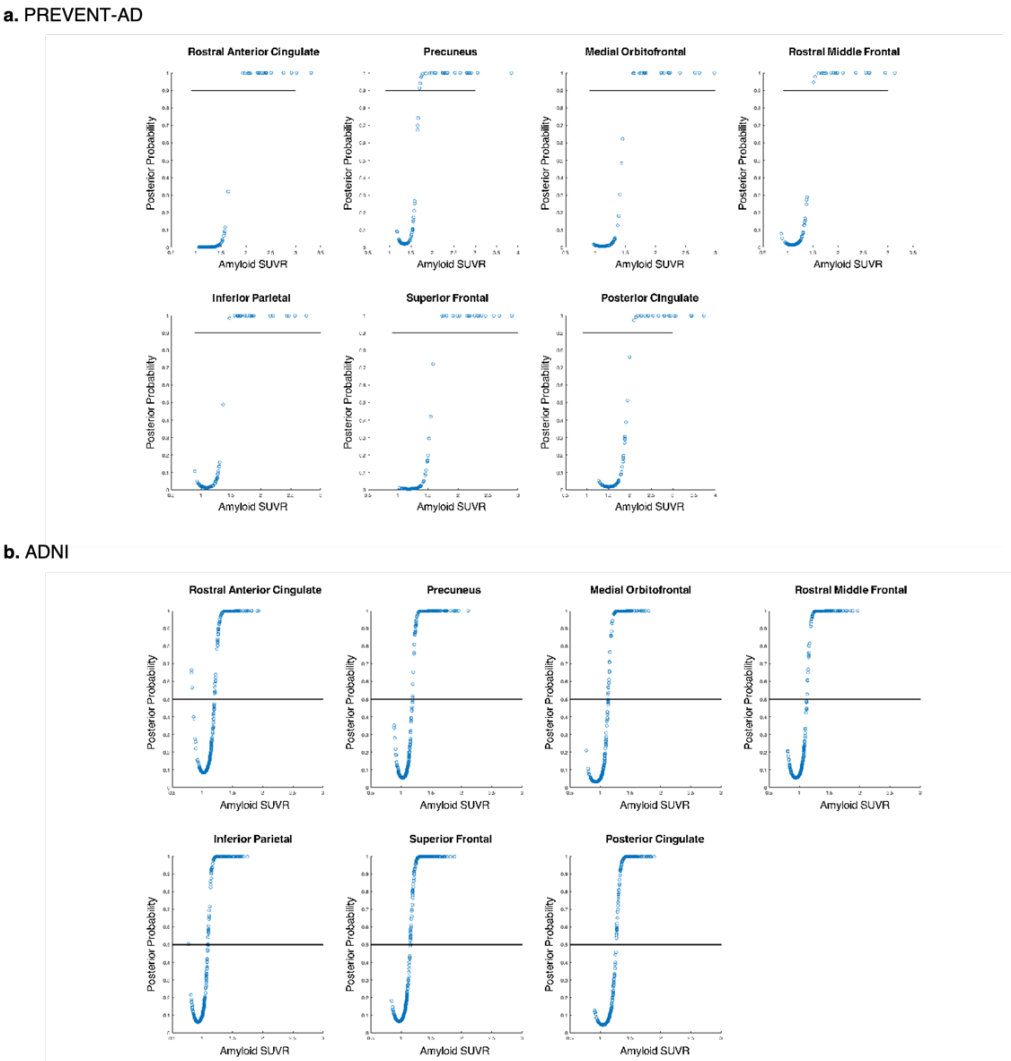

Figure e-1. Amyloid SUVR Distribution in PREVENT-AD and ADNI for Regions of Interest.

Plotted is the association between the posterior probability of the participants according to their Aβ SUVR values for regions of interest. (A) The NAV PET tracer (PREVENT-AD) GMM analyses provided a clear distinction between individuals with and without tracer binding using thresholds for each region which corresponds to a 90% probability of belonging to the low Aβ distribution. (B) Individuals from ADNI followed a more continuous distribution without a distinctive cut-off between lower and higher distributions. Participants had higher uncertainty values which complicate their probability of belonging to the low distribution compared to PREVENT-AD. Prior to defining the groups in ADNI, we, therefore, used a 50-percent probability of belonging to the low- Aβ distribution as cut-off criteria solely depending on the distribution of regional SUVRs as previously reported in other studies (Buckley, 2018; Farrell, 2021; Mormino, 2014).

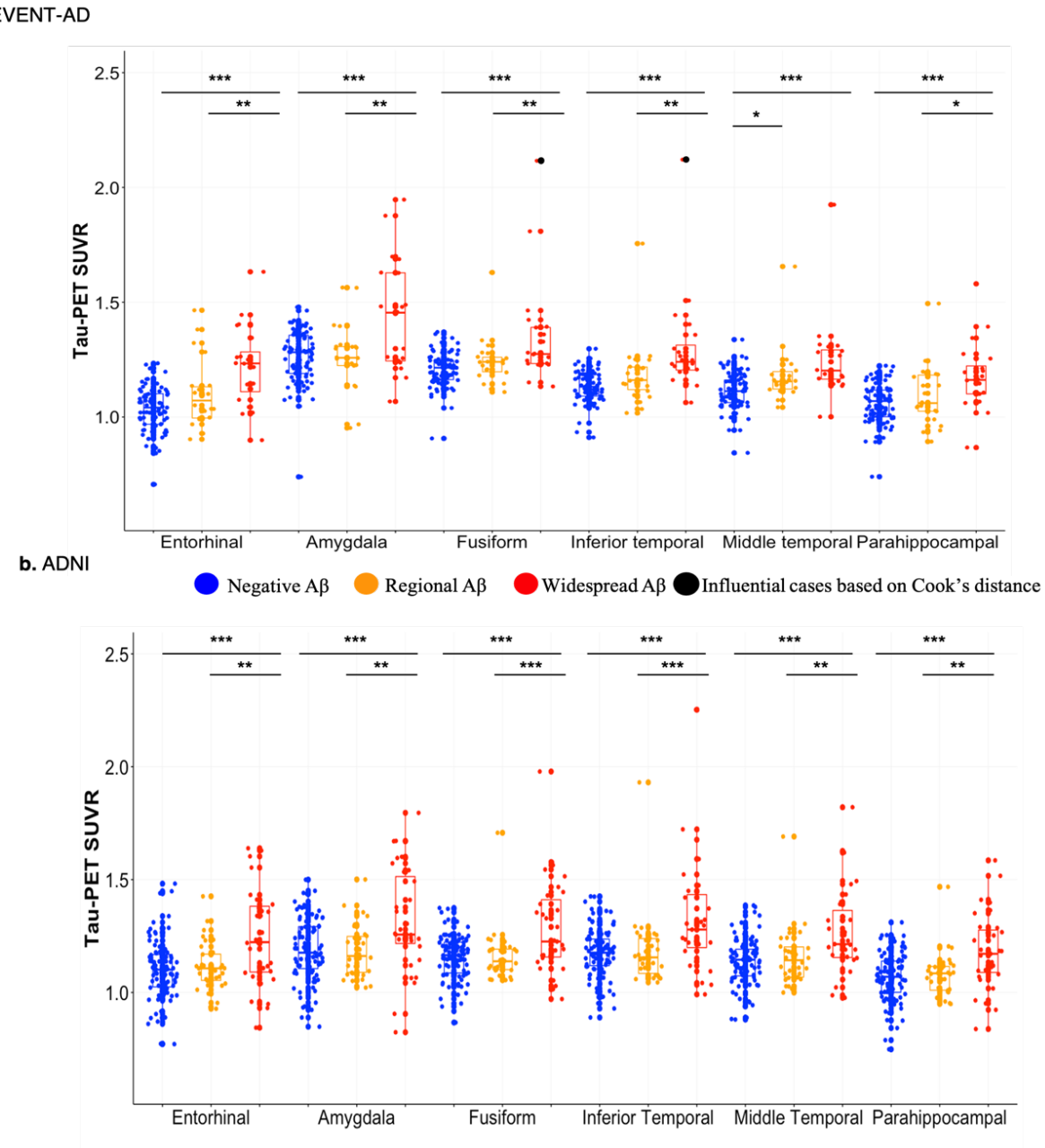

Figure e-2. Tau-PET Uptake Across the 3 Aβ groups.
The original analyses were replicated in both cohorts excluding the Aβ+ participants in Regional Aβ Groups. Six regions were chosen to represent areas of early tau-PET accumulation[31]. Tau-PET scans were available for 123 PREVENT-AD participants and 157 ADNI participants. One PREVENT-AD Widespread participant was considered influential cases based on their Cook's distance. Removing this participant did not influence the results. Analyses were corrected for age and sex. * $p<0.05$; ** $p<0.01$; ***$p<0.001$. *SUVR*: standardized uptake value ratio.

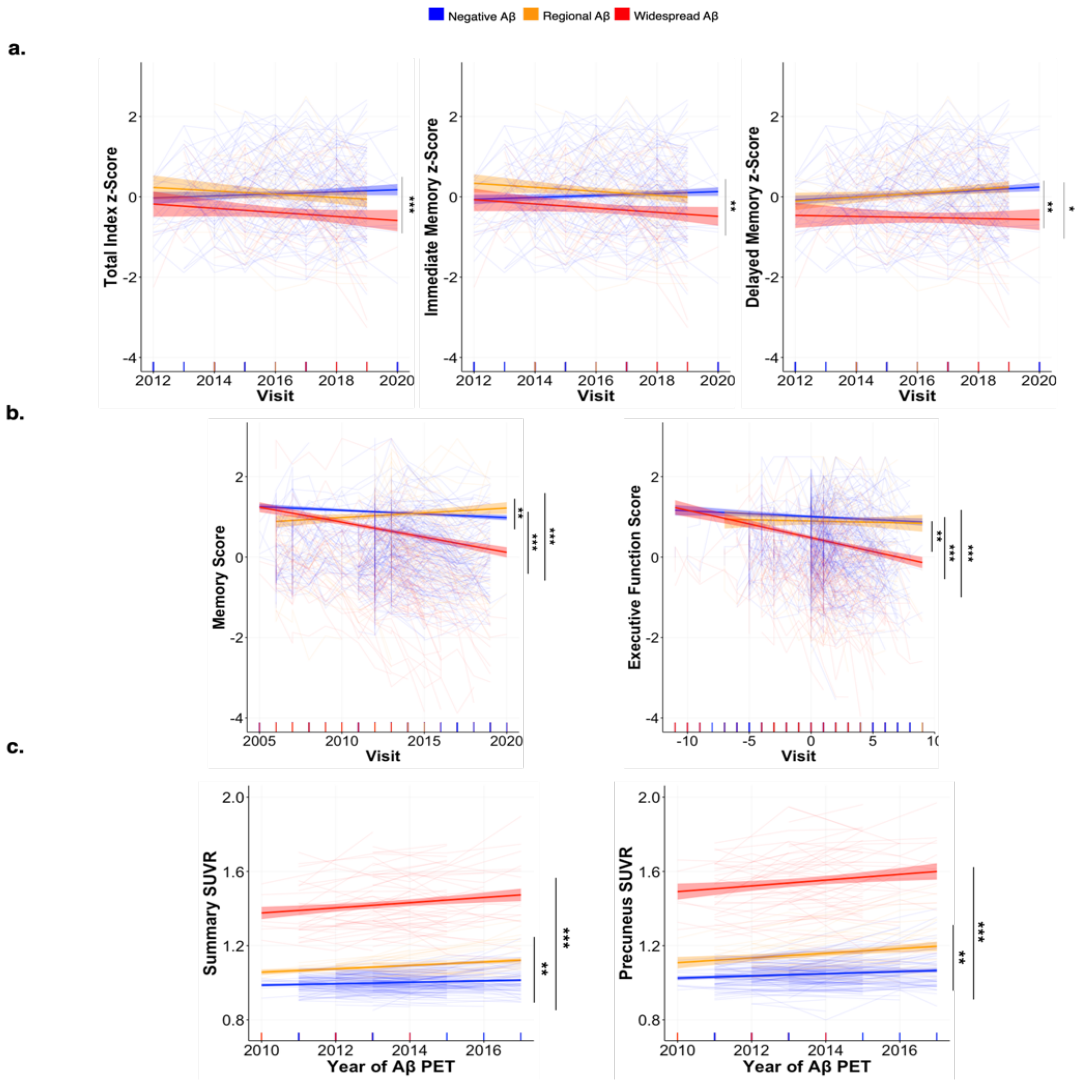

Figure e-3. Change in Cognition and Aβ Uptake Over Time Between the three Aβ Groups. The original analyses were replicated in both cohorts excluding the Aβ+ participants in the Regional Aβ Groups. Linear mixed-effect models were used to assess the effects of Aβ status on longitudinal cognition in both cohorts, and Aβ accumulation rate over time in the ADNI cohort. The longitudinal cognition analyses were corrected for sex and education., while longitudinal Aβ accumulation rate analyses were corrected for age, sex and education. Only significant domains are shown in the figure and the analyses were anchored at the participants' baseline visit date. (a) For PREVENT-AD, cognitive test scores of "Total Score", "Immediate Memory" and "Delayed Memory" on the RBANS. (b) In ADNI, cognitive test scores of "Memory" and "Executive Function" over time in the three different groups. (c) Plotted is the association in the ADNI cohort between Aβ groups and Aβ accumulation rate over time-based on total Aβ summary score and Precuneus SUVR score over the years from their first scan. * $p<0.05$; ** $p<0.01$; ***$p<0.001$; etc. SUVR: standardized uptake value ratio.

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