Artery Research

REVIEW ARTICLE

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Advancing Insights into Large-Artery Stiffening in Humans Through the Application of Multi-omics

Marie-Joe Dib[1*]

Abstract

A healthy aorta limits excess arterial pulsatility and protects the microvasculature from the effects of fluctuating blood flow and pressure. Aging and various pathologic states impair this cushioning function, a phenomenon known as large-artery stiffening (LAS). LAS is a common risk factor for a plethora of chronic diseases, and an important contributor to the conundrum of vascular morbidity. Importantly, LAS is pathologically different from atherosclerosis as it rather occurs primarily from changes in the medial aortic layer, and can manifest itself in the absence of plaque formation. Clinically, LAS is one of the few biological parameters that more than doubles with aging. With the advent of novel highly effective therapies for atherosclerosis, and the likely decline of other causes of death in the next few decades, prevention and treatment of increased LAS may be one of the most relevant strategies for preventing multimorbidity in aging populations in upcoming decades. LAS characterizes a high-priority therapeutic target to improve cardiovascular disease burden and associated comorbidities. This review aims to (i) provide an overview of insights from genetic research on LAS pathophysiology, and explore the scope of next-generation sequencing methods in the field arterial research; (ii) shed light on the utility of emerging state-of-the-art multi-omics approaches to unravel mechanisms underlying LAS to identify candidate therapeutic targets; (iii) highlight the potential of emerging state-of-the-art integrative multi-omics, motivating their use to address current gaps in understanding sex- and ancestry-specific mechanisms of LAS.

Keywords Large-artery stiffening, Target organ damage, Multi-omics, Genetics, Proteomics, Biomarkers, Drug target, Mendelian randomization

1 Introduction

A healthy aorta has the ability to act as a buffer against excess arterial pulsatility and to safeguard the microvasculature from the impact of variation in blood flow and pressure. Aging and various pathologic states are associated with a stiff aorta, which is also referred to as large-artery stiffening (LAS). In fact, LAS measured by carotid-femoral pulse wave velocity (cfPWV), aortic PWV and aortic elastic modulus, is one of the few biological parameters that more than doubles with aging [1–4]. It is characterized by an aorta's impaired "cushioning" function, and is associated with isolated systolic hypertension [5], and excessive pulsatile energy penetrating the microvasculature of organs that require high blood flow and that operate at low pre-capillary arteriolar resistance, as previously described in detail [6]. LAS has a myriad of important consequences on human health, contributing to chronic kidney disease (CKD) [7], cognitive dysfunction [8], microvascular disease in the brain [9], metabolic dysfunction [10], pre-eclampsia [11, 12], and intrauterine

*Correspondence:
Marie-Joe Dib
marie-joe.dib@pennmedicine.upenn.edu
[1] Division of Cardiovascular Medicine, Perelman Center for Advanced Medicine, 3400 Civic Center Blvd, Philadelphia, PA 19104, USA

growth restriction [11]. Additionally, LAS increases left-ventricular afterload and reduces diastolic pressure, contributing to increased left-ventricular mass and heart failure pathogenesis [11, 13]. In addition to its role in disease pathogenesis, several studies have reported that the stiffness of large arteries, measured by cfPWV, is an independent predictor of incident cardiovascular events [14], and cardiovascular mortality and morbidity in patients with hypertension, type 2 diabetes (T2D), and established CKD [15–17].

LAS is therefore a common risk factor for a wide range of chronic diseases, and an important contributor to vascular morbidity. Multimorbidity, defined as the simultaneous presence of two or more chronic diseases [18], has substantial implications on healthcare, with greater rates of disability, healthcare costs, and taxing quality of life [19].

Pathologically, LAS is not similar to atherosclerosis, but rather occurs primarily from changes in the medial aortic layer, and can occur in the absence of plaque formation [11, 20]. With the advent of novel highly effective therapies for atherosclerosis prevention and treatment, as well as the likely decline of other causes of death in the next few decades, prevention and treatment of increased LAS may be one of the most relevant strategies for preventing multimorbidity in aging populations. Given its mechanistic role in disease aetiology and progression, LAS is a high-priority therapeutic target to improve the burden of cardiovascular disease and associated comorbidities.

While exploring LAS as a potential targetable and modifiable risk factor presents an avenue for intervention, its investigation has been mitigated by multiple challenges that require answers from multi-disciplinary approaches. Notably, impediments in traditional therapeutic target identification and drug development for LAS stem from their reliance on research from animal models and challenges for assessing LAS interventions in randomized-controlled trials (RCTs), which call for a high level of prioritization in pre-clinical development, whereby candidate targets are taken forward to RCTs on the basis of having a priori evidence supporting their putative roles in humans. These aforementioned challenges arise from various factors, including first, inter-species differences in aortic structure complicating the translation of findings to human physiology. In fact, whereas in many fields, pre-clinical evidence can be readily applied from animal studies, particularly murine models, there are key inter-species differences in vascular aging [21], encompassing diverse factors including lifespans [22], aortic structure and anatomy (e.g., mice aortas contain a much larger proportion of vascular smooth muscle cells), and intrinsic pulsatile hemodynamic differences related to heart rate and animal body size [21, 23]. Second, arterial aging progresses at a slow pace during the life-course, necessitating lengthy follow-up times in human RCTs. Therefore, a high level of prioritization must be accomplished to enhance the success of any RCTs. This motivates the important quest of identifying a given therapeutic target as a mediator of age-related aortic stiffening in humans.

Inherent challenges in the drug development process contribute to a disconcerting low success rate of drug development programs, estimated to be around 4% [24]. This is attributable to various factors, most prominently the poor translatability of findings from animal models to humans [25, 26]. Given the limitations in identifying molecular targets in animal models, which is particularly prominent for the case of LAS given the inter-species differences mentioned above, as well the formidable challenges for implementing clinical trials to reduce LAS, target identification and prioritization in humans is required to maximize the chances of success in drug development and clinical trials for this purpose. Novel approaches to identify and prioritize molecular mediators of human LAS and its downstream consequences on target organ damage (TOD) are thereby urgently needed.

Although significant advancements have been made to link LAS with CVD progression, the ability to clinically stop or reverse stiffening requires a much deeper understanding of the integrated molecular, cellular, and matrix interactions. The advent of omics technologies marks a pivotal shift in our understanding and management of LAS. These omics platforms offer an unprecedented glimpse into the complex biological networks underpinning LAS, enabling a holistic view of the disease aetiology. This review provides an overview of applications of omics, with an emphasis on genomics, in the field of arterial stiffness. Several future applications of omics in arterial research are highlighted, including the identification of drug targets, and the evaluation of potential risk factors using emerging state-of-the-art methodology.

2 LAS Pathophysiology: Insights from Genetic Research

Relatively little is known about the biological mechanisms and pathways underlying the role of LAS in the development and progression of TOD. Applications of genetic association studies to arterial research has thus far led to the quantification of the proportion of variability in LAS that is attributable to genetic factors, and to the identification of genetic loci involved in LAS pathophysiology. While a few common genetic variants have been associated with measures of LAS, the underlying mechanisms have not yet been elucidated at the cellular and molecular levels [27]. More research will be needed to unravel the genetic architecture of LAS, so that more

effective means of diagnosis, treatment and prevention can be developed.

2.1 Heritability of Determinants of LAS

Heritability (h^2) refers to the proportion of observed variation in a particular trait that can be attributed to inherited genetic factors. Studies on heritability of arterial stiffness using PWV measurements suggest that genes explain a moderate proportion of the variability in arterial stiffness, with heritability estimates ranging between 23 and 50%. This has been reported from populations in a number of studies including: twin studies [28, 29] (h^2 = 53%), and population studies, such as the Framingham Heart Study [30], the Strong Heart Family Study [31], and the Erasmus Rucphen Family Study [32] (h^2 = 26–40%). Most notably, cfPWV, a well-established metric of LAS, exhibits a substantial heritable component, with heritability estimates ranging from 21 to 66% [29, 30]. Findings from these studies have highlighted that the genetic component of arterial stiffening is independent of established cardiovascular risk factors, such as age, blood pressure, height, heart rate, and smoking. Given that LAS measurements harbor moderate heritability, indicating that a moderate proportion of variability in LAS is explained by genetic components, we can leverage this genetic variation for several downstream analyses, that are later described in this review, aimed at gaining insight into the mechanisms underlying LAS pathophysiology.

2.2 Genome-Wide Association Studies of LAS.

A genome-wide association study (GWAS) is a high-throughput statistical method used to identify genetic variants that are statistically associated with a trait or disease of interest, by examining associations with thousands of genetic variants across the genomes of a large number of individuals. The identification of genetic variations related to LAS from GWAS provides a range of applications, including: (1) implementing fine-mapping approaches aimed to identify genetic loci that directly influence LAS development, thereby providing novel insights into its underlying biology and pathophysiology, (2) estimating single-nucleotide polymorphism (SNP) heritability, (3) computing genetic correlations with other relevant phenotypes, (4) computing polygenic risk scores (PRS) to develop potential preventive strategies that benefit those at relatively higher genetic risk of LAS, and (5) employing identified genetic variants as instrumental variables in downstream Mendelian randomization (MR) analyses, allowing us to determine the potentially causal impact of LAS and TOD phenotypes. These applications contribute to the scope of informing drug development programs, namely when integrated with other "omics" data (Fig. 1).

GWAS of arterial stiffness have been performed using PWV primarily as the metric for LAS. However, genetic determinants of LAS, in particular aortic stiffening, remain incompletely characterized given the relatively small number of participants phenotyped in the previous studies (the largest GWAS of cfPWV to date included data from ~ 20,000 participants) [33, 34]. GWAS of carotid-brachial PWV was conducted in participants of the Framingham Heart Study (N = 644), whereby no genetic variants were identified at genome-wide significance [35]. While genomic studies therefore have the potential to provide significant insights into the biologic determinants of age-related LAS in humans, adequately powered studies are needed in cohorts with measured metrics of LAS.

2.3 Opportunities from Next-Generation Sequencing (NGS)

GWAS have identified common genetic variants associated with LAS; however, these account for only a small proportion of the genetic contribution to LAS, prompting investigations into the question of "missing heritability" [36]. GWAS is not designed to capture genetic variants that are rare (i.e., have low frequency in the general population), although such variants may aid in improving our understanding of the pathophysiology of LAS. Such variants may have larger effects on LAS, as they may disrupt gene function more profoundly or alter the expression of genes in the pathways involved in maintaining arterial wall structure and function. NGS methods such as whole-genome sequencing and whole-exome sequencing may enable the detection of rare variants that are necessary to fully understand the genetic underpinnings of LAS and to potentially reveal novel targets of therapeutic intervention [37]. These methods have not been used extensively in the context of LAS, although some applications of whole-exome sequencing on aortic function and atherosclerosis have been reported with mixed results [38, 39]. Translationally, the use of NGS data in LAS has the potential to facilitate the identification of new biomarkers of LAS, diagnostic/prognostic markers, as well as support drug repurposing efforts—i.e., finding new putative uses for existing, licensed drugs. While NGS holds promise for addressing missing heritability, especially with it being more freely accessible, several limitations inherent to the methodology have to be overcome, including sample size, variant interpretation, and functional validation. Importantly, one inherent limitation to whole-genome sequencing is the challenge in interpreting novel results, which will require a combination of "dry" (bioinformatic) and "wet" (laboratory-based)

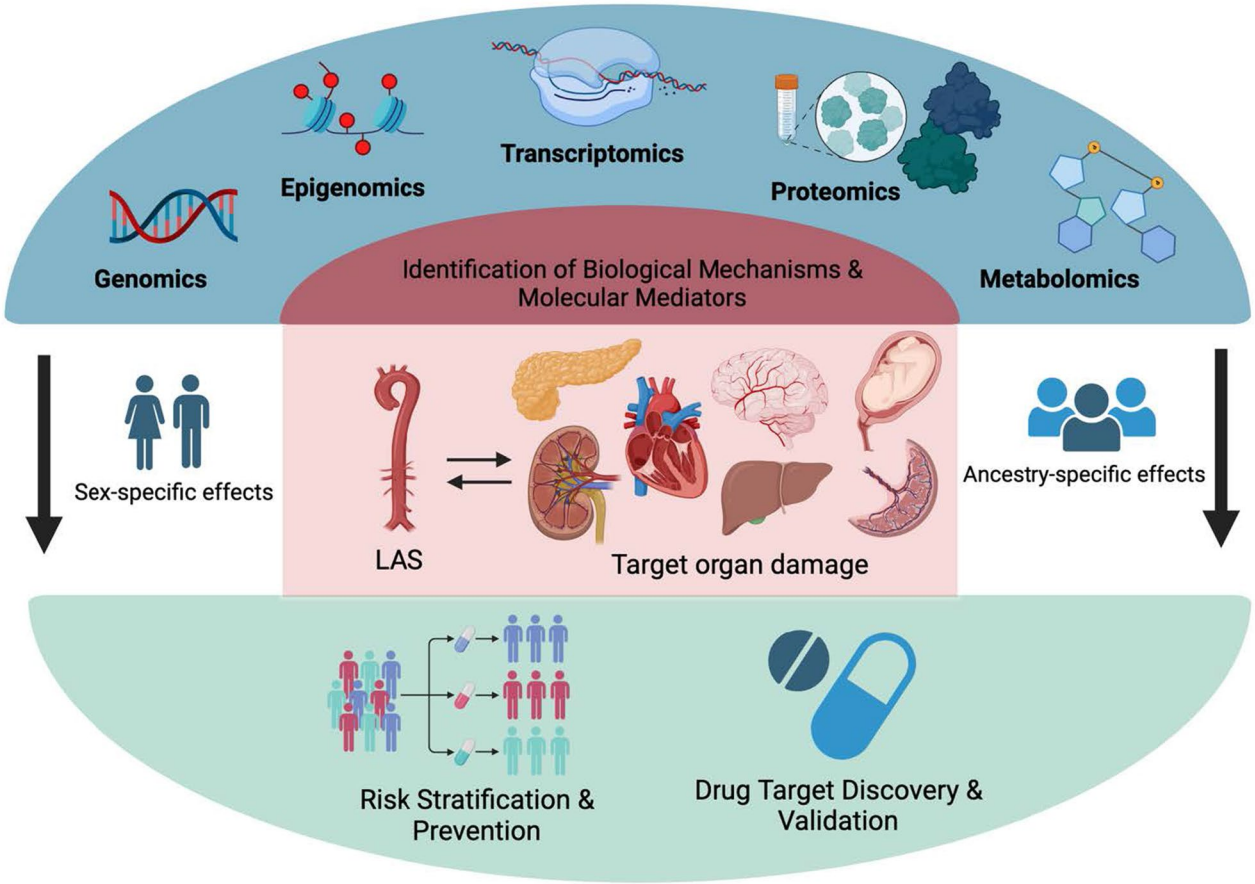

Fig. 1 Applications of multi-omics in providing insights into the mechanisms underlying associations between large-artery stiffening (LAS) and target organ damage (TOD). Emerging state-of-the-art methodology, large-scale datasets, and the advancement of integrative multi-omics can provide unprecedented insights into mechanisms of LAS, thereby informing on drug development, and facilitating strategies for risk stratification and prevention. An emphasis on unraveling sex-specific and ancestry-specific effects of LAS is highlighted. Created with BioRender.com

approaches, integrating functional information from other omics data, such as transcriptomics, metabolomics, and proteomics [40].

3 The Use of Mendelian Randomization (MR) for Unraveling LAS Risk Factors, Mechanistic Pathways, and Therapeutic Drug Targets

MR is a highly informative statistical approach that provides unprecedented opportunities to investigate human biologic causation to gain insight into complex trait mechanisms and identify potential therapeutic drug targets to inform RCTs. MR uses genetic instruments as a natural experiment to investigate causal relationships between potentially modifiable risk factors and health outcomes in observational data, which is particularly useful when RCTs are not feasible [41]. Germline genetic variants are randomly allocated and fixed at conception [42], providing a rationale for using MR to overcome the limitations of traditional epidemiology, including (1) reverse causation, as the genetic variant temporally precedes the outcome, and the genetic sequence of individuals is not altered in the state of disease, and; (2) confounding, as the genetic variant cannot be influenced by confounding factors that act after conception. MR offers a thorough approach for investigating possible causal relationships in observational data under 3 main assumptions that underpin the effective selection of genetic instruments as instrumental variables to facilitate robust MR experiments. As illustrated in Fig. 2, (i) the genetic instrument(s) must be associated with the exposure of interest (the relevance assumption); (ii) there should be no confounding factors influencing the association between the genetic instrument(s) and outcomes of interest (the independence assumption); (iii) the genetic instrument should only be related to the outcome through the exposure, ensuring the absence of pleiotropic effects that may bias MR estimates (the exclusion restriction assumption) [42].

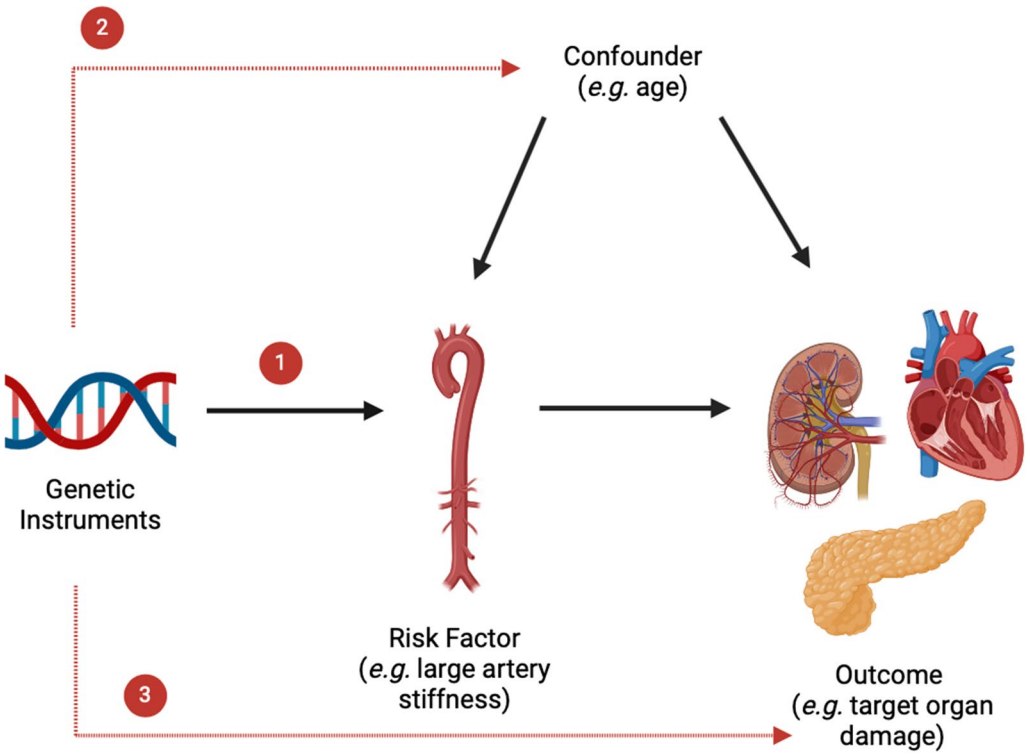

Fig. 2 Assumptions of the paradigm of Mendelian randomization with. MR assumptions: Genetic instruments (1) must be highly associated with the risk factor of interest (e.g., large-artery stiffness); (2) must not be associated with any known or unknown confounders; and (3) must not influence the outcome through pathways distinct from the risk factor. Created with BioRender.com

3.1 Mendelian Randomization (MR) for Unraveling Mechanistic Pathways and Risk Factor Identification

The causal associations between LAS and a number of TOD phenotypes have been assessed using MR analyses. Xu et al. investigated the causal association between T2D and increased brachial–ankle pulse wave velocity (baPWV) in 11,385 participants of East Asian ancestry and highlighted a causal association between increased T2D propensity and the odds of arterial stiffening [43]. Similarly, in a population of the Framingham risk study, Cohen et al. demonstrated that an increased risk of genetically predicted brachial pulse pressure was associated with increased odds of T2D, independently of mean arterial pressure [10]. Cecelja et al. highlighted a bi-directional causal association between blood pressure and PWV using data from the TwinsUK [44]. Simistiras et al. showed that lipoprotein(a) is not causally associated with baPWV and cfPWV [45]. While some genetic evidence supports causal relationships between LAS and some phenotypes of TOD, there remains a need to leverage genetic instruments for more robust markers of LAS.

3.2 MR for the Identification of Candidate Therapeutic Targets

Human genetic evidence has been reported as a surrogate indicator for the success of drugs in the drug development process, primarily due to its ability to discern causal mechanistic relationships between drug targets and diseases. In fact, studies of drug development programs have also shown that targets with genomic support have a higher rate of success [46], and two-thirds of new drugs approved by the FDA in 2021 were supported by genetic evidence [47]. MR has been widely used to repurpose licensed drugs and discover novel therapeutic targets by integrating summary data from disease GWASs, protein quantitative trait loci (pQTL) and expression quantitative trait loci (eQTL) studies. An eQTL refers to a genetic variant that explain variations in gene expression levels [48, 49]. Since gene expression is not necessarily indicative of protein expression, integrating tissue-specific eQTL data from large-scale datasets with pQTL data from proteomics studies is key in determining whether changes in gene and protein expression are potentially causal to LAS—a pivotal step for target identification. In light of this, to

date, there is a paucity of studies that have investigated molecular targets of LAS in humans. Dib et al. have identified 13 novel proteins that appear to have causal roles on LAS, proxied by increased pulse pressure, in humans (data accepted for publication). These may represent novel therapeutic targets for reducing LAS and its deleterious consequences. Further work is required to validate the putative role of these proteins on LAS by integrating tissue-specific expression data, and to assess the clinical impact of targeting these proteins on progression to LAS.

4 Future Directions for the Applications of Omics in Unraveling LAS Aetiology

4.1 Polygenic Risk Scores (PRSs) and Risk Stratification

PRS leverage polygenic models of complex traits and disease by pooling effects from several genes. While the utility of PRSs is being debated at the clinical application level, namely due to their ability to only account for heritable component of traits omitting the role of lifestyle and environment [50], they nonetheless have promising utility in disease risk stratification for lifelong arterial stiffening and pharmacogenomics. Although this has been extensively studied in the context of coronary artery disease, it remains to be explored in the context of arterial stiffening.

4.2 Integrative Multi-omics

While a single-omics approach offers a glimpse into the biological underpinnings of disease, it is insufficient to capture the complexity inherent in biological mechanisms. The discovery of LAS-associated genetic variants has provided some insight into the pathophysiology of LAS; however, alone they are unable to fully explain the landscape of LAS, and whether intervention at the level of gene expression may mitigate risk of LAS. Genomics (*i.e.,* the study of data derived from the DNA sequence), transcriptomics (i.e., the study of RNA transcripts produced by the genome), proteomics (i.e., the study of protein expression), metabolomics (i.e., the study of metabolites such as lipids, amino acids, sugars, and other metabolic intermediates), and epigenomics (i.e., the study of chemical modifications to the DNA sequence, such as DNA methylation, and histone modifications involved in the regulation of gene expression) often have complementary roles in performing life-course biological functions, and they often interact with environmental factors, adding the complexity of the biological landscape. Harnessing the full potential of these integrated datasets involves continuous refinement of analytical methodologies, which have some limitations that have been previously discussed [51]. Finally, an important component of integrative multi-omics is the investigation of multidimensional gene-environment interactions [52]. The latter requires further investigation as they may alter the risk of LAS development and progression, and should be taken into account when applying integrative models and interpreting results.

4.3 Addressing Sex-Specific and Ancestry-Specific Differences in LAS Mechanisms

In addition to established disparities in cardiovascular risk factors across diverse ancestry groups, observational studies have highlighted differences in LAS and aortic hemodynamic LAS age-of-onset [53]. These associations are yet to be explored using genetic data and MR approaches to mitigate confounding and untangle the complexity of the identified relationships. One limitation that has mitigated the advancement of this field is the scarcity of available ancestry-specific genetic data. Notably, 86% of high-throughput genetic association studies that have identified loci for traits and diseases have been carried out in Europeans [54], and there is a call to carry out studies in ancestrally diverse populations to provide insights into disease [55]. The availability of multi-ancestry GWAS is essential for the prioritization of risk factors of disease and subsequent targeted intervention in diverse population groups, and is being facilitated by large-scale datasets. Nonetheless, there is a need to intensify efforts to acquire such data on measures of LAS.

Additionally, while advanced age is associated with LAS in both males and females, evidence supports sex disparities in aging-related LAS [56] and the associated CVD risk, which is more prominent in postmenopausal females [57]. The association between LAS and mortality has been shown to be almost twofold higher in females compared to their male counterparts. Females also develop more pronounced increases in pulse pressure in the life-course, that are independent of body and aortic size [58–60]. Thus, there is a need to identify sex-specific determinants of LAS and their contribution to TOD for tailored prevention and intervention.

5 Conclusions

In conclusion, with the advent of large-scale omics data and the development of integrative analytical methods, we are becoming increasingly equipped to dissect the intricate interplay of genetic, molecular, and environmental factors contributing to LAS. As these technologies and analytical methods continue to be refined, the triangulation of evidence from several epidemiological and multi-omics approaches will enable the scope of preventing, targeting, and treating LAS, transforming patient outcomes and advancing public health. More specifically, this will be pivotal in (1) unraveling the mechanisms of arterial function and the molecular pathways involved in LAS and arterial–ventricular interaction, and

(2) the discovery of novel biomarkers for early detection to inform experimental studies, thereby opening new avenues for targeted prevention strategies.

Abbreviations

baPWV	Brachial–ankle pulse wave velocity
cfPWV	Carotid-femoral pulse wave velocity
CKD	Chronic kidney disease
CVD	Cardiovascular disease
eQTL	Expression quantitative trait loci
GWAS	Genome-wide association study
LAS	Large-artery stiffening
MR	Mendelian randomization
NGS	Next-generation sequencing
PRS	Polygenic risk score
pQTL	Protein quantitative trait loci
RCT	Randomized-controlled trial
T2D	Type 2 diabetes
TOD	Target organ damage

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