Impact of Alzheimer's disease risk factors on white matter hyperintensities and cognition in cognitively unimpaired individuals

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Resum

Les hiperintensitats de substància blanca (HSB) s'han associat a un augment del risc i la progressió de la malaltia d'Alzheimer (MA). No obstant, encara no s'ha explorat completament fins a quin punt aquesta associació pot relacionar-se amb els factors de risc de la MA en persones cognitivament sanes. Per tant, l'objectiu principal d'aquesta tesi és estudiar com els factors de risc de la MA tenen impacte sobre la càrrega de les HSB en participants d'edat mitjana cognitivament sanes. També s'exploraran els mecanismes pels quals les HSB tenen un impacte sobre la cognició. S'han recopilat dades clíniques, hàbits d'estil de vida, avaluacions cognitives i adquisicions de ressonància magnètica en una mostra de més de 500 persones cognitivament sanes de mitjana edat. Els resultats mostren que, tot i la baixa càrrega d'HSB i una baixa prevalença de factors de risc de la MA en els participants, els factors de risc es van associar a una major càrrega d'HSB. A nivell regional, la càrrega d'HBS va mostrar un impacte en la cognició en persones de mitjana edat. Finalment, es va trobar que el volum de les HBS mesurava la relació amb el volum cortical de la substància gris en regions implicades en la funció executiva. La conclusió principal d'aquest estudi és que tot i el baix nivell de factors de risc de la MA, aquests tenen un impacte significatiu en l'estructura cerebral i la cognició en persones cognitivament sanes. Aquestes troballes donen suport al control dels factors de risc modificables de la MA en individus amb un major risc de desenvolupar HSB com una forma pràctica de reduir o retardar l'inici de la demència.

Abstract

White matter hyperintensities (WMH) have been associated with increased risk and progression of Alzheimer's disease (AD). However, the range to which this association can be extended to AD risk factors and WMH burden in cognitively unimpaired (CU) middle-aged individuals has not yet been fully explored. Thus, the main goal of this thesis is to study how AD risk factors impact on WMH burden in CU middle-aged participants. In addition, we aim to explore the mechanisms by which WMH impact on cognition. We gathered clinical data, lifestyle habits, cognitive assessments and MRI acquisitions in a sample of more than 500 CU middle-aged individuals. The results show that despite the low WMH burden and a low prevalence of AD risk factors in the participants, AD risk factors were associated with a higher WMH burden. Regionally, WMH burden showed an impact on cognition in CU middle-aged individuals. Finally, it was found that WMH volume mediates the relationship with cortical gray matter volume in regions involved in executive functioning. The main conclusion of this study is that even very low levels of AD risk factors have a significant impact on brain structure and cognition in CU individuals. These findings support the control of modifiable AD risk factors in individuals at higher risk of developing WMH as a practical way to reduce or delay the onset of dementia.

Preface

An increasing number of clinical and epidemiological studies suggest that multiple biological, behavioural, social and environmental factors could contribute to the risk of developing Alzheimer's disease (AD). Nowadays, a series of non-modifiable and modifiable risk factors have been well-established. The main non-modifiable risk factors of AD are age, genetics and family history. Concerning genetics, Apolipoprotein E (*APOE*) ε4 allele is the major known genetic risk factor for AD. In contrast, the *APOE*-ε2 allele seems to confer resistance towards developing the disease. Modifiable risk factors for AD are mostly related to cardiovascular risk factors (such as diabetes, hypertension and body mass index), psychosocial factors (such as low education) and/or lifestyle habits (including smoking, unhealthy diet and low participation in cognitive and social activities). The moment of exposure to AD risk factors within the lifespan course needs to be considered when weighing up the impact of these risk factors in relation to AD.

On the other hand, white matter hyperintensities (WMH) are among the most common structural neuroimaging findings in the brain of cognitively unimpaired (CU) middle-aged and elderly individuals. They are thought to be associated with axonal loss and demyelination due to chronic ischemia and, therefore, are considered as surrogate markers of cerebral small vessel disease. Multiple risk factors of WMH are shared with AD, such as ageing, hypertension, hypercholesterolemia, and diabetes. Moreover, global WMH load has been shown to exert a negative impact on multiple cognitive domains, on the onset and severity of AD, and to constitute an independent risk factor for cognitive decline.

A better understanding of the physiopathological mechanisms underlying AD risk factors and the time-window where their modification may exert the most beneficial effect is necessary for the rational design of preventive interventions and to derive appropriate surrogate markers of risk reduction. It can be considered that AD risk

factors may directly favour the build-up of AD pathophysiology or indirectly impact on the resilience/vulnerability of the brain to such pathology.

Evidence on the association of AD risk factors with AD pathophysiology and/or markers of brain vulnerability, like WMH, in middle-aged CU individuals would support the hypothesis that control of modifiable AD risk factors is a useful preventive strategy to reduce or delay the onset of dementia.

Taking into account all the stated above, this thesis is addressed to study the impact of AD risk factors on WMH burden in CU middle-aged individuals. In addition, it will explore the mechanisms by which WMH impact on cognition. Through novel methodological approaches, this thesis will contribute to an increase in the understanding of how risk factors render the brain vulnerability to AD pathophysiology.

Abbreviations

AD: Alzheimer's disease
ADNI: Alzheimer's Disease Neuroimaging Initiative
APOE: Apolipoprotein E
AxD: axial diffusivity
Aβ: β-amyloid
BBRC: Barcelonaβeta Brain Research Centre
BMI: body mass index
CAIDE: Cardiovascular Risk Factors, Aging and Dementia
CDR: Clinical Dementia Rating
CI: confidence interval
CSF: cerebrospinal fluid
CU: cognitively unimpaired
CVRF: cardiovascular risk factors
DWI: diffusion-weighted imaging
DWMH: deep white matter hyperintensities
EF: executive function
EM: episodic memory
EMA: European Medicines Agency
FDA: United States Food and Drug Administration
FDG-PET: F-fluorodeoxyglucose positron emission tomography
FH: family history
FLAIR: fluid attenuation inversion recovery
FSRP: Framingham Stroke Risk Profile
GM: gray matter
HTA: hypertension
IF: incidental findings
IWG: International Working Group

JCWMH: juxtacortical white matter hyperintensities
MBT: Memory Binding Test
MCI: mild cognitive impairment
MMSE: Mini Mental State Examination
MRI: magnetic resonance imaging
MTA: Medial Temporal Lobe
NIA: National Institute on Aging
NIA-AA: National Institute on Aging and the Alzheimer's Association
OR: odds ratio
PET: positron emission tomography
PiB: Pittsburgh compound B
PVWMH: periventricular white matter hyperintensities
REGICOR: Registre Gironí del Cor
ROI: region of interest
SNAP: Suspected Non-Alzheimer Pathology
VBM: Voxel-Based Morphometry
WMH: white matter hyperintensities

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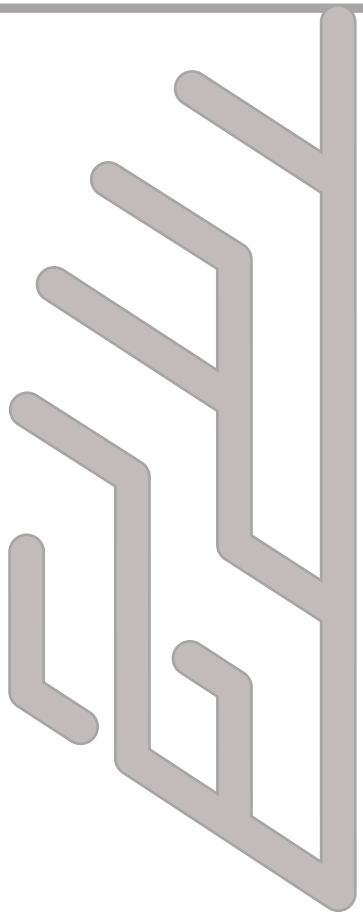
INTRODUCTION



1. Alzheimer's disease

1.1. Background

The increase in average life expectancy during the last 50 years has been accompanied by an increment in the prevalence of age-associated disorders, such as dementia (1). Worldwide, about 50 million people are living with dementia. Alzheimer's disease (AD) is the most common cause of dementia, accounting for 50-70% of cases (2).

AD is a progressive neurodegenerative disorder that, among others, can lead to dementia, characterized by a progressive cognitive decline with early memory impairment followed by other cognitive domains. A pattern of specific neuropathological hallmarks underlie AD which is characterized by deposits of extracellular β-amyloid protein (Aβ) in the form of neuritic plaques and intracellular deposits of tau protein in the form of neuritic strands and neurofibrillary tangles along with neuronal and synaptic loss and glial proliferation (3–5). AD has been classically conceptualized and diagnosed as a clinical-pathological syndrome. Accordingly, initial diagnostic criteria only considered a 'definite' AD diagnosis only when post mortem confirmation was available. Otherwise, a 'probable' AD diagnosis was contemplated that only required the presence of a clinical picture of dementia after ruling out other potential aetiologies. Therefore, a syndromic diagnosis required confirmation by post-mortem examination in order to make a definite AD diagnosis (6). However, in the last decades, biomarker development, including magnetic resonance imaging (MRI), positron emission tomography (PET) and cerebrospinal fluid (CSF) analyses, have enabled the change of AD conceptualization from a clinical-pathological entity to a clinical-biological one. At present, AD is defined as a *continuum* that can be divided into three stages: preclinical (abnormal biomarkers and no or only subtle cognitive impairment), mild cognitive impairment (MCI) or prodromal AD (abnormal pathophysiological biomarkers and episodic memory impairment) and dementia (abnormal biomarkers and clear cognitive and functional impairment).

Two sets of AD criteria have recently been published, one by the International Working Group (IWG) that has later been revised (IWG-2) (7) and the other by working groups assembled by the National Institute on Aging (NIA) and the Alzheimer's Association (AA) in the US (NIA-AA) (8). The most recent NIA-AA research framework defines AD as the presence of both Aβ deposition and pathologic tau deposits defined in vivo by abnormal biomarkers, describing it as a pathological *continuum* (5,9).

There is evidence showing that specific AD neuropathological hallmarks are present in persons with normal cognition up to 20 years before onset of symptoms, referred to as we mentioned before as the preclinical stage of AD (3,4,10). Sperling et al. (2011) proposed a classification of this early AD stage (4): Stage 1; asymptomatic cerebral amyloidosis (positive amyloid imaging, low CSF Aβ); Stage 2; amyloidosis and neurodegeneration (neuronal dysfunction; high CSF tau); and Stage 3; amyloidosis, neurodegeneration and subtle cognitive and behavioural decline that does not yet meet criteria for MCI or dementia due to AD. Later, the preclinical staging has been refined by several authors (7,11,12) adding 2 extra groups: Stage 0; comprises individuals without biomarker abnormalities who are not thought to be on the AD trajectory and the SNAP (Suspected Non-Alzheimer Pathology) group; composed of individuals with biomarker and imaging evidence of neurodegeneration without exceeding the biomarker cut point for amyloidosis (12).

Although the preclinical AD stage entails cognitively unimpaired (CU) individuals, there is ample evidence that they could present subtle cognitive changes that can be detected at this early stage (4). Findings across longitudinal studies, have shown initial cognitive changes consisting of subtle decreases in episodic and semantic memory, as well as executive functions (EF) performance (13,14). Additionally, self-reporting of these cognitive changes increased the likelihood of AD biomarker abnormalities and the risk of future cognitive decline and dementia, even with normal performance in cognitive tests (15–17). Consequently, there is an increasing necessity to develop highly sensitive cognitive assessments tools to detect very subtle cognitive impairment as early as possible in the course of the disease (4).

The number of people living with AD is predicted to increase; however, to date, no disease-modifying therapies have been successful. This lack of success may be partly explained by considering AD pathophysiology heterogeneity and by limitations in clinical trial designs, which have generally enrolled participants later in the course of the disease or with not enrich for Aβ, resulting in substantial misclassification (18–20). In this context, the preclinical stage of AD offers a window of opportunity for prevention and therapeutic success, and provides the opportunity to intervene at early stages of the *continuum*, potentially delaying the onset of cognitive decline and finally dementia (21) (figure 1). Currently, many interventional studies are moving their focus to CU individuals at risk of developing AD to reduce their incidence (22). These interventions could act upon modifiable AD risk factors (referred to as primary prevention) or be based on the early detection of the pathophysiological hallmarks and intervention at the preclinical stage (referred to as secondary prevention).

In conclusion, the detection of CU individuals who harbour the pathological hallmarks of AD is crucial for the assessment of secondary prevention strategies. Likewise, a better understanding of the mechanisms through which AD risk factors lead to the observed increased vulnerability to the disease is critical for the rational design of novel primary prevention interventions (23). In this regard, identification of the optimal timing to apply preventive interventions addressing the different risk factors a question of the utmost importance.

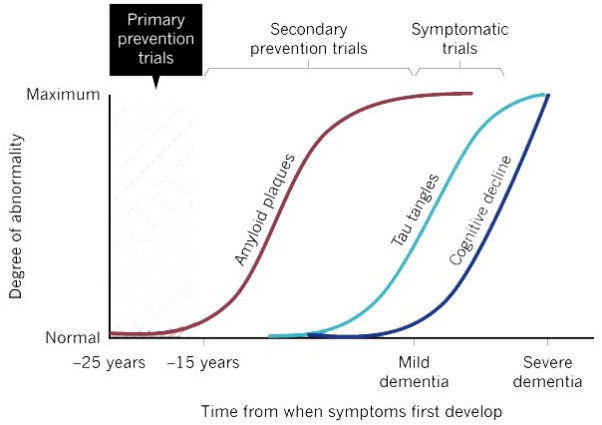


Figure 1. The time frame to develop successful primary and secondary prevention interventions. Preclinical AD, up to 20 years before symptoms onset, offers an opportunity window for prevention and therapeutic success. Primary preventions are based on early detection of the pathophysiological hallmarks before brain pathology or cognitive symptoms develop, whereas secondary prevention are implemented on the preclinical stage. Abbreviations: AD, Alzheimer's disease. From (24).

1.2. Risk factors of Alzheimer's disease

1.2.1. Non-modifiable risk factors

The main non-modifiable risk factors of AD are age (25–27)genetics and family history of AD. Increasing age is the greatest known non-modifiable risk factor for AD, but Alzheimer's is not a normal part of aging. A recent meta-analysis showed that the prevalence of amyloid pathology increased from age 50 to 90 years from 10% to 44% among individuals with normal cognition (27).

Regarding genetics, Apolipoprotein E (*APOE*) ε4 allele is the major known genetic risk factor for AD (28–32). The human *APOE* gene exists as three polymorphic alleles; ε2, ε3, and ε4 (33). Globally, the prevalence of ε2, ε3 and ε4 alleles is estimated as 7, 79 and 14%, respectively in the general population (34). The presence of one ε4 allele increases the risk of developing AD by a factor of 4 approximately (34), and homozygotes for this allele have nearly 14 times higher risk than *APOE*-ε3/ε3 genotype individuals (29). By contrast, the *APOE*-ε2 allele seems to confer resistance towards

developing the disease (28,29) by a factor of 0.62 (34). Previous studies revealed a correlation between aged and *APOE* genotype. The meta-analysis aforementioned reported that *APOE*-ε4 risk allele (comparing with ε3 allele) was associated with greater risk for amyloid positivity and decreased age at onset, while the *APOE*-ε2 allele had the opposite association (figure 2). In addition to the *APOE* locus, 19 loci presented genome-wide significance associated with AD risk (35).

Lastly, the family history of AD also confers an increase of the relative risk of AD, being in those who have at least one first-degree relative affected by dementia of 3.5. The risk of AD is significantly lower in those with a one relative in the first degree (OR= 2.6) compared to those who had two or more affected relatives (OR=7.5). The individuals with maternal history of AD have an increased risk compared to those with a paternal one (36).

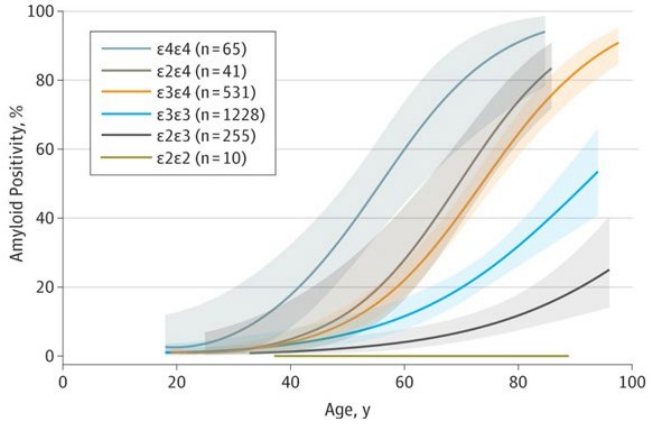


Figure 2. Association of age with prevalence estimates of amyloid pathology according to *APOE* genotype in individuals cognitively unimpaired. Results from Jansen et al. (2015). At the median age of 70 years, the amyloid positive prevalence (measured by PET or by CSF) estimates were different between all *APOE* genotypes in participants with normal cognition (N= 2914, mean aged of 66.8 [13.2] years old), except for those with ε2/ε4 and ε3/ε4 genotypes which did not differ from each other. None of the 10 participants with ε2/ε2 genotype were amyloid positive, therefore no 95% confidence interval is provided for this group. Abbreviations: PET, positron emission tomography; CSF, cerebrospinal fluid. From (27).

1.2.2. Modifiable risk factors

Modifiable risk factors for AD are mostly related to cardiovascular disease, psychosocial factors and/or lifestyle habits. The main cardiovascular risk factors (CVRF) that have shown an increased risk of developing AD include type 2 diabetes (odds ratio [OR]= 1.4), hypertension (HTA) (OR= 1.10), hypercholesterolemia (>251 mg/dl, OR= 1.72) and obesity (meawsured by body mass index (BMI ≥30kg/m2, OR= 2 approximately) (37). Regarding psychosocial factors, individuals with low education (≤6-8 years) had 1.58 times the risk of AD incidence (38). Lastly, the main lifestyle habits associated with the risk of developed AD include heavy smoking, at least >55.5–156 pack-years, (OR= 2 approximately) (38), physical inactivity (OR= 0.65) (39), unhealthy diet (40–42), and low participation in cognitive and social activities (OR= 0.58) (38). Concerning dietary pattern, a previous meta-analysis showed that following a healthy pattern, such as a Mediterranean-type diet, reduced AD risk about 57% (38).

The moment of exposure to AD risk factors within the lifespan needs to be considered when weighing up the impact of these risk factors in relation to AD (3,43) (figure 3). Most major CVRF, including HTA (44,45), hypercholesterolemia (45,46) and obesity (47) in in middle-age increase dementia risk. In contrast, other factors such as diet (40–42), smoking (48,49) and diabetes (50) affect risk across their lifespan.

Finally, after accounting for non-independence between risk factors, it has been suggested that around a third of AD cases are attributable to potentially modifiable risk factors (22).

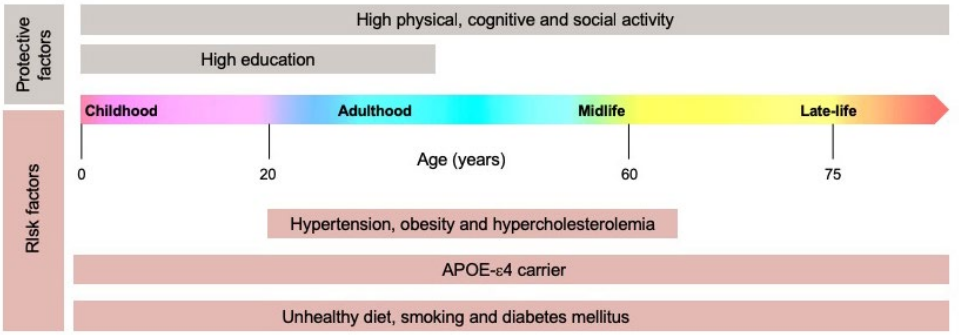


Figure 3. Protective and risk factors for Alzheimer's disease across the lifespan. Some factors can differentially affect the risk of dementia and AD in an individual across the lifespan. For instance, hypertension, obesity and dyslipidemia increase dementia risk when a person is exposed during midlife. Abbreviations: AD, Alzheimer's disease; *APOE*, Apolipoprotein E. Adapted from (43).

1.3. Biomarkers of Alzheimer's disease

It must be noted that, unlike other organs, the brain is rather complex and cannot be easily sampled to obtain living tissue for investigation. In addition, animal models of AD do not recapitulate all the pathophysiological characteristics of the disease in humans. In this context, AD biomarkers are very valuable tools to identify the biological mechanisms of the disease and how AD risk factors impact on them. AD biomarkers can be divided into pathophysiological and topographical. Pathophysiological biomarkers inform of the presence of the pathological hallmarks of the disease (Aβ42, and tau) and can be derived either from CSF sampling or by PET imaging. Topographical biomarkers are imaging biomarkers that probe cerebral characteristics, which are not specific to AD. In these cases, it is not the change in the measured magnitude but the resulting cerebral pattern of affectation, which conveys specificity. Examples of these kind of biomarkers are structural MRI and 18F-fluorodeoxyglucose PET (FDG-PET). In this context, MRI can also be addressed as a marker of comorbid vascular pathology (51), which frequently coexists with AD. Cerebral vascular features seen on MRI include recent small subcortical infarcts, lacunes, white matter hyperintensities (WMH), perivascular spaces and microbleeds.

1.3.1. Pathophysiological biomarkers

The pathophysiological markers of AD are those indicating the specific presence of tau and amyloid pathology characterize AD pathology. Both amyloid and tau PET and AD CSF biomarkers are established Alzheimer's biomarkers that highly correlate with brain biopsy findings (52,53) serving as proxies of in vivo assessment of AD pathology.

In CSF, the AD pathology is detected as a reduced CSF concentration of Aβ related to amyloid plaque pathology, and increased CSF concentrations of p-tau (phosphorylated tau) and t-tau (total tau) related to the presence of neurofibrillary tangles and axonal degeneration respectively (2). Evidence suggests that CSF Aβ42, together with t-tau and p-tau, are biomarkers supportive of an AD diagnosis (7,54) and they may be prognostic of disease progression in CU individuals (55,56) and those with MCI (57–59). Furthermore, CSF Aβ42/p-tau ratio has shown to be highly reliable in the prediction of progression to AD dementia in MCI patients younger than 70 years (58).

In previous longitudinal studies, individuals with normal cognition at baseline and positive AβPET scan showed a higher risk of developing cognitive impairment in later years (60,61). Several PET tracers have been developed to enable in vivo imaging of Aβ. Pittsburgh compound B (PiB) was one of the first PET tracers to be developed to detect in vivo Aβ plaques in the human brain using PET (62) (figure 4). Subsequently, other PET tracers were developed and are approved by European Medicines Agency (EMA) and United States Food and Drug Administration (FDA) which contributed to a widespread use of Aβ PET: ^{18}F-florbetapir, ^{18}F-florbetaben, ^{18}F-flutemetamol has contributed to a widespread use of amyloid PET imaging (figure 5). Even though that aggregation of Aβ in the brain is not specific to AD since it is present in many other degenerative diseases associated to dementia, such as Parkinson's diseases and dementia with Lewy bodies, there is a typical pattern of amyloid distribution in AD. In AD patients, PIB showed a marked retention in regions such as frontal, temporal and parietal cortices, portions of occipital cortex, and the striatum (63) (figure 4)

Both biomarker modalities of Aβ have their advantages and caveats. CSF allows the simultaneous analysis of several biomarkers and, thus, with a single spinal tap, information regarding several biomarkers can be obtained (64,65). The main disadvantage of CSF measurement may be the relative invasiveness of CSF collection by lumbar puncture and the negative attitude among patients and medical doctors (66). However, in these situations or in case of contraindications against lumbar puncture (e.g. anti-coagulant treatment) PET is a good alternative. CSF is cheaper and easier to perform (64,65) than PET. As an advantage, PET allows for the detection of early patterns of regional Aβ deposition that might occur before the global neocortical signal, and the assessment ofthe spread and progression of the pathological AD hallmarks (67).

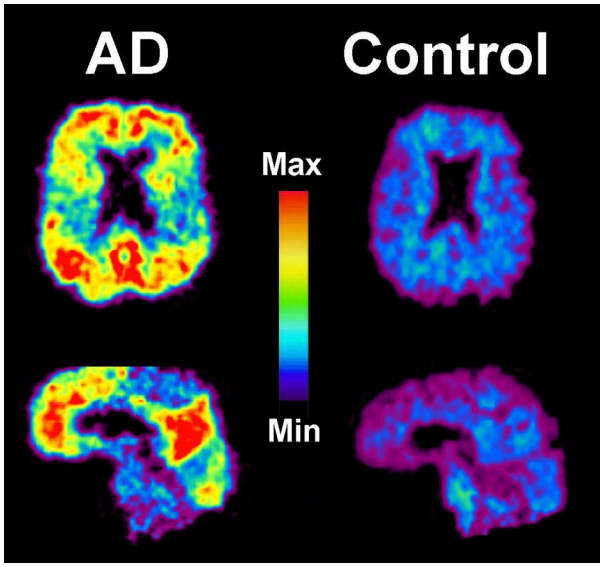


Figure 4. Topographical pattern of PIB retention in Alzheimer's disease. The image reflects the differences in PIB retention observed between controls and AD patients in brain areas known to contain significant amyloid deposits in AD, mainly in frontal and temporoparietal cortices. Read areas show the greatest levels of amyloid, and the dark blue indicates no amyloid. Abbreviations: PIB, Pittsburgh compound B; AD, Alzheimer's disease. Image Credit: University of Pittsburgh Medical Center.

However, CSF and PET measure different aspects of the pathophysiology of a given biomarker. CSF biomarkers are measures of the concentrations of proteins in CSF that reflect the rates of reduction and clearance. On the other hand, imaging measures assess the magnitude of the neuropathological load or damage accumulated over time (5). In this context, discordance with analytical variation is expected to be overcome with fully automated systems, such as the novel Elecsys CSF immunoassay (68) by which allow comparing CSF biomarkers with Aβ PET (69–71).

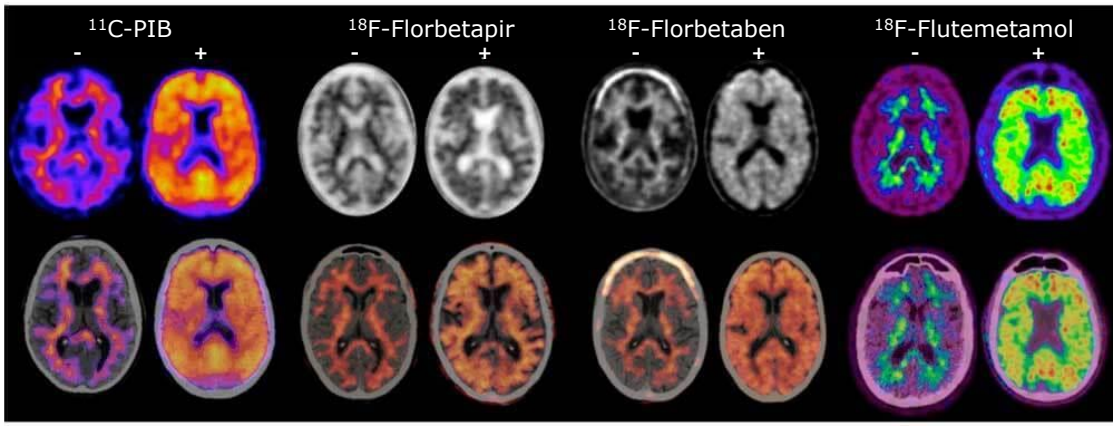


Figure 5. Representative amyloid axial PET images obtained using the three approved amyloid PET radiopharmaceuticals. Top row; PET images, bottom row; PET/CT images. Amyloid negative (-), amyloid-positive (+). Abbreviations: PET, positron emission tomography; CT, computed tomography. From (72).

Notwithstanding differences between both techniques, significant discrepancies have not been found in the estimation of prevalence of amyloid positivity across the lifespan when assessed by CSF versus PET (27). However, there is evidence to support the notion that CSF may be more sensitive in detecting Aβ deposition in the earliest stages (3,73,74) (figure 6). In contrast, AβPET may be more specific for detecting individuals with more advanced pathology (3). Currently, CSF Aβ42, t-tau and p-tau have been validated as core CSF biomarkers for AD pathophysiology (54,75–77).

More recently, blood biomarkers are experiencing rapid progress as a reliable Aβ (78) and neurofilament (79) marker to predict disease progression and brain neurodegeneration at the early presymptomatic stages of AD. Furthermore, CSF and blood may provide a chance for the detection of some biomarkers that cannot be identified by brain imaging (80).

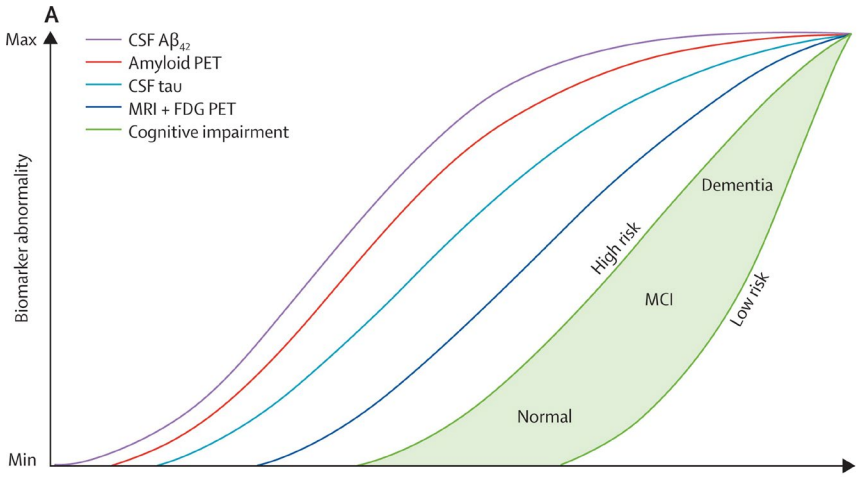


Figure 6. Revised model of dynamic biomarkers of the Alzheimer's disease pathological cascade by Jack et al. 2013. The model supports a general temporal ordering framework in which amyloid biomarkers become abnormal first, followed by biomarkers of neurodegeneration and, finally, clinical symptoms. Neurodegeneration is measured by FDG-PET and structural MRI (dark blue). Cognitive impairment is illustrated as a light green-filled area with low-risk and high-risk borders. Abbreviations: AD, Alzheimer's disease; FDG-PET, 18F-fluorodeoxyglucose positron emission tomography; MRI, magnetic resonance imaging. From (81).

1.3.2. Topographical markers

On top of pathophysiological markers, imaging also allows the measurement of other cerebral features like cortical thinning or reductions in glucose consumption. Although topographical markers are not considered to be specifics for AD pathology (82), they have allowed for the demonstration of a characteristic topographical pattern in AD that includes medial temporal lobe atrophy (75,83,84) (by structural MRI) and reduced glucose metabolism in temporal and parietal regions on ^{18}FDG-PET (85) (figure 7).

Abnormalities in structural MRI become clearly detectable before the first clinical signs of the disease (3). Concerning regional hippocampal volume, previous research showed that CA1 region and subiculum compared with the total volume of the hippocampus were more closely associated with progression to MCI in CU individuals (86,87). Decreased entorhinal cortex volume was shown to precede significant cognitive decline by 4 years in CU elderly individuals (86). Moreover, prefrontal cortex atrophy in CU individuals was found to precede dementia onset by a 6-year period [172]. However, the progression of neuronal injury, determined by FDG-PET, to clinical AD it is not yet well established. In the AD Neuroimaging Initiative (ADNI) cohort, models with baseline features derived from MRI and FDG-PET were capable of successfully predicting with 81.2% accuracy whether an individual will progress and convert to MCI within 48 months or remain cognitively stable (88).

Since under physiological condition glucose represents the brain's main energetic source, ^{18}FDG-PET is used to determine neuronal activity. In AD, metabolic deficiencies have been shown in cortical areas, such as precuneus, posterior cingulate cortex, parieto-occipital regions and the frontal cortex, and hippocampal regions (62,89), in contrast to healthy individuals (90). Longitudinal studies reported the ^{18}FDG-PET capacity to predict the development of MCI and the conversion from MCI to AD (91–93).

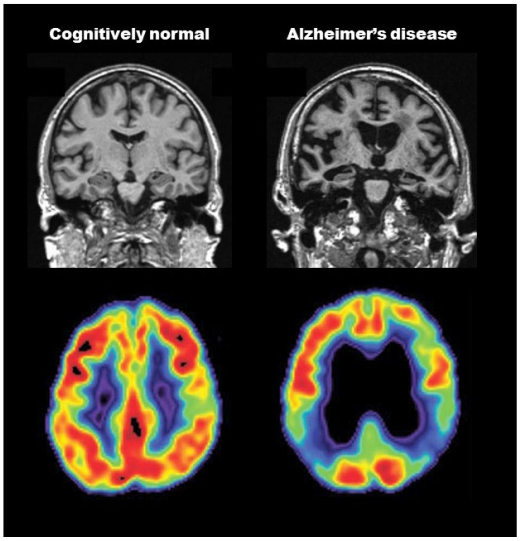


Figure 7. Characteristic topographical pattern in Alzheimer disease by MRI and ^{18}FDG-PET. Top row refers typical MRI scans in CN individuals and AD patients. In AD there is a temporal atrophy (specifically, the hippocampus and ventricular enlargement) compared with CN. Adapted from (166). Below row refers typical ^{18}FDG-PET scans in CN individuals and AD patients. In AD there is a reduced glucose metabolism particularly in temporal and parietal lobes located on the sides and the back of the brain. Green colour indicates decreased levels of glucose metabolism in the brain. Credit image (adapted): Cindee Madison and Susan Landau, UC Berkley. Abbreviations: AD, Alzheimer's disease; MRI, magnetic resonance imaging; 18FDG-PET, 18F-fluorodeoxyglucose positron emission tomography; CN, cognitively normal.

1.3.3. Cerebrovascular pathology

MRI can be addressed as a marker of comorbid vascular pathology (51). MRI allows for the visualization of lacunar infarcts or haemorrhages, WMH, perivascular spaces and microbleeds (94) (figure 8). Among them, WMH are commonly detected in the brain of asymptomatic elderly individuals (95,96).

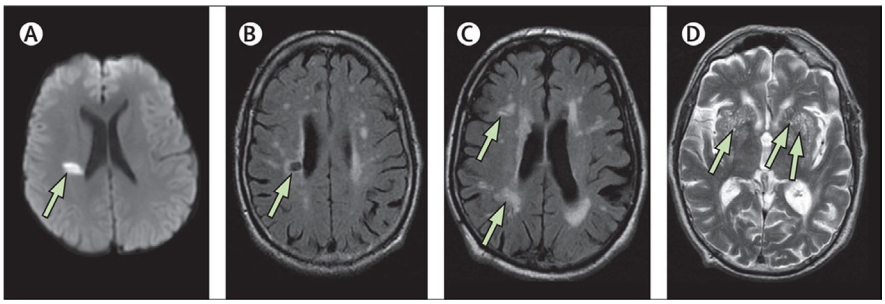


Figure 8. MRI images of characteristics features of vascular pathology. A) DWI image of a lacunar infarct (arrow). B) Lacune on FLAIR imaging (arrow). C) WMH on FLAIR imaging. The top arrow indicates deep WMH and the below arrow shoes periventricular WMH. D) Perivascular spaces on T2-weighted imaging (arrows). Abbreviations: DWI, diffusion-weighted image; FLAIR, fluid-attenuated inversion recovery; WMH, white matter hyperintensities. Adapted from (94).

WMH are considered to reflect small-vessel ischemic damage in the brain (51). Even though they are prevalent in healthy elderly individuals, global WMH load has been shown to exert a negative impact on multiple cognitive domains (97–99), on the onset and severity of AD (100,101), and to constitute an independent risk factor for cognitive decline (98,102). Despite presumed vascular aetiology of WMH (103), the precise underlying pathology is not completely understood (104). Research suggested that these lesions may mostly reflect demyelination and axonal loss (103). Previous studies have shown an association between HTA, which displayed the strongest and more widespread association with WMH load (100,105–109). Additionally, regarding AD genetic risk factors, it has been proposed that the *APOE*-ε4/ε4 genotype could be an independent risk factor for the development of WMH (110,111).

1.4. Impact of Alzheimer disease risk factors on biomarkers

Understanding the mechanisms through which risk factors exert their deleterious or protective effects is critical to rationally design interventions to prevent the onset of AD, as well as which and when individuals may benefit from. To this end, biomarkers offer a window

to understand the biological and physiopathological mechanisms associated to AD risk factors.

In a recent meta-analysis on the prevalence of cerebral Aβpathology (estimated by PET or CSF) in persons without dementia reported that it was determined by age, *APOE* genotype, higher education and presence of cognitive impairment. Concerning *APOE*, the ε4 allele was associated with a greater risk for amyloid positivity and decreased age onset, while ε2 allele showed the opposite association (27). Additionally, in a previous longitudinal study with individuals without dementia (N= 1671, mean age of 71.3 years old) found that the Aβ prevalence (determined by PET) was higher in women than in men (112). With regards to CVRF, the exposure of midlife risk factors is important for amyloid deposition (determined by PET), such as BMI (113) and HTA (114). In agreement with these findings, the Framingham Coronary Risk Profile score (an index counting elevated cholesterol level, diabetes, HTA, and smoking) has been associated with increased amyloid burden (PIB-PET) in a sample of CU individuals, participants with MCI, and participants with AD (115).

As stated before, AD and cardiovascular disease share important risk factors suggesting that vascular disease could interact or overlap with primary AD pathology (110). Increasing evidence demonstrates that cerebrovascular changes have an additive effect on neurodegeneration, accelerate cognitive decline and progression to dementia (103,116,117). Concerning cognition, in general, amyloid deposition is associated with episodic memory (EM) impairment, whereas cerebral small vessel disease is associated with EF dysfunction, and the presence of both may have additive effects on cognitive decline (118).

2. Methodological approaches

2.1. Risk scores

The co-occurrence of modifiable and non-modifiable risk factors across the lifespan of a person, jointly with the increasing research focus on the preclinical stage of AD, have stimulated the development of risk scores to assess risk of cognitive impairment and estimate the overall risk of dementia in individuals (43). Risk scores have generally included some known risk factors that are easily measurable to calculate the consequent risk of an event or disease within a given time frame (23). The main use of them is to detect those individuals with higher risk of disease and who might benefit from prevention interventions (119).

One of dementia risk score used to select at risk individuals for lifestyle studies is the Cardiovascular Risk Factors, Aging and Dementia (CAIDE) risk score. CAIDE risk score is based on population-based data from CAIDE study and provides an estimation of the probability within 20 years in middle-aged individuals of suffering dementia. There are two models of the CAIDE dementia risk score; both including age, sex, education, blood pressure, cholesterol, BMI, and physical inactivity (CAIDE-I). The second one additionally includes *APOE* genotype (CAIDE-II) (23) (figure 9). Both risk scores have been previously associated with cognition in healthy adults, specifically with lower EF performance (120,121).

Concerning cerebrovascular disease, the Framingham Stroke Risk Profile was developed to assess the risk for stroke. This risk score combines the major CVRF for stroke and weights them in such a way as to produce a score giving the 10-year probability, or risk, of stroke. The stroke risk factors included in the profile are age, systolic blood pressure, the use of antihypertensive therapy, diabetes mellitus, smoking, prior cardiovascular disease (coronary heart disease, cardiac failure, or intermittent claudication), atrial fibrillation, and left ventricular hypertrophy by electrocardiogram (122). In Spain, it has been developed an adaptation of the Framingham function called REGICOR (Registre Gironí del Cor). The REGICOR function provide the individual' risk of suffering coronary disease events at 10 years

and include sex, age, total cholesterol and high-density lipoprotein cholesterol, blood pressure, smoking, and diabetes mellitus (123).

Risk factor		Points
Age	<47 years	0
	47–53 years	3
	>53 years	4
Education	≥10 years	0
	7–9 years	2
	<7 years	3
Sex	Female	0
	Male	1
Blood pressure	≤140 mmHg	0
	>140 mmHg	2
BMI	≤30 kg/m ^{2}	0
	>30 kg/m ^{2}	2
Total cholesterol	≤6.5 mmol/l	0
	>6.5 mmol/l	2
Physical activity	Yes	0
	No	1

Figure 9. The CAIDE dementia risk score. CAIDE score provides an estimation of the probability within 20 years in middle-aged individuals of suffering dementia. Exist two models of CAIDE; Model I (presented in the table) and Model II that includes *APOE* genotype as *APOE*-ε4 allele non-carriers scored 0 points and carriers scored 2 points. Abbreviations: CAIDE, Cardiovascular Risk Factors, Aging and Dementia; *APOE*, *Apolipoprotein E.* Adapted from (43).

In summary, risk score draws attention to the role of CVRF in the development of dementia and allow for the identification of individuals who might benefit from primary and secondary prevention interventions.

2.2. White matter hyperintensities: assessment

In their mild form, WMH usually appear as small "caps" on the frontal and/or occipital horns and as branches along the walls of the lateral ventricles on transverse sections (namely periventricular WMH [PVWMH]) or as punctuate foci in subcortical white matter (namely deep WMH [DWMH] or juxtacortical WMH [JCWMH] when located in regions adjacent to the cortex). During the progression of WMH, the PVWMH may extend into DWMH and JCWMH (103).

At present, no reference method has been established for the assessment of WMH. There are various methods to quantify the presence and severity of these lesions, rating to qualitative (generally applying visual rating scales) and quantitative (measuring the lesions volume) methods. Visual rating scales offer the advantage of being quite fast and reliable when employed by an experienced professional, as well as not requiring sophisticated and expensive post-processing facilities. In contrast, automated methods provide exact WMH volumes, which allow the exploration of subtle associations (103). Previous studies have shown that both methods correlate between them (124,125).

Widely used visual rating scales include those introduced by Fazekas and Scheltens. The Fazekas scale (126) is widely used and categorizes separately the severity of deep and periventricular lesions on a scale from 0 to 3 (figure10). The Scheltens scale rates WMH separately in the periventricular region on a 0–6-point scale, and in the subcortical region on a 0–24-point scale, on the basis of the size and number of the lesions (127). Both scales were designed for cross-sectional rating of WMH. Concerning Fazekas, it is important considering that the scale provides a score of WMH load on a pathological level, whereas the quantitative methods provide exact WMH volumes which are required when one is looking for subtle associations. Previous studies showed that Fazekas score ≤ 2 is considered as pathological in individuals younger than 75 years old (128–130).

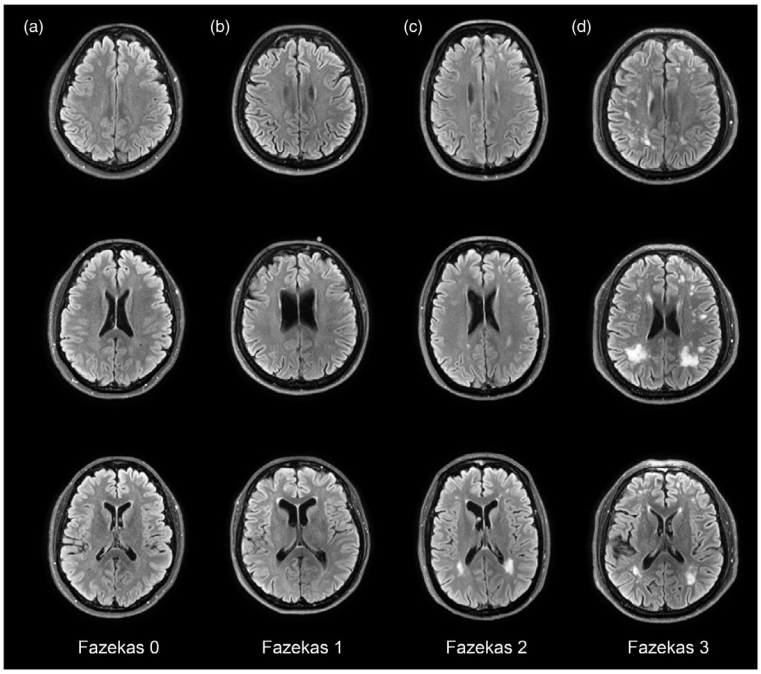


Figure 10. The Fazekas scale. T2-FLAIR images illustrating the Fazekas Scores. The grades of WMH are evaluated separately categorizes the severity of deep and periventricular lesions, on a scale from 0 to 3. Fazekas 0 (a); none or a single punctate WHM lesion, Fazekas 1 (b); multiple punctate lesions, Fazekas 2; beginning confluency of lesions [bridging], and Fazekas 3; large confluent lesions. Abbreviations: WMH, white matter hyperintensities.

Quantitative methods allow for the automatic quantifying of total or regional WMH volume. These technics have been shown to be associated with clinical outcomes, may be due to the ability to better differentiate between clinical subgroups than visual rating scales (103). Spatial distribution of WMH burden assessment have showed more predictive power than global WMH burden (131). Recently, Sudre et al. (2017) has developed a novel approach to show the regional-zonal representation of WMH load (131). In this, WMH are automatically segmented using a previously developed algorithm (132). The lesion frequency per defined spatial local region WMH lesion loads are representing in a bullseyes plot (figure 7). Every sector of the bullseyes represents one lobar white matter segment; frontal, parietal, temporal and occipital lobes were delineated on the right and left side, with another unique region corresponding

to the basal ganglia (including internal capsule and thalamus). The concentric rings in the bullseye plot are defined by dividing the area between the ventricular surface and the cortical sheet in four equidistant layers. The interior layer in the plot represents the most periventricular area, the next two layers corresponds to the deep and, finally, the most external layer corresponds to juxtacortical regions. The bullseyes plot allow for the visualisation of association with risk factors or differences between populations (131).

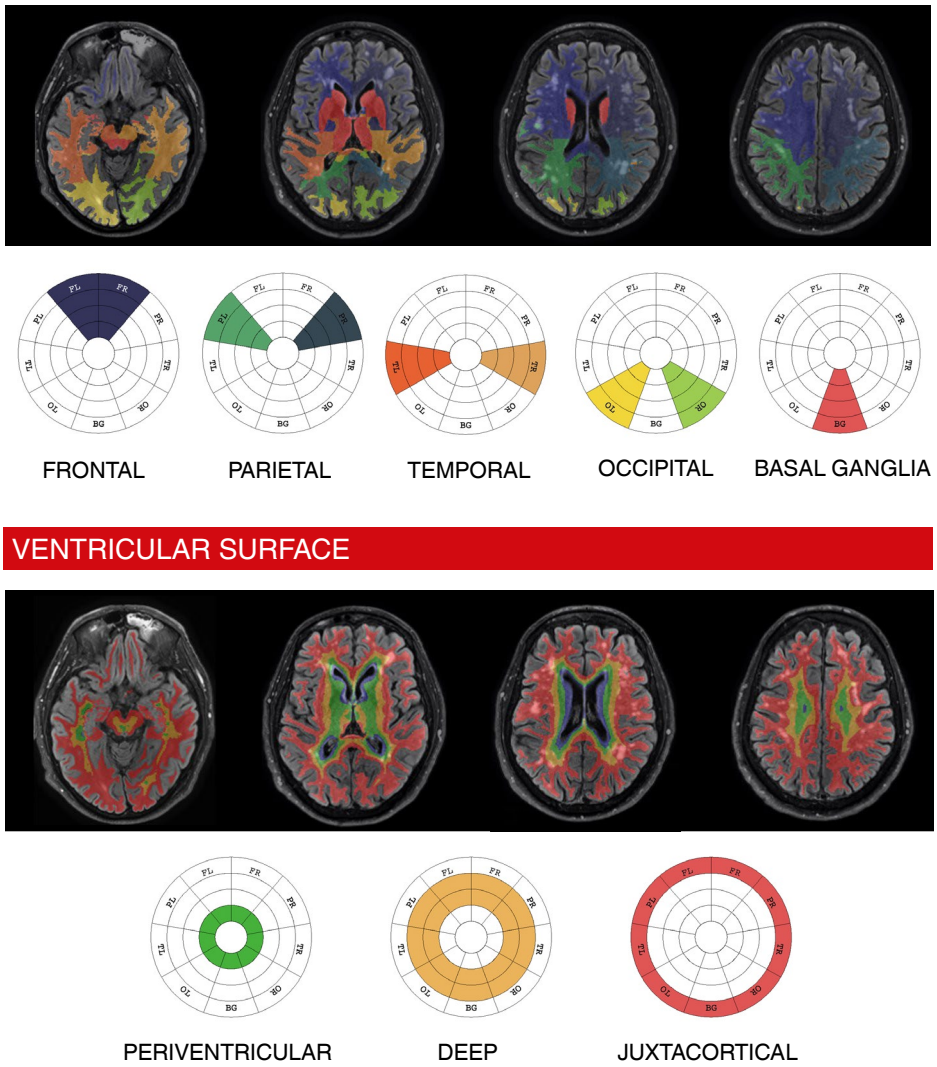


Figure 11. Example of the brain segmentation of one participant and the representation of the building blocks of the WMH lesion loads. Lobar regions: the first row refers to the lobar and the unique region segmentation. The second row shows the lobar bullseyes representation; every sector represents one lobar white matter segment. The each sector color corresponds to the lobe color in the segmentations images. Ventricular surface: the first row refers to the distance based layer separation from the ventricular surface towards the cortical sheet. The second row shows the representation of four layers which each lobe was segmented. Of these layers, the most internal represents periventricular areas, following by two layers of deep white matter and, finally, a juxtacortical layer which is the most external. Abbreviations: WMH, white matter lesions.

2.3. Gray matter volume: quantification and regional patterns associated to Alzheimer's disease

MRI, as a topographical marker, allows for the quantification of gray matter (GM) atrophy. One first level of semi-quantification of AD-related brain atrophy is through neuroradiological scales. Probably, the most widely used scale as visual rating of cerebral atrophy is the Medial Temporal Lobe (MTA) scale (133) As stated before, the medial temporal lobe atrophy is typically in AD and has been shown to be a very strong predictor of the progression of MCI to AD. MTA scale is administered using coronal T1 weighted images through the hippocampus at the level of the anterior pons. It is based on width of the choroid fissure and of the temporal horn of lateral ventricle and the height of the hippocampus. The score (0 to 4) (figure 12) is interpreted in relation to age; a score of 1 can be regarded as normal in patients younger than 75 years, and a score of ≤2 can be considered normal in individuals older than 75 years (134).

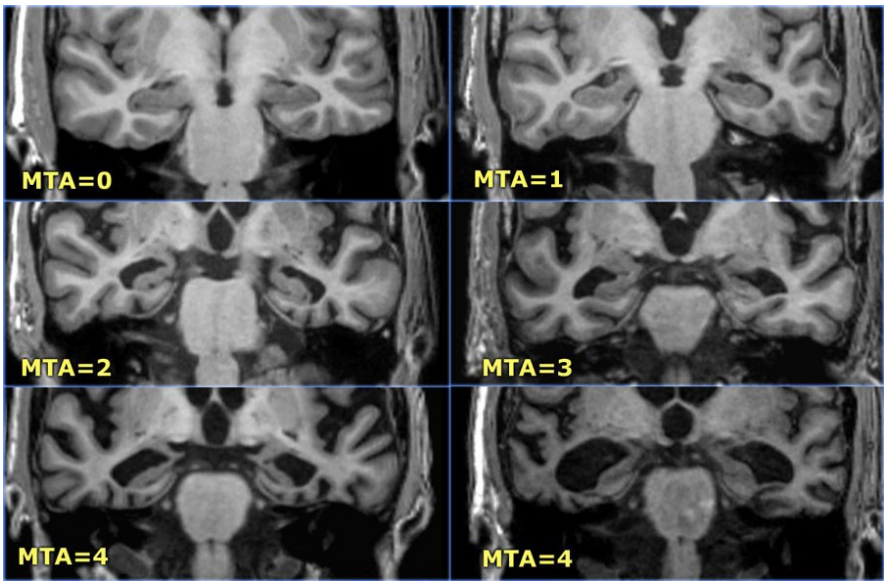


Figure 12. Medial Temporal Atrophy (MTA) scale. The MTA score is based on a visual rating of the width of the choroid fissure, the width of the temporal horn, and the height of the hippocampal formation. Score 0= no atrophy; score 1= only widening of choroid fissure; score 2= also widening of temporal horn of lateral ventricle, score 3= moderate loss of hippocampal volume (decrease in height) and score 4= severe volume loss of hippocampus. Credit image: Radiology Assistant.

The quantification of cortical thickness by GM atrophy in MRI can be used to detect those early brain changes in older individuals (135), considered a marker of neurodegeneration (136). There are two main approaches for quantitative analysis of volumetric brain changes in MRI: manual region of interest (ROI) and voxel-based. The ROI analysis technique allows for the assessment of the volume of specific brain regions, which can be manually traced or automatically derived using a brain atlas. Typically, this approach requires *a priori* hypothesis of the regions that will be affected by the factors under investigation. On the other hand, voxel-based approaches assess GM volume or cortical thickness throughout the whole brain in an unbiased approach. The main drawback of this approach is the inherent risk of detecting false positives, due to massive multiple comparisons and the challenge of inter-subject brain comparability (137,138).

Several neuroimaging software suites are available for use in a voxel-wise approach, such as the Voxel-Based Morphometry (VBM) in Statistical Parametric Mapping software (SPM12; Wellcome Department of Imaging Neuroscience, London, UK) which detects GM density across the whole brain structural images by comparing voxel to voxel across individuals through nonlinear registration of multiple brain images to a standard anatomical template. The value at each voxel in the resulting tissue segments can be thought of as representing the proportion of the corresponding tissue in that voxel. Once the images are aligned into the same coordinate space voxel-wise statistical comparisons of GM volume concentration across different individuals groups can be performed (139) (figure 13). VBM does not require a priori hypothesis and is relatively fast. VBM measured cortical thickness across different individuals requires methods to match corresponding anatomical regions of the cortical surfaces, such as the popular FreeSurfer (http://surfer.nmr.mgh.harvard.edu/), SPM (140) and ANTs (antsCorticalThickness.sh) packages (141).

Concerning cortical thickness, the AD signature of thinning has been found in vulnerable cortical regions related to symptom severity, even in the earliest stages of the disease, which includes atrophy in the medial temporal lobe, especially in the hippocampus and entorhinal cortex, in lateral temporoparietal, midline parietal and frontal regions (84) (figure 14).

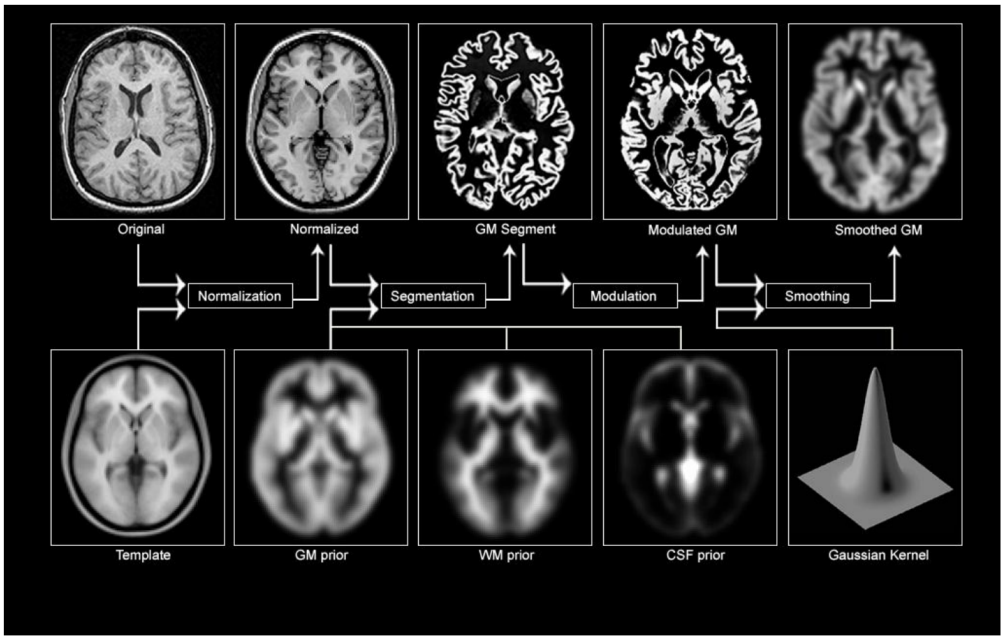


Figure 13. Voxel-Based Morphometry Steps. The standard VBM process typically involves four steps: (1) spatial normalization (applying a nonlinear registration to each individual's T1-weighted MRI); (2) tissue segmentation (based on the intensity in the image); (3) spatial smoothing (intensity in each voxel is a local weighted average generally expressed as GM, white matter or CSF concentration); and (4) statistical analysis–group comparisons or correlations with covariates of interest (139). Abbreviations: VBM, voxel-based morphometry; MRI, magnetic resonance imaging; GM, gray matter; CSF, CSF, cerebrospinal fluid.

Previous cross-sectional studies with CU individuals showed aging strongly associated with lower GM volume in both parietal lobes and prefrontal regions (142). *APOE*-ε4 has also shown to have an additive effect on GM volume in regions relevant for AD pathophysiology already in healthy individuals, especially in hippocampus (143,144), caudate, precentral gyrus and cerebellar crus (144). Concerning non-modifiable risk factors, recent cross-sectional studies with CU individuals have shown that lifestyle factors, such as unhealthy diet (145), smoking (146,147) and low level of physical exercise (148), are associated with cortical thickness in orbitofrontal cortex, prefrontal regions, posterior cingulate cortex, and precuneus. In last, CVRF have also shown a relationship with GM decrease. BMI (149,150), HTA (151) and diabetes (152,153) lead to brain volume reduction

in CU elderly individuals, mainly in temporal areas, specifically hippocampus, frontal regions and in cingulate gyrus (150,154). In this context, cross-sectional studies have revealed a regional relationship between GM volume and cognition in CU individuals (155,156). As a result, GM loss is significantly associated with the onset and progression of AD (157).

Prior research suggested an association between WMH and cortical GM atrophy in non-demented individuals (158). Indeed, high WMH burden is associated with total GM atrophy (159) in the temporal lobe (100) and the frontal cortex (160) in CU individuals. Additionally, previous studies have shown that both presence of WMH and reduced of GM volume contribute to impaired cognition in individuals with CVRF (161) in mixed samples including cognitively healthy, MCI and AD individuals (162,163).

In conclusion, in order to progressively reduce the global burden of dementia by means of prevention, it is of the utmost importance to acquire a better knowledge of the mechanisms linking WMH with neurodegeneration (2,103). However, the regional patterns of brain atrophy associated with higher WMH burden, as well as their simultaneously impact on cognition are still not thoroughly understood in CU young individuals.

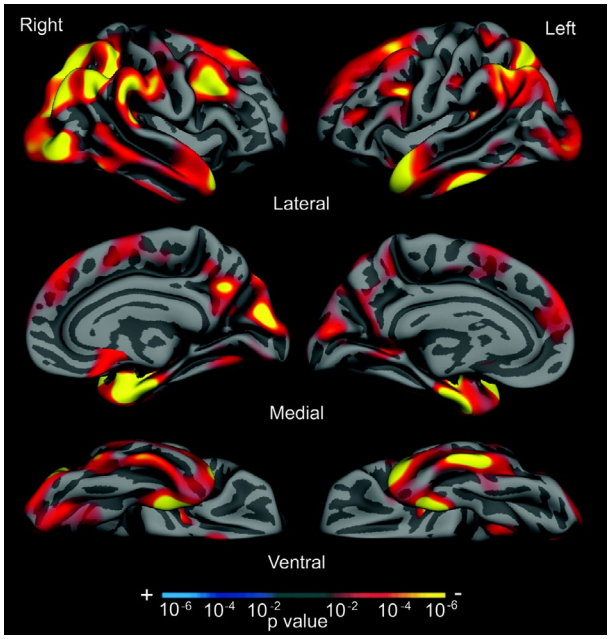


Figure 14. The cortical signature of Alzheimer's disease. The AD significant regions include regions of medial temporal lobe, lateral temporoparietal, midline parietal and frontal. The color scale represents the significance of the thickness difference with yellow referring regions of most significant thinning in AD compared with CU individuals. Abbreviations: AD, Alzheimer's disease, CU, cognitively unimpaired. From (84).

3. The ALFA research platform

As stated before, the setup of preventive studies requires the identification of individuals with an increased risk of developing AD in the near future that are suitable for recruitment as asymptomatic individuals in prevention studies and clinical trials. With this in mind, and aiming at increasing the knowledge of the pathophysiology and pathogenic factors emerging at early preclinical AD stages, the Barcelonaβeta Brain Research Centre (BBRC) started the ALFA (for Alzheimer and Families) programme for the prospective follow-up of a cohort of cognitively unimpaired individuals, most of which are the offspring of AD patients (1). The ALFA parent cohort inclusion criteria are shown in table 1.

Table 1. The ALFA parent cohort inclusion criteria

Inclusion criteria	Exclusion criteria
• Spanish and/or Catalan-speaking men and women • Aged between 45 and 75 years • Agreement with study procedures and tests • Involvement of a close relative for the participant's functional evaluation	• Cognitive impairment • Functional status impairment • Major psychiatric disorders (DSM-IV-TR) or diseases that could affect cognitive abilities • Severe auditory and/or visual, neurodevelopmental and/or psychomotor disorders, significant diseases that could interfere with cognition • Neurological disorders • Brain injury interfering with cognition • Family history of AD with suspected autosomal dominant pattern

Abbreviations: CDR, Clinical Dementia Rating scale; MMSE, Mini-Mental State Examination; MIS, Memory Impairment Screen; TO-BTII, Time- Orientation subtest of the Barcelona Test II; SF, Semantic Fluency

From (1)

The ALFA parent cohort is composed of 2743 CU individuals aged between 45 and 74 years at recruitment (mean age of 55.8 (6.7) years). 36.8% of individuals are men and 63.2% are women with an average years of formal education of 13.3 (3.5) years. Regarding inclusion cognitive criteria, the mean of Mini-Mental State Examination (MMSE) (164) was of 29.0 (1.1).

Many of the ALFA parent cohort members are offspring of AD patients. Family history of AD was considered positive when their mother and/or father, had been diagnosed with AD. When considering a more strict family history encoding (AD patients that had shown signs of cognitive impairment before the age of 75) 47.4% of the ALFA study participants have positive AD family history. Specifically, in 2.2% of the participants both parents had been diagnosed with AD (at least one of them before the age of 75 years) in the 14.3% of the cases was the father and in the 31% was the mother who had been diagnosed with AD before the age of 75 (figure 15).

There is a higher proportion of *APOE*-ε4 carriers in the ALFA parent cohort than in the general population. (19% and 14%, respectively; $p<0.001$) (1). In brief, of 2670 ALFA members whose genotype could be determined, 9 were *APOE*-ε2/ε2, 167 were *APOE*-ε2/ε3, 59 were *APOE*-ε2/ε4, 1.567 were *APOE*-ε3/ε3, 782 were *APOE*-ε3/ε4 and, finally, 86 were *APOE*-ε4/ε4 (figure 15). As a result, BBRC established a research platform enriched in genetic risk factors for AD.

The CVRF and lifestyles data were obtained during the recruitment of participants. Current HTA was the most prevalent cardiovascular comorbidity most prevalent (64.5%). 42.4% of the study participants self-reported endocrino-metabolic comorbidities: of these, 69.8% reported current dyslipidemia and 9.8% were currently diagnosed with diabetes. Lastly, 79.8% of the ALFA cohort members had a BMI≤30 and 73.6% a measured systolic blood pressure ≤140, being both ranges associated with a lower risk of developing cognitive impairment and/or cardiovascular disease. Regarding lifestyles, 65.4% fell in the "active" category (considered as at least 150 minutes per week of moderate exercise or 75 minutes per week of vigorous exercise as recommended by current guidelines), and 34.6% were categorized as inactive. 17.5% of the ALFA population had never smoked, 57.6% had given up smoking for more than a year ago and, finally, 24.9% of them fell in the smokers' category (1).

Consequently, due to exclusion of individuals with relevant medical pathology or neurologic disease at the period of recruitment, the ALFA parent cohort population is healthier than could be expected from an age-matched cohort selected from the general population. The main clinical and lifestyle features of the ALFA cohort compared to those of the Spanish general population (165) are shown in table 2.

Table 2. Clinical features of the ALFA parent cohort participants

	General population		ALFA population	
Descriptor	Men	Women	Men	Women
Hypertension[a]	47.0	39.0	51.6	34.2
Diabetes[b]	13.0	10.0	6.3	3.5
Dyslipidemia[b]	35	32	35.8	29.4
BMI = 25-29.9	51.0	36.0	52.3	36.5
BMI ≥30	29.0	29.0	24.3	18.0
Smoker	33.0	21.0	23.6	25.7
Ex-smoker[c]	36.0	14.0	61.1	55.6
Non-smoker	32.0	66.0	15.3	18.7

Percentages are shown. With the exception of BMI 25-29.9, the rest of comparisons were statistically significantly different (p <.05).

a Self-reported hypertension + systolic/diastolic ≥140/90 mmHg

b Self-reported

c For longer than a year

From (1)

In the current thesis, the sample used in all the presented studies consists of a subset of ALFA parent cohort. After *APOE* genotyping all participants homozygous for the ε4 allele as well as carriers of the ε2 allele were invited to undergo a MRI along with ε4 heterozygous and matched for age and sex. This sampling strategy was designed to maximize the representation of *APOE*-ε4 homozygotes. The final sample and the inclusion and exclusion criteria are specified in each study.

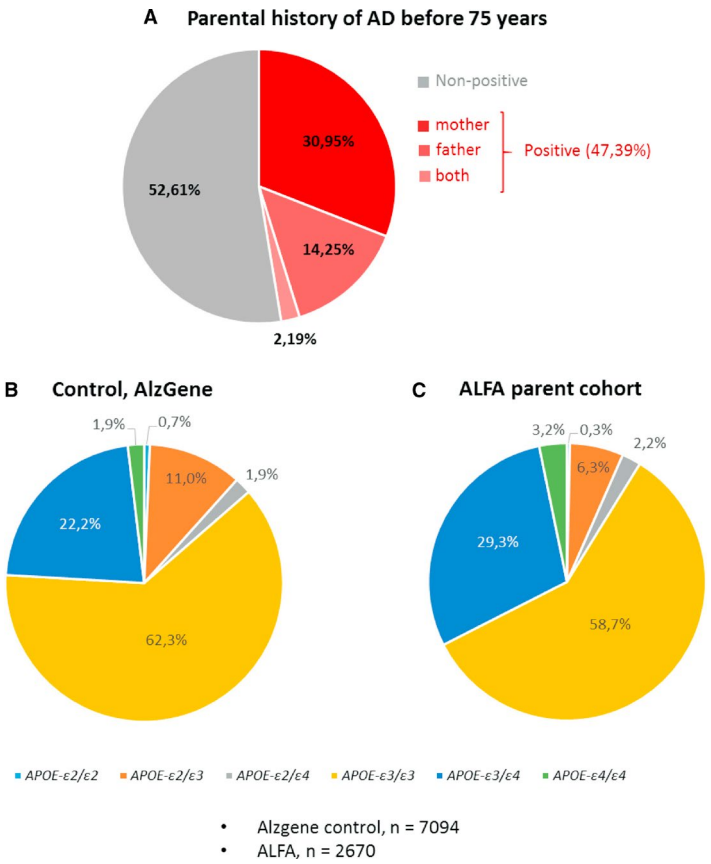


Figure 15. Non-modifiable risk factors for Alzheimer's disease. A) Representation of the ALFA suty participant's parental history of AD before the age of 75 years. Percentage of *APOE* genotypes in the ALFA parent cohort population (C) compared to cognitively normal individuals' taken from the AlzGene database (B). Abbreviations: Alzheimer's disease. From (1).

In summary, the ALFA parent cohort is a valuable infrastructure of middle-aged participants representing the whole spectrum of risk that will leverage with different projects and trials to prevent AD (1) (figure 16). Throughout the ALFA project, biomarkers present in the AD preclinical stage will be detected, which will provide information about the presence of brain Aβ deposition. Furthermore, the longitudinal assessments will be useful to understand early pathological changes together with modeling the preclinical stages to develop successful trials.

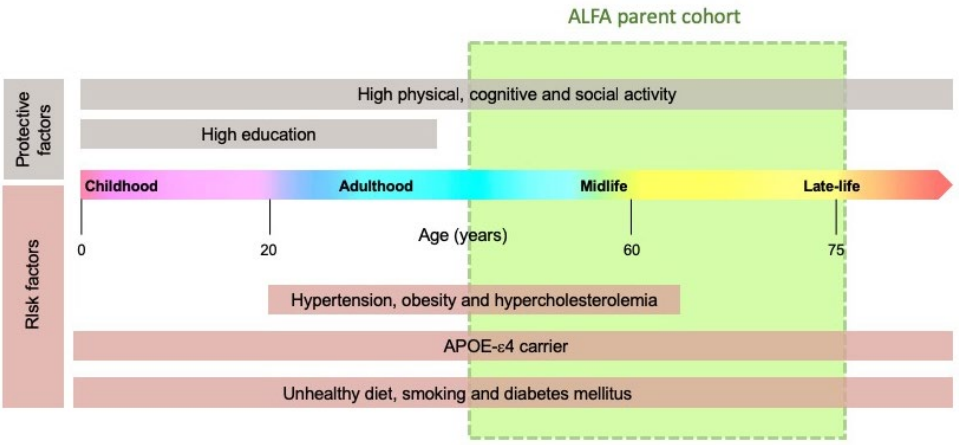


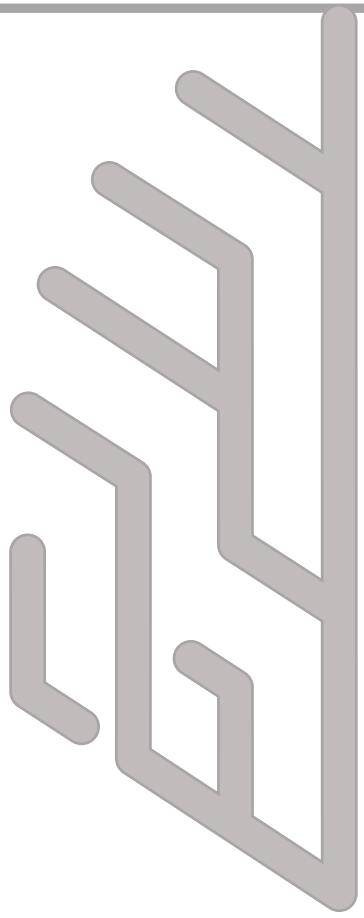
Figure 16. ALFA parent cohort scope. The intervention in CU individuals at risk of developing AD in middle-aged will provide an increased knowledge of pathophysiology and pathogenic risk factors, as well as their biologic mechanisms and impact on the brain, emerging at early preclinical AD stages. As a result, the ALFA parent cohort represents a valuable infrastructure in AD prevention research. Abbreviations: CU, cognitively unimpaired; AD, Alzheimer's disease. Adapted from (43).

4. Outline of the thesis

The main goal of this thesis is to study the impact of AD risk factors on WMH of CU middle-aged individuals. In addition, it will explore the regional patterns of GM volume and specific cognitive performances associated with higher WMH burden. In order to achieve these goals, they were designed five original research studies using data gathered in the ALFA cohort.

This thesis will contribute, through novel methodological approaches, to increase the understanding of the mechanisms through that AD risk factors elicit brain vulnerability to disease. Additionally, the present work will highlight the relevance of middle age as an optimal timing to apply preventive interventions addressing the different risk factors to delay the onset of dementia.

HYPOTHESIS
OBJECTIVES



Hypothesis

1. The middle-aged children of AD patients show a comparable prevalence of pathological levels of white matter hyperintensities (Fazekas score ≥2) to individuals without familiar AD history of the same age ranges.

2. Cognitively unimpaired *APOE*-ε4/ε4 carriers present a higher risk of having pathological levels of white matter hyperintensities. Additionally, cardiovascular risk factors are also associated with pathological white matter hyperintensities but they do not interact with the effect of *APOE*-ε4/ε4.

3. Global and regional volumes of white matter hyperintensities is associated with Alzheimer's disease risk factors even in cognitively unimpaired individuals with very low risk of dementia.

4. Episodic memory and executive function performance are associated with global white matter hyperintensity volumes in cognitively unimpaired middle-aged individuals. Regionally, white matter hyperintensity load in frontal and temporal areas correlate with these cognitive functions.

5. White matter hyperintensity lesions mediate the relationship between gray matter volume and cognition even in cognitively unimpaired middle-aged individuals with low load of white matter hyperintensities.

Objectives

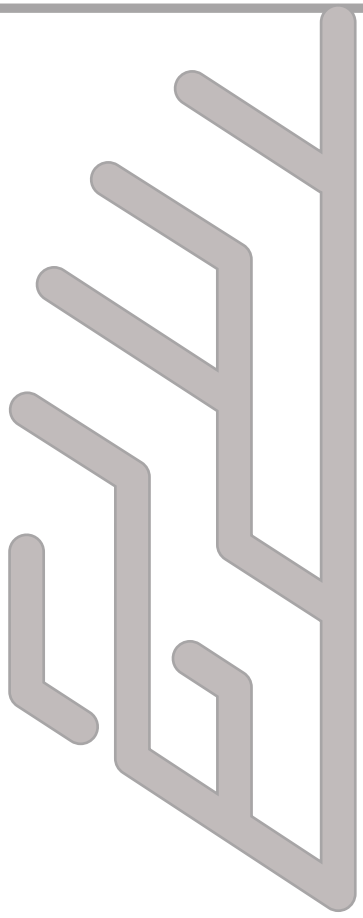
General Objective

The general objective of this thesis is to study the impact of AD risk factors on white matter hyperintensities in cognitively unimpaired middle-aged individuals. In addition, we aim to explore the effect of white matter hyperintensities on cognition by studying the relationship linking simultaneously white matter hyperintensities with neurodegeneration in cognitive performance.

Specific Objectives

1. To compare the prevalence of brain MRI incidental findings between the adult children of AD patients with respect those without familiar history.
2. To investigate the association between the number of *APOE*-ε4 alleles with the presence of pathological levels of white matter hyperintensities (Fazekas score ≥2) and their interaction with cardiovascular risk factors.
3. To assess whether the global volume of white matter hyperintensities is associated with dementia risk estimates and Alzheimer's disease risk factors.
4. To describe the patterns of white matter hyperintensities associated with dementia risk estimates and Alzheimer's disease risk factors.
5. To study the impact of global and regional distribution of white matter hyperintensities on episodic memory and executive function.
6. To explore whether white matter hyperintensity lesion volume mediates the relationship between gray matter volume and cognition.

RESULTS



Chapter 1

Incidental findings on brain MRI of cognitively normal first-degree descendants of patients with Alzheimer's disease: a cross-sectional analysis from the ALFA (Alzheimer and Families) project

Anna Brugulat-Serrat*, Santiago Rojas*, Nuria Bargalló, Gerardo Conesa, Carolina Minguillón, Karine Fauria, Nina Gramunt, José Luis Molinuevo and Juan Domingo Gispert

(*) These authors have equally contributed to this manuscript

The contribution of thesis author to the paper, following the International Committee of Medical Journal Editors (ICMJE), was:

- Substantial contributions to the conception or design the work: acquisition, analysis and interpretation of data of the work.
- Drafting the work and revising it critically for important content.
- Final approval of the version to be published.
- Agreement to be accountable for all aspects of the work in ensuring that questions related to accuracy or integrity of any part of the work have appropriately investigated and resolved.

Brugulat-Serrat A, Rojas S, Bargalló N, Conesa G, Minguillón C, Fauria K, et al. Incidental findings on brain MRI of cognitively normal first-degree descendants of patients with Alzheimer's disease: A cross-sectional analysis from the ALFA (Alzheimer and Families) project. BMJ Open. 2017 Mar 1;7(3). DOI: 10.1136/bmjopen-2016-013215

Chapter 2

Higher prevalence of cerebral white matter hyperintensities in homozygous *APOE*-ε4 allele carriers aged 45–75: Results from the ALFA study

Santiago Rojas*, Anna Brugulat-Serrat*, Nuria Bargalló, Carolina Minguillón, Alan Tucholka, Carles Falcon, Andreia Carvalho, Sebastian Morán, Manel Esteller, Nina Gramunt, Karine Fauria, Jordi Camí, José L Molinuevo and Juan D Gispert

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Rojas S, Brugulat-Serrat A, Bargalló N, Minguillón C, Tucholka A, Falcon C, et al. Higher prevalence of cerebral white matter hyperintensities in homozygous APOE-ε4 allele carriers aged 45–75: Results from the ALFA study. J Cereb Blood Flow Metab. 2018 Feb 1;38(2):250–61. DOI: 10.1177/0271678X17707397

Chapter 3

Spatial patterns of white matter hyperintensities associated with Alzheimer's disease risk factors in a cognitively healthy middle-aged cohort

Gemma Salvadó, Anna Brugulat-Serrat, Carole H. Sudre, Oriol Grau-Rivera, Marc Suárez-Calvet, Carles Falcon, Karine Fauria, M. Jorge Cardoso, Frederik Barkhof, José Luis Molinuevo, Juan Domingo Gispert

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Salvadó G, Brugulat-Serrat A, Sudre CH, Grau-Rivera O, Suárez-Calvet M, Falcon C, et al. Spatial patterns of white matter hyperintensities associated with Alzheimer's disease risk factors in a cognitively healthy middle-aged cohort. Alzheimer's Res Ther. 2019 Jan 24;11(1). DOI: 10.1186/s13195-018-0460-1

Chapter 4

Patterns of white matter hyperintensities associated to cognition in middle-aged cognitively healthy individuals

Anna Brugulat-Serrat*, Gemma Salvadó*, Carole H. Sudre, Oriol Grau-Rivera, Marc Suárez-Calvet, Carles Falcon, Gonzalo Sánchez-Benavides, Nina Gramunt, Karine Fauria, M. Jorge Cardoso, Frederik Barkhof, José Luis Molinuevo, Juan Domingo Gispert

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Chapter 5

White matter hyperintensities mediate gray matter volume and executive function relationship in cognitively unimpaired individuals

Anna Brugulat-Serrat*, Gemma Salvadó*, Grégory Opertoa, Raffaele Cacciagliaa, Carole H. Sudre, Oriol Grau-Riveraa, Marc Suárez-Calveta, Carles Falcona, Gonzalo Sánchez-Benavidesa, Nina Gramunta, Carolina Minguillona, Karine Fauriaa, Frederik Barkhofd, José Luis Molinuevo, Juan Domingo Gispert

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Brugulat-Serrat A, Salvadó G, Sudre CH, Grau-Rivera O, Suárez-Calvet M, et al. Patterns of White matter hyperintensities associated to cognition in middle-aged cognitively healthy individuals. Under review.

WHITE MATTER HYPERINTENSITIES MEDIATE GRAY MATTER VOLUME AND EXECUTIVE FUNCTION RELATIONSHIP IN COGNITIVELY UNIMPAIRED PARTICIPANTS

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Disclosures

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ABSTRACT

White matter hyperintensities (WMH) have been extensively associated with cognitive impairment and reductions in gray matter volume (GMv) independently. This study explored whether WMH lesion volume mediates the relationship between cerebral patterns of GMv and cognition in in 521 middle-aged (mean age 57.7 years) cognitively unimpaired middle-aged participants. Episodic memory (EM) was measured with the Memory Binding Test and executive functions (EF) using five WAIS-IV subtests. WMH were determined from T2 and FLAIR sequences and characterized using diffusion weighted imaging (DWI) parameters. WMH volume was entered as a mediator in a voxel-wise mediation analysis relating GMv and cognitive performance. The mediation model was corrected by sociodemographic factors, *APOE*-ε4 and total intracranial volume. We found that, even though the very low levels of WMH burden (median of 3.2 ml), higher WMH lesion volume was significantly associated to a widespread pattern of lower GMv in temporal, frontal, and cerebellar areas. DWI diffusivity parameters in WMH were compatible with demyelination and axonal loss. Therefore, we found that even in cognitively unimpaired middle-aged participants, higher WMH indirectly impact the GMv and EF relationship, but not EM, suggesting a strong link between axonal injury and neuronal loss. These findings lead to reflect on the relevance of the control of cardiovascular risk factors in individuals at higher risk of developing WMH as a valuable preventive strategy to reduce or delay cognitive decline.

Keywords: aging cognition, white matter lesions, prevention, hypertension, vascular risk factors

INTRODUCTION

White matter hyperintensities (WMH) are commonly detected in the brain elderly individuals through magnetic resonance imaging (MRI) [Longstreth et al., 1996] and are thought to have a vascular aetiology, although their histopathological substrate might be heterogeneous as shown by pathological studies [Mortamais et al., 2014; Prins and Scheltens, 2015] as well as by microstructural characterization of WMH with diffusion-weighted MRI imaging [Bastin et al., 2009; Wardlaw et al., 2015; Zhong and Lou, 2016]. Even they are relatively frequent in asymptomatic individuals [Arvanitakis et al., 2016; Birdsill et al., 2014; Brugulat-Serrat et al., 2017; Kloppenborg et al., 2014], WMH burden has been shown to exert a negative impact on cognition [Arvanitakis et al., 2016; Bolandzadeh et al., 2012; Jiang et al., 2018; Lampe et al., 2017] mainly in executive function (EF) [Desmond, 2002; de Groot et al., 2000; Jiang et al., 2018; Kloppenborg et al., 2014; Ramirez et al., 2014]. WMH also increase the risk of cognitive decline and Alzheimer's Disease (AD), contributing to its progression and severity [Habes et al., 2016; Smith et al., 2008]. Risk factors of WMH overlap with those of AD such as ageing, hypertension, hypercholesterolemia and diabetes [Christiane et al., 2010; Habes et al., 2016; Jeerakathil et al., 2004; Kivipelto et al., 2006; Murray et al., 2005; Salvadó et al., 2019]. Regarding hereditary risk factors for AD, *Apolipoprotein* E (*APOE*) ε4 allele is the major known genetic risk factor for AD [Corder et al., 1993; Farrer et al., 1997; Izaks et al., 2011; Jack et al., 2015; Lim et al., 2015] and has also been associated with increased WMH load [Brickman et al., 2014; Rojas et al., 2017; Schilling et al., 2013].

Normal aging is characterized by gray matter volume (GMv) loss [Ramanoël et al., 2018] being the frontal and temporal lobes the regions with the highest degree of GM loss [Driscoll et al., 2009], which is also considered a marker of neurodegeneration [Dong et al., 2015]. Cross-sectional studies have also revealed a regional relationship between GMv and cognition in cognitively unimpaired individuals [Duarte et al., 2006; Tuladhar et al., 2014], relying EF and episodic memory (EM) on non-overlapping cerebral networks [Cacciaglia et al., 2018b]. As efforts to reduce the global burden of dementia progressively shift towards prevention, a better understanding of

the mechanisms linking WMH with neurodegeneration is of utmost importance [Prins and Scheltens, 2015; Winblad et al., 2016].

Prior studies have shown that high WMH burden are associated with total GM atrophy [Wen et al., 2006] in the temporal lobe [Habes et al., 2016; Rizvi et al., 2018; Swardfager et al., 2018] and the frontal cortex [Raji et al., 2012; Rizvi et al., 2018]. However, the regional patterns of brain atrophy associated with higher WMH burden are still not widely understood in cognitively unimpaired young individuals.

Nonetheless, few studies have explored the effects on cognition on both WMH and GM volume into account simultaneously. A recent cross-sectional study found that global and regional cortical thickness mediated the relationship between WMH and global cognition in a mixed sample of cognitively healthy individuals, mild cognitive impairment (MCI) and AD patients [Rizvi et al., 2018]. Authors also sought for the effect in AD-related regions, such as the entorhinal cortex and the hippocampus and showed an indirect effect on the association of frontal, parietal and occipital WMH with memory performance. Previous studies showed that GMv mediated the association between WMH burden with EF and EM, again in non-demented individuals [Knopman et al., 2015], and in mixed populations of individuals with cardiovascular risk factors and AD patients[Swardfager et al., 2018] (details of these previous studies are shown in Table 1). However, the extent to which these associations are present in cognitively unimpaired individuals has not yet been assessed.

Table 1. Previous cross-sectional studies that apply mediation analysis between WMH, cortical thickness and cognition

Reference (year)	Study sample	Population	N	Age, years (SD)	Women (%)	Education, years (SD)	WMH measurement	WMH volume, cm^3	Cortical thickness measurement	Cortical thickness, mm	Cognition	Mediation
Rizvi, et al. (2018)	Washington Heights-Inwood Columbia Aging Project (WHICAP)	CU MCI AD	519	73.98 (5.6)	56.2	12.8 (4.5)	Volumetric	5.5 (7.1)	Global ROI-based	2.434 (0.115)	Global Memory	X: WMH Y: Cognition M: Cortical thinning[a]
Swardfager, et al. (2018)	Sunnybrook Dementia Study	CU MCI AD	702	70.7 (9.4)	46.2	14.1 (3.7)	Volumetric	7.7 (12.4)	Temporal ROI-based	Inferred as BPF[d]	Memory	X: WMH Y: Verbal recall M1: Left temporal atrophy M2: Verbal learning[b]
Knopman, et al. (2015)	Atherosclerosis Risk in Communities (ARIC)	CU	1906	75.55 (5.21)	58	37% <11 years	Volumetric	17.29 (16.86)	ROI-based	Hippocampal= 6.89 (0.93) Posterior ROI= 59.07 (6.90) Frontal ROI= 150.21 (16.00)	Memory Executive Function Language	X: WMH Y: Cognition M: Cortical thinning[c]

Abbreviations: AD, Alzheimer's disease; BPF, CU, cognitively normal; MCI, mild cognitive impairment; M, mediation; ROI, region of interest; TIV, total intracranial volume; WMH; white matter hyperintensities

[a] Model adjusted by age, education, TIV

[b] BPF, brain parenchymal fraction. Left temporal atrophy= 0.78 (0.06)

[c] Model adjusted by age, education, sex and TIV

[d] Model adjusted by age, sex, race, education, history of diabetes mellitus, history of hypertension, history of alcohol use, history of smoking, *APOE*-ε4 genotype and TIV

In this work, we examine in cognitively unimpaired middle-aged participants the mediating role of WMH lesion volume in the relationship between cognition and topographical patterns of GMv. To this end, we used the Multilevel Mediation and Moderation (M3) toolbox [Wager et al., 2008; Wager et al., 2009] for voxel-based morphometry (VBM) imaging data. This method enables the evaluation of mediation effects in a topographically unbiased way. To better understand the pathological substrate of WMH in our healthy sample, we further characterized WMH lesions by means of diffusion–weighted magnetic resonance (DWI) parameters.

METHODS

Participants

The ALFA (for ALzheimer and FAmilies) cohort, established by the Barcelonaβeta Brain Research Center (BBRC), is composed by 2743 cognitively normal participants aged between 45 and 75 years [Molinuevo et al., 2016]. A subset of 608 participants was selected to participate in the present study (Clinicaltrials.gov Identifier: NCT02198586) that was approved by the Ethics Committee of the ''Parc de Salut Mar'' (Barcelona, Spain; MRI/ FBB2014v1.0). A detailed description of the inclusion criteria can be found in [Cacciaglia et al., 2018a] but in brief, those subjects carriers of *APOE*-ε4 and *APOE*-ε2 and those with family history of AD were prioritized. All participants accepted the study procedures by signing the informed consent. 576 participants provided with valid MRI scans, out of which 32 had to be discarded due to MRI incidental findings and 11 due to poor image quality. From 533 remaining participants, 12 were excluded due to WMH segmentation failure, rendering a final sample of 521 participants. The mean age of the final sample was 57.7 years and 60.5% were women. Sixty-four participants (12.3%) were *APOE*-ε4 homozygotes, 201 (38.6%) were *APOE*-ε4 heterozygotes and 256 (49.2%) were ε4 allele non-carriers. The median WMH load was 3.2 ml. The main characteristics of the participants are displayed in Table 2.

Table 2. Characteristics of the study population (N=521)	
Age,years, mean (SD), [range]	57.7 (7.4) [44-75 years]
Sex, female, No. (%)	315 (60.5)
Education, years, mean (SD)	13.7 (3.6)
Number of *APOE*-ε4 alleles, No. (%)	
None	256 (49.2)
One *APOE*- ε4 alleles	201 (38.6)
Two *APOE*- ε4 alleles	64 (12.3)
TIV, ml, (Q1-Q3)	1416 (1328-1490)
WMH volume, ml, (Q1-Q3)	3.2 [1.09-3.69]
Periventricular WMH volume, ml, (Q1-Q3)	1.23 [0.53-1.55]
Deep WMH volume, ml, (Q1-Q3)	1.10 [0.25-1.14]
Juxtacortical WMH, ml, (Q1-Q3)	0.68 [0.16-0.75]
Cognitive evaluation, mean (SD)	
Episodic Memory	0.01 (0.9)
Executive Function	0.01 (0.6)
Memory Binding Test	
Total Paired Recall (0-32)	24.1 (4.6)
Total Free Recall (0-32)	16.5 (5.2)
Paired Recall Pairs (0-16)	9.2 (3.4)
Total Delayed Free Recall (0-32)	16.9 (5.3)
Total Delayed Paired Recall (0-32)	23.9 (4.7)
Pairs in Delayed Free Recall (0-16)	6.4 (3.1)
Semantic Proactive Interference (%)	75.3 (18.8)
WAIS-IV subtests	
Visual Puzzles (0-26)	13.3 (4.3)
Digit Span Forward (0-16)	8.5 (2.1)
Digit Span Backward (0-16)	8.0 (2.1)
Digit Span Sequencing (0-16)	8.4 (2.1)
Matrix Reasoning (0-26)	16.4 (4.3)
Similarities (0-36)	22.6 (4.7)
Coding (0-135)	65.6 (15.1)

Cognitive measures

The Memory Binding Test (MBT) [Buschke, 2014] was used to evaluate verbal EM. This test assesses immediate and delayed retention of verbal information (after a lapse of 25 to 35 minutes) through a controlled learning process of two lists of 16 words belonging to 16 different semantic categories presented in the same order. Further detail on the administration procedure of the MBT and an exhaustive description of each of the variables can be found in [Gramunt et al., 2015]. We analyzed seven MBT main outcomes corresponding to two main areas: learning and immediate recall, and delayed recall. EF was assessed by means of five WAIS-IV subtests: the Digit span (immediate and working memory): forward, backward and sequencing; Coding subtest (processing speed and attention); Matrix reasoning and Visual puzzles (fluid intelligence); and Similarities (abstract verbal reasoning).

MRI acquisition and processing

MRI scans for all participants were acquired on the same3.0 T scanner (GE Discovery MR750 W 3T) using the same protocol that included a T1-, three T2-weighted sequences (fluid-attenuated inversion recovery [FLAIR], fast spin echo [FSE] and gradient echo [GRE]) and diffusion-weighted (DWI) imaging sequence. The T1-weighted sequence had an isotropic voxel size of $1mm^3$ with a matrix size of 256 × 256 × 160 (TR/TE/TI = 8.0/3.7/450ms, NSA= 1, flip angle = 8°). T2 and T2*-weighted sequences, with a voxel size of 1 × 1 × 3 mm, were as follows: fluid attenuation inversion recovery (FLAIR: TR/TE/TI = 11,000/90/2600 ms, flip angle = 160°), fast spin echo (TR/TE = 5000/85 ms, flip angle = 110°), and gradient echo (GRE: TR/TE = 1300/23 ms, flip angle = 15°). Finally, DW volumes acquired with 64 distinct diffusion-encoding directions ($b = 1000$ $s \cdot mm^{-2}$). The field of view was 256 × 256 mm, and the imaging matrix was 128 × 128 with 56 slices and slice thickness 2 mm, giving 2-mm isotropic voxels.

The 3D-T1w images were segmented into GM and WM tissue using the new segment function implemented in Statistical Parametric Mapping software (SPM 12, Wellcome Department of Imaging Neuroscience, London, UK), and located into a common space for

subsequent normalization using a 9-affine parameter transformation. Segmented images were then used to generate a reference template object of the sample, which was warped into a standard Montreal Neurological Institute (MNI) space using the high dimensional DARTEL toolbox [Ashburner, 2007]. The generated flow fields and normalization parameters were then implemented to normalize the native GM and WM images to the MNI space. In order to preserve the native local amount of GM as well as WM volume, we applied a modulation step, where each voxel signal's intensity was multiplied by the Jacobian determinants derived from the normalization procedure [Good et al., 2001]. Quality control of normalization was assured by checking the sample homogeneity with the computational anatomy toolbox (CAT12) (http://dbm.neuro.uni-jena.de/cat/) using non-smoothed data, which did not return errors in the registration procedure in any subject. Finally, images were spatially smoothed with a 8 mm full-width at half maximum (FWHM) Gaussian kernel. Total intracranial volume (TIV) was computed by summing the segmented GM, WM, and CSF for each individual.

WMH were automatically segmented using a Bayesian algorithm [Sudre et al., 2015] and quality control of this segmentation was performed visually for each participant by a trained rater. In short, T1-weighted, T2-weighted and T2-FLAIR images are rigidly coregistered using the NiftyReg package [Modat et al., 2014]. The data is then modelled as a multivariate Gaussian mixture model that simultaneously accounts for healthy tissue and unexpected observations and is constrained by participant-specific statistical tissue priors derived from the Geodesic Information Flows (GIF) algorithm [Cardoso et al., 2015]. The number of required Gaussian components is dynamically determined on a patient level to ensure a balance between model fit and complexity using the Bayesian Inference Criterion. Once the model has converged, a post-processing step is applied to extract probability maps of candidate lesion voxels that are then further corrected for spurious false positive detection using the output of the parcellation algorithm to avoid regions prone to artefacts. Volumetric measurements are derived as the sum of this probability map over a region of interest. WMH volumes were also calculated in periventricular WMH (PVWMH), deep WMH (DWMH) and juxtacortical WMH (JCWMH) as described previously [Sudre et al., 2017].

The WMH probability maps first registered to T1-space were then registered to DWI space using ANTs' non-linear algorithm [Avants et al., 2009]. DWI images were used to generate fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AxD), and radial diffusivity (RD) maps using the FSL Diffusion Toolbox [Jenkinson et al., 2012]. DWI images were normalized to the MNI standard space by coregistering all participants' FA data to a common space using FMRIB's software Library (http://www.fmrib.ox.ac.uk/fsl) Non-linear Image Registration Tool. These same parameters were applied to T1-space WMH probability maps to warp them to the same MNI-space group template. Then, DWI parameters were recorded in voxels categorized as WMH as well as in normally appearing white matter (NAWM) in equivalent white matter locations across the group. This last step is explained further in section Statistical analysis / DWI parameters.

Statistical analysis

Cognitive outcomes

First, we computed two global z-scores for the cognitive measures: EM from MBT and EF from WAIS-IV subtests. These global measures were calculated by averaging normalized raw scores of all subtests in each domain. Supporting Information Figure 1 shows the cross-correlation and statistical significance between pairs of cognitive scores.

DWI parameters

To better characterize WMH in our sample, we compared DWI parameters (FA, MD, AxD and RD) between WMH and NAWM in the same brain locations. In order to restrict the subsequent analysis only to voxels with the highest-class probability (of being WMH or NAWM) and to ensure that interclass comparisons would be performed on sufficient participants across the group, we filtered voxels based on the two following rules. First, voxels with a probability of being WMH higher than 0.9 (lower than 0.1) were classified as WMH (NAWM, respectively). Voxels with probabilities between 0.1 and 0.9 were left out of this analysis. Then, we counted the numbers of participants in which every voxel was classified either as a WMH

or as NAWM and included a voxel only if these two numbers were both higher than 10 subjects. In these voxels only, values of the DWI parameters were compared between WMH and NAWM using t-test. Differences in DWI parameters in WMH tissue were calculated as the percentage of change with respect NAWM: (DWI_{WMH}-DWI_{NAWM})/ DWINAWM; and a two-sample t-test and a threshold for significance of $p<0.05$ was carried out.

Mediation analysis

First, we tested the normality distribution of WMH load (Supporting information Figure 2). Given the great skewness of the distribution of WMH load, which log-transformation did not solve all the following analysis were performed with non-parametric techniques.

In the mediation analysis, we defined GMv as the predictor (X), WMH (global and divided by distance to the ventricle; PVWMH, DWMH and JCWMH, or by lobes) as the mediator (M) and cognitive performance as outcome (Y). See Figure 1 for a schematic representation of the mediation analysis and accompanying definitions. The VBM pairwise and mediation analyses were performed using the Multilevel Mediation and Moderation (M3) Toolbox of SPM [Wager et al., 2008; Wager et al., 2009] with bootstrapping techniques to address the non-normal distribution of WMH volumes. This method partitions the variance shared by X and Y (total effect, denoted as *c*) into two components: one mediated by M (indirect effect; *ab*) and another one independent from M (direct effect; *c*'). Cognitive performance was analyzed by both global z-scores (EM and EF) and all the outcomes of MBT and WAIS-IV individually. Age, sex, education, TIV and *APOE* (number of ε4 alleles) were introduced as confounders in the VBM analysis adjusting all the paths associations between mediators and outcomes.

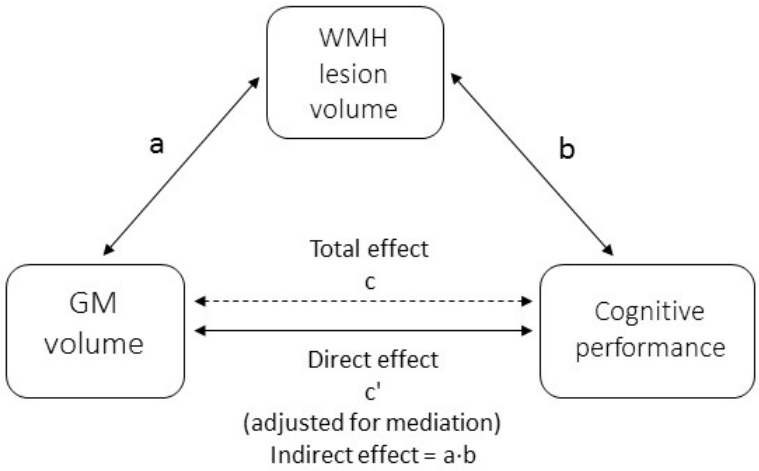

Figure 1. Schematic illustration of the mediation statistical model running in the study. All paths were adjusted for age, sex, education, TIV and number of *APOE*-ε4 alleles. Abbreviations: WMH, white matter hyperintensities; GMv, gray matter volume; TIV, total intracranial volume.

We looked at all the relationships or paths as they all give some relevant information. First, we looked at the total association between GMv and cognition (total effect; *c*) by the VBM analysis. Secondly, we evaluated pairwise associations between GMv-WMH burden (path a) and WMH burden-cognition (path b) independently. The correlation between WMH burden and cognition was the only path not to include GMv, for this reason we tested it independently, with Spearman's rank test (adjusting for the same covariates as in the rest of the analysis). Next, we tested the mediation effect (path *ab*). And, finally, we assessed the association between GMv and cognition after removing the mediation effect (direct effect; *c*'). Statistical significance threshold was set to $p<0.005$. This threshold is the most commonly one used with the bootstrapping implementation in the M3 toolbox and provides a good balance between control of false positives and sensitivity [Wager et al., 2008; Wager et al., 2009]. For total, direct and indirect effects we computed the average β coefficient of significant clusters found by voxel-wise analysis within brain regions defined by Neuromorphometrics atlas (www.

neuromorphometrics.com). Afterward, the proportion mediated was calculated; $1-\left(\frac{\hat{c}'}{\hat{c}}\right)=\frac{\hat{a}\hat{b}}{\hat{a}\hat{b}+\hat{c}'}$ (being $\wedge$ the absolute value) [Fleming and DeMets, 1996].

For all paths that included GMv, we only reported as significant areas those that were bigger than 5% of the region of interest (ROI) from Neuromorphometrics atlas, and that included more than 100 voxels. The maximum Z effect was computed for each significant brain regions.

Additional analysis

As complementary analyses, we replicated the mediation analysis defining WMH as predictor (X) and GM volume as mediator (M) to assess the impact of the interaction between WMH and EF on GMv, after correction by the previously described confounders.

RESULTS

WMH characterization by DWI parameters

The mean percentage of change of DWI metrics compared for all voxels in equivalent brain locations between WMH and NAWM is represented in Figure 2. WMH showed lower FA (-8.46%; 95%CI: [-7.84%, -9.08%]; $p<0.001$) and increased diffusivity, particularly in the radial direction (MD: +4.40%; 95%CI: [3.68%, 5.12%]; $p<0.001$; AxD: +1.40%; 95%CI: [0.82%, 1.97%]; $p<0.001$; RD: +7.08%; 95%CI: [6.23%, 7.93%]; $p<0.001$).

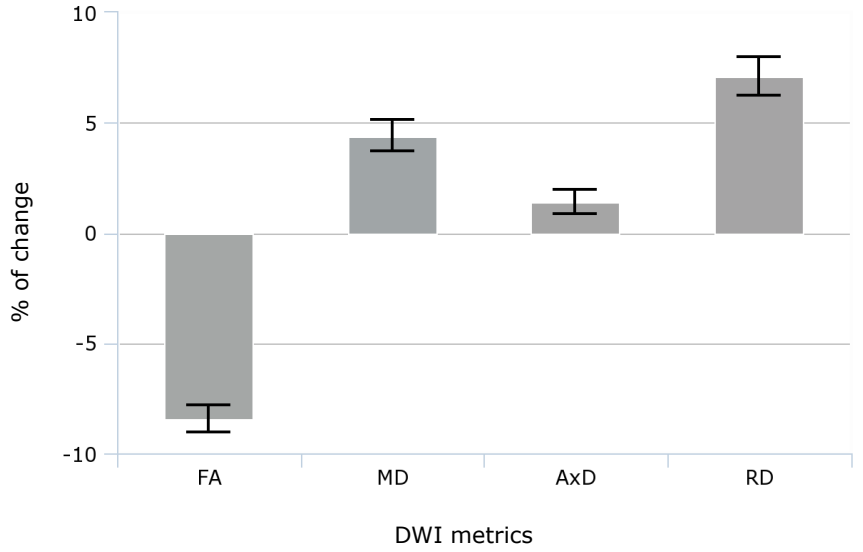

Figure 2. Mean percentage of change of DWI metrics compared in equivalent brain locations between WMH and NAWM. WMH showed significant lower FA and increased diffusivity. RD shows significantly larger changes than in AxD in WMH. All the differences were statistically significant at $p<0.001$. Error bars show 95% of CI. Abbreviations: FA, fractional anisotropy; MD, mean diffusivity; AxD, axial diffusivity; RD, radial diffusivity.

Pairwise Associations

GM vs WMH load (path *a*)

We found that a greater WMH lesion volume was associated with lower GMv (Figure 3) in specific brain regions, mainly in temporal and in frontal areas (Supporting Information Table 2). The regions with greater ROI percentage of this direct effect were the bilateral nucleus accumbens (R=59.0%, L=55.98%), right amygdala (55.02%), and right caudate nucleus (48.41%).

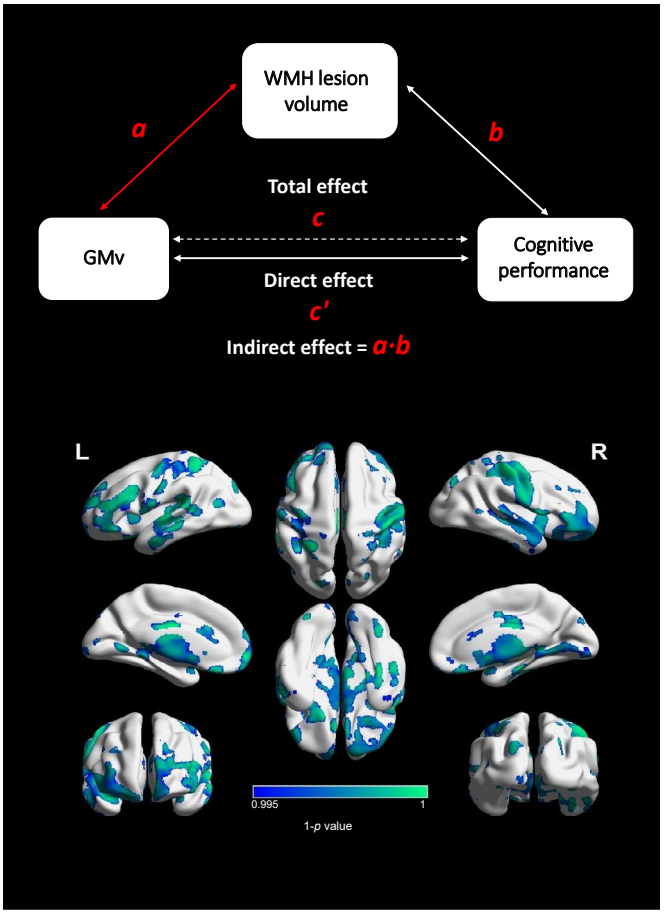

Figure 3. Association between GM volume and WMH burden (path *a*). A greater WMH lesion volume was associated with lower GMv in specific brain regions, mainly in parietal, temporal, and in frontal areas. Cold colorbar = negative relationship. Abbreviations: WMH, white matter hyperintensities; GMv, gray matter volume.

WMH load vs Cognition (path *b*)

Global WMH was significantly associated with lower performance on EM (Rho=-0.07, p=0.04) and EF (Rho=-0.07, p=0.04) (Table 3). Some subtests showed also a significant correlation with WMH: Digit Span Backwards (Rho=-0.09, p=0.01), Total Delayed Free recall (Rho=-0.07, p=0.04), Total Delayed Paired Recall (Rho=-0.07, p=0.04), Pairs in Delayed Free Recall (Rho=-0.08, p=0.03) and Semantic Proactive Interference (Rho=-0.08, p=0.03). Spearman's

rho values for correlations between regional WMH load divided distance to the ventricles and cognition are shown in Supporting Information Table 1.

Table 3. Correlation between global WMH load and cognition [a]

	Rho [95% CI]	***p***
Cognitive z-score composites		
Episodic Memory	-0.07 [-0.156 - 0.001]	**0.04***
Executive Function	-0.07 [-0.152 - 0.012]	**0.04***
Memory Binding Test		
Total Free Recall	-0.05 [-0.131 - 0.029]	0.12
Total Delayed Free Recall	-0.07 [-0.156 - 0.001]	**0.04***
Total Paired Recall	-0.05 [-0.130 - 0.037]	0.14
Total Delayed Paired Recall	-0.07 [-0.155 - -0.001]	**0.04***
Paired Recall Pairs	-0.06 [-0.146 - 0.019]	0.07
Pairs in Delayed Free Recall	-0.08 [-0.166 - -0.001]	**0.03***
Semantic Proactive Interference	-0.08 [-0.161 - 5.5e-05]	**0.03***
Subtests of WAIS-IV		
Digit Span Forward	-0.01[-0.090 – 0.079]	0.42
Digit Span Backward	-0.09 [-0.174 - -0.001]	**0.01***
Digit Span Sequencing	0.03 [-0.056 - 0.110]	0.28
Coding	-0.06 [-0.135 - 0.023]	0.08
Visual Puzzles	-0.01 [-0.095 - 0.070]	0.39
Matrix Reasoning	-0.05 [-0.129 - 0.031]	0.12
Similarities	-0.06 [-0.143 - 0.024]	0.08

[a] Cognition adjusted by age, sex, education and number of APOE-ε4 alleles. WMH also adjusted by TIV.
* $p < 0.05$.

GM vs Cognition (Total effect; path *c*)

A significant association between GMv and cognition was found with non-overlapping patterns linked to EF and EM (Figure 4). We found a significant total effect between GMv and EF (β average=0.4247), but not in EM (Table 4).

Table 4. Average β of total (*c*), direct (*c'*) and mediated (*ab*) effect of significant brain regions

	Total effect (*c*)	Direct effect (*c'*)	Indirect effect (*ab*)	PM[a]
	β	β	β	%
Cognition Composites				
Episodic Memory	NS	NS	NS	-
Executive Function	0.4247	0.2296	0.1952	45.95
Executive Function Outcomes				
Coding	4.9412	0.2462	4.6950	95.02
Digit Span Backward	-0.3666	-0.8253	0.4587	IMM[b]
Matrix Reasoning	-4.8647	-7.0463	2.1817	IMM[b]
Similarities	-6.4325	-8.9388	2.5063	IMM[b]
Mediation effect of WMH on EF by distance to ventricles				
PVWMH	0.0961	-0.1006	0.1968	IMM[b]
DWMH	0.6436	0.4531	0.1950	29.60
JCWMH	-0.9364	-1.0921	0.1557	IMM[b]

Abbreviations: PVWM, periventricular white matter hyperintensities; DWMH, deep white matter hyperintensities; JCWMH, juxtacortical white matter hyperintensities; PM, percentage of mediation; NS, Non-Significant; IMM, inconsistent mediation model.

[a] PM; percentage of mediation= indirect effect β coefficient/total effect β coefficient

[b] IMM; inconsistent mediation model [MacKinnon et al., 2000]

All average β values were significant (p<0.005)

Mediation effects across GMv and cognitive performance through WMH load

Indirect effect (*ab*)

We found a significant partial mediation effect (averageβ=0.1952) of WMH lesion volume in the relationship between GMv and brain regions involved in EF performance (Figure 5). The significant brain

regions with greater ROI percentage of mediation effect were right frontal regions (orbital gyrus: anterior; 84.7%, medial; 64.7%, lateral; 75.0% and posterior; 60.8%), but also temporal (right subcallosal area (65.8%) and right transverse temporal gyrus (62.7%)), and parietal regions (parietal operculum (53.6%)) (Supporting Information Table 3). On the other hand, we did not find a significant mediation effect of WMH in GMv of regions involved in EM performance (Figure 6).

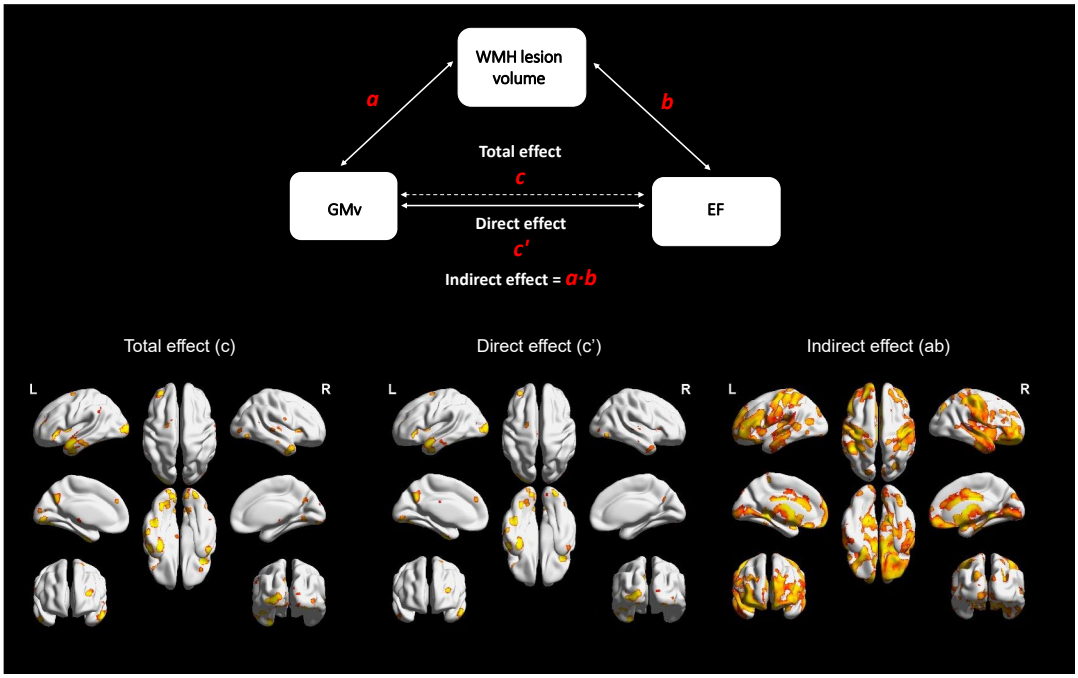

Figure 5. Mediation results from executive function performance. Total effect (path *c*) = GMv-EF relationship. Direct effect (path *c*') = pure effect of GMv in cognitive domain performance (removing the mediation effect). Indirect effect (path *ab*) = mediation effect across GMv and EF performance through WMH load. After discounting the mediation effect, a direct significant association (path *c*') remained between GMv in the temporal pole, inferior temporal and the insular in EF. Statistical significance was set at $p<0.005$. Paths were adjusted for mediator-outcomes confounders: age, sex, education TIV and number of *APOE*-ε4 alleles. Hot colorbar = positive relationship. Abbreviations: WMH, white matter hyperintensities; GMv, gray matter volume; EF, executive function.

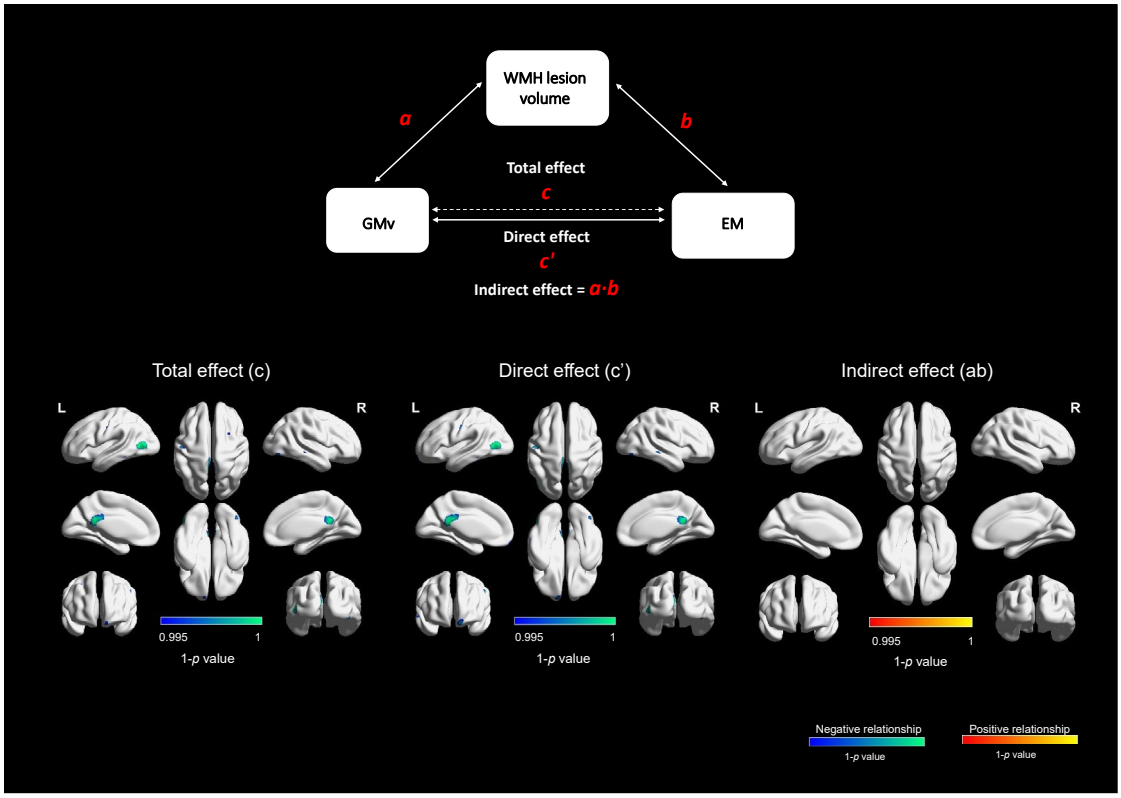

Figure 6. Mediation results from episodic memory performance. Total effect (path *c*) = GMv-EM relationship. Direct effect (path *c'*) = pure effect of GMv in EM (removing the mediation effect). Indirect effect (path *ab*) = mediation effect across GMv and EM performance through WMH load. We did not find any significant mediation effect in EM performance. Statistical significance was set at $p<0.005$. Paths were adjusted for mediator-outcomes confounders: age, sex, education TIV and number of *APOE*-ε4 alleles. Cold colorbar = negative relationship Abbreviations: WMH, white matter hyperintensities; GMv, gray matter volume, executive function; EM, episodic memory; TIV, total intracranial volume.

When we repeated the mediation analysis for EF including as mediator WMH as distance to the ventricle, we found that the indirect effect of WMH on EF through GMv showed more significant regions for DWMH (β=0.1950) than for PVWMH (β average=0.1969) and JCWMH (average β=0.1557) (Supporting Information Figure 3). Specifically, about 29.60% of the total effect of GMv on EF was mediated by DWMH load mainly in temporal and frontal regions (Table 4).

Regarding specific cognitive tests, we found significant mediation effect of WMH in 4/7 outcomes of EF: Coding (average β=4.6950), Digit Span Backward (DSB) (average β=0.4587), (Supporting Information Figure 4), Matrix reasoning (average β=2.1817) and Similarities (average β=2.5063) (Table 4). Coding displayed an effect size that was one order of magnitude higher than the rest of outcomes; about 95.02% of the total effect of GMv on Coding was mediated by WMH load (Table 4), specifically, in temporal and frontal regions was mainly driven by the mediation effects (*ab*) of DWMH (Table 4 and Figure 5 of Supporting Information). On the other hand, the results of PVWMH and JCWMH mediation effect on DSB, Matrix and Similarities performance showed inconsistent mediation models [MacKinnon et al., 2000].

Direct effect (*c*')

After discounting the mediation effect, a direct significant association remained between GMv and EF performance in the temporal pole, inferior temporal and the insulae (average β=0.2296) (Figure 5). The total effect found between GMv and EM remained significant in the same locations as direct effect (Figure 6).

Additional analysis

In the mediation analysis defining WMH as predictor (X) and GMv as mediator (M), we did not find a GMv mediation effect across the relationship between WMH load and EF performance (Supporting Information Figure 6).

DISCUSSION

In this work, we have studied whether WMH volume mediates the relationship between GMv and cognition in cognitively unimpaired middle-aged participants to extend previous findings [Knopman et al., 2015; Rizvi et al., 2018; Swardfager et al., 2018; Tuladhar et al., 2014], to a younger and healthier cohort both from the cognitive and cerebrovascular standpoint. Our results show that, even in such a low risk population, higher WMH lesion volume is significantly

associated to a widespread pattern of lower GMv in frontal, occipital and in temporal regions, including the hippocampus, as well as in the thalamus and cerebellum. In turn, WMH volume mediates the relationship between GMv and EF in frontal and temporal regions, including the hippocampus, as well as in the thalamus. Instead, regions associated with EM were spared from the effect of WMH and the mediation effect was not found to be significant. As has been suggested in previous works, changes in DWI metrics may be related to biological interpretations [Alexander et al., 2007; Melhem et al., 2002; Song et al., 2002; Zhang et al., 2012]. In our results, characterization of WMH lesions through DWI metrics was found compatible with axonal loss (suggested by significantly lower FA values and increased diffusivity in WMH vs NAWM in equivalent brain locations) and demyelination (significantly larger changes in RD than in AxD in WMH). Nevertheless, interpretation of the microstructural alterations leading to altered DWI parameters should be done with caution as they can be related to multiple causes [Scholz et al., 2013].

Previous cross-sectional studies that used mediation pathway analysis selected WMH as predictor, whose association with cognition was mediated by GMv [Rizvi et al., 2018; Tuladhar et al., 2014]. These are in line with longitudinal studies in unimpaired individuals that showed that WMH might be associated to subsequent GM atrophy, independently of AD biomarkers [Barnes et al., 2013] or vascular risk factors [Kloppenborg et al., 2012]. However, in the present study, we did not find significant mediation effects with this model; due to the low incidence of WMH load in our healthy population, the behaviour of indirect effect (*ab*) was maybe driven by the higher association of GMv and EF relationship (path *b*).

For mediation to be considered as significant, paths a, b and ab are required to show significant associations [MacKinnon et al., 2007]. These requirements are met in our own findings and are also supported by previous literature. We found that greater WMH lesion volume related to lower GMv in extensive brain regions comprising temporal, frontal and occipital areas, thalamus and cerebellum. Previous studies showed a relationship between WMH and global GM atrophy also in the temporal [Habes et al., 2016; Swardfager et al., 2018], and

frontal cortex [Raji et al., 2012], and in the bilateral hippocampus [Habes et al., 2016]. However, to the best of our knowledge, no previous research has sought for the joint impact of both WMH and GMv on cognition using voxel-wise approach [Rizvi et al., 2018; Wen et al., 2006]. Regarding path c, we had previously established in this cohort that EM and EF rely on non-overlapping cerebral structural networks [Cacciaglia et al., 2018b]. In the present study, the mediation analysis (path *ab*) showed a significant indirect effect of GMv on EF performance through WMH lesion volume regionally, such as in frontal and temporal areas. This regional pattern on the mediation effect resulted from the concatenation of the associations between WMH and GMv and from this to EF. Nevertheless, after discounting the mediation effect, a direct significant association (path *c*') remained between GMv and EF in the temporal pole, inferior temporal and the insulae, thus confirming our previously reported results [Cacciaglia et al., 2018b].

There might be a number of possible explanations for these findings. On the one hand, ischemic injury to the axons might impair the connectivity of distal cortical and subcortical regions and eventually lead to neuronal loss. On the other hand, there is also ample evidence that WMH may be the result of degenerative axonal loss secondary to neuronal damage due to Wallerian degeneration. Alternatively, ischemia could be leading to both vascular brain injury and neurodegeneration. Even though implicit in the mediation pathway model, analysis of cross-sectional data cannot establish a causal link between the studied variables.

When we look at the regional WMH effect, we see that the mediation effect was significant in more regions for DWMH than for PVWMH. Particularly, we found that the behavior of the EF composite was mainly driven by the indirect effects (*ab*) of DWMH in Coding. Previous literature has determined that WMH progression extends from the PVWMH to DWMH into subcortical white matter [Prins and Scheltens, 2015], and that DWMH is functionally more relevant [Wen et al., 2006] and related to reduced cognitive function than PVWMH. Coding results are consistent with an earlier study that found that psychomotor speed was partially mediated by posterior cortical regions atrophy, such as hippocampus, parahippocampus

gyrus and entorhinal cortex [Knopman et al., 2015]. In this line, we found significant mediation effects in regions belonging to the executive control network (ECN), such as the dorsolateral prefrontal cortex, the anterior cingulate gyrus [Vincent et al., 2008] and the thalamus [Marzinzik et al., 2008].

In contrast to earlier findings [Rizvi et al., 2018; Swardfager et al., 2018], we did not find significant mediation effects in regions involved with EM performance or its outcomes. The discrepant results may be explained by the fact that participants in previous studies were older than our sample and included patients with MCI and/or AD. In addition, such a discrepancy with preceding findings might also stem from the different characteristics of the test that we used to measure EM (the MBT), which may differ from those of the other memory tests used in similar reports. We previously showed that, in cognitively unimpaired middle-aged participants, better EM performance was significantly associated with lower GMv in several brain regions modulated by aging. [Cacciaglia et al., 2018b]. This result is consistent with earlier research in that WMH are mainly related to frontal-type dysfunction including impairments in attention, EF and processing speed [Desmond, 2002; Kloppenborg et al., 2014].

One of the main strengths of this study is that even our participants are younger and cognitively unimpaired with a lower WMH burden than in previous studies, we found an impact of WMH and GMv over cognition on those participants. This result highlights the importance of an early control of modifiable risk factors in those individuals at higher risk of developing WMH as a useful preventive strategy to reduce or delay the onset of dementia. Another strength is the application of a voxel-wise approach which allowed us to detect topographical patterns of GMv in an unbiased fashion compared with previous studies applying a ROI-based approach.

However, our work is not free of limitations. The highly non-normal distribution of WMH load, due to the high percentage of WMH-free participants, prevented us from using parametric statistics. Even though, the bootstrap method implemented in the M3 toolbox is robust against deviations from normality, the use of a non-parametric test is expected to have lowered the statistical power in this study.

Another limitation is the lack of core AD biomarkers. Prior research suggested that WMH load might interact with the hallmarks of AD pathology, such as abnormal deposition of β-amyloid (Aβ) [Gold et al., 2017] and tau-protein [McAleese et al., 2017] in cognitively unimpaired participants. Therefore, the study of the impact of core AD pathology on the mediation effects here described represents an important topic for future research.

CONCLUSION

We found in middle-aged cognitively unimpaired participants that WMH volume significantly mediated the association between EF, but not EM, and GMv in extensive brain regions encompassing frontal and temporal regions, including the hippocampus, as well as in the thalamus. Higher WMH lesion volume was significantly associated with a widespread pattern of lower GMv in temporal, frontal and occipital areas as well as in the thalamus and cerebellum. This study provides novel evidence of the impact of WMH on GM and cognition even in a healthy middle-aged population. In light of these results, the control of modifiable risk factors in individuals at higher risk of developing WMH might represent a valuable preventive strategy to reduce or delay the onset cognitive decline.

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Supplementary material

Table 1. Spearman's rho values from the correlation between cognition and total periventricular, deep and juxtacortical WMH

Cognitive z-score composites	**Periventricular**	**Deep**	**Juxtacortical**
Episodic Memory	-0.06	-0.06	-0.06
Executive Function	-0.06	-0.07	-0.05
Memory Binding Test			
Total Paired Recall	-0.03	-0.03	-0.05
Total Free Recall	-0.06	-0.05	-0.06
Paired Recall Pairs	-0.06	-0.06	-0.07
Total Delayed Free Recall	-0.07	-0.06	-0.05
Total Delayed Paired Recall	-0.06	-0.06	-0.08
Pairs in Delayed Free Recall	-0.09*	-0.08	-0.07
Semantic Proactive Interference	-0.08	-0.08	-0.08*
Subtests of WAIS-IV			
Visual Puzzles	0.02	-0.00	-0.02
Digit Span Forward	-0.01	-0.02	0.03
Digit Span Backward	-0.09*	-0.08	-0.02
Digit Span Sequencing	0.02	0.04	0.03
Matrix Reasoning	-0.03	-0.06	-0.04
Similarities	-0.02	-0.05	-0.08
Coding	-0.07	-0.09*	-0.10*

Cognition adjusted by age, sex, education and number of *APOE*-ε4

WMH adjusted by TIV

* $p<0.05$

Table 2. Brain regions with significant association between WMH burden and GMv (path *a*)

Number of cluster voxels	Z[a]	Brain Region[b]	Laterality	% of ROI
20465	2.60	Accumbens area	L	55.98
	2.58	Amygdala	R	55.02
	2.58	Cerebellum exterior	L	41.54
	2.58	Hippocampus	R	14.25
	2.58	Cerebellar Vermal Lobules I-V	-	12.55
	2.61	Anterior insula	L	8.80
	2.58	Angular gyrus	R	8.57
	2.58	Calcarine cortex	R	8.80
	2.58	Frontal operculum	R	5.80
9643	2.60f	Accumbens area	R	59.00
	2.58	Cerebellum exterior	L	42.72
	2.60	Anterior insula	R	9.05
5373	2.58	Cerebellum exterior	R	47.61
	2.66	Anterior cingulate gyrus	R	10.20
	2.58	Anterior cingulate gyrus	L	9.47
5186	2.58	Anterior orbital gyrus	L	23.63
4310	2.78	Caudate	R	48.41
	2.58	Hippocampus	L	15.43
	2.58	Thalamus	R	13.76
	2.58	Anterior cingulate gyrus	L	9.59
1213	2.66	Anterior cingulate gyrus	R	9.65
	2.62	Cuneus	R	7.15
1203	2.58	Thalamus	L	13.48
865	2.58	Anterior insula	R	9.29
861	2.58	Central operculum	R	7.40
	2.58	Cuneus	L	6.58
156	2.62	Cuneus	R	7.04

Abbreviations: ROI, Region of interest.

[a] Maximum Z effect for each significant brain region

[b] Brain regions with >5% of ROI and >100 voxels number

Table 3. Brain regions with significant WMH mediation effect in the association between GM volume and EF performance (path *ab*)

Number of cluster voxels	Zmax	Brain Region	Laterality	% of ROI
Executive Function				
84666	2.58	Anterior orbital gyrus	R	84.7
	2.58	Frontal Pole	L	77.3
	2.58	Lateral orbital gyrus	R	75.0
	2.58	Planum polare	R	72.4
	2.60	Accumbens	R	71.5
	2.58	Subcallosal area	R	65.8
	2.58	Medial orbital gyrus	R	64.7
	2.58	Transverse temporal gyrus	R	62.7
	2.58	Posterior orbital gyrus	R	60.8
	2.58	Central operculum	R	58.4
	2.58	Posterior orbital gyrus	L	59.3
	2.58	Medial frontal cortex	R	58.4
	2.58	Triangular part of the inferior frontal gyrus	L	57.3
	2.58	Postcentral gyrus	R	53.6
	2.58	Parahippocampal gyrus	R	52.2
	2.58	Lateral orbital gyrus	L	50.6
	2.58	Orbital part of the inferior frontal gyrus	L	49.7
	2.58	Transverse temporal gyrus	L	48.5
	2.58	Posterior insula	R	45.9
	2.58	Orbital part of the inferior frontal gyrus	R	41.8
	2.58	Triangular part of the inferior frontal gyrus	R	41.8
	2.58	Planum polare	L	41.1
	2.58	Occipital fusiform gyrus	R	40.9
	2.58	Thalamus	L	40.1
	2.58	Superior temporal gyrus	R	40.0
	2.58	Medial frontal cortex	L	38.3
	2.58	Opercular part of the inferior frontal gyrus	L	38.0
	2.58	Calcarine cortex	L	37.4
	2.58	Precentral gyrus	L	36.2
	2.58	Lingual gyrus	R	34.6
	2.58	Planum temporale	R	34.5
	2.58	Planum temporale	L	34.1
	2.58	Calcarine cortex	R	32.8
	2.58	Thalamus	R	32.1
	2.58	Frontal operculum	L	31.9
	2.58	Parietal operculum	L	31.7
	2.58	Parahippocampal gyrus	L	31.2
	2.58	Amygdala	R	30.5
	2.58	Middle frontal gyrus	R	30.0
	2.58	Anterior insula	R	27.3
	2.58	Parietal operculum	R	26.9
	2.58	Fusiform gyrus	R	26.1

	2.58	Hippocampus	R	25.6
	2.58	Superior parietal lobule	L	25.4
	2.58	Postcentral gyrus	L	25.0
	2.58	Inferior temporal gyrus	R	25.0
	2.58	Cerebellar vermal lobules VI-VIII	-	24.7
	2.58	Superior occipital gyrus	L	24.3
	2.58	Precentral gyrus	R	23.9
	2.58	Supramarginal gyrus	R	23.4
	2.58	Lingual gyrus	R	22.2
	2.68	Middle temporal gyrus	L	21.4
	2.58	Entorhinal area	R	20.9
	2.58	Opercular part of the inferior frontal gyrus	R	20.8
	2.58	Posterior insula	L	20.2
	2.58	Central operculum	L	18.1
	2.58	Middle frontal gyrus	L	18.0
	2.58	Medial orbital gyrus	L	18.0
	2.58	Superior frontal gyrus	L	17.7
	2.58	Temporal pole	R	16.1
	2.58	Anterior orbital gyrus	L	116.1
	2.59	Frontal pole	R	15.7
	2.58	Anterior insula	L	15.6
	2.58	Inferior temporal gyrus	L	14.9
	2.58	Hippocampus	L	14.3
	2.58	Cuneus	L	14.1
	2.68	Middle temporal gyrus	R	13.4
	2.58	Supramarginal gyrus	L	12.0
	2.58	Cuneus	R	11.5
	2.58	Inferior occipital gyrus	L	11.3
	2.58	Superior parietal gyrus	L	11.2
	2.58	Posterior cingulate gyrus	R	11.0
	2.58	Occipital fusiform gyrus	L	9.0
2527	2.58	Middle cingulate gyrus	R	28.4
	2.58	Middle cingulate gyrus	L	16.2
399	2.58	Superior occipital gyrus	R	11.3
Coding				
70193	2.58	Lateral orbital gyrus	R	87.6
	2.58	Anterior orbital gyrus	R	87.2
	2.58	Frontal pole	L	76.9
	2.58	Planum polare	R	69.5
	2.59	Accumbens area	R	68.0
	2.58	Central operculum	R	63.9
	2.58	Posterior orbital gyrus	R	61.8
	2.58	Medial orbital gyrus	R	57.1
	2.58	Triangular part of the inferior frontal gyrus	L	49.3
	2.58	Planum temporale	R	48.6
	2.58	Orbital part of the inferior frontal gyrus	L	47.6
	2.58	Occipital fusiform gyrus	R	45.6
	2.58	Posterior orbital gyrus	L	45.1
	2.58	Posterior insula	R	44.0
	2.58	Medial frontal cortex	R	42.9

2.58	Postcentral gyrus	R	42.6
2.58	Planum polare	L	40.1
2.58	Orbital part of the inferior frontal gyrus	R	38.8
2.60	Superior temporal gyrus	R	38.2
2.58	Medial frontal cortex	L	35.3
2.58	Parahippocampal gyrus	R	35.3
2.58	Triangular part of the inferior frontal gyrus	R	35.1
2.58	Frontal operculum	L	34.5
2.58	Calcarine cortex	R	33.3
2.58	Transverse temporal gyrus	R	33.1
2.58	Opercular part of the inferior frontal gyrus	L	33.1
2.58	Anterior Insula	R	32.3
2.58	Lateral orbital gyrus	L	32.1
2.58	Parietal operculum	L	31.3
2.58	Calcarine cortex	L	31.2
2.58	Thalamus	L	30.7
2.58	Lingual gyrus	R	30.3
2.58	Subcallosal area	L	28.3
2.58	Middle frontal gyrus	R	26.7
2.58	Lingual gyrus	L	25.3
2.58	Transverse temporal gyrus	L	25.0
2.58	Precentral gyrus	L	23.8
2.58	Central operculum	L	23.8
2.58	Parietal operculum	R	23.2
2.58	Temporal pole	R	23.1
2.58	Inferior temporal gyrus	R	22.4
2.58	Fusiform gyrus	R	22.2
2.58	Inferior temporal gyrus	L	21.7
2.58	Thalamus	R	20.5
2.58	Supramarginal gyrus	R	20.3
2.61	Middle temporal gyrus	L	20.0
2.58	Precentral gyrus	R	19.7
2.58	Parahippocampal gyrus	L	19.7
2.58	Cerebellar Vermal Lobules VI-VIII	-	18.7
2.58	Superior frontal gyrus	L	18.6
2.58	Middle frontal gyrus	L	17.8
2.58	Postcentral gyrus	L	17.5
2.58	Inferior occipital gyrus	L	17.2
2.58	Planum temporale	L	17.0
2.58	Medial orbital gyrus	L	16.3
2.58	Occipital fusiform gyrus	L	16.2
2.58	Middle temporal gyrus	R	15.1
2.58	Frontal pole	R	14.2
2.58	Posterior insula	L	13.6
2.58	Opercular part of the inferior frontal gyrus	R	13.1
2.58	Supramarginal gyrus	L	11.8
2.58	Superior frontal gyrus medial segment	L	11.2
2.58	Superior parietal gyrus	R	10.6
2.58	Cuneus	R	10.0
2.58	Posterior cingulate gyrus	R	9.8
2.70	Posterior cingulate gyrus	L	9.5

	2.58	Cuneus	L	8.9
	2.58	Hippocampus	R	7.4
	2.58	Inferior occipital gyrus	R	6.6
	2.58	Fusiform gyrus	L	5.3
1926	2.58	Middle cingulate gyrus	R	22.9
	2.58	Anterior cingulate gyrus	R	20.1
	2.58	Anterior cingulate gyrus	L	9.8
	2.58	Middle Cingulate gyrus	L	8.3
366	2.58	Opercular part of the inferior frontal gyrus	R	7.2
336	2.58	Superior occipital gyrus	R	10.4
299	2.58	Anterior insula	L	11.8
182	2.61	Superior occipital gyrus	L	16.1
Digit Span Backward				
4189	2.58	Posterior orbital gyrus	R	26.2
	2.58	Thalamus	L	9.9
	2.58	Anterior insula	R	8.5
1932	2.58	Supramarginal gyrus	R	10.1
	2.58	Postcentral gyrus	R	10.1
	2.58	Superior parietal gyrus	R	5.4
1845	2.58	Supramarginal gyrus	L	9.0
	2.58	Precentral gyrus	L	6.1
	2.58	Superior parietal lobe	L	5.3
1591	2.58	Middle cingulate gyrus	R/L	18.0/10.6
	2.58	Anterior cingulate gyrus	R/L	7.7/11.1
1591	2.58	Superior frontal gyrus	L	9.2
862	2.58	Postcentral gyrus	L	7.0
	2.58	Central operculum	L	6.5
507	2.58	Posterior orbital gyrus	L	26.1
505	2.58	Parietal operculum	R	15.2
	2.58	Central operculum	R	11.1
221	2.58	Frontal pole	L	9.3
Matrix				
163	2.58	Anterior orbital gyrus	R	6.2
Similarities				
540	2.58	Planum polare	R	15.3

Abbreviations: ROI = Region of interest

[a] Maximum Z effect for each significant brain region

[b] Brain regions with >5% of ROI and >100 voxels number

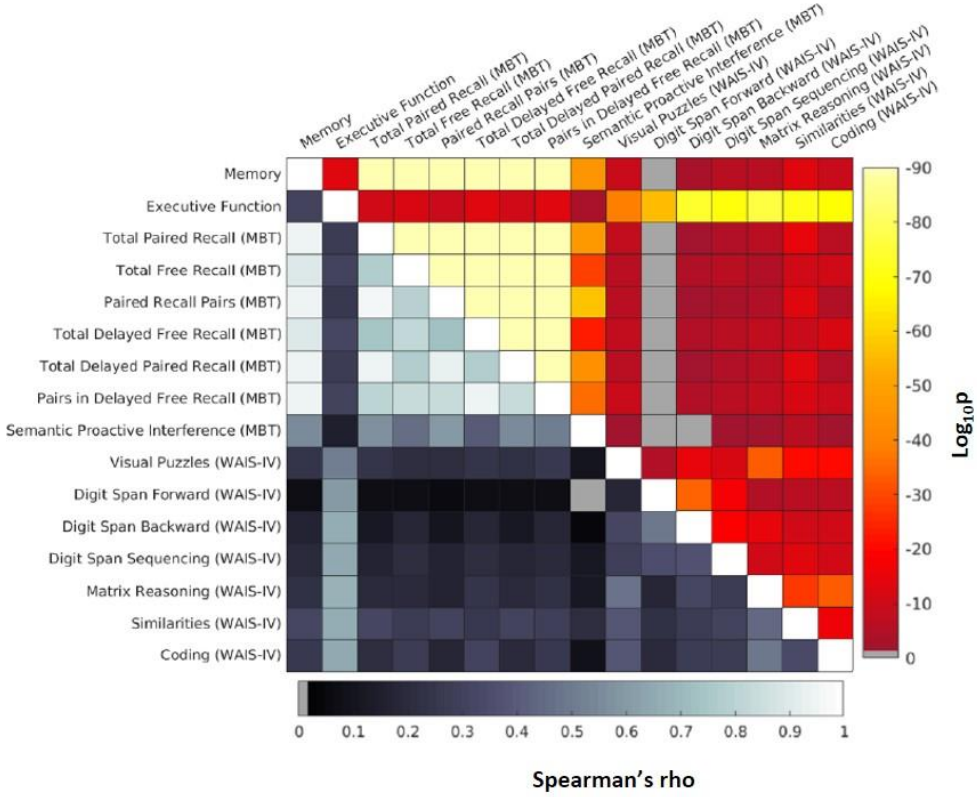
Table 4. Brain regions with significant DWMH mediation effect in the association between GM volume and Coding (path *ab*)

Number of cluster voxels	Z[a]	Brain Region[b]	Laterality	% of ROI
21427	2.58	Lateral orbital gyrus	R	73.16
	2.58	Anterior orbital gyrus	R	68.87
	2.58	Posterior orbital gyrus	R	51.64
	2.58	Central operculum	R	44.87
	2.58	Frontal pole	L	44.76
	2.58	Planum polare	R	39.21
	2.58	Posterior insula	R	37.85
	2.58	Anterior insula	R	32.23
	2.58	Medial orbital gyrus	R	25.77
	2.58	Opercular part of the inferior frontal gyrus	L	25.05
	2.58	Triangular part of the inferior frontal gyrus	L	23.36
	2.58	Postcentral gyrus	R	22.41
	2.58	Frontal operculum	L	20.35
	2.58	Orbital part of the inferior frontal gyrus	R	17.95
	2.58	Medial frontal cortex	L	15.54
	2.58	Superior frontal gyrus	L	14.17
	2.58	Precentral gyrus	R	12.37
	2.58	Temporal pole	R	12.19
	2.58	Frontal pole	R	12.10
	2.58	Middle frontal gyrus	L	12.00
	2.58	Middle frontal gyrus	R	11.86
	2.58	Supramarginal gyrus	R	10.58
	2.58	Inferior temporal gyrus	R	7.84
	2.58	Superior temporal gyrus	R	7.76
	2.58	Middle temporal gyrus	R	7.44
	2.58	Fusiform gyrus	R	6.81
	2.58	Superior frontal gyrus medial segment	L	6.74
3408	2.58	Inferior occipital gyrus	L	14.81
	2.58	Inferior temporal gyrus	L	15.99
	2.58	Middle temporal gyrus	L	15.50
	2.58	Occipital fusiform gyrus	L	8.38
3055	2.58	Thalamus proper	L	22.59
	2.66	Thalamus proper	R	6.66
2450	2.59	Accumbens	R	62.21
	2.58	Subcallosal area	R	31.13
	2.58	Medial frontal cortex	L	12.99
1733	2.58	Superior parietal lobule	L	10.89
	2.58	Supramarginal gyrus	L	9.76
1649	2.58	Central operculum	L	18.40
	2.58	Parietal operculum	L	9.62
	2.58	Postcentral gyrus	L	8.15
1203	2.58	Occipital fusiform gyrus	R	30.07
892	2.58	Anterior cingulate gyrus	R	22.08
	2.58	Middle cingulate gyrus	R	11.17
692	2.67	Calcarine cortex	R	20.25
586	2.58	Inferior temporal gyrus	R	9.56

180	2.58	Planum polare	L	16.69

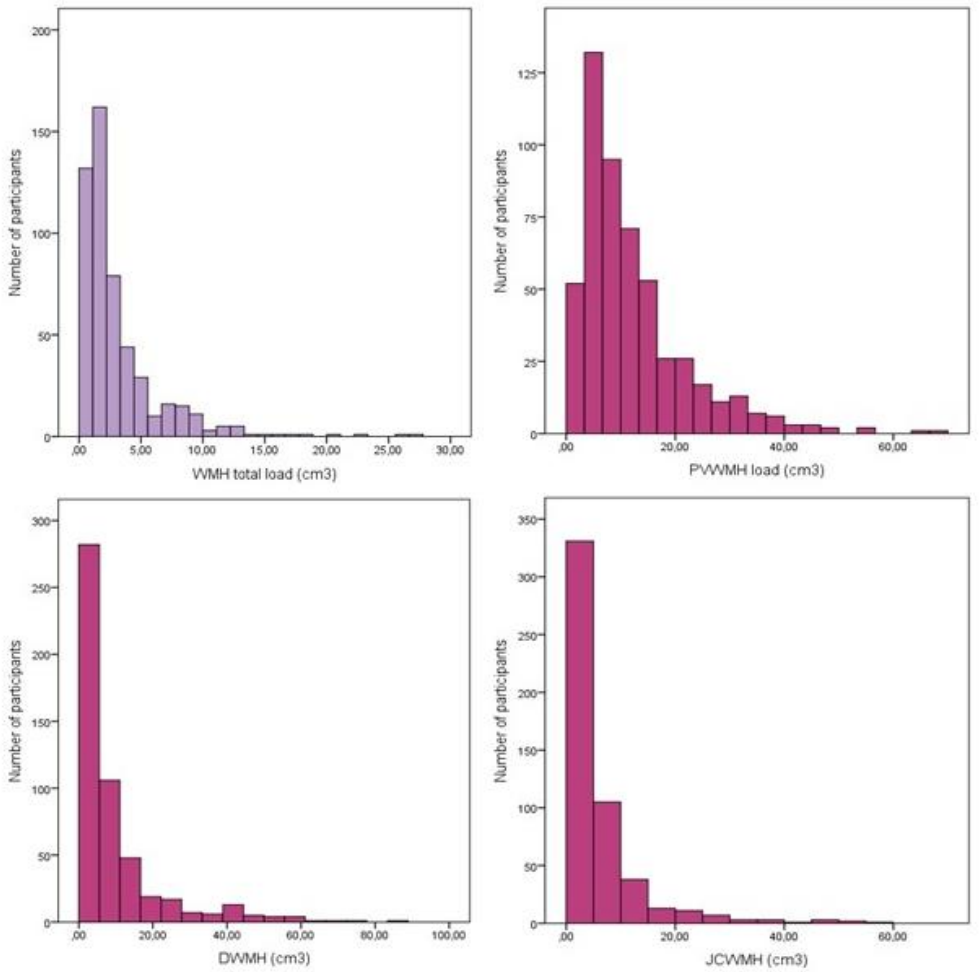
Abbreviations: ROI = Region of interest
[a] Maximum Z effect for each significant brain region
[b] Brain regions with >5% of ROI and >100 voxels number

Figure 1. Cross-correlations and statistical significance between pairs of cognitive scores



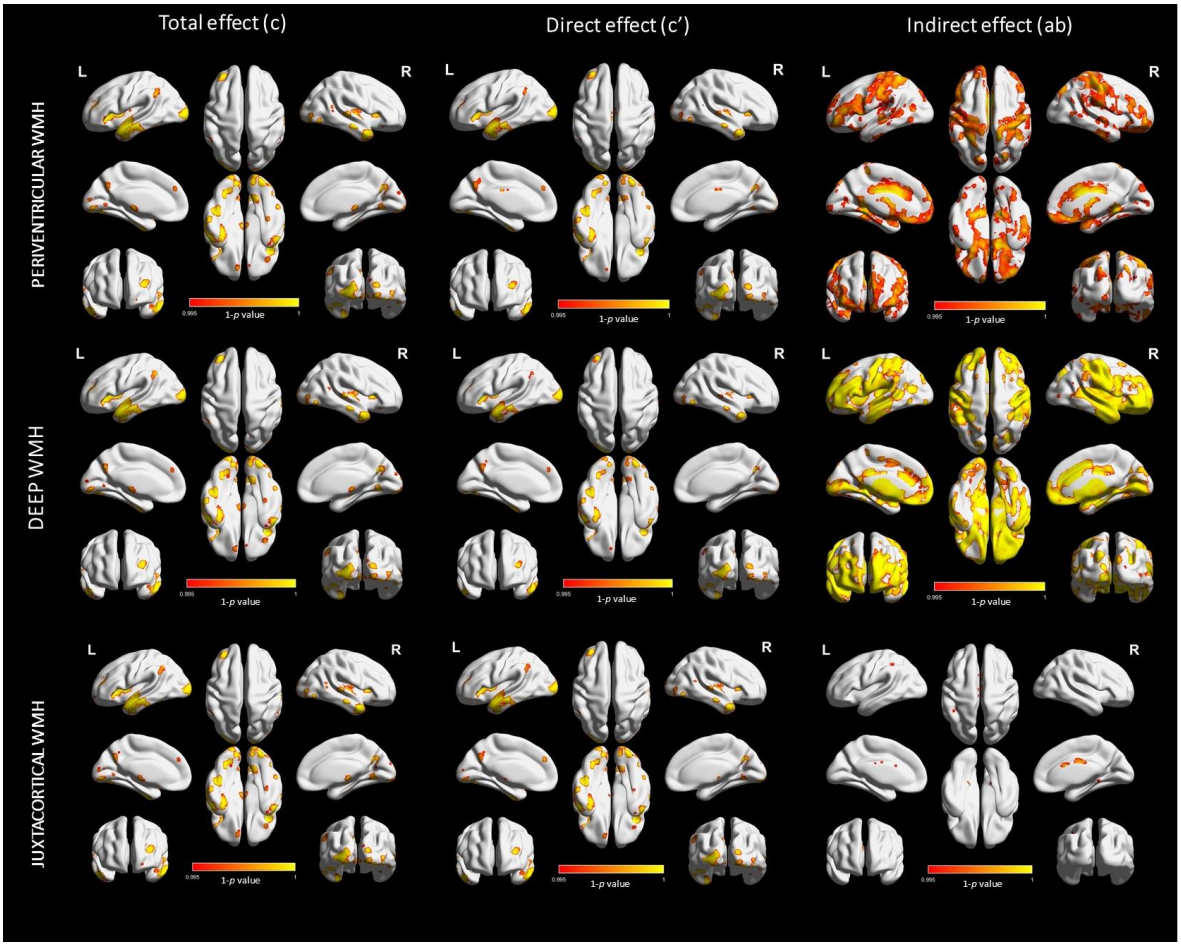
MBT subtest scores were highly correlated among them whereas the correlation between pairs of EF scales was only modest. Logically, memory and EF z-scores were highly correlated with their respective scales. Correlation between cognitive functions was modest but yet statistically significant (p<0.001). MBT = Memory Binding Test; EF = executive function.

Figure 2. Histogram of WMH load; total, PVWMH, DWMH and JCWMH (N=521)



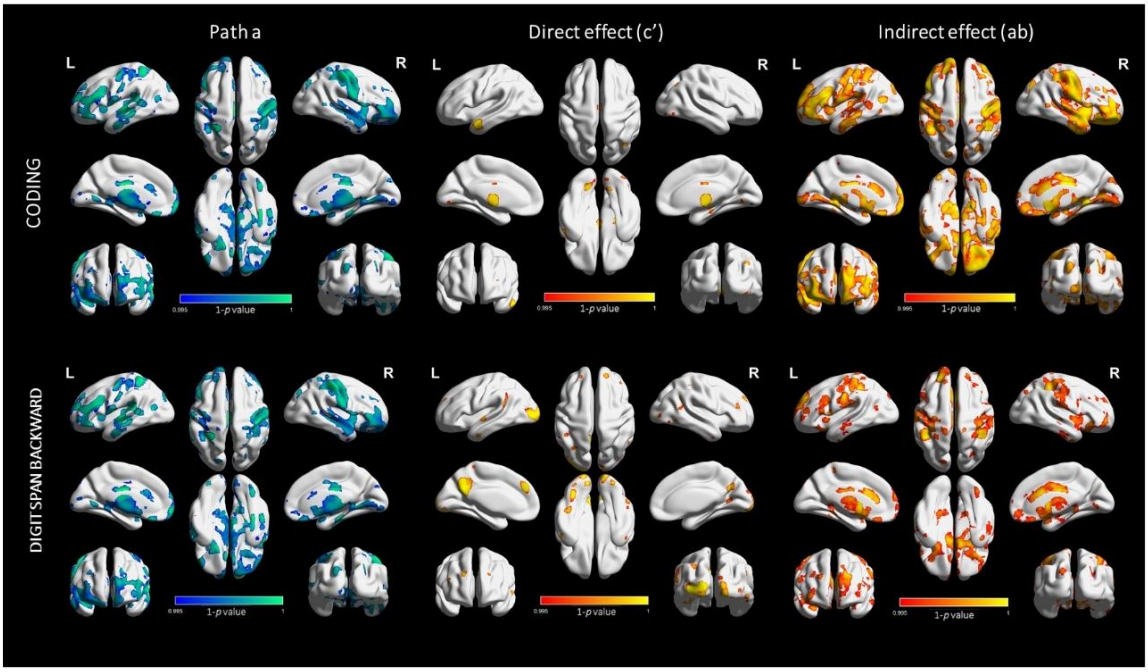
Abbreviations: WMH, white matter hyperintensities; PVWMH, periventricular white matter hyperintensities; DWMH, deep white matter hyperintensities; JCWMH, juxtacortical white matter hyperintensities.

Figure 3. Mediation effect of WMH per distance to the ventricles on the relationship between GM volume and EF.



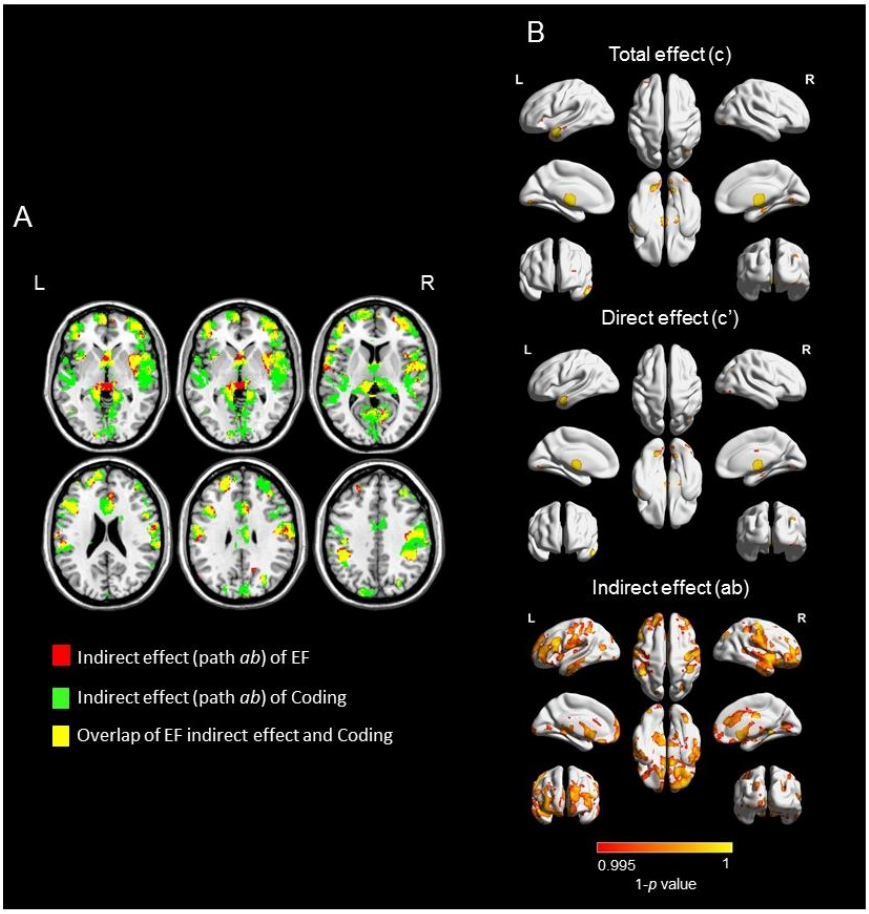
Total effect (path *c*) = relationship between GM volume and EF. Direct effect (path *c'*) = pure effect of GM volume in EF (removing the mediation effect). Indirect effect (path *ab*) = mediation effect across GM volume and cognitive performance through WMH load. The indirect effect of DWMH was more significant than PVWMH and JCWMH in the GM volume of regions involved in EF performance, mainly located in temporal (e.g. hippocampus, fusiform gyrus) and frontal (e.g. frontal pole and orbital gyrus (last column). Statistical significance was set at $p<0.005$. Paths were adjusted for mediator-outcomes confounders: age, sex, education TIV and number of *APOE*-ε4 alleles. Hot colorbar = positive relationship.

Figure 4. Mediation effect of WMH load on the relationship between GM volume and Coding and DSB performance



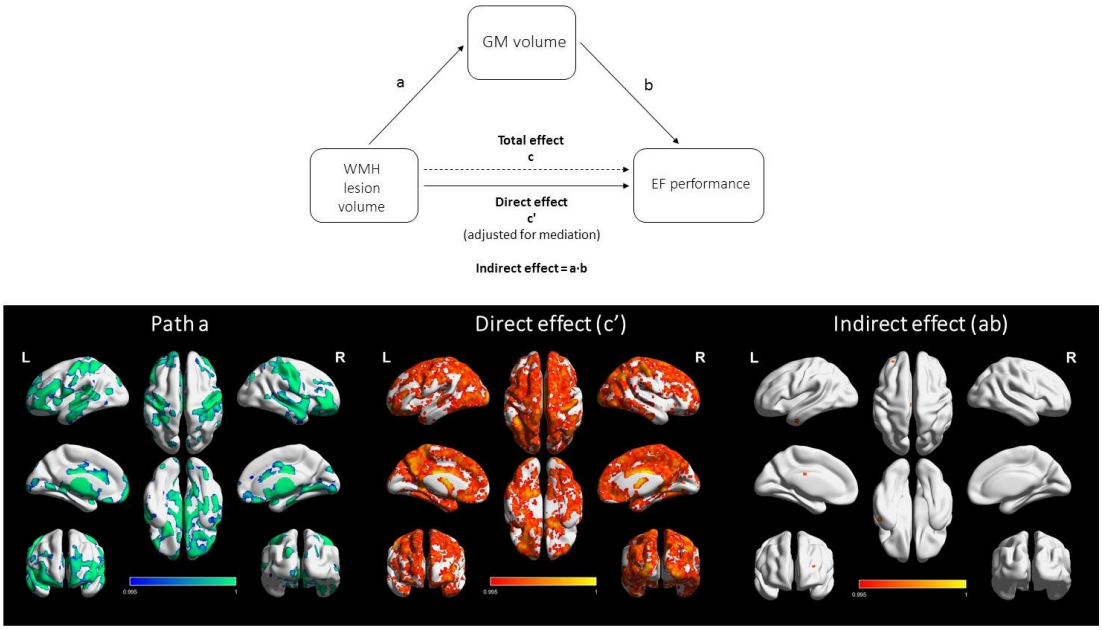
WMH load mediates the relationship between GM volume and Coding and DSB performance. These relationships were found in the same regions previously described being involved in those tests (1). Path *a*; relationship between GM volume and WMH load. Direct effect (path *c'*) = pure effect of GM volume on cognitive test performance (removing the mediation effect). Indirect effect (path *ab*) = mediation effect across GM volume and cognitive performance through WMH load. Lower GM volume is associated with greater WMH burden mainly in temporal and frontal regions (left column). Positive correlations between GM volume and cognition were still significant after removing mediation effect mainly in frontal and temporal regions (middle column). Lastly, WMH mediation effect were significant widespread brain regions (right column). Statistical significance was set at $p<0.005$. Paths were adjusted for mediator-outcomes confounders: age, sex, education TIV and number of *APOE*-ε4 alleles. Cold colorbar = negative relationship. Hot colorbar = positive relationship.

Figure 5. Mediation effect of DWMH load on the relationship between GM volume and Coding



(A) Regions with significant mediation effect on EF and Coding. Red = indirect effect (path *ab*) of DWMH burden over GMv and EF relationship, yellow = indirect effect (path *ab*) of DWMH burden over GMv and Coding relationship, green = overlap between indirect effect of EF and Coding performance. The behavior of the EF composite was driven by the indirect effect (*ab*) in Coding. (B) Mediation results on Coding performance. Total effect (path *c*) = GMv-Coding relationship. Direct effect (path *c'*) = pure effect of GMv in Coding (removing the mediation effect). Indirect effect (path *ab*) = mediation effect across GMv and Coding performance through DWMH load. After discounting the mediation effect, a direct significant association (path *c'*) remained between GMv in the temporal pole and inferior temporal in Coding. Statistical significance was set at $p<0.005$. Paths were adjusted for mediator-outcomes confounders: age, sex, education TIV and number of *APOE*-ε4 alleles. Hot colorbar = positive relationship. WMH = white matter hyperintensities; GMv = gray matter volume: EF= executive function.

Figure 6. Significant brain regions in the relationship between WMH load and GM volume (path *a*), and direct and indirect effect of WMH load and EF relationship.

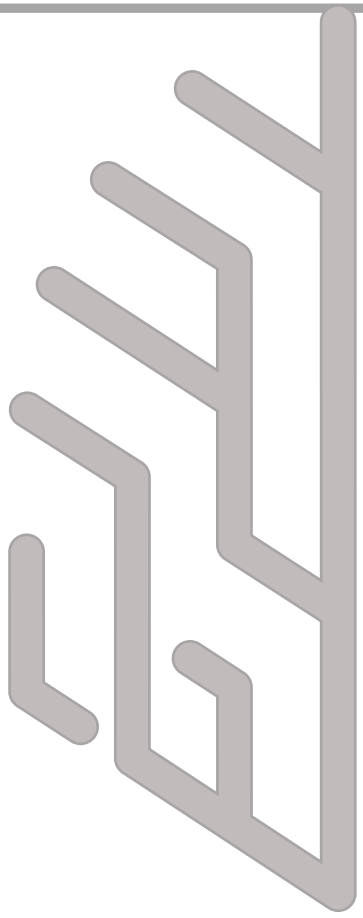


Path *a* = relationship between WMH load and GM volume. Direct effect (path *c'*) = pure effect of WMH load in EF performance (removing the mediation effect). Indirect effect (path *ab*) = mediation effect across WMH load and EF performance through GM volume. Lower GM volume is associated with greater WMH burden mainly in temporal and frontal regions (path *a*). According to the figure, the relationship between WMH load and EF was no mediated by GM volume (last two columns). Statistical significance was set at $p<0.005$. Paths were adjusted for mediator-outcomes confounders: age, sex, education TIV and number of *APOE*-ε4 alleles. Cold colorbar = negative relationship. Hot colorbar = positive relationship.

References

1. Knopman DS., Griswold ME., Lirette ST., et al. Vascular Imaging Abnormalities and Cognition. Stroke 2015;46(2):433–40.

DISCUSSION



In this thesis, we sought to extend previous findings reporting on the impact of AD risk factors on WMH burden in a relatively young CU population. In addition, we have explored the mechanisms by which WMH impact on cognition.

The main conclusion of this study is that even though our middle-aged participants displayed lower WMH burden compared with previously studied clinical cohorts (108,166,167), and also low prevalence of AD risk factors, a significant association was found between AD risk factors and WMH load. Furthermore, higher WMH lesion volume was significantly associated with a widespread pattern of cortical atrophy. In turn, WMH mediates the relationship between this atrophy in regions involved in EF. Therefore, our results highlight that AD risk factors impact early on brain structures and cognition.

The strongest genetic risk factor for AD is the *APOE*-ε4 allele. In the second study, we reported that *APOE*-ε4 homozygotes show a higher risk of presenting pathological WMH compared to their heterozygous and non-carriers' counterparts. In addition, this higher effect is independent of the presence of CVRF, which are strongly associated with the prevalence and extension of WMH. However, when we analysed the association with WMH volume in *APOE*-ε4 homozygotes we did not find a statistically significant increase of WMH burden. At first sight, this finding might seem in contradiction with our finding about higher risk of presenting pathological levels of WMH in ε4 homozygotes. With this in mind, there are several considerations to be taken into account. First, it is important to note that even the Fakezas scores and WMH volumes are certainly correlated measures (125,168), they do not exactly capure the same WMH features. While the Fazekas also contemplates the volume of WMH, it also reflects the location, morphology and confluence of lesions (103). A second consideration is that even though the regional WMH volumes of *APOE*-ε4 homozygotes did not reach statistical significance against age-matched 3ε homozygotes, we found that a regional pattern involving parietal regions showed a tendency to significance. Therefore, in this case the use of non-parametric statistics may have prevented us from achieving the sufficient statistical power to detect this effect in *APOE*-ε4 homozygotes.

Therefore, we believe that *APOE*-ε4 homozygosity exerts a deleterious effect on WMH volumes which cannot be interpreted as significantly different from our observation when assessed by means of the Fazekas score. Still, one potential explanation to reconcile both findings would be that white matter lesions in homozygotes progress faster to pathological stages. This would be in line with several reports that support the hypothesis that the *APOE*-ε4 genotype confers a higher vulnerability to brain insults, including but not limited to those associated with CVRF (33). For example, a previous longitudinal population-based study (169) showed a higher rate of WMH increases in ε4 homozygotes than heterozygotes and non-carriers, suggesting that the former reach pathological WMH levels earlier.

The biological mechanisms through which the *APOE*-ε4 genotype might increases the risk of AD have been typically classified into Aε-dependent and Aε-independent pathways (33). *APOE* isoforms differently impact the deposition of Aε to form amyloid plaques, one of the neuropathological hallmarks of AD. As stated before, the *APOE*-ε4 allele has been associated with a greater risk for amyloid positivity and younger age at onset in persons without dementia (27). Given the age of our *APOE*-ε4 homozygote group, we expect about half of them to harbour abnormally elevated amyloid levels. Therefore, to assess the precise mechanism that underlies the observed relationship between *APOE*-ε4 and WMH in our results we would need to factor out the effect of Aε deposition. However, due to the unavailability of amyloid status of participants at the moment of the study, it will be important to include core AD biomarkers to assess their contribution to WMH load in future research. This analysis would provide information about the amyloid pathology role in the results found, whether it predicts or additively contributes with WMH burden or, otherwise, it interacts with *APOE*-ε4 allele to develop WMH burden. On the other hand, independently of Aβ, it must be noted that *APOE* plays a critical role as the primary carrier in the brain of cholesterol and other lipids, which are an essential component of the myelin sheaths and indispensable for axonal growth, synaptic formation, and remodelling (170). In this context, the ε4 isoform of the *APOE* has been shown to be less efficient than the ε3 and ε2 ones in transporting cholesterol in the brain (33). Therefore, this Aβ-independent

mechanism might explain in part why *APOE*-ε4 homozygotes may be less resilient to WMH lesions and progress faster to pathological stages. The *APOE*-ε2 allele showed a protective effect against global and regional WMH load. These results could also be mediated by the impact of the *APOE* genotype on serum cholesterol levels and, with ε4 carriers showing a higher prevalence of hypercholesterolemia and ε2 carriers allele a lower one. Nevertheless, a previous meta-analysis reported that the *APOE*-ε2 allele was associated to increasing WMH load in CU older participants (111). This discrepancy could be due to the fact that all studies included older participants with comorbidities.

Regarding non-modifiable risk factors other than *APOE*-ε4, age, sex and family history of AD were associated with distinct regional patterns of WMH burden. As expected, age was positively associated with a pathological degree of WMH and displayed the strongest and most widespread association with WMH load. Sex, female, was associated with higher juxtacortical WMH load. This sex difference has been related in the literature with a higher arterial stiffness in women than in men, especially in the external layers of white matter (108). Another possible explanation of this result is the sex differences in white matter microstructure previously reported, probably due to hormonal differences (171). Concerning family history of AD, our results from the first study reflected that this non-modifiable risk factor did not increase the risk of presenting pathological WMH in middle-aged CU individuals. However, in the third study, we found that participants with a maternal, but not paternal, history of AD presented higher global and regional WMH loads than those without any familiar history. In those participants, WMH burden increased in AD-vulnerable regions, such as the temporal lobe, which is expected to contribute to the observed higher AD prevalence in individuals with a maternal history compared with those with a paternal one (36). Although family history of AD is also expected to be associated with a higher prevalence of *APOE*-ε4 alleles, the stronger observed effect of maternal AD history, suggests the possible existence of additional hereditary factors contributing to the presence of higher WMH burden. Further analysis with additional risk *loci* for AD will provide us with a broader picture of the genetic underpinnings of WMH. The genetic basis of WMH is still unclear. Previous studies explored the heritability for total WMH in CU older adults twins (172) and

in a family-based sample (173), showing that the heritability was higher in women and declined with age. Still, additional non-genetic heritable factors could also be envisaged to have an impact on WMH load, such as food habits or socioeconomic status that may be passed on through generations, as well as the shared environmental risk that family members may be exposed to. Therefore, there is a need for future research, stratifying for the *APOE*-ε4 genotype, to further detail familial lifestyle habits and their underlying risk mechanisms. For instance, the specific regional pattern of WMH load we observed in participants with maternal AD history is similar to the one observed in participants with hypercholesterolemia, which may be also partially accounted by the *APOE* genotype.

Regarding modifiable risk factors, even though our middle-age participants displayed very low WMH burden, a significant association was found between the main modifiable AD risk factors and WMH burden. Therefore, we extended previously reported associations between AD risk estimates and WMH burden to younger and healthier individuals. As expected, HTA was strongly associated with pathological WMH levels and both global and regional WMH volume. Globally, we showed an association between HTA that survived correction by age. Together with age, HTA was a main driver of WMH load in regional analysis, showing a significant association with WMH in almost all regions analysed. Hypercholesterolemia and BMI also displayed a minor, albeit significant, influence in global and regional WMH. Finally, we observed that even though the CAIDE dementia risk of population was low (23,121,174,175), it was positively correlated with increased global and regional WMH burden. Furthermore, we found that even small WMH loads on strategic brain locations exert a negative impact on cognition even in CU middle-aged individuals. In summary, these results suggest that tight control of modifiable risk factors in middle-aged could have a significant impact on late life dementia. In this line, recently published results from a randomized clinical trial (SPRINT-MIND) (176) in individuals with increased cardiovascular risk, but without diabetes, showed that control of systolic blood pressure below 120 mmHg resulted in 19% fewer cases of mild cognitive decline over 3 years (177). Indeed, additional evidence supports that effect of cardiovascular disease on brain health is stronger in midlife (178,179).

Still, the mechanisms through which WMH burden impacts on cognition are not yet fully understood in CU young individuals. In our last study, we found that, as was mentioned before, even in such a low risk population, higher WMH lesion volume is associated to widespread changes on cerebral cortex in regions associated to EF in CU middle-aged participants. In turn, WMH volume mediates the relationship between GM volume and EF. There might be a number of possible explanations for these findings. On the one hand, ischemic injury to the axons might impair the connectivity of distal cortical and subcortical regions and eventually lead to neuronal loss. On the other hand, there is also ample evidence that WMH may be the result of degenerative axonal loss secondary to neuronal damage due to Wallerian degeneration. Alternatively, ischemia could be leading to both vascular brain injury and neurodegeneration. Even though it is implicit in the mediation pathway model, analysis of cross-sectional data cannot establish a causal link between the studied variables. Only a randomized intervention that could effectively modify WMH burden could establish the causality of such associations. Still, our findings from the mediation analysis suggest the existence of a direct mechanism via which WMH load mediates the relationship between GM volume and brain regions involved with EF. On the other hand, the fact that we did not find significant effects of WMH load on GM volume in regions involved with EM performance might suggest the presence of an indirect effect of WMH in cognition. WMH may reflect or be related to separate processes such as inflammation or other forms of vascular pathology and thus may affect cognition via other mechanisms (180). Future research is needed to clarify the basis of the association of WMH with cognitive performance in middle-aged individuals.

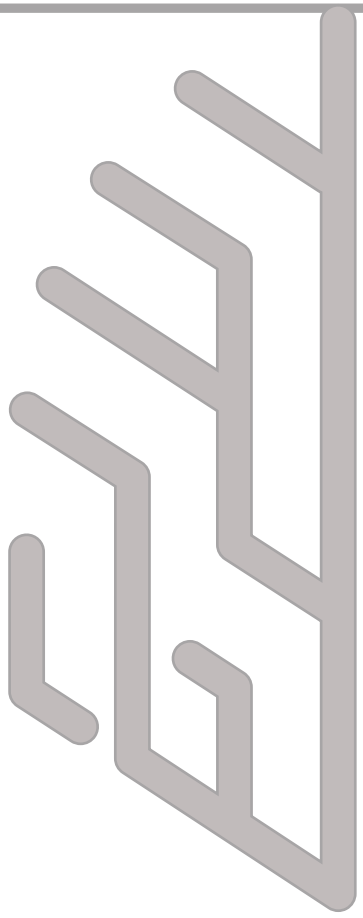
In summary, in line with previous studies (95,96),our results showed that WMH are commonly detected in the brain of asymptomatic middle-aged individuals. Still, previous research has reported that WMH have important clinical and risk factor associations (103) underlining that they should not be mistreated as inevitable ‘silent’ consequences of the physiological aging of the brain. Controlling CVRF may reduce the risk of developed WMH burden in any person, however, the brain of individuals *APOE*-ε4/ε4 carriers are

more vulnerable to their impact. Although the high risk of *APOE*-ε4 homozygous to present pathological WMH, it is important to note that such risk can be also related to CVRF, just as in non-carriers. However, the control of CVRF could be particularly relevant for *APOE*-e4 homozygous, which in turn are at higher risk of developing AD. All *APOE* alleles alter, in a dose-dependent manner, the likelihood and the age onset of clinical symptoms of AD (181) still, *APOE*-ε4/ε 4 individuals can benefit more from the early CVRF control. In those, the resulting final WMH load is a combination of the additive effect of CVRF and *APOE*-ε4 homozygosity. All these results together and given the known association between WMH and future cognitive decline, the early prevention of CVRF risk factors in individuals at higher risk of developing WMH appears as a useful preventive strategy to reduce or delay the onset of dementia.

Future work will address some of the limitations of the present studies and extending the findings here reported. The longitudinal long-term study of the ALFA parent cohort will allow increasing relevant knowledge in the field addressed here. As mentioned above, it is important to include core AD biomarkers to assess their contribution to WMH load and their joint impact on cognition. These AD biomarkers could also be useful to assess the impact of WMH load prevalence on Aβ pathology independently of *APOE*-ε4, HTA and age. This analysis will also contribute to extend the results concerning the mechanism through which *APOE*-ε4/ε4 genotype might increase the risk of presenting pathological WMH. In this context, it will also be interesting to explore whether AD pathology mediates or moderates the relationship found between GM volume and regions involved in EF performance. In addition, to have AD biomarkers available may make possible to determine whether the impact of WMH on EM performance is through an indirect mechanism. It will be also interesting to explore whether the longitudinal progression of WMH predicts cognitive decline and whether there is a specific regional WMH pattern that predict cognitive impairment. Moreover, it should also allow the exploration of which non-modifiable and modifiable

AD risk factors are associated with cognitive decline. Finally, due to the fact that WMH commonly reflect small vessel cerebrovascular disease, it would be interesting to explore the role of vascular disease with AD pathology in the preclinical stage of the disease and whether it interacts or has an additive effect on neurodegeneration.

CONCLUSIONS



- Cognitively unimpaired middle-aged individuals presented pathological WMH and their prevalence significantly increased with advancing age. The prevalence found in adults with a family history of AD was comparable with that of those without in the same age range.

- Risk factors of AD were associated with a higher prevalence of pathological, global and regional WMH in cognitively unimpaired participants with low risk of dementia:

    - Modifiable and non-modifiable risk factors of AD risk factors showed and independent effect over WMH load.

    - Some specific non-modifiable AD risk factors (age, sex, *APOE*-ε2, and family history of AD) and modifiable AD risk factors (HTA, BMI, and hypercholesterolemia), were significantly associated with distinct regional patterns of WMH burden in cognitively unimpaired middle-aged individuals. In addition, both non-modifiable and modifiable risk factors showed and additive effect.

    - *APOE*-ε4 homozygotes showed a higher risk of presenting pathological WMH compared to their heterozygous and non-carrier' counterparts, independently of the presence of cardiovascular risk factors.

- Both global and regional WMH had an impact on cognition even in CU middle-aged individuals with low WMH burden and low risk of dementia.

- Even in such a low risk population, higher WMH volume was associated with widespread structural changes on cerebral cortex.

- One of the mechanisms by which WMH impact on cognition in cognitively unimpaired middle-aged participants is through an association with a cortical atrophy pattern. This pattern is specific of regions involved in executive function.

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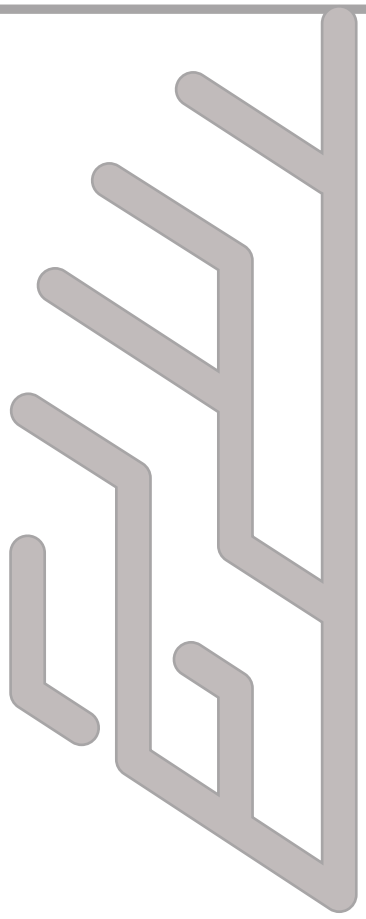

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