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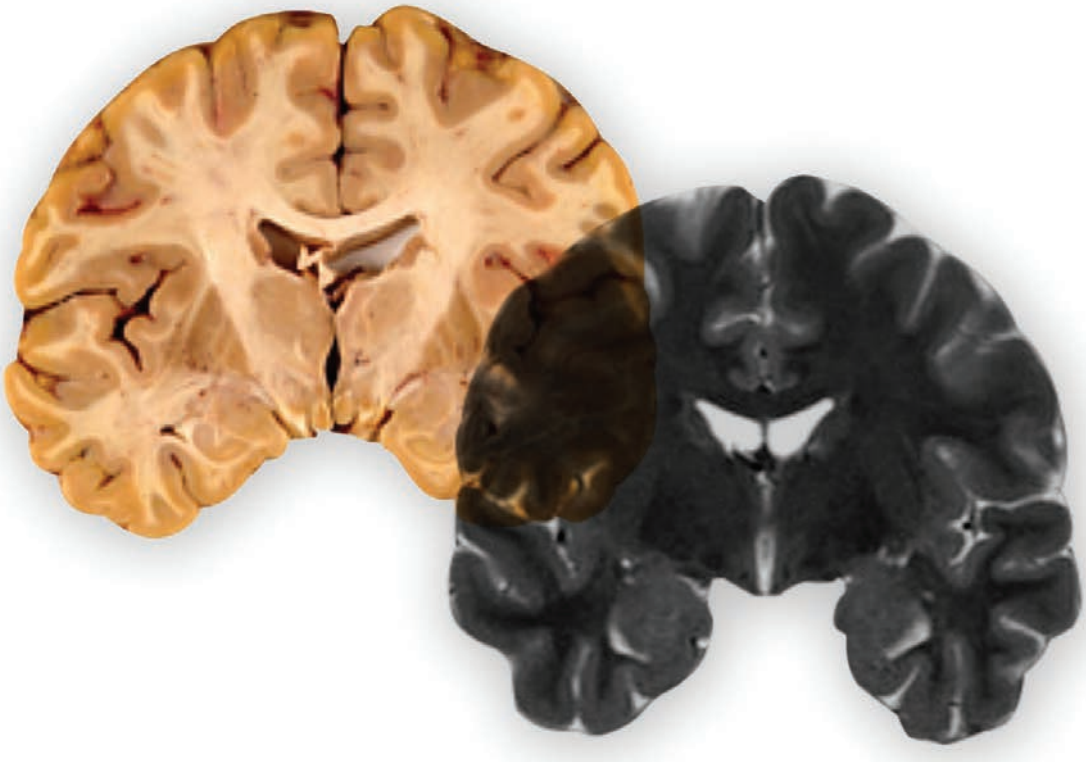
Uncovering the mysteries of brain regional susceptibility to neurodegeneration in Alzheimer's disease: from neuropathology to brain magnetic resonance imaging

TIAGO GIL OLIVEIRA



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Biography / Author

Tiago Gil Oliveira

Tiago Gil Oliveira is Associate Professor at the School of Medicine, at the University of Minho, research line coordinator at the Life and Health Sciences Research Institute (ICVS), neuroradiologist at Hospital de Braga, and President of the Portuguese Society for Neuroscience.

He was one of the first students to graduate from the joint Minho MD/PhD program with Columbia University, NYC, USA, the first of its kind in Portugal. He carried out his PhD studies at Columbia University, between 2007 and 2010, and MD studies at the University of Minho. In his PhD, he studied the role of lipid signaling in Alzheimer's disease pathogenesis with the goal of finding new treatment strategies. He is now using lipidomic approaches together with brain imaging to study neurodegenerative disorders.

In the last few years he has led multiple research projects as principal investigator, being funded by national and international competitive grants, such as the ones from the Brain and Behavior Research Foundation, the Portuguese Foundation for Science and Technology and Bial Foundation. He has been the leading author in key publications in some of the most prestigious international scientific journals, for his work on the role of lipids in cognition and Alzheimer's disease, and on the identification of brain regional signatures of susceptibility or resistance to Alzheimer's disease using brain imaging approaches. His work has been recognized with various awards, including the 2022 Pfizer Award and the Prémio Bial de Medicina Clínica 2024. He is an active physician-scientist and a devoted mentor, with many of his trainees working themselves as physician-scientists in various hospitals throughout Portugal.

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Acknowledgements

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Abbreviations

AC - anterior cingulate
AD - Alzheimer's disease
ADNC - Alzheimer's disease neuropathologic change
ADNC+P - Alzheimer's disease neuropathologic change with psychosis
AGD - argyrophilic grain disease
APOE - apolipoprotein E
APP - amyloid precursor protein
ARIA - amyloid-related imaging abnormalities
ARIA-E - amyloid-related imaging abnormalities - edema and/or effusion
ARIA-H - amyloid-related imaging abnormalities - hemorrhage
AT - anterior temporal
Aβ - amyloid-beta
BMP - bis(monoacylglycero)phosphate
CAA - cerebral amyloid angiopathy
CADASIL - cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy
CBD - corticobasal degeneration
CDR-SB - cognitive dementia rating sum of boxes
CE - cholesteryl ester
Cer - ceramide
CERAD - consortium to establish a registry for Alzheimer's disease
CSF - cerebrospinal fluid
CTE - chronic traumatic encephalopathy
DAG/DG - diacylglycerol
dhSM - dihydrosphingomyelin
DIGIB - digit span backwards test
DIGIF - digit span forwards test
EC - entorhinal cortex
EPVS - enlarged perivascular spaces
FAD - familial Alzheimer's disease
FC - free cholesterol
FI - fronto-insular
FTD - frontotemporal dementia
GM3 - monosialodihexosylganglioside
GWAS - genome wide association study

GWC - gray matter white matter contrast
HexCer - hexosylceramide
ICH - intracerebral hemorrhage
LacCer - lactosylceramide
LATE - limbic-predominant age-related TAR DNA binding protein-43 encephalopathy
LB - Lewy bodies
LBD - Lewy-body dementia
LPC - lysophosphatidylcholine
LPCe - ether lysophosphatidylcholine
LPE - lysophosphatidylethanolamine
LPEp - plasmalogen lysophosphatidylethanolamine
LPI - lysophosphatidylinositol
MCI - mild cognitive impairment
MMSE - mini-mental state examination
MRI - magnetic resonance imaging
MT - medial temporal
MTA - medial temporal lobe atrophy
N-acyl-PS - N-acylphosphatidylserine
NACC - National Alzheimer's Coordinating Center
NFT - neurofibrillary tangles
oAβ - Aβ oligomers
OF - orbito-frontal
p-tau - phosphorylated tau
PA - phosphatidic acid
PART - primary age-related tauopathy
PC - phosphatidylcholine
PCe - ether phosphatidylcholine
PD - Parkinson's disease
PE - phosphatidylethanolamine
PEp - plasmalogen phosphatidylethanolamine
PET - positron emission tomography
PG - phosphatidylglycerol
PI - phosphatidylinositol
PiD - Pick's disease

PLD1 - phospholipase D1
PLD2 - phospholipase D2
Post - posterior
PS - phosphatidylserine
PSP - progressive supranuclear paralysis
PVC - primary visual cortex
SAD - sporadic Alzheimer's disease
SM - sphingomyelin
SNAP - suspected non-Alzheimer's disease pathology
Sulf - sulfatides
Sulf(2OH) - 2-hydroxy N-acyl sulfatide
SwAPP - Swedish familial Alzheimer's disease amyloid precursor protein mutation
SWI - susceptibility weighted imaging
TDP-43 - TAR DNA binding protein-43
TG - triacylglycerol
TMT - trail making test
WAIS - Wechsler adult intelligence scale digit symbol test
WMH - white matter T2 hyperintensities

Summary

The main questions I have been addressing as a researcher have been focused on the identification of which brain regions are differentially affected by Alzheimer's disease (AD) pathologies and why that happens. To do that I have leveraged my training as a physician-scientist and neuroradiologist, using brain magnetic resonance imaging (MRI) to map brain alterations in AD patients, and rodent models to understand the molecular changes underlying regional brain pathologies.

AD is the most prevalent neurodegenerative disorder and the most common cause of dementia. Pathologically, it is defined by the presence of specific protein accumulations in the brain, namely amyloid-beta (Aβ), the major constituent of Aβ plaques, and hyperphosphorylated forms of tau, constituting neurofibrillary tangles (NFTs). By studying familial genetic forms of AD, mutations were identified in genes that contribute to Aβ formation, providing the basis to the "amyloid cascade hypothesis". As the most widely accepted explanation for the pathological events occurring in the brains of AD patients, it proposes that Aβ accumulation is an early pathological event, subsequently promoting tau accumulation and spreading throughout the brain. Even though memory impairment is the most common symptom, other disease processes can lead to impairment of memory circuits. Therefore, patient selection based on biomarkers that depict Aβ and tau pathologies are crucial for enrolment in clinical trials and initiation of treatment with anti-Aβ antibodies. However, even with these new therapies, clinical progression is only slightly delayed.

With the aging of populations worldwide, the number of AD cases that will need treatment is expected to increase dramatically, but anti-Aβ antibodies are clearly insufficient since they only target one type of pathology. Therefore, I decided to target this major gap in the management of AD patients by trying to understand the impact of the diversity of brain pathologies that contribute to neurodegeneration. To do that I have resorted to the use of assembled cohorts where *post mortem* assessment has been performed in the brains of AD patients. I have then used *ante mortem* clinical and imaging data to gather information while patients were still alive. Using this *post mortem* vs *ante mortem* innovative analytical strategy, I have focused on translating definite neuropathological evidence to a new understanding of the neuroimaging and pathophysiology of AD.

We started by studying a condition called primary age-related tauopathy (PART), diagnosed at brain autopsy, and defined by the presence of NFTs, although in the absence of Aβ plaques. We showed that PART had atrophy of temporal lobe regions that depended predominantly on NFTs and that was associated with semantic memory deficits. We then compared atrophy patterns in PART to AD and found that while in AD there is a gradual increase in atrophy of medial temporal lobe regions due to an increase in the density of Aβ plaques, PART surprisingly did not follow this tendency, showing intermediate levels of atrophy. This indicated that other brain co-pathologies could be contributing to these atrophy patterns. To tackle this, we conducted a number of studies showing that cerebrovascular lesion burden was more correlated with brain regional atrophy in PART than AD; that TDP-43 accumulations were a major determinant of medial temporal lobe atrophy; and that the presence of Lewy bodies (LBs) contributed to worse cognition and brain regional atrophy. Our studies on the impact of LBs as a co-pathology in AD further highlighted that the presence of Aβ plaques promotes both the spread of NFTs and LBs throughout the neocortex. These results are particularly relevant since the impact of co-pathologies, such as LBs, in AD has not been accounted in the results from clinical trials where Aβ plaques have been cleared from the brain.

Additionally, we expanded these co-pathology concepts to a population in our local hospital in Braga where we studied the impact of having cerebral amyloid angiopathy (CAA) diagnosed by MRI criteria. We observed that long-term re-hemorrhaging events are frequent in CAA patients and we found that medial temporal lobe atrophy was higher in men with dementia, while women showed a higher number of enlarged perivascular spaces in the centrum semiovale. Since CAA was found to be a major risk factor for side-effects in anti-Aβ treatments, our results underscore the need to explore differential pathophysiologic mechanisms with sex-specific neuroimaging patterns in CAA.

We further studied the underexplored but prevalent presence of psychotic symptoms in neuropathologically confirmed AD. We observed that psychosis is present in about 40% of patients, associating with worse cognitive deficits and regional brain atrophy predominantly on the right temporal lobe. Therefore, this is an important clinical presentation that

indicates a more severe form of AD, which needs directed treatments to be developed.

Finally, I complemented our clinical studies with basic science approaches studying the role of lipids in brain regional susceptibility to AD. I showed that the genetic ablation of the lipid modifying enzyme, phospholipase D2, conferred resistance to Aβ pathology in mice. We then showed that mice that express the AD genetic risk factor *APOE4* have lipidomic signatures in the entorhinal cortex indicating endo-lysosomal dysfunction. Since the hippocampus is another region affected early in AD, using a lipidomic approach we showed specific lipid signatures along the hippocampal longitudinal axis indicating a differentia,l regulation of the phospholipase D pathway. This led us to study rodents with phospholipase D1 ablation, which showed predominant impairment of the hippocampus along its longitudinal axis. Altogether, these lipid-focused studies showed that lipid signaling is a key factor in AD pathophysiology, and has the potential to be the basis for new disease biomarkers or lipid-targeting therapeutic strategies.

Overall, in this book, I provide a window to my scientific approach. With the body of work that I present here, I identified key regions impacted by pathologies that occur in the brains of AD patients and combined this with molecular approaches using animal models, that could then fuel new questions to be pursued in patients. Our work on brain co-pathologies in AD has major implications for clinical practice since it explains why certain patients might not respond well to anti-Aβ therapies, and our molecular mapping of the brain provides a path to develop therapies to confer resistance to AD pathologies. With all my studies I hope to not only contribute to improve the treatment of AD patients, but also to inspire others to follow this fascinating adventure of being a physician-scientist.

1. Introduction

1. Introduction

Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder and the most common cause of dementia [1]. According to the World Health Organization dementia report, 55 million people suffered from dementia in 2019, and this number is predicted to grow to 78 million in 2030 and 139 million in 2050 [1]. Therefore, it is urgent to invest in research to better understand the pathophysiology of AD, to develop strategies to diagnose AD as early as possible, and to discover therapeutic approaches directed to its etiology and to its frequent co-pathologies that can affect treatment outcomes.

1.1. A biological definition of Alzheimer's disease

In order to tackle AD, it is crucial to establish the difference between AD and dementia. While dementia refers to the clinical presentation that includes deficits in memory-related tasks, spatial-orientation, language or problem-solving with an impact in daily life activities, AD, albeit the most common cause of dementia, is only one of many other causes that can lead to dementia [2, 3]. Other potential causes of dementia include cerebrovascular disease, Lewy-body dementia (LBD), limbic-predominant age-related TAR DNA binding protein-43 encephalopathy (LATE) or frontotemporal dementia (FTD) [2, 3].

With this in mind, many efforts from the scientific community led to the development of a biological definition of AD that relies on an objective assessment of biomarkers that reflect ongoing pathophysiological processes specifically related to AD. This is somewhat similar to what happened with other disorders, such as hypertension, which is now objectively defined by cut-off values of blood pressure, even in asymptomatic patients. Extensive research has shown that pharmacologically reducing high levels of blood pressure decisively contributes to prevent vascular related organ-lesions, including the brain [1]. Importantly, when symptoms related with this hypertension-related damage occur, the multiple organ-lesions can already be established up to the point of irreversibility, further emphasizing the

major impact of targeting a pathophysiological process as early as possible, based on reliable disease biomarkers.

This led to many efforts from the scientific community to establish a system to diagnose AD independently of the clinical presentation, based on a biological definition. The most updated criteria for diagnosis and staging AD incorporate the recent advances in the understanding of AD pathogenesis and its application to the newly developed fluid and imaging biomarkers [3].

AD is then defined as a biological process that initiates with the appearance of AD neuropathologic change (ADNC), even in asymptomatic individuals. The current understanding of the evolution of ADNC highlights that in the majority of cases, these patients will eventually progress to the development of clinical symptoms [3]. Brain regions affected in AD typically have deposits of amyloid plaques and neurofibrillary tangles (NFTs), which are constituted by amyloid-beta (Aβ) and hyperphosphorylated tau, respectively, which constitute the pathological hallmarks of AD (more detailed below). Aβ is a peptide produced by the sequential cleavage of amyloid precursor protein (APP), by BACE1 and β-secretase [4]. ADNC then is defined by presence of Aβ pathology, which can coexist with tau pathology, particularly in more advanced cases. This pathophysiologic knowledge of AD led to the development of the "ATXN system", where "A" stands for biomarker evidence of Aβ pathology; "T" for tau pathology, "X" for new biomarkers beyond A, T or N, which can be inflammatory / immune mechanisms "I", vascular brain injury "V" and α-synucleinopathy "S"; and "N" stands for neurodegeneration, which includes all sources of evidence for ongoing brain neurodegeneration due to a variety of causes, such as AD. This system has been proposed years ago [5], and is constantly being updated with the advances of our understanding of AD pathogenesis [3].

1.2. Diversity of clinical presentations

Importantly, symptoms due to AD are a consequence of AD-related pathological processes and, therefore, are not necessary for AD diagnosis. In many cases, these symptoms can appear in about 20 years after AD brain

pathology starts. Additionally, clinical syndromes, such as dementia, that occur in AD, can also be due to other diseases beyond AD, and for this reason, even though the clinical presentation can be of very high importance for clinical decisions, it should not impact the diagnosis and biomarker-based AD staging [3, 6]. Therefore, a key concept is that clinical presentations are a consequence of synaptic dysfunction and neurodegeneration of specific circuits and regions of the brain that encode specific functions, responsible for behavioral outputs, that then become impaired in this context. There is a high level of variability from person-to-person, due to brain plasticity, brain reserve and genetically-determined resilience, with some patients even withstanding high levels of AD pathology, delaying clinical presentations, until the point of "collapse" of compensatory mechanisms.

However, it is the clinical presentation and behavioral deficits that impact patients and families, and any interventions that can prevent, ameliorate or delay the progression of these deficits are of extreme clinical value. AD presents as a clinical continuum with three main stages considered: (1) pre-clinical AD, with no symptoms, but with evidence of AD pathology by positivity in the biomarker profile; (2) mild cognitive impairment (MCI) due to AD, with mild symptoms that may not interfere with daily life activities; and (3) dementia due to AD, with symptoms that affect daily life activities with varying degrees [1, 7].

The typical clinical AD presentations in MCI include subtle symptoms such as problems with memory, language, spatial orientation and thinking, which in the setting of AD, precede the onset of dementia presentations. These presentations can be unnoticed in some patients, and many efforts are being pursued in order to identify patients in early AD stages, mostly pre-symptomatic or MCI in order to implement treatment strategies as early as possible [1].

In early stages of AD dementia, patients might still function adequately, although they might need assistance in their activities or longer time to complete tasks. However, complex behaviors that implicate executive function, such as handling personal or family finances can be particularly challenging. As the disease progresses, other memory and language functions become more impaired leading to the setting of confusion, inability to complete daily tasks, such as the ones related with personal hygiene, inability

to recognize known people, personality changes and even agitation. In even more advanced stages, communication and movement are greatly impaired, eventually leading to a bed-ridden condition and inability to self-feed [1].

There are various risk factors that have been identified as contributing to either the predisposition or resistance to the manifestation of clinical presentations. The major identified AD-risk factor is age, even though there is high variability between patients. Other ones are: education, with more years of engagement in academic training contributing to delay clinical AD presentations; exercise, with various studies highlighting the protective effect of life-time regular exercise; diet, even though there is high variability among cultures and world regions, as an example, the Mediterranean diet, rich in omega-3 fatty acids, has been shown to have a protective effect; or depression, which has been shown to either predispose or contribute to an earlier onset of clinical AD [8].

Within the realm of clinical presentations, I am particularly interested in the ones that enlighten us about brain regional or circuitry susceptibility to AD pathology. The hippocampus and the entorhinal cortex (EC) are brain regions crucial for encoding space in the brain and for spatial memory tasks, and interestingly, they are affected early in AD [9]. Using basic science approaches, my team has studied the hippocampus at the molecular level, focusing on lipid composition and we developed methods to segment magnetic resonance imaging (MRI) acquisitions of the hippocampus along its longitudinal axis. We have found that specific hippocampal sub-regions (dorsal in rodents and posterior in humans) are affected in early AD and have a specific lipidomic constitution **(Chapter 6)**.

Additionally, I am interested in "niche" clinical AD presentations. Neuropsychiatric symptoms, such as anxiety, depression or aggression are common in AD patients. My team got particularly interested in studying psychosis in AD, since it is present in about 40% of AD patients at some point in their disease course. Importantly, psychosis is associated with higher levels of AD-pathology in the brains of patients and atrophy of specific brain regions that impact specific brain regions **(Chapter 5)**.

1.3. Alzheimer's disease post-mortem neuropathology

While the "ATXN system" has major implications due to its utility in clinical practice, AD is a disorder that has been, and still is, defined by its neuropathological processes which are assessed in a standardized manner at the *post-mortem* level. Therefore, the *gold-standard* for AD diagnosis is still *post-mortem* assessment by autopsy neuropathology assessment by a clinical neuropathologist. In order to establish a final diagnosis, the hallmarks of AD neuropathology are identified by histological processing techniques and graded according to the "ABC" clinical system that includes: (A) direct assessment of amyloid pathology by the Thal staging, from 0 to 5, that identifies the regions that show Aβ deposition; (B) identification of NFT brain regional disease, by using the Braak staging scheme, from none to VI, where tau accumulations are observed in different regions starting in the EC in earlier stages, than the hippocampus and eventual, in more advanced Braak stages, throughout the neocortex; and (C) the identification of neuritic plaques (NPs) by specific stainings, where the density of these NPs, are classified according to the Consortium to Establish a Registry for AD (CERAD) score (CERAD = 0, no NPs; CERAD = 1, sparse; CERAD = 2, moderate; CERAD = 3, frequent). Importantly, while Thal staging and CERAD both classify amyloid pathology, they differ since the NPs identified in CERAD have an additional dystrophic neurite component [10].

While the identification of ADNC can be identified by the presence of amyloid pathology (assessed by Thal and CERAD scores) with varying degrees of NFTs pathology (Braak staging), clinicians have characterized an intriguing neuropathologic condition named primary age-related tauopathy (PART), which is defined by the absence of NPs (CERAD = 0), with varying degrees of tau pathology. Since the NFTs that occur in PART and AD are structurally similar, I am particularly interested in identifying what distinguishes these two disorders, namely at the clinical and imaging levels, while the patients are still alive, with the goal to understand why PART patients do not develop amyloid pathology and therefore unlock the secrets of resilience to AD pathology **(Chapter 2)**.

The implications of using this "ABC" system go beyond the standardization of AD diagnosis. Additionally, it provides a method to

stage the disease processes and to identify the factors that contribute to higher or lower pathology, or resistance or susceptibility to AD, since other morphological features can be assessed as brain weight, atrophy of different brain regions and, most importantly, other co-pathologies that can be at the basis of other disorders that could also be affecting AD brains [10]. In the *post-mortem* assessment of an AD case, besides classifying according to the "ABC" system, (1) staining for Aβ can also lead to identification of Aβ in brain vessels and the diagnosis of cerebral amyloid angiopathy (CAA); (2) staining for α-synuclein can provide the identification of Lewy bodies (LBs), which frequently occur in LBD or Parkinson's disease (PD); (3) staining for TAR DNA binding protein (TDP)-43 inclusions can lead to the identification of this additional co-pathology, commonly present in LATE or certain FTD cases; (4) and finally, cerebrovascular lesions can be identified *post-mortem* x as small or major infarcts in the brain [10]. Understanding how these co-pathologies impact the brain has been one of my main interests and my team has been addressing this question in a multitude of studies **(Chapters 2 and 3)**.

1.4. Alzheimer's disease genetics

The understanding of AD pathogenesis has evolved immensely by the discovery of genetic mutations that give rise to familial forms of AD (FAD). These correspond to about 1% of all cases of AD, and the genes implicated have been *APP*, and *PSEN1* and *PSEN2*, which stand for presenilin. Due to the Aβ peptide amino acid sequence being found in the middle of the APP amino acid sequence, and *PSEN1* and *PSEN2* being found to be part of the protein complex that cleaves APP to produce Aβ, this provided a connection between what was observed characteristically *post-mortem*, with the amyloid plaques, and the etiologically defining mutations. Further links come from families that carry *APP* duplications and since the *APP* gene is located in the chromosome 21, from Down Syndrome patients, with both genetic conditions developing ADNC at young age [11, 12]. The study of these FAD cases led to the development of the "amyloid cascade hypothesis" in AD, which is still the most accepted one in AD. Furthermore, by knowing

that FAD carriers will eventually develop AD brain pathology, these patients can be longitudinally followed even before symptomatic presentations, which led to the development of biomarker curves. Knowing that specific forms of Aβ with 42 amino acid length can predominantly aggregate in AD, and that pathological forms of tau suffer post-translation modifications with predominant pathological phosphorylation at specific phospho-sites (p-tau), these can be measured in body fluids, such as the cerebrospinal fluid (CSF) or blood, by tracking the levels of Aβ 42 or p-tau, or alternatively track Aβ and/or tau aggregates directly, with positron emission tomography (PET) [13]. Interestingly, these biomarker curves have been not only characterized in detail in FAD patients, but have also been more recently reproduced in non-FAD cases [13]. Quite interestingly, while there are no FAD cases with mutations in the tau gene, *MAPT*, there are actually genetically determined neurodegenerative disorders, with *MAPT* mutations, such as some forms of FTD, corticobasal degeneration (CBD) or progressive supranuclear paralysis (PSP). While these are considered primary tauopathies, AD is considered a secondary tauopathy [14].

Since the big majority of AD cases occur *sporadically* (SAD), with no genetic etiologic identified mutations, these SAD cases, when studied as a whole, in a big population setting with thousands of patients identified, can be the basis for population genetic studies, such as genome wide association studies (GWAS). Along the years, with increasing power, due to more patients being included in studies, it has led to the identification of a variety of genetic risk factors. The most recent major GWAS led to the identification of genetic risk variants in 35 already known genes and 42 new implicated genes, for a total of 79 different genes. The most consistent genetic hit over the years, and with the strongest risk effect, has been the *APOE* gene, which stands for apolipoprotein E. This gene encodes a lipid transport protein, which in the brain is predominantly produced in astrocytes and microglia in a reactive state, and has been implicated in shuttling lipids between neurons and glial cells. The majority of individuals carry the most frequent *APOE3* allelic variant, but others that carry either one or two copies of the *APOE4* variant have a dose-dependent risk effect to develop AD. This risk has been deemed so high that a recent study has proposed that virtually all patients with two *APOE4* copies will develop ADNC [15]. I am intrigued by the

mechanisms underlying the pathologic effects of *APOE4*, which are poorly elucidated. To contribute to solving this puzzle, my team has performed a lipidomic analysis of susceptible brain regions to early AD pathology in mice that carry human *APOE4* variants **(Chapter 6)**.

As a whole, these 79 different genes have been grouped based on their overall functions, and it has been observed that the main molecular pathways implicated in AD pathogenesis are: (1) lipid pathways, (2) membrane trafficking, implicating the endo-lysosome system, and (3) immune system. Aligned with my interests in unravelling APOE biology, and knowing that lipids are the major constituents of the brain (following water), I have been studying the role of lipid signaling in the brain for many years now, and I have performed lipidomic profiling of different brain regions in human and rodent brains in the context of AD-linked conditions **(Chapter 6)**.

Finally, there are also genetic variants that confer resistance to the development of AD. One of the major protective risk factors is another *APOE* variant, *APOE2*, although it is somewhat uncommon in the general population. Quite interestingly, another even rarer *APOE* variant, *APOE Christchurch*, conferred resistance to neurodegeneration and to the development of tau pathology in a patient carrying the FAD *PSEN1* mutation [16]. Additionally, besides being implicated as a risk gene, a mutation in *APP* has also been implicated as having a protective effect [17]. Overall, these studies highlight that understanding the mechanisms that confer protection can potentially lead to future clinical therapeutic strategies. Fascinated by this possibility, I have been using genetic rodent models to study these processes. I previously used rodents that carry human FAD-linked *APP* variants, leading to high levels of Aβ in the brain and spatial memory deficits, and I showed that the ablation of a specific lipid modulating gene, *Pld2*, confers protection in memory tasks independently of Aβ brain clearing **(Chapter 6)**, mimicking the effects of *APOE* variants.

1.5. How to diagnose Alzheimer's disease in patients

The Alzheimer's Association Workgroup has proposed a staging-criteria based on the imaging and fluid biomarkers. Importantly, one of the main

implications is that detecting ADNC in patients is now proposed to be equivalent to diagnosing the disease. Another important concept is that asymptomatic individuals that have altered biomarker AD-profile are at risk to eventually develop AD-linked symptoms [3].

Beyond the "ATXN classifying system", biomarkers can be further grouped into (1) "core AD biomarkers" of ADNC, (2) non-specific biomarkers that are relevant for AD as for other disorders, and (3) biomarkers of common non-AD co-pathologies. "Core AD biomarkers" can be further divided into: (1.1) "core 1", which depict early pathological processes, closely related with Aβ pathology, such as Aβ42 in CSF or plasma, amyloid PET, or p-tau217, p-tau181 or p-tau231; and (1.2) "core 2", which depict later pathological processes, more closely correlated with symptomatic presentations and tau accumulation, such as MTBR-tau243, p-tau 205, or tau-PET; biomarkers of non-specific processes involved in AD pathophysiology that denote (2.1) injury, dysfunction, or degeneration of neuropil, which classifies the "N" category, include NfL levels in either CSF or blood, brain MRI or fludeoxyglucose-PET; (2.2) the "I" category that depicts inflammation, such as astrocytic activation, includes GFAP fluid levels; and biomarkers of non-AD co-pathology that (3.1) associated with the "V", from vascular brain injury, observed for instance through brain MRI, and finally, (3.2) linked to the "S" category, manifested as β-synuclein pathology, can be detected in the CSF with α-synuclein seed amplification assay.

Using these set of biomarkers such as amyloid PET or fluid markers of Aβ42 levels or p-tau determines the AD diagnosis ("Core 1"), then "Core 2" biomarkers can be further used to stage the disease for more advanced additional AD pathology, with other markers contributing for assessing other co-pathologies that impact brain degeneration and/or clinical presentation. Importantly, when "Core 2" biomarkers are found positive, with "Core 1" negative – "A-T+" – these cases were previously considered within the scope of the umbrella term "suspected non-AD pathology" (SNAP). These SNAP cases could also be considered as the biomarker equivalent of PART. However, due to the nature of "Core 2" biomarkers being considered AD-linked, some authors have considered PART as potentially being an atypical AD-presentation where tau-pathology could be appearing "first" with additional amyloid pathology occurring later on and leading to more typical "A+T+" pathological presentations [18].

Overall, the future usage of all these biomarkers will provide the possibility to screen, diagnose and stage AD, as well as to assess co-pathologies and to monitor treatment effects, in all settings of clinical practice, from primary care to specialized memory clinics [19].

1.6. Current treatments in Alzheimer's disease

In the last few years there has been a revolution in the approach to AD treatment with the approval of the first antibody-based anti-Aβ therapies in the United States, Japan, United Kingdom, among other countries, with a reported effect of slowing the progression of cognitive decline [20]. This family of drugs have the goal of clearing Aβ plaques from the brain, and are based on the assumption that indeed the "amyloid hypothesis" is at the basis of AD pathogenesis [21]. Among the different drugs developed, they differ among other pharmacokinetic characteristics, on their binding properties to different amyloid species: solenazumab binds predominantly to monomers; crenezumab to a range of monomers, oligomers and fibrils; gantenerumab to fibrils; aducanumab to aggregates; lecanemab to protofibrils; and donanemab to pyro-Aβ and plaques [22]. Impressively, the majority of these drugs were very effective in clearing Aβ plaques from the brain of AD patients [22]. But most importantly, only a few of them passed the main clinical trial goal, which was significant slowing of clinical cognitive deficits, assessed by standardized neuropsychological tests [23-25]. When all clinical trials are pooled together a greater extent of amyloid removal correlates best with cognitive decline protection [23]. Among the specific drugs, most had negative results, and while aducanumab had mixed effects, lecanemab and donanemab were the only drugs with consistent positive effects, consequently being the only currently ongoing approved drugs [23-25].

However, it should be noted that this class of drugs is still not approved in Europe at the date of writing and this is due to the major concerns by the regulatory agencies regarding the reported side-effects during clinical trials, such as amyloid-related imaging abnormalities (ARIA) and decreased brain volume [22]. ARIA is identified in brain MRI in patients undergoing anti-Aβ antibody therapies in up to around a third of the patients and can be of two

types, (1) ARIA-E, with "E" standing for edema and/or effusion, depicting inflammatory alterations ongoing in response to Aβ mobilization; and (2) ARIA-H, with "H" standing for hemorrhage, depicting microhemorrhages and cortical superficial siderosis [22, 26]. Brain volume loss occurring after anti-Aβ antibody treatment is still intriguing to the scientific community and clearance of the volume occupied by Aβ or other potentially Aβ-linked fluid mobilization have been some of the hypotheses proposed to explain these changes [24, 25]. For this reason, understanding the mechanisms underlying ARIA and other side-effects, and the factors that predispose to them, is an active area of research in order to make this class of drugs safer. Indeed, it should be noted that ARIA is reported with differing frequencies between the different drugs, and since ARIA occurs early-on upon starting the treatment scheme, a closely monitored titration has been proposed as a key pharmacokinetic safety approach [27]. Additionally, the presence of CAA identified at baseline scan before treatment and patients being *APOE4* positive, have been identified as the main factors that predispose to ARIA [27].

Since the success of anti-Aβ antibody approaches is only partial, with significant side-effects, efforts are being actively implemented to test other disease-modifying treatments. These ongoing approaches in clinical trials are targeting a variety of alternative pathways, such as neurotransmitter receptors, neuroinflammation, other Aβ-related processes, synaptic plasticity neuroprotection, tau-related processes, metabolism, APOE-related processes, lipids, proteostasis, oxidative stress, circadian rhythm or vascular factors [20], which really highlights the complexity of AD pathogenesis and the multiple biological angles that are being tested.

1.7. The importance of co-pathologies in Alzheimer's disease management

While AD is the most prevalent neurodegenerative disease, with specific neuropathologic features such as Aβ plaques and NFTs, other somewhat prevalent pathologic features characteristic of other neurodegenerative disorders can exist independently, and very frequently co-occur with AD pathology.

The second most frequent cause of dementia after AD is cerebrovascular disease, leading to vascular dementia [28]. Since cerebrovascular disease is very frequent and increases and accumulates with aging, evidence of vascular damage in the brain can also be present in the brains of AD patients. For this reason, one term that has often been used is "mixed dementia", when it is a challenge to tease apart the contributions of either etiology. In patients, the main features of cerebrovascular disease can be identified by brain MRI as major brain infarcts, lacunar ischemic lesions, small cortical infarcts, white matter T2 hyperintensities (WMH), or vascular damage leading to hypoperfusion, all contributing to progressive neurodegeneration. My team addressed the impact of cerebrovascular disease assessed by MRI in pathologically confirmed PART and AD cases **(Chapter 3)**.

An additional feature which is in the border between AD pathology and cerebrovascular disease is CAA, due to the deposition of Aβ in brain vessels. These vessels can be damaged leading to microhemorrhages and major hemorrhagic brain infarcts, with a predominant lobar location. Importantly, these pathologic features can be identified not only *post-mortem*, but also in patients in brain MRI with T2* or susceptibility weighted imaging (SWI) acquisitions, contributing to the diagnosis of CAA. The identification of CAA is crucial for AD management, since it is frequently present in AD cases [29], and the presence of CAA is now considered a counterindication for the treatment with anti-Aβ antibodies, since it increases the risk of severe ARIA events [30]. With the development of criteria that contribute to the diagnosis of CAA by MRI, neuroradiologists can play a central role in patient decision-making steps [31]. This led us to study a local population diagnosed with CAA in the Hospital de Braga and we identified the clinical and imaging features that associate with re-hemorrhage and brain damage in the setting of dementia **(Chapter 4)**.

As in vascular and AD mixed-dementia cases, another frequent reported co-occurrence is between AD and LB pathology, with clinicians struggling whether to consider either AD or LBD diagnosis in the setting of a dementia case [32-45]. With the advent of recent biomarkers to address with some degree of specificity AD and LB pathologies, the future will likely pass through a joint approach handling both disorders. As is the case for tau pathology, α-synuclein pathology has been proposed to progress along different brain

regions in a given order of LB stages according to the analysis of *post-mortem* cases in either olfactory bulb, brainstem, limbic, amygdala-predominant and neocortical locations [46]. Basic science studies have shown over the years that α-synuclein and tau pathologies can synergize in their mechanisms leading to neurodegeneration [47] and we further show here the presence of Aβ pathology can contribute in the spreading of both α-synuclein and tau co-pathologies to the neocortex **(Chapter 3)**.

TDP-43 pathological aggregates have recently been found to also co-occur in either the setting of PART and to correlate with hippocampal, amygdala and anterior temporal atrophy, which in the setting of AD is even of higher magnitude [48-50]. The previously neuropathologically described condition, hippocampal sclerosis, now known to most likely be frequently representing the recently described LATE, can be anatomically tracked by brain MRI, depicting the characteristic features of higher relative hippocampal atrophy when compared to the whole brain, which in the framework of the "ATXN system", in an AD biomarker confirmed case, can indicate a condition of ADNC with concomitant non-AD pathological change. We have explored the contribution of TDP-43 pathology contribution to medial temporal atrophy in both PART and AD **(Chapter 3)**.

In the process of understanding how these different pathologies impact the brain we can infer some global concepts concerning neurodegenerative disorders. For instance, specific proteins tend to aggregate leading to neurodegeneration of specific brain regions. In AD there are two different defining pathologies that start their aggregation processes in different brain regions, namely Aβ predominantly aggregates early-on in the parietal lobe, while tau aggregates in the temporal lobe, and then their co-existence contributes for the propagation of tau aggregates throughout the brain, with NFTs more closely correlating with atrophy [51]. One proposed mechanism of α-synuclein aggregates propagation implicates the sequential neurodegeneration from the brainstem, to limbic regions and then to the neocortex [52]. And in the case of LATE it predominantly impacts limbic regions, such as the hippocampus [53]. Potential common cell biological processes that contribute to the pathological aggregation of these proteins, could be due to a failure in proteostasis mechanisms, due to either acceleration of the processes that lead to aggregation or dysfunction of the processes that

could contribute to the degradation and clearance of these aggregates [54]. For this reason, cell biology pathways that converge on the lysosome, such as endo-lysosomal flux or autophagy, have been shown to be particularly implicated in the pathogenesis of neurodegenerative disorders as a whole, and further explain why these co-pathologies can co-occur due to common cell-biological impaired processes [54].

Importantly, the study of the impact of co-pathologies in the era of disease-modifying therapies in AD is taking center stage because it can (1) partly explain why certain patients respond clinically differently at the symptomatic level even when Aβ plaques are completely cleared from the brain, and (2) highlights the need to develop directed therapies specifically directed to these co-pathologies, such as α-synuclein and TDP-43 regional brain accumulation.

1.8. The role of brain magnetic resonance imaging in Alzheimer's disease

One of the main roles of brain MRI in the management of patients with dementia is to diagnose and/or exclude causes that can either be treated, such as normal pressure hydrocephalus, or that have pathognomonic imaging features, such as Creuzfeldt-Jakob disease or cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy – CADASIL [55]. Since neurodegenerative-linked protein aggregates have been described to affect predominantly certain regions of the brain, consequently this can lead to characteristic spatial patterns of neurodegeneration and atrophy, that can contribute to a given diagnosis [55]. For instance, when complemented with a concordant clinical presentation, a focal medial temporal or frontal lobe atrophy can indicate a behavioral variant FTD, a predominant left temporal lobe atrophy can indicate a semantic FTD, or a midbrain atrophy with a "hummingbird" appearance can indicate a progressive supranuclear palsy [55]. In the imaging analytical methodologies in our studies we have two approaches to identify regional atrophy patterns that could be depicting ongoing neurodegeneration processes, (1) a visual rating approach, widely validated in the literature, which could be applied to both clinical and research scans [56], and (2) a quantitative volumetric approach, which requires

volumetric acquisitions [57]. This set of quantitative and semi-quantitative approaches then provides a framework to assess different contributions to neurodegeneration, which in the "ATXN system" corresponds to the "N" component. In my clinical practice and in the studies by my team, I have extensively applied them to inquire about the differential impact of various co-pathologies in the setting of AD.

Moreover, brain MRI, due to its multimodal nature that allows the possibility to assess various components of brain structure and function, also provides the possibility to assess steady-state cerebrovascular damage burden, which in the setting of AD is essential for patient management [55]. WMH of presumed vascular lesional origin can be computed as a surrogate of cerebrovascular disease burden and classified according to the Fazekas scale **(Figure 1)**, and specific features of CAA, according to the new Boston 2.0 criteria, such as lobar hemorrhagic lesions on T2* / SWI, high levels of perivascular spaces in the centrum semiovale or WMH in a multispot pattern also in the centrum semiovale [31]. Beyond the potential contributions of CAA pathology to regional atrophy, its identification on MRI is now of even more crucial clinical significance since patients with CAA are at high risk to develop ARIA in the context of anti-Aβ antibody treatments [30].

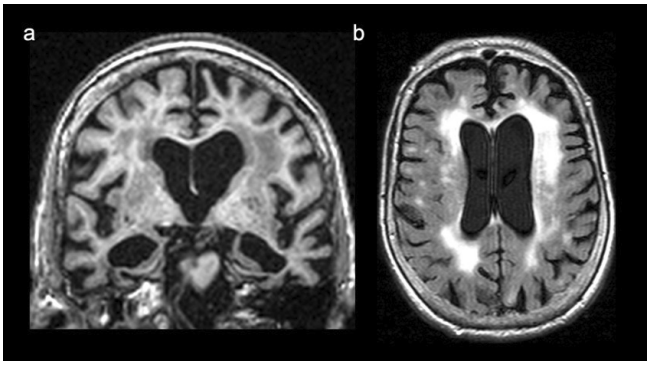

Figure 1. Representative case of a 91-year-old male patient with Alzheimer's disease. (a) Coronal reformat of a 3D T1-weighted sequence shows severe widening of the choroid fissures and severe enlargement of the temporal horns, with marked atrophy of both hippocampi (Medial temporal lobe atrophy – MTA – score of 4). (b) Axial T2-FLAIR sequence reveals hyperintensities involving the periventricular and the deep white matter, with irregular periventricular signal extending to the deep white matter in the former and severe confluence in the latter (periventricular and deep Fazekas score of 3).

1.9. Comparing ante-mortem brain imaging signatures with post-mortem neuropathology subtypes

One of the main revolutions of modern science has been the assembly of large datasets with multiple levels of information, such as brain MRIs, from patients with different disorders. A major advantage is the potential immediate clinical applications that can be derived from identifying meaningful features in these datasets. Over the last few years my team has been collaborating with the National Alzheimer's Coordinating Center (NACC), in Seattle, in the US, with the goal to explore a unique research angle. What differentiates the NACC dataset from others is that it pools together all cases from various AD Research Centers in the US where a standardized brain autopsy was performed covering the analysis of core neuropathologies, such as amyloid pathology, mainly with the CERAD score; tau pathology, with Braak staging; and other AD co-pathologies such as LBs or TDP-43 accumulations [58]. Quite interestingly for me, a fraction of these autopsied patients also has available brain MRIs and clinical information while the patients were still alive. Therefore, although we have the drawback of a certain lag time between age of MRI acquisition and age of death, this strategy allows us the possibility of studying the imaging and clinical signatures of neurodegenerative disorders diagnosed with gold-standard *post mortem* methods [44, 59, 60].

1.10. Uncovering the mysteries of Alzheimer's disease pathogenesis by exploring neuropathology, clinical and molecular niches

In the following chapters I describe the findings that my team has contributed to some of the most relevant ongoing questions in the AD field. In **Chapter 2**, we use our approach of unravelling neuroimaging-clinical signatures that distinguish PART from AD. In **Chapter 3**, we continue exploring neuroimaging-clinical signatures of co-pathologies such as cerebrovascular lesion burden, TDP-43, and LBs in the context of PART and AD. In **Chapter 4**, we expand these co-pathology concepts to a population in our local hospital in Braga, and we study the impact of having

CAA diagnosis on long-term re-hemorrhaging events and on neuroimaging-clinical signatures. In **Chapter 5**, we study an underexplored clinical presentation in AD, based on the presence of psychosis clinical symptoms in AD neuropathologically confirmed cases. In **Chapter 6**, we connect our clinical based questions to basic science approaches with the goal to better understand at the molecular level why some circuits and brain regions are predominantly affected in AD.

Overall, my intention here is to provide a window into my scientific approach as a physician-scientist. With this body of work, I have identified key regions impacted by pathologies that occur in the brains of AD patients and I then combined this with molecular approaches using animal models, that could then fuel new questions to be pursued in patients. I am mutually inspired by the groundbreaking advances in the understanding of AD pathogenesis by the scientific community and by the tireless efforts of all clinicians that follow these patients and collect valuable data that can be used for future discoveries, and I hope my work can contribute to better treat AD patients in Portugal and worldwide.

2. Studying primary age-related tauopathy to better understand Alzheimer's disease

2. Studying primary age-related tauopathy to better understand Alzheimer's disease

PART was described in 2014 by my colleague and collaborator John Crary [61] as a neuropathological condition that shows NFT accumulation predominantly confined to the temporal lobe, in the absence of Aβ plaques. PART is part of big group of brain pathologies called tauopathies, which are characterized by the accumulation of pathologically phosphorylated forms of tau, that eventually lead to the formation of NFTs [14]. These NFTs are the aggregates identified by neuropathologists at the *post mortem* level, and they can differ on their composition based on the tau isoforms that constitute them. The human gene that encodes the tau protein, *MAPT*, can lead to six isoforms due to alternative splicing of exons 2, 3, and 10, and in particular the presence of exon 10 determines 4R tau isoforms, and its absence, 3R tau isoforms [62]. Among the various known tauopathies, while in CBD, PSP or argyrophilic grain disease (AGD), NFTs with a predominant 4R isoform composition predominate; Pick's disease (PiD), which presents as an FTD clinical syndrome, is characteristically composed by 3R isoform NFTs; and in AD, PART and chronic traumatic encephalopathy (CTE), all present with tangles with a 3R / 4R mixed composition [14]. Interestingly, from the subset of tauopathies mentioned above, cryo-electron microscopy based structural analysis of CBD, PSP, AGD, PiD and CTE, showed that these all present different structural NFT organization, but AD and PART, quite interestingly, share the same structure [63].

Therefore, what exactly distinguishes PART from AD is the presence of Aβ plaques, which then provides a framework to study PART as a means to understand AD. Many outcomes can be derived by studying PART in the era of anti-Aβ antibody therapies, because upon plaque clearance AD treated cases are *"pharmacologically converted to PART"*: (1) we will be able to better understand why some patients do not clinically respond well to Aβ clearance; (2) since in PART, NFTs are mainly confined to the temporal lobe, while in advanced AD they are throughout the neocortex, we can mechanistically inquire what are the factors that promote tau pathological propagation.

With this in mind, I started to collaborate with John Crary and the NACC to understand how did *post mortem* diagnosed PART looked like

ante mortem at the clinical and brain imaging levels. Since at the point of our initial studies the NACC had only a very limited number of volumetric MRI acquisitions from PART cases, we decided to use previously validated visual atrophy scales that evaluate six different brain regions, namely anterior cingulate, orbito-frontal, anterior temporal, fronto-insular, medial temporal and posterior regions [64] to assess the correlation between regional atrophy with either the degree of NFT pathology progression as assessed by the Braak staging or age. Since both NFT pathology and age are known to independently impact atrophy, we statistically controlled for either Braak staging or age in each analysis. Upon correction for age, the relative atrophy of the medial temporal lobe significantly correlated with Braak staging **(Figure 2)** [59]. Upon correcting for Braak staging, we observed a trend to increasing atrophy with age, predominantly on the anterior temporal and medial temporal regions **(Figure 3)** [59].

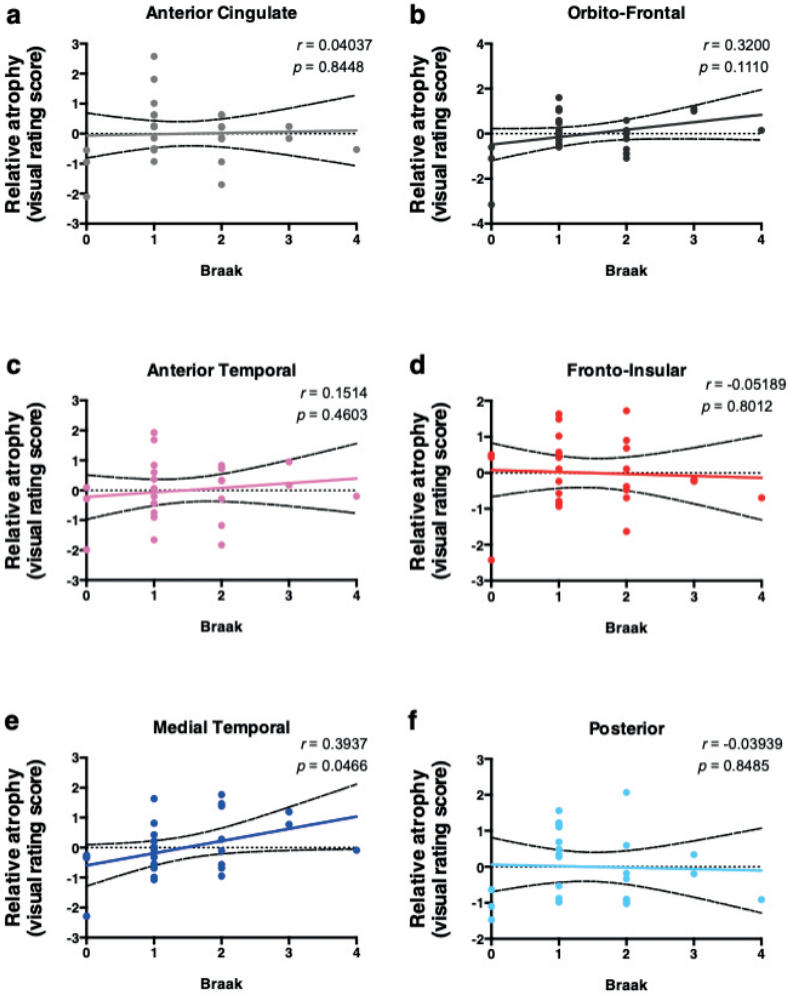

Figure 2. Medial temporal lobe atrophy correlates with increasing Braak staging in PART. The values represented are residuals corrected for age, with Pearson correlation analysis between Braak staging and atrophy of different brain regions based on a previously validated imaging rating scale. The regions evaluated are (a) anterior cingulate, (b) orbito-frontal, (c) anterior temporal, (d) fronto-insular, (e) medial temporal and (f) posterior brain regions. Statistical significance was considered as $p<0.05$. (adapted from Quintas-Neves et al, Acta Neuropathologica Comm, 2019 [59])

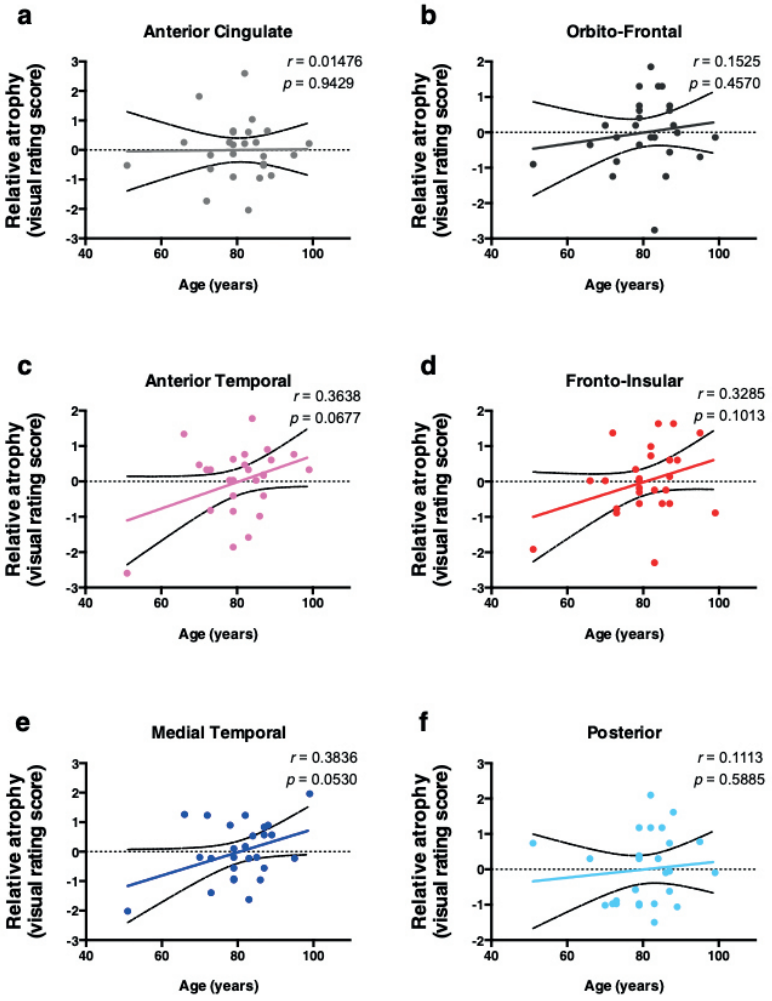

Figure 3. Anterior and medial temporal lobe atrophy increases with increasing age in PART. The values represented are residuals corrected for Braak staging, with Pearson correlation analysis between age and atrophy of different brain regions based on a previously validated imaging rating scale. The regions evaluated are (a) anterior cingulate, (b) orbito-frontal, (c) anterior temporal, (d) fronto-insular, (e) medial temporal and (f) posterior brain regions. Since statistical significance was considered as $p<0.05$, no significant correlations were found, although both anterior temporal ($r = 0.3638$, $p = 0.0677$), and medial temporal ($r = 0.3836$, $p = 0.053$) regions were found to be close to this statistical threshold. (adapted from Quintas-Neves et al, Acta Neuropathologica Comm, 2019 [59])

From these studies on PART cases, the majority had also performed an extensive neuropsychological battery testing. We then tested for an association between regional brain atrophy and performance in each of the cognitive domains assessed and we observed that atrophy of the anterior temporal lobe was associated with decreased semantic memory/language, given by the animals and vegetables naming tests, as was atrophy of the medial temporal lobe, given by the vegetables naming test **(Figure 4)** [59].

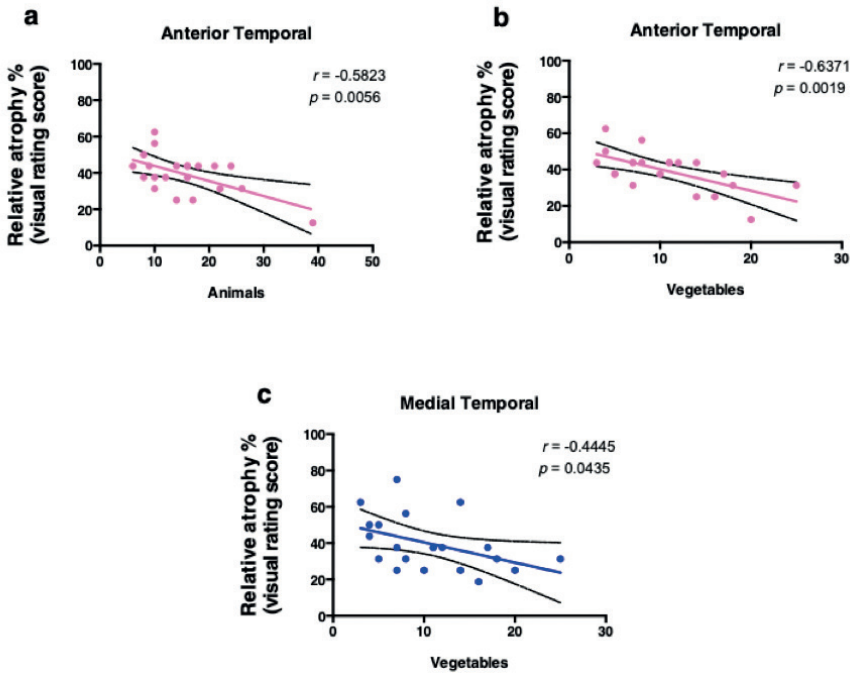

Figure 4. Semantic memory/language impairment correlates with anterior and medial temporal atrophy in PART. Pearson correlation analysis of neuropsychological test performance versus temporal brain atrophy regions evaluated with a previously validated imaging rating scale for patients with definite PART was performed and only the statistically significant correlations are shown ($p<0.05$). "Vegetables" represents the number of vegetables a subject can name in 1 minute. "Animals" represents the number of animals a subject can name in 1 minute. (a) Anterior temporal lobe atrophy is associated with decreased semantic memory/language, given by the animals (r=-0.5823, p=0.0056) and (b) vegetables naming tests (r=-0.6371, p=0.0019). (c) Medial temporal lobe atrophy is also associated with decreased semantic memory/language, given by the vegetables naming test (r=-0.4445, p=0.0435). (adapted from Quintas-Neves et al, Acta Neuropathologica Comm, 2019 [59])

These results showed an association between Braak staging and atrophy of the medial temporal lobe on PART upon correction for age, which was consistent with another imaging study, on which left hippocampal volume was found to be decreased in PART [65], and previous anatomopathological studies describing PART, where NFTs accumulation is predominantly confined to the temporal lobe [61]. Concordantly, medial temporal lobe atrophy is commonly found in typical cases of AD, namely with decreased hippocampal volume [66]. While this initial PART study did not help to confidently distinguish PART from "early AD", it instead contributed to distinguish it from advanced cases of AD where atrophy is extensive in other brain regions, such as the neocortex. For instance, these findings partly explained why more than 50% of PART cases were clinically diagnosed as AD [67]. In order to clinically distinguish PART from AD, we further inquired as to which neuropsychological domains could be impaired with the observed atrophy in the temporal lobe, and we observed that regional temporal lobe atrophy significantly correlated with worse performance on semantic memory/language domain tests. These findings were consistent with previous observations on the importance of temporal lobe on semantic verbal fluency [68], and with a previous study that showed predominant anterior hippocampus atrophy on patients with PART, which also presented semantic memory/language domain deficits [65].

These results supported the hypothesis that PART could be a form of pathological brain aging, with preferential atrophy in the medial temporal lobes, differing from AD.

While various other observations contribute to the concept of PART as a separate pathological entity from AD, further supported by the absence of an association between PART and the *APOE4* allele, the strongest risk factor for AD [69, 70], and the presence of "ghost" tangles, i.e. extracellular tangles, being more frequently found on PART than AD patients [71], it fuelled us to directly compare AD and PART using a similar approach as we had done before, by assessing brain regional atrophy with a 6 region semi-quantitative scale based analysis. We segregated cases based on CERAD scores (0-3), with CERAD = 0 being considered PART, and CERAD 1-3 along the "AD spectrum", and we calculated the standardized residuals for age, sex and difference between age of death and age at MRI. We observed in

the medial temporal lobe region, that brain atrophy was higher in CERAD 3 versus CERAD 2, in CERAD 3 versus CERAD 1, and there was a non-significant trend in CERAD 3 versus CERAD 0 **(Figure 5)**. For the other regions, there were no significant differences in relative atrophy between groups. Upon correction for Braak stage, no significant differences in relative atrophy between groups were observed. Overall, this shows that while there is a stepwise increase in medial temporal atrophy within the AD-continuum from CERAD 1 to 3, PART cases do not follow this continuum. While these observations support a more benign nature of PART when compared to severe AD, it also suggests that there could be other co-pathologies contributing to atrophy, particularly in PART. This is a question we address in detail in **Chapter 3**.

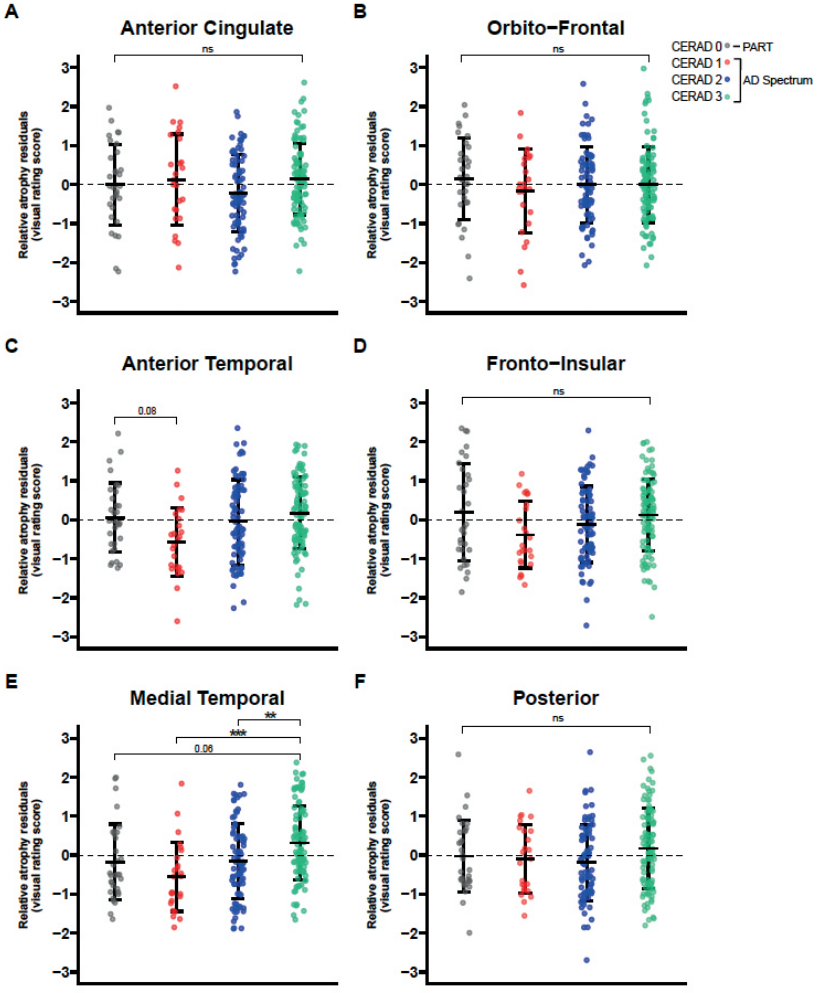

Figure 5. Age-corrected relative regional brain atrophy shows differential patterns in PART and Alzheimer's disease spectrum CERAD groups. Standardized residuals corrected for age for each specific region of brain atrophy among 4 groups of participants, distributed according to PART (CERAD 0 and BRAAK stage higher than 0) or Alzheimer's disease spectrum with presence (CERAD 1 – mild; CERAD 2 – moderate; CERAD 3 – severe) of neocortical neuritic plaques after neuropathological evaluation. The regions evaluated are (A) Anterior Cingulate, (B) Orbito-Frontal, (C) Anterior Temporal, (D) Fronto-Insular, (E) Medial Temporal, and (F) Posterior. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. 'ns' represents non-significant differences between groups. Total $n = 233$ participants. (adapted from Quintas-Neves et al, Neurobiology of Aging, 2022 [60])

Within this comparison between PART and AD, we also inquired about what could be distinguishing these two conditions at the clinical level when taking into account the different brain atrophy patterns. After correcting for age, brain atrophy scores were correlated with z-scores for the neuropsychological tests. We found that AD shows a widespread pattern of negative correlations between regional atrophy and z-scores of most neuropsychological tests **(Figure 6)**. On the other hand, PART showed a specific negative correlation between anterior temporal atrophy and language function scores **(Figure 6)**. Previous studies assessing cognitive status found that within comparable global cognitive levels, patients with PART had relative sparing of semantic memory/language when comparing to AD [67].

While these regional based analyses are highly informative, with the evolution and updates in the NACC database, we had the chance to complement our semi-quantitative studies with volumetric based approaches.

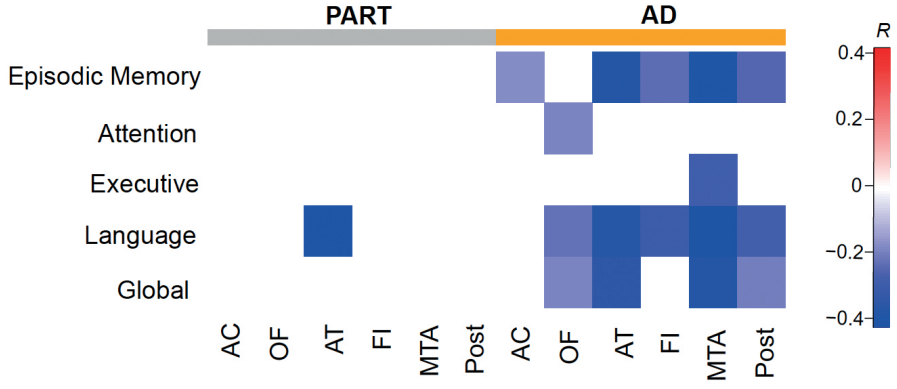

Figure 6. Correlation analysis between relative regional brain atrophy and neuropsychological assessment shows differential patterns in Alzheimer's disease and PART. Pearson correlation analysis of relative brain atrophy residuals after linear regression with age per region versus z-scores of neuropsychological test composites among 2 groups of participants, distributed according to the absence (PART) or presence (Alzheimer's disease) of neocortical neuritic plaques after neuropathological evaluation. Only showing pairs with $p < 0.05$ in the correlational analysis. Colour indicates *R* pearson coefficient. Anterior Cingulate (AC), Orbito-Frontal (OF), Anterior Temporal (AT), Fronto-Insular (FI), Medial Temporal (MT) and Posterior (Post). (adapted from Quintas-Neves et al, Neurobiology of Aging, 2022 [60])

We knew from the literature that both ADNC and PART present with preferential deposition of NFTs along a well-described brain topographical pattern described by the Braak stage progression in AD. Aligned with previous studies, we expected that cortical atrophy in PART should be confined predominantly to the medial temporal lobe when compared to AD [59]. On top of that, the hippocampus and amygdala are subcortical structures that visually contribute to medial temporal lobe atrophy assessment, and which are well known to be affected in both ADNC and PART. We then compared ADNC with PART cases, in the absence of other confusing factors such as LB, and we observed that ADNC led to lower volume of multiple temporal (left fusiform, banks of the superior temporal sulcus, inferior, middle and superior temporal gyri; and right fusiform, banks of the superior temporal sulcus, inferior, middle and superior temporal gyri), parietal (left inferior and superior parietal, precuneus gyri; and right precuneus and inferior parietal gyri), frontal (left and right rostral middle and medial orbitofrontal; and right superior gyri), left posterior and caudal anterior cingulate regions and higher atrophy of the amygdala bilaterally and right hippocampus when compared with PART **(Figure 7)**.

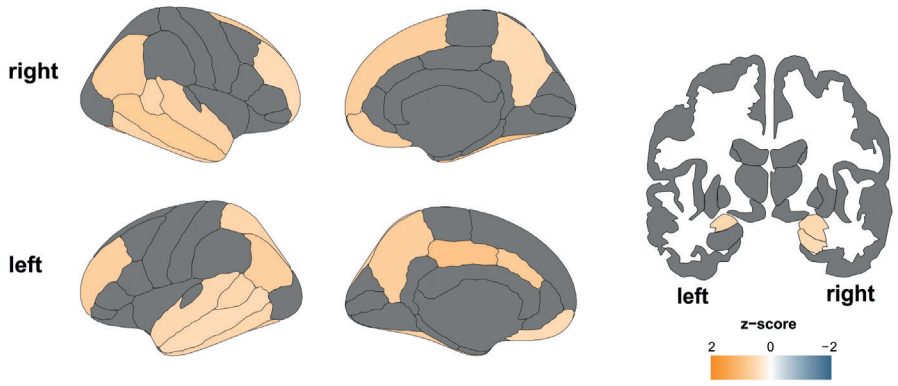

Figure 7. Comparison of cortical and subcortical volumes for ADNC and PART. Blue regions represent lower volumes in PART, whereas yellow represents higher volumes in PART, compared to ADNC. Only significantly different comparisons are shown ($p < 0.05$ after FDR correction for Welch's ANOVA or Kruskall-Wallis Test and $p < 0.05$ after TukeyHSD or Dunn's test with Holm correction). When not significant, group comparisons are not shown. Sample size, PART w/o LB, $n = 38$; ADNC w/o LB, $n = 157$. (adapted from Almeida et al, Alzheimer's Dementia, 2024 [72])

These volumetric approaches propelled us to a more detailed analysis of the differences between PART and AD. Overall, PART (with excluded presence of LB) represented a more benign group regarding dementia severity and this is reflected in a pattern of lower atrophy compared with AD (with excluded LBs). We explore in more detail these clinical phenotypes below, in the context of inquiring about the effects of LBs on ADNC and PART **(Chapter 3)**. Considering the areas that we observed to be more atrophied in ADNC vs PART we found the areas more impacted are known to be early on affected by NPs deposition, such as the precuneus [4] , and temporal lobe regions, known to be early on impacted by NFTs deposition in AD [73]. Even though PART also shows deposition of NFTs in similar temporal lobe regions, one potential hypothesis is that the brain inflammatory environment conferred by NPs could be inducing a higher-level accumulation of NFTs in those same regions, leading then to more atrophy, even if similar regions both show presence of tau pathology. While NFT Braak staging only takes into account if certain regions are affected, as a surrogate for the spatial progression of tau pathology, it does not consider the regional levels of tau. Indeed, this is starting to be assessed *in vivo* by tau PET approaches and recent studies show that local high levels of tau radiotracer binding, indicating high levels of tau aggregates, correlate with regional atrophy [74]. Therefore, these new concepts that we propose here, should be applied to future clinical strategies that monitor AD and PART cases diagnosed *ante mortem*, with amyloid and tau PET, combined with MRI, and this multimodal imaging approach inspired by neuropathology evidence should be developed as a gold-standard to be implemented in clinical practice.

3. The differential impact of neurodegenerative co-pathologies in Alzheimer's disease

3. The differential impact of neurodegenerative co-pathologies in Alzheimer's disease

Besides the diagnostic hallmarks of AD, such as NPs and NFTs, patients often present with other additional co-pathologies, such as cerebrovascular lesions, CAA, LBs, or TDP-43 inclusions [32]. Various factors could be contributing to develop ADNC and also these other co-pathologies, and many of the factors have been implicated as also contributing to increase the risk to develop AD. These include genetic risk factors, traumatic brain injury or vascular disease [75].

For instance, cerebrovascular disease burden can be assessed in the form of WMH in brain MRI. These WMH have been found to be a predictor of AD progression, and can be clinically assessed by a grading score, the Fazekas scale [76]. This is a three-point based scale [77] that assesses the rating of deep and periventricular white matter lesions related to leukoaraiosis, with the deep white matter lesions being more frequently associated with small vessel disease. Moreover, it is known that the amount of Aβ deposition and white matter lesions independently predict cognitive impairment, which supports the diagnostic usefulness of assessing white matter damage [78]. Teasing apart the differential effects of amyloid pathology and cerebrovascular lesion on cognition is challenging, but by studying patients with a confirmed diagnosis of PART can be highly informative. Indeed, a recent neuropathological study found a significant correlation between cognitive impairment and cerebrovascular disease in PART patients [79], raising the hypothesis that brain vascular pathology as a co-morbidity could have a differential impact in PART compared to AD, concerning the *ante mortem* clinical impact brain atrophy, assessed by MRI [59, 60, 64].

To study this question regarding the impact of cerebrovascular lesion as co-pathology within the AD-continuum, we applied the Fazekas scale in both PART and AD cases. Although statistically significant differences were found in periventricular and deep Fazekas scores between PART and AD cases, no significant differences remained after correcting for age and Braak **(Figure 8)**. We then performed a correlation analysis between the Fazekas scores and regional atrophy assessed by the semi-quantitative 6 region method we described above. In AD patients, significant positive correlations were found between the Fazekas score (periventricular and deep) and atrophy in the

medial temporal, anterior temporal and fronto-insular regions, and between the periventricular Fazekas and the orbito-frontal and the anterior cingulate regions, while in PART patients, significant positive correlations were found between the Fazekas score (periventricular and deep) and atrophy in the medial temporal and fronto-insular regions **(Figure 9)**.

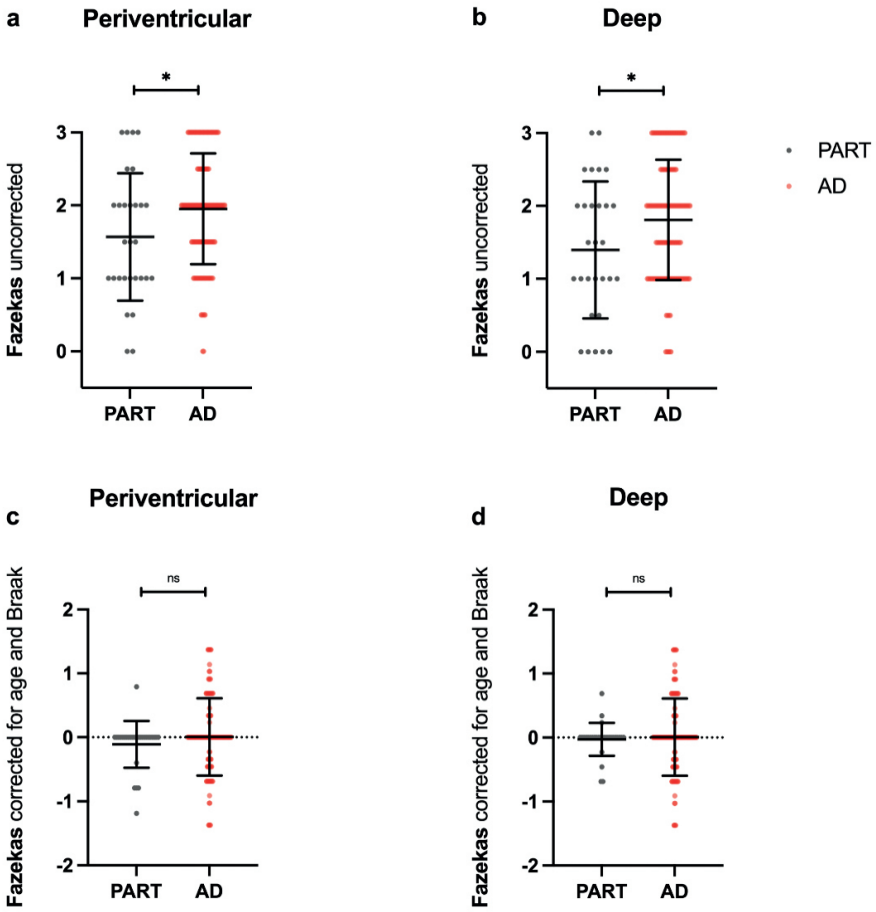

Figure 8. No significant differences were found in the Fazekas grading between patients with PART and AD. Uncorrected (a,b) and standardized residuals for age and Braak (c,d) for deep and periventricular Fazekas scores among patients with absence (ie PART) or presence (ie AD) of neocortical neuritic plaques after neuropathological evaluation. * $p < 0.05$. 'ns' represents non-significant differences between groups. *N* = 167 (adapted from Quintas-Neves et al, under review [80])

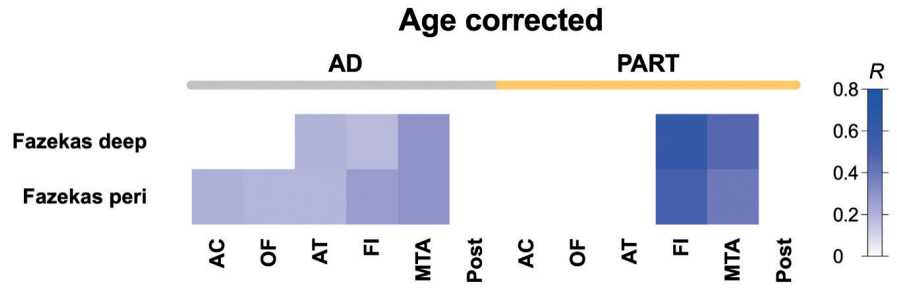

Figure 9. Correlation analysis between relative regional brain atrophy and Fazekas scores, corrected for age, shows differential patterns in AD and PART. Pearson correlation analysis of relative brain atrophy residuals after linear regression with age per region versus Fazekas scores (periventricular and deep) among 2 groups of participants, distributed according to the absence (ie PART) or presence (ie AD) of neocortical neuritic plaques after neuropathological evaluation. The regions evaluated are Anterior Cingulate (AC), Orbito-frontal (OF), Fronto-insular (FI), Medial temporal (MTA) and Posterior (Post). Only showing pairs with $p < 0.05$ in the correlational analysis. Color indicates R Pearson coefficient. (adapted from Quintas-Neves et al, under review [80])

Another potential major contributor to neurodegeneration in both AD and PART is the deposition of TDP-43, which was initially described as a main component of ubiquitinated inclusions found at autopsy in patients with FTD [81]. It was then described that TDP-43 was accumulating in other pathologies, also named TDP-43 proteinopathies. In AD, TDP-43 associates with memory deficits [82], atrophy of the hippocampus [82], and faster rates of hippocampal atrophy [49]. In PART, TDP-43 is associated with amygdala and hippocampal atrophy [48], supporting TDP-43 as being associated with temporal lobe atrophy across the entire range of Aβ and NFT deposition. Moreover, high TDP-43 levels in PART was associated with smaller brain volumes, faster rates of brain atrophy and acceleration of atrophy rates, however TDP-43-linked brain atrophy in PART occurred 3 years later than in AD [50]. Since the NACC only partially provided with TDP-43 pathology assessment at the time we initially tackled this question, we were only able to analyse the impact of TDP-43 in a small number of cases. That said, we still observed that TDP-43 pathology contributes to atrophy of the medial temporal lobe region in PART, and to a lesser extent in AD **(Figure 10)**. Our hypothesis is that these PART/TDP-43+ cases

correspond most likely to patients with LATE [53]. With the expansion of the NACC database and with the increased number of cases with extended co-pathologies assessment, such as TPD-43, we will be able to readdress this question in more detail in the future. Overall, these initial observations suggest that tau and TDP-43 contribute differently to the rate of brain atrophy over time in patients with PART and AD.

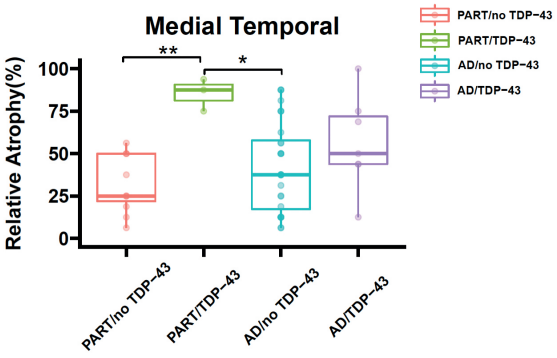

Figure 10. TDP-43 aggregates contributes to atrophy in the medial temporal lobe regions. Relative atrophy was assessed in 4 groups of participants, PART/no TDP-43, PART/TDP-43, AD/no TDP-43 and AD/TDP-43. * $p < 0.05$ and ** $p < 0.01$. (adapted from Quintas-Neves et al, Neurobiology of Aging, 2022 [60])

The other co-pathology we addressed in detail was regarding the effects of LB in AD and PART, since this anatomopathological feature has been systematically assessed in the NACC providing us the possibility to tackle this question. LBs are frequently found in AD neuropathological assessments and AD pathologies are also often detected in clinically considered LBD cases [32-45]. Although tauopathy without amyloidosis is known to occur in patients with LB [33, 36, 83], its implications to disease are not well studied as this group appears to be uncommon. This is relevant since it might reveal possible protein contributions to cognitive impairment and neurodegeneration among these mixed-pathology groups.

With the available data at the NACC we considered 7595 participants with available Braak stage, CERAD score and LB stage of any type, ADNC without LB (ADNC w/o LB) was the most frequent group, accounting

for 40.2%, ADNC with LB (ADNC+LB) for 26.4%, PART without LB (PART w/o LB) for 9.0% and PART with LB (PART+LB) for 2.6%, with other pathologies accounting for 21.8% **(Figure 11)**. Our further analyses revealed that ADNC+LB and PART+LB had a higher proportion of male participants compared with the respective groups without LB, that ADNC+LB had a significantly earlier age at death compared with the other groups, and that ADNC w/o LB and PART+LB had an earlier age at death compared with PART w/o LB.

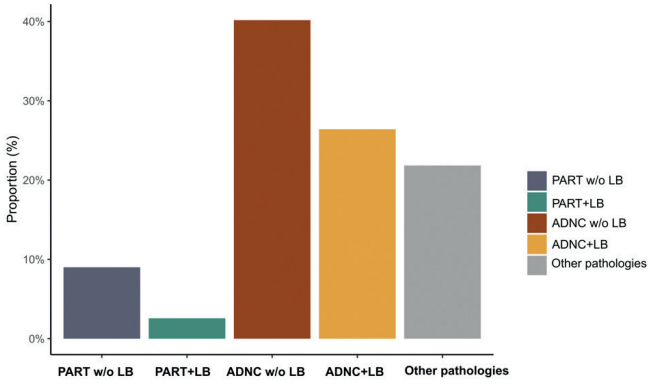

Figure 11. Proportion of each neuropathological group in the prevalence cohort. ADNC w/o LB was the most frequent group (40.2%), followed by ADNC+LB (26.4%), other pathologies (21.8%), PART w/o LB (9.0%) and PART+LB (2.6%). Other pathologies correspond to the presence of neuropathological or clinical evidence of 3R or 4R tauopathy, frontotemporal degeneration, argyrophylic grain disease, amyotrophic lateral sclerosis, prion disease, multiple system atrophy and Parkinson's disease. Sample size, *n* = 7595 (PART w/o LB, *n* = 684; PART+LB, *n* = 195; ADNC w/o LB, *n* = 3051; ADNC+LB, *n* = 2006; Other pathologies, *n* = 1659). (adapted from Almeida et al, Alzheimer's and Dementia, 2024 [72])

Out of the neuropathological cohort we analysed, 1955 participants had a neuropsychological assessment less than 2 years before death. We first analysed the effects of co-pathologies on cognitive decline, quantified by the cognitive dementia rating sum of boxes (CDR-SB) scale, which takes into consideration the following six cognitive categories: memory, orientation, judgement and problem solving, community affairs, home and hobbies, and personal care. The CDR-SB is calculated by summing the ratings of the six cognitive domains, ranging from 0 (normal) to 18 (severe dementia), and

provides the possibility to divide cases into three dementia severity groups based on CDR global scores: 0 – normal cognition (NC); 0.5 – MCI; > 0.5 – dementia (Dem). Cross-sectionally, ADNC+LB presented with the highest median CDR-SB score corrected for age, followed by ADNC w/o LB and PART+LB and PART w/o LB **(Figure 12)**. The proportion of dementia severity based on CDR global scores (NC, MCI and Dem) also differed significantly between all groups in the same gradient of dementia severity **(Figure 12)**.

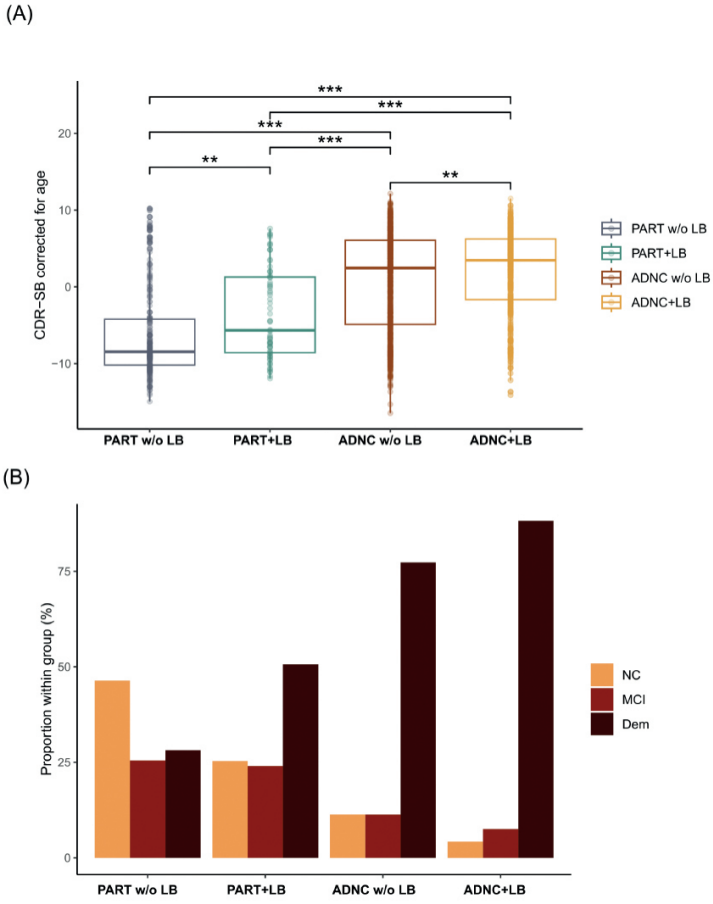

Figure 12. The presence of LBs associates with cognitive impairment in PART. In (A) it is shown the comparison of CDR-SB residuals after linear regression with age across the four groups.

ADNC+LB showed the highest dementia severity followed by ADNC w/o LB, PART+LB and PART w/o LB, with all pairwise comparisons statistically significant (*p < .05; **p < .01; ***p < .001, with Dunn's test after Holm correction). The proportion of dementia severity based on CDR global (B) showed the same gradient of dementia severity across groups. All pairwise comparisons were statistically significant (p < 0.05 with chi-square test after Holm correction). Sample size, n = 1955 (PART w/o LB, n = 263; PART+LB, n = 75; ADNC w/o LB, n = 953; ADNC+LB, n = 664). NC – normal cognition; MCI – mild cognitive impairment; Dem – dementia. (adapted from Almeida et al, Alzheimer's and Dementia, 2024 [72])

Even though the proportion of Braak stages did not differ significantly within PART and ADNC groups, the groups with co-pathology showed a higher proportion of more advanced Braak stages. In order to test whether differences in cognitive impairment within PART and ADNC were due to Braak stage imbalances, we corrected CDR-SB for age and Braak stage and compared PART w/o LB with PART+LB and ADNC w/o LB with ADNC+LB. We observed a significant lower CDR-SB score in PART w/o LB compared with PART+LB. Division according to Braak stage within PART groups also showed statistically significant differences between PART w/o LB and PART+LB within the same Braak stage **(Figure 13)**. ADNC w/o LB also showed a lower CDR-SB score compared with ADNC+LB after age and Braak correction. Division according to Braak stage within ADNC groups showed statistically significant differences between ADNC w/o LB and ADNC+LB in Braak III-IV stages, but not on I-II or on V-VI, suggesting a possible ceiling effect as measured by the CDR-SB **(Figure 13)**.

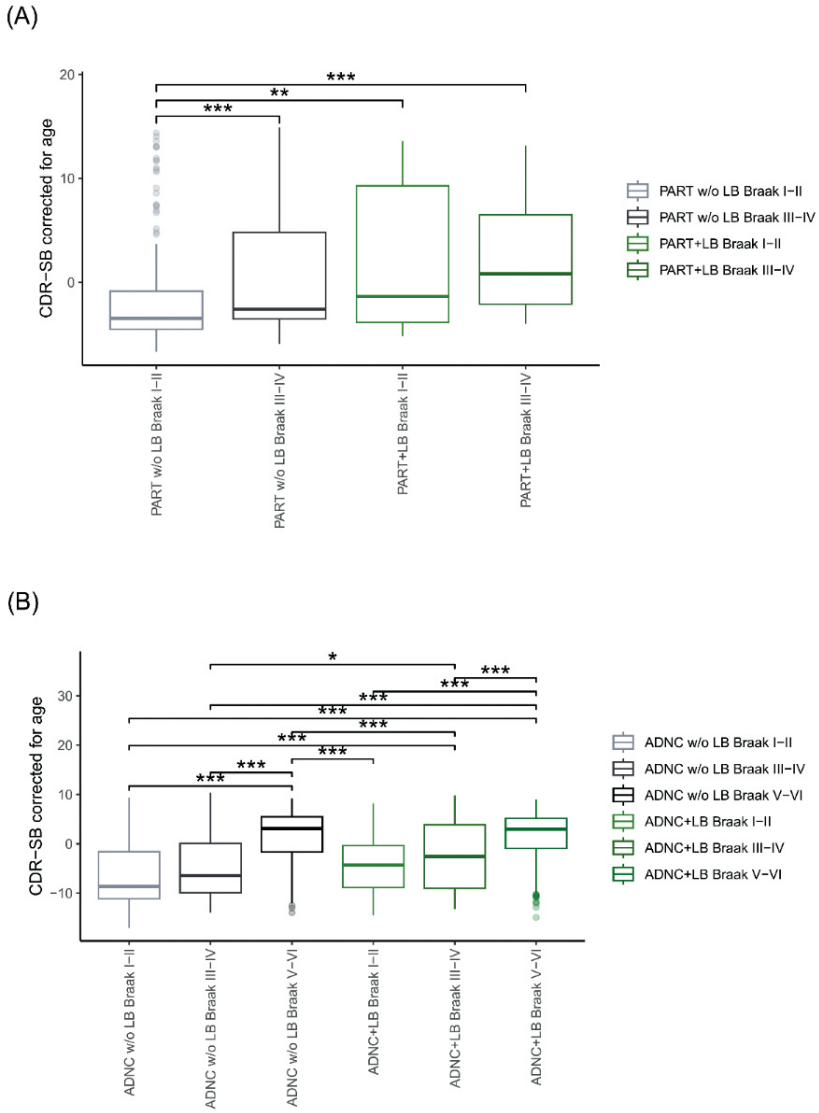

Figure 13. Lewy body co-pathology contributes to cognitive impairment independently of Braak stage. Supplementary Figure 3 shows (A) gradient increase of CDR-SB residuals after age correction with Braak stage and LB stage. Corrected CDR-SB was higher in PART+LB with Braak I-II and III-IV stages compared to PART w/o LB Braak I-II ($p < 0.05$ after Dunn's test with Holm correction). Braak stage V-VI cases in both groups were excluded from the analysis due to low sample size. Sample size, $n = 331$ (PART w/o LB, Braak I-II, $n = 173$; PART w/o LB, Braak III-IV, $n = 87$; PART+LB,

Braak I-II, n = 37; PART+LB, Braak III-IV, n = 34). (B) shows a similar analysis in ADNC groups, showing a gradient effect according to both Braak stage and LB stage. Note that ADNC+LB with Braak stage III-IV was significantly different from ADNC w/o LB with Braak stage III-IV. Sample size, n = 1612 (ADNC w/o LB, Braak I-II, n = 80; ADNC w/o LB, Braak III-IV, n = 224; ADNC w/o LB, Braak V-VI, n = 646; ADNC+LB, Braak I-II, n = 31; ADNC+LB, Braak III-IV, n = 93; ADNC+LB, Braak V-VI, n = 538). $^{*}p < .05$; $^{**}p < .01$; $^{***}p < .001$. (adapted from Almeida et al, Alzheimer's and Dementia, 2024 [72])

We next tested the longitudinal progression of cognitive impairment with a battery of neuropsychological tests with a retrospective follow-up period of 2000 days. Executive function was assessed by the Trail Making Test (TMT) A and B, which globally tests attention, visual scanning and search skills, and psychomotor speed and coordination [84]; TMT A can independently assess processing speed, while TMT B assesses set switching; on both parts of this test (i.e., A and B), the total number of seconds to complete the test, the number of commission errors, and number of correct lines were recorded; the Wechsler Adult Intelligence Scale digit symbol (WAIS) test was also considered to provide an estimate of processing speed [85]. Semantic memory/language was assessed by category (vegetables and animals) verbal fluency [86], consisting of a test on registering the total number of vegetables and animals named in 60 s; the Boston naming test [26], which also assesses the effect of language function, more precisely the confrontational word retrieval, was included in this evaluation, and consisted of showing pictures (up to 60) to the patient, and wait up to 20 s for the patients to name them. Attention and working memory was evaluated by Digit span forwards (DIGIF) and backwards test (DIGIB) [87], consisting on registering the ability of recalling a sequence of numbers shown, and the total length of numbers successfully achieved (DIGIFL and DIGIBL, respectively). Logical memory was evaluated using Logical Memory Immediate and Delayed Recall tests (Logical Memory; Memory Unit), in which an orally presented verbal story is asked to be recalled immediately and 20 minutes after [88]. Mini-Mental State Examination (MMSE) [89] was performed as a brief cognitive screening instrument that provides a measure of global cognition. Using multiple linear regression mixed-effects models, ADNC+LB and ADNC w/o LB progressed faster in cognitive impairment in all neuropsychological tests compared with PART w/o LB. PART+LB progressed faster compared

with PART-LB in CDR-SB and TMT-A. ADNC w/o LB declined faster in CDR-SB, MMSE and Boston Naming Test compared with PART+LB. ADNC+LB declined faster compared with PART+LB in CDR-SB, MMSE, WAIS, Animals, Vegetables, Boston Naming Test, TMT-A, TMT-B and DIGIF-L and Logical Memory and faster than ADNC w/o LB in CDR-SB, MMSE, WAIS, Animals, Boston Naming Test, TMT-A, DIGIF and DIGIF-L. These results suggest a worse progression in ADNC+LB compared with other groups across multiple cognitive domains, followed by ADNC w/o LB, PART+LB and PART-LB **(Figure 14)**.

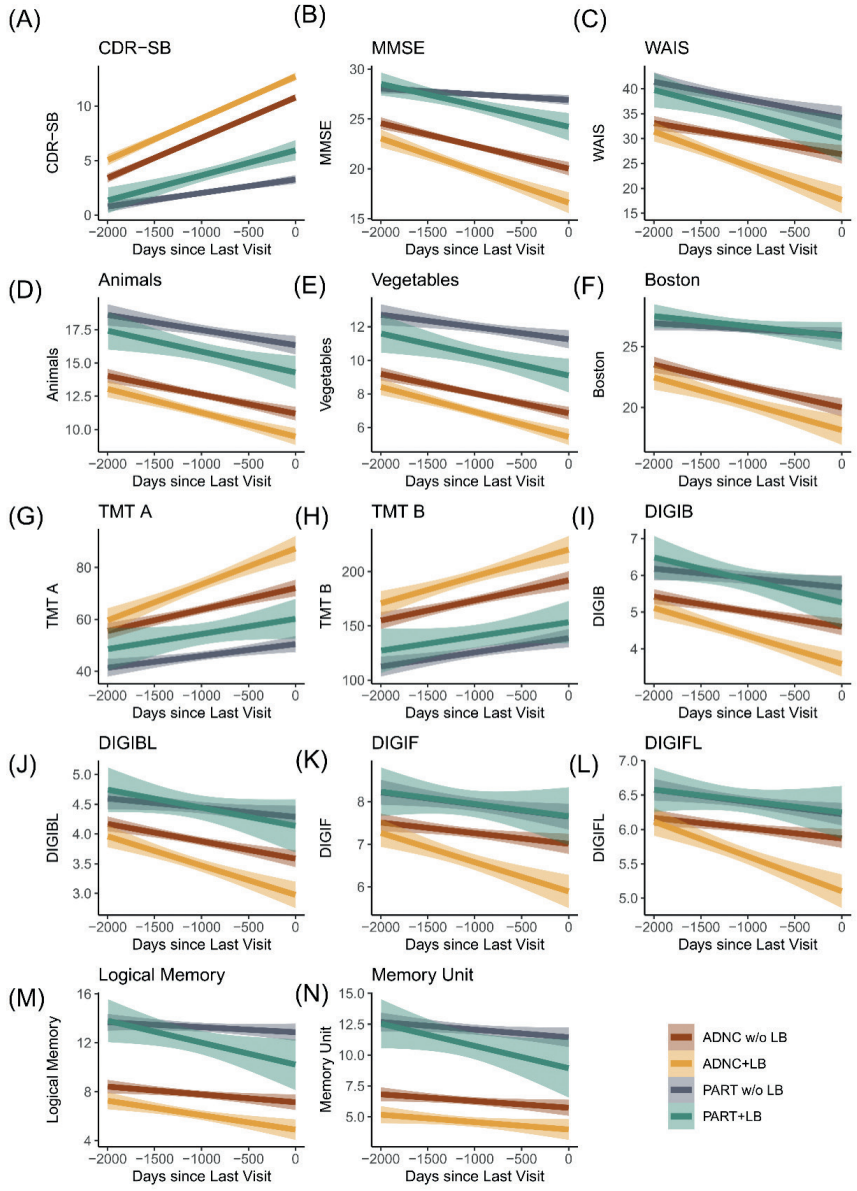

Figure 14. Longitudinal progression of cognitive testing across groups. Here it is shown the progression of cognitive impairment of each group across a range of neuropsychological tests (A-N). WAIS - Wechsler Adult Intelligence Scale; TMT – Trail Making Test; DIGIB – Digit Span Backwards; DIGIBL - Digit Span Backwards Length; DIGIF – Digit Span Forwards; DIGIFL – Digit Span

Forwards Length. Follow-up period of 2000 days before last visit. Exclusion criteria: less than two longitudinal datapoints. *N* = 1808 participants included in this analysis. (adapted from Almeida et al, Alzheimer's and Dementia, 2024 [72])

Since ADNC and PART present with preferential deposition of NFTs along a well-described brain topographical pattern described by the Braak stage progression in AD, we then inquired what was the impact of LB co-pathology in either PART or ADNC. After exclusion criteria, we considered only cases that had volumetric brain MRI acquisitions, which were then processed with the Freesurfer software for standardized quantification of volumes in predefined brain regional atlas. We compared cortical and subcortical volumes between each group after obtaining residuals from linear regression with age. PART+LB presented lower volume of the left rostral middle frontal, right putamen and right amygdala compared with PART w/o LB **(Figure 15)**. Additionally, PART w/o LB represented a more benign group regarding dementia severity and this is reflected in a pattern of lower atrophy compared with the other groups. The presence of LBs in PART resulted in significant cognitive impairment and a pattern of atrophy localizing to the frontal lobe, the putamen and the amygdala. Interestingly, higher atrophy of the putamen had previously been shown in LBD compared with AD [90], suggesting a role of LB pathology in atrophy of the basal ganglia independent of AD pathology. To our knowledge, this was the first report to show a differential pattern of atrophy according to the presence of LBs in PART. Overall, these findings support the concept that LB co-pathology is a determining factor of disease severity and degeneration in PART.

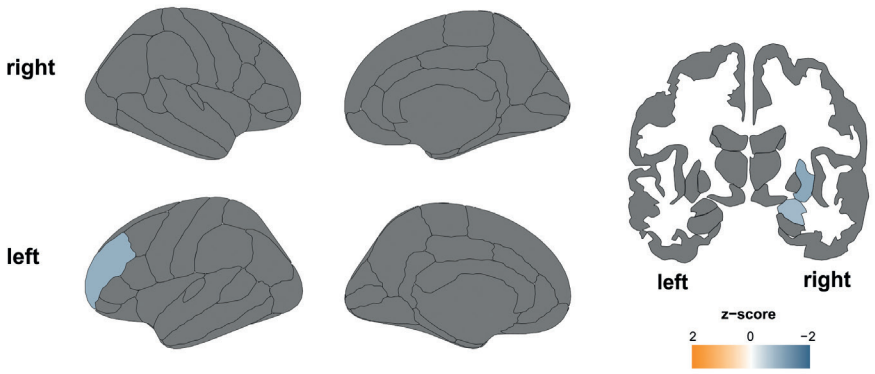

Figure 15. Comparison of cortical and subcortical volumes for the effect of LB in PART. Blue regions represent lower volumes in PART+LB, whereas yellow represents higher volumes in PART+LB, compared to PART without LB. Only significantly different comparisons are shown ($p < 0.05$ after FDR correction for Welch's ANOVA or Kruskall-Wallis Test and $p < 0.05$ after TukeyHSD or Dunn's test with Holm correction). When not significant, group comparisons are not shown. Sample size, PART w/o LB, $n = 38$; ADNC+LB, $n = 13$. (adapted from Almeida et al, Alzheimer's and Dementia, 2024 [72])

ADNC+LB presented higher atrophy of the right pars opercularis, transverse temporal gyri and right amygdala compared with ADNC w/o LB **(Figure 16)**. ADNC w/o LB and ADNC+LB showed a widespread pattern of higher cortical and subcortical atrophy, which ranged from the hippocampus to the frontal lobe, compared with PART. This is in line with the progression of tauopathy to neocortical regions and likely with detrimental effects of tau and Aβ in the shared territorial brain areas of ADNC and PART. Interestingly, the volume of EC was not found to be different between ADNC w/o LB and PART groups, supporting the possibility that, in PART, an initial tau-initiated neurodegeneration might be in place.

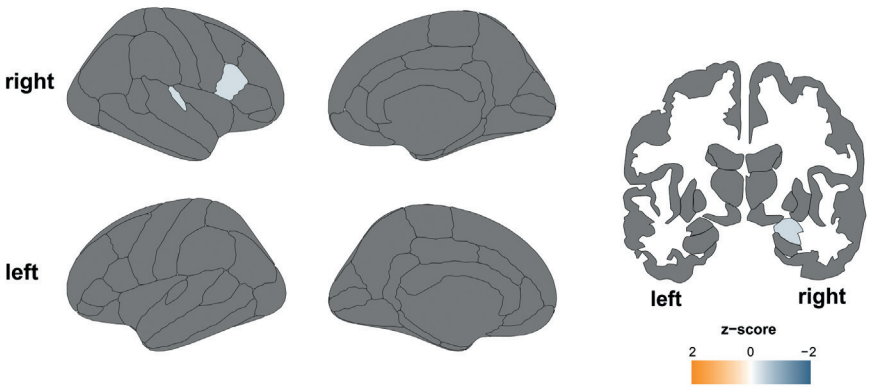

Figure 16. Comparison of cortical and subcortical volumes for the effect of LB in ADNC. Blue regions represent lower volumes in ADNC+LB, whereas yellow represents higher volumes in ADNC+LB, compared to ADNC without LB. Only significantly different comparisons are shown ($p < 0.05$ after FDR correction for Welch's ANOVA or Kruskall-Wallis Test and $p < 0.05$ after TukeyHSD or Dunn's test with Holm correction). When not significant, group comparisons are not shown. Sample size, ADNC w/o LB, $n = 157$; ADNC+LB, $n = 114$. (adapted from Almeida et al, Alzheimer's and Dementia, 2024 [72])

Taking into account the effects of LBs on cognitive domains and regional atrophy, it was quite interesting to observe that higher atrophy of the right amygdala was found in ADNC+LB versus ADNC w/o LB, which was maintained even after correction for Braak stage. This region is a known locus of co-occurring pathology, thus supporting the concept that atrophy might be a marker of the interaction of these proteinopathies in this region [91].

Given the differential distribution of NFTs in PART and ADNC, we inspected the anatomical location of LBs, to test whether LB pathology accompanies NFT pathology progression between PART and ADNC. A higher proportion of neocortical LB pathology and lower brainstem LB pathology is observed in Moderate NPs+LB (54.5%, 14.2%; respectively) and Severe NPs+LB (50.5%, 6.4%; respectively) compared with PART+LB (40.3%, 25.4%; respectively) and Mild NPs+LB (37.6%, 30.2%; respectively). Pairwise Fisher tests with Holm correction were used to compare total LB distribution (sum of Braak stages) across PART+LB and the different CERAD scores in ADNC+LB. PART+LB was not significantly different from Mild NPs+LB (p = 0.75). Both Moderate NPs+LB and Severe NPs+LB differed significantly from PART+LB and Mild NPs+LB (Moderate NPs+LB vs PART+LB, p = 0.048; Moderate NPs+LB vs Mild NPs+LB, $p < 0.01$; Severe NPs+LB vs PART+LB, $p < 0.001$; Severe NPs+LB vs PART+LB, $p < 0.001$). Severe NPs+LB also differed significantly from Moderate NPs+LB ($p < 0.01$) **(Figure 17)**.

Overall, our findings show that the distribution pattern of NFTs and LBs in PART+LB and ADNC+LB was different. With the increase in NP load, LB pathology progressed from brainstem to neocortical regions, accompanying the evolution of NFT pathology across its Braak stages. This pattern has also been hinted at previously in a larger sample size study [83]. This raises the hypothesis that the presence of NPs might associate not only with the progression of tauopathy, but also with the progression of LBs into neocortical stages. Interestingly, injection of α-synuclein pre-formed fibrils in an amyloidogenesis mouse model exacerbates α-synuclein inclusion pathology spread compared with a model without Aβ [92]. Further mechanistic research is needed to assess this relationship.

Our studies on co-pathologies in PART and AD shows differential

patterns of dementia severity and cortical and subcortical atrophy being influenced by cerebrovascular disease, TDP-43 and LBs. These results emphasize the growing importance of co-pathologies in AD-related disease with implications for clinical trial design, selection of patients for specific treatments and pathophysiological research.

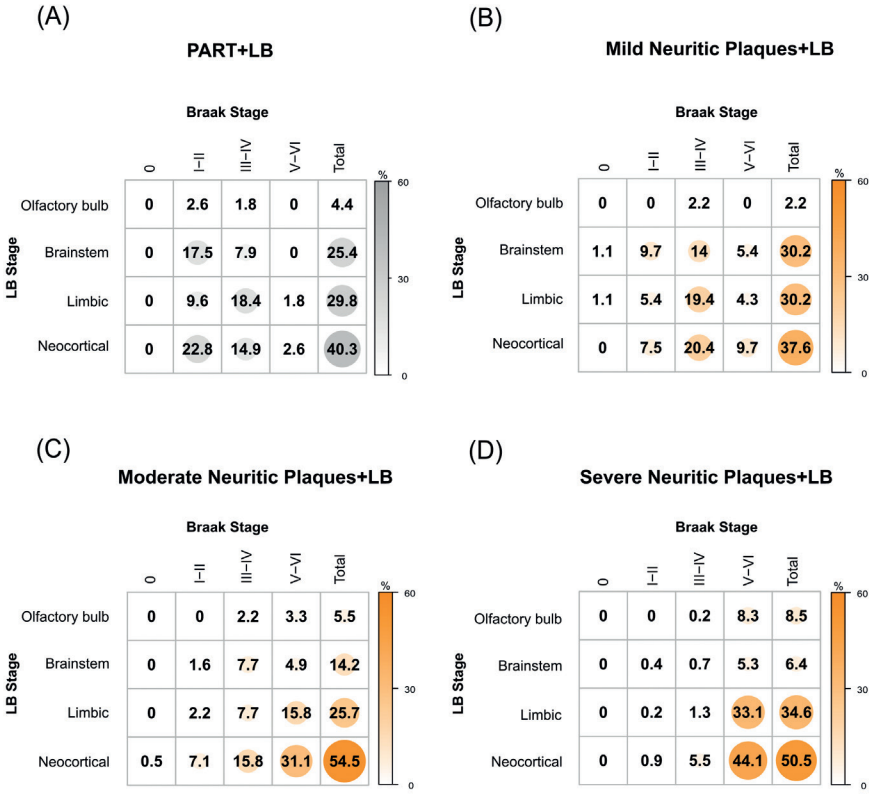

Figure 17. ADNC+LB and PART+LB show different topographical patterns of LB deposition. Here it is shown the proportion of participants according to LB location and Braak stage in (A) - PART+LB; (B) – Mild Neuritic Plaques+LB; (C) – Moderate Neuritic Plaques+LB; (D) – Severe Neuritic Plaques+LB. In PART+LB and Mild Neuritic Plaques+LB, most patients located in early tau Braak stages (I-IV) and balanced LB stages, whereas in Moderate to Severe Neuritic Plaques+LB, most patients localized to neocortical tau Braak stages (V-VI) and limbic and neocortical LB stages. Sample size, *n* = 1955 (PART w/o LB, *n* = 263; PART+LB, *n* = 75; ADNC w/o LB, *n* = 953; ADNC+LB, *n* = 664). (adapted from Almeida et al, Alzheimer's and Dementia, 2024 [72])

4. Studying cerebral amyloid angiopathy in a Portuguese population

4. Studying cerebral amyloid angiopathy in a Portuguese population

As a neuroradiologist, in most situations, my clinical role is in providing a complementary approach to other clinicians in the diagnosis of neurodegenerative disorders, which rely on clinical neurological and psychiatric observations, fluid biomarker quantification or imaging assessments beyond the scope of neuroradiology such as PET scans by nuclear medicine specialists. In the particular case of AD, neuroradiology is crucial, not necessarily for the diagnosis, which is done with evidence from fluid and/or PET biomarkers, but for the assessment of co-pathologies, such as cerebrovascular lesions, or the impact of co-pathologies, such as LBs or TDP-43 accumulations, by assessing regional brain atrophy, or in the diagnosis of complications of anti-Aβ antibody therapies, such as ARIA.

CAA is another very frequent co-pathology in AD, being present in about half of cases [29]. CAA is pathologically characterized by Aβ deposition in the walls of leptomeningeal and cortical small vessels [29]. In the context of neuroradiological practice, CAA got increased attention with the publication of the Boston criteria for the identification of probable and possible CAA cases [93], which were further updated recently [31]. These rely in the identification of evidence of lobar hemorrhage, including microhemorrhages or cortical siderosis, which can be identified by SWI acquisitions; enlarged perivascular spaces (EPVS) in the centrum semiovale, which can be identified on T2 weighted images; and confluent WHM in the centrum semiovale, in the context of likely CAA-linked cerebrovascular lesions **(Figure 18)**.

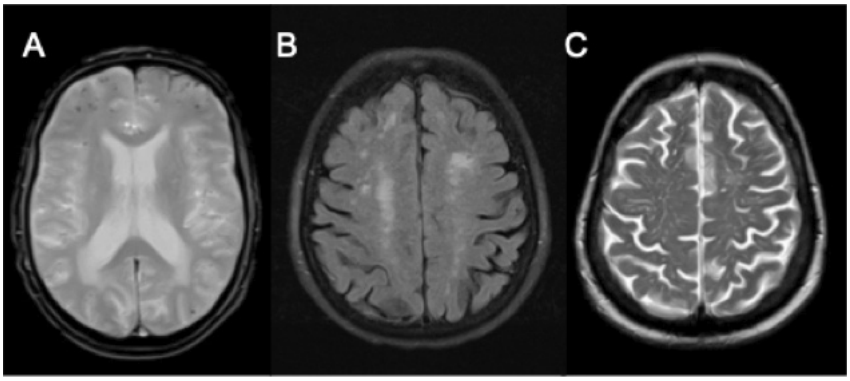

Figure 18. Brain MRI pathological features of cerebral amyloid angiopathy pathology. (A) Axial T2* image from a 76-year-old male patient showing left frontal cortical siderosis and multiple foci of microhemorrhages of predominant frontal location bilaterally. (B) Axial T2-FLAIR image from a 73-year-old female patient showing centrum semiovale white matter T2 hyperintense lesions attributed to chronic small vessel ischemia. (C) Axial T2 image from a 78-year-old female patient showing enlarged perivascular spaces in the centrum semiovale. (Adapted from Pinho et al, AJNR, 2023 [94])

This paves the way for neuroradiology to take a central stage in the diagnosis and management of CAA cases. Therefore, I set out to assemble and study a CAA cohort in my local hospital in Braga, in collaboration with my former colleague from the Neurology Department, João Diogo Pinho.

From big populations studies, it is known that both CAA and arteriolosclerotic microangiopathy are among the most frequent causes of non-traumatic intracerebral hemorrhage (ICH) [94], that CAA is associated with a high hemorrhage recurrence risk [96], and that this risk is significantly higher when compared to patients with ICH related to other causes [97]. The distribution of cerebral microbleeds is thought to reflect the etiology of the underlying microangiopathy, which translates into different hemorrhage recurrence risks, but the role of other *in vivo* biomarkers of recurrence has also been highlighted in more recent studies [98]. This distribution of cerebral microbleeds is highlighted in the Boston criteria 2.0, since strictly lobar microhemmorhages are considered a criterium for CAA, while in the basal ganglia the etiology is rather attributed to arteriolosclerotic microangiopathy [31]. So, the characterization of new imaging biomarkers of ICH recurrence may not only provide better prognostic information in patients presenting

with ICH, but also help us to better understand the pathophysiology of the underlying cause. Our goal was to study ICH recurrence in patients CAA-related and CAA-unrelated ICH in our local cohort of patients in Braga.

Among 448 consecutive patients with non-traumatic ICH admitted during the study period, 126 patients underwent brain MRI. From these, 22 patients were excluded due to missing MRI sequences, insufficient quality or unavailability of images for analysis. The final study population consisted of 104 patients, with a mean age of 64 years, 59.6% males and a median follow-up time of 27 months. Overall, ICH recurrence during follow-up was 12.5%. As expected, patients with CAA-related ICH more frequently presented lobar hemorrhages at the index event (93.8% vs. 19.6%). None of the patients with CAA-unrelated ICH experienced hemorrhage recurrence, while 13 patients with CAA-related ICH had at least a hemorrhage recurrence during the follow-up period (27.1%, 12.7 per 100 person-years). The Kaplan-Meier curves showed no difference concerning mortality between groups, but showed significantly higher ICH recurrence in the group of patients with CAA-related ICH **(Figure 19)**. Our further analyses revealed that age, presence of disseminated cortical siderosis, and burden of EPVS in the centrum semiovale were associated with ICH recurrence.

The incidence of ICH recurrence in patients with CAA-related ICH is similar to larger cohort studies, which reported incidences between 8-10% per year [99-101]. Even though an increasing burden of microbleeds has been associated with ICH recurrence both in CAA-related and CAA-unrelated ICH [97], more recent cohorts of CAA patients did not find this association and highlighted the importance of cortical siderosis as a more robust predictor for ICH recurrence [98]. In a meta-analysis of studies which included patients with CAA with and without ICH, even though cortical siderosis was found to be associated with occurrence of future ICH, disseminated cortical siderosis conferred a higher independent future ICH risk when compared to focal cortical siderosis [98]. Our results concerning the association of disseminated cortical siderosis with ICH recurrence are in line with these reported observations.

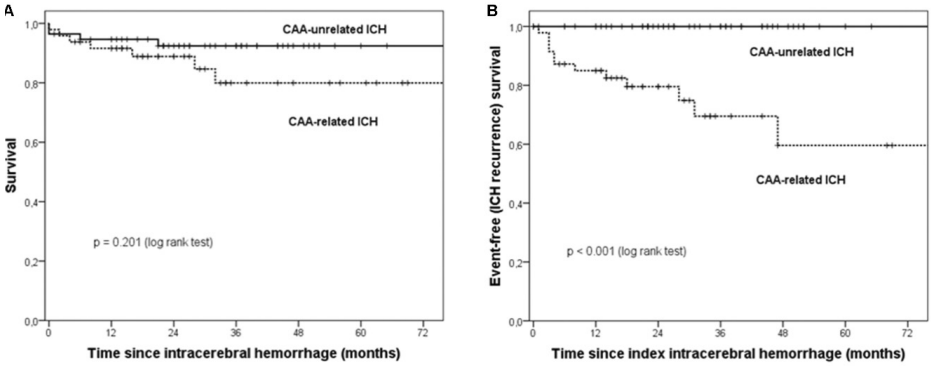

Figure 19. Kaplan-Meier curves for survival (A) and for ICH recurrence (B) according to the presence of CAA-related and CAA-unrelated ICH. Total number of patients included in the analysis – n = 47, CAA-related ICH; and n = 54, CAA-unrelated ICH. (Adapted from Pinho et al, Cerebrovasc Dis Extra, 2021 [102])

This initial study led us to go deeper in our brain imaging analyses and we sought to understand the contribution of CAA to neurodegeneration by assessing regional atrophy using a brain regional assessment, as we had done previously.

Structural MRI in CAA show intermediate level of atrophy between individuals with no cognitive deficits and AD, which is a frequent known association [103, 104]. CAA pathology was found to be present in >80% of AD patients at autopsy [105]. Also, the impact of CAA on cognition indicates that it could play its own role in cognitive dysfunction, in a potentially distinct manner than typical AD [106-110]. Surprisingly, the connection between cognitive impairment and brain atrophy in CAA patients has been scarcely explored, and while sex-specific effects have been observed in AD, it is still underexplored in CAA [111]. The recent inclusion of non-hemorrhagic imaging features in Boston 2.0 criteria [31], such as EPVS in the centrum semiovale, is aligned with previous reports connecting them with increased cortical vascular Aβ in the overlying cortex [112, 113] and as indirect measures of CAA severity, such as cortical microbleeds and cortical superficial siderosis [114-116]. We then studied in our local Braga cohort of 58 patients, brain MRI features in CAA, such as cerebral atrophy and frequency of EPVS, and their relationship with severity of cognitive impairment and sex in patients with CAA.

Regarding visual rating scores for each brain region, increased medial temporal lobe atrophy was found in patients with dementia compared to patients with normal cognition **(Figure 20)**, with no other significant differences across groups in the assessed brain regions. When we inquired about the mediators of medial temporal lobe atrophy, we found that it was more severe in male patients with dementia compared with female patients with dementia and compared with males without dementia, and females without dementia **(Figure 21)**. More severe medial temporal lobe atrophy was observed in patients with cortical siderosis **(Figure 22)**. We also observed more severe atrophy of the posterior and anterior cingulate regions in patients with cortical siderosis. More severe medial temporal lobe atrophy was also associated with increased WMH lesions both in the basal ganglia and in the periventricular white matter **(Figure 22)**, although we also found this same pattern of association for all brain regions.

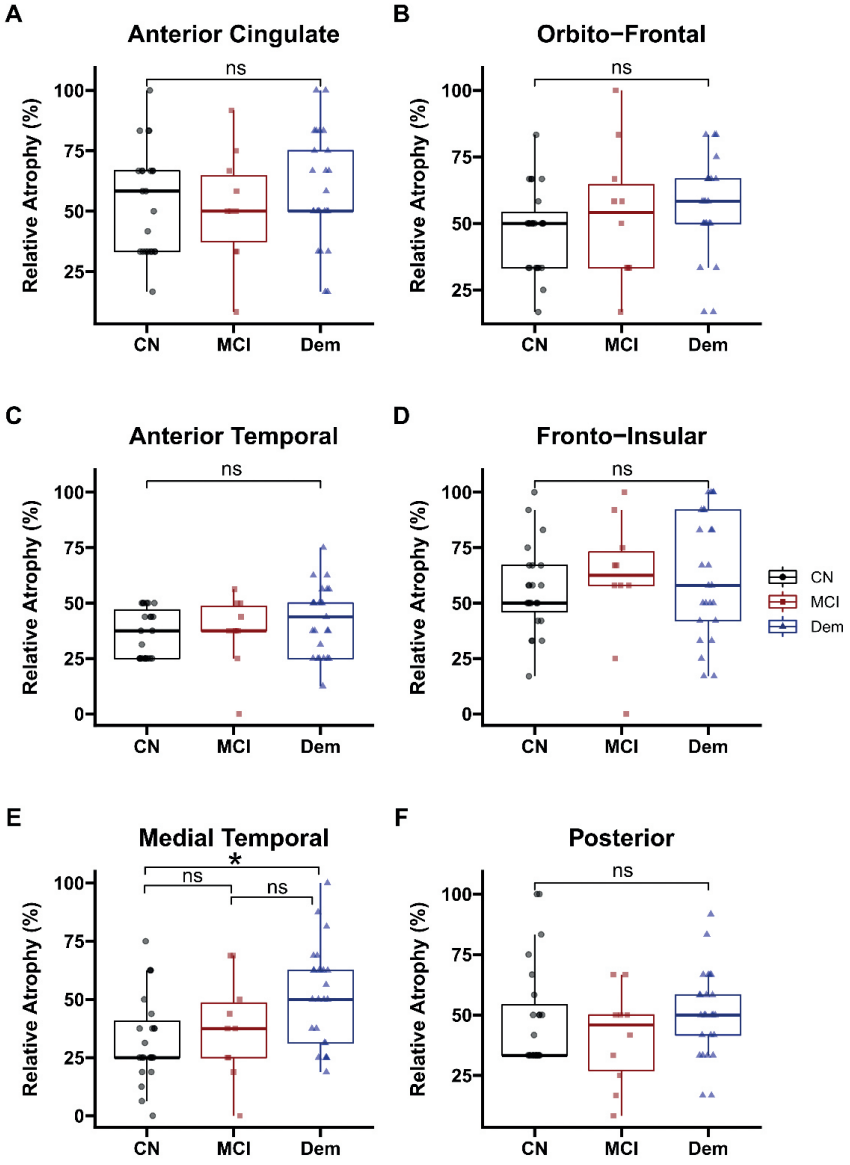

Figure 20. Regional brain atrophy as measured by the visual rating score compared across cognition levels: normal; mild cognitive impairment (MCI); Dementia. The regions evaluated are (A) Anterior Cingulate (AC), (B) Orbito-Frontal (OF), (C) Anterior Temporal (AT), (D) Fronto-Insular (FI), (E) Medial Temporal, and (F) Posterior. Statistical significance considered at p values < 0.05. * $p < 0.05$, ** $p < 0.01$ and *** < 0.001. (Adapted from Pinho et al, AJNR, 2023 [94])

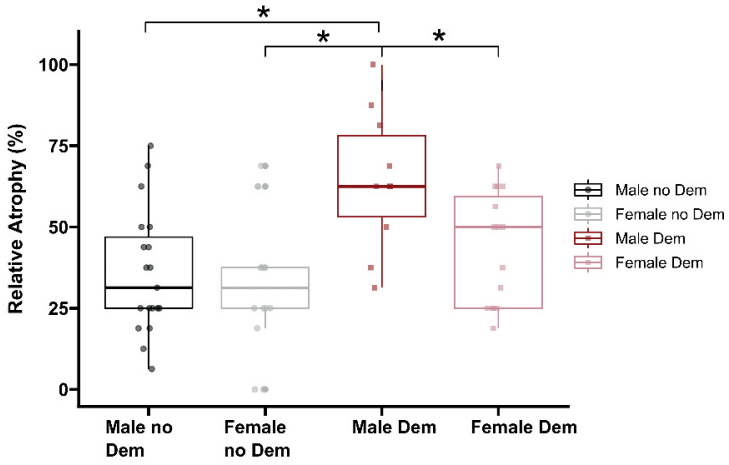

Figure 21. Medial temporal atrophy as measured by the visual rating across dementia groups and sex. Male no Dem: Male, no dementia; Female no Dem: Female, no dementia; Male Dem: Male, dementia; Female Dem: Female, dementia. Statistical significance considered at p values < 0.05. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. (Adapted from Pinho et al, AJNR, 2023 [94])

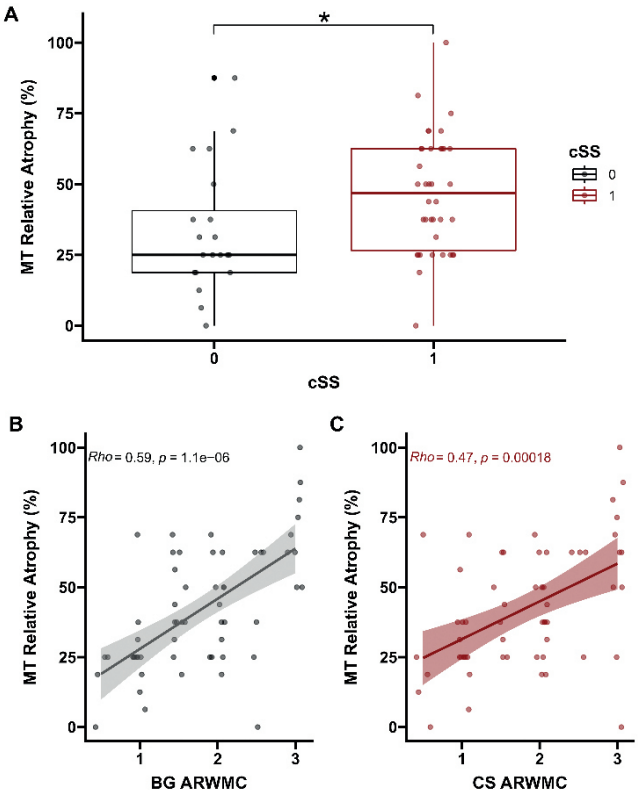

Figure 22. Determinants of medial temporal atrophy in CAA. (A) Medial temporal atrophy as measured by the visual rating across the presence (1) or absence (0) of cortical superficial siderosis (cSS). (B) Spearman correlation analysis of medial temporal atrophy with basal ganglia age-related white matter changes (BG ARWMC). (C) Spearman correlation analysis of medial temporal atrophy with centrum semiovale age-related white matter changes (CS ARWMC). Spearman correlation coefficient (rho). Statistical significance considered at p values < 0.05. * p < 0.05. (Adapted from Pinho et al, AJNR, 2023 [94])

We then assessed whether the frequency of EPVS was different according to the cognitive status of the patient. We found that the burden of EPVS is increased in the centrum semiovale, but not in the basal ganglia, in patients with dementia versus those with normal cognition **(Figure 23)**. When we divided the groups according to sex and presence of dementia, we found that females with dementia showed a higher burden of EPVS in the centrum

semiovale compared with males with dementia and with females without dementia and males without dementia **(Figure 23)**. These observations are in line with the general dichotomy of basal ganglia EPVS being associated with arteriolosclerotic small vessel disease and the centrum semiovale with CAA [117]. Indeed, the presence of these EPVS has been associated with vascular Aβ deposition [112, 113], which is in accordance with the hypothesis that centrum semiovale EPVS are associated with impaired perivascular drainage of Aβ.

Overall, our results indicate a sex-specific pattern of cerebral atrophy and EPVS in patients with CAA and dementia. More severe MTA was associated with dementia in male patients with CAA, but not in females, whereas higher frequency of EPVS in the centrum semiovale was associated with dementia in female patients, but not in males.

AD and CAA are frequently associated and share common pathophysiological pathways [29, 118]. For instance, a decrease in the volume of the hippocampus is not only a known feature of AD, but it is also found in sporadic CAA, albeit to a lesser degree [104]. Additionally, medial temporal lobe atrophy has been implicated as a predictor of incidental dementia in CAA patients [119]. Quite interestingly, and in accordance with these studies, we found that the medial temporal lobe was the only brain region assessed by us to be correlated with dementia.

Sex-specific differences have also been reported, with increased severity of CAA pathology found in male patients with AD, independently of age and AD neuropathological severity [111].

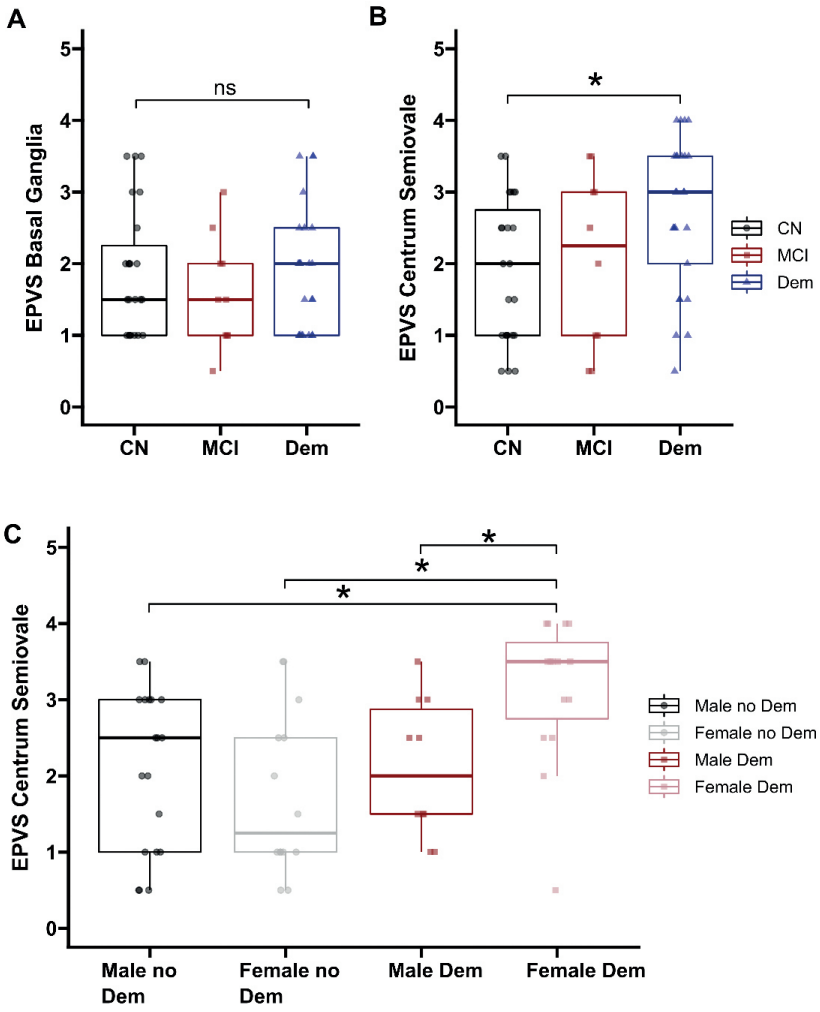

Figure 23. Enlarged perivascular spaces (EPVS) across cognition groups. (A) Basal ganglia (BG) EPVS; (B) Centrum semiovale (CS) EVPS. (C) CS EPVS across dementia by sex groups. Statistical significance considered at p values < 0.05. * p < 0.05. (Adapted from Pinho et al, AJNR, 2023 [94])

The mechanisms underlying brain atrophy in patients with CAA are not fully understood. It is thought, however, that the consequences of the amyloidotic small vessel disease, such as cortical microinfarcts, white matter

lesions and ischemic demyelination are important contributors to cortical thinning in CAA [118]. Concomitant AD pathology is likely an additional contributor for brain atrophy in some CAA patients, but how often and to what extent, remains unclear [29]. Interestingly, increased severity of CAA in male AD patients has been interpreted as a sign of higher susceptibility to vascular damage [111]. Accordingly, this sex-related small vessel disease susceptibility might explain why males with dementia have higher atrophy than females with dementia, whereas putative contribution from parenchymal amyloid might contribute to increased atrophy relative to males without dementia. Recently, men with sporadic CAA were shown to present with earlier onset of disease and a more hemorrhagic phenotype compared with females, supporting the hypothesis of increased vascular burden in these patients [120]. Since dementia in females was not significantly associated with more severe MTA compared to females without dementia could be indicating that different factors in the pathophysiology of the disease may play a role.

In the future we hope to expand our approaches using quantitative volumetric assessments to study atrophy patterns, perivascular spaces, WMH, and haemorrhages for localization, quantification, and classification in patients with CAA. Future work implementing quantitative techniques is needed to confirm these results and potentially explore new associations with more detailed analysis of these neuroimaging findings. We plan to assess the association of CAA pathology identified and classified at the *post mortem* level with *ante mortem* clinical and imaging features, as we did in other studies. Finally, the interest to study CAA mechanisms in AD patients has recently increased since ARIA-E and ARIA-H, two types of frequent side effects that occur upon initiating anti-Aβ antibody treatment, have been considered some sort of *"pharmacological CAA-type"*, since both edema and hemorrhages occur in inflammatory forms of CAA, also known as CAA-related inflammation – CAA-RI [121], prompting the need to understand how to tackle therapeutically both CAA and ARIA.

5. Psychosis in Alzheimer's disease indicates more severe presentations

5. Psychosis in Alzheimer's disease indicates more severe presentations

The motivation for this study came directly from clinical practice, from an observation from my PhD student, Francisco Almeida, who observed a patient with amnestic AD-typical dementia that presented in the emergency room with concomitant symptoms of psychosis. It was then apparent that these symptoms caused marked suffering to the patient and the direct family, and were remarkably difficult to treat. This was therefore a major clinical challenge with potential immediate implications from understanding the underlying mechanisms of this niche, but impactful presentation in AD.

Psychosis in AD is defined as the presence of delusions, i.e. fixed false beliefs, or visual or auditory hallucinations [122, 123]. These symptoms are present in ≈41% of AD patients [124] and associate with worse cognitive function, greater decline of cognition over time and increased mortality [124-132]. As Francisco had observed in his patient, these psychotic clinical presentations have indeed been reported to be highly distressing to both patients and caregivers, resulting in increased rates of patient institutionalization [127].

Clinical and research criteria have been recently developed to guide both the diagnosis of psychosis in a neurodegenerative context and to anchor research in a neurobiological framework based on currently available biomarkers [122, 123]. Indeed, the neuropathological mechanisms underlying the emergence of these symptoms are not yet well understood and therapies for these symptoms are lacking.

One possibility is that psychotic symptoms are a consequence of impaired cognition, resulting from an inability to remember things or orient oneself in space and time, without necessarily reflecting a different pathophysiology. On the other hand, it has been shown that psychotic symptoms are more frequent in AD patients with increased typical neuropathology load, especially tau accumulations [133], CAA [134], concomitant LB pathology [135] or arteriosclerotic leukoencephalopathy [134, 136]. These studies suggest that the presence of psychosis reflects either the presence of co-pathologies, such as LBs, which associate with psychotic symptoms in LBD, or an increase in typical AD neuropathology burden, namely NFTs and NPs. Importantly, neuropsychiatric-neuropathology studies have found that

psychosis is more common in AD+LBs versus those with pure LBD and pure AD [45, 137, 138], suggesting relevant interactions between these two pathologies. Differential associations between different neuropsychiatric symptoms and specific neuropathology has been supported not only in these neuropathology studies, but also by the recent finding of specific genetic risk loci for psychosis in AD in a genome-wide association study [139]. These findings suggest there is pathophysiological specificity to psychosis and open an avenue to explore the underlying mechanisms associated with these symptoms.

Using our previously successful approach of tackling the role of co-pathologies with *ante mortem* vs *post mortem*, using the NACC dataset, we decided to explore the impact of having psychosis on the cognitive profiles, cortical and subcortical volumes and concomitant neuropathology in a population of patients with ADNC, identified at *post mortem*.

Our cohort, with ADNC confirmed at autopsy, was composed of 178 participants, of which 75 (42%) had psychotic symptoms over the follow-up period of 3000 days. ADNC cases with psychosis (ADNC+P) had statistically significant earlier age at first visit, earlier age at MRI and earlier death. The ADNC+P group showed a higher prevalence of CERAD 3 score, of V-VI Braak stage and a higher proportion of patients with LBs in the neocortex, but no significant differences were found for CAA or *APOE* genotype. In patients with available data for presence of TDP-43 and white matter rarefaction, we did not find significant differences between groups. In the ADNC+P group, 34 patients (45.3%) presented with both hallucinations and delusions, 25 solely with delusions (33.3%) and 16 (21.3%) solely with hallucinations. We then inquired whether our cohort replicates previous findings of worse cognition over time in ADNC+P, by analyzing individual and smoothed group trajectories of CDR-SB in ADNC+P and ADNC-P groups over time since first visit, indicating a higher baseline CDR-SB value and steeper slope for the ADNC+P group **(Figure 24)**.

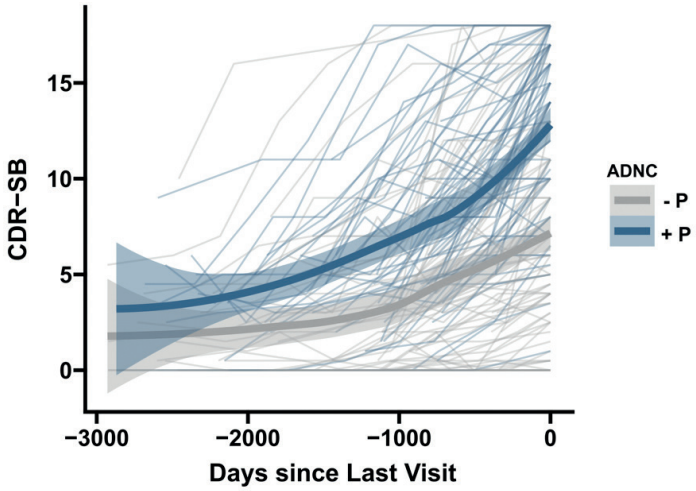

Figure 24. The presence of psychosis has differential cognitive and volumetric underpinnings. Plot shows results for ADNC+P versus ADNC-P comparisons. Individual CDR-SB trajectories (transparent) and grouped smoothed trajectories (bold) across time from first visit in ADNC+P (blue) and ADNC-P (grey). Total *n* = 178, n = 75 with psychotic symptoms. (adapted from Almeida et al, Neurobiology of Aging, 2024 [44])

In order to understand what could be at the basis for the worse cognitive profile in ADNC+P, we investigated whether this group was associated with volumetric differences compared to the ADNC-P group. After correcting for age at MRI, we found lower volumes in the ADNC+P group for right fusiform, inferior temporal, middle temporal, parahippocampal gyri and temporal pole **(Figure 25)**. We did not find any significant differences between groups in subcortical structures.

We next studied in deeper detail the potential cognitive differences between both groups by calculating the z-scores of a battery of neuropsychological tests in the visit closest to the MRI of each subject. We found that the ADNC+P group showed a widespread multi-domain cognitive dysfunction **(Figure 25)**.

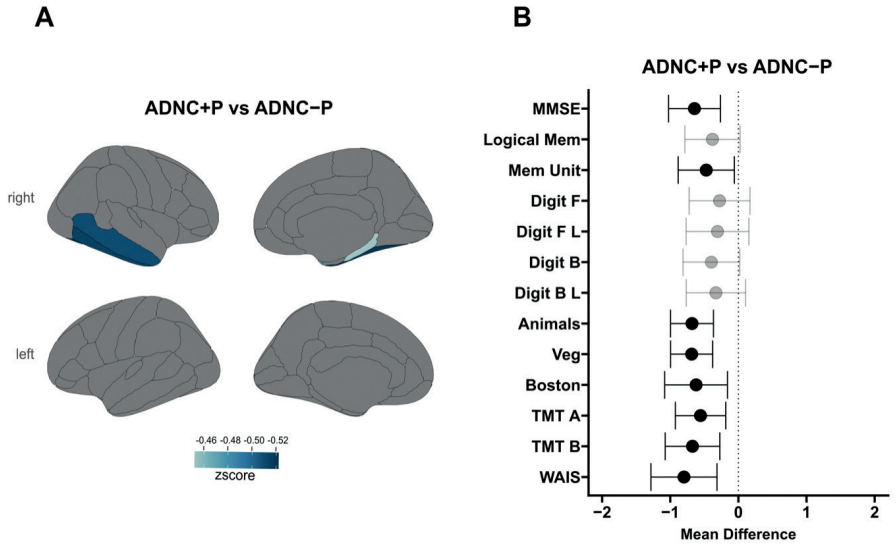

Figure 25. Psychosis is associated with a differential pattern of cortical brain volume and neuropsychological test performance. Plots show results for volumetric cortical comparisons and neuropsychological testing between ADNC+P and ADNC-P. (A) Z-scores based on mean difference in brain volume residuals after linear regression with age at MRI in ADNC+P versus ADNC-P, using Welch's t-test. Only significantly different comparisons are shown ($p < 0.05$ after FDR correction). (B) Mean differences and 95% confidence interval in neuropsychological tests z-scores in ADNC+P versus ADNC-P, using Welch's t-test. Significantly different comparisons are shown in bold ($p < 0.05$ after FDR correction). (adapted from Almeida et al, Neurobiology of Aging, 2024 [44])

Since psychosis can be classified by either the presence of delusions or hallucinations, we then assessed whether participants with only delusions, only hallucinations, or both, differed in regional brain volume compared to participants without psychosis. We found that participants with delusions-only had significantly higher atrophy in lateral temporal lobe regions bilaterally, which was of higher magnitude on the right hemisphere, where it additionally included the parahippocampal gyrus, insula and supramarginal gyrus, as well as the cingulate gyrus on the left hemisphere **(Figure 26)**. We did not find statistically significant differences in the hallucinations-only and both delusions and hallucinations groups compared to the group without psychosis. Moreover, we did not find statistically significant differences in regional brain volume between symptom groups.

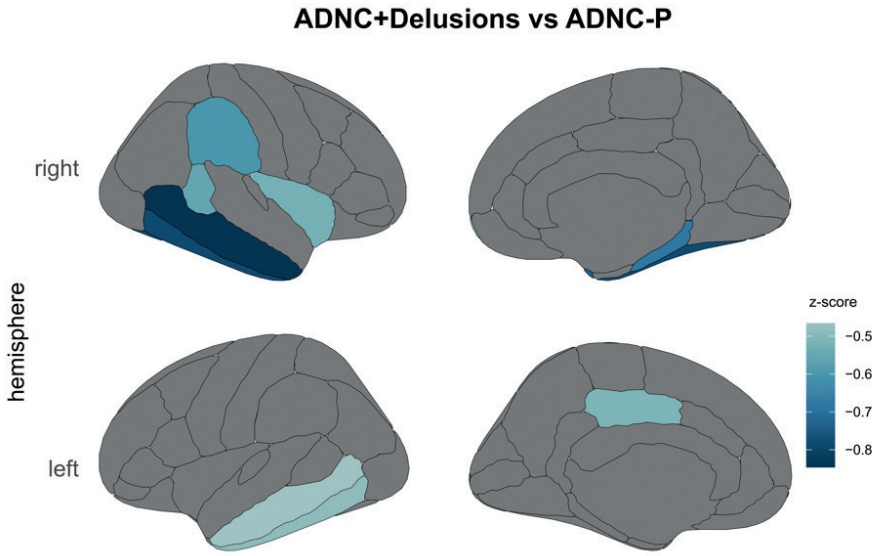

Figure 26. Delusions-only are associated with higher temporal cortical atrophy compared to participants without psychosis. Regional brain volume residuals were calculated after linear regression with age and sex and compared between each symptom group (delusions, hallucinations, both) and the group without psychosis (ADNC-P). We found higher temporal cortical atrophy bilaterally, and of the right insula, right supramarginal gyrus and left posterior cingulate gyrus in the delusions-only participants (ADNC+Delusions) compared to ADNC-P. We did not find statistically significant differences in the remaining comparisons. Groups were compared using one-way ANOVA followed by TukeyHSD and FDR correction for the number of brain regions assessed. Only regions significant after FDR correction are shown. (adapted from Almeida et al, Neurobiology of Aging, 2024 [44])

In order to establish which neuropathological components were most likely associated with the presence of psychosis in this cohort, we used a logistic regression model with the presence of psychosis as the dependent variable and the grouped Braak stages, CERAD scores and the presence of LB as independent categorical variables, and only the presence of LB was associated with higher odds of presenting psychosis. We thus explored how this co-pathology contributes to cognitive impairment and atrophy in ADNC with and without psychosis. ADNC+P and ADNC-P with LB (ADNC+P+LB; ADNC-P+LB) showed a higher percentage of male patients. Mean age at MRI and mean age at death were higher for ADNC-P without

LB (ADNC-P-LB). CDR-SB was significantly different between groups, with ADNC+P+LB and ADNC+P-LB showing higher scores.

We compared each group regarding their scores on neuropsychological tests, after age at MRI and sex correction. ADNC+P-LB performed significantly worse in the Animals and Vegetables tests compared to ADNC+P+LB. Thus, in the groups with psychosis, not having LB pathology was associated with more dysfunction in memory tests. ADNC-P+LB performed worse on TMT A than ADNC-P-LB, suggesting higher executive dysfunction in the LB patients within the groups without psychosis. ADNC+P+LB was not significantly different from ADNC-P+LB. The group with psychosis and without LB, ADNC+P-LB, performed worse on multiple domain cognitive tests compared with ADNC-P-LB, namely memory tests (Animals, Vegetables and Boston) and executive tests (TMT A and TMT B). A similar finding was present in ADNC+P+LB versus ADNC-P-LB, but less affected on memory tests (Vegetables) and more on executive and processing speed tests (TMT A, TMT B and WAIS). Finally, ADNC+P-LB performed worse on memory tests (Animals, Vegetables) compared with ADNC-P+LB **(Figure 27)**.

Next, we compared cortical and subcortical volumes between groups after correction for age at MRI and sex. Interestingly, we found a gradient pattern of increased atrophy of the right fusiform and inferior temporal gyri, most severe for ADNC+P-LB followed by ADNC+P+LB compared with ADNC-P-LB **(Figure 27)**. As for subcortical structures, we did not find statistically significant differences.

Overall, our results replicate and reinforce previous literature showing that the presence of psychosis in AD neuropathology reflects a worse prognostic group, with higher cognitive impairment, at baseline and on follow-up, and an earlier death [125]. Importantly, this was the first study to disentangle the effects of LB co-pathology on neuropsychological and neuroimaging correlates of psychosis in AD.

Concerning our observations that ADNC+P had a higher density of NPs and a more advanced distribution of NFTs, it was aligned with previous studies have showed that psychosis in AD could be associated with higher tau load, in the form of hyperphosphorylated aggregates [140], higher levels of tau in the cerebrospinal fluid [141] and higher load of NFTs [135]. This

was further supported by a recent tau-PET study, where the AD+P was associated with higher levels of tau aggregate detection compared with AD-P in the same Braak stages [142].

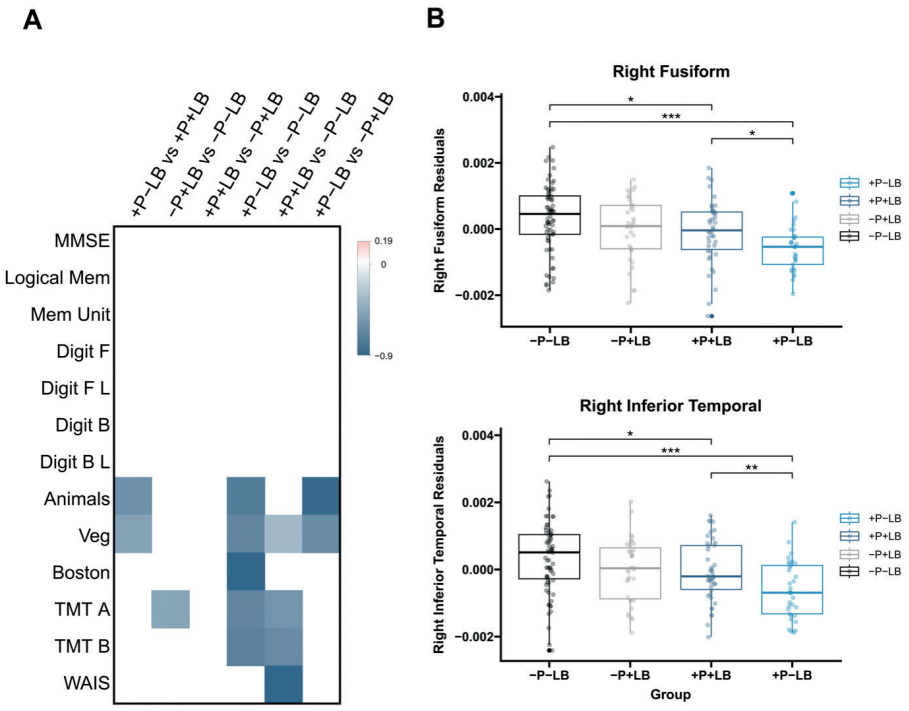

Figure 27. The presence of Lewy Bodies influences cognitive and volumetric differences. Plots show results for ADNC with and without Psychosis with and without LB. (A) Heatmap shows mean differences in neuropsychological tests residuals after linear regression with age and sex in ADNC±P±LB groups, using Welch's t-test. Significantly different comparisons are shown in colour ($p < 0.05$ after FDR correction within each pairwise comparison). (B) Boxplot of significant brain region comparisons across groups. Brain volume after linear regression with age and sex in ADNC±P±LB groups, using Welch's t-test. Only significantly different comparisons are shown ($p < 0.05$ after FDR correction within each pairwise comparison). (adapted from Almeida et al, Neurobiology of Aging, 2024 [44])

Altogether, our observations suggest that a higher NP load may serve as a trigger for higher tau deposition, which might contribute to more prominent atrophy and cognitive deficits in AD patients with psychosis. Indeed, the inferior temporal lobe has been posited as a key hub for tau

deposition, where tau and Aβ first coexist, promoting as a consequence tau propagation into other neocortical areas [51, 143, 144].

Interestingly, we show that the right temporal lobe is more atrophied in the ADNC+P group compared with the ADNC-P group. At this timepoint, ADNC+P showed a multi-domain worse cognitive performance than ADNC-P. The pattern of atrophy is in line with a right lateralization of affected regions in psychosis in AD and post-stroke observed in previous studies [125, 145, 146]. The temporal lobe has been implicated in psychosis in AD [125, 145], which may be part of a network of regions that when disrupted might culminate in psychosis [147, 148]. Thus, lateralization of atrophy in regions which are known to be at the transition of tau pathology to the neocortex might suggest brain regional susceptibility in these patients.

Considering the differential impact of having either delusions or hallucinations has been observed to differentially impact cerebral perfusion patterns in LBD [149]. While in AD, further research is needed to elucidate the underlying pathophysiology across symptom groups [123], our observations indicate that participants with delusions-only showed higher atrophy of both lateral temporal cortices, although more prominent on the right hemisphere, where it also included higher atrophy of the insula, parahippocampus and the supramarginal gyrus. Interestingly, this suggests that delusions are more associated with atrophy than hallucinations. Predominant temporal atrophy has been previously found in AD patients with delusions-only compared with those without psychosis [150, 151].

Our data indicated that LB pathology was associated with increased odds of presenting psychotic symptoms, which is aligned with previous studies reporting an association of LBs in AD with psychosis [135, 140]. We further explored this co-pathology impact and found that within ADNC+P, the absence of LB pathology was associated with lower performance on semantic memory tests compared with those who had LB pathology, suggesting that psychosis in the absence of LB pathology might reflect a more severe AD pathophysiological process. On the other hand, in those without psychosis, the presence of LB pathology led to a worse score on TMT A, a test of visuospatial attention and processing speed. Indeed, previous studies have found correlations between the presence of LBs and deficits in Trail Making Tests in AD [152], which have also been shown to help differentiate LBD

from clinical AD [153]. The presence of psychosis did not lead to statistically significant differences in patients with LB co-pathology, suggesting that ADNC+P+LB and ADNC-P+LB might be a relatively similar group. On the other hand, the presence of psychosis in those without LB pathology led to a higher multi-domain cognitive impairment, in both memory and executive function. A similar pattern was found when comparing those with both psychosis and LB pathology to those without any, ADNC+P+LB versus ADNC-P-LB, but with more impairment on tests of executive dysfunction rather than semantic memory.

Thus, when put together, these results suggest that LB pathology contributes to executive dysfunction in the presence and absence of psychosis, whereas AD pathology severity associates with semantic memory, executive impairment and psychosis. This "division of dysfunction" according to co-pathology and the presence of psychosis was further reflected in the gradient degree of atrophy found across groups. ADNC+P-LB showed the highest atrophy of the right fusiform and inferior temporal gyri. This corroborates the concept that in ADNC+P-LB, a higher load of AD typical neuropathology might lead to more temporal lobe atrophy, decline in semantic and executive cognition and psychosis. In ADNC+P+LB, we found LBs might play a role in the emergence of psychotic symptoms through the interaction with AD pathology, resulting in an existent, but less severe pattern of atrophy and cognitive impairment. These differences across co-pathology, cognitive function and brain atrophy might suggest there are differential mechanisms underlying these symptoms. Interestingly, α-synuclein has been shown to interact with both Aβ and tau in a synergistic way [154, 155] and co-pathology increases the risk for psychosis [45, 137, 138]. Overall, our results confirm that psychosis in AD is associated with LB pathology, but these symptoms can occur independently of it.

Nonetheless, our results provide evidence that psychosis is an emergent symptom of a neuropathological mechanism which acts over the course of AD pathophysiology. Longitudinal studies are needed to assess structural and functional brain changes in ADNC patients in correlation to these symptoms. Future PET studies mapping tau, Aβ and α-synuclein regional brain deposition across time are needed to test this hypothesis *in vivo*.

6. Molecular signatures of Alzheimer's disease brain regional susceptibility to neurodegeneration – the case for lipids

6. Molecular signatures of Alzheimer's disease brain regional susceptibility to neurodegeneration – the case for lipids

The studies from my team with MRI approaches showed that specific combinations of pathological signatures converge to lead to neurodegeneration unravelling patterns of brain regional susceptibility or resistance in the context of AD. The understanding of the molecular basis underlying the mechanisms of neurodegeneration can potentially lead to a better understanding of AD pathophysiology, to the discovery of new biomarkers for diagnosis, prognosis and disease monitoring, and to the development of novel therapeutic approaches.

Brain regional atrophy (as a surrogate for neurodegeneration), Aβ deposition and tau accumulations can be monitored *in vivo* by brain imaging approaches using MRI and PET [156]. However, while Aβ accumulation appears to be an early pathogenic event in AD, it fails to correlate with the onset of disease. On the other hand, tau tangles correlate well with both regional atrophy and cognitive decline in patients [157]. Interestingly, in murine models tau appears to act downstream of Aβ accumulation, as the genetic ablation of tau protects from cognitive impairment without altering Aβ brain levels [158]. Since the most recent trials that targeted Aβ accumulation only showed minor effects on cognitive decline in AD, this prompted new strategies to block Aβ-tau crosstalk based on population genetics and neuropathological studies. However, our current understanding of AD progression misses a molecular mechanism linking Aβ and tau deposition.

While Aβ plaques have been identified as a central diagnostic AD hallmark, many research groups showed that soluble Aβ oligomers (oAβ) are the main toxic species, supported by observations showing that APP transgenic models present behavioural deficits and synaptic loss in the absence of plaques [159], that increased oAβ levels correlate with cognitive deficits and synaptic loss [160] and that purified oAβ treatment lead to altered neuroplasticity and memory impairment in rodents [161]. Moreover, soluble Aβ species lead to microglia and astrocyte activation and to dystrophic neurites [162]. Importantly, oAβ were shown to lead to induction of tau pathology and specifically blocking oAβ reduced tau pathological

phosphorylation [163, 164]. Even though many putative oAβ receptors have been proposed, such as PrPc [165], α7nAChR [166] or NMDAR [167], consequently leading to increased pathological intraneuronal calcium levels [160], the downstream mediators that lead to tau impairment remain unknown. Importantly, population genetics and patient neuropathological studies have provided new insights into the pathogenic mechanisms of AD. Amongst the many genes that predispose to SAD, the majority are related to lipid metabolism and inflammatory pathways [168]. Interestingly, a relevant fraction of cytosolic tau directly interacts with membranes, which are mainly constituted of lipids [169].

In a healthy brain the diverse functions of neurons are at least in part dependent on particular lipid profiles, which include a variety of lipid species from the main membrane lipid categories, such as sphingolipids, glycerolipids and sterols. In fact, the lipidome of specific brain regions has been associated with differing vulnerability to neurodegenerative diseases, such as AD [170]. In fact, lipidomic studies in brains of AD patients and animal models uncovered pathological lipid signatures [171] and manipulation of brain AD-related genes can lead to an altered lipidome and impaired inflammatory response [172]. While there is extensive evidence of lipid signalling contributing to inflammatory processes, the mechanisms in how lipid imbalances contribute to AD-linked Aβ and tau pathology remain largely unexplored [170], therefore it is possible that lipid signalling links Aβ and tau accumulation. Supporting evidence comes from a FAD patient, who carried a *PENS1* mutation that leads to high Aβ plaque burden. However, this patient harbored an additional mutation (known as Christchurch mutation) in the lipid-linked gene, *APOE*, which restricted tau accumulation, blocked pathological tau spreading in the brain, and protected from atrophy and associated cognitive deficits [16]. Remarkably, the major AD genetic risk factor is the ε4 variant of *APOE* [168]. ApoE is a lipid transport protein that shuttles cholesterol and other lipids to neurons, and even though it is predominantly expressed in astrocytes, its levels are also upregulated in disease associated microglia [173]. Interestingly, lipidomic analysis of microglia from *ApoE* KO mice, upon myelin clearing challenges, showed similar increases in cholesteryl esters levels, in a similar way as in AD patients and mice, suggestive of lipid droplet accumulation

and indicating a mechanistic basis for lipid metabolism in mediating the role of these genes in a stress cellular response [172]. Further connections, beyond *APOE*, between inflammatory mechanisms and lipid metabolism, are provided by human AD-linked genetic variants in lipid associated genes that impact microglial pathways and phagocytosis, such as *ABCA7*, which is involved in lipid efflux; *BIN1*, a membrane trafficking regulator, known to bind tau [174], phospholipase D1 (PLD1) and PLD2 [175]; and *INPP5D* and *PLCG2*, both regulating phosphoinositide lipid signaling [173].

Phosphatidic acid (PA) has emerged as a class of lipid mediators involved in diverse cellular functions by transmitting, amplifying, and regulating a number of signalling pathways [176]. Today we know that PA serves as a major lipid second messenger conveying signals by recruiting specific proteins through protein-lipid interactions at restricted areas of membranes. Accordingly, PA has been reported to bind to a multitude of proteins, including mTORC1 [176]. Interestingly, tau was reported to bind to lipids and in particular with high affinity to PA [177]. One main enzymatic source of PA is the ubiquitously expressed PLD [178]. The two canonical PLD isoenzymes in mammals, PLD1 and PLD2, differ in their subcellular localization [179]. While PLD1 is mainly localized in the Golgi complex, endosomes, autophagosomes and nucleus [180, 181], PLD2 is preferentially found in the vicinity of the plasma membrane [178], a key location to be mediating Aβ's effects [182]. Interestingly, Aβ accumulation coincides with increased PLD activity, and I showed that reduced PLD2 levels not only blocks Aβ synaptotoxicity, but also prevents behavioral deficits induced by Aβ overproduction in rodents, without affecting Aβ brain levels, in mice that carry a FAD *APP* mutation (SwAPP) [182]. Among the various tests I used, in a radial-arm water maze task while *Pld2*$^{+}$/$^{+}$*/SwAPP* animals did not properly learn how to perform or remember the goal in a spatial task, *Pld2*$^{-}$/$^{+}$*/SwAPP* and *Pld*$^{+}$/$^{+}$*/SwAPP* were protected from the deleterious effects of high levels of Aβ in rodents **(Figure 28)**.

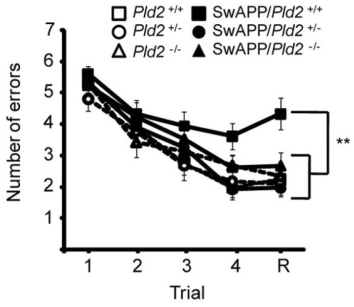

Figure 28. PLD2 ablation improves learning and memory in SwAPP mice. Twelve-month-old mice were subjected to RAWM testing. Errors were scored in the last 3 d of testing. The n value was 8 for all the genotypes, except for *Pld2* $^{-/-}$ */ SwAPP* (n=7) and *Pld2* $^{+/+}$ */ SwAPP* (n=6). **p<0.01. Values denote means ±SEM. (adapted from Oliveira et al, Journal of Neuroscience, 2010 [182])

Additionally, I showed this protection effect could be at least partly due to preserved synaptic functioning, since *Pld2* ablation in *SwAPP* mice led to preserved levels comparable to control mice, while *Pld2*$^{+}$/$^{+}$*/SwAPP* showed significantly decreased levels of the predominantly pre-synaptic protein, synaptophysin, and the predominantly post-synaptic protein, PSD95 **(Figure 29)**.

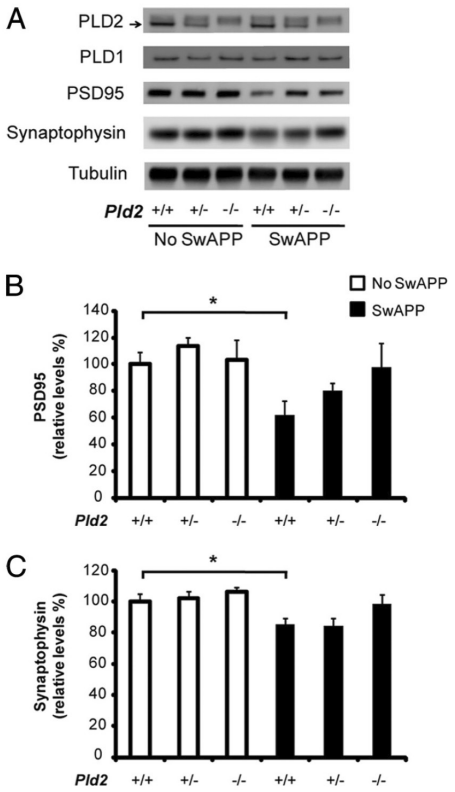

Figure 29. PLD2 ablation confers synaptic protection in the forebrain of SwAPP mice. After RAWM testing, forebrains from 12-month-old *Pld2/SwAPP* mice were processed for biochemical analysis. A, Protein levels were evaluated by Western blot analysis of PLD2, PLD1, PSD95, synaptophysin, and tubulin (representative blots are shown). B, Quantification of PSD95 levels by densitometric analysis. C, Quantification of synaptophysin levels by densitometric analysis. Values denote means ±SEM. n=4, *p<0.05. (adapted from Oliveira et al, Journal of Neuroscience, 2010 [182])

Thus, in an APP-based animal model of AD, PLD2 ablation effectively phenocopies tau ablation and it is reasonable to presume PLD2 ablation could ameliorate cognitive impairment through tau modulation [158]. While pharmacologic inhibition of PLD1 provided synaptic protection in an AD model [183], further showing the relevance of the PLD pathway in AD, PLD1 ablation dramatically decreased PA hippocampal levels and disrupted

synaptic plasticity in the dorsal hippocampus [184]. On the other hand, PLD2 ablation does not alter total PA levels, which indicates that PLD2 could be a more specific and safer target to reduce aberrant PLD activity. Overall, this raises the possibility that PLD2 mediated PA production provides the mechanistic link between Aβ and tau deposition.

Connected with the mechanisms of protection conferred by PLD2 ablation there is also evidence supporting a connection between PA metabolism, mainly driven by PLD enzymes, and APOE, the major risk factor for AD. A recent look into the lipidome of APOE4-expressing astrocytes revealed an increased accumulation of unsaturated triacylglycerol (TG) species, which reside in lipid droplets, when compared to APOE3 astrocytes [185]. A similar phenotype was observed in APOE4-expressing yeast, accompanied by a growth defect. A genetic screen for suppressors of the APOE4-mediated defects found OPI1 as one of the main hits. Quite interestingly, OPI1 is transcription factor that binds and is activated by PA. Additionally, supplementation with choline, also a direct enzymatic product of PLD activity upon cleavage from phosphatidylcholine (PC), rescued the APOE4-induced defects in both yeast and human induced pluripotent stem cell-derived astrocytes [185]. Additionally, in humans, a *post-mortem* lipidomic analysis of the inferior parietal lobule from AD patients showed that *APOE4* carriers had decreased levels of glycerophospholipids, most notably in PA [186].

Therefore, I became interested in expanding these molecular approaches to a rodent model that expresses APOE4, to assess the impact of this AD-risk condition on the lipidome of brain regions differentially involved in AD pathogenesis. It was previously shown that multi-omic analysis of the EC of aged *APOE4* impacted several important biological pathways, including neuronal activity [187], endosomal-lysosomal processing [188], and bioenergetics [189].

In order to understand the effects of differential *APOE* isoform expression on lipid signatures in the brain, we performed a targeted analysis of lipid metabolites extracted from the brains of aged *APOE* mice. Specifically, we utilized male 14-15 month-old *APOE* targeted replacement mice, which express the human *APOE* gene in place of the mouse *Apoe* gene. In order to understand the regional and dosage effects of *APOE4* allelic expression, we

studied *APOE3/3*, *APOE3/4* and *APOE4/4* mice and extracted lipids from two brain regions, namely the EC, which is one of the first brain regions where NFTs accumulate in AD, and the primary visual cortex (PVC), which develops tangle pathology at a later stage [190]. The lipidomic analysis was performed using a liquid chromatography - mass spectrometry platform [171], allowing for the detection of 28 lipid classes and over 300 distinct lipid species.

In the EC, 35 lipid species were differentially expressed in aged *APOE* mice, whereas only 9 lipid species were differentially expressed in the PVC between the different genotype groups **(Figure 30)**. Specifically, we observe that in the EC, several neutral lipid species were affected by *APOE4* expression, including numerous short-length DAG species, which showed decreasing levels with *APOE4* expression, as did several longer DAG species. CE species were also reduced in the EC with increasing number of *APOE4* alleles. We detected differential effects in sphingolipid species, namely APOE4-associated increases in ceramide (Cer), hexosylceramide (HexCer), lactosylceramide (LacCer) and monosialodihexosylganglioside (GM3) species, while SM and dhSM were decreased with *APOE4* expression. Interestingly, multiple species of the atypical phospholipid bis(monoacylglycero)phosphate (BMP) were elevated with increasing *APOE4* alleles. Importantly, while BMP is exclusively found in the intraluminal vesicles of late endocytic compartments [191], we have also previously observed increased levels of BMP in the EC of human AD patients [171] **(Figure 30)**.

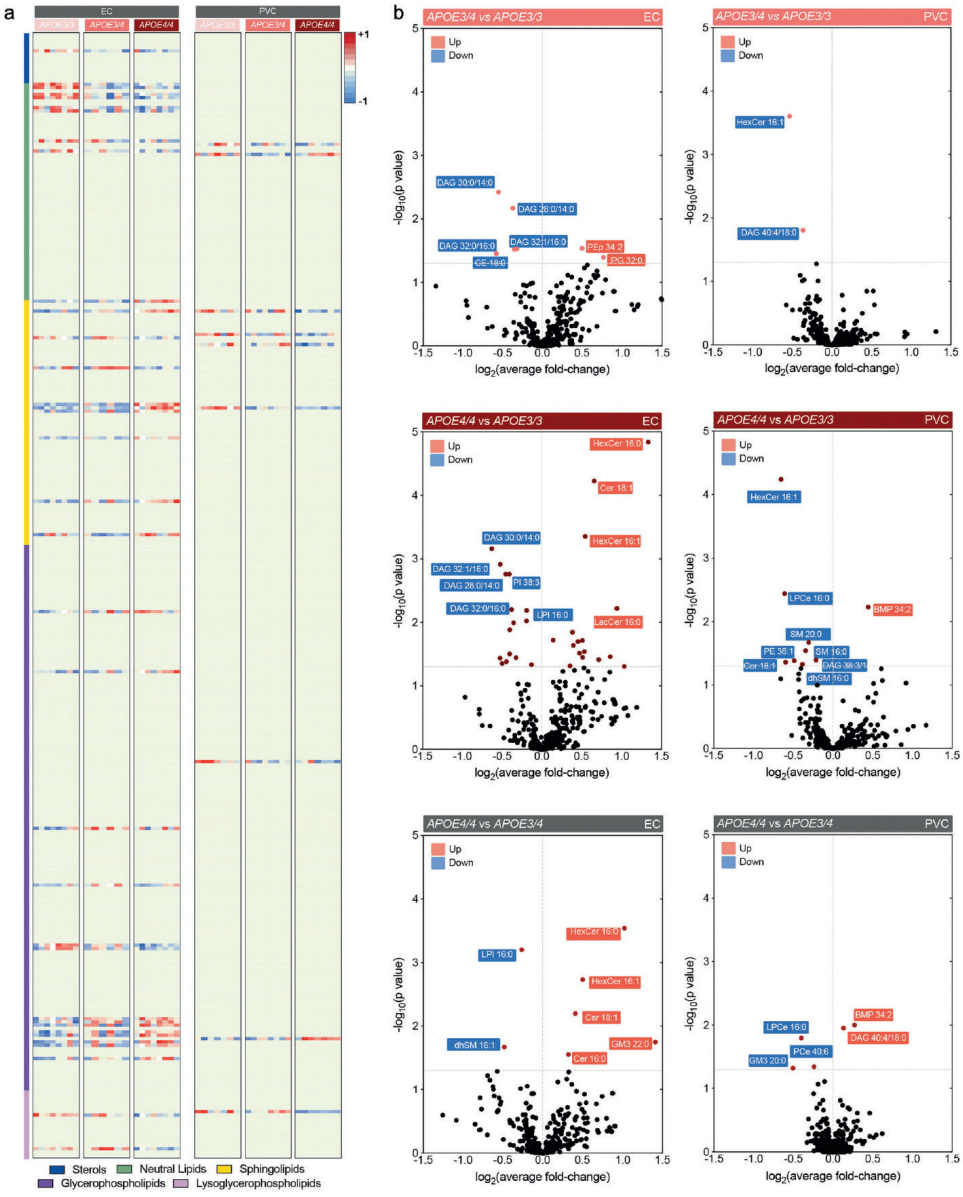

Figure 30. Comparative lipid profile of EC and PVC of aged APOE targeted replacement mice. (a) Heatmap of lipids significantly altered by *APOE3/4*, *APOE4/4* and *APOE3/3* genotype in EC and PVC at 14–15 months of age. Each row represents a lipid species of a given category (see color

legends); each column represents a sample. Results are expressed as Z-score [(average mol% of lipid species per genotype–average mol% of lipid species in all genotypes)/standard deviation of average mol% lipid species] represented in gradient color; blue and red indicate negative and positive Z-score, respectively. One-way ANOVA was performed and results thresholded by $p < 0.05$; n = 8, 6, and 8 for EC and n = 7, 8, and 7 for PVC in *APOE3/3*, *APOE3/4*, and APOE4/4 mice, respectively. (b) Volcano plots of differentially modulated lipid species in EC and PVC between *APOE3/4* vs. *APOE3/3*, *APOE4/4* vs. *APOE3/3* and *APOE4/4* vs. *APOE3/4*. Colored plots denote significantly affected species at Tukey's post-hoc $p < 0.05$, black plots denote unaltered species ($p > 0.05$). (adapted from Miranda et al, Transl Psychiatry, 2022 [190])

In the PVC, we found minor lipidomic alterations supporting a reduced effect of *APOE4* on the lipid composition of the PVC, as compared to the EC, which could play a role in regional vulnerability during AD pathogenesis, although we still highlight the concordance of lipid subclasses affected in both the EC and the PVC, namely DAG, Cer, SM and BMP **(Figure 30)**. Remarkably, each of these lipid subclasses are associated with the endosomal-lysosomal pathway, as either bioactive signaling molecules (DAG) [192], substrates of lysosomal degradation (Cer, SM) [193], or lysosomal resident lipids (BMP) [191]. This suggests that while some effects of *APOE4* expression may be regionally restricted, others are common throughout the brain, in line with previously described effects of *APOE4* expression on endosomal-lysosomal processing [188, 194].

My interest in the lipidomic characterization of AD-relevant brain regions was not only limited to the EC, but also included the hippocampus. Understanding the common lipid-related signatures linked to these regions could be highly informative since the EC is a major projection region to the hippocampus [195], both contribute to spatial memory [9], both are early on impacted by NFT accumulation [73], and NFTs in typical AD progression has been proposed to occur from the EC to the hippocampus [11]. The EC-hippocampal projections are also organized along the longitudinal axis of the hippocampus. These functions are believed to be differentially regulated by subregions along the longitudinal axis of the hippocampus, from dorsal to ventral poles in rodents, or from posterior to anterior poles in humans, respectively [9]. A dominant view in the field implicates the dorsal hippocampus particularly in cognitive function (*e.g.* spatial memory), while the ventral hippocampus mediates emotional responses [196-198]. The implementation of genomic-scale tools redefined the anatomical

boundaries of the hippocampus along its longitudinal axis [199-201]. While the transcriptomic and proteomic landscapes of hippocampal poles have since been linked to hippocampal regional functionality [173], we decided to expand these studies to a lipidomic characterization of the longitudinal hippocampal axis, by performing a broad-scale lipid analysis of dorsal, intermediate and ventral hippocampus in rodents and compared it to other brain areas, namely the prefrontal cortex, amygdala and cerebellum **(Figure 31)**. We plotted a heatmap of standard scores (Z score) of the average mol% of each lipid class per region, taking as reference value the mean of all regions pooled. First, we observed a high degree of similarity, expressed as lower modular Z scores (lighter blue and red shades), between the three hippocampal regions (dorsal, intermediate and ventral) relatively to the other three brain regions (prefrontal cortex - PFC, amygdala and cerebellum) under study. More specifically, we found the dorsal hippocampus to show higher similarity with the intermediate than the ventral hippocampus, consistent with the proposed continuous gradient of molecular identity along dorsal-ventral axis, where distinctions are more obvious between the poles [199, 200]. The other three brain regions showed a high degree of differentiation comparatively to the hippocampal subregions and between themselves **(Figure 31)**. Altogether, our results highlight the heterogeneity of lipid composition between distinct brain regions and identify a continuous molecular gradient along the longitudinal axis of the hippocampus.

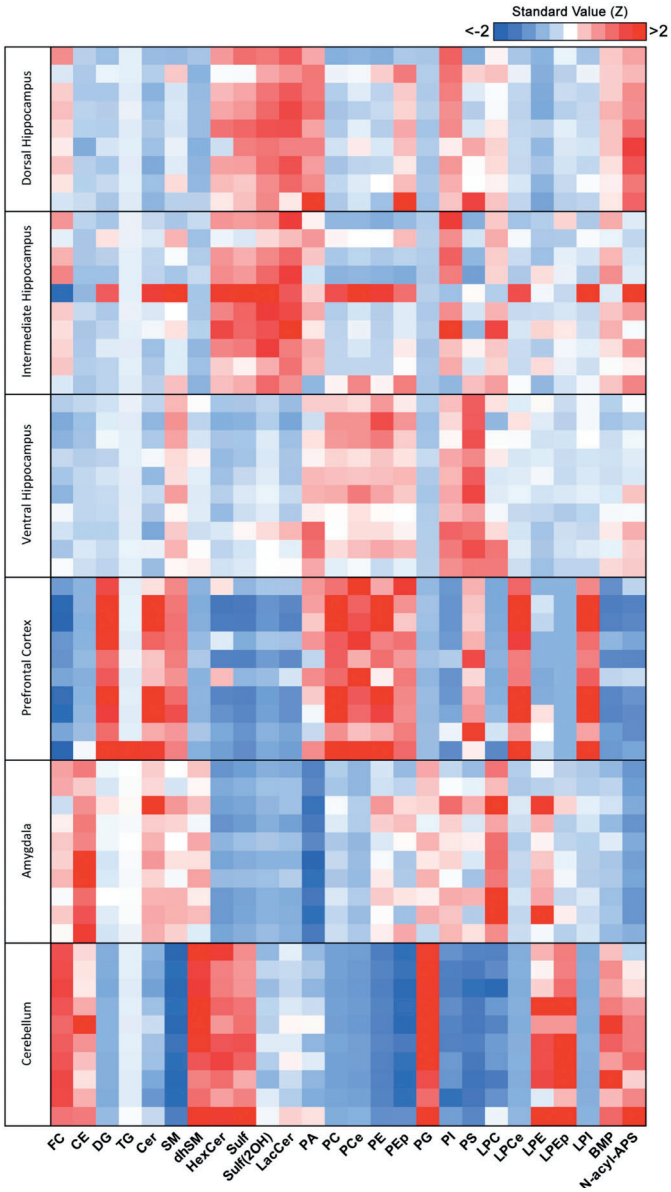

Figure 31. Lipidomic analysis of brain regions from adult rats. Adult rats were subjected to daily injections of vehicle (VEH) and brain regions were macrodissected prior to analysis by LC-MS (see Methods for details). Heatmap generated by calculating the standard value of each lipid class (column) using as reference the pooled average relative mol% of all brain regions. Z scores [(mol% of

lipid class of each animal–average mol% of lipid class of all brain regions of pool of animals)/standard deviation of average mol% lipid class of all brain regions of pool of animals] represented in gradient color; blue indicates negative Z value; red indicates positive Z value (lower and higher than reference average, respectively). Each row indicates an individual animal, per brain region (N = 9 for dorsal hippocampus, N =10 for all other regions). Nomenclature abbreviations are FC, free cholesterol; CE, cholesteryl ester; DG, diacylglycerol; TG, triacylglycerol; Cer, ceramide; SM, sphingomyelin; dhSM, dihydrosphingomyelin; HexCer, hexosylceramide; Sulf, sulfatides; Sulf(2OH), 2-hydroxy N-acyl sulfatide; LacCer, lactosylceramide; PA, phosphatidic acid; PC, phosphatidyl choline; PCe, ether phosphatidylcholine; PE, phosphatidylethanolamine; PEp, plasmalogen phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; LPC, lysophosphatidylcholine; LPCe, ether lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; LPEp, plasmalogen lysophosphatidylethanolamine; LPI, lysophosphatidylinositol; BMP, bis(monoacylglycero)phosphate; N-acyl-PS, N-acylphosphatidylserine. (adapted from Miranda et al, Transl Psychiatry, 2019 [203])

This lipidomic analysis along the longitudinal axis provided the possibility to inquire which specific pathways could be differentially regulated. Among the various signatures observed, we found that comparatively with the ventral hippocampus, the dorsal counterpart showed higher levels of PA and lower levels of PC, indicating the activity of the enzymes that convert PC into PA, PLD1 and PLD2, could be differentially increased in steady-state conditions [203]. Aligned with this observation, we found that the levels of PLD1 were also higher in the dorsal compared with the ventral hippocampus [184]. Additionally, our lipidomic analyses of both PLD1 and PLD2 KO mice of dorsal and ventral hippocampal subdivisions revealed that the ablation of PLD1 impacted more significantly the lipidome.

This led my team to study the impact of ablating PLD1, focusing our attention in the functions that rely on the longitudinal hippocampal axis organization. In our detailed characterization of *Pld1* KO mice we found that (1) the lipidome of the dorsal hippocampus is more affected than the ventral hippocampus, (2) there was an impairment in synaptic plasticity at the level of long-term depression specifically in the dorsal hippocampus, (3) synaptic proteins were specifically decreased in the dorsal hippocampus, and (4) only behaviors related with recognition memory were altered **(Figure 32)**. In summary, these results show that PLD1 ablation is detrimental, affecting memory-related circuitry and impacting the dorsal hippocampus in a more predominant manner. Interestingly, we generated further evidence supporting the concept that upon PLD1 reduction there

is compensation by PLD2, and that upon PLD2 ablation there could even be an overcompensation by PLD1 [184]. Also knowing, from my previous studies that PLD2 ablation conferred resistance to AD pathology effects, I propose that a condition with PLD1 reduction leads to susceptibility to pathology, while PLD2 reduction could be conferring protection.

Knowing that the PLD pathway, previously implicated in AD-mechanisms by my team and others, impacted the hippocampal longitudinal axis, my team developed an algorithm to be applied in volumetric brain MRI acquisitions to segment the hippocampus along its longitudinal axis and to divide in a standardized manner the hippocampus in anterior, intermediate and posterior subdivisions **(Figure 33)**. Therefore, we go full circle applying a concept from our basic science approaches where we found that PLD1 ablation affected predominantly the rodent dorsal hippocampus, which is equivalent to the posterior hippocampus.

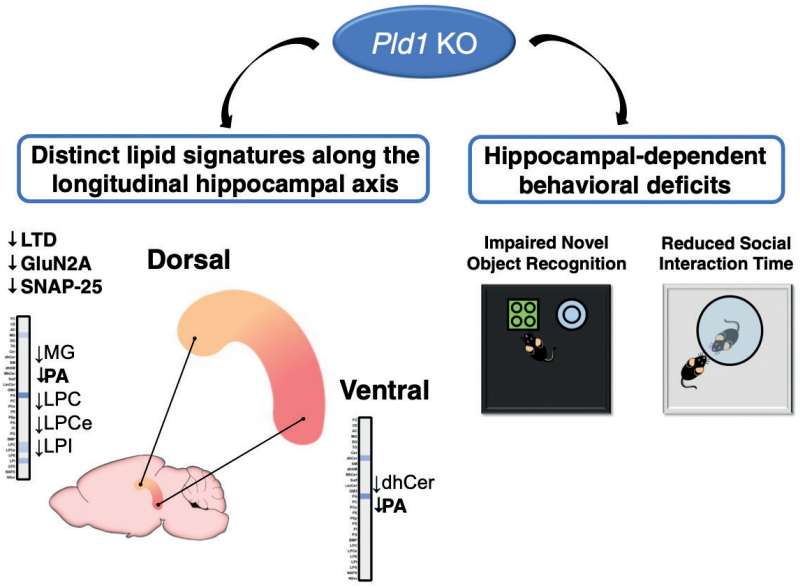

Figure 32. PLD1 Ablation Disrupts Mouse Longitudinal Hippocampal Axis Organization and Functioning. This summary figure depicts the main alterations observed upon PLD1 ablation on hippocampal functioning in mice: (1) lipidomic changes along the longitudinal hippocampal axis, (2) synaptic plasticity and synaptic protein level deficits in the dorsal hippocampus, and (3) impairment in recognition memory tasks. (adapted from Santa-Marinha and Castanho et al, Cell Reports, 2020 [184])

To test and validate our algorithm we used T1 volumetric acquisitions from the Alzheimer's Disease Neuroimage Initiative, an open-access US database with brain MRI scans that can be used for research. We studied 583 patients (205 cognitively normal, 204 MCI, and 174 AD), which we processed with Freesurfer to segment the hippocampus, which were then segmented with our algorithm to compute the volume of each hippocampal sub-division along the longitudinal hippocampal axis. We then calculated the ratio between the anterior and posterior hippocampus volumes to use a metric that encompasses proper organization of the longitudinal hippocampal axis. Remarkably, we found that along aging, while CN subjects significantly evolve towards an "aging" signature, AD subjects do not show these physiologic aging adaptations, suggesting that the longitudinal hippocampal axis ratio is reflecting early disfunction likely related with AD-pathology **(Figure 33)**. Interestingly, we found that the early alterations in this ratio are mainly at the expense of the posterior hippocampus, which is in line with what we observed with dorsal hippocampus disfunction susceptibility conferred by PLD1 ablation [184].

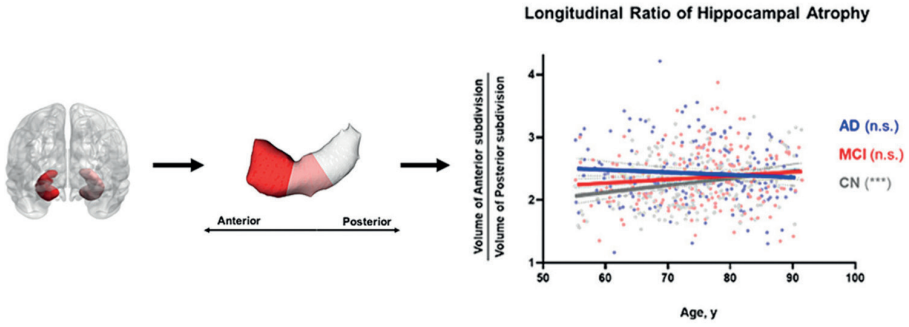

Figure 33. Anterior–posterior volumetric hippocampal ratio is abnormally maintained throughout ageing in AD. Longitudinal axis hippocampal ratios of atrophy with age were obtained by dividing the absolute volumes of the anterior by the posterior subdivisions. This highlighted an increase in the anterior–posterior ratio of CN group, while in MCI and AD subjects this ratio did not significantly change with age. Results shown from the left hippocampus. N = 583 subjects (205 CN, 204 MCI, 174 AD). AD, Alzheimer's disease; CN, cognitively normal; MCI, mild cognitive impairment; n.s., not significant; y, years. $^{**}p < .01$; $^{***}p < .001$. (adapted from Morais-Ribeiro and Almeida et al, Eur J Neuroscience, 2024 [204])

Among the future goals of my team, we are currently applying this longitudinal hippocampal axis segmentation to the NACC dataset to understand the neuropathological AD-related determinants of neurodegeneration, verified at the *post mortem* level, and using longitudinal *in vivo* cohorts we would like to identify the fluid biomarkers that better depict these sub-regional hippocampal signatures based on brain MRI acquisitions.

7. Applying brain regional susceptibility to Alzheimer's disease pathology concepts to patients – future directions

7. Applying brain regional susceptibility to Alzheimer's disease pathology concepts to patients – future directions

The final goal of the research efforts of my team is to apply these research concepts at the clinical level in the management of AD patients. We believe we have made significant advances at various levels, using our brain MRI-based approaches to uncover brain regional susceptibility to AD pathology, and using rodent models to find mechanistic signatures at the lipid level that partly explain the underlying neurodegeneration in AD.

Having in mind the ongoing revolution in the management of AD patients with the treatments with anti-Aβ antibody treatments, our research has therefore major implications in clinical practice. By studying PART patients, we can understand the factors that make these patients "resistant" to develop amyloid pathology. Aligned with this, it has been shown that in AD the genetic risk variant *APOE4* is more frequent, while PART patients more commonly carry the protective variant *APOE2* [205]. We further characterized PART patients at the imaging level showing that atrophy is predominantly confined to the temporal lobe, aligned with NFT pathology [59, 60], and that other factors contribute to the neurodegeneration and cognitive deficit in the "*PART continuum*" in the medial temporal lobe region, compared to the "*AD continuum*" that follows an increased atrophy from CERAD1 to CERAD3 [60]. We expand on the factors that could be explaining theses *continuums* and we show that additional co-pathologies could be playing a major role in both AD and PART. In fact, the high variability in clinical trial results to anti-Aβ antibody therapies does not delve in detail in the potential role of these co-pathologies. Our results show that cerebrovascular lesion burden [80], TDP-43 [60] and LB [44] pathologies impact neurodegeneration patterns and clinical outcomes. This clearly shows that these are factors should be taken into account in patient management and highlight that novel treatments for these co-pathologies should be envisioned for the future treatment of AD patients.

Another interesting point of connection with our studies regarding PART signatures, stems from the finding that PART patients show an NFT deposition pattern in the hippocampus different from AD patients. Quite interestingly, PART patients have predominant tau accumulation in

the hippocampal subregion CA2 [206]. CA2 is an intriguing region with recently allocated specific functions for social behavior in rodents [207] and with a circuit organization that implicates the longitudinal hippocampal axis with projections from dorsal to ventral hippocampus [208]. While in humans CA2 functions are still being explored, it is interesting to observe that we found that PART patients show a somewhat specific susceptibility to language and semantic memory tasks [59, 60], raising the question whether CA2's functions could be related with these semantic more complex tasks in a global framework for social circuit organization in humans. This would be an interesting question to tackle using functional MRI tasks both in subjects with no pathology to understand the function of the regions in humans, and in pathological conditions, such as AD and PART patients identified *in vivo* with recent biomarker approaches. It is also interesting to see that our new approaches with an algorithm directed to study the longitudinal hippocampal axis in AD finds already regional dysfunction [204], raising the question whether this could be coupled with CA2 based studies since CA2 projects along this axis to ventral CA1 [208].

My team is not only interested in discovering new concepts to be applied in the management in AD patients, but also in contributing to improving clinical practice in our local hospitals. With that goal in mind we studied a cohort of patients with CAA from the Hospital de Braga. CAA can now be identified with high probability using MRI-based diagnostic criteria which allowed us to assemble our database. We found that CAA patients are more prone to have re-hemorrhagic events [102] and we showed with imaging approaches that medial temporal lobe atrophy was more prominent in men with dementia, whereas women showed a higher number of enlarged perivascular spaces in the centrum semiovale [94]. This shows that these patients identified by brain MRI need special attention. For instance, CAA has recently been shown to be one of the main risk factors to develop ARIA upon initiating anti-Aβ antibody treatment [20, 30]. Therefore, it is crucial we have in our Portuguese population a detailed identification of patients with CAA, in order to know which ones can potentially be eligible for anti-Aβ antibody treatment if they eventually are approved in Europe.

The other major factor known to predispose to ARIA is the presence of *APOE4* [22], which is also the main genetic risk factor to develop AD

[209]. We found lipidomic signatures showing that the endolysosomal pathway is dysfunctional upon *APOE4* expression [190], which can lead to specific biomarkers to be used in the clinic assessing endolysosomal pathway dysfunction, such as the levels of BMP or sphingolipids usually degraded in the lysosome, which could then accumulate in either CSF or blood. It also emphasizes mechanistic alterations that could be at the basis for future treatments of ARIA, targeting lipid signaling, when the patients are identified by brain MRI.

Aligned with our studies on the role of lipids in AD and our interest in understanding the mechanisms that explain why AD patients with psychosis have a more severe clinical presentation, a recent genome wide association study in AD patients with psychosis implicated two risk genes, *ENPP6* and *SUMF1* [210]. Remarkably, both encode for lipid modulating enzymes, with *ENPP6* encoding for a choline-specific glycerophosphodiesterase that hydrolyzes glycerophosphocholine and LPC and contributes to supplying choline to the cells [211], and *SUMF1* encoding for an enzyme that catalyzes the hydrolysis of sulfate esters, with reported mutations that cause sulfatase deficiency, and lysosomal storage disorder. Connecting with other lines of our research as ENPP6 contributes to choline production, also PLD1 and PLD2 both produce choline and our work has shown they differentially impact neurodegenerative mechanisms [182, 184]; and SUMF1 being involved in lysosomal dysfunction is globally aligned with the endolysosomal lipidomic signatures we observed in *APOE4* mice [190].

Finally, surprising connections with our work came from the revolutionary studies stemming from big data genetic-imaging initiatives, such as the UKBiobank. It was recently shown that a brain MRI metric that can be computed a standardized manner, namely the contrast between the gray and white matter (GWC) has specific genetic determinants, which among them the major one is *PLD1* [212, 213]. Our team has been studying this gene for many years now, and we have shown that rodents with *Pld1* ablation have synaptic plasticity deficits and behavioral alterations connected with hippocampal dysfunction [184]. Therefore, my team has not only acquired deep knowledge in this pathway, but it also has the expertise to explore these molecular connections at the brain imaging technical level. Since PLD1 has been implicated in cell processes affecting membrane trafficking with

potential implications for amyloid plaque and NFT turnover, one major question that my team will explore is the connection between GWC, which is signature that relies on *PLD1*, and pathological AD processes, as assessed by AD-related biomarkers **(Figure 34)**. The GWC computation relies on both the gray and white matter, but since *PLD1* is predominantly enriched in olidodendrocytes [214], we will additionally focus on assessing myelin-based MRI metrics in AD cohorts.

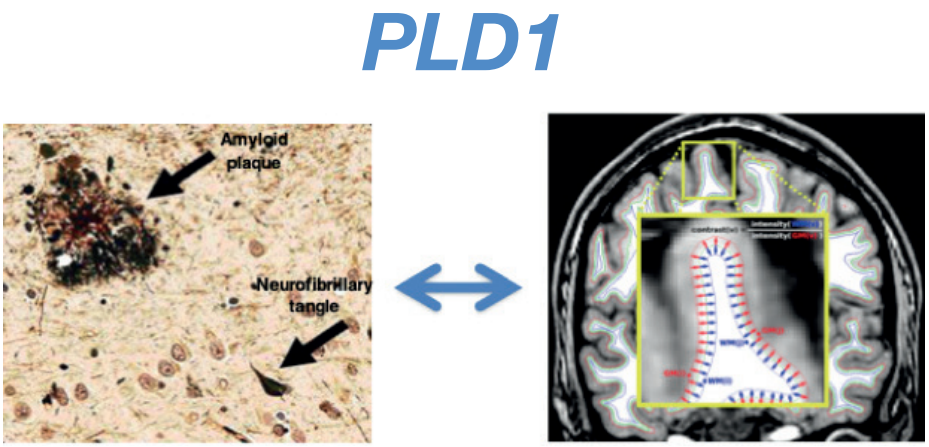

Figure 34. PLD1 impacts both Alzheimer's disease neuropathological hallmark features and gray-white matter tissue contrast on T1-MRI. Neuropathology image adapted from Jack et al, Radiology, 2012 [215], and T1-MRI GWC calculation example adapted from Lewis et al, Neuroimage, 2018 [216].

I hope our investment over the years in understanding these lipid complex pathways at the basic science level can lead to a new understanding in some of the mysteries underlying the functioning of the brain and the pathogenesis of neurodegenerative disorders and inspire others to go down the road of exploring a career as a physician-scientist.

8. Bibliography

8. Bibliography

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Prémio **Bial** de Medicina Clínica 2024

A Fundação Bial foi criada em 1994 pela Bial, em conjunto com o Conselho de Reitores das Universidades Portuguesas, com o objetivo de promover o estudo científico do ser humano, tanto do ponto de vista físico como espiritual.

No contexto da sua missão, a Fundação Bial atribui Prémios no âmbito da investigação em ciências da saúde, nomeadamente o PRÉMIO BIAL DE MEDICINA CLÍNICA, o BIAL AWARD IN BIOMEDICINE, e o PRÉMIO MARIA DE SOUSA, este último em parceria com a Ordem dos Médicos.

O PRÉMIO BIAL DE MEDICINA CLÍNICA 2024 foi entregue a Tiago Gil Oliveira pelo trabalho "Uncovering the mysteries of brain regional susceptibility to neurodegeneration in Alzheimer's disease: from neuropathology to brain magnetic resonance imaging".

Nesta edição, foram também distinguidas duas obras com Menções Honrosas: "Screening & Eye Examination Centre using AI resources", de autoria de Luís Abegão Pinto, Joana Tavares Ferreira e Quirina Tavares Ferreira, e "Degenerescência Macular da Idade - A Primeira Causa de Cegueira Irreversível em Portugal", de José Paulo Andrade e Ângela Carneiro.

O júri do PRÉMIO BIAL DE MEDICINA CLÍNICA 2024 foi presidido por José Melo Cristino e constituído por Jaime Branco, Miguel Castelo-Branco, Henrique Cyrne Carvalho, João Forjaz Lacerda, Helena Leitão, José Miguel Pêgo, Carlos Robalo Cordeiro e Amândio Rocha Sousa. Em 2026 a Fundação Bial realiza uma nova edição do concurso PRÉMIO BIAL DE MEDICINA CLÍNICA, que conta com o Alto Patrocínio do Senhor Presidente da República, e os patrocínios do Conselho de Reitores das Universidades Portuguesas e da Ordem dos Médicos.

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The PRÉMIO BIAL DE MEDICINA CLÍNICA 2024 was awarded to Tiago Gil Oliveira for the work "Uncovering the mysteries of brain regional susceptibility to neurodegeneration in Alzheimer's disease: from neuropathology to brain magnetic resonance imaging".

In this edition, two other works were also distinguished with Honourable Mentions: "Screening & Eye Examination Centre using AI resources", by Luís Abegão Pinto, Joana Tavares Ferreira and Quirina Tavares Ferreira, and "Degenerescência Macular da Idade - A Primeira Causa de Cegueira Irreversível em Portugal", by José Paulo Andrade and Ângela Carneiro.

The jury of the PRÉMIO BIAL DE MEDICINA CLÍNICA 2024 was presided by José Melo Cristino and composed by Jaime Branco, Miguel Castelo-Branco, Henrique Cyrne Carvalho, João Forjaz Lacerda, Helena Leitão, José Miguel Pêgo, Carlos Robalo Cordeiro, and Amândio Rocha Sousa. In 2026, the Bial Foundation will launch a new edition of the PRÉMIO BIAL DE MEDICINA CLÍNICA, under the High Patronage of the President of the Portuguese Republic, with the sponsorship of the Council of Rectors of Portuguese Universities and the Portuguese Medical Association.