Subclinical atherosclerosis and brain health in midlife: Rationale and design of the PESA-Brain study

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ABSTRACT

Rationale Cognitive decline and dementia have been reportedly linked to atherosclerosis, the main cause of cardiovascular disease. Cohort studies identifying early brain alterations associated with subclinical atherosclerosis are warranted to understand the potential of prevention strategies before cerebral damage becomes symptomatic and irreversible.

Methods & design The Progression of Early Subclinical Atherosclerosis (PESA) study is a longitudinal observational cohort study that recruited 4,184 asymptomatic middle-aged individuals (40-54 years) in 2010 in Madrid (Spain) to thoroughly characterize subclinical atherosclerosis development over time. In this framework, the PESA-Brain study has been designed to identify early structural, functional and vascular brain changes associated with midlife atherosclerosis and cardiovascular risk factors. The PESA-Brain study targets 1,000 participants at the 10-year follow-up PESA visit and consists of thorough neuropsychological testing, advanced multimodal neuroimaging, and quantification of blood-based neuropathological biomarkers.

Primary hypothesis We hypothesize that, in middle-age, the presence of cardiovascular risk factors and a high burden of subclinical atherosclerosis will be associated with structural, functional and vascular brain alterations, greater amyloid burden and subtle cognitive impairment. We further hypothesize that the link between subclinical atherosclerosis and poor brain health in midlife will be mediated by cerebrovascular pathology and intracranial atherosclerosis.

Enrollment dates The PESA-Brain study started in October 2020 and is estimated to be completed by December 2024.

Conclusion This study is in a unique position to unveil novel relationships between cardiovascular and brain alterations in the health-to-disease transition, which may have important implications for interventional and therapeutic approaches. *ClinicalTrials.gov identifier:* NCT01410318. (Am Heart J 2024;278:195–207.)

Background

Dementia and atherosclerosis–travel fellows

Dementia is 1 of the greatest global health challenges of the century due to an unprecedented incidence growth and a substantial socio-economic impact.[1] The most common form of dementia is Alzheimer's disease (AD), accounting for approximately two-thirds of dementia cases. AD is a multifactorial neurodegenerative disorder characterized by accumulation of amyloid-β (Aβ) plaques and tau tangles in the brain which ultimately leads to neurodegeneration together with cognitive and functional decline.[2] AD has a long-lasting asymptomatic stage, during which a cascade of pathological events unfolds including neuronal injury, neuroinflammation, hypoperfusion, hypometabolism and brain atrophy.[2] Following AD, vascular dementia accounts for 15% of de-

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mentia cases and refers to any form of cognitive decline related to the progressive accumulation of cerebrovascular pathological changes, with most cases attributable to cerebral small vessel disease.[3]

However, most dementia patients present mixed pathology and the majority of AD cases exhibit alterations across the brain vasculature, such as reduced cerebral blood flow (CBF), microbleeds and microinfarcts, cerebral amyloid angiopathy, altered blood-brain barrier permeability and a pro-coagulant state.[4] Among these, the presence of intracranial and extracranial atherosclerosis has been also estimated to contribute to the risk of developing AD, being associated with lower executive and memory performance,[5,6] Aβ load, brain atrophy and vascular lesions across the AD spectrum.[7-10]

Atherosclerosis consists in the progressive buildup of fatty and fibrous material in the artery walls, with lipid deposition and vascular inflammation playing major roles.[11,12] Cerebral circulation is not likely to be spared (directly or indirectly) by such changes. One of the links between atherosclerosis and AD lies in the accumulation of brain Aβ as a result of vessel damage with concomitant CBF reduction and hindered brain clearance.[13] In turn, the presence of Aβ may promote atherogenesis through endothelial dysfunction, oxidative stress and inflammation.[14] As the dementia field searches for biomarkers to track disease progression, atherosclerosis may be a potential predictor of future cognitive decline and a target for early intervention.

Shared risk factors in dementia and atherosclerosis

CVD and dementia also share a similar age-dependence and a strong genetic predisposition. The different apolipoprotein E (APOE) isoforms have been associated with changes in blood cholesterol levels and differential risk of developing atherosclerosis and CVD, even at subclinical stages.[15] APOE also binds to Aβ in an isoform-dependent manner, being responsible for its aggregation and clearance and determining the risk of developing AD.[16] Notably, *APOE-ε4* carriers display a higher prevalence of both atherosclerosis and AD, while the *APOE-ε3* isoform is considered neutral and the *APOE-ε2* is potentially protective.[16]

Other determinants also contribute to the expression of these diseases apart from genetic factors. Indeed, atherosclerosis and cardiovascular disease (CVD) burden are highly attributable to modifiable cardiovascular risk factors (CVRFs), such as hypertension, high triglycerides, smoking, low physical activity and unhealthy diets.[11] Strikingly, 1 in every 3 cases of AD may also be accounted for by the presence of CVRFs across the lifespan,[17,18] with the risk of dementia increasing by 20% in the presence of 1 risk factor and by 65% in the presence of 2.[19] In fact, population-based analyses have shown that a decreased prevalence of CVRFs leads to a reduction in expected AD and preserves cognitive functioning in the elderly.[17,20,21] Less conventional risk factors, for example environmental and psychosocial factors, such as depression and social isolation, have also been shown to play a role in atherosclerosis and AD, representing other modifiable disease targets.[18,22] Thus, although the underlying mechanisms are not well understood, growing evidence suggests that vascular dysfunction plays a role from the silent onset of AD, potentially being part of the earliest manifestations of disease.

Silent progression of atherosclerosis and dementia-Need for midlife cohort studies

Another common feature of atherosclerosis and dementia is the presence of prolonged asymptomatic stages. However, scarce information is available on how early these diseases start co-developing and whether there is a cause-effect relationship. The first neuropathological alterations associated with AD may start 20-30 years before the first cognitive complaints,[23] which has led to a research shift into asymptomatic phases, when individuals are still cognitively unimpaired and there might be an opportunity to intervene in the progression of disease. Concurrently, atherosclerosis develops silently decades before manifesting as CVD and is highly prevalent in midlife, thus identifying subclinical atherosclerosis using noninvasive vascular imaging tools is central to CVD prevention.[24]

Cohort studies are therefore paramount to elucidate how early atherosclerosis may be implicated in the disruption of brain health in midlife. The CARDIA (Coronary Artery Risk Development in Young Adults) Brain study has reported that greater carotid intima-media thickness associates with lower CBF but not with brain volumes or white matter lesions in a population aged 51 $\pm$ 3 years old.[25] In turn, carotid intima-media thickness and carotid stenosis were associated with greater brain atrophy and cognitive decline in 58 $\pm$ 10 year-old individuals with manifest arterial disease in the SMART-MR (Second Manifestations of ARTerial Disease) study.[26,27] Moreover, greater carotid intima-media thickness and presence of carotid plaque in 57 $\pm$ 6 year-old individuals of the ARIC (Atherosclerosis Risk in Communities) study also showed significant correlations with smaller deep gray matter and cortical volumes 20 years later, but not with cerebrovascular lesions.[28] Beyond carotid atherosclerosis markers, aortic stiffness has also been associated with poor cognitive function and white matter lesions in individuals aged 46 $\pm$ 9 years old from the Framingham Cohort Study[29]; and presence and volume of coronary artery plaque with larger white matter lesion volumes in the 51 $\pm$ 10 year-old GeneSTAR (Genetic Study of Atherosclerosis Risk) cohort.[30] Despite some discrepancies, all ARIC, KALS (Kuopio Atherosclerosis and Longevity Study) and MESA (Multiethnic Study of Atherosclerosis) studies have shown that different markers of atherosclerosis including greater

carotid intima-media thickness, carotid interadventitial diameter, carotid stiffness and coronary artery calcium score and lower carotid distensibility are predictive of later dementia.[7,31,32]

Most of these cohorts are multicenter and ethnically diverse and recruited individuals with varying levels of education, leading to representative and generalizable findings that have greatly contributed to the existing literature. However, despite being middle-aged, none of these cohort studies had inclusion criteria restrictive enough to capture truly subclinical stages since they either recruited individuals with manifest arterial disease, or widely broad in terms of age.[26,27,29,30,33] Likewise, the ARIC study included brain scans 20 years after cardiovascular assessment in midlife, providing useful predictive data but not data on potentially co-occurring phenotypes earlier in life.[28,32] Also, most of these cohort studies are limited to standard brain magnetic resonance imaging (MRI) as used in clinical practice and do not employ other functional or vascular brain measures that may be more sensitive to subclinical stages. Lastly, these studies generally focus on a single vascular territory, which is typically the carotid arteries, and lack highly accurate measures of subclinical atherosclerosis that better reflect cardiovascular risk in low-risk populations.

Thus, more observational cohorts are warranted to extensively address the gaps in current knowledge at asymptomatic stages. Accurate quantification of multiterritorial subclinical atherosclerosis and comprehensive assessment of early CVRFs are pressingly needed in middle-aged healthy individuals as early predictors of accelerated vascular aging. Moreover, complementary multimodal neuroimaging and fluid-based biomarkers could help disentangle the underlying neuropathological mechanisms associated with vascular changes and determine how early brain health is compromised, providing a specific window for potential lifestyle and medical interventions that could delay the development of dementia.

PESA study design and main outcomes

The Progression of Early Subclinical Atherosclerosis (PESA) - CNIC-Santander study (NCT01410318) is a longitudinal prospective cohort study that aims to examine the presence and progression of subclinical atherosclerosis and decipher the determinants associated with its development in midlife.[34] To that end, in 2010 the PESA study recruited 4,184 asymptomatic middle-aged White individuals in Madrid, Spain (40-54 years old, 37% females).[34] The PESA study initially included 3 follow-up visits between 2010 and 2019. In each visit, the CVRFs were determined from clinical interviews, physical examinations and blood and urine samples.[35] A variety of lifestyle questionnaires was also collected in order to evaluate the influence of dietary habits, sleeping patterns and physical activity on subclinical atherosclerosis, while incorporating the most recent advances in omics.[34] In addition, individual psychosocial scales of depression, anxiety, perceived social support, perceived stress and job strain have been measured in all PESA follow-up visits. As a key assessment, PESA participants also underwent multiterritorial noninvasive 2/3-dimensional vascular ultrasound (2D/3D-VUS) of the carotid, abdominal and femoral arteries to identify plaque presence and burden, as well as a computed tomography (CT) scan to estimate coronary artery calcium score.[34] Of note, plaque volume as measured by 3D-VUS has shown greater sensitivity as a reflection of the cumulative effect of atherosclerosis exposure compared to standard plaque metrics used in most observational studies,[36] providing a closer match with global cardiovascular risk.[37] The baseline PESA study showed that over 60% of these asymptomatic middle-aged individuals already had some extent of subclinical atherosclerosis.[35] Moreover, subclinical atherosclerosis progressed in 33% of participants - either *de novo* plaque development or significant increase in size of pre-existing plaque - at the 6-year follow-up visit.[38]

First evidence of midlife brain changes in the PESA study

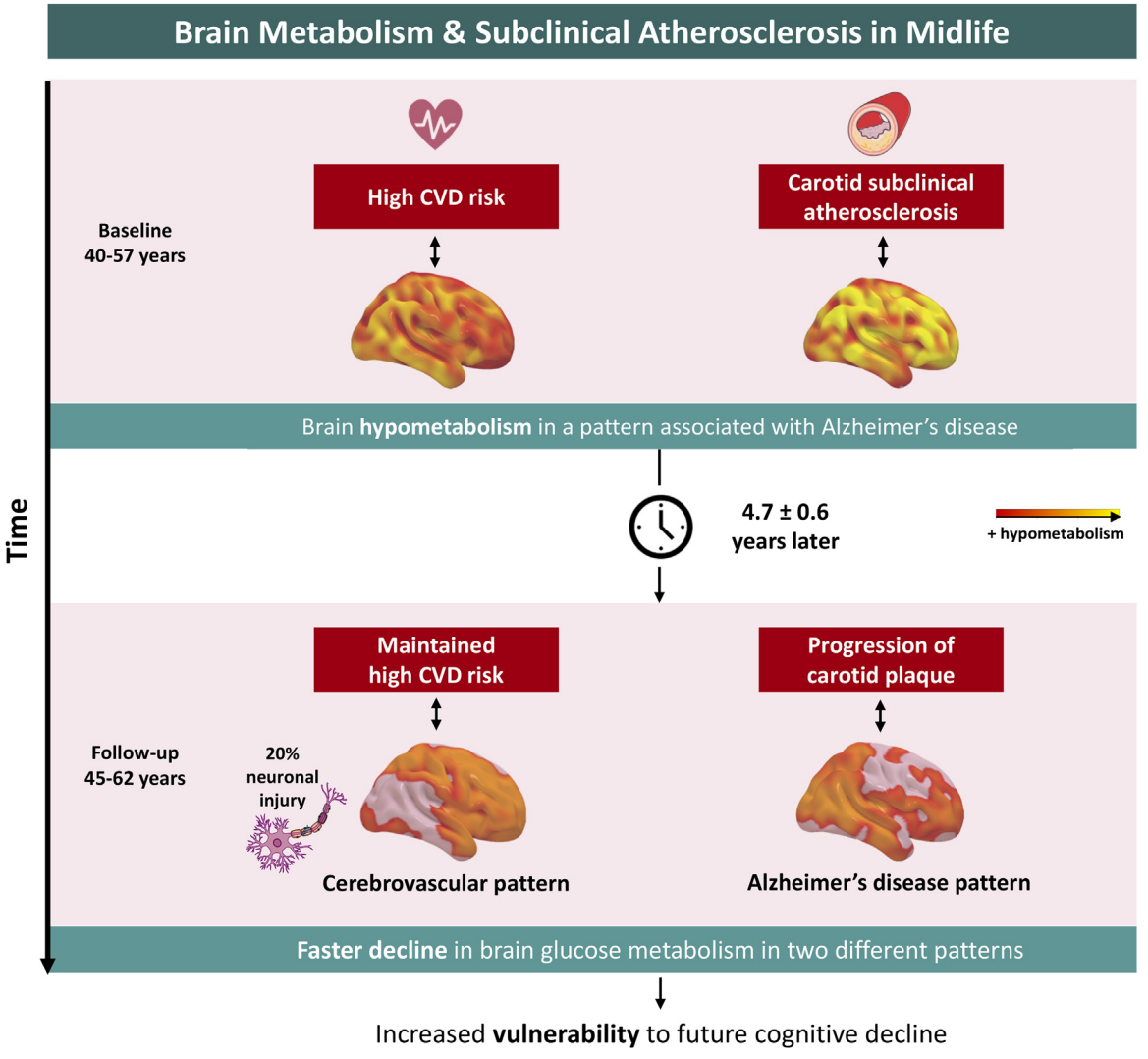
PESA participants with a high burden of subclinical atherosclerosis at the baseline visit were included in the Advanced Imaging PESA subcohort and underwent [^{18}F]fluorodeoxyglucose-positron emission tomography (FDG-PET) scans at the baseline and 6-year follow-up visits.[34] From this middle-aged subcohort with available brain FDG-PET data, our group obtained the first evidence that subclinical atherosclerosis in the carotid arteries was associated with cerebral hypometabolism in regions vulnerable to AD,[39] and that longitudinal increments in atheroma plaque were further associated with an accelerated decline in cerebral metabolism (Figure 1).[40] In this subcohort, we also quantified standard blood-based biomarkers of neuropathology, which have recently demonstrated great staging potential and promising applicability in clinical settings.[41] We further observed that this decline in brain health was partially mediated by plasma levels of neurofilament light chain, a biomarker of neurodegeneration, in middle-aged individuals who remained at high CVD risk over time (Figure 1),[40] suggesting that subclinical atherosclerosis and CVRFs in midlife may result in a more vulnerable and less resilient brain to cope with the pathological burden of neurodegenerative or cerebrovascular diseases later in life.

Methods

PESA-Brain study rationale

The PESA study was extended to contemplate 2 additional visits between 2019 and 2029 (PESA-Health

Figure 1. First evidence of brain health disruption in the PESA study. Cerebral hypometabolism was associated with high cardiovascular risk and subclinical carotid atherosclerosis at the baseline Advanced Imaging PESA visit in middle-aged asymptomatic individuals aged 40-57 (n = 547).[39] The longitudinal analysis revealed that maintenance of high cardiovascular risk and increments in carotid plaque are also linked with a faster decline in cerebral metabolism in midlife (n = 370).[40]

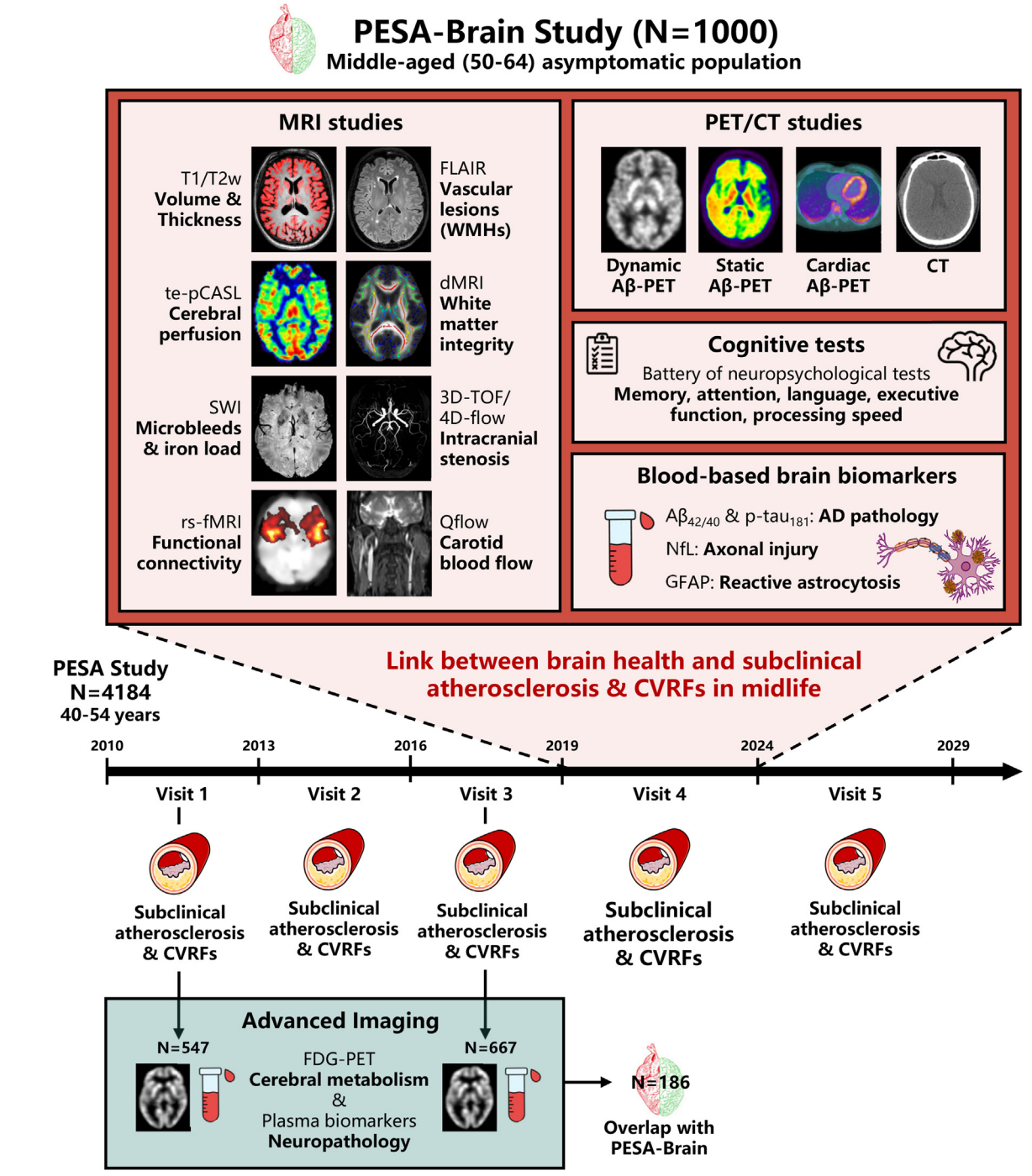


study).[42] The uniqueness of the PESA study, allied to our previous FDG-PET findings, renders this population ideal to study the effects of subclinical atherosclerosis and CVRFs on brain health in midlife. Furthermore, it offers the opportunity to determine whether brain changes predictive of future cognitive decline occur early in life in response to cardiovascular burden and whether there is an interplay between subclinical cerebrovascular disease and neurodegeneration. Thus, the PESA-Brain study was designed to address current research needs.

The PESA-Brain study selected 1,000 individuals from the PESA cohort to additionally undergo extensive cognitive testing; comprehensive multimodal neuroimaging studies, including MRI to study brain structure, function and vasculature and amyloid PET/CT to evaluate cerebral and cardiac Aβ deposition; and quantification of standard blood-based biomarkers of neuropathology (Figure 2). This cross-sectional study falls within the 10-year follow-up visit of the PESA study (visit 4), will span between October 2020 and December 2024, and will nurture from all longitudinal clinical data gathered during the 20-year span of the parent PESA cohort.

The novelty of the PESA-Brain study lies in capturing truly subclinical stages of atherosclerosis before cardiovascular events occur, thanks to the narrow age range of

Figure 2. PESA-Brain Study Design. FLAIR, fluid-attenuated inversion recovery; te-pCASL, time-encoded pseudo-continuous arterial spin labelling; mS-DTI, multishell diffusion tensor imaging; SWI, susceptibility-weighted MRI; TOF, time-of-flight; rs-fMRI, resting-state functional MRI; $A\beta_{42/40}$, amyloid-β ratio 42/40; p-tau_{181}, phosphorylated tau 181; NfL, neurofilament light chain; GFAP, glial fibrillary acidic protein; FDG, [^{18}F]fluorodeoxyglucose.



this population, the large sample size and the thorough multiterritorial longitudinal assessment of atherosclerosis using leading-edge imaging tools such as the 3D-VUS. This, together with the state-of-the-art comprehensive brain assessments represents a unique opportunity to find the earliest brain signatures associated to the cardiovascular profile and to propose targets for prevention of brain health disruption before injuries are irreversible in cognitively unimpaired individuals. Such an exhaustive brain health description using multiple head-to-head quantification techniques as well as available longitudinal data will further allow to explore the underlying pathways from subclinical atherosclerosis and CVRFs to cognitive decline.

Study hypothesis

We hypothesize that in middle-aged PESA-Brain participants the presence of CVRFs and a high burden of subclinical atherosclerosis in the carotid, femoral, aorta and coronary arteries will be associated with structural, functional and vascular brain changes, greater Aβ burden and subtle cognitive impairment. We expect the link between subclinical atherosclerosis and poor brain health in midlife to be mediated by cerebrovascular pathology and possibly intracranial atherosclerosis. We further hypothesize that these associations will be *APOE*-dependent, being aggravated in $\varepsilon 4$ carriers and ameliorated in $\varepsilon 2$ carriers.

Study objectives

The general objective of the PESA-Brain study is to determine whether CVRFs and multiterritorial subclinical atherosclerosis are associated with subtle cognitive alterations and brain changes in midlife. Specific objectives are represented in the Graphical Abstract and are as follows: (1) to determine whether peripheric vascular burden, such as multiterritorial subclinical atherosclerosis and the presence of CVRFs, is associated with cerebral small and/or large vessel disease in midlife, as well as with intracranial atherosclerosis, (2) to determine the extent to which cerebrovascular pathology mediates the association between vascular burden and neurodegeneration in midlife, (3) to determine which cognitive domains are most affected by neurodegeneration secondary to vascular burden and cerebrovascular pathology in midlife, and (4) the extent to which those associations are mediated by AD pathology, (5) are APOE isoform-dependent, or (6) are mediated by peripheral inflammatory status or neuro-inflammation. Finally, longitudinal vascular data from the PESA study will allow us to study whether the progression of subclinical atherosclerosis and the maintenance of a high CVD risk over time predict any of the brain-related abnormalities found in midlife and, in turn, whether preserving a healthy cardiovascular profile over 10 years is associated with preserved brain health later in life.

Secondary objectives will be developed based on primary findings and may include studying sex differences and menopause changes in brain health outcomes or determining how lifestyle behaviors such as sleep, physical activity or nutrition may impact those outcomes. In addition, psychosocial factors derived from scales of depression, anxiety, perceived social support, perceived stress and job strain will be used as exposures. Omics data will be available in a subgroup of the PESA-Brain study and may also provide insights on the mechanisms through which subclinical atherosclerosis and poor brain health are linked in midlife.

Study population

All individuals enrolled in the PESA study at the 10-year follow-up visit were invited to participate in the PESA-Brain study. Out of over 2,000 individuals who agreed to participate, 1,000 were selected based on *APOE* genotype, given the well-established role of *APOE* as a genetic risk factor for both AD and atherosclerosis.[16] All $\varepsilon 2$- and $\varepsilon 4$-*APOE* carriers who consented to participate were included in the PESA-Brain study for increased sensitivity, while $\varepsilon 3/\varepsilon 3$ carriers were randomly selected ensuring a balance in age, sex and subclinical atherosclerosis until reaching 1,000 participants. The initial selection included 163 low-risk individuals (4 $\varepsilon 2/\varepsilon 2$ and 159 $\varepsilon 2/\varepsilon 3$); 579 neutral-risk individuals ($\varepsilon 3/\varepsilon 3$); and 239 high-risk individuals (222 $\varepsilon 3/\varepsilon 4$ and 17 $\varepsilon 4/\varepsilon 4$). Additionally, 19 $\varepsilon 2/\varepsilon 4$ carriers were included even though its conferred risk to both diseases remains uncertain.[43,44] All PESA-Brain participants were offered genetic counselling by experts at the Fundación Jiménez Díaz University Hospital (Madrid, Spain). Detailed inclusion and exclusion criteria are shown in Table 1.

The initially selected PESA-Brain population included 405 females and 595 males and the mean age was 52.0 $\pm$ 4.5 years old at the 6-year follow-up visit. All PESA-Brain participants are White and highly educated (949 with high school and 747 of those with a college degree, 51 missing). Population characteristics may slightly change as data collection is still ongoing and the participants initially selected may withdraw or be excluded before or during enrollment. All participants provide written informed consent to all study assessments. The PESA-Brain study is part of the PESA study (NCT01410318) and both have been approved by the ethics committee of the *Instituto de Salud Carlos III* in Madrid.

Study assessments

Cognitive tests - In the PESA-Brain study, global cognitive function is initially assessed using the Clinical Dementia Rating (CDR) test followed by a battery of 10 neuropsychological tests, carefully designed to detect subtle deficits in multiple cognitive domains in middle-aged individuals (Table 2).[45,46] The Montreal Cognitive Assessment (MoCA) is administered at the discretion of

Table 1. PESA-Brain study - Inclusion, exclusion and withdrawal criteria.

Inclusion criteria

1. Enrollment in the parent PESA study and consent to use biological material for omics and to undergo all neuropsychological and neuroimaging studies.
2. Priority given to participants not included in the Advanced Imaging PESA sub-cohort* or in the interventional TANSNIP-PESA study#.

Exclusion Criteria

1. Family history of early-onset AD due to monogenic forms.
2. Neurological disorder (Parkinson's disease, Huntington's disease, normal pressure hydrocephalus, progressive supra-nuclear palsy, seizure disorder, subdural hematoma, multiple sclerosis, history of significant head trauma) or evidence of mental retardation.
3. Visual and/or hearing impairment inadequate for neuropsychological testing.
4. Active treatment for cancer, history of transplant with active immunosuppressive or immunomodulator treatment, or with clinically relevant renal or hepatic insufficiency.
5. Clinically significant condition that decreases life expectancy to $\leq$ 3 years.
6. Pregnant or breast-feeding females at the time of the neuroimaging assessments.
7. Having pacemakers, aneurysm grafts, artificial heart valves, auditory implants, cerebral shunts, metal fragments or any objects that contraindicate MRI studies.
8. Intolerance or hypersensitivity to any amyloid PET tracer.
9. Current or recent participation in any procedure involving radioactive agents such that the total radiation dose exposure in a given year exceeds the limits of annual or total recommended dose.[59]
10. Claustrophobia.
11. Active drug or alcohol abuse.

Withdrawal criteria

1. Participant's request at any time.
2. PESA team's decision for security reasons.
3. Participant's death.

* Participants not included in the Advanced Imaging PESA sub-cohort were prioritized to reduce radiation exposure.
# 1020 PESA participants were enrolled in the 30-month worksite-based healthy lifestyle interventional TANSNIP-PESA sub-study.[60] These participants will only be included in the PESA-Brain study if their incorporation is needed after the initial selection, in which case TANSNIP-PESA participants allocated in the Control Group will be prioritized.

Table 2. PESA-Brain study - Cognitive assessment.

Neuropsychological test	Assessment
Clinical Dementia Rating (CDR)	Memory, orientation, judgement, problem solving, community affairs, home, hobbies, personal care.
Subjective Cognitive Decline (SCD) Assessment	Subjective memory perception.
Montreal Cognitive Assessment (MoCA)	Attention, memory, language, visuospatial abilities, executive functions, orientation.
Hospital Anxiety and Depression Scale (HADS)	Anxiety, depression.
Logical Memory (Immediate Recall)	Short-term episodic memory.
FCSRT (Immediate Recall)	Short-term verbal memory.
WAIS-IV Coding Subtest	Processing speed.
WAIS-IV Visual Puzzles Subtest	Nonverbal reasoning, visual learning.
WAIS-IV Digit Span Subtest	Working memory, auditory processing, executive attention.
Logical Memory (Delayed Recall)	Long-term episodic memory.
FCSRT (Delayed Recall)	Long-term verbal memory.
Semantic Fluency Test	Verbal fluency, semantic memory.

WAIS, Wechsler Adult Intelligence Scale; FCSRT, Free and Cued Selective Reminding Test. A detailed description of cognitive tests can be found in Supplementary Table 1.

the neurologists when previous tests are inconclusive or score below average. All cognitive tests are conducted by trained neurologists and neuropsychologists and an inter-rater agreement index will be calculated to account for any discrepancies. Both the established normative data validated for the Spanish adult population[45] and direct scores retrieved from the individual tests will be employed to assess cognitive performance status. Moreover, we will use an adapted version of the Preclinical Alzheimer Cognitive Composite (PACC) score, which combines different tests to estimate Aβ-related cognitive decline in cognitively normal individuals.[47] A detailed description of the neuropsychological tests can be found in Supplementary Table 1.

Table 3. PESA-Brain study - Brain MRI protocol.

MRI	Assessment	Acquisition parameters
3D-T1	Global and regional brain volume and thickness	MPRAGE; acquired voxel size: 0.90 × 0.90 × 0.90 mm^3; FOV: 250 × 250 × 190 mm^3; TR/TE/TI: 3000/4.5/900; AT: 3:32.
3D-T2	Radiological assessment of vascular pathology	Spin echo; acquired voxel size: 0.90 × 0.90 × 0.90 mm^3; FOV: 250 × 250 × 190 mm^3; TR/TE/TE equivalent: 3000/300/143; AT: 3:27.
3D-FLAIR	Global and regional volume of white matter lesions	Inversion recovery; acquired voxel size: 1.00 × 1.00 × 1.00 mm^3; FOV: 250 × 250 × 190 mm^3; TR/TE/TE equivalent/TI: 5000/370/152/1700; AT: 4:20.
te-pCASL	Global and regional cerebral blood flow and arrival transit time	Gradient echo EPI; acquired voxel size: 3.10 × 3.10 × 6.00 mm^3; FOV: 220 × 220 × 126 mm^3; TR/TE: 4600/12; total label duration: 3600 ms; PLD block: 200, 350, 500, 700, 1000, 1400, 2000; proton density with TR/TE: 2000/8.6 ms for calibration; AT: 5:31+0:16.
mS-DTI	Local white matter structure integrity	Spin echo EPI; acquired voxel size: 2.00 × 2.00 × 2.00 mm^3; FOV: 240 × 240 × 140 mm^3; TR/TE: 5607/114; PEd: AP; N_{B0}:1; N_{B1000}:16; N_{B2500}:32; AT: 4:47.
3D-SWI	Radiological assessment of microbleeds and vascular pathology; global and regional iron accumulation	Gradient echo; acquired voxel size: 0.80 × 0.80 × 2.00 mm^3; FOV: 240 × 180 × 140 mm^3; TR/TE1/ΔTE: 50/5.1/7; 6 echoes; AT: 4:15.
3D-TOF-MRA	Radiological assessment of vascular pathology; intracranial arterial stenosis	Gradient echo; acquired voxel size: 0.45 × 0.65 × 1.00 mm^3; FOV: 200 × 200 × 90 mm^3; TR/TE: 23/3.5; AT: 3:42.
4D-flow	Global and regional cerebral blood flow velocity	Gradient echo; acquired voxel size: 1.10 × 1.10 × 1.10 mm^3; FOV: 200 × 200 × 80 mm^3; TR/TE: 5.0/2.9; time-series with 15 volumes per cardiac cycle; AT: 12:43.
2D-Qflow	Carotid blood flow velocity	Gradient echo; acquired voxel size: 0.80 × 0.90 × 6.00 mm^3; FOV: 150 × 150 mm^2; TR/TE: 6.6/4.1; time-series with 20 volumes; AT: 1:39.
rs-fMRI	Functional connectivity across brain networks in resting-state	Gradient echo EPI; acquired voxel size: 2.50 × 2.50 × 2.50 mm^3; FOV: 240 × 240 × 127.5 mm^3; TR/TE: 1062/30; time-series with 450 volumes; PEd: AP; AT: 8:07.

FLAIR, fluid-attenuated inversion recovery; te-pCASL, time-encoded pseudo-continuous arterial spin labelling; mS-DTI, multishell diffusion tensor imaging; SWI, susceptibility-weighted MRI; TOF-MRA, time-of-flight magnetic resonance angiography; Qflow, quantitative flow; rs-fMRI, resting-state functional MRI; TR, repetition time (ms); TE, echo time (ms); AT, acquisition time (min); TI, inversion time (ms); EPI, echo-planar imaging; PLD, postlabelling delay (ms).

Blood samples collection and quantification of plasma biomarkers - Fasting blood samples are drawn in the PESA-Brain study before neuroimaging assessment to measure potential physiological confounders of functional signal from MRI acquisitions, such as blood oxygenation levels and gases, which are known to alter CBF.[48] Within 30 minutes of collection, all blood samples are centrifuged and plasma is aliquoted and stored at -80ºC (sample collection of Dr. Valentin Fuster: C.0000800). Detailed blood collection procedure is described in Supplementary Methods. Aliquots of these plasma samples are also used for quantification of standard blood-based biomarkers of neuropathology such as $A\beta_{40}$, $A\beta_{42}$, neurofilament light chain, glial fibrillary acidic protein and phosphorylated tau using ultrasensitive assays such as Simoa® kits (Quanterix Corporation, Billerica, USA).[40] Other novel plasma biomarkers and multiomics analyses may be additionally measured and performed for further exploration.

Brain MRI - A comprehensive brain MRI is acquired to evaluate brain structure, function and vasculature in this middle-aged asymptomatic population. MRI studies include a 1-hour protocol of advanced sequences on a Philips Ingenia Elition X 3T scanner (Philips Healthcare, Best, The Netherlands) (Table 3). All PESA-Brain participants undergo T1- and T2-weighted MRI acquisition for the quantification of global and regional brain volumes and cortical thickness using volume- or surface-based morphometry. Structural data will also be used for brain age estimates and prediction of accelerated aging.[49] Fluid-attenuated inversion recovery (FLAIR) scans are also acquired to detect white matter hyperintensities, which represent lesions of presumed vascular origin and will be segmented and quantified in terms of volume and distribution.[50] Moreover, a cutting-edge version of the arterial spin labelling (ASL) technique, denominated time-encoded pseudo-continuous ASL (te-pCASL), is also implemented in the PESA-Brain study and allows for the retrieval of accurate global and regional CBF and arterial arrival transit time metrics by using multipost labeling delays.[51] This te-pCASL sequence has shown high sensitivity at detecting early CBF changes in preclinical stages of AD.[52] Multishell diffusion tensor imaging (mS-DTI) scans are additionally acquired to obtain standard measures of white matter integrity from tensor computation, such as mean diffusivity or fractional anisotropy, mathematical modelling such as neurite orientation dispersion and density and structural tract connectivity.

Susceptibility-weighted MRI (SWI) scans are further obtained to quantify brain iron and calcium accumulation, whereas a time-of-flight angiography (TOF-MRA) without contrast is acquired to detect changes in the cerebral vasculature, such as intracranial arterial stenosis, aneurysms or blood flow disturbances.

Other sequences, such as a 4D-flow acquisition to characterize brain hemodynamics and a quantitative flow (Qflow) acquisition to measure blood flow in the carotids were added to the MRI protocol after the beginning of recruitment and are not available for all 1,000 PESA-Brain participants. Lastly, a resting-state functional MRI (rs-fMRI) scan is offered to all participants at the end of the MRI protocol to explore functional connectivity networks at the voxel-level using bias-free approaches.[53] Additional information on the MRI protocol can be found in Supplementary Methods.

In parallel to quantification, T2-weighted, mS-DTI, SWI and TOF-MRA data are used for radiological assessment of cerebrovascular pathology, such as identification and grading of lacunar and cortical infarcts, microbleeds, hemorrhages, enlarged perivascular spaces, arterial stenosis and occlusion, ischemic lesions and white matter lesions. All MRI images are visually inspected by trained radiologists for a report of clinically relevant incidental findings.

Amyloid PET - In order to assess 1 of the validated biomarkers for AD diagnosis, a subset of PESA-Brain participants also underwent amyloid PET. Between October 2020 and June 2022, the first 278 PESA-Brain participants recruited received a bolus injection of [^{18}F]flutemetamol (185 MBq $\pm$ 10% of Vizamyl, GE Healthcare, Chicago, Illinois, USA) and were imaged on a Philips Vereos Digital PET/CT scanner (Philips Healthcare, Cleveland, Ohio, USA). A dual-time window PET protocol was implemented to allow for the acquisition of early frames (dynamic scan), which provide a more accurate measurement of Aβ and relative CBF through pharmacokinetic modelling of the amyloid radiotracer, without the need of extra radiation; and late frames (static scan), when tracer binding has reached a pseudoequilibrium in the brain, which allows to measure the standardized uptake value ratio and the centiloid scale.[54] The first dynamic scan is acquired from 0 to 12.5 minutes postinjection, followed by a second 1-minute dynamic scan at minute 15 postinjection and, lastly, a 20-minute static scan at approximately 100 minutes postinjection (Table 4). Static PET scans were visually assessed by trained nuclear medicine physicians and classified as either *positive* (tracer binding in at least 1 brain region) or *negative* (predominantly white matter uptake).

Additionally, scan time was taken full advantage of with an additional cardiac PET study in-between dynamic brain PET acquisitions, since Aβ has been found to aggregate in the heart in AD patients.[55] The PET protocol was slightly altered over time to improve quantification (Supplementary Table 2).

All scans were corrected for attenuation using a low-dose CT attenuation map, acquired prior to each PET scan. The CT scan may also be used for the detection of intracranial calcifications, which could have different vascular etiologies such as atherosclerosis.[56]

Study data storage and management

After acquisition, imaging data are exported to an XNAT server that enables a secure storage while providing a platform where data can be easily assessed.[57] The processing pipelines of the different imaging modalities are automatized through Docker containers, allowing to control the exact environment and software versions used to process data, increasing reproducibility and ensuring the usability of images before analysis.

Statistical methods

Statistical analysis will initially focus on traditional methods such as multivariable regression to study the associations between quantitative brain health biomarkers (imaging and blood-based biomarkers and cognitive scores) and subclinical atheroma plaque volumes, CVD risk and CVRFs, depending on each specific study objective. In addition, neuroimaging analyses will include region-of-interest and voxelwise approaches, in order to identify specific brain regions at early risk and regional patterns of brain vulnerability. More advanced methods will also be used to achieve translational findings and increase statistical power, namely predictive modeling and other pattern recognition techniques to predict clinical outcomes based on integrative brain features.[58] Correction for multiple comparisons and dimensionality reduction techniques will be used to bypass multiple statistical testing and model overfitting. Additionally, we aim to use causal inference models including longitudinal models of mediation analysis and structural equation modelling to build a unique framework that will potentially identify pathways from subclinical atherosclerosis and CVRFs to brain injury and cognitive decline in midlife, as well as to simulate the effect of lifestyle interventions on brain health. If possible, replication in independent cohorts will be performed to overcome the lack of diversity in terms of race/ethnicity and education level of the PESA study.

With a sample size of n = 1,000, these analyses will allow us to detect as significant ($\alpha = 0.05$, 1-$\beta = 0.95$ and 4-10 predictors) effect sizes larger than $f^2 = 0.018$-0.025, which are typically considered very small. Statistical power calculations were performed using the R package *pwr*.

Advanced imaging PESA & PESA-Brain studies

The Advanced Imaging PESA sub-cohort partially overlaps with the PESA-Brain cohort, with 222 PESA-Brain

Table 4. PESA-Brain study - Amyloid PET protocol.

	PET scan	Scan time	PET frames	Assessment	Acquisition parameters
Brain	1st dynamic PET	0-12.5 min	25 frames (30s/frame)	Radiotracer binding and relative CBF	Voxel size: 2.0 × 2.0 × 2.0 mm^3; matrix: 128 × 128 × 82; 3D iterative RAMLA reconstruction algorithm.
	2nd dynamic PET	15-16 min	2 frames (30s/frame)		
	Static PET	100-120 min	-	Aβ accumulation	
Heart	Static PET	12.5-15 min	-	Cardiac Aβ accumulation	Voxel size: 4.0 × 4.0 × 4.0 mm^3; matrix: 144 × 144 × 41; 3D iterative RAMLA reconstruction algorithm.
Brain/Heart	Low-dose CT	-	-	Intracranial calcifications	Voxel size: 1.2 × 1.2 × 2.0 mm^3; matrix: 512 × 512 × 82; 120 kVP; 190 mAs; filtered-back projection reconstruction.

Scan time refers to minutes after injection of 185 MBq of [^{18}F]flutemetamol.
The PET protocol was slightly altered over time to improve quantification (Supplementary Table 2).
RAMLA, row-action maximum-likelihood algorithm.

participants having brain FDG-PET and blood-based biomarkers available from the baseline PESA visit[39] and 186 participants from both baseline and 6-year follow-up visits (Figure 2).[40] This subgroup of individuals will be crucial to further determine the longitudinal interplay between cerebral glucose metabolism and other brain changes in midlife, specifically whether hypometabolism may be partially explained by lower CBF; and whether the accelerated decline in metabolism in individuals at sustained high CVD risk may have a clinical impact on cognition. We will also be able to study the trajectories of plasma biomarkers of neuropathology over 10 years in 3 different timepoints and their correlates with other brain health markers, subclinical atherosclerosis and CVRFs. Analyses in this subcohort (n = 186) will allow us to detect as significant ($\alpha = 0.05$, 1-$\beta = 0.95$ and 4-10 predictors) effect sizes larger than $f^2 = 0.10$-0.14, which are considered medium. Statistical power calculations were performed using the R package *pwr*.

Conclusions

The PESA-Brain study will be a valuable asset to understand the interplay between subclinical vascular pathology and brain health in midlife which may further support strategies for the prevention of cognitive decline and dementia through the early screening of atherosclerosis and lifestyle interventions aimed at reducing CVRFs and CVD. This population cohort study will collect and exploit a myriad of clinical, cognitive and imaging data from middle-aged asymptomatic individuals to contribute to the growing evidence that cognitive impairment may be a result of the continuous exposure to cardiovascular burden allied to the interplay between cerebrovascular disease and neurodegeneration, ultimately determining whether maintaining cardiovascular health early in life may be key to reducing burden of dementia later in life.

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Conflict of interest

JSG is a Philips employee. JDG is currently an AstraZeneca employee and has received research support from GE healthcare, Roche Diagnostics and Hoffmann-La Roche, has given lectures in symposia sponsored by General Electric, Philips, Life Molecular Imaging, and Biogen, has served at scientific advisory boards and/or as a consultant for Prothena and Roche Diagnostics, and is the inventor, founder and co-owner of BetaScreen. All other authors have nothing to disclose.

CRediT authorship contribution statement

Catarina Tristão-Pereira: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Valentin Fuster:** Writing – review & editing, Supervision, Investigation, Funding acquisition, Conceptualization. **Alejandro Lopez-Jimenez:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Alberto Fernández-Pena:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Aurora Semerano:** Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation. **Irene Fernandez-Nueda:** Writing – review & editing, Resources, Project administration, Methodology. **Ines Garcia-Lunar:** Writing – review & editing, Project administration, Investigation. **Carmen Ayuso:** Writing – review & editing, Investigation, Conceptualization. **Javier Sanchez-Gonzalez:** Writing – review & editing, Software, Methodology, Formal analysis. **Borja Ibanez:** Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Conceptualization. **Juan Domingo Gispert:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marta Cortes-Canteli:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ahj.2024.09.028.

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