Evaluation of cortical microinfarcts in patients with Down syndrome

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Thesis Doctoral

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LIST OF ABBREVIATIONS AND ACRONYMS

AD	- Alzheimer's Disease
aDS	- DS participant without AD symptoms
APOE	- Apolipoprotein E
APP	- Amyloid precursor protein
Aβ	- Amyloid-β
CAA	- Cerebral amyloid angiopathy
CAMCOG-DS	- Cambridge Examination for Mental Disorders of Older People with Down's Syndrome and others with intellectual disabilities
CDR	- Clinical Dementia Rating
CSF	- Cerebrospinal fluid
CSVD	- Cerebral small vessel disease
DABNI	- Down-Alzheimer Barcelona Neuroimaging Initiative
dAD	- Symptomatic sporadic AD patient with dementia
dDS	- DS participant with AD dementia
DS	- Down syndrome
DTI	- Diffusion-tensor imaging
FCSRT	- Free and Cued Selective Reminding Test
FLAIR	- Fluid-attenuated inversion recovery
GRE	- Gradient echo images
ID	- Intellectual disability
mCRT	- Modified Cued Recall Test
MMSE	- Mini–Mental State Examination
MRI	- Magnetic resonance imaging
NfL	- Neurofilament light
pAD	- Symptomatic sporadic AD patient with prodromal symptoms
pDS	- DS participant with prodromal AD symptoms
PET	- Positron emission tomography
pTau	- Phosphorylated tau
SPIN	- Sant Pau Initiative on Neurodegeneration
SWI	- Susceptibility weighted imaging
T1	- T1-weighted sequence
T2	- T2-weighted sequence
T2*	- T2*-weighted sequence
WMH	- White matter hyperintensities

LIST OF FIGURES

LIST OF TABLES

SUMMARY

RESUMO

Introdução

Microinfartos corticais (MIC) são pequenas lesões isquêmicas no córtex cerebral que até recentemente eram invisíveis em imagens de ressonância magnética (RM) e apenas detectáveis em estudos *post-mortem*. No entanto, o desenvolvimento da RM 7T permitiu sua detecção *in-vivo*. Recentemente, diretrizes estabelecidas para a detecção visual de MIC em RM 3T permitiram o estudo dessas lesões em grandes coortes, relacionando MIC a fatores de risco vascular, disfunção cardíaca, hipoperfusão cerebral, acidente vascular cerebral e demência vascular. Além disso, alguns estudos sugerem que MIC são lesões comuns na angiopatia amilóide cerebral, juntamente com micro-hemorragias cerebrais e siderose superficial. Diferenciar as possíveis causas de MIC na população em geral é desafiador devido à frequente sobreposição de patologias. No entanto, na síndrome de Down (SD), a prevalência de fatores de risco vascular é baixa, enquanto a prevalência de angiopatia amiloide cerebral é alta (devido à produção excessiva de proteína precursora amiloide e maior deposição de peptídeo β-amiloide no parênquima e capilares cerebrais). A combinação dessas características faz da SD um ótimo modelo para estudar a patologia amiloide como possível causa de MIC. No entanto, nenhum estudo avaliando MIC nesta população foi realizado até o momento.

Hipótese

Hipotetizamos que em SD os MIC estão relacionados à angiopatia amiloide cerebral, dada a amiloidose cerebral onipresente e a baixa prevalência de fatores de risco vasculares nesta população.

Objetivos

Nosso objetivo foi caracterizar os MIC na SD investigando sua a prevalência de acordo com a idade e ao longo do espectro clínico da doença de Alzheimer (DA), sua distribuição topográfica e sua associação com fatores de risco vascular, outros achados vasculares por neuroimagem, sexo, haplotipo APOE, biomarcadores AT(N) no líquido cefalorraquidiano e no plasma, e desempenho cognitivo.

Métodos

Este estudo transversal com RM 3T incluiu participantes das coortes DABNI e SPIN. A coorte SPIN é uma coorte para descoberta e validação de biomarcadores que inclui voluntários saudáveis e participantes com diferentes doenças neurodegenerativas. A coorte DABNI faz parte do estudo SPIN e é uma coorte de base populacional de adultos com SD e biomarcadores multimodais da DA. O presente estudo incluiu 364 participantes com RM 3T, sendo 195 adultos com SD (126 assintomáticos, 29 com DA prodrômica e 40 com demência por DA), 63 com pacientes DA esporádica sintomática (43 com DA prodrômica e 20 com demência por DA) e 106 controles cognitivamente normais. Um neurorradiologista (cego aos dados e diagnósticos dos participantes) analisou visualmente imagens de RM 3T

para detectar MIC (usando um previamente protocolo validado), avaliar a carga de hiperintensidades de substância branca em FLAIR, a presença de lacunas, de grandes infartos corticossubcorticais, de micro-hemorragias e de siderose superficial.

Resultados

MIC foram observados em 11,8% dos participantes com SD (6.7% de indivíduos assintomáticos e 17.4% de indivíduos com DA sintomática), 4,7% dos controles e 17,5% dos participantes com DA esporádica (p=0.061). A prevalência de MIC aumentou com a idade e ao longo do espectro clínico da DA em SD. Em SD, os MIC foram observados predominantemente nos lobos parietais, enquanto em participantes euploides, os MIC se observam ao longo das linhas parassagitais. Em SD, participantes com MIC apresentaram maiores níveis plasmáticos de NfL do que participantes sem MIC (p=0,044). Neste grupo, participantes com MIC apresentam maior número de lacunas (p=0,026) e de infartos corticossubcorticais (p=0,004) comparados com participantes sem MIC. Foi observada ainda uma tendência de maior prevalência de hiperintensidades de substância branca em FLAIR (Fazekas≥2) em participantes com MIC (p=0,054). Entretanto, a presença de MIC não apresentou relação com fatores de risco vasculares, lesões hemorrágicas, sexo, haplotipo APOE ou desempenho cognitivo em participantes com SD, com DA esporádica ou em controles cognitivamente normais.

Conclusão

Os resultados do presente estudo apoiam nossa hipótese principal de que MIC em SD estão relacionados a angiopatia amiloide cerebral, possivelmente associados a um fenótipo de imagem não hemorrágico da doença.

Palavras-chave: Síndrome de Down. Doença de Alzheimer. Angiopatia amiloide cerebral. Microinfartos corticais. Imageamento por ressonância magnética. Demência. Disfunção cognitiva.

ABSTRACT

Introduction

Cortical microinfarcts (CMI) are small ischemic lesions in the brain cortex that were, until recently, invisible on magnetic resonance imaging (MRI) and only detected in post-mortem studies. However, the development of ultra-high field 7T-MRI allowed their detection *in-vivo*. Recently, established guidelines for CMI visual detection on 3T-MRI enabled the study of such lesions in large cohorts, linking CMI to vascular risk factors, cardiac dysfunction, reduced cerebral perfusion, stroke, and vascular cognitive impairment. However, a few studies have suggested that CMI is also a common feature of cerebral amyloid angiopathy (CAA), together with cerebral microbleeds and superficial siderosis. Disentangling the potential underlying causes of CMI in the general population is challenging due to the frequent overlap between vascular risk factors and Alzheimer's disease (AD). In Down syndrome (DS), however, the prevalence of classic vascular risk factors is low, while CAA is highly prevalent due to the overproduction of amyloid-precursor protein and increased amyloid-β (Aβ) deposition in the brain parenchyma and vasculature. The combination of these features makes DS a great model for studying amyloid pathology as a possible cause of CMI. However, to our knowledge, no studies assessing CMI in this population have been conducted so far.

Hypothesis

We hypothesize that, in DS, CMI are related to CAA pathology, given the ubiquitous brain amyloidosis and low prevalence of vascular risk factors in this population.

Objectives

We aimed at characterizing CMI in DS by assessing their prevalence with age and along the AD continuum, their topographic distribution and their association with vascular risk factors, vascular neuroimaging findings, sex, *APOE* haplotype, fluid AT(N) biomarkers, and cognitive performance.

Methods

This single-center cross-sectional study with 3T-MRI scans included participants from a population-based cohort of adults with DS with multimodal AD biomarkers and from a cohort for multimodal biomarker discovery and validation that includes cognitively euploid healthy volunteers and participants with different neurodegenerative diseases. A total of 364 participants with 3T-MRI were included: 195 adults with DS (126 asymptomatic, 29 prodromal AD, and 40 AD-dementia patients), 63 with symptomatic sporadic AD (sAD: 43 prodromal AD and 20 AD-dementia patients), and 106 cognitively unimpaired controls. A neuroradiologist (blind to participant's data and diagnosis) visually analyzed 3T-MRI images to assess the presence and location of CMI (using a validated protocol), the burden of white matter FLAIR hyperintensities, the presence of lacunar infarcts, large corticosubcortical infarcts, microbleeds, and superficial siderosis.

Results

CMI were present in 11.8% of participants with DS (6.7% in asymptomatic individuals and 17.4% in patients with symptomatic AD), 4.7% of controls, and 17.5% of symptomatic sporadic AD patients (p=0.061). CMI prevalence increased with age and along the AD clinical continuum in DS. CMI were predominantly located in the parietal lobes in DS and along the frontoparietal parasagittal lines in euploid participants. CMI were significantly related to lacunes (p=0.026), and corticosubcortical infarcts (p=0.004). Also, a trend towards significance was observed in the higher prevalence of WMH (Fazekas≥2) in DS participants with CMI (p=0.054). However, no association of CMI with vascular risk factors, hemorrhagic lesions, sex, APOE haplotype or cognitive performance was observed in DS, symptomatic sporadic AD or cognitively unimpaired controls.

Conclusion

Our data support our main hypothesis that cortical microinfarcts in Down syndrome are related to CAA, possibly related to a non-hemorrhagic imaging phenotype of the disease.

Key words: Down Syndrome, Alzheimer Disease, Cerebral Amyloid Angiopathy, Cortical Microinfarcts, Magnetic Resonance Imaging, Dementia, Cognitive Impairment

RESUMEN

Introducción

Los microinfartos corticales (MIC) son pequeñas lesiones isquémicas en la corteza cerebral que hasta hace poco eran invisibles en imágenes de resonancia magnética (RM) y solo detectables en estudios post mortem. Sin embargo, el desarrollo de la RM 7T ha permitido su detección in vivo. Recientemente, las directrices establecidas para la detección visual de MIC en RM 3T han permitido el estudio de estas lesiones en grandes cohortes, relacionando los MIC con factores de riesgo vascular, disfunción cardíaca, hipoperfusión cerebral, ictus y demencia vascular. Además, algunos estudios sugieren que los MIC son lesiones comunes en la angiopatía amiloide cerebral, junto con microhemorragias cerebrales y siderosis superficial. Diferenciar las posibles causas de MIC en la población general es un desafío debido a la frecuente superposición de patologías. Sin embargo, en el síndrome de Down (SD), la prevalencia de factores de riesgo vascular es baja, mientras que la prevalencia de angiopatía amiloide cerebral es alta (debido a la sobreproducción de proteína precursora amiloide y mayor deposición de péptido β-amiloide en el parénquima y los capilares cerebrales). La combinación de estas características hace que el SD sea un modelo óptimo para estudiar la patología amiloide como posible causa de los MIC. Sin embargo, hasta la fecha, no se ha realizado ningún estudio que evalúe los MIC en esta población.

Hipótesis

Hipotetizamos que en el SD, los MIC están relacionados con la angiopatía amiloide cerebral, dada la omnipresencia de la amiloidosis cerebral y la baja prevalencia de factores de riesgo vascular en esta población.

Objetivos

Nuestro objetivo fue caracterizar los MIC en el SD a través de la investigación de su prevalencia según la edad y a lo largo del espectro clínico de la enfermedad de Alzheimer (EA), de su distribución topográfica y de su asociación con factores de riesgo vascular, otros hallazgos vasculares por neuroimagen, sexo, haplotipo APOE, biomarcadores AT(N) en el líquido cefalorraquídeo y plasma, y rendimiento cognitivo.

Métodos

Este estudio transversal con RM 3T incluyó participantes de las cohortes DABNI y SPIN. La cohorte SPIN es una cohorte para el descubrimiento y validación de biomarcadores que incluye voluntarios saludables y participantes con diferentes enfermedades neurodegenerativas. La cohorte DABNI forma parte del estudio SPIN y es una cohorte poblacional de adultos con SD y biomarcadores multimodales de la EA. Este estudio incluyó a 364 participantes con RM 3T, de los cuales 195 eran adultos con SD (126 asintomáticos, 29 con EA prodrómica y 40 con demencia por EA), 63 con EA esporádica (43 con EA prodromal y 20 con demencia por EA) y 106 controles cognitivamente normales. Un neurorradiólogo (ciego a los datos y

diagnósticos de los participantes) analizó visualmente las imágenes de RM 3T para detectar los MIC (utilizando un protocolo previamente validado), evaluar la carga de hiperintensidades de sustancia blanca en FLAIR, la presencia de lacunas, infartos corticosubcorticales grandes, microhemorragias y siderosis superficial.

Resultados

Se observaron MIC en el 11,8% de los participantes con SD (6,7% de individuos asintomáticos y 17,4% de individuos con EA sintomática), en el 4,7% de los controles y en el 17,5% de los participantes con EA esporádica (p=0,061). La prevalencia de MIC aumentó con la edad y a lo largo del espectro clínico de la EA en el SD. En el SD, los MIC se observaron predominantemente en los lóbulos parietales, mientras que en los participantes euploides, los MIC se observaron a lo largo de las líneas parasagitales en las regiones frontoparietales. En el SD, los participantes con MIC presentaron niveles plasmáticos más altos de NfL que los participantes sin MIC (p=0,044). En este grupo, los participantes con MIC presentaron un mayor número de lagunas (p=0,026) e infartos corticosubcorticales (p=0,004) en comparación con los participantes sin MIC. También se observó una tendencia a una mayor prevalencia de hiperintensidades de sustancia blanca en FLAIR (Fazekas≥2) en los participantes con MIC (p=0,054). Sin embargo, la presencia de MIC no se relacionó con factores de riesgo vascular, lesiones hemorrágicas, sexo, haplotipo APOE o rendimiento cognitivo en los participantes con SD, con EA esporádica o en los controles cognitivamente normales.

Conclusión

Los resultados de este estudio respaldan nuestra hipótesis principal de que los MIC en el SD están relacionados con la angiopatía amiloide cerebral, posiblemente asociados con un fenotipo de imagen no hemorrágica de la enfermedad.

Palabras clave: Síndrome de Down, Enfermedad de Alzheimer, Angiopatía Amiloide Cerebral, Microinfartos Corticales, Imagen por Resonancia Magnética, Demencia, Disfunción Cognitiva

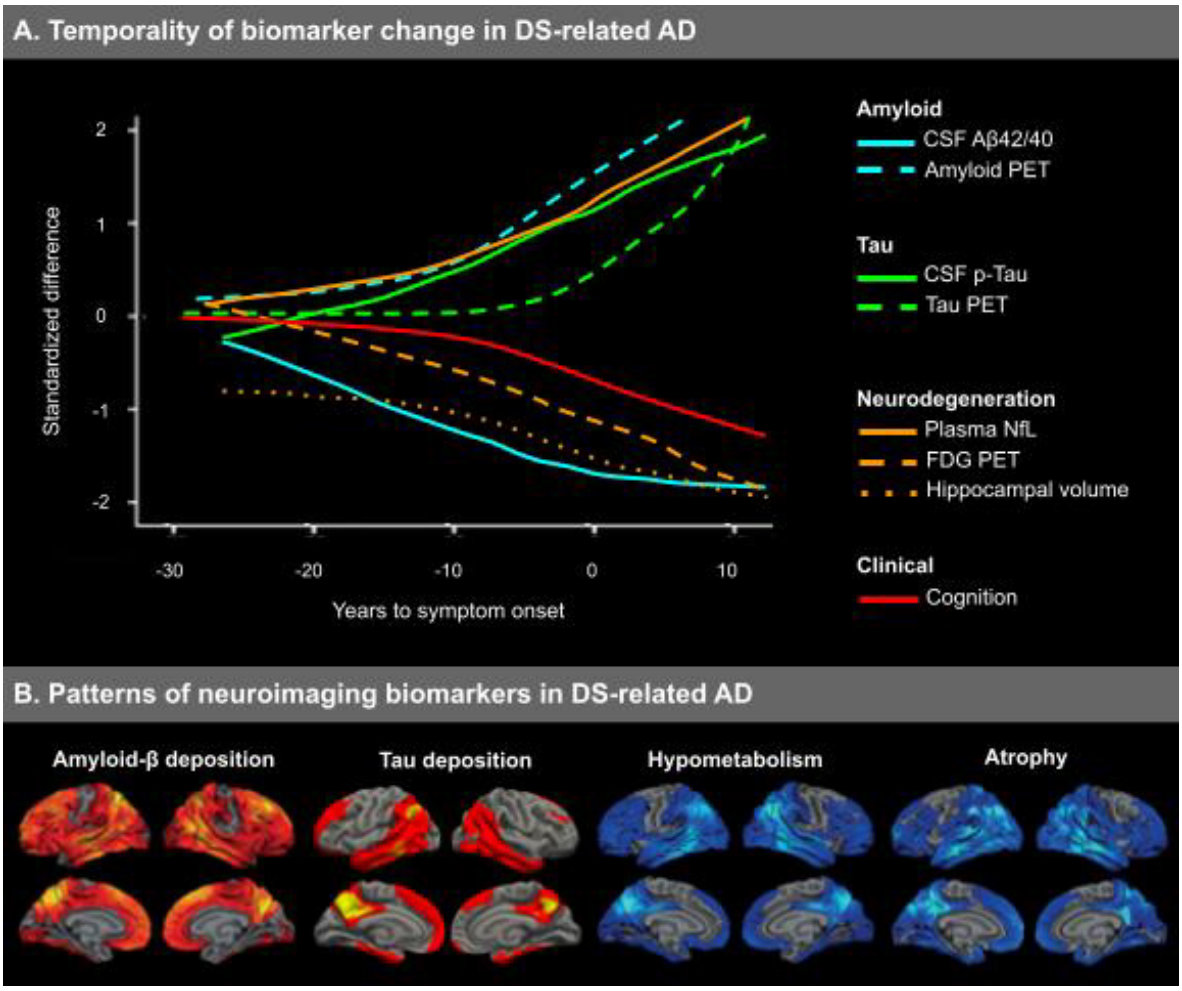
1 INTRODUCTION

1.1 Down Syndrome and Alzheimer's disease

Down syndrome (DS) or trisomy of chromosome 21, is the most common chromosomal abnormality[1] and the leading genetic cause of intellectual disability (ID) in the world[2]. Individuals with DS experience a unique set of comorbidities that often vary with age. During childhood, they are more susceptible to congenital heart defects, gastrointestinal issues, and frequent respiratory infections. As they transition into adulthood, the risk of obesity, diabetes, and thyroid disorders tends to increase. However, one of the most critical concerns in aging individuals with DS is the markedly elevated risk for Alzheimer's Disease (AD). In this respect, McCarron et al.[3] have followed people with DS for over 20 years and showed that AD dementia affects nearly 100% of participants older than 65 years.

Although most (>99%) of AD cases are sporadic, some genetic mutations can cause early onset AD with full penetrance. The familial (or autosomal dominant) forms of AD are related to mutations in the presenilin (PSEN1 and PSEN2) and amyloid precursor protein (APP) encoding genes[4]. Similarly, the triplication of chromosome 21 leads to an extra copy of the gene encoding the APP, increasing the amount of APP protein in the neuronal membrane and the amyloid-β (Aβ) peptide aggregates in the extracellular space[5]. APP gene triplication caused by the trisomy of chromosome 21 is thus the main driver of AD in DS and is considered to be both a necessary and sufficient condition to cause AD pathology and early onset dementia in this population[6]. Indeed, two case reports have shown that people with trisomy of chromosome 21 but without APP gene triplication do not have a higher risk of developing AD pathology than the general population, while patients with isolated triplication of the APP gene do not have DS phenotype but develop AD[7]. Therefore, DS is now considered a genetic form of dementia[8,9].

AD is characterized pathologically by extracellular deposits of Aβ peptide in the form of diffuse plaques, together with intracellular tau neurofibrillary tangles[10]. The neuropathological hallmarks in DS-related AD are strikingly similar in appearance and distribution to those in autosomal dominant and sporadic AD[11]. The similarities in pathology reflect in similarities in biomarkers between the different forms of AD. Therefore, the fluid AT(N) biomarker profile and the patterns of amyloid and tau deposition, glucose hypometabolism, and cortical atrophy in DS-related AD are strikingly similar to those in autosomal dominant and sporadic AD (Figure 1)[12].

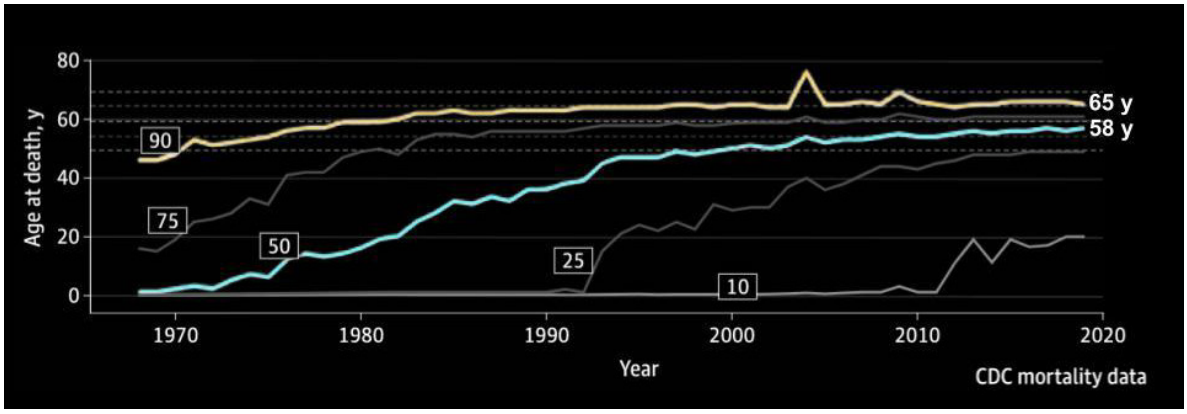


Temporal changes in pathophysiological AD biomarkers in Down syndrome. Adapted from Fortea et al.[9] and Fortea et al.[12].

Figure 1. Temporality of biomarker's changes and patterns of amyloid and tau deposition, glucose hypometabolism, and cortical atrophy in DS-related AD

Given its near full penetrance, AD crucially impacts the lifespan of people with DS. While the life expectancy of the general population showed a mild and steady increase in the 20th century, the life expectancy of people with DS increased exponentially in the same period (due to improvements in healthcare for this population, notably in cardiac surgery)[13]. However, a recent meta-analysis by Iulita et al.[14] with DS mortality data from the United States' Center for Disease Control and Prevention (CDC) showed that, despite the increase in the age at death over the past 50 years, it has reached a plateau in the past decade for most individuals. Currently, 50% of people with DS die before age 60, and 90% die before age 70, about 20 years earlier than the general population (Figure 2). This study provides data to suggest that AD limits the life expectancy of people with DS and that this population

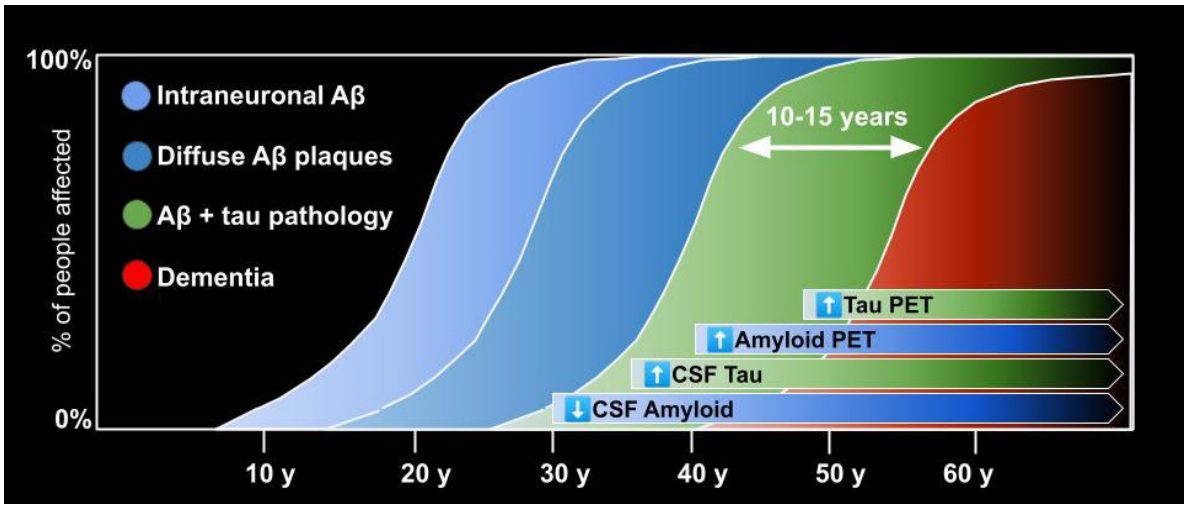
will not extend further their life expectancy until effective AD therapies become available for this population.



Center for Disease Control and Prevention (CDC) mortality data of people with DS over the past 50 years. The blue curve shows the average age at death of 50% of people with DS, while the yellow curve shows the average age at death of 90% of people with DS (adapted from Iulita et al.[14]).

Figure 2. The evolution of life expectancy in DS

DS-related AD represents a unique model for studying the natural history of AD neuropathology. Due to the different co-occurring conditions associated with DS, people with DS die earlier from non-neurodegenerative diseases, and a relatively large amount of neuropathological data across the entire lifespan is available. Neuropathological studies have shown that the amyloid cascade is a process that happens over decades and follows a predictable sequence of events, starting with intraneuronal Aβ deposits, followed by diffuse extracellular Aβ plaques formation and, lastly, tau pathology in the form of neurofibrillary tangles. In DS, Aβ pathology in the form of intraneuronal deposits starts already in childhood, while the deposition of diffuse Aβ plaques in the brain begins in teenagers. Aβ pathology happens many years before changes in cerebrospinal fluid (CSF) or positron emission tomography (PET) can be detected. The formation of tau neurofibrillary tangles follows Aβ pathology. Between 40-45 years old, people with DS tend to have full-blown AD pathology, and after 10-15 years, will develop symptoms, with a median age at prodromal AD diagnosis of 50.8 years and AD dementia of 53.8 (Figure 3)[9].

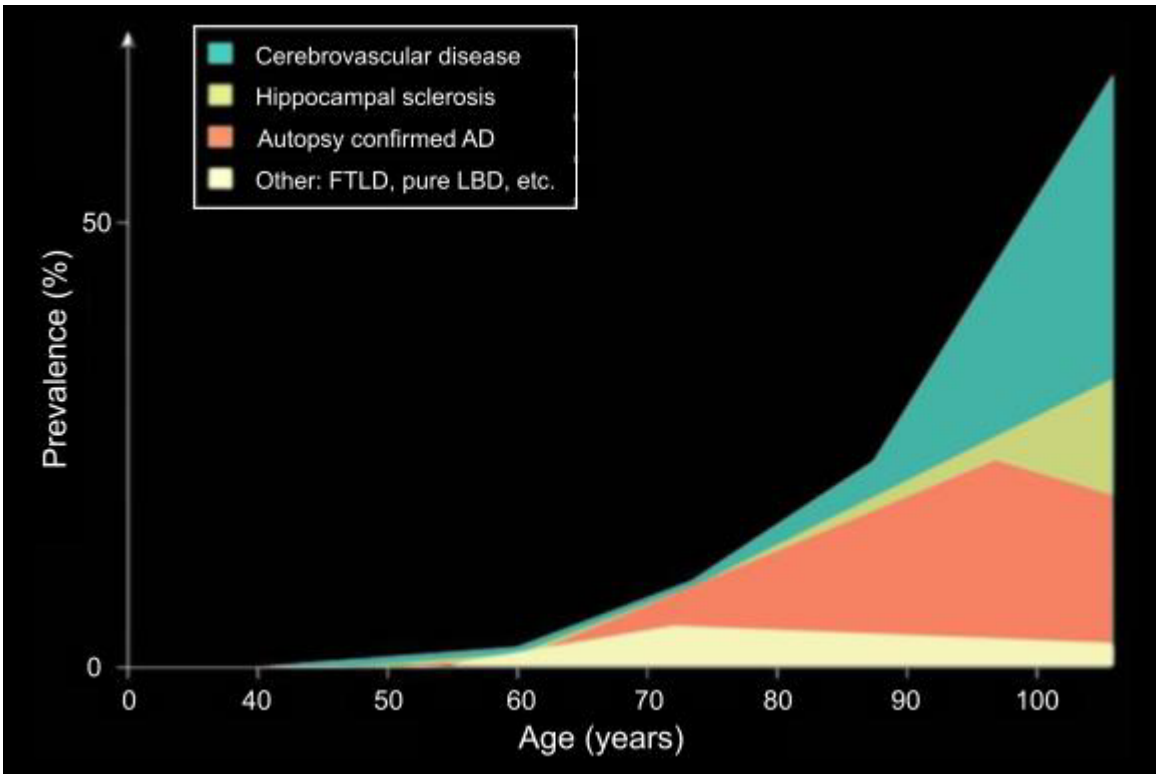


Natural history of AD neuropathology in DS (adapted from Fortea et al.[9])

Figure 3. Natural history of AD neuropathology in DS

1.2 Down syndrome and cerebral small vessel disease

Age is the leading risk factor for sporadic AD. However, several other age-related causes of cognitive decline may overlap in older adults in the general population, the most important of those being cerebrovascular disease (Figure 4)[10]. Thus, sporadic AD pathology is often associated with cerebrovascular changes (mainly cerebral small vessel disease - CSVD), frequent in the general population. The pathophysiology of CSVD is not entirely understood, but hemodynamic and wall changes in intraparenchymal and leptomeningeal blood vessels appear to be key factors in explaining the histological and neuroimaging findings.



Causes of cognitive decline in the elderly euploid population (adapted from Knopman et al.[10]).

Figure 4. Neuropathological diagnoses leading to cognitive decline as a function of age

CSVD is currently considered the vascular component that contributes most to cognitive decline in elderly patients followed up in memory clinics[15]. Classically, CSVD has been associated with symptoms related to the disconnection of white matter tracts, which include reduced mental processing speed, cognitive, executive, and motor functions, and altered mood regulation[16]. These symptoms are related to lacunes and white matter hyperintensities (WMH). However, cortical damage-related symptoms, such as deficits in language, memory, attention, and visuospatial abilities, have only recently been related to CSVD[16,17] and may be associated with cortical microbleeds and CMI.

Pantoni et al.[18] describe six distinct types of CSVD:

- **Type 1: arteriolosclerosis** (or age-related and vascular risk-factor-related small vessel disease)
- **Type 2: cerebral amyloid angiopathy (CAA)** (sporadic and hereditary)
- **Type 3: inherited or genetic small vessel diseases** distinct from cerebral amyloid angiopathy (e.g. CARASIL, CADASIL, MELAS, and others)

- **Type 4: inflammatory and immunologically mediated small vessel diseases** (e.g. Wegener's granulomatosis, Churg-Strauss syndrome, microscopic polyangiitis, Henoch-Schönlein purpura, and others)
- **Type 5: venous collagenosis**
- **Type 6: other small vessel diseases** (e.g. post-radiation angiopathy)

The most prevalent type is related to age and classic vascular risk factors (dyslipidemia, arterial hypertension, and diabetes mellitus type 2), while the second most common type is amyloid-related (CAA).

In patients with sporadic AD and neuroimaging findings of CSVD, it is impossible to disentangle if CSVD findings are related to vascular risk factors, amyloid pathology, or a combination of both. However, in DS, the prevalence of classic vascular risk factors is reduced[19,20]. Also, in this population, the APP overproduction increases the deposition of β-amyloid peptide not only in the brain parenchyma but also in the cerebral and meningeal vasculature, leading to a higher prevalence of CAA in DS[21]. In the context of sporadic AD, the prevalence of CAA is 72% in neuropathological studies[22] and 24% in neuroimaging studies[21], while in DS, CAA's prevalence is 87% in pathological studies[22] and 31% in magnetic resonance imaging (MRI) studies[21]. In sporadic CAA, reduced CSF Aβ42 and Aβ40 concentrations have been reported[23]. However, in AD-related CAA, core AD fluid biomarkers are not good predictors of in-vivo CAA diagnosis[21].

From a neuroimaging perspective, CAA is classically related to hemorrhagic manifestations, namely cerebral microbleeds and superficial siderosis. In fact, the Boston criteria[24] and the modified Boston criteria[25] for CAA diagnosis are partially based on these neuroimaging findings. However, other non-hemorrhagic findings have been recognized as part of the neuroimaging spectrum of CAA. In 2022, enlarged perivascular spaces and white matter hyperintensities were included in the modified Boston criteria 2.0[26], and a growing number of neuropathological and neuroimaging studies are recognizing CMI as an ischemic manifestation of CAA[27-29].

From a neuropathology standpoint, two phenotypes of CAA have been proposed: CAA-type 1, which affects parenchymal and meningeal capillaries and is associated with CMI, and CAA-type 2, which affects parenchymal and meningeal arteries (but not capillaries) and is associated to hemorrhagic phenomena, namely cortical microbleeds and superficial siderosis[30,31].

1.3 Cortical microinfarcts

CMI are small ischemic lesions in the brain cortex that were, until recently, invisible on MRI and only detected in post-mortem studies[32]. In fact, these lesions were, defined by the *National Institute of Neurologic Disorders and Stroke - Canadian Stroke Network Vascular Cognitive Impairment (NINSD-CSN VCI)* as "not visible to the naked eye but detectable on histological studies"[33].

However, the development of high and ultra-high field MRI enabled *in-vivo* CMI detection. In 2013, van Veluw et al.[34] detected chronic CMI using *in-vivo* 7T MRI and validated their findings based on *ex-vivo* 7T MRI and pathology. Further studies from the same group showed that lesions detectable with *in-vivo* 7T MRI were also seen with 3T MRI magnetic field[34].

In subsequent work, van Veluw et al.[35] defined three subtypes of chronic CMI on 7T and 3T scans:

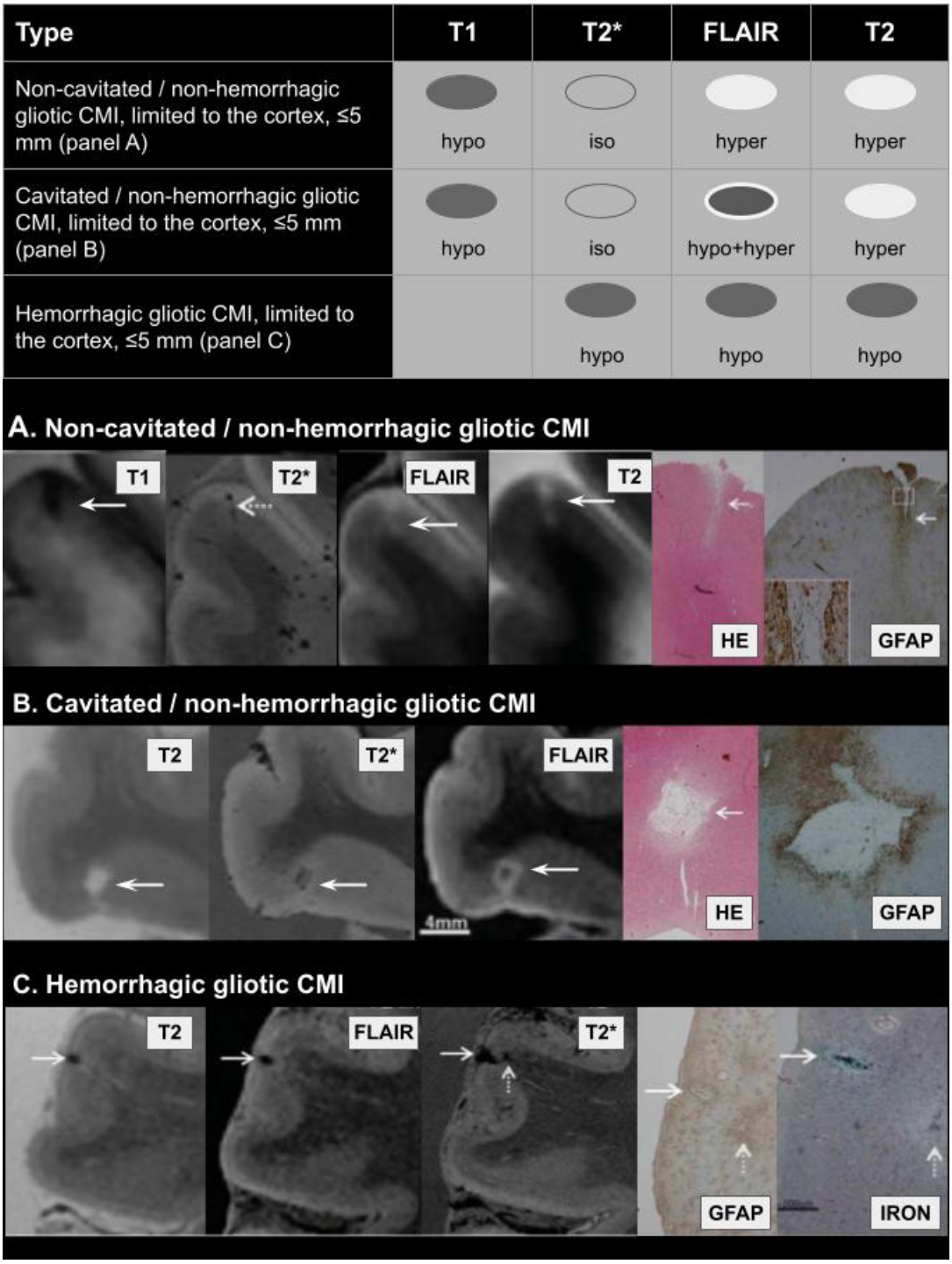
1. Non-cavitated/non-hemorrhagic;
2. Cavitated/non-hemorrhagic;
3. Non-cavitated and hemorrhagic.

Each subtype presents different signal characteristics on T1, T2, T2*, and FLAIR sequences (Figure 5). The same authors further established protocols for visually detection of these lesions on 3T and 7T MRI scans (detailed in the Methods section)[36].

While several studies have associated CMI with heart failure, carotid stenosis, and stroke, recent pathological and neuroimaging data suggest CMI to be also related to CAA, a CSVD caused by Aβ deposition in the brain capillaries walls, as previously discussed[27,28,37,38].

Moreover, studies suggest that CMI topographic distribution in the brain might reflect different pathophysiological processes. For example, a study by Kövari et al.[29] showed that CMI located in the occipital lobes were related to CAA pathology, whereas CMI in the hippocampus and frontal lobes had no relationship with CAA pathological changes in the pial vessels and cortical capillaries. Another study by Ferro et al.[39] showed that in patients with vascular dementia in this population, CMI were predominantly concentrated in the parasagittal lines of the frontal and parietal lobes, compatible with vascular watershed areas[39], which are meeting zones of terminal capillaries of large arterial trunks and are subject to hypoperfusion[40]. In a later study, the same group[41] showed that these watershed CMI are related to cerebral hypoperfusion measured with the Arterial Spin Labeling, a non-invasive MRI

technique to access cerebral perfusion by magnetically tagging the water molecules in the blood that irrigates the brain[42].



MRI and pathological features of chronic cortical microinfarcts. HE: hematoxilin & eosin; GFAP: glial fibrillary acidic protein. In panels A, B and C, GFAP staining depicts gliosis around the CMI. In panel C, iron staining depicts blood residues filling the CMI.

Figure 5. MRI and pathological features of chronic cortical microinfarcts (adapted from van Veluw et al.[35])

1.4 Rationale and relevance of the present study

CMI are small ischemic lesions in the brain cortex associated with small vessel diseases, most importantly with CAA, and with cardiac or large vessel diseases leading to cerebral hypoperfusion (e.g. heart failure and atherosclerosis). In fact, CMI have been considered a common feature of CAA, together with cerebral microbleeds and superficial siderosis[27,28,43]. MRI findings of CAA are crucial for the diagnosis, since other good biomarkers are still lacking. Disentangling the potential underlying causes of CMI in the general population is challenging due to the common overlap of vascular factors and CAA. In DS, however, the prevalence of arterial hypertension, diabetes mellitus type 2, dyslipidemia, and atheromatosis is low[20,44-47]; while the prevalence of CAA is very high, due to the overproduction of amyloid-precursor protein and increased Aβ deposition in the brain parenchyma and capillaries[48]. These features make DS a great model for studying CAA as a possible cause of CMI, with a lesser influence of vascular risk factors. However, to our knowledge, no studies assessing CMI in this population have been conducted so far.

This work is focused on adults with DS distributed along the clinical spectrum of AD (asymptomatic individuals and patients with prodromal AD or AD dementia)[8], benefiting from the largest population-based cohort of adults with DS and multimodal AD biomarkers in the world (Down Alzheimer Barcelona Neuroimaging Initiative-DABNI). This study is the first to characterize CMI in DS, and assess their relationship with age, sex, apolipoprotein E (APOE) haplotype, vascular risk factors, AD clinical continuum, AT(N) biomarkers, and cognitive performance in this population. For comparison purposes, we also included a group of symptomatic sporadic AD patients and cognitively unimpaired euploid controls.

2 HYPOTHESIS

The main hypothesis of the present work is that, given the ubiquitous brain amyloidosis and low prevalence of vascular risk factors in Down syndrome, cortical microinfarcts are related to CAA pathology in this population.

3 AIMS

Main Aim: To characterize CMI prevalence, number, and topographic distribution in adults with DS, symptomatic sporadic AD, and cognitively unimpaired controls.

Secondary Aims:

1. To assess CMI association with age, clinical AD stage, sex, APOE haplotype, and vascular risk factors in adults with DS.
2. To investigate CMI's relationship with other vascular neuroimaging findings (WMH, cerebral microbleeds, superficial siderosis, corticosubcortical infarcts, and lacunes), and fluid AT(N) biomarkers (CSF Aβ42, CSF Aβ40, CSF Aβ42/40 ratio, CSF and plasma phosphorylated tau [pTau], CSF and plasma neurofilament light [NfL]) in adults with DS.
3. To evaluate CMI's impact on cognitive performance in adults with DS (assessed with the Cambridge Examination for Mental Disorders of Older People with Down's Syndrome and others with intellectual disabilities [CAMCOG-DS] and the modified Cued Recall Test [mCRT]).

4 METHODS

4.1 Study design and setting

This is a single-center cross-sectional study in adults with DS, symptomatic sporadic AD patients and cognitively unimpaired controls, performed at the Hospital de la Santa Creu i Sant Pau in Barcelona, Spain, approved by the local Research Ethics Committee, following the human medical research standards recommended by the Declaration of Helsinki. All participants and/or their legally authorized representatives gave written informed consent. Participants were recruited between January/2011 and July/2021. MRI data were acquired at Hospital del Mar and Hospital Clínic, in Barcelona, Spain, in the same period.

4.2 Participants

This study included participants with DS, symptomatic sporadic AD patients and cognitively unimpaired euploid controls of both sexes (+18 years). Adults with DS were recruited from the Down-Alzheimer Barcelona Neuroimaging Initiative (DABNI) cohort. Participants with symptomatic sporadic AD and controls were recruited from the Sant Pau Initiative on Neurodegeneration (SPIN) cohort.

4.2.1 The SPIN and DABNI cohorts

The SPIN project was launched in 2011 as an umbrella project to group the observational clinical studies of the Sant Pau Memory Unit (Hospital de la Santa Creu i Sant Pau, Barcelona, Spain)[49]. The primary objective of the SPIN cohort is the discovery and validation of plasma, CSF, and neuroimaging biomarkers in neurodegenerative diseases. It includes cognitively normal controls, participants with subjective cognitive decline, mild cognitive decline, sporadic AD, dementia with Lewy bodies, frontotemporal lobar degeneration, and DS (DABNI cohort).

The inclusion criteria for cognitively unimpaired controls, symptomatic sporadic AD patients, and Down syndrome participants in the SPIN cohort are:

- Cognitively normal controls:
    - No memory complaints;
    - Mini–Mental State Examination (MMSE): 27-30;

    - Total Clinical Dementia Rating (CDR) global score = 0;
    - Free and Cued Selective Reminding Test (FCSRT) total immediate score (education-adjusted score at the age of 62 years [EAS62]) ≥ 7;
    - Absence of significant impairment in other domains or in daily living activities.

- Prodromal AD:
    - MMSE: 24-30;
    - CDR global score = 0.5;
    - Absence of a clinical diagnosis of dementia;
    - CSF biomarkers supporting AD pathophysiology;

- Typical AD dementia:
    - CDR global score ≥ 0.5;
    - FCSRT total immediate score (EAS62) ≤ 6;
    - Clinical criteria of “probable AD dementia with evidence of the AD pathophysiological process”[50].

- Down syndrome:
    - Presence of trisomy at chromosome 21.

The exclusion criteria are:
- Inability to complete neuropsychological tests and questionnaires (illiteracy, blindness, hearing impairment).
- Contraindication for MRI (claustrophobia, pacemaker, aneurysm clips, cardiac mechanical valve).
- Contraindication for lumbar puncture (anticoagulation, coagulation disease): Must not be taking anticoagulant treatment such as acenocoumarol, heparin, warfarin, dabigatran, rivaroxaban, apixaban.
- Current treatment with drugs that can impair cognition.
- Medical history of:
    - Neurological disease (major stroke, brain lesions, epilepsy)
    - Psychiatric disease (psychosis or major depression)
    - Drug abuse in the last year
    - Medical history of cancer is an exclusion criterion when:

- It affects the central nervous system
- It has not been in complete remission for 5 years or longer
- Patient has received potentially neurotoxic chemotherapy
- Patient has received cranial radiotherapy

The DABNI cohort was launched in 2014 by the Sant Pau Memory Unit in cooperation with the Catalan Down Syndrome Foundation. It is the world's largest population-based cohort of adults with DS with assessment for AD and aims to understand the mechanisms related to AD in patients with DS[12,51]. DABNI includes adults (≥18 years) with DS screened for AD dementia in a health plan in Catalonia, Spain, run at the Barcelona Down Medical Center (Fundació Catalana Síndrome de Down and Hospital de la Santa Creu i Sant Pau). Participants undergo yearly neurological and neuropsycho-logical assessments and optional neuroimaging, plasma, CSF, and genetic biomarker assessments[51].

4.2.2 Patient's selection for the current project

For the current project, the following inclusion criterion was established:

1. 3 Tesla MRI available with:
   a. Volumetric T1-weighted sequence (T1).
   b. T2*-weighted sequence (T2*) (SWI-susceptibility weighted imaging or GRE-gradient echo images).

The exclusion criteria were:

1. Low T1 image quality (automated Image Quality Rating < 80%, described below) limiting visual analysis of T1 volumetric images.
2. Unavailability of T1 or T2* images.

4.3 Neuropsychological evaluation and study subgroups

Neuropsychological assessment in DS is challenging and requires adapted cognitive tests. In this population, the baseline intellectual disability (ID) importantly affects the neuropsychological evaluation[52].

In participants with DS, ID was categorized into mild, moderate, severe, or profound according to the Diagnostic and Statistical Manual of Mental Disorders -

Fifth Edition (DSM-V). This assessment was based on caregivers' reports of the individuals' best-ever level of functioning and the Intelligence Quotient score of the Kaufman Brief Intelligence Test Spanish version when possible[53]. In this population, neuropsychological evaluation is only reliable in participants with mild or moderate ID, because subjects with severe or profound ID often score at the minimum (floor effect), mitigating the test's capacity of detecting cognitive decline. In DS, neuropsychological evaluation was performed with the Cambridge Examination for Mental Disorders of Older People with Down's Syndrome and others with intellectual disabilities (CAMCOG-DS, Spanish version)[54,55] for assessment of global cognition and with the modified Cued Recall Test (mCRT) for assessment of episodic memory[56]. In euploid participants, global cognitive assessment was performed with the Mini-Mental State Examination (MMSE)[57].

Regarding AD symptoms in DS, after independent neurological and neuropsychological evaluations (blinded to biomarker data), each participant with DS was classified in a consensus meeting as asymptomatic (aDS) when there was no clinical or neuropsychological suspicion of AD (i.e., absence of cognitive or functional decline compared to the previous functioning), prodromal AD (pDS) when there was cognitive impairment, but symptoms did not fulfill the criteria for dementia (i.e., cognitive impairment without functional changes), and AD dementia (dDS) when there was a functional decline compared to the previous functioning. The assessment of functional status for differentiating pDS and dDS was based on anamnesis, the Dementia Questionnaire for People with Learning Disabilities, and the CAMDEX-DS[58].

Participants with symptomatic sporadic AD were also divided into prodromal AD (pAD) when there was cognitive impairment without functional changes and AD dementia (dAD) when cognitive impairment impacted daily activities.

Lastly, cognitively unimpaired controls were divided into young controls (age<60 years) to serve as a control group for participants with DS; and old controls (age≥60 years) to serve as a control group for participants with symptomatic sporadic AD.

4.4 CSF AND Plasma AT(N) Biomarkers Acquisition and analyses

A subset of participants underwent lumbar puncture with CSF tap and/or blood collection by venipuncture. Samples were processed as previously described[51] and stored at -80° C before analysis. The CSF levels of Aβ40 and Aβ42 peptides and pTau 181 were measured using the Lumipulse G600II fully automated platform (Lumipulse, Fujirebio-Europe, Ghent, Belgium)[49]. CSF NfL concentration was quantified with an enzyme-linked immunosorbent assay (ELISA; NF-Light Assay; UmanDiagnostics, Umeå, Sweden), following the manufacturer's recommendations. All CSF samples were analyzed at Hospital Sant Pau, Spain. Plasma concentrations of pTau and NfL were measured with Single molecule Array (Simoa) technology (Quanterix, Billerica, MA, USA). Plasma pTauwas analyzed at the University of Gothenburg, Sweden, and NfL at Hospital Sant Pau, Spain, following established protocols[12,51,59]. APOE haplotype was determined by polymerase chain reaction amplification of DNA from blood samples[60]. The Alzheimer's laboratory at Hospital Sant Pau integrates the Alzheimer's Association external quality control program for CSF biomarkers[61].

The clinical cutoffs for core CSF AD biomarkers were initially obtained from a group of 70 patients clinically diagnosed with AD dementia (whose clinical diagnoses were made blind to biomarker results) and 45 age-matched cognitively normal controls. Internal cutoffs were calculated using ELISA[62], transferred to fully automated platforms and validated in a sample of patients that underwent amyloid PET[63]. The corresponding cutoffs for positivity were[63]:

- Aβ42 ≤ 916 pg/mL
- Aβ42/40 ≤ 0.062
- pTau ≥ 63 pg/mL
- pTau/Aβ42 ≤ 0.068

The number of participants with available data on APOE haplotype, vascular risk factors, CSF and plasma AT(N) biomarkers and cognitive performance is presented on Table 1.

Table 1. Number of participants with demographic data, APOE haplotype, vascular risk factors, other vascular neuroimaging findings, CSF, plasma, andneuropsychological data in each study group

	Down syndrome (N=195)	Young Controls (N=76)	Old Controls (N=30)	Sporadic AD (N=63)
N with APOE haplotype	182	75	29	55
N with vascular risk factors data	193	53	26	28
N with CSF Aß42 and Aß40	117	50	20	52
N with CSF pTau	117	51	20	52
N with CSF NfL	124	64	22	53
N with Plasma pTau	117	38	14	50
N with Plasma NfL	119	70	29	22
N with Cognitive assessment	162	75	30	62

4.5 MR Image acquisition and preprocessing

4.5.1 Acquisition

MRI was performed on a Philips Achieva 3T (Philips Healthcare) at Hospital del Mar or a Siemens TrioTim 3T scanner (Siemens Healthcare) at Hospital Clínic, in Barcelona, Spain. The neuroimaging protocol performed at Hospital del Mar includes volumetric T1, fluid-attenuated inversion recovery (FLAIR), T2*, non-volumetric T2, and diffusion-tensor imaging (DTI) acquisitions. The protocol performed at Hospital Clínic does not include the T2 sequence. These images are stored in DICOM format on a server of the Memory Unit of the Hospital de la Santa Creu i Sant Pau. Only T1, SWI, FLAIR, and T2 (when available) sequences were used for the present study. The image acquisition parameters of the MRI protocols used are detailed in Table 2.

Table 2. MRI protocols

	Hospital del Mar (3T Philips Achieva)				
	T1	**T2**	**T2***	**FLAIR**	**DTI**
TE (ms)	3.81	80	25.5	140	78
TR (ms)	8.3	3000	17.2	10000	10800
Voxel dimensions (mm)	0.93x0.93x1.0	0.43x0.42x5.4	0.44x0.44x1.0	0.9x0.9x1.0	1.6x1.3x2.0
Matrix	256x256x160	480x480x25	512x512x130	256x256x140	160x160x80
bval	NA	NA	NA	NA	1000
#Gradients	NA	NA	NA	NA	32
#B0	NA	NA	NA	NA	1
	Hospital Clínic (3T Siemens TrioTim)				
	T1	**T2**	**T2***	**FLAIR**	**DTI**
TE (ms)	2.98	NA	20	128	89
TR (ms)	2300	NA	26	9000	7700
Voxel dimensions (mm)	1.0x1.0x1.0	NA	0.75x0.75x0.75	0.8x0.8x3.0	2.0x2.0x2.0
Matrix	240x256x256	NA	240x320x192	192x256x45	122x122x60
bval	NA	NA	NA	NA	1000
#Gradients	NA	NA	NA	NA	30
#B0	NA	NA	NA	NA	1

#Gradients: number of gradients / directions b=1000 used in the acquisition of the diffusion tensor-DTI images. #B0: number of gradients / directions b=0 used in the DTI acquisition. Matrix: dimensions of the image forming matrix, TE: echo time. TR: repetition time. Voxel: dimensions of the image forming voxel.

4.5.2 Preprocessing

The preprocessing pipeline of the MR images was composed of the following procedures:

Image anonymization: All image files were anonymized to ensure the image rater had no information regarding the participant's study group.

Image conversion: Conversion of MR images from DICOM (original format generated at image acquisition) to NIFTI format (used to optimize image data storage and processing) using DCM2NIIX software (https://github.com/rordenlab/dcm2niix)

Correction of field inhomogeneity artifacts: The magnetic field field inside the MRI scanner is not homogeneous, because of interference of structural components of the scanner and the examination room (e.g., coil, stretcher, and electrical components). Thus, the acquired image usually presents heterogeneous signal intensity along the different image axes axes, generating lower and higher

signal intensity areas. Although this artifact has little impact on the visual assessment of images in clinical practice, its correction is essential to improve the anatomical registration between the different sequences (Figure 6). In this study, magnetic field inhomogeneity artifacts were corrected with the *N4 Bias Field Correction* algorithm implemented in ANTs software (Advanced Neuroimaging Tools - https://github.com/ANTsX/ANTs)[64].

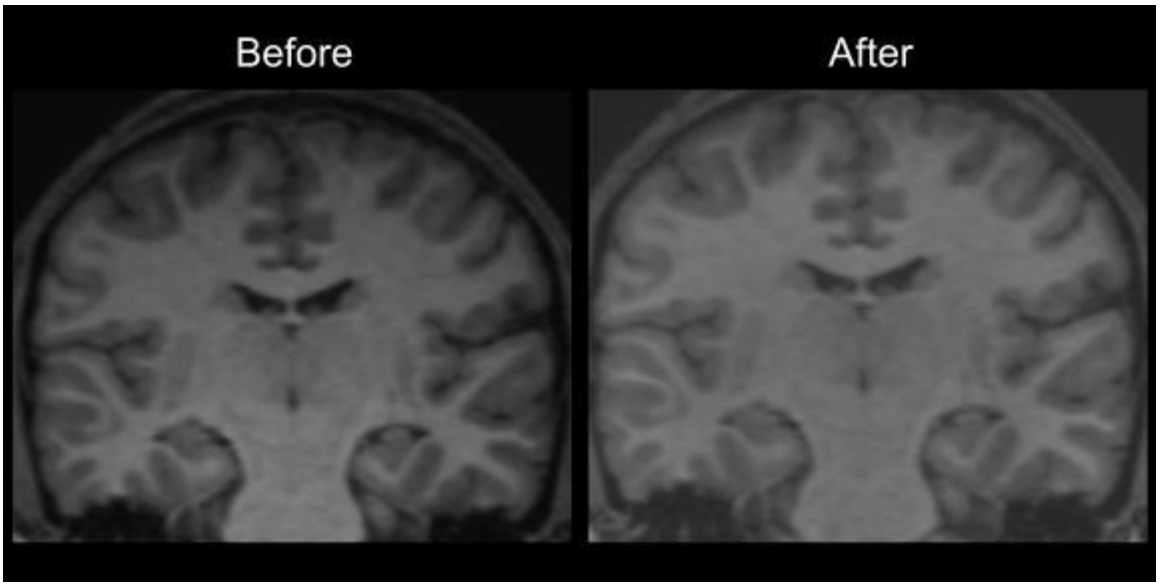


Figure 6. Correction of field inhomogeneity artifacts

Correction of motion artifacts in FLAIR sequence: Part of the FLAIR images included in the current study were acquired as a thin-slice *multishot* FLAIR acquisition, which, in practice, consists of the isolated acquisition of 256 thin slices that are grouped into a single image. This acquisition is very sensitive to motion, and minor head movement misaligns the slices, generating a noisy image. The motion artifacts in these images were solved by isolating (using FSL software - https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/) and realigning (using rigid coregistration [detailed in item 5] with ANTs software) each of the slices that compose the image (Figure 7).

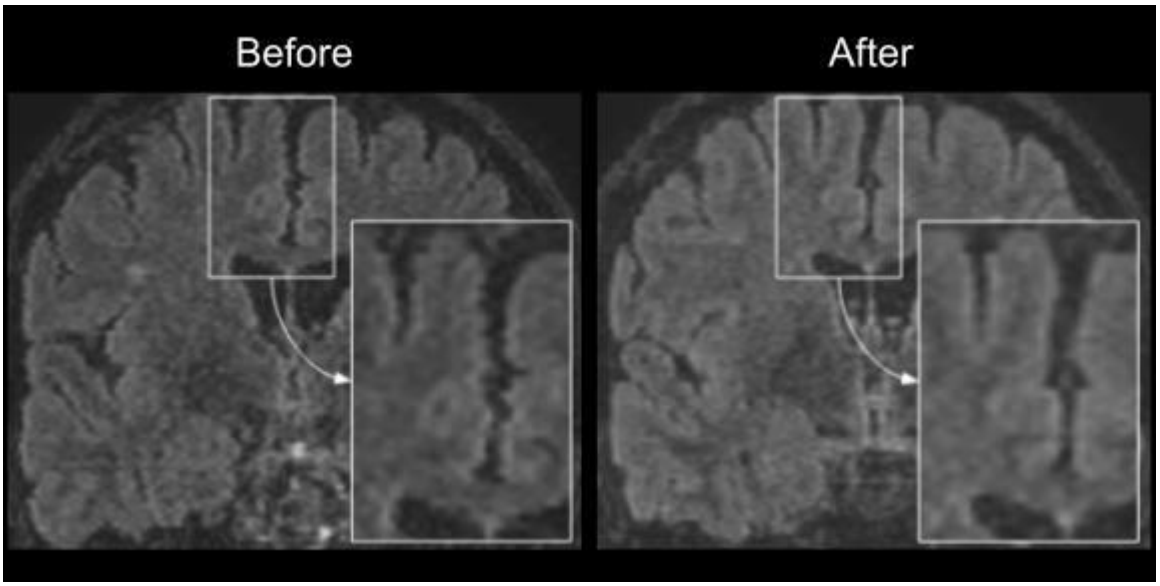


Figure 7. Correction of motion artifacts in FLAIR sequences

Image coregistration: To ensure perfect anatomical correspondence between the different sequences, T1, FLAIR, SWI, and T2 images were corregistered with a rigid registration method (6 dimensions), using T1 as the reference image. The choice of rigid registration over affine (12 dimensions) or non-linear registration modalities was made because they are images of the same patient performed in a single session. Rigid registration aligns the images through rotation and displacement in the X, Y and Z axes, avoiding greater degrees of image deformation to ensure satisfactory anatomical alignment and allowing greater processing speed. Registration was performed with Advanced Neuroimaging Tools (ANTs) software, using the *antsRegistration* function with the "Mutual Information (MI)" interpolation option (Figure 8). Achieving the best possible alignment between the different images is crucial for this project because we are dealing with millimetric lesions, and small misalignments between the images may make it impossible to evaluate the CMI on the different MRI sequences.

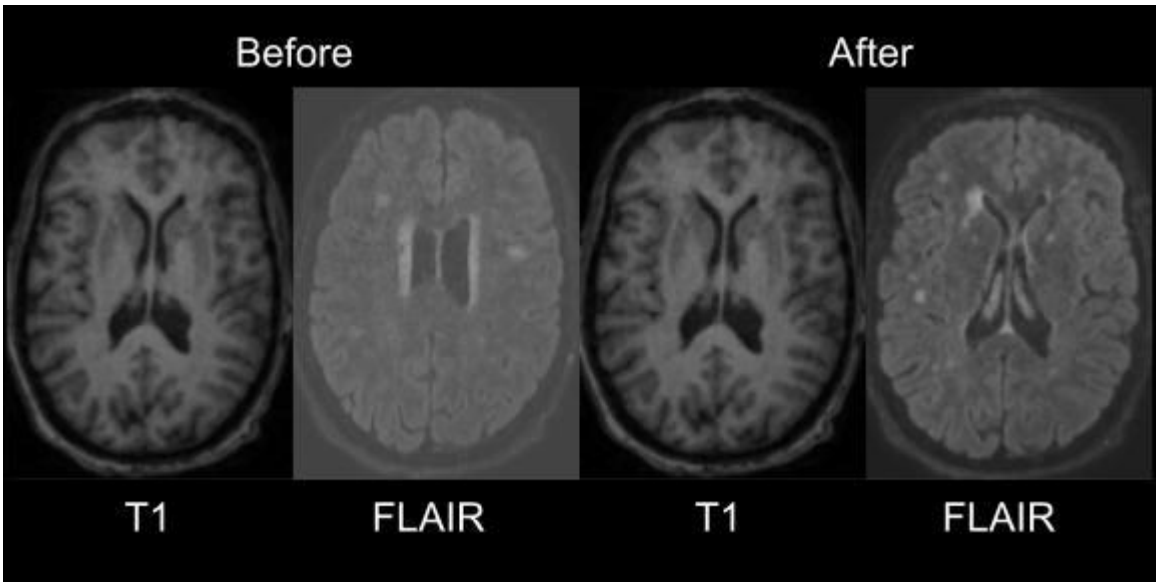


Figure 8. Image coregistration

4.6 MRI Visual Analysis

4.6.1 Identification and manual segmentation of chronic CMI

CMI assessment was performed by the Ph.D. candidate (MRA), blinded to the participant's clinical information and the status of the other imaging, CSF, and plasma biomarkers. The Ph.D. candidate has been trained for CMI visual detection in 3T MRI on an official test set available at the University Medical Center Utrecht, the Netherlands, with good reliability results on the examination scans (Dice's similarity index=0.83).

Lesion detection was performed based on the protocol previously established by van Veluw et al.[34,36] minimally modified to meet the specific needs of this project. This group has published guidelines for detecting chronic CMI on 3T and 7T MRI images, in which chronic non-hemorrhagic CMI are defined as T1 hypointense cortical lesions with hyper- or iso signal on T2, FLAIR, and T2*.

The distinction between chronic hemorrhagic cortical microinfarcts, calcifications, and cortical microhemorrhagic foci related to cerebral amyloid angiopathy is impossible based on T1-, T2-, FLAIR, and T2* sequences alone. Therefore, in this study and the protocol of Veluw et al.[36], only the chronic, non-hemorrhagic subtype of CMI will be considered.

The original protocol proposed by van Veluw et al.[36] for CMI detection on in-vivo 3T MR images consists of the following:

1. Acquire brain images of the population of interest on a 3T MRI scanner. Existing data can be analyzed if the protocol includes at least 3D T1, FLAIR, and T2 images.
2. On a visualization platform that allows simultaneous analysis of the three orthogonal image planes (e.g., MeVisLab), access the CMI on the 3T MR images as detailed below, using the following detection criteria:
    a. T1 hypointense lesions;
    b. Detectable in at least two orthogonal planes;
    c. Restricted to the cortex;
    d. Distinct from perivascular spaces;
    e. With dimension ≤4 mm.
3. Access each hemisphere on T1 in the sagittal plane:
    a. Evaluate the entire cerebral cortex for focal T1 hypointense lesions. Upon finding a lesion that meets the above criteria, mark it as a possible CMI.
    b. Explore the topography of the possible CMI on FLAIR and T2 images:
        i. Mark the lesion as probable CMI if the location is hyper- or isointense with the gray matter on FLAIR and T2;
        ii. Discard the lesion if a T2 or FLAIR hypointense focus is observed in its location, indicating that the T1 hypointense lesion is a hemorrhagic lesion, a pial vessel, or an artifact. If in doubt, evaluate the area on T2* images.
4. Re-access each hemisphere in the axial plane and simultaneously check all marked locations on the FLAIR and T2 sequences:
    a. Consider the location as probable CMI if it is hyper- or isointense on T2 and FLAIR;
    b. Discard the location if it seems related to an artifact or anatomical structure;
    c. Discard the location presenting a hypointense signal on T2.
5. Beware of artifacts that mimic CMI on T1 images:
    a. Motion artifacts in the brain margins (also appear in adjacent gyri);
    b. Folding of cortical gyri;

c. Larger caliber pial vessels;
d. Penetrating vessels.

6. Rule out possible CMI near larger infarct zones.
7. Save CMI locations and segmentation.

Some adaptations in the protocol by van Veluw et al.[36] were made to adapt it to the current project better:

1. According to van Veluw et al.[36], the detection of hemorrhagic components in cortical lesions can be done with or without T2* sequence if T2 and FLAIR sequences are available. Unlike van Veluw et al [36], in this project, we prioritized the evaluation of T2* images (SWI) over T2/FLAIR images to differentiate chronic non-hemorrhagic CMI from other lesions for two reasons:
    a. The T2 sequence is not part of the MRI protocol used for the examination of some patients in the DABNI cohort (examinations performed at Hospital Clínic)
    b. Exploratory analysis of the available images evidenced cases of foci of cortical microbleeds that mimicked CMI, showed marked hypointense signal on T2* sequence, but were not observed on T2 sequence (given its acquisition in thick slices, non-volumetric) and showed iso signal on FLAIR so that the combination of the findings on T2 and FLAIR sequences would not be able to detect these microhemorrhage foci.
2. In addition to marking the location of probable CMI, the protocol used in this project includes manual segmentation of these lesions, generating a binary image that corresponds to the location map of CMI. The binary maps will be used to assess the distribution of CMI across the brain surface.

Following the protocol suggested by van Veluw et al.[36], visual analysis was performed using the open source medical image processing and visualization platform MeVisLab (https://www.mevislab.de/), using a pipeline developed specifically for this project that allows for manual annotation and segmentation of chronic CMI. On average, the whole analysis process for a single subject takes 35 minutes.

Examples of chronic non-hemorrhagic CMI and findings that mimic these lesions are listed in the APPENDIX section.

In Figure 9, we present the criteria defined for microinfarct identification, while in Figure 10, we illustrate CMI identification, annotation, and segmentation.

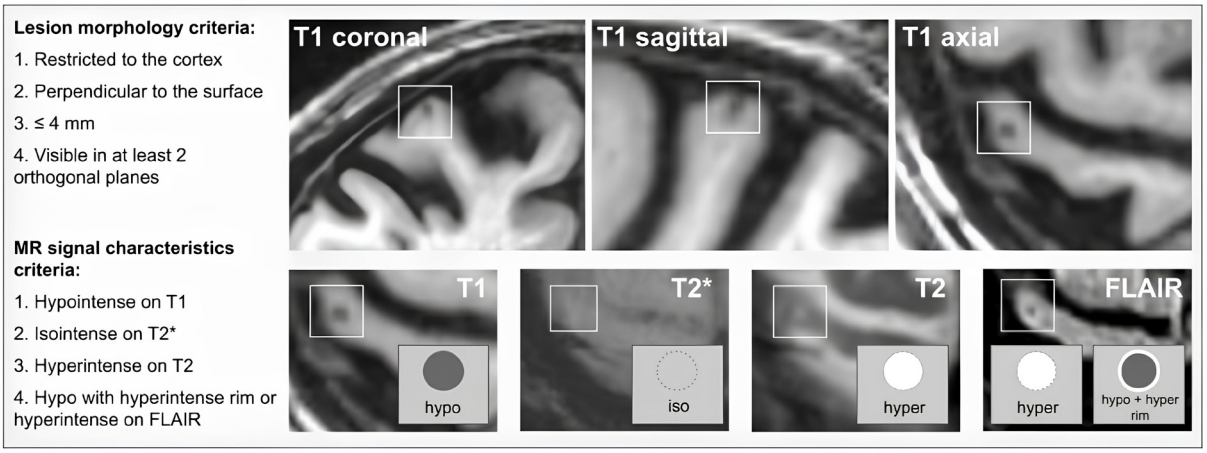


Figure 9. Identification criteria for chronic non-hemorrhagic CMI

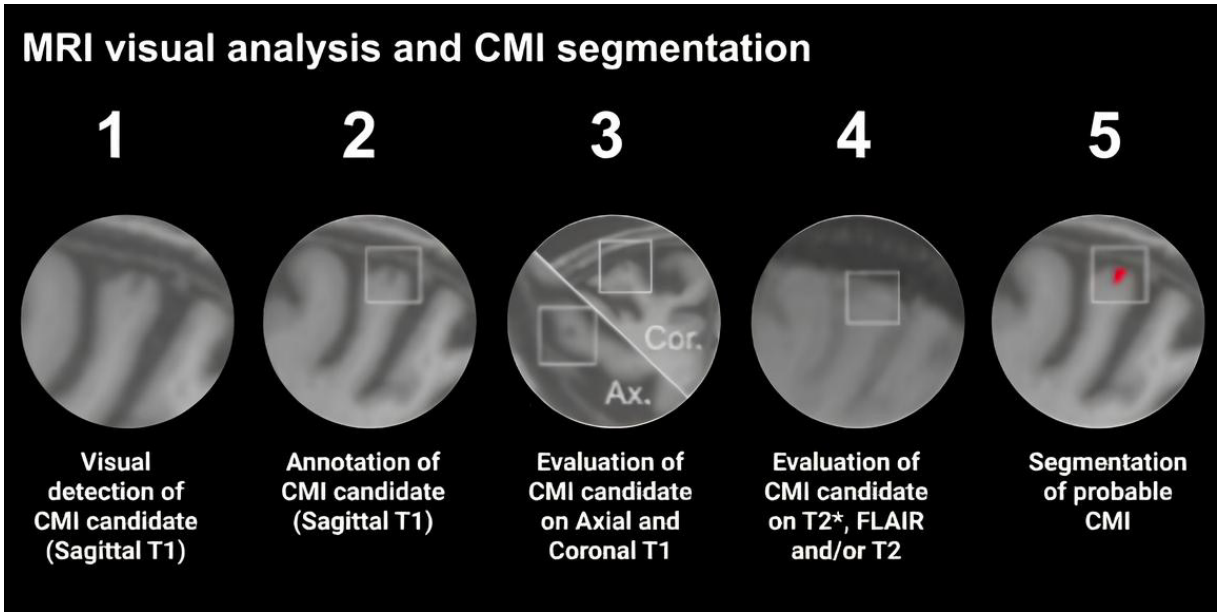


Figure 10. Manual CMI identification, annotation, and segmentation

4.6.2 Identification of other neuroimaging findings

Lacunar infarcts, cortico-subcortical infarcts, WMH, cerebral microbleeds, and superficial siderosis are exemplified in Figure 11 and were rated as follows:

- **Lacunes**: A round or ovoid subcortical cavity (3-15 mm), with a central signal similar to CSF, consistent with chronic subcortical infarct or hemorrhage in the territory of perforating arterioles[65].
- **Corticosubcortical infarcts**: Infarcts affecting the cortex and the subcortical white matter (>5 mm)[66].
- **WMH**: Foci of hyperintensity signal on FLAIR images, without cavitation, in the cerebral white matter[65].
- **Microbleeds**: Areas of low signal with the associated blooming effect seen on T2* sequences (usually 2–5 mm, but sometimes up to 10 mm[65].
- **Superficial siderosis**: a rim of low signal delineating the brain surface, particularly noted with the gradient echo or susceptibility-weighted sequences, representing hemosiderin deposition in the subarachnoid space[67].

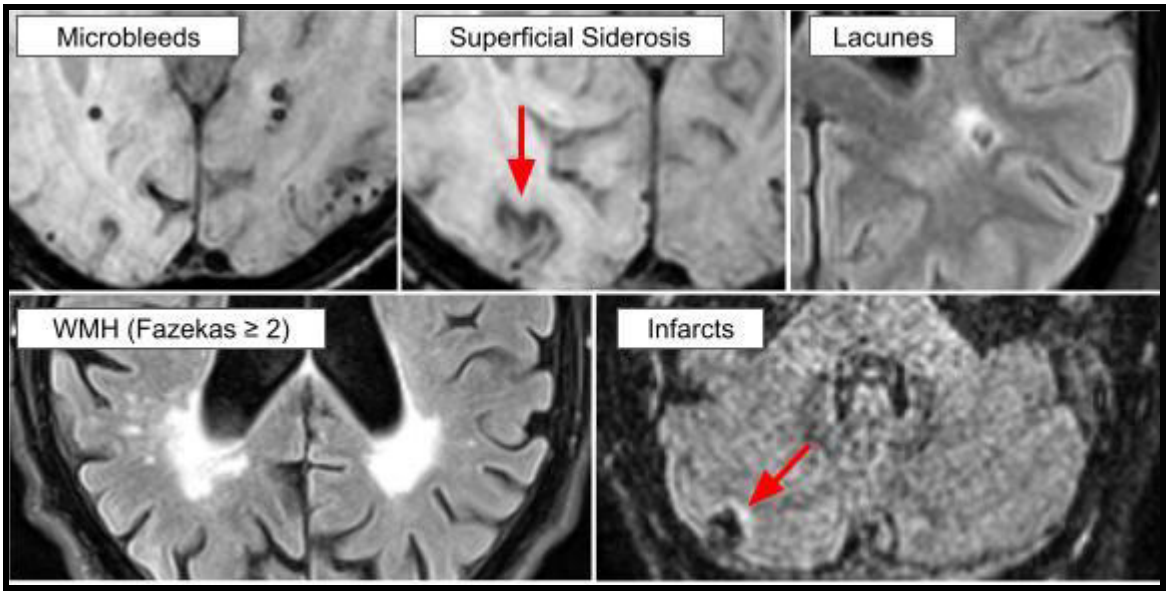


Figure 11. Examples of other vascular neuroimaging findings

4.7 Statistical analysis

Data statistical analyses were performed with R software, version 3.6.3 (www.R-project.org). Significance was set at $p<0.05$.

Statistical approach to aim 1: To investigate the CMI prevalence along the age span, DS, symptomatic sporadic AD patients, young controls, and old controls were divided into age tertiles. To assess CMI prevalence within the AD clinical continuum, DS participants were divided into aDS, pDS, and dDS, and symptomatic sporadic AD participants were divided into pAD and dAD. Arterial hypertension, dyslipdemia and diabetes mellitus type 2 were categorized as present or absent. Group comparisons were performed using the chi-squared test.

Statistical approach to aim 2: To assess the association of CMI presence and number with other vascular neuroimaging findings, participants were dichotomized by the presence of CMI and further categorized according to the number of CMI into: 1 CMI, 2-5 CMI and >5 CMI. As for the vascular neuroimaging findings, WMH (Fazekas≥ 2[68]), cerebral microbleeds, superficial siderosis, corticosubcortical infarcts, and lacunes were categorized as present or absent. Fluid AT(N) (CSF Aβ42, CSF Aβ40, CSF and plasma pTau, CSF and plasma NfL) were treated as continuous numeric variables. Group comparisons were performed using the chi-squared test for categorical data, t-test or ANOVA for continuous data with normal distribution, and the Kruskal-Wallis test for continuous data with non-normal distribution.

Statistical approach to aim 3: To investigate the association of CMI presence and number with cognitive performance, participants were dichotomized by the presence of CMI and further categorized according to the number of CMI into: 1 CMI, 2-5 CMI and >5 CMI. Neuropsychological scores were treated as continuous numeric variables. Participants with DS were stratified according to ID (mild or moderate). Group comparisons were performed using t-test or ANOVA for continuous data with normal distribution, and the Kruskal-Wallis test for continuous data with non-normal distribution.

5 RESULTS

5.1 CMI prevalence, number, topographic distribution, and association with age, clinical ad stage, sex, apoe haplotype, and vascular risk factors IN adults with DS, symptomatic sporadic AD, and cognitively unimpaired controls

5.1.1 Participants and demographics

Table 3 summarizes the demographic data, APOE haplotype, and vascular risk factors in each study group. A total of 364 participants were included: 195 adults with DS (126 aDS, 29 pDS, and 40 dDS), 63 symptomatic sporadic AD patients (43 pAD and 20 dAD), and 106 controls (76 young and 30 old controls). Figure 12 shows the flow-chart with the reasons for excluding participants from the study.

Participants with DS were younger than young controls (median age [IQR], in years: 44.4 [35.0;50.9] vs. 52.6 [46.9;56.2], years, p<0.001), had a lower proportion of females (42.6% vs. 69.7%; p=0.001) and *APOE* ε4 carriers (19.2% vs. 34.7%, p=0.013). Participants with symptomatic sporadic AD were older (mean±standard deviation, 70.1±6.8 vs. 66±5, years, p=0.001), and had a higher proportion of *APOE* ε4 carriers than old controls (54.5% vs. 20.7%, p=0.006) (Table 3).

In DS, the prevalence of arterial hypertension and dyslipidemia was lower than in young controls (2.1% vs. 13.2% [p=0.002] and 19.2% vs. 34% [p=0.035], respectively), while the prevalence of diabetes mellitus type 2 in both groups were not statistically different (p=0.345). No differences between the prevalence of vascular risk factors were observed between symptomatic sporadic AD patients and old controls (Table 3).

Table 3. Demographic data, APOE haplotype, and vascular risk factors in each study group

	Young Controls	Down syndrome				p	Old Controls	Sporadic AD			p
		All DS	aDS	pDS	dDS			All AD	pAD	dAD	
Age (years)	52.6 [46.9;56.2]	44.4 [35;50.9]	39.0 [30;44.4]	49.6 [47.7;52.3]	54.2 [49;56.6]	<0.001[a]	66±5	70.1±6.8	70.3±6.7	69.7±7	0.001[c]
Sex (female)	69.7%	42.6%	42.1%	44.8%	42.5%	<0.001[b]	46.7%	63.5%	65.1%	60.0%	0.189[b]
APOE ε4 +	34.7%	19.2%	19.0%	26.9%	15.0%	0.013[b]	20.7%	54.5%	62.2%	38.9%	0.006[b]
APOE ε2 +	5.3%	10.4%	9.5%	23.1%	5.0%	0.288[b]	10.3%	3.6%	5.4%	0.0%	0.335[b]
Hypertension	13.2%	2.1%	0.8%	0.0%	7.9%	0.002[b]	38.5%	44.8%	41.2%	50.0%	0.838[b]
Dyslipidemia	34.0%	19.2%	15.1%	31.0%	23.7%	0.035[b]	38.5%	60.0%	61.1%	58.3%	0.180[b]
D. Mellitus 2	0.0%	3.1%	3.2%	3.4%	2.6%	0.345[b]	7.7%	14.3%	18.8%	8.3%	0.670[b]

Data presented as: frequency (%), median [Interquartile Range] or mean±standard deviation; [a]Kruskall-Wallis test; [b]chi-squared test; [c]t-test

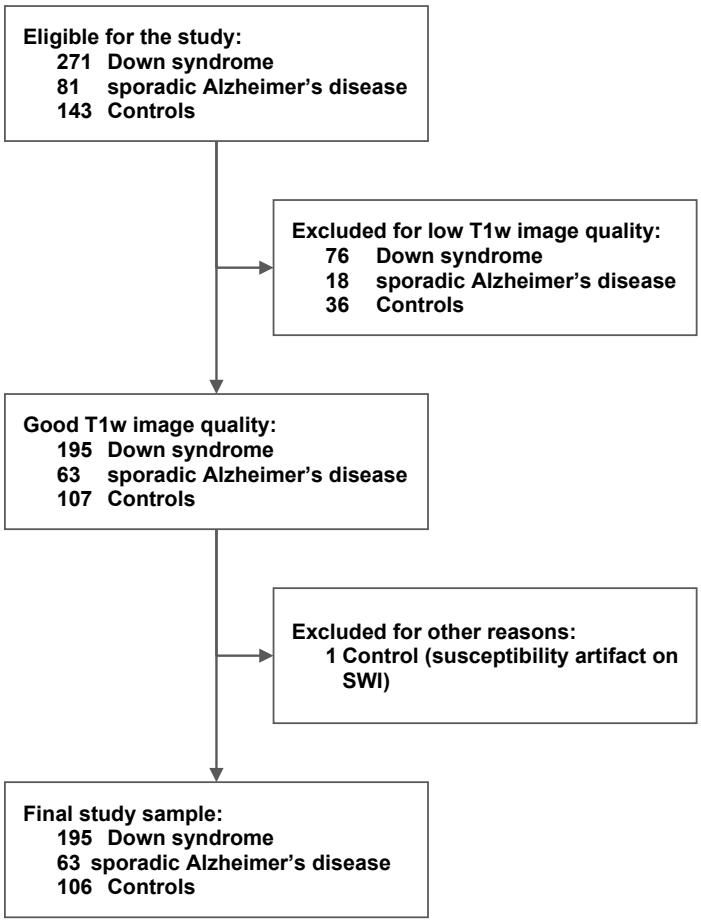


Figure 12. Flowchart of the reasons for exclusion of participants from the study

5.1.2 CMI prevalence, number and topographic distribution in each study group

The prevalence of CMI was 11.8% in DS overall compared with 2.6% in young controls (p=0.035). In symptomatic sporadic AD patients, the prevalence of CMI was 17.5%, while 10.0% in old controls (p=0.717) (Table 4).

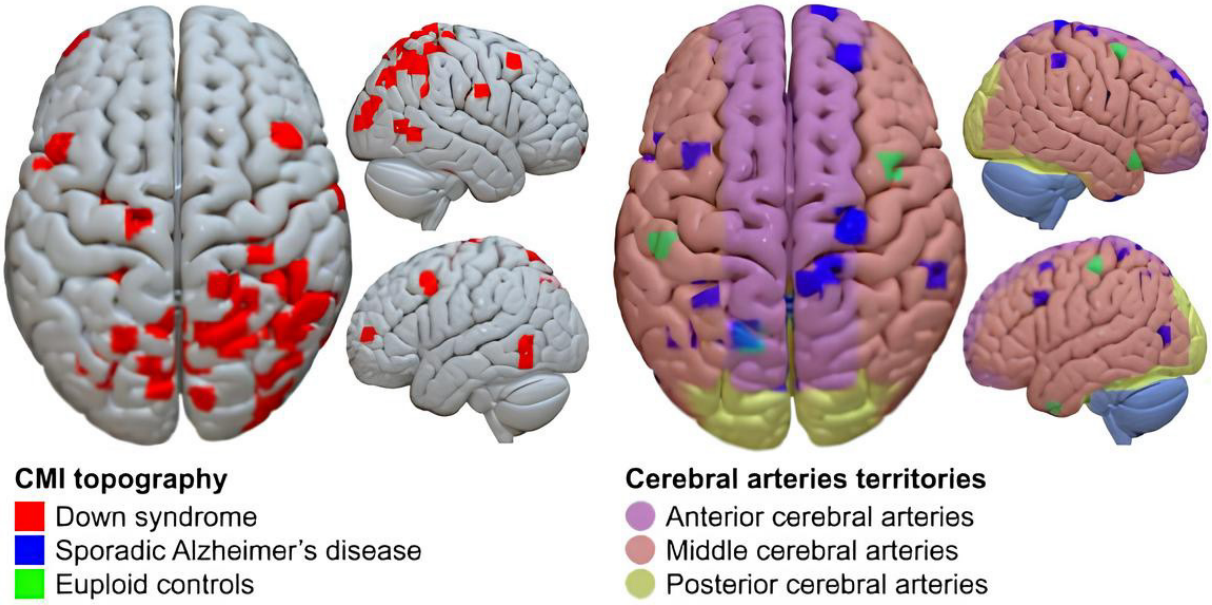
We identified 74 CMI in 23 adults with DS, located mainly in posterior regions and predominantly in the parietal lobes. In euploid participants, we found 22 CMI in 11 sporadic AD patients and five CMI in five controls, predominantly distributed along parasagittal lines in frontoparietal regions (Figure 13).

Considering only the participants with CMI, the average number of CMI per participant was nominally higher in DS than in controls (3.1 vs. 1.0, d=0.570, p=0.132) and sporadic AD (3.1 vs. 2.0, d=0.340, p=0.866), but statistical significance was not reached (Table 4).

Table 4. Prevalence and number of cortical microinfarcts in each study group

	Young Controls	Down syndrome				p	Old Controls	Sporadic AD			p
		All DS	aDS	pDS	dDS			All AD	pAD	dAD	
CMI prevalence											
Overall	2.6%	11.8%	8.7%	6.9%	25.0%	0.035[a]	10.0%	17.5%	20.9%	10.0%	0.717[a]
1st age tertile	3.8%	4.6%	-	-	-	0.783[a]	10.0%	19%	-	-	0.095[a]
2nd age tertile	4.0%	10.8%	-	-	-	0.077[a]	10.0%	14.3%	-	-	0.383[a]
3rd age tertile	0%	20.0%	-	-	-	<0.001[a]	10.0%	19%	-	-	0.095[a]
CMI number (only subjects with CMI were considered)											
Average/subj.	1.0	3.1	3.1	2.0	3.6	0.132[b]	1.0	2.0	2.1	1.5	0.866[b]
1st age tertile	1.0	2.3	-	-	-	0.239[b]	1.0	1.8	-	-	0.633[b]
2nd age tertile	1.0	4.6	-	-	-	0.128[b]	1.0	3.0	-	-	0.317[b]
3rd age tertile	0	2.7	-	-	-	0.100[b]	1.0	1.0	-	-	1.000[b]

Data presented as frequency (%) or numeric average. [a]chi-squared test; [b]t-test



Spatial distribution of CMI. CMI were individually annotated with MeVisLab to create a binary map in the native space with all CMI locations for each subject. Then each CMI map was then transformed from native to MNI space using ANTs software. Finally, all transformed CMI maps were plotted as an overlay in an MNI brain 3D model using Surf Ice software (https://www.nitrc.org/projects/surfice/). Additionally, for the figures in the right panel row, a mask of the vascular territories of cerebral arteries was plotted on the brain model to depict the vascular watershed area.

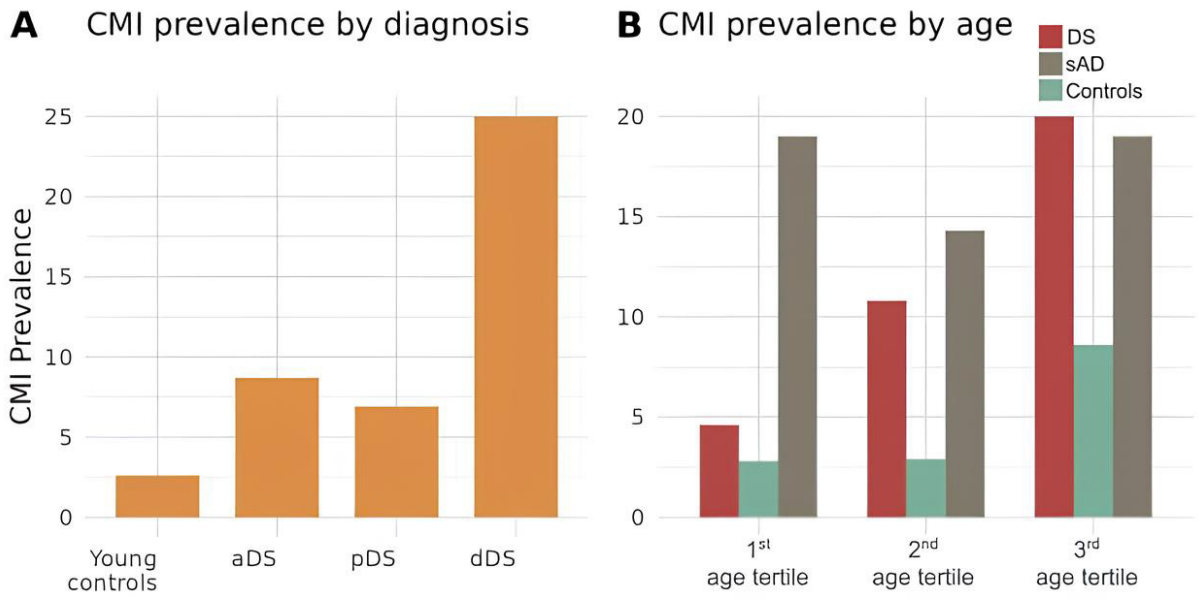
Figure 13. Spatial distribution of CMI

5.1.3 CMI prevalence and number with age and along the AD clinical continuum

In DS, CMI prevalence increased along the AD continuum from 8.7% in aDS and 6.9% in pDS to 25% in dDS (p=0.005) (Table 4, Figure 2A). In symptomatic sporadic AD patients, no statistically significant differences on CMI prevalence were observed between prodromal and demented patients (20.9% in pAD and 10% in dAD, p=0.478) (Table 4).

Considering only the participants with CMI, the average number of CMI per participant did not increase along the AD clinical continuum in DS (3.1 in aDS, 2.0 in pDS and 3.6 in dDS, p=0.889) or in symptomatic sporadic AD (1.5 in pAD and 2.1 in dAD, p=0.445).

CMI prevalence, but not number, increased with age in DS and controls. In DS, CMI prevalence was 4.6% in the first, 10.8% in the second, and 20.0% in the third age tertiles. Among controls (young and old controls together), CMI prevalence was 2.8% in the first, 2.9% in the second, and 8.6% in the third age tertiles. However, in sporadic AD patients, neither CMI prevalence or number increased with age (Table 4, Figure 14B).



CMI: cortical microinfarcts; DS: Down syndrome; aDS: asymptomatic Down syndrome; pDS: prodromal Down syndrome-related Alzheimer's disease; dDS: Down syndrome-related Alzheimer's disease dementia; sAD: symptomatic sporadic Alzheimer's disease

Figure 14. Prevalence of CMI with age and along the AD clinical continuum in Down syndrome

5.1.4 CMI presence and number with sex, APOE haplotype, and vascular risk factors

CMI presence was not associated with sex, APOE ε4 or ε2 haplotypes, arterial hypertension, diabetes mellitus type 2, or dyslipidemia in adults with DS, symptomatic sporadic AD patients or controls (Table 5). CMI number was also not associated with these variables in DS and symptomatic sporadic AD (Table 6). Since cognitively unimpaired controls with CMI only had one lesion per participant, the relationship of CMI number and these variables was not assessed in this group.

Table 5. Differences in demographic data between participants with and without CMI in each study group

	Down syndrome			Sporadic AD			Controls		
	CMI - (N=172)	CMI + (N=23)	p	CMI - (N=52)	CMI + (N=11)	p	CMI - (N=101)	CMI + (N=05)	p
Demographics									
Age, years	43.9 [32.3;50.2]	49.9 [41.6;54.9]	0.015[a]	70.1±7.0	70.0±5.9	0.935[c]	55.3 [49.2;60.3]	63.4 [53.8;63.7]	0.344[a]
Sex (female)	43.6%	34.8%	0.562[b]	67.3%	45.5%	0.189[b]	62.4%	80.0%	0.650[b]
APOE ε4 +	19.3%	19.0%	1.000[b]	50.0%	77.8%	0.160[b]	31.0%	25.0%	1.000[b]
APOE ε2 +	11.2%	4.8%	0.703[b]	2.2%	11.1%	0.303[b]	7.0%	0.0%	1.000[b]
Hypertension	2.3%	0.0%	1.000[b]	48.0%	25.0%	0.606[b]	20.0%	50.0%	0.201[b]
Dyslipidemia	18.7%	22.7%	0.773[b]	61.5%	50.0%	1.000[b]	33.3%	75.0%	0.125[b]
D. Mellitus 2	2.9%	4.5%	0.521[b]	16.7%	0.0%	1.000[b]	2.7%	0.0%	1.000[b]

Data presented as: frequency (%), median [Interquartile Range] or mean±standard deviation; [a]Kruskall-Wallis test; [b]chi-squared test.

Table 6. Relationship of CMI number with demographic data, APOE haplotype, and vascular risk factors in Down syndrome and symptomatic sporadic Alzheimer's disease

	Down syndrome				Sporadic Alzheimer's disease		
	1 CMI (N=15)	2-5 CMI (N=4)	>5 CMI (N=4)	p-value	1 CMI (N=6)	2-5 CMI (N=5)	p-value
Demographics							
Age, years	46.3 [40.0;54.8]	54.5 [50.5;58.4]	50.1 [47.1;52.0]	0.252[a]	70.4±7.6	69.4±4.0	0.791[c]
Sex (female)	33.3%	25.0%	50.0%	0.837[b]	50.0%	40.0%	1.000[b]
APOE ε4 +	21.4%	33.3%	0.0%	0.757[b]	66.7%	100.0%	0.500[b]
APOE ε2 +	0.0%	33.3%	0.0%	0.143[b]	0.0%	33.3%	0.333[b]
hypertension	0.0%	0.0%	0.0%	-	0.0%	50.0%	1.000[b]
Dyslipidemia	26.7%	25.0%	0.0%	1.000[b]	50.0%	50.0%	1.000[b]
D. Mellitus 2	6.7%	0.0%	0.0%	1.000[b]	0.0%	0.0%	-

Data presented as: frequency (%), median [Interquartile Range] or mean±standard deviation; [a]Kruskall-Wallis test; [b]chi-squared test; [c]t-test.

5.2 Relationship between cmi presence and number with other vascular neuroimaging findings, and fluid AT(N) biomarkers

5.2.1 CMI presence and number with other vascular neuroimaging findings

Microbleeds, superficial siderosis and lacunes were more prevalent in DS than in young controls (p=0.001, p=0.022, and p=0.047, respectively). WMH (Fazekas≥2) were more prevalent in symptomatic sporadic AD patients compared to old controls (p=0.023) (Table 7).

In DS, lacunes and corticosubcortical infarcts were more prevalent among participants with CMI than in those without CMI (p=0.026 and p=0.004, respectively). A trend toward a higher prevalence of WMH (Fazekas≥2) was also observed in participants with CMI (p=0.054). We found no associations of CMI presence with cerebral microbleeds or superficial siderosis (Table 8).

In symptomatic sporadic AD patients, CMI presence was related to corticosubcortical infarcts (p=0.028). In controls, no association of CMI presence and other neuroimaging findings was observed. No relationship between CMI and cerebral microbleeds or superficial siderosis was observed among euploid participants (Table 8).

Increased number of CMI per participant with CMI was not associated with higher prevalence of lacunes, corticosubcortical infarcts, WMH, microbleeds or superficial siderosis in adults with DS or symptomatic sporadic AD patients (Table 9). Since cognitively unimpaired controls with CMI only had one lesion per participant, the relationship of CMI number and these variables was not assessed in this group.

Table 7. Prevalence of vascular neuroimaging findings in each study group

	Young Controls	Down syndrome				p	Old Controls	Sporadic AD			p
		All DS	aDS	pDS	dDS			All AD	pAD	dAD	
Neuroimaging findings											
Cerebral microbleeds	7.9%	27.2%	15.4%	41.4%	53.8%	0.001[a]	20%	27%	34.9%	10%	0.636[a]
Superficial siderosis	0%	6.3%	0.8%	10.7%	20.5%	0.022[a]	0%	4.8%	7%	0%	0.548[a]
WMH (Fazekas≥2)	0%	4.1%	3.2%	3.4%	7.5%	0.111[a]	6.7%	30.2%	30.2%	30%	0.023[a]
Lacunes	1.3%	8.2%	8.7%	3.4%	10%	0.047[a]	10%	12.7%	14%	10%	1.000[a]
Infarcts (>4mm)	0%	3.6%	2.4%	3.4%	7.5%	0.196[a]	6.7%	3.2%	2.3%	5%	0.592[a]

Data presented as: frequency (%); [a]chi-squared test.

Table 8. Differences in vascular neuroimaging findings between participants with and without CMI in each study group

	Down syndrome			Sporadic AD			Controls		
	CMI - (N=172)	CMI + (N=23)	p	CMI - (N=52)	CMI + (N=11)	p	CMI - (N=101)	CMI + (N=05)	p
Neuroimaging findings									
Cerebral microbleeds	26.0%	36.4%	0.442[a]	23.1%	45.5%	0.149[a]	11.3%	20.0%	0.458[a]
Superficial siderosis	5.4%	13.6%	0.148[a]	3.8%	9.1%	0.443[a]	0%	0%	-
WMH (Fazekas≥2)	2.9%	13.0%	0.054[a]	26.9%)	45.5%)	0.283[a]	1.0%	20.0%	0.093[a]
Lacunes	6.4%	21.7%	0.026[a]	11.5%	18.2%	0.620[a]	4.0%	0%	1.000[a]
Infarcts (>4mm)	1.7%	17.4%	0.004[a]	0.0%	18.2%)	0.028[a]	2.0%	0%	1.000[a]

Data presented as: frequency (%); [a]chi-squared test.

Table 9. Relationship of cortical microinfarct's number with vascular neuroimaging findings in Down syndrome and symptomatic sporadic Alzheimer's disease

	Down syndrome			p	Sporadic Alzheimer's disease		p
	1 CMI (N=15)	2-5 CMI (N=4)	>5 CMI (N=4)		1 CMI (N=6)	2-5 CMI (N=5)	
Neuroimaging findings							
Cerebral microbleeds	35.7%	75.0%	0.0%	0.083[a]	16.7%	80.0%	0.080[a]
Superficial siderosis	21.4%	0.0%	0.0%	1.000[a]	0.0%	20.0%	0.455[a]
WMH (Fazekas≥2)	13.3%	25.0%	0.0%	0.743[a]	33.3%	60.0%	0.567[a]
Lacunes	13.3%	50.0%	25.0%	0.208[a]	0.0%	40.0%	0.182[a]
Infarcts (>4mm)	6.7%	25.0%	50.0%	0.103[a]	16.7%	20.0%	1.000[a]

Data presented as: frequency (%); [a]chi-squared test.

5.2.2 CMI presence and number with fluid AT(N) biomarkers

Fluid AT(N) biomarker levels are presented in Table 10.

Fluid amyloid and tau biomarkers were not associated with CMI's presence in the overall DS sample or any of the three clinical subgroups. We only found a higher plasma concentration of pTau in symptomatic sporadic AD patients with CMI compared to those without (p=0.047) (Table 11 and Figure 15). No relationship was observed between the number of CMI and amyloid or tau biomarkers in adults with DS or in symptomatic sporadic AD patients. Also, no relationship between the presence of CMI and fluid amyloid or tau biomarker was observed in controls (Table 12 and Figure 15).

Plasma NfL concentration was higher in the overall sample of DS participants with CMI than those without (p=0.044), but such difference was not observed within each stage of the AD clinical continuum (Table 10 and Figure 15). No relationship was observed between the number of CMI and CSF or plasma NfL in adults with DS or in symptomatic sporadic AD patients (Table 12).

Table 10. Fluid AT(N) biomarkers in each study group

	Young Controls	Down syndrome				p	Old Controls	Sporadic AD			p
		All DS	aDS	pDS	dDS			All AD	pAD	dAD	
Fluid AT(N) biomarkers											
CSF Aβ40	11.6±2.9	11.6±4.2	12.1±4.3	10.6±4.2	11.1±3.7	0.925[a]	13.4±4.1	12.5±2.8	12.3±2.9	12.4±2.7	0.374[a]
CSF Aβ42	1.1 [1.0;1.4]	0.7 [0.5;0.9]	0.9 [0.7;1.3]	0.5 [0.4;0.6]	0.5 [0.4;0.6]	<0.001[b]	1.3 [1.1;1.6]	0.5 [0.4;0.6]	0.6 [0.5;0.6]	0. [0.4;0.6]	<0.001[a]
CSF Aβ42/40	0.1 [0.1;0.1]	0.1 [<0.1;0.1]	0.1 [0.1;0.1]	<0.1 [<0.1;0.1]	<0.1 [<0.1;<0.1]	<0.001[b]	0.1 [0.1;0.1]	<0.1 [<0.1;<0.1]	<0.1 [<0.1;<0.1]	<0.1 [<0.1;<0.1]	<0.001[a]
CSF pTau	3.2 [2.6;4.1]	4.4 [2.1;10.7]	2.6 [1.6;4.2]	9.1 [5.0;14.3]	14.3 [9.3;18.2]	0.038[b]	4.2 [3.3;4.8]	10.6 [7.8;16.2]	10.1 [7.5;13.8]	16.3 [9.3;22.6]	<0.001[a]
Plasma pTau	1.3 [1.0;1.8]	1.5 [0.9;2.4]	1.1 [0.8;1.6]	2.0 [1.3;2.3]	2.4 [20;3.9]	0.459[b]	1.0 [0.8;1.5]	1.9 [1.5;2.6]	1.9 [1.5;2.4]	1.8 [1.5;2.8]	<0.001[a]
CSF NfL	0.4 [0.3;0.4]	0.5 [0.3;0.8]	0.3 [0.2;0.5]	0.7 [0.6;0.8]	1.1 [0.7;1.6]	<0.001[b]	0.5 [0.4;0.6]	0.9 [0.7;1.1]	0.8 [0.6;1.0]	1.0 [0.8;1.2]	<0.001[b]
Plasma NfL	0.8 [0.6;1.1]	1.2 [0.7;2.0]	0.9 [0.6;1.3]	1.3 [1.2;1.9]	2.5 [2.0;3.9]	0.006[b]	1.1 [0.8;1.3]	1.5 [1.2;1.8]	1.4 [1.0;1.7]	1.8 [1.4;2.6]	0.010[b]

Data presented as median [Interquartile Range]; [a]t-test; [b]Kruskall-Wallis test. CSF Aβ40 and CSF Aβ42 expressed in $.10^3$pg/mL; CSF and plasma pTau expressed in $.10^1$pg/mL

Table 11. Differences in fluid AT(N) biomarkers between participants with and without CMI in each study group

	Down syndrome		p	Sporadic AD		p	Controls		p
	CMI - (N=172)	CMI + (N=23)		CMI - (N=52)	CMI + (N=11)		CMI - (N=101)	CMI + (N=05)	
Fluid AT(N) biomarkers									
CSF Aβ40	11.8±4.2	9.9±3.1	0.078[a]	12.3±2.5	13.2±4.0	0.530[a]	12±3.1	15±5.7	0.356[a]
CSF Aβ42	0.7 [0.5;0.9]	0.6 [0.4;0.9]	0.341[b]	0.5±0.1	0.6±0.2	0.476[a]	1.2±0.4	1.3±0.2	0.221[a]
CSF Aβ42/40	0.1 [<0.1;0.1]	0.1 [0.1;0.1]	0.957[b]	<0.1±<0.1	<0.1±<0.1	0.886[a]	0.1 [0.1;0.1]	0.1 [0.1;0.1]	0.288[b]
CSF pTau	4.2 [2.1;10.7]	5.2 [3.4;10.7]	0.512[b]	10.5 [7.8;16.0]	14.1 [8.6;18.8]	0.365[b]	3.3 [2.7;4.3]	4.1 [3.7;6.4]	0.201[b]
Plasma pTau	1.4 [0.9;2.3]	2.4 [1.1;3.7]	0.245[b]	1.8 [1.4;2.6]	2.2 [2.1;2.6]	0.047[b]	1.2 [0.9;1.8]	1.6 [1.6;1.6]	0.571[b]
CSF NfL	0.5 [0.2;0.7]	0.9 [0.5;1.3]	0.056[b]	0.9 [0.7;1.0]	0.9 [0.7;1.3]	0.492[b]	0.4 [0.3;0.5]	0.4 [0.4;0.5]	0.688[b]
Plasma NfL	1.2 [0.7;1.2]	1.9 [1.0;3.4]	0.044[b]	1.4 [1.2;2.1]	1.6 [1.4;1.7]	1.000[b]	1.0 [0.7;1.2]	0.7 [0.6;0.8]	0.129[b]

Data presented as: median [Interquartile Range] or mean±standard deviation; [a]t-test, [b]Kruskall-Wallis test. CSF Aβ40 and CSF Aβ42 expressed in $.10^3$pg/mL; CSF and plasma pTau expressed in $.10^1$pg/mL

Table 12. Relationship of cortical microinfarct's number with fluid AT(N) biomarkers in Down syndrome and symptomatic sporadic Alzheimer's disease

	Down syndrome			p	Sporadic Alzheimer's disease		p
	1 CMI (N=15)	2-5 CMI (N=4)	>5 CMI (N=4)		1 CMI (N=6)	2-5 CMI (N=5)	
Fluid AT(N) biomarkers							
CSF Aβ40	10.5±3.1	4.7±0	10.2±2.3	0.221[a]	14.7±2.6	10.9±4.9	0.223[c]
CSF Aβ42	0.6 [0.4;0.9]	0.2 [0.2;0.2]	0.6 [0.6;0.7]	0.265[b]	0.6±0.2	0.5±0.2	0.521[c]
CSF Aβ42/40	0.1±<0.1	0.1±0	0.1±<0.1	0.829[a]	<0.1±<0.1	<0.1±<0.1	0.214[c]
CSF pTau	4.7 [3.0;9.5]	10.0 [10.0;10.0]	7.0 [3.2;13.3]	0.767[b]	18.6±10.4	12.6±11.9	0.450[c]
Plasma pTau	1.8±1.1	3.7±0	2.9±1.7	0.288[a]	2.3±0.3	2.4±0.7	0.875[c]
CSF NfL	0.9±0.8	2.0±0	0.8±0.3	0.374[a]	0.8 [0.7;1.6]	0.9 [0.8;1.1]	0.796[b]
Plasma NfL	2.4±1.9	5.3±0	1.8±0.4	0.203[a]	1.4 [1.2; 1.5]	1.6 [1.6; 1.7]	1.000[b]

Data presented as: median [Interquartile Range] or mean±standard deviation; [a]ANOVA, [b]Kruskall-Wallis test, [c]t-test. CSF Aβ40 and CSF Aβ42 expressed in $.10^3$pg/mL; CSF and plasma pTau expressed in $.10^1$pg/mL

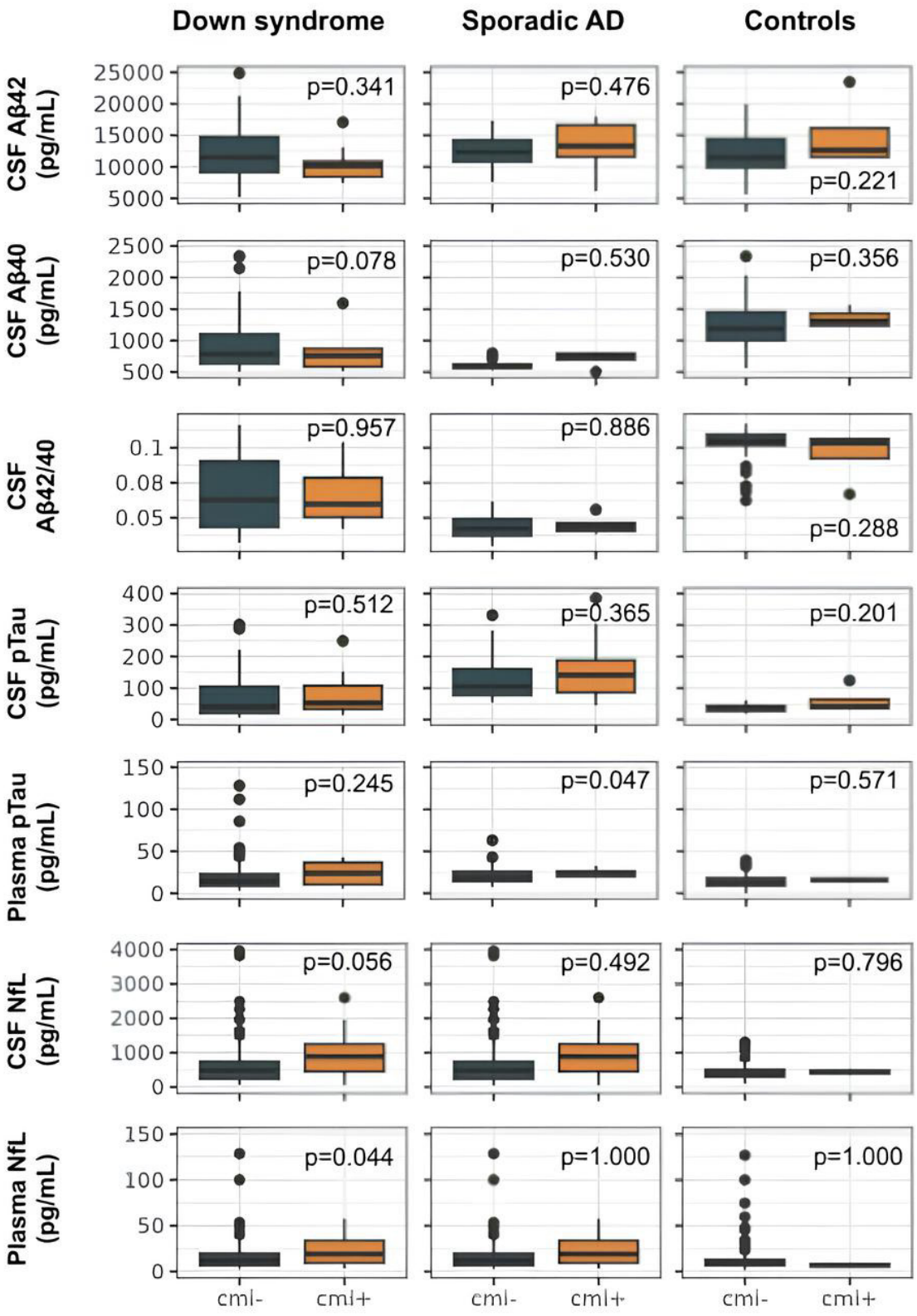


Figure 15. Fluid AT(N) biomarkers in participants with and without CMI within each study group

5.3 Relationship between cmi presence and number with cognitive performance

Cognitive scores in each group are presented in Table 13. We found no significant association between CMI presence and number with cognitive performance in DS (participants with mild and moderate ID analyzed separately), symptomatic sporadic AD, or controls (Tables 14 and 15).

Table 13. Cognitive performance in each study group

	Young Controls	Down syndrome				p	Old Controls	Sporadic AD			p
		All DS	aDS	pDS	dDS			All AD	pAD	dAD	
DS - mild and moderate ID											
CAMCOG-DS	-	72.0 [60.0;83.0]	78.0 [66.5;86.5]	72.0 [63.0;76.0]	55.0 [46.0;64.5]	<0.001[a]	-	-	-	-	-
mCRT	-	34.0 [29.0;36.0]	36.0 [34.0;36.0]	28.0 [20.5;35.0]	18.5 [12.5;25.5]	<0.001[a]	-	-	-	-	-
DS - mild ID											
CAMCOG-DS	-	84.0 [79.5;91.5]	88.0 [82.0;93.0]	77.5 [75.2;79.8]	71.0 [58.0;73.0]	<0.001[a]	-	-	-	-	-
mCRT	-	36.0 [33.2;36.0]	36.0 [35.0;36.0]	33.5 [27.5;36.0]	16.0 [13.0;25.0]	<0.001[a]	-	-	-	-	-
DS - moderate ID											
CAMCOG-DS	-	63.0 [55.0;72.0]	68.5 [58.8;74.2]	64.0 [62.0;72.5]	54.5 [42.8;61.8]	0.001[b]	-	-	-	-	-
mCRT	-	33.0 [24.0;35.0]	34.0 [33.0;36.0]	24.0 [20.5;33.0]	20.0 [13.0;25.5]	<0.001[a]	-	-	-	-	-
Euploids											
MMSE	30.0 [29.0;30.0]	-	-	-	-	-	29.0 [29.0;30.0]	26.0 [24.0;27.0]	27.0 [25.0;28.0]	23.0 [19.5;25.0]	<0.001[b]

Data presented as median [Interquartile Range]; [a]Kruskall-Wallis test, [b]. CSF NfL expressed in $.10^3$pg/mL and plasma NfL expressed in $.10^1$pg/mL.

Table 14. Differences cognitive performance between participants with and without CMI in each study group

	Down syndrome		p	Sporadic AD		p	Controls		p
	CMI -	CMI +		CMI -	CMI +		CMI -	CMI +	
DS - mild and moderate ID									
CAMCOG-DS	72.0 [60.0;83.0]	73.0 [61.0;85.2]	0.583[a]	-	-	-	-	-	-
mCRT	34.0 [30.0;36.0]	35.0 [23.0;36.0]	0.880[a]	-	-	-	-	-	-
DS - mild ID									
CAMCOG-DS	83.0 [79.5;91.0]	86.5 [77.5;94.2]	0.587[a]	-	-	-	-	-	-
mCRT	36.0 [33.2;36.0]	36.0 [33.8;36.0]	0.775[a]	-	-	-	-	-	-
DS - moderate ID									
CAMCOG-DS	63.0 [55.0;72.0]	69.0 [60.8;72.5]	0.506[a]	-	-	-	-	-	-
mCRT	33.0 [26.0;35.0]	28.5 [22.2;34.5]	0.690[a]	-	-	-	-	-	-
Euploids									
MMSE	-	-	-	26.0 [24.0;27.5]	26.0 [24.5;26.0]	0.623[a]	30.0 [29.0;30.0]	29.0 [29.0;29.0]	0.172[a]

Data presented as: median [Interquartile Range] or mean±standard deviation; [a]Kruskall-Wallis test, [b]t-test. CSF NfL expressed in $.10^3$pg/mL and plasma NfL expressed in $.10^1$pg/mL.

Table 15. Relationship of cortical microinfarct's number with cognitive performance in Down syndrome and symptomatic sporadic Alzheimer's disease

	Down syndrome			p	Sporadic Alzheimer's disease		p
	1 CMI	2-5 CMI	>5 CMI		1 CMI	2-5 CMI	
DS - mild and moderate ID							
CAMCOG-DS	74.6±17.9	65.5±4.9	76.7±17.2	0.754[a]	-	-	-
mCRT	36.0 [30.5;36.0]	20.0 [20.0;20.0]	29.5 [23.0;36.0]	0.435[b]	-	-	-
DS - mild ID							
CAMCOG-DS	80.2±21.1	-	86.5±3.5	0.508[c]	-	-	-
mCRT	36.0[0;0]	-	36.0[0;0]	1.000[b]	-	-	-
DS - moderate ID							
CAMCOG-DS	68.0±12.2	65.5±4.9	57.0±0	0.684[a]	-	-	-
mCRT	31.0±9.0	20.0±0	23.0±0.0	0.371[a]	-	-	-
Euploids							
MMSE	-	-	-	-	25.5±1.4	25.2±1.9	0.779[c]

Data presented as: median [Interquartile Range] or mean±standard deviation; [a]ANOVA, [b]Kruskall-Wallis test, [c]t-test. CSF NfL expressed in $.10^3$pg/mL and plasma NfL expressed in $.10^1$pg/mL.

6 DISCUSSION

This is the first study to assess CMI in adults with DS and their association with age, sex, APOE haplotype, vascular risk factors, AD clinical continuum, fluid AT(N) biomarkers, other vascular neuroimaging findings, and cognition. We showed that the CMI prevalence is higher in DS than in young euploid controls (<60 years) and increases with age and along the AD clinical continuum in this population. Also, in DS, CMI are predominantly located in the parietal lobes and are associated with other ischemic neuroimaging findings, namely lacunes, large corticosubcortical infarcts, and WMH. However, they seem to be unrelated to vascular risk factors or hemorrhagic CAA manifestations such as lobar microbleeds and superficial siderosis.

6.1 CMI Prevalence according to age and along the ad clinical spectrum in DS

In the current work, we aimed to assess for the first time the prevalence and number of CMI according to age and along the AD clinical spectrum in DS in vivo through MRI. We found an overall CMI prevalence of 11.8%, which is higher than in cognitively unimpaired controls aged <60 years (2.6%), despite the overall younger age of the DS participants. However, the prevalence of CMI in euploid controls aged ≥60 years was higher (10%) and comparable to that observed by other groups in similar controls recruited from the general population[28,41,66,69-71].

The prevalence in symptomatic AD patients is more variable in the literature. Data of CMI prevalence in cognitively impaired subjects has been published in studies performed mainly in two large cohorts: from the National University Health System Memory Ageing and Cognition Centre, in Singapore, and the TRACE-VCI cohort of the University Medical Center (UMC) Utrecht, the Netherlands. The first is a cohort from a multiethnic Asian memory clinic with high vascular burden[66], while the second is a cohort of patients with vascular brain injury on MRI[72]. We found the prevalence of CMI to be 17.5% in symptomatic sporadic AD patients, while the prevalence found in the Singaporean and the Dutch cohorts were 31.9% and 21.3%, respectively. This difference is likely explained by the lower prevalence of cerebrovascular disease and vascular risk factors among our subjects due to our exclusion criteria that precluded individuals with high vascular burden to enter the SPIN cohort. This was in contrast with the high likelihood of vascular co-pathology in symptomatic sporadic AD patients from the Utrecht and Singapore cohorts.

Finally, we aimed to assess how CMI prevalence evolves along the AD clinical continuum in DS. As our main hypothesis is that CMI are related to amyloid pathology in this population, we expected CMI prevalence to increase along the AD clinical spectrum, given the life-long cumulative amyloid deposition in the brain parenchyma and cerebral vasculature. We found CMI prevalence to increase from 8.7% and 6.9% in aDS and pDS participants to 25% in dDS, in accordance with our hypothesis.

6.2 CMI topographic distribution

We also aimed to investigate the spatial distribution of CMI. We observed different patterns in adults with DS and in euploid participants (symptomatic sporadic AD patients and cognitively unimpaired controls). While CMI had a posterior distribution (mainly in the parietal lobes) in DS, in euploid participants CMI were predominantly distributed along the parasagittal lines in the frontoparietal regions.

A few studies mapping the topographic distribution of CMI throughout the brain suggest that the spatial location of these lesions might reflect differences in their underlying pathology[29,39]. Ferro et al.[73] and ter Telgte et al.[74], analyzing cohorts of patients with cerebrovascular disease, have shown chronic CMI distributed in vascular watershed areas. However, studies on a Singaporean memory clinic cohort[41,66], a cohort that is also characterized by a high vascular disease burden, showed that CMI were concentrated in frontoparietal regions but without a clear watershed distribution. In our cohort, the small number of CMI in euploid participants is insufficient to make relevant inferences about their distribution, but their topography slightly resembles vascular watershed areas. However, the pattern observed in our DS participants strikingly resembles the posterior distribution of cerebral microbleeds in CAA[31,75].

6.3 Association of CMI with sex, apoe haplotype, vascular risk factors, fluid AT(N) Biomarkers, and other vascular neuroimaging findings

6.3.1 CMI relationship with sex, APOE haplotype and vascular risk factors

Another aim of the study was to investigate relationship of CMI with sex, *APOE* haplotype, vascular risk factors, fluid AT(N) biomarkers, and other vascular neuroimaging findings, namely WMH, cerebral microbleeds, superficial siderosis, corticosubcortical infarcts, and lacunes.

We found no differences in CMI prevalence between men and women, which is aligned with studies of CMI in different CAA cohorts[28,76] and in a cohort of patients from a memory clinic with diverse neurodegenerative and cerebrovascular diseases[66]. However, when studying cohorts of vascular cognitive impairment, a higher prevalence of CMI was found in men[71,73], which was expected, since in this population, CMI are likely a manifestation of cardiac and large vessel disease, known to be more prevalent in men[77]. On the other hand, a recent study addressing specifically the sex differences in CAA-associated MRI markers found no differences in CAA prevalence between men and women[78]. Hence, we did not expect sex differences in the CMI prevalence in DS, and our findings met our expectations.

As for the association between CMI and APOE haplotype, we expected CMI to be associated with APOEε4 haplotype based on neuropathology studies showing that CAA have a higher burden of CMI and a strong association with APOEε4 allele in euploid subjects[30,31,79]. However, we observed no such association. Despite the lack of MRI studies addressing the relationship between APOE haplotype and CMI, Carmona et al.[21] and Lao et al.[44] found no association between CAA neuroimaging markers and APOE haplotype in DS[21,44]. Since our main hypothesis is that CMI are related to CAA pathology in DS, our results are aligned with the findings published by those authors. A possible explanation for the lack of association of CAA and APOE haplotype in DS is that other genetic factors, such as the APP gene triplication, might overcome the role of APOEε4 haplotype in increasing the risk for developing CAA.

Lastly, as expected, we found no association between CMI and vascular risk factors. Studies on cohorts with high cerebrovascular disease burden have found associations of CMI with arterial hypertension and dyslipidemia[41,66]. However, studies of CMI on euploid subjects with CAA found no such relationship[28,76]. In DS, as previously mentioned, we hypothesized that CMI reflects CAA pathology. Besides,

DS is known for a low prevalence of vascular risk factors. Therefore, our findings are aligned with our initial expectations.

6.3.2 CMI relationship with fluid AT(N) biomarkers.

We found no CMI association with fluid amyloid or tau biomarkers in DS. Regarding the relationship of CMI with neurodegeneration biomarkers, we found only a weak association of CMI presence with higher CSF and plasma NfL in DS. Although previous studies reported reduced CSF Aβ42 levels in subjects with acute CMI[72], data on the relationship between chronic CMI and fluid AT(N) biomarkers are lacking. In sporadic CAA, reduced CSF Aβ42 and Aβ40 concentrations have been reported[23]. However, in AD-related CAA, core AD fluid biomarkers are not good predictors of in-vivo CAA diagnosis[21]. We are still lacking good biomarkers for CAA beyond the manifestations found in MRI.

6.3.3 CMI relationship with other vascular neuroimaging findings

In DS, we found CMI to be associated with other ischemic neuroimaging findings of cerebral small vessel disease, namely lacunes, corticosubcortical infarcts, and WMH, but not with hemorrhagic lesions such as cortical microbleeds or superficial siderosis. In this population, the ubiquitous brain amyloidosis that leads to a higher CAA prevalence than in the general population is the most likely cause of CMI[21]. Yet, we observed no associations between CMI and classic CAA hemorrhagic lesions. Recently, Gokcal et al.[80] have also found an association of CMI with WMH and lacunes in CAA. However, the relationship of CMI with CAA hemorrhagic manifestations remains controversial. While studies have shown associations of CMI with cerebral microbleeds[43] and superficial siderosis[76], van den Brink et al.[28] reported a lack of relationship between CMI and hemorrhagic findings in CAA. A recent neuropathological study has shown that microbleeds and CMI likely result from two different pathological mechanisms in CAA[27]. This study has shown that the area surrounding a cortical microbleed contains fewer Aβ-positive vessels than a simulated control lesion, whereas CMI were associated with higher number of

adjacent Aβ-positive vessels. This study also found that microbleeds seem to be associated with vessel wall remodeling with aneurism-like formation in Aβ-poor vessel segments, while CMI seem related to stiffening of Aβ-rich vessels. Hence, the lack of association between these lesions in our study could be explained by differences in their underlying pathological changes in the same context of CAA.

Another possible reason contributing to the lack of association between microbleeds and CMI in our study is the discrepancy in the 3T-MRI sensitivity to detect these lesions. Van Veluw et al. have shown that, while the number of microbleeds detected on ex-vivo 3T-MRI is correlated with the number of microbleeds detected in histopathology, the number of CMI is largely underestimated in neuroimaging studies compared to neuropathology analysis[27].

In summary, the lack of relationship of CMI presence with sex or vascular risk factors is in accordance with our initial expectations. However, we also expected that CMI would be related to APOEε4 haplotype, given the strong relationship between APOEε4 allele and CAA, but such relationship was not confirmed by our results. Regarding the relationship between CMI and AD neuropathological biomarkers, no CMI association with amyloid or tau biomarkers was found. As for the relationship of CMI and other vascular imaging abnormalities, o we found CMI to be associated with other ischemic but not with hemorrhagic neuroimaging findings.

6.4 CMI's Impact on cognition

Lastly, we aimed to evaluate CMI's impact on cognitive performance in adults with DS.

The current study found no associations between CMI and worse global cognition or episodic memory in DS. This finding is discordant with the literature reports of worse cognitive performance in large population-based[81] and memory clinic cohorts of euploid participants[66,82]. One study in a small cohort of CAA subjects found no associations between CMI and cognitive performance[28]. In DS, the intellectual disability highly impacts neuropsychological assessment. In our cohort, only scores of subjects with mild or moderate ID were included in the anaylsis of cognitive performance, but the limited neuropsychological battery adapted for people with ID might not capture the potential impact of CMI on cognition. In euploid

participants, we were probably unable to detect impaired cognitive performance due to the relatively low number of CMI found and the fewer participants compared to studies that assessed CMI's relationship with cognition in larger cohorts[66,82,83].

We initially expected adults with DS and CMI to present poorer cognitive performance on neuropsychological tests than those without CMI. However, this was confirmed by our findings.

6.5 Strengths And weaknesses of the study

This is the first study to assess CMI in adults with DS and their association with vascular risk factors, AD clinical continuum, fluid AT(N) biomarkers, other neuroimaging findings, and cognition. The main strength of our work is the large, well-characterized, and population-based cohort of adults with DS with clinical and multimodal AD biomarkers, allowing us to analyze the relationship of CMI with age, the AD clinical continuum, AT(N) biomarkers, vascular risk factors, other neuroimaging findings, and cognitive performance in this population. Despite basing our analysis on well-established criteria for visual CMI detection on 3T-MRI, the low sensitivity of *in-vivo* neuroimaging for detecting CMI compared to neuropathology limits our study. CMI detected by *in-vivo* MRI reflect only a fraction of the total CMI burden[27]. The low rate of CMI detection through *in-vivo* 3T-MRI limits the statistical analysis to a binary approach according to the presence or absence of CMI, missing the nuances of CMI load on the correlations with neuroimaging, fluid AT(N) biomarkers and neuropsychological evaluation. Also, the challenges imposed by the intellectual disability to neuropsychological assessment in DS, together with the relative scarcity of cognitive batteries validated for this population should also be accounted as a limitation for investigating the impact of CMI on cognitive performance. Lastly, the presence of motion artifacts in a population with intellectual disability and cognitive decline is inevitable and decreases the sensitivity of visual analysis for detecting CMI. Therefore, despite subjects' inclusion based on the MRI quality, some degree of motion artifact had to be tolerated to ensure sufficient sample size.

7 CONCLUSIONS

1. CMI prevalence increases with age and along the AD clinical continuum in DS.

2. In DS, CMI are predominantly located in the parietal lobes and are associated with other ischemic neuroimaging findings, namely lacunes, large corticosubcortical infarcts, and WMH. However, they seem to be unrelated to vascular risk factors or hemorrhagic CAA manifestations, i.e. lobar micro or macrobleeds and superficial siderosis.

3. The presence of CMI does not impact cognitive performance in DS.

4. Our findings suggest that, in Down syndrome, cortical microinfarcts might select a subgroup of individuals with a non-hemorrhagic CAA imaging phenotype.

8 FUTURE DIRECTIONS

Currently, the labor-intensive process of visual identification and manual segmentation of CMI limits the size of cohorts with available CMI data. In addition, there is an enourmous miss-match between the number of CMI detected visually with in-vivo MRI and the number detected in neuropathological studies. These conditions combined hinder the investigation of CMI association with other biomarkers or the assessment of their clinical impact. Hence, from a technicnological perspective, the field would greatly benefit from new MRI sequences able to enhance CMI visualization, from increased availability of 7T MRI in the clinical setting, and from the development of artificial intelligence (AI) powered tools for automated identification and segmentation of CMI, allowing the assessment of these lesions in large cohorts to increase statistical power in biomarker analysis.

From a neuroimaging perspective, CAA has been largely recognized by its hemorrhagic manifestations. Identifying new ischemic markers potentially related to CAA helps characterize the spectrum of neuroimaging findings associated with this entity. Hence, future research should focus on the relationship between CMI and hemorrhagic manifestations of CAA. Exploring such relationship in DS might facilitate the identification of different underlying pathways and mechanisms of CAA pathology. Besides, understanding this relationship can affect the assessment of inflammatory and hemorrhagic amyloid-related imaging abnormalities (ARIA-E and ARIA-H, respectively) in the context of anti-amyloid immunotherapy for AD.

Also, future studies should delve into the clinical implications of CMI in DS. This might involve longitudinal research to track the progression of CMI in DS individuals along the Alzheimer's disease (AD) continuum. Long-term follow-up assessments could help determine whether CMI impacts AD-related cognitive decline in DS.

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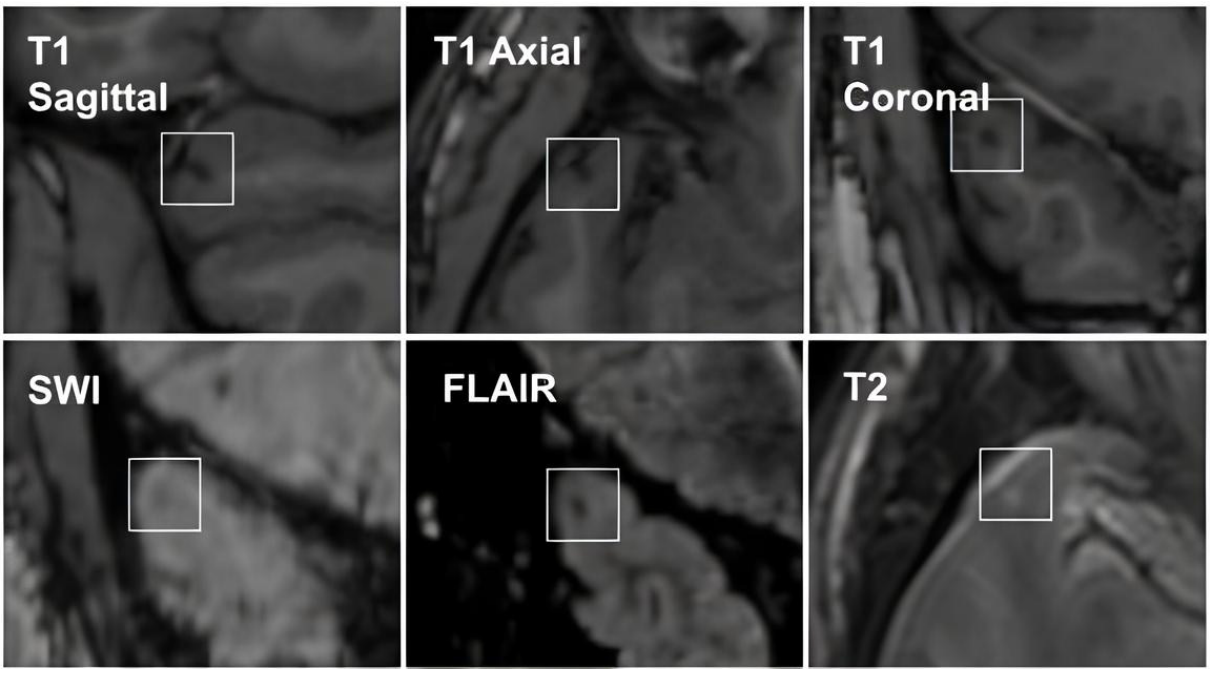
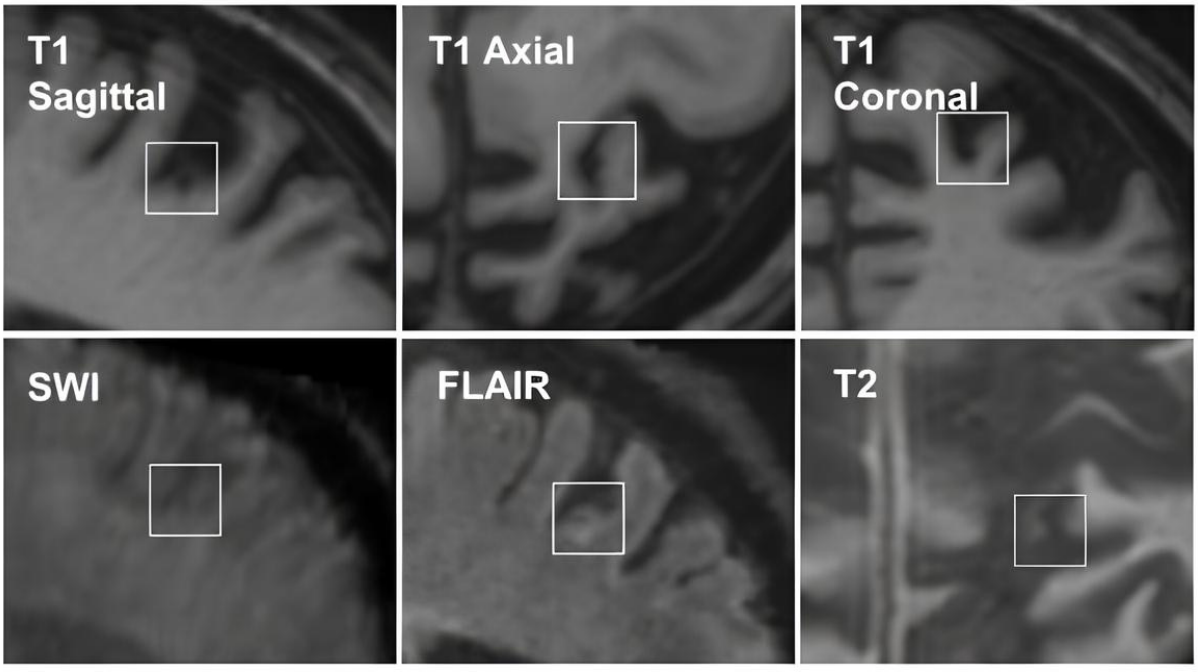
10 APPENDICES

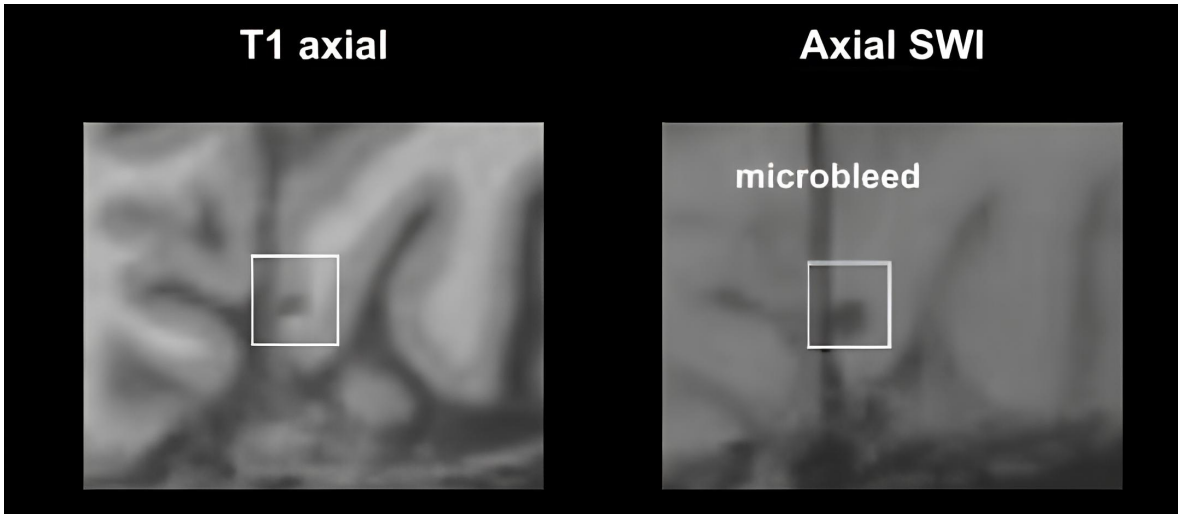
10.1 Examples of cortical microinfarcts on 3T MRI images



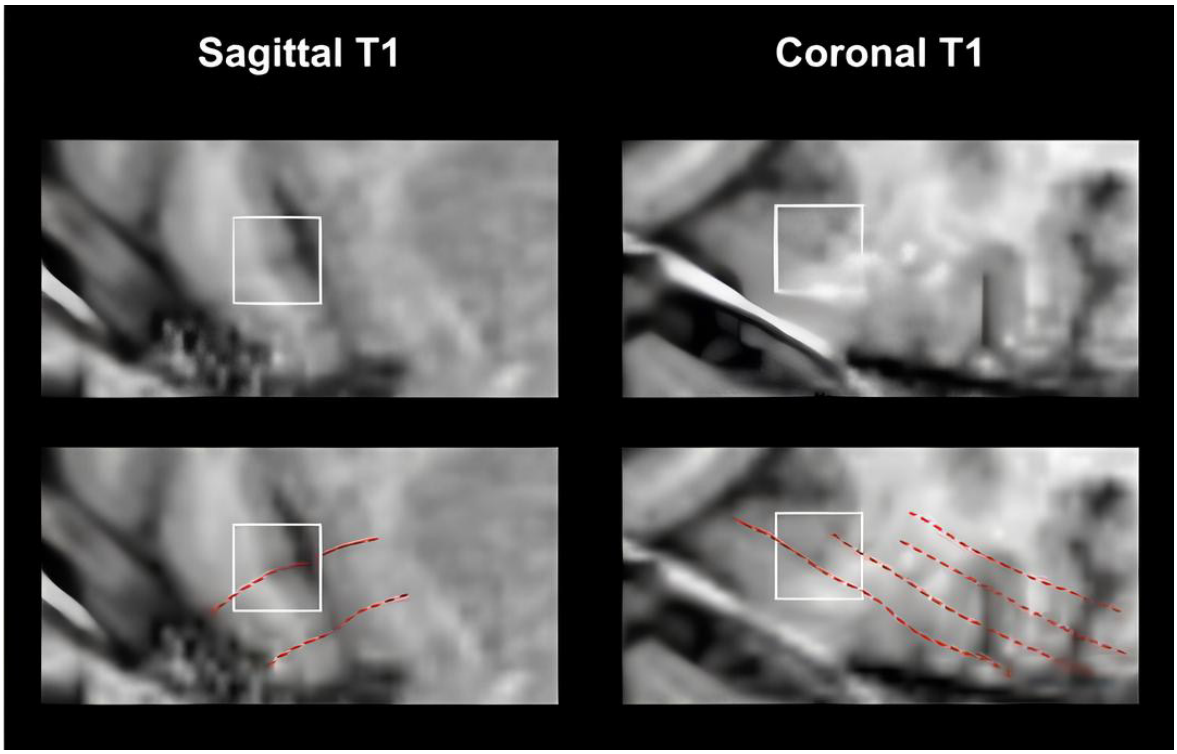


10.2 Examples of findings that mimic cortical microinfarcts on 3T MRI images

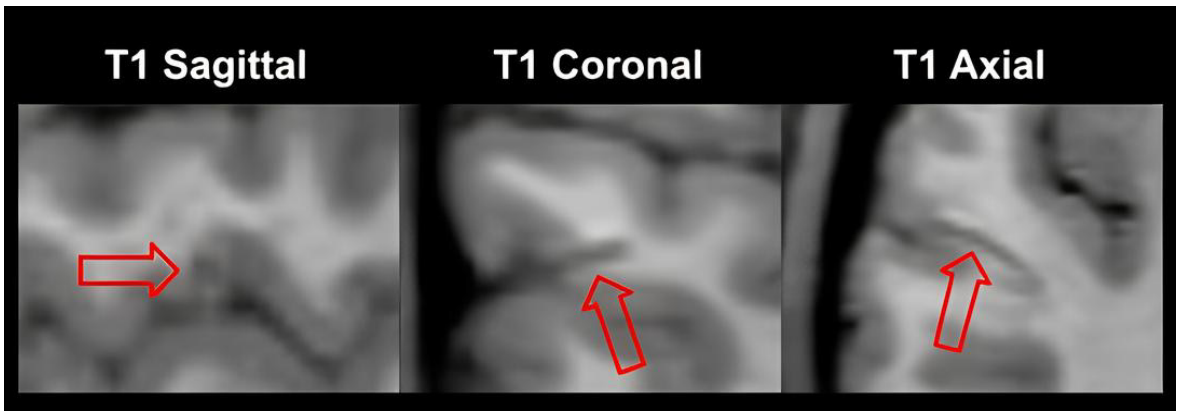
1. Cortical microbleeds:



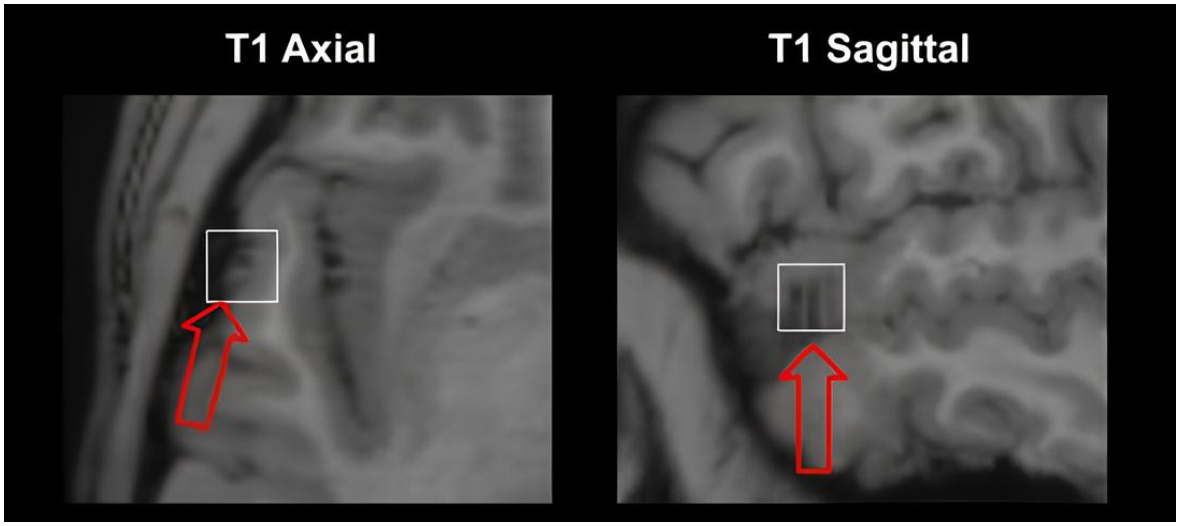
2. Motion artifacts:



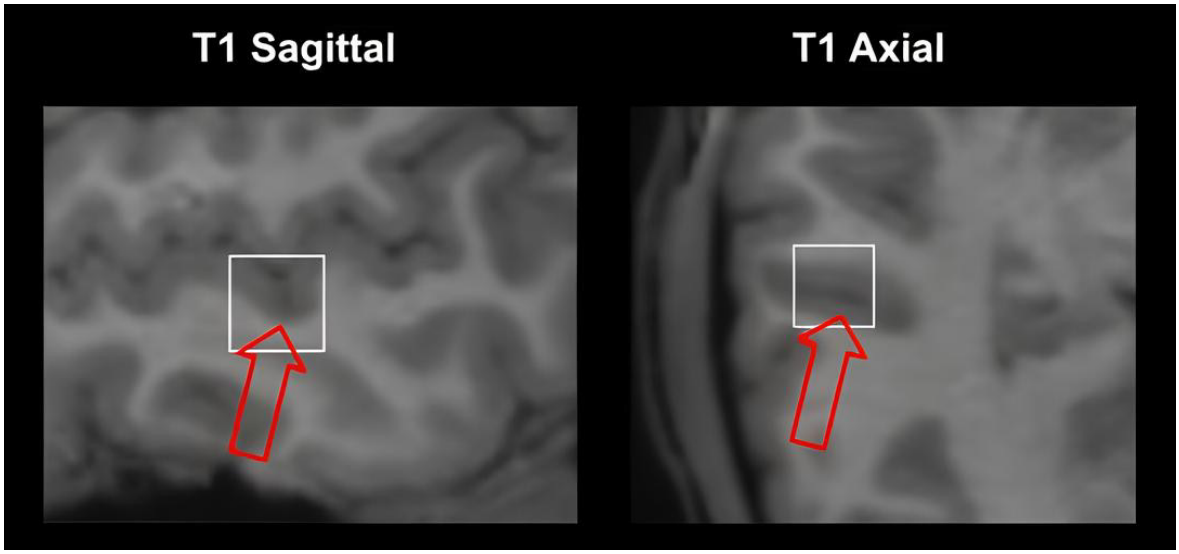
3. **Enlarged cortical vessels / perivascular spaces:**



4. **Larger caliber pial vessels:**



5. **Folds in the cortex / depth of cortical grooves:**



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