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A *Grim* link: the association between subclinical atherosclerosis and epigenetic age

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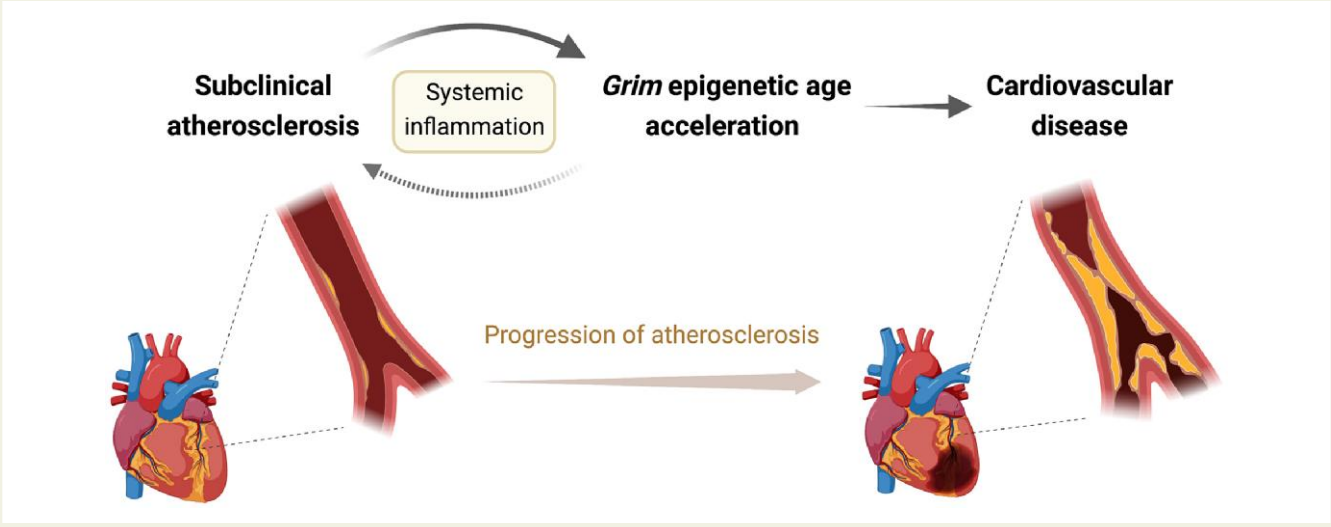
This editorial refers to 'Subclinical atherosclerosis and accelerated epigenetic age mediated by inflammation: a multi-omics study', by F. Sanchez-Cabo *et al.*, https://doi.org/10.1093/eurheartj/ehad361.

Graphical Abstract



A potential mediatory role for systemic inflammation in the association between subclinical atherosclerosis and *Grim* epigenetic age acceleration, identified by multi-omics analyses utilizing participants in the Progression of Early Subclinical Atherosclerosis (PESA) study.

Ageing and cardiovascular disease

Atherosclerosis and many other cardiovascular diseases are very age dependent, with chronological age a major risk factor for cardiac disease.[1,2] Atherosclerotic plaques can accumulate in otherwise healthy individuals with age, remaining clinically silent for years as subclinical atherosclerosis (SA).[3] The highest prevalence of SA is in middle-aged individuals. Understanding the interplay of ageing and pathogenesis of SA and uncovering early biomarkers of SA are important, as clinical manifestation of initial symptoms can be lethal.

Biological ageing seems to progress at different rates amongst individuals with the same chronological age, with significant variation in physiological and functional hallmarks of ageing.[4] Epigenetic parameters such as DNA methylation patterns vary significantly between individuals of the same chronological age and are demonstrably linked to human ageing.[4,5] Rigorous study of the dynamic landscape of epigenetic changes has established 'epigenetic clocks', which can provide biological vs. chronological age of an individual, and thus a prediction of health and life span. Further, epigenetic age acceleration (EAA) defines the difference between epigenetic age and chronological age, and is strongly associated with many variables of health, disease, and ageing in humans.[5] The emerging association between cardiovascular diseases and EAA highlights the potential usefulness of EAA as a biomarker of cardiovascular disease.[6]

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Association between *Grim* epigenetic age acceleration and subclinical atherosclerosis

The study by Sanchez-Cabo *et al*. in the current issue of the *European Heart Journal* establishes a strong association between SA and EAA, and describes a potential role for proinflammatory mechanisms in this association.[7] The authors utilized a subset of participants of the Progression of Early Subclinical Atherosclerosis (PESA) study, which established the prevalence of SA in middle-aged individuals.[3,8] Participants were scored on the extent of SA based on the presence of plaques in key territories (aorta, carotid, and ilio-femoral arteries) as well as the Coronary Artery Calcification (CAC) score. Whole blood methylomics, transcriptomics, and plasma proteomics were obtained from 391 asymptomatic participants to estimate EAA.

Sanchez-Cabo *et al*. report an association between *Grim* EAA and the presence, extension, and progression of SA.[7] The authors calculated epigenetic age based on four different epigenetic clocks: two predictors of life span, *Grim* and *Pheno*; and two predictors of chronological age known as Horvath's and Hannum's clocks. The presence of SA and extension of SA were both found to be associated with predictors of life span such as *Grim* EAA and *Pheno* EAA, independent of traditional cardiovascular risk factors, by 2D/3D vascular ultrasound. By computed tomography measurements, however, only *Grim* EAA was associated with a positive CAC score and its extension, independent of traditional cardiovascular risk factors. Further, by three different imaging techniques, predictors of chronological age such as Horvath's and Hannum's clocks were not significantly associated with the presence or extension of SA. The authors thus show that *Grim* EAA occurs with an increase in SA, independent of traditional cardiovascular risk factors. To confirm these findings, the authors also conducted an analysis that categorized participants based on *Grim* EAA, to estimate SA progression and cardiovascular risk among these categories. By this analysis, the authors further show a trend for increasing SA burden with *Grim* EAA. Remarkably, the association of *Grim* EAA with the progression of CAC remained significant even after adjustment for cardiovascular risk factors, including smoking. This is despite individuals with *Grim* EAA having a higher prevalence of cardiovascular risk factors than other groups, and also exhibiting increased cardiovascular age. The association between SA and *Grim* EAA was further validated using publicly available CD14+ methylomics data from a multiethnic study of atherosclerosis.[9]

Proinflammatory pathways mediate epigenetic age acceleration

Sanchez-Cabo *et al*. report a mediatory role for proinflammatory pathways in association between *Grim* EAA and SA.[7] By RNA-seq analysis, the top canonical pathways associated with EAA were immune response pathways, including Th1/2 activation pathways, the STAT3 pathway, interleukin (IL)-10 signalling, and Toll-like receptor signalling. Key biomarkers for increased systemic inflammation, including an increase in total number of platelets and white blood cells, plasma C-reactive protein level, and neutrophil to lymphocyte ratio, were found to be strongly correlated with EAA. The INFLA-Score, a measure of chronic low-grade inflammation, was also significantly associated with *Grim* EAA.[10] Further, the INFLA-Score was found to partially mediate the effect of SA on EAA, but not of EAA on SA. The role of inflammation as a critical mediator of all phases of atherosclerosis is well established.[11] The results of Sanchez-Cabo *et al*. expand upon this, and suggest an effect of SA and chronic inflammation on overall reduced health and life span of individuals.[7]

Inflammation in subclinical atherosclerosis and cardiovascular disease risk

Early detection at the subclinical stages of atherosclerosis is a critical goal.[3] Further, while the link between initiation of SA and inflammation is known,[12] an exploration of the direct impact of SA on health and life span of individuals, as well as the mediatory mechanisms underlying it, were not established. The study by Sanchez-Cabo *et al*. addresses these gaps in knowledge by identifying the robust association between *Grim* EAA and SA.[7] They also describe a mediatory role for systemic inflammation in the effect of SA on *Grim* EAA. Thus, they show a detrimental impact of SA on human health and life span, alongside increased cardiovascular age and increased *Grim* EAA both being associated with SA (*Graphical Abstract*).

Understanding these associations is valuable to establish effective biomarkers for identifying early atherosclerosis, as well as developing therapeutic approaches for preventing progression of atherosclerosis beyond the subclinical stages. The authors highlight the potential for attenuating systemic inflammation to reduce the detrimental effect of SA on epigenetic age. By mediation analysis of omics data, they identified proinflammatory molecules such as *IL1B*, *CLEC10A*, and *OSM* which mediate key mechanisms such as *IL10* signalling and inflammasome pathways. *OSM* was recently reported to have a role in progression of atherosclerosis as well as survival probability in humans.[13] The CANTOS study also highlighted the importance of targeting proinflammatory IL-1β signalling with canakinumab to reduce atherosclerotic disease and cardiovascular risk, although a recent study found no reduction in plaque burden with a canakinumab treatment strategy in peripheral artery disease.[14,15]

This study by Sanchez-Cabo *et al*. certainly does not exclude other factors beyond known inflammatory pathways in the presence and progression of SA. Also, while the authors identified a strong association between SA and *Grim* EAA, a causal effect cannot be established due to the study design. Further longitudinal studies designed to assess effect and directionality of SA on health and life span, as well as the viability of modulating inflammatory signalling to prevent progression of SA, are needed. Nevertheless, the findings of Sanchez-Cabo *et al*. strengthen the role of inflammatory mechanisms in SA and its impact on EAA. The study also provides more mechanistic evidence for strategies aiming at attenuating systemic inflammation for the delay of atherosclerosis progression in cardiovascular disease.

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The graphical abstract was created with BioRender.com.

Data availability

No new data were generated or analysed in support of this work.

Conflict of interest

N.V. declares that she has no conflicts of interest. R.T.L. is a co-founder, scientific advisory board member, and private equity holder of Elevian, Inc., a member of the scientific advisory board of Revidia Therapeutics, Inc., and a consultant to BlueRock Therapeutics. The Lee laboratory has received funding support from Elevian, Inc. and BlueRock Therapeutics.

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