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Progression of Early Subclinical Atherosclerosis (PESA) Study

JACC Focus Seminar 7/8

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ABSTRACT

Atherosclerosis starts early in life and progresses silently for decades. Considering atherosclerosis as a "systemic disease" invites the use of noninvasive methodologies to detect disease in various regions before symptoms appear. The PESA- (Progression of Early Subclinical Atherosclerosis) CNIC-SANTANDER study is an ongoing prospective cohort study examining imaging, biological, and behavioral parameters associated with the presence and progression of early subclinical atherosclerosis. Between 2010 and 2014, PESA enrolled 4,184 asymptomatic middle-aged participants who undergo serial 3-yearly follow-up examinations including clinical interviews, lifestyle questionnaires, sampling, and noninvasive imaging assessment of multiterritorial subclinical atherosclerosis (carotids, iliofemorals, aorta, and coronaries). PESA tracks the trajectories of atherosclerosis and associated disorders from early stages to the transition to symptomatic phases. A joint venture between the CNIC and the Santander Bank, PESA is expected to run until at least 2029, and its significant contributions to date are presented in this review paper. (J Am Coll Cardiol 2021;78:156–79) © 2021 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Cardiovascular disease (CVD) is the leading cause of mortality and morbidity worldwide and represents the highest proportion of costs to health care systems (1,2). Global CVD prevalence is growing, and if this trend continues, it will threaten the ability of health care systems to cope with its consequences (3). Most forms of CVD originate in atherosclerosis, which is thus the main driver of this terrible scenario. One of the major challenges to addressing this health care crisis is that atherosclerosis starts early in life and progresses silently for decades, eventually manifesting as debilitating or fatal cardiovascular (CV) events (4).

Although atherosclerosis is a systemic disease, different vascular territories vary in their vulnerability to the disease. This recognition of atherosclerosis as a "systemic disease" invites the use of noninvasive methods to identify disease in accessible regions before clinical symptoms appear.

Until very recently, CV risk was calculated according to the presence of traditional risk factors. Although this approach gives a good estimate of risk

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HIGHLIGHTS

- PESA is an ongoing longitudinal cohort study integrating serial imaging, biological, and behavioral parameters associated with the progression of subclinical atherosclerosis in a large, middle-aged, asymptomatic population.
- Comprehensive serial multimodality imaging of multiple vascular territories provides detailed characterization of the extent and regional distribution of early asymptomatic atherosclerosis before and during transition to clinical events.
- Brain imaging and assessments of cognitive function are assessed in association with CV risk factors and subclinical atherosclerosis.
- The PESA cohort has provided data supporting guideline recommendations for plaque detection and primary prevention, but because these derive predominantly from Caucasian Spanish participants, caution is necessary in generalizing findings to other populations.

at the population level, it is often inaccurate at predicting individual risk. More recent evidence has shown that directly visualizing the disease (by quantifying coronary calcium or detecting peripheral artery plaques) can restratify the risk of individuals with a borderline indication for intervention according to traditional risk scales (5,6). This breakthrough heralds the beginning of a paradigm change that will revolutionize the way CV risk is estimated in asymptomatic individuals over the next few years. To get to that stage, it will first be necessary to identify and track atherosclerosis and associated disorders from early subclinical stages through the transition to symptomatic phases. This is already possible using current imaging technology, which can identify key processes in atherosclerosis such as cholesterol deposition in the vessel wall, inflammation and active metabolism, and calcification (7). The PESA (Progression of Early Subclinical Atherosclerosis) Centro Nacional de Investigaciones Cardiovasculares (CNIC)-Santander study was launched in 2010 under the leadership of Valentín Fuster, with the aim of harnessing the power of modern imaging technologies to map the progression of atherosclerosis from its earliest stages.

PESA DESIGN AND OBJECTIVES

The PESA-CNIC-SANTANDER study is a joint venture between the Spanish National Center for Cardiovascular Research (CNIC) and Santander Bank. This ongoing prospective cohort study examines imaging, biological, and behavioral parameters associated with the presence and progression of early subclinical atherosclerosis (8). Middle-aged (40 to 54 years) male and female employees of *Santander Bank* in Madrid were recruited between June 2010 and February 2014. Exclusion criteria included known CVD, cancer or immunological disorders, morbid obesity, chronic kidney disease, the presence of any disease that significantly decreased life expectancy, and any condition that could affect adherence to the study procedures.

PESA recruited 4,184 participants (mean age on enrollment 46 years; 37% women). The original study design (8) schedules each participant to undergo 3 study visits 3 years apart (baseline and 3- and 6-year follow-up, termed visits 1, 2, and 3, respectively). All study visits include a clinical interview, a physical examination, collection of blood and urine samples for analysis and storage, lifestyle and diet questionnaires, measurement of physical activity by accelerometry, a 12-lead electrocardiogram (ECG), and assessment of subclinical atherosclerosis by noninvasive vascular imaging tests. These imaging tests include 2-dimensional (2D) and 3-dimensional (3D) vascular ultrasound (VUS) of the aorta and iliofemoral and carotid arteries, and noncontrast computed tomography (CT) to determine the coronary artery calcium score (CACS). A subgroup of participants with documented atherosclerosis, defined as being in the highest plaque thickness tertile on 2D ultrasound or having any coronary artery calcium (CAC) on CT, were scheduled for additional examination by vascular hybrid ^{18}F-fluorodeoxy-glucose position emission tomography (^{18}FDG PET)-magnetic resonance imaging at baseline (visit 1) and at the 6-year follow-up (visit 3). This "advanced imaging subcohort" was also scheduled for examination by cardiac magnetic resonance (CMR) with pharmacological stress on visit 2.

In 2019, PESA was extended for 10 additional years (PESA-HEALTH, discussed in detail in the final section of this paper). The extended protocol includes 2 additional visits 5 years apart, 10 and 15 years after

ABBREVIATIONS AND ACRONYMS

^{18}FDG PET = ^{18}F-fluorodeoxy-glucose position emission tomography

CACS = coronary artery calcium score

CMR = cardiac magnetic resonance

CNIC = Centro Nacional de Investigaciones Cardiovasculares (Spanish National Center for Cardiovascular Research)

CT = computed tomography

CVD = cardiovascular disease

CVRF = cardiovascular risk factor

EN = elastic net

FBS = Fuster BEWAT score

ICHS = ideal cardiovascular health score

LDL-C = low-density lipoprotein cholesterol

LTL = leukocyte telomere length

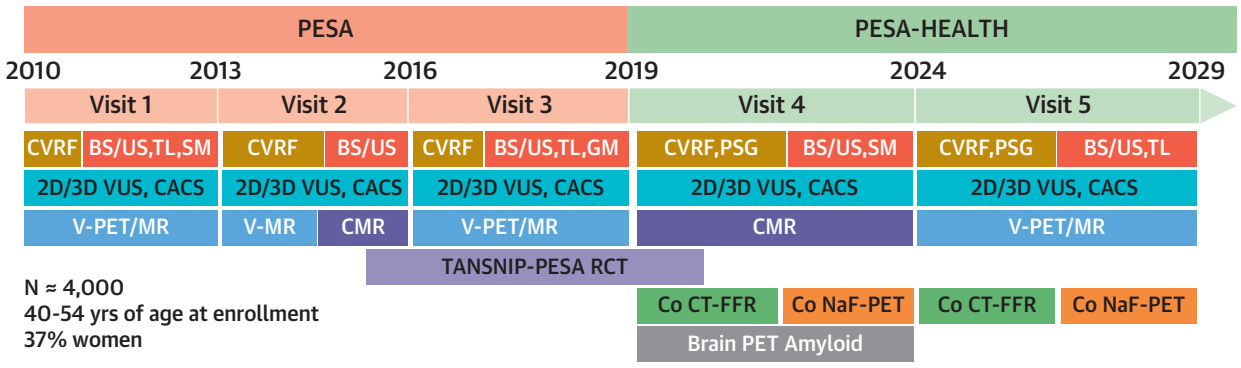
MetS = metabolic syndrome

MR = magnetic resonance

oxLDL = oxidized low-density lipoprotein

VPA = vigorous physical activity

FIGURE 1 PESA Study Design



CVRF,PSG:	Cardiovascular risk factors (CVRF), lifestyle-diet questionnaires, accelerometer (physical activity, sleep), ECG, spirometry, clinical events, cardiorespiratory polysomnography [PSG] **(N ≈ 4,000)**
BS/US,TL,GM,SM:	Blood sample [BS] (DNA*, RNA, buffy coat, plasma, serum)/Urine sample [US] **(N ≈ 4,000)**, Telomere length [TL] **(N ≈ 1,500)**, Gut microbiome [GM] **(N ≈ 450)**, somatic mutations [SM] **(N ≈ 4,000)**
2D/3D VUS, CACS:	Vascular basic imaging (carotid, femoral, aorta 2D/3D ultrasound [VUS], coronary artery calcium score [CACS]) **(N ≈ 4,000)**
V-PET/MR, V-MR:	**Advanced imaging subcohort** (carotid, femoral, aorta ^{18}FDG-PET and vascular MR) **(N ≈ 800)**
CMR:	**Advanced imaging subcohort** (stress cardiac magnetic resonance [CMR]) **(N ≈ 750)**
TANSNIP-PESA RCT:	**TANSNIP-PESA substudy** (lifestyle intervention *vs.* standard of care) **(N ≈ 850)**
Co CT-FFR:	**Coronary subcohort** (CT coronary angiography / CT-Fractional Flow Reserve [FFR]) **(N ≈ 700)**
Co NaF-PET:	**Coronary subcohort** (Coronary sodium fluoride PET study) **(N ≈ 300)**
Brain PET Amyloid:	**Brain subcohort** (Brain PET Amyloid study / neuropsychological tests) **(N ≈ 1,000)**

Visits timetable summarizing the procedures performed in PESA-CNIC-SANTANDER participants during visits 1 to 3 and scheduled for PESA-HEALTH participants during visits 4 and 5. *DNA extraction was limited to visit 1. ^{18}FDG-PET = 18-fluorodeoxy-glucose positron emission tomography; Co CT-FFR = computed tomography coronary angiography / Fractional flow reserve; Co NaF-PET = coronary sodium fluoride positron emission tomography; ECG = electrocardiogram; PESA = Progression of Early Subclinical Atherosclerosis; TANSNIP = Trans-Atlantic Network to study Stepwise Non-invasive Imaging as a tool for cardiovascular prognosis and Prevention; V-PET/MR = 18F-Fluorodeoxyglucose positron emission tomography and vascular magnetic resonance.

enrollment (visits 4 and 5). These visits will continue the approaches used in the first phase and add several new approaches to tackle novel scientific questions emerging from this evolving project. The follow-up visits and tests in PESA (2010-2019) and PESA-HEALTH (2019-2029) are summarized in Figure 1.

The PESA-CNIC-SANTANDER study has a range of core objectives: 1) The overarching goal is to characterize the presence of subclinical atherosclerosis and its progression over 16 years in apparently healthy middle-aged individuals. 2) Within this population, the study is characterizing plaque composition and vascular inflammation in the advanced imaging subcohort. Other areas of study examine how the presence and progression of subclinical atherosclerosis predict or are influenced by key parameters: 3) traditional and emerging risk factors identified by high-throughput omics approaches; 4) lifestyle behaviors (dietary habits, physical activity, and sleep patterns) and psychosocial factors; and 5) the risk of future CVD events. The study also investigates the influence of subclinical atherosclerosis and its risk factors on 6) myocardial anatomy, function, and composition and 7) cognitive function and brain structure, connectivity, and metabolism. 8) Another goal is to analyze the relationship between coronary artery subclinical disease (including active coronary microcalcification) and incident CVD events. 9) Finally, PESA is testing the effectiveness of a worksite-based lifestyle program to promote CV health.

PESA IN CONTEXT: BENCHMARKING AGAINST PRIMARY PREVENTION STUDIES WORLDWIDE

Even though several population studies have investigated the prevalence of atherosclerosis in asymptomatic middle-aged individuals, PESA has some unique features:

1. Inclusion and long-term follow-up of relatively young individuals (age 40-54 years) with low baseline CV risk according to classical equations. The MESA (Multi-Ethnic Study of Atherosclerosis), Heinz Nixdorf Recall, Cardiovascular Health, HRP (High-Risk Plaque), and Rotterdam studies all enrolled older participants with a higher risk profile than those in PESA (4,9,10). Although the CARDIA (Coronary Artery Risk Development in Young Adults) study included young individuals (age 18-30 years), its design mainly aimed to assess the influence of lifestyle and healthy behaviors on CV risk factors and not an assessment of baseline subclinical disease with imaging techniques (11). The Dallas Heart Study included >6,000 individuals (age 18-65 years) to assess CVD, but vascular ultrasound to visualize atherosclerotic plaque was not included in the study protocol (12). The presence of subclinical atherosclerosis is also explored in another Spanish independent cohort study, AWHS (Aragon Workers' Health Study) (13); however, this study included only men, and with a slightly older age profile than PESA (aged 40-59 years). Other major CVD cohort studies include the international Seven Countries, MONICA (Monitoring Trends and Determinants in Cardiovascular Disease), INTERHEART (The Effect of Potentially Modifiable Risk Factors Associated with Myocardial Infarction), and PURE (Prospective Urban Rural Epidemiology Study) studies, as well as the British WHITEHALL and the German PROCAM (The Prospective Cardiovascular Münster) studies; however, these studies are not specifically designed to assess subclinical atherosclerosis.
2. PESA is the most ambitious study in terms of systemic atherosclerosis characterization, including serial multiterritorial imaging assessment of the carotid, iliofemoral, aortic, and coronary territories. This approach includes identification of both atherosclerotic plaques and CAC. Although atherosclerosis is a systemic disease that might affect the entire arterial tree, most of previous studies have assessed at baseline disease in only 1 or 2 territories, potentially missing the presence of the disease in many subjects. For example, in the MESA and CARDIA studies, evaluation of atherosclerosis was initially limited to carotid intima-media thickness (which is not a good surrogate for actual atherosclerosis) and CAC, while ARIC (Atherosclerosis Risk In Communities) (14) only focused on atherosclerosis in the carotid and popliteal territories. The multiterritorial approach in PESA is designed to characterize the distribution of the disease in its early stages and to track its trajectory in these different territories.
3. PESA uses more advanced modern imaging technology, including 3DVUS (discussed in detail in the next section). The use of cutting-edge imaging technology is yet another unique feature of PESA. Plaque characterization by ultrasound and vascular MR, together with the identification of arterial inflammation by FDG PET, is a key feature of the study. **Table 1** shows the similarities and differences between different international primary prevention studies.

THE 10 MOST IMPORTANT FINDINGS OF THE PESA STUDY

A summary of the 10 most important findings can be found in the **Central Illustration**.

PREVALENCE OF SUBCLINICAL ATHEROSCLEROSIS IN MIDDLE AGE. The role of noninvasive imaging in improving the stratification of lifetime risk. In the baseline evaluation of the PESA cohort, 63% of the asymptomatic participants (mean age 46 years) showed evidence of atherosclerosis, defined as the presence of atherosclerotic plaque in any of the screened territories by ultrasound (carotids, iliofemorals, aorta) or evidence of CAC in the coronary tree by CT (CACS ≥1) (15). Atherosclerosis was more prevalent in men (71% vs 48% in women). CAC was detected in only 18% of the cohort (CACS 1-99 in 14%, 100-399 in 3%, and ≥400 in 0.7%), in line with previous data from the CARDIA and MESA studies (16). The PESA data show that CACS = 0 should not be considered an indicator of the absence of atherosclerotic disease, since ≈60% of PESA participants without CAC had plaques at other vascular sites.

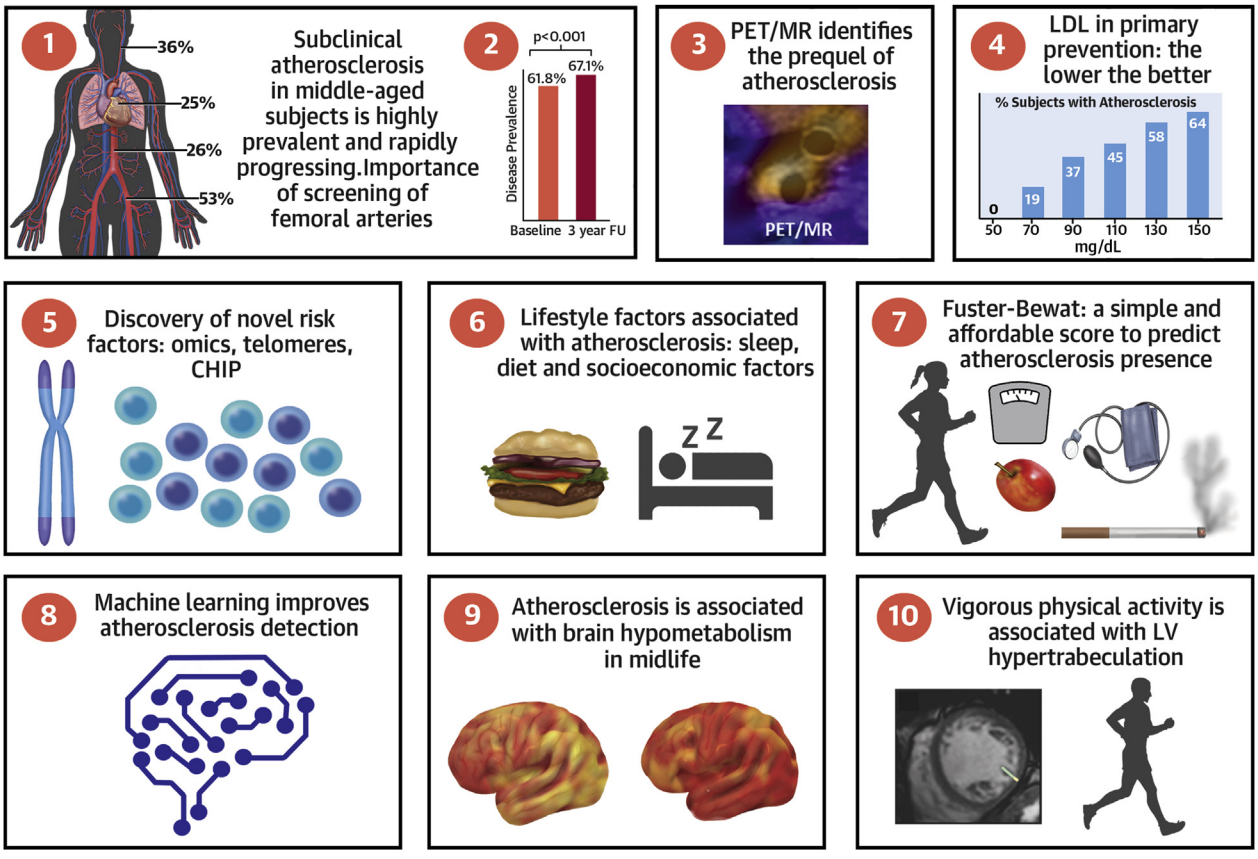
Plaques were more frequent in the iliofemoral territory (44% of subjects), followed by the carotids (31%) and the aorta (25%). Given that the femoral territory is seldom explored in patient screenings, these data have implications for strategies in this area. Indeed, the absence of disease in the iliofemoral territory was strongly associated with the absence of atherosclerosis at other vascular sites. These data are consistent with findings of the AWHS study, which found that atherosclerotic plaques in apparently healthy individuals were more frequent in the femoral than in the carotid arteries and also demonstrated that femoral disease was more strongly associated with cardiovascular risk factors (CVRFs) (13). Moreover, the CAFES-CAVE (Carotid and Femoral Ultrasound Morphology Screening and Cardiovascular Events in Low Risk Subjects) study demonstrated an association between subclinical

TABLE 1 Population Studies Assessing Subclinical Atherosclerosis

	PESA	CARDIA	ARIC	Rotterdam	Dallas Health	MESA	Heinz Nixdorf Recall	High Risk Plaque
Study population and environment	4,184 (2010-present) Madrid	5,116 (1985-present) 4 U.S. centers	15,800 (1987-present) 4 U.S. centers	14,926 (1990-2016) Rotterdam	6,101 (2000-present) Dallas County	6,814 (2000-present) 6 U.S. centers	4,814 (2000-2013) 3 sites, Germany	7,687 (2008 up to 600 events) Chicago, Florida
Age, y	40-54	18-30	45-64	>45	18-65	45-84	45-75	55-80
Ethnicity	White	Black and White	Black and White	White	White, African-American, Hispanic, other	White, African-American, Hispanic, or Chinese	White	74% White
Male, %	63.5	45.5	48	38	34	47	47	44
Imaging techniques	Carotids: • 2DVUS, 3DVUS (n = 4,184; 0, 3, 6, 10, 15 y) • MRI (n = 946; 0, 3, 6, 15 y) • ^{18}F-FDG PET/MRI (n = 946; 0, 6, 15 y) Aorta: • 2DVUS (n = 4,184; 0, 3, 6, 10, 15 y) • ^{18}F-FDG PET (n = 946; 0, 6, 15 y) Femorals: • 2DVUS, 3DVUS (n = 4,184; 0, 3, 6, 10, 15 y) • MRI (n = 946; 0, 3, 6, 15 y) • ^{18}F-FDG PET/MRI (n = 946; 0, 6, 15 y) Cardiac: • CACS (n = 4,184; 0, 3, 6, 10, 15 y) • Coronary CTA (n = 700; 10, 15 y) • NaF PET/CT (n = 300, 10, 15 y) • CMR (n = 946; stress n = 550; 3, 15 y) • Aortic valve-based echo (n = 4,184; 10, 15 y) Brain: • Comprehensive MRI (n = 1,000; 10 y) • Amyloid PET/CT (n = 1,000; 10 y) • Perfusion CMR (n = 946; 10, 15 y)	Carotids: • IMT (n = 3,650; 20 y) Cardiac: • Echo (n = 4,352; 5, 10, 25, 30 y) • CACS (n = 3,100; 15, 20 y) Brain: • MRI (n = 600; 25, 30 y) Abdomen: • CT (n = 3,500; 25 y)	Carotids: • 2DVUS (n = 15,792) • MRI (n = 2,066) Cardiac: Echo (n = 12,887) Brain: • MRI (n = 1,974) • Amyloid PET (n = 329) Abdomen: • CT (n = 6,538)	Carotids: • 2DVUS (n = 14,926) • MRI (n = 7,983) Cardiac: • CACS (n = 2,349) Brain: • MRI (n = 5,886)	Carotids: • MRI (n = 3,401) Aorta: • MRI (n = 3,072) Cardiac: • Echo (n = 3,401) • CACS (n = 3,072) • CMR (n = 3,072) Brain: • MRI (n = 3,401) Liver: • MRI (n = 3,072)	Carotids: • 2DVUS (n = 6,814) • MRI (n = 1,000) • PET/MRI (n = 350) Cardiac: • Echo (n = 3,303) • Coronary CTA (n = 6,814) • CMR (n = 5,000) • Coronary MR (n = 300) Aortic: • CT (n = 1,000) Brain: • Amyloid PET (n = 216) • MRI (n = 1,896)	Carotids: • 2DVUS (n = 4,814) Cardiac: • Echo (n = 4,814) • CACS (n = 4,814)	Carotids: • 2DVUS, 3DVUS (n = 6,104) Cardiac: • CACS (n = 6,104)

^{18}FDG PET = ^{18}F-fluorodeoxy-glucose position emission tomography; 2D = 2-dimensional; 3D = 3-dimensional; CACS = coronary artery calcium score; CMR = cardiac magnetic resonance; CT = computed tomography; CTA = computed tomography angiography; IMT = intima-media thickness; MRI = magnetic resonance imaging; PET = positron emission tomography; VUS = vascular ultrasound.

CENTRAL ILLUSTRATION **The 10 Most Important Findings of the PESA Study: Key Messages From 10 Selected Contributions of PESA**



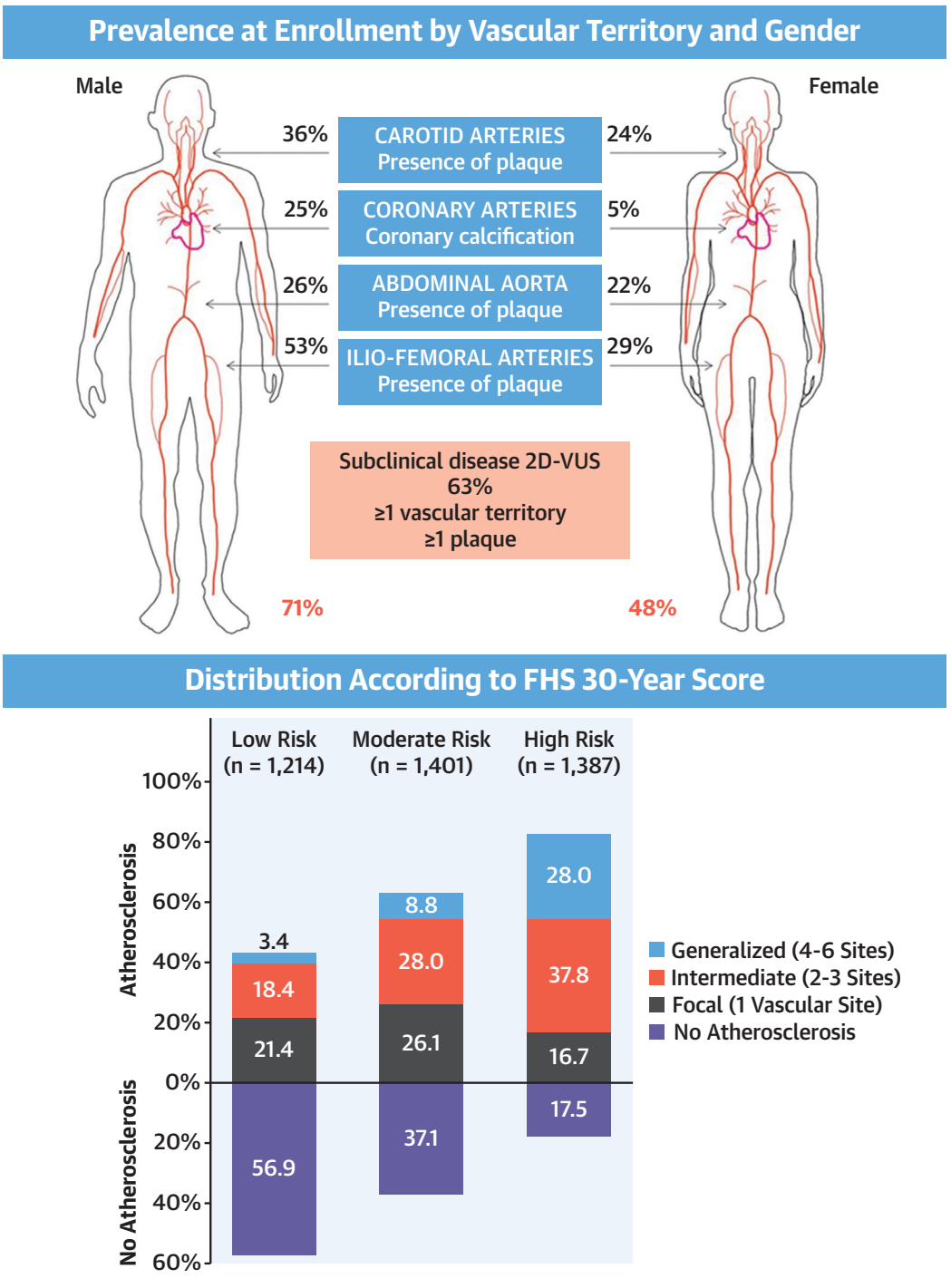
Ibanez, B. et al. J Am Coll Cardiol. 2021;78(2):156–79.

Image in panel 1 is reproduced from Fernandez-Friera L, Penalvo JL, Fernandez-Ortiz A et al. Prevalence, Vascular Distribution, and Multiterritorial Extent of Subclinical Atherosclerosis in a Middle-Aged Cohort: The PESA (Progression of Early Subclinical Atherosclerosis) Study. Circulation 2015;131:2104-13. Graph in panel 2 is reproduced from Lopez-Melgar B, Fernandez-Friera L, Oliva B et al. Short-Term Progression of Multiterritorial Subclinical Atherosclerosis. J Am Coll Cardiol 2020;75:1617-1627. Image in panel 3 is reproduced from Fernandez-Friera L, Fuster V, Lopez-Melgar B et al. Vascular Inflammation in Subclinical Atherosclerosis Detected by Hybrid PET/MRI. J Am Coll Cardiol 2019;73:1371-1382. Graph in panel 4 is reproduced from Fernandez-Friera L, Fuster V, Lopez-Melgar B et al. Normal LDL-Cholesterol Levels Are Associated With Subclinical Atherosclerosis in the Absence of Risk Factors. J Am Coll Cardiol 2017;70:2979-2991. The images in panel 9 are reproduced from Cortes-Canteli M, Gispert JD, Salvado G et al. Subclinical Atherosclerosis and Brain Metabolism in Middle-Aged Individuals: The PESA Study. J Am Coll Cardiol 2021;77:888-898. The image in panel 10 is reproduced from de la Chica JA, Gomez-Talavera S, Garcia-Ruiz JM et al. Association Between Left Ventricular Noncompaction and Vigorous Physical Activity. J Am Coll Cardiol 2020;76:1723-1733. CHIP = clonal hematopoiesis of indeterminate potential; FU = follow-up; LDL = low-density lipoprotein-cholesterol; LV = left ventricular; PET/MR = positron emission tomography/magnetic resonance.

femoral disease and CV events along 10 years of follow-up (17). Imaging of the iliofemoral arteries may thus be a useful population-wide screening tool for detecting atherosclerosis in its early asymptomatic stages. Based on these and other findings, recent dyslipidemia guidelines recommend carotid or femoral screening as a risk modifier in individuals at low or moderate risk (5). It is expected that, sooner than later, these recommendations affect decisions made by health care providers, policy makers, and patients.

It is enormously relevant that the PESA baseline analysis detected evidence of atherosclerosis in ≈40% of participants classified at low long-term risk and ≈60% of those at intermediate risk according to classical equations; moreover, a significant proportion of the affected individuals had extensive disease (with several territories affected) (**Figure 2**) (15). This high rate of detection of disease in low-intermediate risk groups is probably the result of the comprehensive screening of many territories using different imaging techniques in PESA, and these results could explain to

FIGURE 2 **Prevalence and Territorial Distribution of Subclinical Atherosclerosis in Middle-Aged Individuals**



(Upper panel) Prevalence of subclinical atherosclerosis at enrollment (visit 1) by vascular territory and sex. **(Lower panel)** Distribution of subclinical atherosclerosis according to FHS 30-year risk categories; the vascular sites examined were the right and left carotids, the abdominal aorta, and the right and left iliofemoral arteries (presence of plaque), as well as the coronary vessels (coronary artery calcification). FHS scores were classified as low (<10%), moderate (≥10%-20%), or high (>20%) risk (9). Reprinted with permission from Fernandez-Friera et al. (15). FHS = Framingham Heart Study; 2D-VUS = 2-dimensional vascular ultrasound.

some extent the discrepancy between traditional population-based risk scoring systems and the occurrence of clinical events at the individual level.

PROGRESSION OF SUBCLINICAL ATHEROSCLEROSIS BY 3DVUS. One of the key innovations in PESA is the inclusion, in addition to traditional 2DVUS screening, of new 3DVUS technology validated by our group (18). After scanning nearly 4,000 PESA participants, we found that plaque burden assessed by 3DVUS correlates strongly with CVRFs and reflects estimated CV risk more closely than 2D plaque detection alone (19), suggesting the potential value of 3DVUS imaging for improving risk stratification strategies. The importance of plaque burden quantification is also suggested by previous studies from other groups. For example, the BioImage study in the HRP cohort showed that quantifying carotid burden by 3DVUS (albeit with more primitive technology than that available in PESA) is comparable to CACS in terms of improved risk prediction, risk reclassification, and the definition of statin eligibility for primary prevention (9,20). Likewise, 2D plaque area quantification has been shown to significantly improve the prediction of the risk of myocardial infarction, stroke risk, and even CV death (21). The PESA results also show that addition of femoral plaque burden quantification could improve the prediction of CV risk at early stages of atherosclerosis, especially in low-risk populations (19).

Changes in atherosclerosis are monitored in PESA through frequent follow-up visits (every 3 years). Although atherosclerosis is known to progress over time, the rate of progression is unknown. Unexpectedly, at the 3-year follow-up visit (visit 2), >40% of the apparently healthy middle-age PESA cohort showed evidence of disease progression (**Figure 3**) (22). Overall, peripheral plaque progression at visit 2 detected by VUS was more common than an increase in coronary calcification. However, the relative frequency of these parameters depended on participants' prior history: participants with no evidence of disease at baseline more frequently showed peripheral plaque progression, whereas CACS progression was more frequent in individuals who already had disease at visit 1. These data reinforce the idea that CAC is a later manifestation of atherosclerosis. Short-term atherosclerosis progression was associated with most conventional CVRFs, especially age, sex, and dyslipidemia, in agreement with findings from the REFINE (Risk Evaluation For INfarct Estimates) study (23).

HYBRID PET/MR IDENTIFIES VASCULAR INFLAMMATION ASSOCIATED WITH ATHEROSCLEROTIC RISK. Animal models and classical pathology studies have shown that atherosclerotic plaque development is essentially an inflammatory process (24). The inclusion of the "advanced imaging" subcohort uniquely enables PESA to go beyond plaque identification to deeply characterize plaque inflammation and composition. Hybrid ^{18}FDG PET/MR was scheduled for visit 1 (baseline) and visit 3 (6 years later) in a subcohort of 946 participants showing baseline evidence of plaques or coronary calcification; this analysis examines arterial ^{18}F-FDG uptake, which is an index of high metabolic activity, a surrogate of inflammation (25). In this subcohort of apparently healthy middle-aged PESA participants (mean age 50 years), one-half of the participants showed evidence of arterial inflammation in the baseline examination, most often in the femoral territory (**Figure 4**) (26). Arterial inflammation was independently associated with male sex, age, smoking, and obesity. The association of elevated arterial ^{18}F-FDG uptake with smoking and obesity is in line with previous findings. Smoking is known to promote immune-mediated vascular injury (27), and obesity triggers a pro-inflammatory effect caused by adipocyte-mediated release of proinflammatory cytokines, which play a central role in vascular inflammation (28).

A fascinating finding of the hybrid ^{18}FDG PET/MR analysis is that most arterial FDG uptake occurred in plaque-free vascular segments, with FDG uptake detected in only 11% of plaques identified by MR (**Figure 4**) (26). This raises the intriguing possibility that active arterial inflammation precedes the development of atherosclerotic plaque, in line with previous data demonstrating that focal arterial inflammation precedes subsequent atherosclerotic calcification (29). At present, we cannot establish temporality or causation because the results reported so far are cross-sectional. We are currently analyzing data from the second ^{18}FDG PET/MR examination, performed at visit 3, and a third vascular PET study is scheduled for visit 5 (>10 years after the baseline inflammation scan) (**Figure 1**).

Although at the time of writing, our conclusions remain speculative, these findings suggest the possibility of using imaging to identify a high-risk population that could benefit from preventive anti-inflammatory interventions. The CANTOS (Canakinumab Anti-Inflammatory Thrombosis Outcomes Study) trial recently showed the potential benefits of anti-inflammatory therapies in patients at advanced stages of atherosclerosis with a high inflammatory component (30). If, as suggested by PESA and other studies (31), inflammation plays a critical role in early stages of atherosclerosis, early intervention applied

to at-risk individuals might have a significantly stronger beneficial impact on preventing future CVD events.

LOW-DENSITY LIPOPROTEIN AND SUBCLINICAL ATHEROSCLEROSIS. The lower, the better even in asymptomatic subjects. For CVRFs that are continuous variables (cholesterol, blood pressure, glycated hemoglobin, and so on), the assignment of individual risk is based on fixed, arbitrary thresholds. Although such cutoffs are needed to guide intervention strategies, they give the false impression that individuals with values below these thresholds are free of CV risk. Indeed, a below-threshold value for a classical CVRF is enough to assign a given individual a low or very low risk of atherosclerosis (32). However, individuals without CVRFs, as currently defined, still have subclinical atherosclerosis and experience CV events (16,33). For example, 60% of PESA participants classified as low risk according to traditional risk scales showed evidence of subclinical atherosclerosis, and the disease was generalized (≥4 vascular territories affected) in >40% (**Figure 2**) (3). These findings underline the already mentioned failure of traditional CVRFs to predict individual risk.

A standout illustration of this issue is the detection in the PESA study of subclinical atherosclerosis in one-half of apparently healthy middle-aged participants with "normal" values for all CVRFs (34). More importantly, even in individuals with "optimal" values for all CVRFs, there was a significant correlation between low-density lipoprotein cholesterol (LDL-C) concentration and the presence of atherosclerosis, with each 10-mg/dL increase in LDL-C associated with an OR for having multiterritorial atherosclerosis of 1.18 (**Figure 5**) (34). These data reinforce the idea that desirable LDL-C concentrations are probably much lower than those currently recommended, and suggest that atherosclerosis in both men and women develops above an LDL-C threshold concentration of approximately 50-60 mg/dL (35), similar to the level associated with disease regression in other studies (36). This hypothesis is consistent with recent lipid-lowering trials, in which adverse clinical outcomes were significantly reduced when LDL-C levels were lowered below current targets (37). Indeed, recent guidelines (5,38,39) have defined more restrictive levels for normal LDL-C. Together, these data support the extension of the "*the lower LDL-C, the better*" concept to individuals classified as at low risk.

Through these advances, PESA is contributing to the notion that the risk entailed by CVRFs should be viewed as a continuum, and that cutoffs, although necessary to guide clinical cardiologists in decision-making, should not be interpreted as meaning that individuals below them are without risk. The PESA data to date establish that this is the case for LDL-C (34), and Hb1c (40), suggesting that the key to tackling the atherosclerosis epidemic may be a more aggressive control of risk factors.

PESA has also recently advanced knowledge about the interplay between LDL-C and atherosclerosis. High plasma concentrations of oxidized low-density lipoprotein (oxLDL) are associated with all stages of atherosclerosis as well as with alterations linked to its onset, progression, and complications, including insulin resistance, diabetes mellitus, obesity, and metabolic syndrome (MetS) (41). It is therefore critically important to shed light on the association between oxLDL and other CVRFs in CVD-free individuals without diabetes. The PESA data show a direct association between plasma oxLDL and MetS (42). Furthermore, modern mediation analysis demonstrated that plasma oxLDL in individuals without diabetes is associated with all individual MetS components except for fasting glucose concentration, and that this association was independent of central obesity and insulin resistance (42). These data suggest that elevated plasma oxLDL may be a predictor of MetS, reflecting a core mechanism through which MetS components develop and progress in the absence of central obesity and before the appearance of insulin resistance.

Another critical research challenge is to identify new oxLDL mediators. In vascular cells, the main receptor of oxLDL is lectin-like oxLDL receptor-1 (LOX-1), but a putative receptor on T lymphocytes long remained elusive despite a suggested role for oxLDL in adaptive immune responses. CNIC researchers addressed this question by combining elegant molecular and animal studies with human validation in the PESA cohort (43). For the first time, it was demonstrated that CD69 on the surface of human (and rodent) T cells acts as a specific receptor for oxLDL. CD69-oxLDL interaction on T cells activates the expression of NR4A transcription factors, dampening pro-atherogenic Th17 and Th1 responses. Remarkably, CD69 and NR4A1 mRNA levels were significantly down-regulated in peripheral blood leukocytes from PESA participants with subclinical atherosclerosis, even after adjusting for traditional risk factors, NR4A1 expression, and plasma oxLDL concentration. These results highlight a novel role of CD69 as an oxLDL receptor on T cells that protects against atherosclerosis by regulating T-cell-mediated adaptive immune responses.

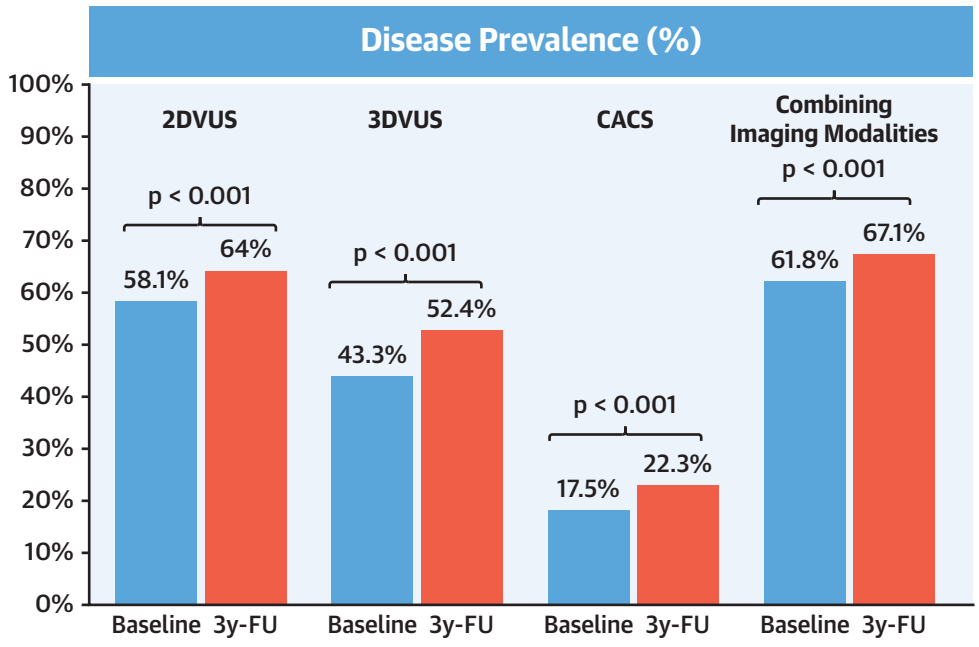
DISCOVERY OF NOVEL RISK FACTORS. From omics to telomeres. The quest to develop new predictive scores that identify asymptomatic individuals with a high risk of future CVD events, particularly those without traditional risk factors, can be assisted by high-throughput "omics," a powerful tool that can be exploited for the unbiased identification of novel biomarkers of subclinical atherosclerosis and mechanisms underlying its progression and clinical complications. Ongoing omics studies in PESA include high-throughput proteomics and metabolomics/lipidomics in plasma samples, and RNAseq (microRNA and mRNA profiling) and epigenomics in peripheral blood leukocytes (**Figure 6**). Integrative analysis of multiomics data obtained at different time points in the same PESA participants should help identify novel biomarkers and mechanisms underlying atherosclerosis progression and to improve our understanding of the dynamics of disease manifestation. Future studies will be necessary to validate in independent human cohorts the candidate factors identified in PESA. Moreover, functional studies in preclinical models, ideally using multispecies models of disease, will be necessary to establish causal relationships and thus identify new therapeutic targets to treat atherosclerosis (44).

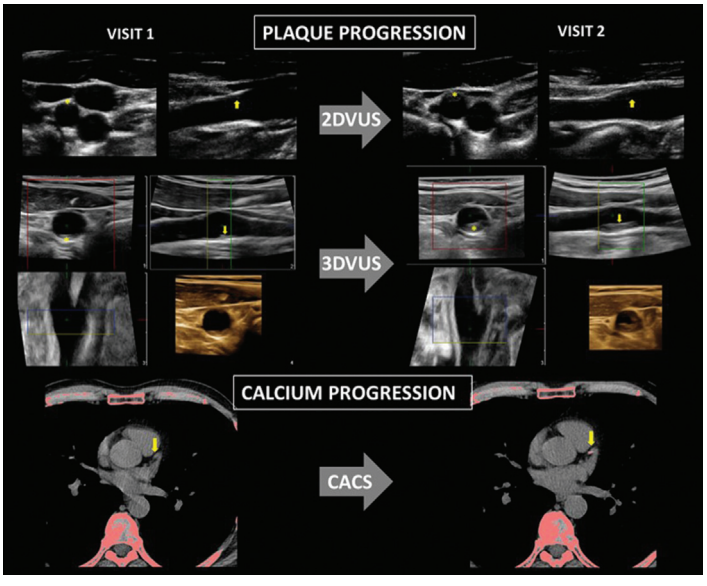
Recent studies illustrate the omics strategies adopted in PESA, as well as how PESA data are being used for independent external validation of results generated in other studies. A recent deep quantitative multiplexed proteomics analysis of human aorta detected increased expression of lipid transport, complement system, immunoglobulin superfamily, and hemostasis-related proteins in early atherosclerotic plaques (45). This analysis of autopsy tissue found an association between increased complement C5 content and complement activation in human plaques. Validation analysis showed that C5 plasma levels in PESA volunteers positively correlated with global plaque burden and CACS, and a similar relationship was found in the NEFRONA cohort (45,46). Future epidemiological and clinical studies are warranted to confirm the accuracy and reliability of plasma C5 as a biomarker for CV risk prediction, perhaps in combination with other recently reported circulating biomarkers of subclinical atherosclerosis.

PESA has also proved its value as an independent external replication tool through our collaboration with the FHS (Framingham Heart Study) investigators, who used liquid chromatography-tandem mass spectrometry to detect cross-sectional and longitudinal lipidomic signatures of MetS components (obesity, dysglycemia, and dyslipidemia) (47). In an analysis of 154 circulating lipid species in 658 study participants, the FHS team identified 39 lipids associated with obesity and 8 with dysglycemia. In independent lipidomic studies, the PESA, Erasmus Rucfen Family, and San Antonio Heart Family Study cohorts independently replicated 5 of 6 lipids (83%) available for validation for dyslipidemia and 28 of 32 lipids available for obesity (88%) (47). This collaborative effort has identified and replicated numerous circulating lipid species, which are associated cross-sectionally with MetS components and with their longitudinal alterations. Future studies are warranted to explore potential prognostic and therapeutic applications of these lipids.

PESA is also ideally positioned to resolve apparent discrepancies between studies examining the links between age-related CVD and telomere length. Telomeres are complexes of DNA, RNA, and proteins located at chromosome ends that protect chromosomes against injury (48). Preservation of functional telomeres is therefore essential for maintaining genetic stability. Human telomeres shorten with age, in large part because of the end-replication problem and the inactivation of telomerase, the enzyme that replicates telomere DNA (48). There is a remarkably high degree of interindividual variability in both mean telomere length at birth (ranging from 5 to 12 kb) and the rate of age-related telomere shortening (~70-150 bp/year). The accumulation of critically short telomeres triggers cellular senescence and is thought to be a pro-aging mechanism (48), and telomere attrition has therefore been considered a marker of biological aging, rather than chronological aging (49). Moreover, because age is the main CV risk factor, there has been tremendous interest in the possibility that telomere ablation might contribute to age-dependent CVD, particularly after seminal work by Samani et al. (50) demonstrating shorter mean leukocyte telomere length (LTL) in patients with advanced atherosclerotic disease than in disease-free individuals. These observations were confirmed in a recent meta-analysis (51), and results from prospective and genome-wide association studies suggest a causal link between short LTL and atherothrombotic events (52,53). However, in apolipoprotein E-null mice with diet-induced atherosclerosis, short telomeres did not exacerbate the disease but rather protected against it (54); moreover, the Asklepios (55) and Bruneck (56) human studies both revealed a lack of association between mean LTL and early subclinical atherosclerosis. In PESA, we have examined telomeres by high-throughput quantitative in situ hybridization, which allowed us to simultaneously quantify mean LTL and the accumulation of critically

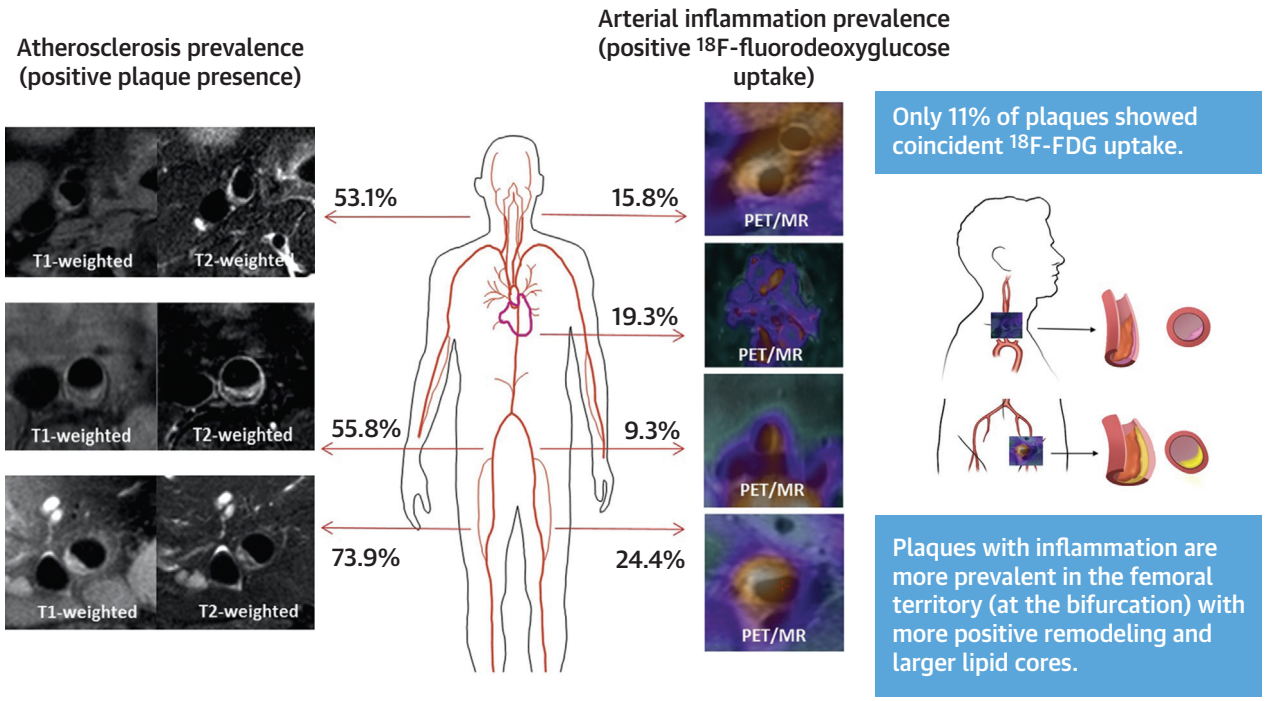
FIGURE 3 Short-Term (3-Year) Progression of Subclinical Atherosclerosis





(Upper panel) Subclinical atherosclerosis prevalence at baseline (visit 1) and 3-year follow-up (visit 2) by 2DVUS, 3DVUS, CACS, and the 3 imaging modalities combined. **(Lower panel)** Representative figures illustrating disease progression. Intimal irregularities in the carotid bulb were detected on visit 1 by 2DVUS. By visit 2, this had progressed to a hypoechogenic atherosclerotic plaque **(arrow in the upper right panel)** extending into the external carotid artery **(asterisk in the upper right panel).** Analysis by 3DVUS detected and quantified significant growth of a plaque already present in the carotid bulb on visit 1 **(arrow and asterisk in middle panels).** Computed tomography images show new onset of a calcified lesion in the left anterior descending coronary artery on visit 2 **(arrow in bottom panels).** Adapted with permission from López-Melgar et al. (22). 2DVUS = 2-dimensional vascular ultrasound; 3DVUS = 3-dimensional vascular ultrasound; CACS = coronary artery calcium score; FU = follow-up.

FIGURE 4 Distribution and Inflammation by Positron Emission Tomography/Magnetic Resonance in Subclinical Atherosclerosis



(Left) Prevalence of atherosclerosis and arterial inflammation by hybrid ^{18}F-FDG PET/MR across several vascular territories in the PESA participants. **(Right)** Typical phenotypic features of plaques with or without inflammation. Reprinted with permission from Fernandez-Friera et al. (26). ^{18}F-FDG = ^{18}F-fluorodeoxyglucose; PESA = Progression of Early Subclinical Atherosclerosis; PET/MR = positron emission tomography/magnetic resonance.

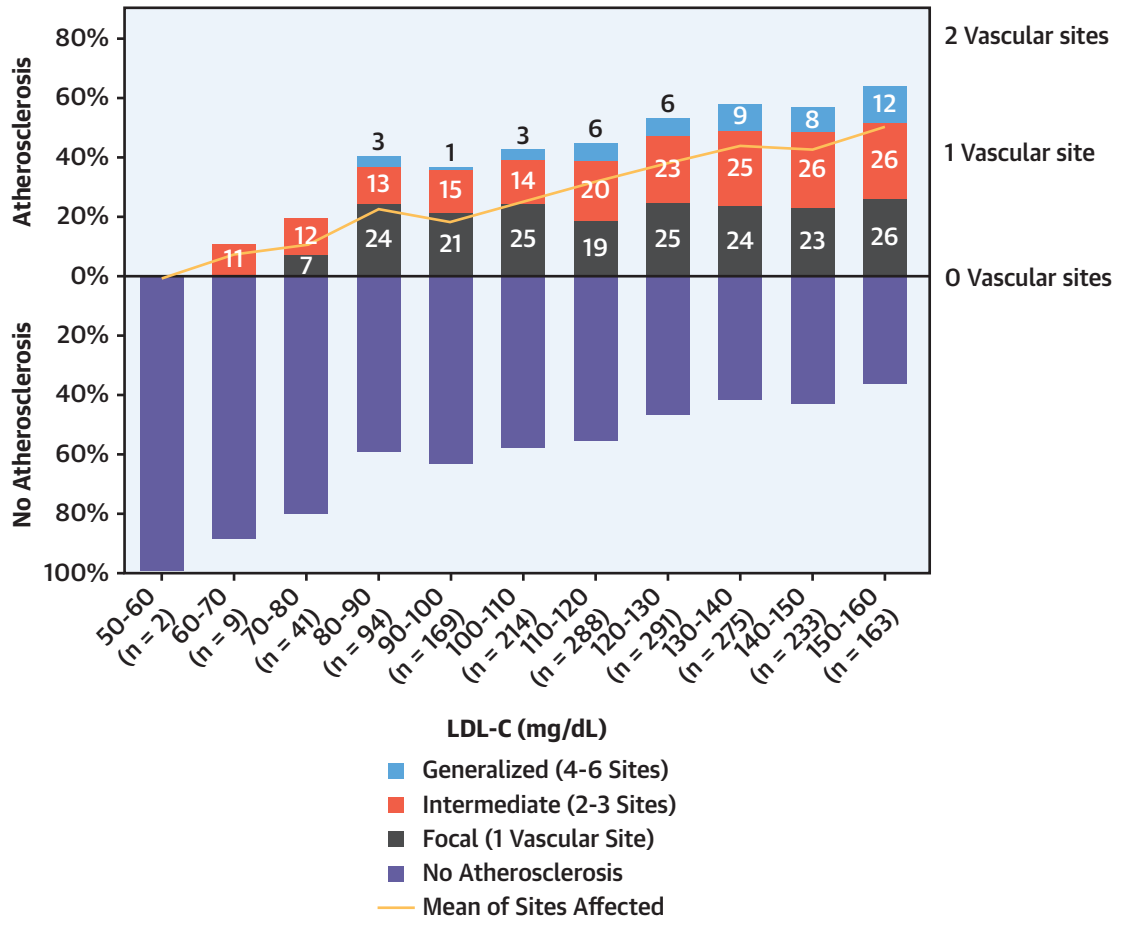
short telomeres. In this substudy, the possible association between LTL and subclinical atherosclerosis was assessed at baseline in a subgroup of ≈1,500 PESA participants (58% men; age 40 to 54 years) (57). As expected, age correlated inversely with LTL and directly with critically short telomere length. However, neither mean LTL nor critically short telomere load showed an association with subclinical atherosclerosis (57). Ongoing studies in the PESA cohort will assess whether the rate of telomere attrition is an independent determinant of subclinical atherosclerosis progression during aging (**Figure 6**).

LIFESTYLE FACTORS AND SUBCLINICAL ATHEROSCLEROSIS. Atherosclerosis is driven by a complex interaction among genetic, environmental, and lifestyle factors. In a recent analysis of data from 4 studies (≈55,000 participants), a healthier lifestyle was associated with a 46% lower relative risk of coronary events among participants with a high genetic risk of coronary artery disease (58).

Among lifestyle factors, diet has been recognized as one of the main influences on CV risk. In the PESA cohort, a cross-sectional analysis of the baseline data has identified 2 dietary patterns associated with an increased prevalence of subclinical atherosclerosis. The first dietary pattern is skipping breakfast, which had already been linked to increased rates of coronary heart disease and stroke in prior observational studies (59,60). In the PESA cohort, regularly skipping breakfast was associated with a 2.6-fold higher odds of generalized atherosclerosis, independently of the presence of conventional CVRFs (**Figure 7**) (61). Consistent with this observation, a subsequent analysis of a large prospective US cohort (62) concluded that skipping breakfast was independently associated with a higher risk of CV death, suggesting that a high rate of subclinical atherosclerosis, as observed in the PESA cohort, may explain the higher incidence of CV events later in life (63). Evidence from recent experimental and early clinical studies has shown a beneficial effect of intermittent fasting on aging and CV health (64). A future challenge will be to investigate whether these strategies can be reconciled (combining an energy-rich breakfast with fasting periods) to produce additional health effects.

The second dietary pattern studied in PESA is a "social-business eating pattern." Described for the first time in this cohort, this pattern is characterized

FIGURE 5 Relation Between LDL-C Levels and Subclinical Atherosclerosis

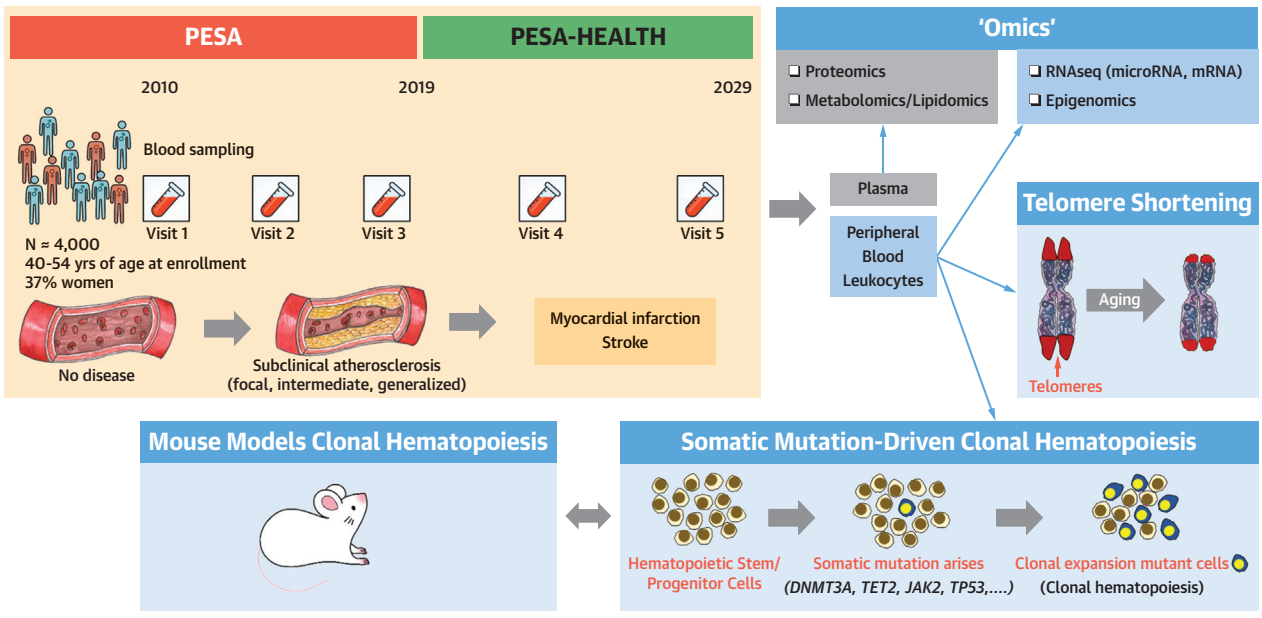


Increases in plasma LDL-C concentration are accompanied by a linear and significant increase in the prevalence of atherosclerosis, ranging from 11% in the 60-70 mg/dL category to 64% in the 150-160 mg/dL subgroup ($P < 0.001$). A similar pattern is observed for the multiterritorial extent of atherosclerosis (focal, intermediate, or generalized disease) as well as for the mean number of vascular sites affected **(yellow line)**. Reprinted with permission from Fernandez-Friera et al. (34). LDL-C = low-density lipoprotein cholesterol.

by a high consumption of red and processed meat, pre-prepared meals, appetizers, snacks, and alcoholic and sugar-sweetened beverages, together with frequent eating out (65). Compared with PESA participants following a Mediterranean diet (40% of the cohort), those following the social-business eating pattern (19%) had a 1.3-fold higher prevalence of subclinical atherosclerosis after adjustment for CVRFs. Moreover, the PESA subgroup following the social-business eating pattern had the highest proportion of individuals with intermediate atherosclerosis (2 or 3 vascular territories affected) or generalized atherosclerosis (≥4 territories) (31% and 37%, respectively). In contrast, most individuals with a Mediterranean dietary pattern were free of disease.

In another important contribution to understanding how lifestyle affects CV risk, the PESA study has provided new insights into the influence of sleep duration and quality on subclinical atherosclerosis (66). Compared with PESA participants sleeping 7 to 8 h/night, those sleeping <6 h/night had an adjusted OR of 1.3 for being in the highest plaque burden tertile in the baseline VUS study (**Figure 7**). This analysis also showed an association between plaque burden and sleep fragmentation. However, the baseline data showed no association between sleep characteristics and CACS. This discrepancy might reflect the different disease stages represented by atherosclerotic plaques and CAC, suggesting that sleep disturbances might be associated with early atherosclerosis;

FIGURE 6 Discovery of Novel Risk Factors in PESA: From Omics to Telomeres



Ongoing omics studies in PESA include high-throughput RNAseq (microRNA and mRNA profiling), proteomics, metabolomics, and epigenomics. PESA-HEALTH will also assess whether the rate of telomere attrition is an independent determinant of subclinical atherosclerosis progression during aging and will investigate the role of clonal hematopoiesis as a driver of atherosclerosis. Research in mouse models of somatic mutation-driven clonal hematopoiesis tailored to the human scenario will identify mechanisms that determine whether mutant hematopoietic clones remain indolent or expand to dominate hematopoiesis, and how somatic mutation-driven CH can promote atherosclerosis. CH = clonal hematopoiesis; PESA = Progression of Early Subclinical Atherosclerosis.

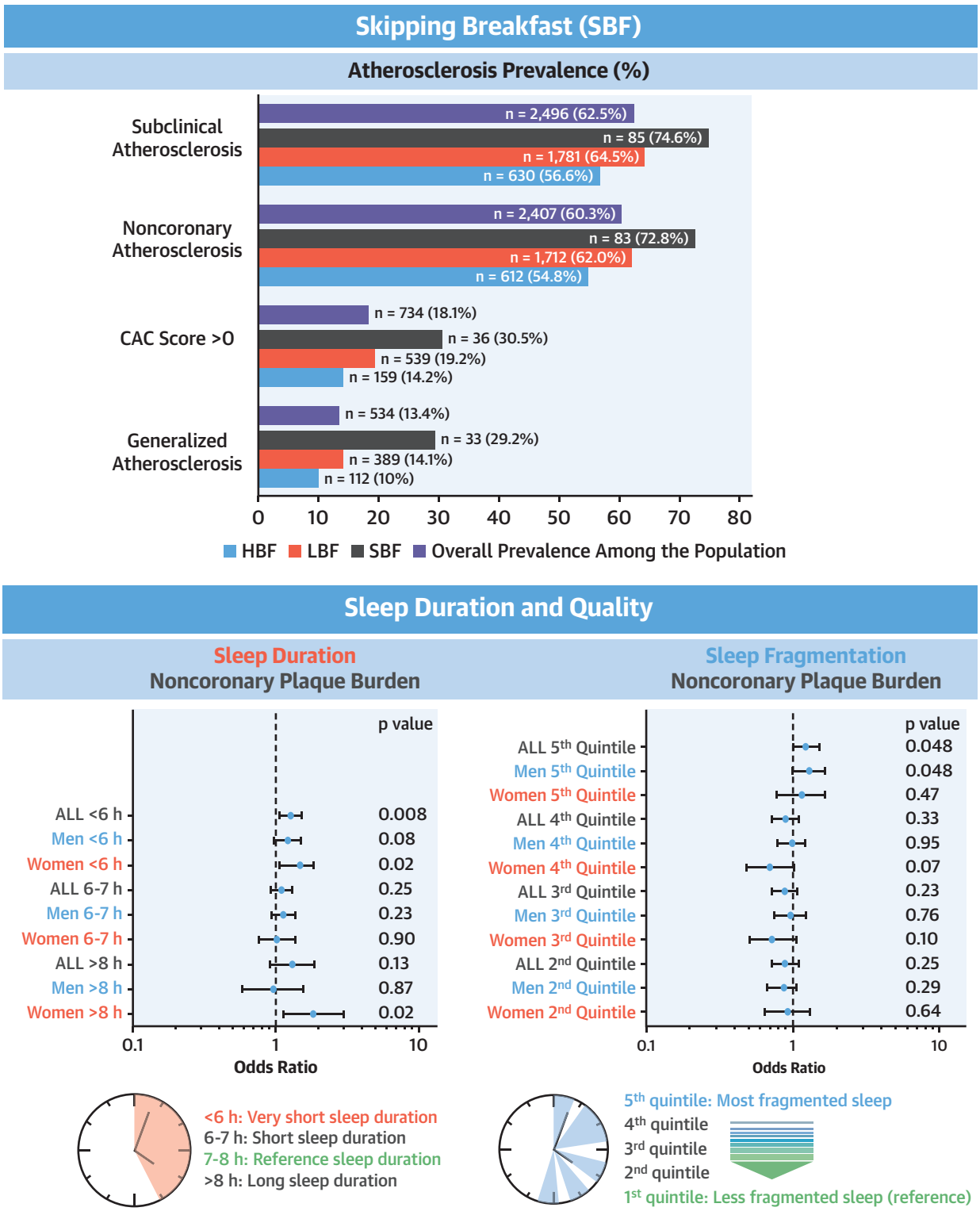
however, the mechanism underlying the link between sleep behavior and atherosclerosis remains unknown. Prior observational studies had already reported an association of both short and long sleep duration with CVRFs, death, and stroke (67,68). One of the strengths of PESA is the objective measurement of sleep duration and sleep fragmentation (with accelerometry), whereas most studies have used self-reported sleep duration (assessed through questionnaires). From a mechanistic point of view, in small-animal models, circadian rhythm disruption or misalignment has been found to affect circulating leukocytes and lipids (both drivers of systemic atherosclerosis) and also to influence endothelial, smooth muscle cell, and macrophage activation within the vessel wall (69). Thus, the alteration of circadian rhythmicity is a potential mechanism explaining the association between short or fragmented sleep and atherosclerosis (and this may also apply to breakfast skipping).

Further studies are needed to determine whether sleep behavior modifications can alter CVD outcomes, perhaps providing an indication that recommendations for healthy sleep habits should be incorporated into CVD prevention programs. Longitudinal sleep evaluation in the PESA and PESA-HEALTH visits will provide valuable information about the links between sleep patterns, atherosclerosis progression and incident CV events.

As well as continuing the objective evaluation of sleep patterns, PESA-HEALTH will assess participants for obstructive sleep apnea (OSA). OSA is a common sleep disorder that has been classically associated with CVRFs, but recent research has questioned its relationship to incident CVD and the effectiveness of continuous airway positive pressure therapy on CV outcomes (70,71). In a comprehensive sleep analysis, PESA-HEALTH participants will be studied by cardiorespiratory polysomnography to clarify the longitudinal associations among OSA patterns, CVRFs, lifestyle habits, brain neuroimaging (in the PESA-brain subcohort), and incident CV events.

The most recent contribution of PESA to understanding the influence of lifestyle factors addressed the impact on atherosclerosis of socioeconomic status (72). Socioeconomic status combines education, income level, and occupation, and previous studies have reported contradictory effects on CVD in different countries. The PESA data show no association between income level and subclinical

FIGURE 7 **Lifestyle Patterns Associated With Subclinical Atherosclerosis: Diet and Sleep**



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atherosclerosis, likely because the PESA cohort is a homogeneous group of bank workers with a relatively high income. However, despite this favorable economic situation, PESA participants with a lower education level were at higher risk of prevalent generalized atherosclerosis in the baseline analysis (OR: 1.5). A mediation model revealed that the main mediator of this association was tobacco smoking.

One inherent limitation to all the aforementioned PESA analyses addressing the association between lifestyle factors and subclinical atherosclerosis is the cross-sectional nature of the examinations that allows evaluation for association but not causality. Indeed, PESA subjects at the highest risk for subclinical atherosclerosis (ie, breakfast skippers, social-business eaters, or short sleepers) also had a worse CV risk profile at baseline. Despite statistical efforts for full baseline covariate adjustment, residual confounders may still be present. This is also a common limitation of most of the existing published reports on this field. Lifestyle factors are powerful risk-modifiers that interact with an individual's genetic predisposition for CVD (58). Either as predictors or as markers of subclinical atherosclerosis presence, the clusters of behaviors identified in the PESA cohort provide valuable information to identify an at-risk population and may help to improve CV risk stratification and tailor prevention strategies in the future.

FUSTER-BEWAT SCORE PREDICTS ATHEROSCLEROSIS PRESENCE IN LOW-RISK INDIVIDUALS. There is a growing interest in devising simple equations that assess CV health rather than CV risk, especially for primary prevention. In line with this idea, the American Heart Association has proposed a shift from the historical focus on reducing CVD prevalence to a national goal to improve CV health. This shift is reflected in the recommendation for primary prevention strategies to incorporate health metrics, such as the ideal cardiovascular health score (ICHS) (73). The ICHS includes several lifestyle factors (smoking, body weight, physical activity, and diet) and 3 established CVRFs (blood cholesterol, blood glucose, and blood pressure). Most studies assessing the association between ICHS metrics and subclinical atherosclerosis have used CACS as the disease marker. However, the PESA study data clearly show that the absence of CAC does not indicate that an individual is free of atherosclerotic disease (15).

The PESA study has made an important contribution in this area by showing a strong inverse relationship between the ICHS and the prevalence of multiterritorial disease in a large cohort of healthy individuals (74). Moreover, PESA obtained comparable predictive value using the Fuster-BEWAT score (FBS), which includes blood pressure (B), exercise (E), weight (W), alimentation (A), and tobacco smoking (T); this tool is simpler than the ICHS as it requires no laboratory tests (75). Compared with individuals with poor ICHS and FBS values, PESA participants with ideal values on these scales had a lower adjusted OR of having atherosclerotic plaques and CAC. Moreover, the ICHS and FBS showed similar levels of significantly discriminating accuracy for the prediction of plaque presence (C-statistic: 0.694) and CACS ≥1 (C-statistic: 0.782).

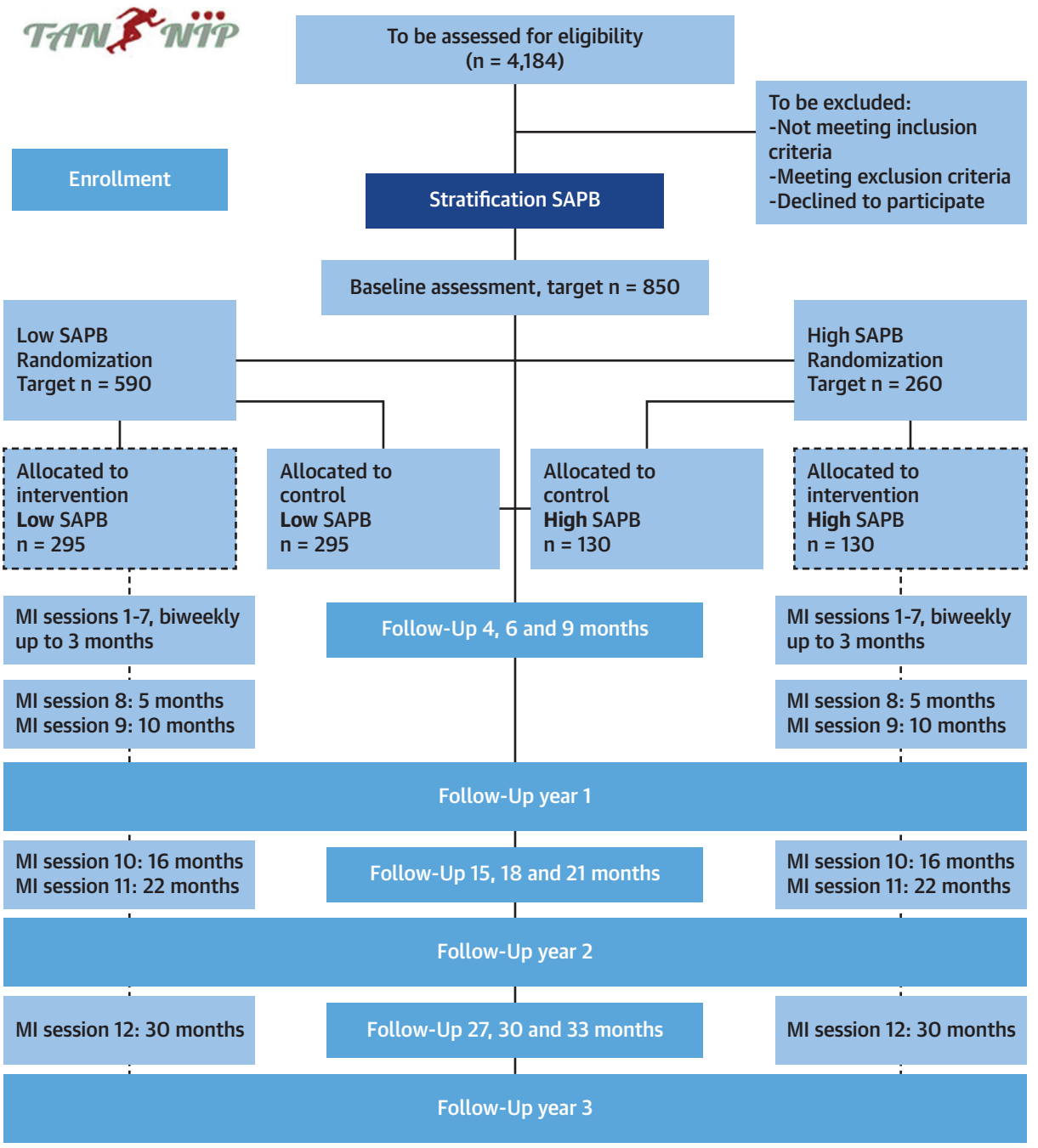
These PESA findings provide good evidence that lifestyle and risk factors affect the early asymptomatic phases of atherosclerosis. Better CV health behavior and risk factor profiles, indexed by higher ICHS and FBS values, are strongly associated with a lower prevalence and a lesser extent of subclinical atherosclerosis in middle-aged healthy individuals. A highly practical message from these data is that the FBS is an easy, pain-free, inexpensive tool that could be implemented in resource-constrained health care settings to identify individuals with a high likelihood of subclinical atherosclerosis who would benefit from focused preventive management strategies. One limitation of this approach is that it still needs to be proven that for a given level of CV risk, subjects with presence of subclinical atherosclerosis detected by imaging are at higher risk of later clinical events. The ongoing PESA-H follow-up will provide very valuable information on incident clinical CV events in this cohort.

The FBS is being used as the primary outcome measure in a randomized controlled clinical trial nested within the PESA cohort (TANSNIP-PESA

FIGURE 7 Continued

(Left) Skipping breakfast (SBF): atherosclerosis prevalence is presented for the total population and stratified by breakfast habits. The SBF group has the highest proportions of individuals with subclinical, noncoronary, and generalized atherosclerosis, as well as CACS ≥1. **(Right)** Sleep duration and quality: Forest plots showing the ORs and 95% CIs of total plaque burden measured by 3-dimensional ultrasound (carotid and femoral territories) and affected territories in the different groups according to actigraphic sleep duration and fragmentation compared with the reference group (7-8 hours of sleep, quintile 1). Adapted with permission from Uzhova et al. (61) and Dominguez et al. (66). CACS = coronary artery calcium score; HBF = high-energy breakfast; LBF = low-energy breakfast; PESA = Progression of Early Subclinical Atherosclerosis.

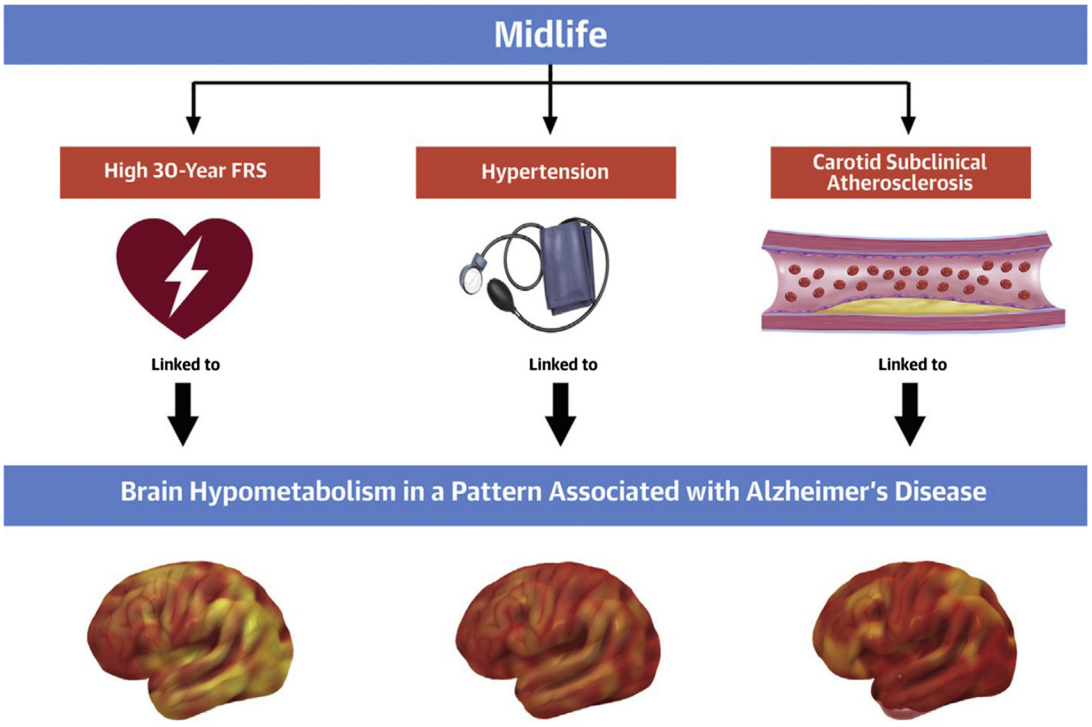
FIGURE 8 **TANSNIP-PESA Randomized Clinical Trial: Lifestyle Intervention Versus Standard of Care**



Study design of the TANSNIP (Trans-Atlantic Network to study Stepwise Non-invasive Imaging as a Tool for Cardiovascular Prognosis and Prevention) randomized clinical trial nested within the PESA cohort. Reprinted with permission from Coffeng et al. (76). SAPB = subclinical atherosclerotic plaque burden.

[Trans-Atlantic Network to study Stepwise Non-invasive Imaging as a tool for cardiovascular prognosis and Prevention-PESA]; NCT02561065). Launched in 2015, TANSNIP-PESA investigates whether a 30-month worksite-based lifestyle intervention can improve CV health metrics and studies if there is a differential effect by baseline subclinical atherosclerosis plaque burden (76). TANSNIP-PESA

FIGURE 9 **Cardiovascular Risk Factors and Subclinical Atherosclerosis Are Associated With Brain Hypometabolism in Middle-Aged Apparently Healthy Individuals**



PESA (Progression of Early Subclinical Atherosclerosis) participants with evidence of subclinical atherosclerosis underwent ^{18}F-fluorodeoxyglucose (FDG)-positron emission tomography (mean age 50 years, 82% men). Cerebral FDG uptake showed an inverse correlation with 30-year Framingham Risk Score (FRS), with the presence of hypertension, and with carotid plaque burden, even after adjusting for 30-year FRS. The brain areas most strongly affected by hypometabolism were dementia-relevant brain regions, especially of the Alzheimer's disease type, such as parietotemporal areas (angular, supramarginal, and temporal gyri) and the cingulate gyrus. The **color** in the tridimensional brain images represents the actual magnitude of the association, with **yellow** areas indicating the lowest p values. Reprinted with permission from Cortes-Canteli et al. (88).

randomized 1,020 PESA participants 1:1 to receive a comprehensive workplace-delivered intervention program or standard of care (Figure 8). The 3 program objectives were to increase daily physical activity, reduce sedentary time, and promote a healthier (Mediterranean) diet. The intervention consisted of 12 individual motivational interviews with a psychologist over a 30-month period, wearing a wrist-worn physical activity tracker, and the use of a sit-stand workstation. The primary outcome measure is an adapted version of the FBS, measured at enrollment and at annual follow-up over 3 years (Figure 8). Follow-up was completed early in 2020, and the primary outcome results will be reported soon.

MACHINE LEARNING IMPROVES CV RISK DEFINITION IN YOUNG, ASYMPTOMATIC INDIVIDUALS. The deep phenotyping of the PESA participants and the longitudinal design of the study continues to generate vast amounts of data that are being exploited to extract the parameters that best define and predict the extent and progression of subclinical atherosclerosis. Machine learning (ML) approaches analyze massive data sets to build complex models and have recently been applied to imaging analysis and language processing (77). However, data need to be accurately collected and curated, and this hurdle has hindered faster and more extensive uptake of ML in medicine. Despite this limitation, ML has been shown to improve the prediction of CV events (78–80).

In PESA, we used an unbiased, data-driven approach to develop an Elastic Net (EN) model (EN-PESA) that predicts the extent and 3-year progression of subclinical atherosclerosis from quantitative variables easily obtained from routine tests (81). A

simplified EN-PESA model based in 12 variables (age, hemoglobin A1C, total hemoglobin, the ratio of total cholesterol to high-density lipoprotein cholesterol, total low-density lipoprotein cholesterol, leukocyte count, isoprostane/creatinine ratio, systolic blood pressure, urinary urate, phosphorus levels, and intake of vitamin B12 and ethanol) (82) performed very well in terms of discriminative ability and calibration, with a c-statistic of 0.88, and was validated in the AWHS cohort (13), where participants underwent multiterritorial noninvasive imaging of subclinical atherosclerosis (81). More than 86% of the individuals identified by EN-PESA as at increased risk showed evidence of subclinical atherosclerosis at baseline or significant progression over 3 years. Unlike traditional risk scores, EN-PESA does not need to categorize predictors, instead using the whole range of values, thus providing a much more personalized assessment of CV risk.

Despite its good performance, the drawback of the EN-PESA score is that some of the 12 quantitative variables that are included in the model might be difficult to obtain in clinical practice (eg, phosphorus levels and intake of vitamin B12 and ethanol). However, based on the parsimony shown by our model, average population-based values can be used for nonroutine variables if their value is unknown for a given individual, with little loss of predictive power (77). By incorporating in the algorithm new longitudinal data that are being continuously generated in the PESA study, the EN-PESA score will be refined in the near future to be even more accurate in CV risk prediction and more applicable in routine clinical practice. With these improvements and upon validation in additional cohorts, EN-PESA could be included in clinical guidelines as a way to personalize CV risk, enabling more tailored treatments and follow-up plans, especially for individuals with an inconclusive risk score according to traditional scales.

BRAIN HYPOMETABOLISM IS ASSOCIATED WITH ATHEROSCLEROSIS IN MIDLIFE. Potential impact on cognitive dysfunction later in life. Dementia is among the top causes of disability and death worldwide and is one of the global challenges of the century (83). The presence of atherosclerosis has been linked to cognitive impairment at advanced stages (84) and to higher odds of Alzheimer's disease in the very old (85). Dementia and atherosclerosis both have a long asymptomatic phase and share risk factors such as hypertension, diabetes, cholesterol, sedentary lifestyle, and smoking (86). However, little is known about how atherosclerosis and cognitive decline influence each other or whether they share a common trajectory during their long asymptomatic stages (87). Understanding the extent to which CVRFs influence brain performance early in the course of atherosclerosis (during midlife) may help to refine preventive strategies and reduce the incidence of dementia later in life.

In the advanced imaging PESA subcohort, the baseline and 6-year follow-up vascular ^{18}FDG PET/MR analysis includes the brain in the carotid PET scan. We have taken advantage of these images to assess associations between brain metabolism and atherosclerosis and its risk factors. Brain hypometabolism (low ^{18}FDG uptake) is an indicator of cerebrovascular deficiency and is a feature of the early stages of neurodegeneration (88). PESA offers a unique and untried possibility to study these associations in middle-aged asymptomatic individuals.

Very recent data from PESA show that the CV risk profile of middle-aged asymptomatic individuals is associated with global brain hypometabolism, and this association is mainly driven by the presence of hypertension. We also observed an association of carotid atherosclerotic plaque burden with global brain hypometabolism even after adjustment for CV risk. Interestingly, the hypometabolic brain regions associated with subclinical atherosclerosis include areas known to be affected in dementia (89) (**Figure 9**). This is the first study to show an association between hypometabolism in dementia-relevant brain regions and CVD risk and atherosclerosis in a cohort of young asymptomatic individuals, long before clinical symptoms are present. These data are of the highest relevance, because, by challenging the prevailing notion that cognitive decline is a late effect of long-term CVRF exposure, they suggest that early intervention strategies could help to prevent dementia. We hypothesize that midlife CVRFs and subclinical atherosclerosis result in a more vulnerable and less resilient brain, unable to cope with the pathological burden associated with Alzheimer's disease later in life.

LEFT VENTRICULAR NONCOMPACTION PHENOTYPE IS ASSOCIATED WITH VIGOROUS PHYSICAL ACTIVITY IN NONATHLETE SUBJECTS. The design of PESA includes longitudinal phenotyping of the myocardium by serial CMR studies (**Figure 1**). One recent report from PESA deals with the prevalence of left ventricular (LV) hypertrabeculation and its association with physical activity in the general population. Left ventricular noncompaction (LVNC) cardiomyopathy is a condition with a genetic basis characterized by

extensive LV trabeculation and associated with an increased risk of heart failure, thromboembolic events, and ventricular arrhythmia (90). The diagnosis of LVNC cardiomyopathy is based on imaging, but current criteria are nonspecific, and there is a risk for over diagnosis. Certain physiological conditions, such as pregnancy or vigorous training in athletes, have been associated with a high prevalence of LVNC imaging criteria (91). However, there is an ongoing controversy whether this may also occur in a general (nonathletic) population. In PESA, we studied the LVNC phenotype using 4 widely available CMR criteria and its relationship with vigorous physical activity (VPA) measured by serial accelerometry (average of 2 7-day measurements, 3 years apart) (92). We found that in those participants at the highest quintile of VPA, ie, performing around 2 h of VPA/week, the prevalence of a LVNC phenotype (based on conventional CMR criteria) was doubled compared with those subjects with no or Q1-Q4 VPA (OR [95% CI] for Petersen criteria: 2.29 [1.38-3.80]). Moreover, we showed for the first time that increased trabeculation is an independent phenomenon from LV dilatation. Our global LVNC prevalence (17%) in a general middle-aged population is similar to the values found previously in the TASCFORCE (The tayside screening for the prevention of cardiac events) study and lower than that reported in MESA, but these studies did not specifically address the relationship of an LVNC pattern with physical activity. Conversely, in the UK Biobank study (93), no association was found between the extremes of physical activity and the extent of LV trabeculation measured by CMR. The differences with PESA results may be explained because subjects included in the UK Biobank study were older and had a more heterogeneous physical activity profile, probably making them less prone to hypertrabeculation in response to vigorous exercise than PESA participants. Our results support the argument that LVNC is a morphological expression of different underlying diseases, ranging from a real myocardial disorder to an epiphenomenon of a pathological pressure/volume load or a physiological response to loading conditions (94). According to our findings, the amount of VPA should be included as a significant parameter when evaluating patients with suspected LVNC. The scheduled very long-term follow-up along PESA-H will be key to analyze the clinical and imaging longitudinal evolution of LVNC phenotype as well as its potential reversibility with decreasing training intensity.

PESA IN THE INTERNATIONAL CONTEXT: MAJOR COLLABORATIONS

PESA is the result of a very active and fruitful collaboration between the CNIC and Santander Bank. As part of its strong commitment to corporate social responsibility, Santander Bank is deeply invested in contributing to improved health of society in general and of its workers in particular. The PESA study also enjoys the full support of Santander bank employees, who rank it as one of the institution's best activities.

PESA actively encourages its investigators to take part in research collaborations and provides support at all stages of the necessary interactions. As part of this collaborative approach, PESA is linked to the TANSNIP Project through partnerships between the CNIC and the Icahn School of Medicine at Mount Sinai, the Framingham Heart Study, and the VU (Free University) Medical Center in Amsterdam. PESA has also collaborated fruitfully with the FHS to allow the use of both cohorts for cross-validations and with UT Southwestern Medical Center in the study of reverse cholesterol transport and subclinical atherosclerosis. At a national level, the CNIC collaborates with the IMIM Center in Barcelona in the CORDELIA initiative (Collaborative cOhorts Reassembled Data to study mEchansims and Longterm Incidence of cArdiovascular diseases). CORDELIA will gather data from PESA and other major Spanish cohorts (≈170,000 participants in total) to study the population-level association among biochemical, genetic, and epigenetic parameters; noninvasive imaging; lifestyle factors; and incident CVD. PESA also collaborates with the Barcelonaβeta (Brain Research Center)/Pasqual Maragall Foundation in the PESA-brain study.

PESA will also contribute to the iMAP Project (95), one of the finalists competing for a single €35 million award with the British Heart Foundation's Big Beat Challenge program for CVD research. The iMAP project is led by Ziad Mallat at the University of Cambridge, United Kingdom, and the CNIC, through the PESA study, is a prominent consortium member. The aim of iMAP is to construct a 3D map of human atherosclerosis and to determine how and why the immune system malfunctions to cause the disease.

PESA continues to invite new internal and external partners from across the scientific community to participate in this unique endeavor. The PESA scientific committee accepts proposals for data exploitation from external researchers that meet high scientific standards and do not overlap with ongoing studies.

PESA-HEALTH: FUTURE PESA RESEARCH

In 2019, the CNIC and Santander Bank signed an agreement to extend PESA for a further 10 years. The 2019-2029 extension, called PESA-HEALTH, will continue the exploitation of the data gathered and will keep collecting clinical events in this population, which in this period will enter an age frame in which adverse CV (and non-CV) events increase in frequency. The highest scientific value of PESA lies in the possibility to retrospectively evaluate imaging and biological data gathered over 20 years. This will allow the trajectories of these parameters to be mapped and will place them in the perspective of incident CV events over a very long follow-up. PESA-HEALTH participants will undergo 2 additional visits 5 years apart (visits 4 and 5) (**Figure 1**). On these visits, blood samples will be collected, and the same "basic" imaging modalities will be used, with screening for atherosclerosis in the carotids, iliofemorals, and aorta by 2D and 3DVUS and in the coronary arteries by noncontrast coronary CT scan to quantify calcium. Additionally, the entire cohort will undergo a comprehensive sleep evaluation by cardiorespiratory polysomnography.

In the advanced imaging subcohort, participants with completed vascular ^{18}FDG PET examinations on visits 1 and 3 will have an additional ^{18}FDG PET examination on visit 5 (14 years after first vascular PET examination). In addition, participants who underwent CMR on visit 2 will have a follow-up CMR examination on visit 4 (6 years after the first scan), including a pharmacological test to evaluate myocardial perfusion.

PESA-HEALTH will build on findings from the 10 years of the PESA study and other scientific discoveries in the field to open new avenues of research. Brain imaging will be expanded to gain further insight into the association between atherosclerosis and cognitive function. Related to this effort, the entire PESA cohort has been genotyped for apolipoprotein E to identify participants who are at high genetic risk of developing Alzheimer's disease (carriers of the E4 allele) or at very low risk (E2 carriers). Based on this genetic information, 1,000 participants at low, intermediate, or high Alzheimer's disease risk will undergo a brain PET-amyloid scan and comprehensive brain magnetic resonance imaging (including advanced procedures such as arterial spin labeling, functional connectivity assessment, and anatomical tract imaging). These participants will also undergo highly sensitive cognitive tests. In addition, because the PESA participants are now entering the age range in which clinical events are more frequent, a participant subset will undergo more detailed coronary imaging, including contrast CT angiography, as well as ^{18}F-NaF-PET cardiac imaging to identify active calcification in the coronary arteries and valves.

The deep phenotyping of the PESA cohort will also be exploited to explore the role of clonal hematopoiesis (CH) as a driver of atherosclerosis and its interplay with CV risk factors. Human aging is associated with the accumulation of somatic DNA mutations, a random process starting during embryonic development that has been suggested to give raise to trillions of genetically distinct somatic cells in healthy adults (96). In CH, a single founding hematopoietic cell acquires a mutation and establishes a subpopulation of cells sharing the same mutation. However, the factors determining whether a mutant hematopoietic clone remains stable or expands remain incompletely characterized. Likewise, while human and mouse studies suggest that somatic mutation-driven CH may play an important role in atherosclerosis and its association with aging (97,98), the mechanisms driving age-related transition from CH to atherosclerosis remain ill-defined. A causal link has been established between somatic mutations in the *TET2* gene and experimental atherosclerosis, and human and animal studies suggest that this connection is mainly driven through overactivation of the pro-inflammatory NLRP3/interleukin-1β/interleukin-6 pathway (97-100). Sequencing studies in the context of clinical trials with drugs that target this pathway (such as the use of canakinumab in the CANTOS trial) (30) are warranted to examine whether targeting these pro-inflammatory mediators may be of particular preventive/therapeutic interest in *TET2* mutation carriers. Further studies are also needed to unveil factors controlling the clonal selection/expansion of mutant cells and to assess whether the association between CVD and other CH-causing somatic mutations could simply reflect shared, but independent, consequences of aging, or be secondary to confounding factors. Taking advantage of the longitudinal nature of PESA-HEALTH, we will address some of these issues by analyzing the presence and progression of CH and atherosclerosis (**Figures 1 and 6**). These human studies will be combined with research in mouse models of somatic mutation-driven CH to unveil cell-intrinsic and extrinsic factors that determine whether mutant hematopoietic clones remain indolent or expand to dominate hematopoiesis, and how CH can modulate atherosclerosis development and its manifestations.

PESA-HEALTH will also investigate the cues leading to supervulnerability to or superprotection against atherosclerosis. Supervulnerability describes

participants without traditional CVRFs but with evidence of atherosclerosis, whereas superprotection refers to those with a high CVRF load but no evidence of atherosclerosis. These subgroups have already been identified in the PESA cohort (15) and are the focus of intense research.

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