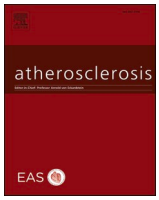
Atherosclerosis 408 (2025) 120414

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis



Multiomics in atherosclerotic cardiovascular disease

Liv Tybjærg Nordestgaard [a,b,*], Brooke N. Wolford [c], David de Gonzalo-Calvo [d,e], Miron Sopić [f,g], Yvan Devaux [g], Ljubica Matic [h], Stephanie Bezzina Wettinger [i], Johannes A. Schmid [j], Núria Amigó [k,l], Lluís Masana [m], Alberico L. Catapano [n,p], Dimitris Kardassis [o], Paolo Magni [n,p], on behalf of EU-AtheroNET COST Action CA21153

[a] *Department of Clinical Biochemistry, Copenhagen University Hospital - Herlev and Gentofte, Borgmester Ib Juuls Vej 1, 2730, Herlev, Denmark*
[b] *Medical Research Council Integrative Epidemiology Unit, Population Health Sciences, University of Bristol, BS8 2BN, United Kingdom*
[c] *HUNT Center for Molecular and Clinical Epidemiology, Department of Public Health and Nursing, Norwegian University of Science and Technology, Trondheim, Norway*
[d] *Translational Research in Respiratory Medicine, University Hospital Arnau de Vilanova and Santa Maria, IRBLleida, Lleida, Spain*
[e] *CIBER of Respiratory Diseases (CIBERES), Institute of Health Carlos III, Madrid, Spain*
[f] *Department of Medical Biochemistry, Faculty of Pharmacy, University of Belgrade, Serbia*
[g] *Cardiovascular Research Unit, Department of Precision Health, Luxembourg Institute of Health, Strassen, Luxembourg*
[h] *Vascular Surgery, Department of Molecular Medicine and Surgery, Karolinska Institutet and Karolinska University Hospital, Sweden*
[i] *Department of Applied Biomedical Science, Faculty of Health Sciences and Centre for Molecular Medicine and Biobanking, University of Malta, Msida 2080, MSD, Malta*
[j] *Institute of Vascular Biology and Thrombosis Research, Comprehensive Center for Cardiovascular Medicine, Medical University of Vienna, Austria*
[k] *Biosfer Teslab, Reus, Spain*
[l] *CIBER of Diabetes and Associated Metabolic Disease (CIBERDEM), Instituto de Salud Carlos III, Madrid, Spain*
[m] *Department of the Vascular Medicine and Metabolism Unit, University Hospital "Sant Joan", IISPV, CIBERDEM, Universitat Rovira I Virgili, Reus, Spain*
[n] *IRCCS MultiMedica, Sesto S. Giovanni, 20099, Italy*
[o] *Department of Basic Sciences, University of Crete Medical School and Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, 71003, Greece*
[p] *Department of Pharmacological and Biomolecular Sciences, Università Degli Studi di Milano, 20133 Milan, Italy*

Abbreviations: ACS, acute coronary syndrome; AI, artificial intelligence; ASCVD, atherosclerotic cardiovascular disease; CAD, coronary artery disease; circRNAs, circular RNAs; cTn, cardiac troponine; DNA, Deoxyribonucleic acid; RP, fat radiomic profile; HRP, high risk plaque; HUNT, The Trøndelag Health Study; lncRNAs, long non-coding; MACE, Major adverse cardiovascular events; ML, Machine learning; miRNAs, microRNAs; MS, mass spectrometry; ncRNAs, non-coding RNAs; and NGS, Next generation sequencing; NMR, nuclear magnetic resonance; PCSK9, proprotein convertase subtilisin/kexin type 9 gene; RNA, ribonucleic acid; VSMC, vascular smooth muscle cell.

* Corresponding author. Medical Research Council Integrative Epidemiology Unit, University of Bristol, Oakfield House, Oakfield Grove, BS8 2BN, United Kingdom
E-mail address: qs20306@bristol.ac.uk (L.T. Nordestgaard).

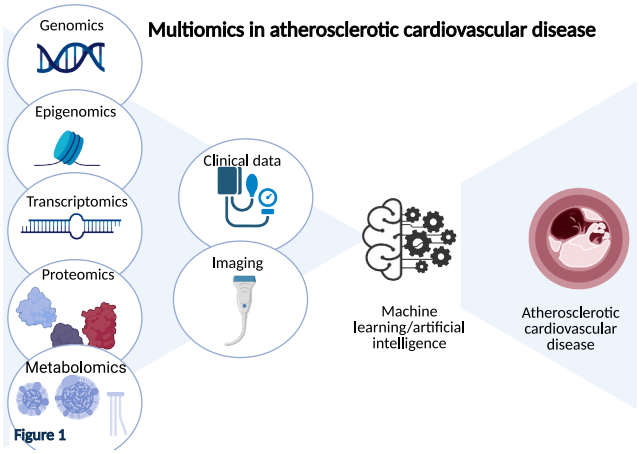
https://doi.org/10.1016/j.atherosclerosis.2025.120414

Available online 26 June 2025

KEY POINTS

- Current methods for diagnosis and treatment of atherosclerotic cardiovascular disease (ASCVD) lack precision.
- The combination of multiomics with MLand AI has emerged as a useful tool for the molecular phenotyping of ASCVD.
- Future possibilitities include using omics data in drug development.
- Omics-based analyses will likely be routinely used in precision diagnostics within the ASCVD field in the future.

GRAPHICAL ABSTRACT



ABSTRACT

Background and aims: Atherosclerotic cardiovascular disease (ASCVD) is among the leading causes of death worldwide, and technological advances have made it possible to expand the repertoire of biomarkers used in diagnostics and treatment of ASCVD. These include different omics (genomics, epigenomics, transcriptomics, proteomics, and metabolomics). We introduce the various layers of omic data and how they can be used in diagnostics and treatment of ASCVD. Further, we discuss future possibilities of combining multiomic data with machine learning (ML) and artificial intelligence (AI) to develop algorithms for facilitating precision medicine.
Methods: we reviewed the current literature on omic data in ASCVD and its integration with ML/AI.
Results: Genomics has been used to generate polygenic risk scores (PRS), which have shown promising results in risk prediction of ASCVD. Key epigenetic changes implicated in atherosclerosis include deoxyribonucleic acid (DNA) methylation. Transcriptomics has been used to identify transcripts, including micro ribonucleic acid (miRNAs), implicated in atherosclerosis progression. Proteomic risk scores have shown independent predictive information and outperformed clinical risk models, and within the metabolomics field, lipidomics has emerged as a promising predictive tool. The combination of multiomic data analysis with ML and AI methods has already demonstrated potential in the development of clinical models.
Conclusions: A major effort is necessary to bring omic data and technologies to the clinical field. Further support will be offered by the generation of clinically applicable/approved AI/ML algorithms able to translate large datasets into valuable information for accurate precision medicine approaches.

1. Introduction

Atherosclerotic cardiovascular disease (ASCVD), including acute coronary syndrome (ACS), is among the most common causes of death worldwide [1]. However, accurate risk stratification for ASCVD and personalized treatment remains challenging as currently available risk charts provide imprecise information [2]. Technological advances, that allow the identification and validation of novel biomarkers may contribute to the improvement of risk stratification in ASCVD and personalized treatment.

These technologies, collectively known as "omics" approaches—such as next-generation sequencing (NGS), mass spectrometry (MS), and nuclear magnetic resonance (NMR)—are crucial for the discovery and validation of novel biomarkers. In combination with multiomic data (proteomics, metabolomics, etc.) they can lead to the generation of large and comprehensive databases. They can be applied to a variety of human-derived biological matrices, including blood, plasma, serum, urine, and saliva, and allow the detection of analytes at very low concentration. Therefore, their application in the clinical setting in a standardized fashion could ameliorate the quality of collected clinical data. However, robust standardization of techniques is still required for reliable clinical applications and future research should be devoted to fill this gap. Furthermore, the integration of multiomic data with traditional clinical information into artificial intelligence/machine learning (AI/ML)-based algorithms could improve ASCVD risk stratification, diagnostics, prognostics, and treatment.

Genomics - the study of genes - has evolved immensely since the first sequencing techniques were developed but whether adding information about genetic risk to traditional clinical risk factors will improve disease prediction remains uncertain (see Table 1). Although advances have been made within the field of epigenomics the exact role it could play in discovering underlying mechanisms in atherosclerosis and in risk prediction remains unknown. Transcriptomics is an emerging field within research but its utility in ASCVD still is unclear – especially the utility of transcriptomic data in combination with ML, with radiomics and the insights provided by single-cell ribonucleic acid (RNA) sequencing needs further scrutiny. Proteomics is a promising field for development of new predictive tools but how proteomic risk scores perform compared

Table 1
Explanation of the most important concepts.

Concept	Description
Genomics	The study of genes, their expression, and functions.
Epigenomics	The study of the chemical modifications and histone proteins that regulate gene expression.
Transcriptomics	The study of RNA molecules transcribed from DNA in a cell, tissue or organism.
Proteomics	The large-scale study of proteins including their structure, function, modifications, and interactions.
Metabolomics	The study of metabolites, small molecules typically less than 1 kDa in size.
Machine learning	A field of study in artificial intelligence that uses statistical algorithms to learn from data and generalize to unseen data.
Artificial intelligence	The capability of computational systems to perform tasks associated with human intelligence.

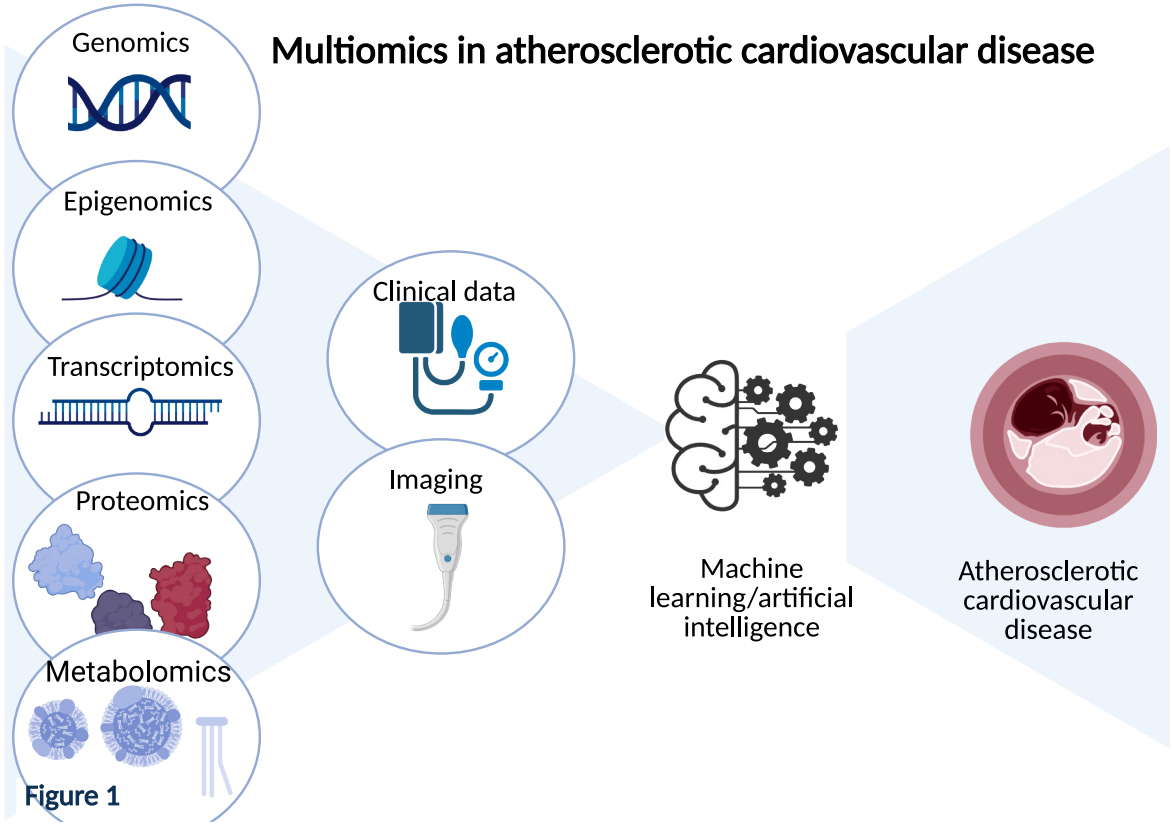


Fig. 1. (Graphical abstract)
Omics is used to describe how genetic variation (genomics) leads to changes in gene expression (transcriptomics) that affects protein expression (proteomics). Genomics are modified by epigenetic modifications (epigenomics) and protein variation determines enzymatic activity and metabolic variation (metabolomics). "Multiomics" is used when referring to multiple different omics data. In this review we summarize how multiomics can be used in the diagnostics and treatment of atherosclerotic cardiovascular disease (ASCVD), and how the integration of this data with clinical data, imaging data, and machine learning (ML)/artificial intelligence (AI) can improve risk prediction and personalized treatment.

to more traditional genetic risk scores and clinical risk factors needs further exploration. Within the fields of metabolomics further understanding of the technologies used as well as the most important metabolites for ASCVD risk prediction needs further clarification. This review discusses the usefulness of these different omic data and their integration by AI/ML strategies for accurate identification, diagnosis, and treatment of subjects at risk of ACSVD and patients with ACSVD (Fig. 1, graphical abstract). Further, the review discusses the strengths and limitations of each of these omic technologies (Fig. 2), how they can be integrated with ML/AI, and how they can be used in the development of new drug targets for ASCVD.

2. Methods

We reviewed the current literature on omic data in ASCVD and its integration with ML/AI.

3. Results

3.1. Genomics

Genomics is the study of genes, their expression, and functions. Deoxyribonucleic acid (DNA) sequencing is the process of determining the nucleic acid sequence – the order of nucleotides in DNA. Single genetic variants have been shown to predict disease risk since the development of DNA sequencing methods (See Table 2). Within the field of ASCVD especially genetic variants involved in lipid metabolism have been shown to be associated with increased risk [3]. More recently, up to millions of genetic variants have been combined into polygenic risk scores (PRS) to predict risk of complex conditions.

3.1.1. Single genetic variants

Genetic variation in genes important in lipid metabolism has been important in the understanding of the pathogenesis of atherosclerosis and in the discovery of drug targets. Variants in genes important in LDL metabolism such as the LDL receptor gene (*LDLR*), the HMG-CoA reductase gene *(HMGCR),* the proprotein convertase subtilisin/kexin type 9 gene *(PCSK9),* the apolipoprotein B gene *(APOB),* and lipoprotein a (*LPA)* have all been associated to risk of ASCVD[4–6]. Likewise, genetic variation in genes known to be important in triglyceride metabolism have also been shown to be important for drug discovery in ASCVD[7–9]. Further, new sequencing methods have enabled more precise diagnostics of genetic susceptibility to ASCVD. In a study by Sturm et al. gene sequencing was used to show that 69 % of individuals with a pathogenic or likely pathogenic variant in a familial hypercholesterolaemia associated gene would have been missed when using a limited variant-screen compared to a comprehensive NGS screening [10]. Further, individuals with high genetic risk have been shown to benefit from treatment with statins and other lipid lowering therapies such as PCSK9 inhibitors [11,12].

3.1.2. Polygenic risk scores

There are several PRS for ASCVD using different numbers of genetic loci and variants, from 27 [11] to over 6.6 million single nucleotide polymorphisms (SNPs) [13]. One genome-wide PRS has been shown to identify 8 % of the population with a 3-fold increased risk for ASCVD, equivalent to the risk associated with strong Mendelian variants such as those for familial hypercholesterolaemia [13]. Another PRS for ASCVD

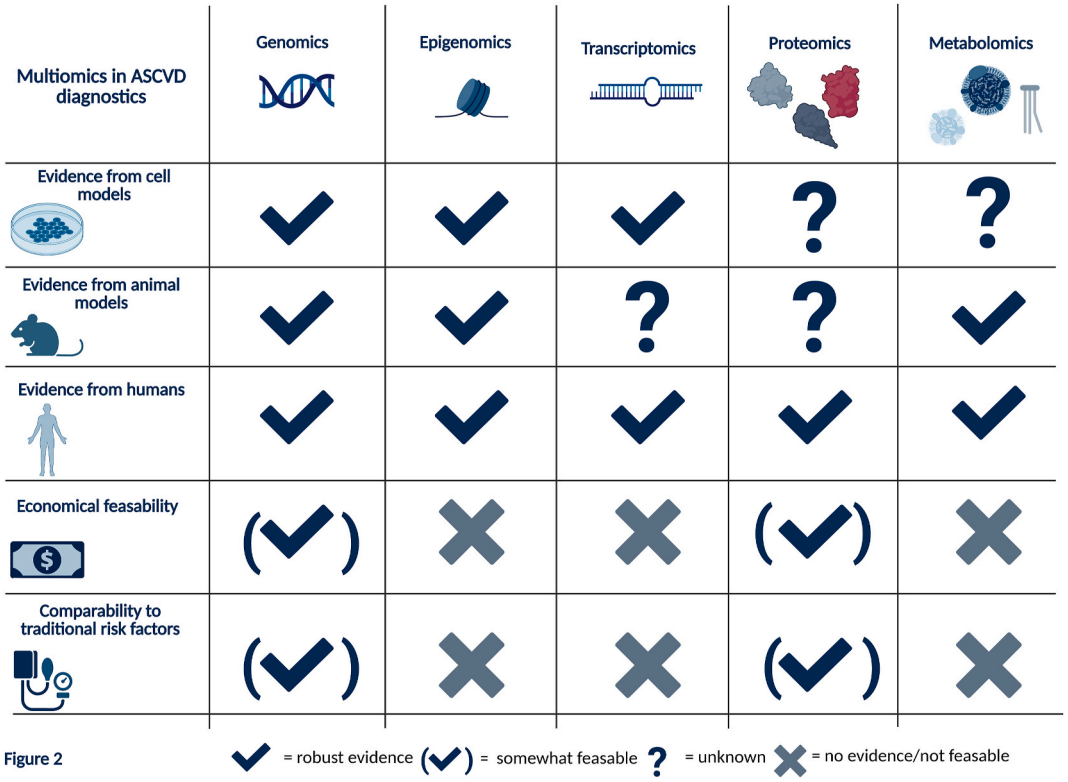


Fig. 2. Summarized evidence of multiomics in atherosclerotic cardiovascular disease
The scientific evidence for and economic feasibility for each of the omic data is summarized in this figure. ✔ = robust evidence, (✔) = some evidence/somewhat feasible, ? = unknown, X = no evidence/not feasible.

includes 1.7 million variants [14], and the addition of PRS to conventional risk factors has been shown to improve prediction accuracy [15]. In a study by Vassy et al. net reclassification improvement for risk of cardiovascular disease (CVD) from adding PRS to a traditional risk model was 0.38 %; 95 % CI, 0.07 %–0.68 % for men and 6.79 %; 95 % CI, 3.01 %–10.58 % for women [16]. In a study by Mars et al. they found that especially in patients under 55 years of age testing for PRS would be beneficial for detecting risk of coronary heart disease [17]. However, although PRS provide additional prognostic information compared to traditional risk factors for ASCVD, the clinical significance of this is still debated [15].

3.1.3. *Strengths*

Genomic testing can happen from birth and may be performed only once in a lifetime, making it advantageous for risk prediction of ASCVD. The sequencing methods have been developed over decades and have become very accurate and enable a very high throughput. In addition, microarrays allow reliable testing of large numbers of SNPs at a reasonable price.

3.1.4. *Limitations*

However, the utility of PRS as a screening tool has been questioned as the increase in relative risk in high risk groups is relatively small compared to other screening tests [18]. Thus, clinical utility is still uncertain [15], particularly since they are prone to a high proportion of false positive results [18].

Further, current available PRS could be improved. Genetic studies and PRS still do not equitably generalize to all global populations [19,20], and the influence of sex needs further investigation [21]. Additionally, the effect of structural variation is still understudied and requires better understanding.

Whilst genetic testing may be conducted from birth, ethical issues, insurability, and insurance premiums are challenges for genomic testing in younger people. The precise age when it is best to conduct such testing is still unclear, as is the influence of disclosure of being at genetic risk on lifestyle modifications [15,22]. Alone, PRS does not allow monitoring of improvements in risk due to medication or lifestyle changes, and the groups that could benefit from PRS still need to be identified. Further, disclosure of genetic risk can be challenging [23], and pressure on genetic counselling service is expected to increase. Direct-to-consumer testing, which is gaining popularity, needs regulation [15], and more research is required to determine the follow up and therapeutic changes required by those at high genetic risk [24]. In a recent clinical consensus statement European Society of Cardiology recommended further research to determine the impact of disclosing PRS results, and to identify specific risk categories that may benefit from PRS testing [25].

3.2. *Epigenomics*

The technological advances in omics technologies (NGS, transcriptomics, epitranscriptomics) have allowed the mapping of epigenetic modifications in DNA/RNA with astonishing accuracy (see Table 3) [26]. Cardiovascular epigenomics is a rapidly advancing field that has already provided critical information regarding the epigenetic landscape of atherosclerosis in animal/cell models and in humans and their predictive value for ASCVD. Key epigenetic alterations that are implicated in the pathogenesis of atherosclerosis include DNA methylation (binding of a methyl group to the 5′ carbon of cytosine in cytosine-guanine dinucleotide sequences) and histone tail modifications (acetylation, methylation, or phosphorylation). In addition non-coding RNAs (ncRNAs) (such as micro RNAs (miRNAs), long non-coding (lncRNAs) and circular RNAs (circRNAs)) are also considered to be part of the epigenetic regulatory machinery [27,28].

Table 2
Methods used for DNA sequencing.

DNA sequencing method	Description	Strengths	Limitations	Specific use cases in genomic studies of ASCVD
Sanger Sequencing	uses chain termination with fluorescently labeled nucleotides [132,133] uses capillary electrophoresis to separate DNA fragments Sequence determined by detecting fluorescent signals	Good for smaller-scale projects Good for validation of genotyping results High accuracy	Slow for high-scale sequencing (>20 samples) Expensive for large-scale sequencing Only one read per sample Low sensitivity Can only read 350 to 800 base pairs per sample	Can be used to confirm already known mutations Can be used for small-scale targeted sequencing
Illumina Sequencing	Amplification of DNA to solid surface [134], generation of single-stranded DNA, incorporation of nucleotides using polymerase, detection of incorporated nucleotides [135]	Extremely high throughput High accuracy [135] Highly scalable Allowing entire genome to be sequenced at once billions of reads per sample	Can be very expensive Requires high concentrations of DNA Relatively long run time [135]	Can be used in the generation of polygenic risk scores for prediction of ASCVD Can be used in the discovery of new ASCVD causing mutations
Ion torrent Sequencing	Micelle droplets loaded with primer, template, dNTP, polymerase [134] DNA fragments amplified Changes in pH are detected when a nucleotide is added, and this is detected by a chip and the type of nucleotide can be determined [135]	Fast Low cost for small-scale sequencing	Low accuracy for certain regions [135] Short read lengths	Can be used in the generation of polygenic risk scores for prediction of ASCVD Can be used in the discovery of new ASCVD causing mutations
Pacific Biosciences Sequencing	Single stranded circular DNA is template Sequencing in "Single molecule real time" cell chip with small pores called zero-mode waveguides Replication by DNA polymerase: binding of a nucleotide produces light pulse Light pulses are interpreted as nucleotide sequences [135,136]	Reads can be very long (10,000 to 100,000 base pairs per run) [135] Can detect epigenetic modifications Does not require amplification [135]	High error rate although improving rapidly [135] Expensive Large size of instruments [135] Low throughput	Detecting large structural variations determining whether genetic variants are inherited together on the same chromosome or are located on opposite chromosomes [136]
Oxford Nanopore Sequencing	DNA strands pass through nanopore Changes in electrical conductance are detected to determine DNA sequence [136]	Long reads The device is portable [135] Inexpensive Real-time data	Is error-prone [135] Requires advanced computational analyses	Studying structural variants Characterizing epigenetic modifications

Table 3
Methods used for detecting epigenetic modifications.

Method for detecting epigenetic changes	Description	Strengths	Limitations
Bisulfate sequencing	Used to identify DNA methylation [135] Sodium bisulfate and alkaline treatment converts unmethylated cytosine to uracil Methylation sites identified by amplification and sequencing of untreated DNA and sodium bisulphite treated DNA	Genome-wide coverage	Expensive Computationally intensive DNA degradation can occur
ChIP-Seq	Used to identify DNA binding proteins particularly histone modifications and transcription factors [135] DNA fragmentation Antibodies used to isolate DNA with histone modifications and transcription factors Followed by sequencing and mapping of DNA binding proteins to genome	Simple Inexpensive Good for targeted analyses	Limited to certain sites Cannot provide genome-wide data
Chromatin Immunoprecipitation	Used to identify histone modifications Histones are cross-linked to DNA The cells are lysed Antibodies are added DNA is purified hybridization of DNA Analysis of the relative signal intensity allows the sites of histone modification to be determined	Can identify histone modifications associated with gene regulation	requires high-quality antibodies requires large number of cells

3.2.1. *DNA methylation*

Aberrant DNA methylation patterns have been found at the single gene level or at genome scale. For instance, hypermethylation of the ATP-binding cassette transporter A1 (*ABCA1*) in patients with atherosclerosis [29,30] and hypomethylation of the pro-inflammatory gene interleukin-6 (IL-6) enhances the inflammatory response and accelerate atherosclerosis [31]. Other DNA methylation alterations that have been associated with cardiovascular disease are in the *FOXP3, ABCG1, AHRR,* and CPT1A genes [32]. These are genes involved in inflammation, cholesterol transport, toxin degradation, and fatty acid oxidation. At the genome level, Silvio Zaina et al. [33] have examined DNA methylation levels using whole-genome bisulfite sequencing. They observed that the atherosclerotic portion of the aorta was hypermethylated across many genomic loci in comparison with the matched healthy counterpart. Computational methods are increasingly being used in DNA methylation research [34], including in the prediction of gene expression [35].

Further, efforts are being made of modeling the entire methylome using generative AI [36].

3.2.2. Histone tail methylation

Histone tail methylation can either activate or repress transcription. Greiβel et al. [37] have analyzed histone methylation marks in human patients with differing stages of atherosclerosis. They found decreased expression of H3K9me3 and H3K27me3 in vascular smooth muscle cells (VSMCs) and inflammatory cells of whole atherosclerotic plaques. Moreover, the expression of H3K4me3 was strongly associated with the severity of atherosclerosis [37].

3.2.3. Strengths

Epigenetic changes can function as predictors of atherosclerosis as evidenced by several studies. The Multi-Ethnic Study of Atherosclerosis (MESA) identified 3 candidate DNA methylation loci (corresponding to genes *VPS13D*, *PIK3CD* and *VPS45*) to be associated with both physical activity and CVD [38]. The GrimAge and PhenoAge studies found that epigenetic clocks incorporating methylation data can predict cardiovascular mortality [39]. These approaches are helpful for the identification of subjects at high risk of ASCVD and can be used alongside traditional risk factors to improve predictive accuracy.

3.2.4. Limitations

Limitations of epigenomics as predictive tools for ASCVD include context dependency (cell type, age, disease state, etc.), heterogeneity among ethnic groups, absence of longitudinal data (how epigenetic markers change over time in a disease) and influence of environmental factors that can act as confounders [40,41]. All these challenges should be addressed before epigenetics is integrated into clinical practice.

3.3. Transcriptomics

Transcriptomics represents the comprehensive analysis of the transcriptome—all the ribonucleic acids (RNAs) within a specific compartment, such as a cell, tissue, organ, or body fluid. While only a minor fraction of these RNAs encode proteins, (messenger RNAs (mRNAs) representing 2–3 % of the entire transcriptome) [42], most RNAs are not protein-coding. These so-called non-coding RNAs (ncRNAs) play crucial roles in regulating transcriptional mechanisms and cellular functions. Among ncRNAs, microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs have been relatively well studied in the context of atherosclerosis [43]. RNA splicing, modifications, and editing, collectively known under the term “epitranscriptomics“, further add to the complexity of the transcriptome [43]. Together with quantitative polymerase chain reaction (qPCR), RNA sequencing is the gold-standard method for transcriptomic analysis. A comprehensive description of the different RNA sequencing technologies currently available, together with their strengths and weaknesses, has been recently published [44].

3.3.1. Epitranscriptomic profiling

Direct sequencing using nanopores shows promise to help in epitranscriptomic profiling. These techniques have allowed the identification of genes implicated in atherosclerosis progression, the exploration of new biomarkers, and the identification of new therapeutic targets [45–48]. This has led to the discovery of new regulators of smooth muscle cell lipid transdifferentiation [49], different signatures in plaques of various origins [45], differences between stable and unstable plaques [46], and sexual dimorphism in plaques [47]. Mokry et al. utilized transcriptomic data from 654 advanced human carotid plaques combined with clinical data and ML [48]. They clustered human atherosclerotic plaques and identified subgroups with distinct underlying biology and clinical presentation. Plaques with the most severe clinical symptoms expressed numerous genes related to inflammation, neutrophil degranulation, matrix changes, and metabolism. Validation in 162 coronary artery plaque samples confirmed that fibro-inflammatory plaques were strongly associated with coronary ischemic events. Combining transcriptomic data with proteomics analysis in blood samples, authors identified 28 circulating biomarkers with significantly different levels among the transcriptomic plaque clusters, supporting the usefulness of multiomic approaches [48].

Table 4
Comparison of proteomics platforms.

	Mass Spectrometry (MS)	Olink	SomaScan
Type of assay	Untargeted or targeted	Targeted (proximity extension assay)	Targeted (aptamer-based binding)
Proteins quantified	Thousands (untargeted); tens to hundreds (targeted)	Up to 3000 (predefined panels)	>7000 (predefined panel)
Need for prior target selection	No (untargeted); Yes (targeted)	Yes	Yes
Sensitivity	Moderate to high (higher in targeted modes)	Very high	Very high
Reproducibility	Moderate (improving with standardization)	High	High
Sample volume required	Moderate (typically >10 μL)	Low (1 μL)	Low (50 μL)
PTM detection (Post-Translational Modifications)	Yes	No	Limited
Main limitations	Complex workflows, instrumentation, data analysis	Dependent on antibody quality, limited to known targets	Potential cross-reactivity, aptamer biases
Common use cases	Biomarker discovery, PTM analysis, validation	Large-scale population studies, clinical prediction	High-throughput screening, early biomarker studies

3.3.2. Transcriptomics and radiomics

Significant efforts have been made to combine transcriptomics with radiomics (the conversion of images into mineable data and the subsequent analysis of these data for decision support) [50], creating a radiotranscriptomic approach for high-risk patient stratification. This approach has been developed and validated for detecting inflammation in perivascular adipose tissue, a risk factor for adverse cardiovascular events. Transcriptomic profiling of adipose tissue combined with radiomics, and ML has led to models that improve cardiac risk prediction [51]. In the SCOT-HEART study, fat radiomic profile (FRP) was found to significantly increase the risk of major adverse cardiovascular events (MACE), with an adjusted hazard ratio of 1.12 per 0.01 increment ($P < 0.001$), and individuals with FRP ≥0.63 had a 10.8-fold higher risk of MACE [51].

3.3.3. Single-cell RNA sequencing

Single-cell RNA sequencing has provided deeper insights into the complex cellular landscape of atherosclerotic plaques, revealing extensive cellular heterogeneity [52], identifying multiple endothelial cell types within plaques, some of them acquiring osteoblast, smooth muscle cell, macrophage, or fibroblast-like phenotypes [53,54]. Additionally, VSMCs can exhibit various phenotypes [55,56] with some being linked to either vulnerable or stable plaques. Multiple subpopulations of macrophages [57,58] and T cells [59] have also been discovered, with some playing detrimental roles in atherosclerosis.

3.3.4. Limitations

Despite these advancements, (single cell) RNA sequencing has barely been translated to the clinic due to high costs, methodological challenges, and data complexity. Targeted sequencing (i.e., sequencing of

only a portion of known genes) may be an alternative and has shown promise for clinical application [60]. Cost reduction, standardization and simplification of experimental procedures may help bridge the gap between the use of RNA sequencing for research purposes and clinical application. Automatized analytical and bioinformatics pipelines such as the Firalink pipeline for targeted RNA sequencing analysis [61] could be further developed and applied in routine labs. An expanded discussion of the challenges and solutions for the translation of RNA sequencing to clinical application can be found in Ref. [44]. Combining transcriptomic-based patient stratification methods with other omics approaches and ML may help developing accurate models for primary and secondary prevention.

3.4. Proteomics

Proteomics is the characterization of protein function, structure, location, and quantification of expression in biological samples—often blood plasma or other tissues of interest for a given disease. For CVD diagnostics and treatment leading proteomics methodologies are mass spectrometry, antibody-based Olink and aptamer-based SomaScan platforms. Heterogeneities between the latter two platforms have been described [62]. They require *a priori* knowledge of the several thousand proteins to be measured, as opposed to mass spectrometry (MS) which has limited sensitivity but can quantify an unlimited number of proteins.

3.4.1. Proteomics methodologies and workflows

While each method has distinct strengths and limitations, MS-based proteomics remains the most widely used approach, particularly in discovery-based research settings (see Table 4).

Mass spectrometry workflows generally fall into two categories: untargeted (discovery) and targeted proteomics. Untargeted approaches aim to identify and quantify thousands of proteins without prior selection. In contrast, targeted methods such as Selected Reaction Monitoring (SRM) or Parallel Reaction Monitoring (PRM) are used to quantify predefined sets of proteins with high precision and reproducibility. The standard MS workflow includes protein extraction and digestion, peptide separation by liquid chromatography, followed by ionization and mass detection. This allows for deep proteome coverage and post-translational modification analysis but requires complex sample preparation and advanced computational tools for data analysis.

Affinity-based platforms like Olink use proximity extension assays to detect proteins through dual antibodies linked to DNA oligonucleotides, while SomaScan employs modified aptamers to bind target proteins. Both platforms are high-throughput and require minimal sample volume.

3.4.2. Proteomic risk scores

Within ASCVD specifically, several studies have attempted to use targeted proteomics data to make a Proteomic Risk Score (ProtRS) [63], akin to the PRS [64] used in genomics. In 2016, a 9-protein risk score was developed for predicting secondary events in a coronary artery disease (CAD) population [63]. As high-throughput proteomics technologies developed, these models included dozens of proteins. A 27-protein risk model for secondary events was developed and validated in The Trøndelag Health Study (The HUNT Study) [65] and a ProtRS with 70 proteins was developed in an Icelandic population for primary prevention of ASCVD [66]. This ProtRS provided independent predictive information from the polygenic score and the inclusion of both "omics" predictors provided significant but modest improvement in discrimination over clinical risk factors. A panel of 50 proteins was shown to outperform a clinical risk model in prediction of recurrent ASCVD [67]. However, a 44 protein model was not better than traditional risk factors at predicting subclinical coronary artery calcifications in a primary prevention setting [68] due to most of these proteins being highly correlated with the traditional risk factors. The Olink data on 54,219 UK Biobank participants [69] available through the Pharma Proteomics Project is currently driving the field of predictive proteomics studies across diseases and provides a valuable training set for studies with Olink data. In this dataset, a sparse protein model identifying 20 proteins did not perform better than the clinical model for coronary heart disease [70].

3.4.3. Strengths and limitations

Proteomic predictors are likely to be more accurate closer to disease onset and indeed may be flagging subclinical symptoms before a diagnosis would traditionally be made [68]. It remains to be established whether proteomic predictors can stratify high-risk individuals 5, 10, or even 20 years before disease onset, as is done with current ASCVD risk predictors (e.g. QRISK3, SCORE2). Because primary prevention aims to reduce exposure to high cholesterol over the lifetime [71], the temporality of proteomics may limit its application in ASCVD prediction. Further, proteomics has unique challenges not seen in measurements of nucleic acids due to the diverse posttranslational modifications, high dynamic range of protein concentrations, and the inability to amplify proteins to aid measurement, among other limitations.

3.4.4. Clinical implementation and challenges

To improve the long-term predictive capacity of proteomic models, several strategies are under active investigation. With regards to clinical implementation of predictive proteomics, it is likely that a sparsely predictive proteomics model is the best [72]. This could include one or several proteins that demonstrate high sensitivity and specificity. For example, cardiac troponin (cTn) is a single plasma protein important for the specific diagnosis acute coronary syndrome [73], and also appears to predict mortality in hospital patients [74]. Lipoprotein (a) is another singular protein proven as a causal risk factor for ASCVD that has not been widely adopted clinically [75]. A high dimensional ProtRS requiring a high throughput proteomics assay is unlikely to be financially feasible for population screening purposes but perhaps in the individual diagnostic setting. Technological advancements in protein quantification are necessary for routine annual screening for ASCVD as may be currently implemented with clinical risk predictors and cholesterol measurements. Complementary, multiomic integration—combining proteomic data with genomics, metabolomics, and clinical information—may enhance model robustness and capture complementary aspects of disease risk. Longitudinal proteomics, involving repeated sampling over time, is another promising approach that could detect temporal shifts in protein expression preceding clinical onset. These strategies could help address the current limitations of proteomic temporality in ASCVD risk prediction.

3.5. Metabolomics

Metabolomics is the comprehensive study of metabolites, small molecules typically less than 1 kDa in size, which are involved in metabolic reactions within cells, tissues, or organisms [76]. This field encompasses the analysis of the entire set of aqueous molecules and lipids (the latter often studied under the subfield of lipidomics) present in a biological sample. Metabolomics also investigates the pathways and enzymes responsible for the synthesis, degradation, and modification of these metabolites [3].

Compared to other "omics" techniques like genomics and proteomics, metabolomics presents a higher level of complexity due to the vast structural diversity of metabolites. This diversity includes both simple and complex molecules, resulting in a wide array of aqueous and lipid molecules and their carriers, such as lipoproteins. Consequently, metabolomic analysis, particularly in the realm of lipidomics, poses significant technical challenges.

3.5.1. Metabolomics in the context of ASCVD

In the context of ASCVD, lipidomics has emerged as the most promising predictive metabolomic approach. The ability to identify

specific biomarkers linked to ASCVD has been greatly enhanced by advanced analytical techniques such as liquid chromatography-mass spectrometry (LC-MS) and NMR spectroscopy [77,78]. These methods have proven crucial in tracking changes in lipid species within biological samples. LC-MS combines liquid chromatography for compound separation with mass spectrometry for accurate mass detection, offering high sensitivity and the ability to detect a broad range of metabolites, though it requires complex sample preparation and may suffer from ion suppression effects. LC-MS, for example, can monitor over 400 different lipid species in serum and tissues like the liver and heart, offering robust data on the lipidomic signatures associated with various stages of cardiometabolic diseases [76]. In contrast, NMR spectroscopy provides highly reproducible and quantitative data with minimal sample preparation, and while it has lower sensitivity compared to LC-MS, it allows for non-destructive analysis and precise structural elucidation of metabolites. NMR spectroscopy further enables comprehensive metabolic profiling, including advanced lipoprotein testing that assesses parameters such as lipoprotein size, composition, and particle concentration—all factors closely associated with ASCVD [76]. Additionally, emerging technologies such as mass spectrometry imaging (MSI), which enables spatial mapping of metabolites in tissue sections, and high-resolution accurate mass (HRAM) metabolomics, which provides ultra-precise mass measurements, are gaining traction and expanding the scope of metabolic phenotyping.

Recent studies have identified specific lipid species, such as ceramides, sphingomyelins, and oxidized phospholipids, as key biomarkers associated with increased ASCVD risk[79–81] linked to plaque instability and adverse cardiovascular outcomes. Moreover, lipidomic profiles have been shown to vary across disease states and populations; for instance, individuals with type 2 diabetes mellitus or metabolic syndrome often display altered triglyceride-rich lipoproteins [82].

3.5.2. Strengths

Metabolomics offers several strengths as a predictive tool in the study of diseases like ASCVD. One of its primary advantages is its diagnostic performance; metabolomics can detect subtle changes in metabolic pathways, providing early indicators of disease before clinical symptoms appear [83,84]. Additionally, metabolomics allows for comprehensive analysis. Techniques such as LC-MS and NMR can simultaneously quantify and structurally analyze a wide range of metabolites, offering a broad and detailed perspective on metabolic health [76].

3.5.3. Limitations

However, metabolomics also presents several limitations. One significant challenge is the cost associated with metabolomic analysis. The need for sophisticated instruments and reagents makes the process expensive, which can be a barrier to its widespread application. Throughput is another limitation, as analyzing large sample sizes can be time-consuming, particularly when using techniques like LC-MS. This can impact the scalability of studies. Furthermore, the reproducibility and robustness of metabolomic results can be affected by variability in sample preparation and analysis, especially with LC-MS, potentially limiting the reliability of metabolomics in clinical settings [85]. However, recent advances in instrumentation, such as higher-sensitivity detectors and faster acquisition systems, are helping to reduce analysis time and improve data quality. Moreover, automation in sample processing and the adoption of standardized normalization protocols are increasingly used to enhance reproducibility and scalability across metabolomics studies.

3.6. Multiomic data in the discovery and validation of novel drug targets

Despite large Research and Development (R&D) investments and numerous proof-of-concept studies, the output of new drugs and biomarkers in the CVD space has remained largely static over the past few decades. The widespread use of lipid-lowering and anti-hypertensive medication has contributed to decreasing CVD mortality since their introduction, by approximately 30 % [86]. Thus, there is still a need for deeper understanding of molecular pathways underlying the disease to elucidate novel therapeutic targets and biomarkers.

Key challenges associated with prioritization of therapeutic targets were highlighted in a study by Cook et al. [87], where establishment of a link between the target and the disease *via* genetic causal association coupled to the proof-of-mechanism was shown to be crucial in early stages of drug development to ensure successful clinical translation. Indeed, recent therapeutic approaches in atherosclerosis include reducing the residual risk by targeting IL-1β or IL-6 [57], or decreasing lipoprotein (a) levels [88], which all show a genetic link with the disease [89]. Yet, there is a paucity of assets in clinical development that directly target vessel wall-specific mechanisms, and a very limited number that are at pre-clinical stage. In this respect, local, vessel drug delivery is beneficial because it mitigates safety concerns associated with systemic targeting, e.g., unwanted effect on blood pressure in patients already prescribed extensive medications.

3.6.1. Validation of ASCVD drug targets

Various approaches are emerging to facilitate the discovery of attractive therapeutic targets, based on the growing human CVD multiomics data contained within established global biobanks and in the public dataspace. A defined, comprehensive framework for target assessment could promote informed decision-making, enabling either the determination of targets based on clearly defined go/no-go criteria, or further investment in those projects which demonstrate the attributes and clinical signals that lead to increased confidence. Some studies [90] suggested a 'molecular' 5D framework for early target assessment, dedicated to examining the following points: i) genetic link with the disease, ii) target tissue expression levels *via* transcriptomic and/or proteomic data, iii) target cell type association *via* scRNAseq data, iv) insight into its function and mechanism *via* pathway enrichment analyses, and v) target associations with patient clinical data. This 'molecular' 5D framework was designed to challenge, validate, or invalidate the scientific hypothesis around a certain target at its inception, placing a focus on a strong target rationale [87]. To exemplify their pipeline, Suur BE et al. conducted the first integrative study of the proprotein convertase subtilisin/kexin (PCSK) family in the CVD context, consisting of nine related proteases: PCSK1-7, MBTPS1, and PCSK9. Apart from PCSK9, little was known about other PCSKs in CVD prior to this publication, while the family has been extensively explored in cancers both as biomarkers and therapeutic targets. The Authors applied an integrative approach, combining multiomic data from three vascular biobanks, with patient clinical parameters and immunohistochemistry of vascular biopsies. Results revealed primarily PCSK6, followed by PCSK5, PCSK7 and FURIN, as proprotein convertases with the highest novel therapeutic potential. The limitation of this 'molecular' 5D framework is that it does not directly address potential safety concerns that would be associated with systemic targeting, such as unwanted effects on blood pressure, coagulation, or lipid levels, especially in patients already prescribed appropriate medications. An additional relevant dimension to be considered is the overall IP-status surveyed from patent databases. Also, independent validations across human cohorts, engaging diseases that share common molecular mechanisms, are warranted, and would lead to more effective translation from molecular targets into medicines.

3.6.2. Limitations

The limitation of this 'molecular' 5D framework is that it does not directly address potential safety concerns that would be associated with systemic targeting, such as unwanted effects on blood pressure, coagulation, or lipid levels, especially in patients already prescribed appropriate medications. An additional relevant dimension to be considered is the overall IP-status surveyed from patent databases. Also, independent validations across human cohorts, engaging diseases that share common

molecular mechanisms, are warranted, and would lead to more effective translation from molecular targets into medicines.

3.6.3. Computational models and multiomics data

Prevention of myocardial infarction and ischemic stroke by tailoring treatment for individual patients with atherosclerosis, may be achieved using computational modeling of multiomics data, containing detailed disease-specific information at various analytical scales. Given the multi-factorial biology of atherosclerosis, such modeling is envisioned to capture the complex molecular interactions estimated to drive disease progression.

Despite its promise, the multiomic approach faces significant challenges. These include data heterogeneity, integration, analysis, interpretation, and validation. The high dimensionality, complexity, and diversity of multiomic data introduce substantial computational and statistical obstacles for effective integration and analysis. Moreover, interpreting and validating findings from multiomic studies requires both domain-specific expertise and experimental confirmation [91].

In the context of cardiovascular disease (CVD), the application of multiomics is still in its early stages. Although it holds great potential, it has not yet led to the development of novel clinical therapies or risk prediction tools. In contrast, multiomic integration has been more extensively applied in oncology. For example, it has enabled the development of deep learning frameworks to predict survival and drug response in breast cancer [92].

3.6.4. Simulation of drug response

In silico simulation of drug response has been another important area of development, with applications including evaluation of side effects, consideration of drug combinations, assessment of drug mechanism, and prediction modeling for drug re-purposing [93]. Some vascular models have tried to utilize biomechanical computations to simulate physical behavior and interaction between vessel tissue and blood, using idealized or patient-specific vessel geometries [94].

3.6.5. Models of plaque instability

In atherosclerosis, it has been observed that, to obtain sufficiently granular information, incorporating molecular analysis is necessary and requires an appreciation of the numerous structural and biological features characterizing the unstable lesions [95]. To this end, clinically relevant transcriptomics- and proteomics-informed, systems biology models of atherosclerotic plaque instability coupled with publicly curated networks of molecular pathways have been developed, and even calibrated, with individual patient data [95,96]. The models' potential was evaluated by simulating the effects of different pharmacological treatments on molecular processes relevant to stabilizing atherosclerotic lesions, optimized for personal pharmacotherapy. The study suggested that predicting individualized pharmacological effects may be possible, highlighting a potential for tailoring therapy as part of future strategies to prevent adverse events.

3.6.6. Novel mechanisms, risk prediction, and precision medicine

Regarding novel mechanistic insights, spatial omics techniques have recently revealed key processes such as lymphocyte recruitment *via* ACKR1-expressing endothelial cells of the vasa vasorum, migration of VSMCs transforming into fibromyocytes, and intricate cell–cell interactions in plaque regions. These findings highlight complex cellular dynamics within the adventitia and subendothelial space in human atherosclerosis [97]. In terms of risk prediction, recent multiomic studies have validated the clinical potential of PRSs [98]. Additionally, integration of demographic, clinical, and molecular data with imaging—using neural networks and hierarchical clustering—has enabled identification of distinct atherosclerosis endotypes, advancing precision medicine approaches [99]. The field of multiomics and multi-modal data integration is evolving rapidly. It is likely that previously unrecognized targets and pathways from recent studies will soon be investigated further by academic and industry players through proof-of-concept studies, paving the way for translation into clinical practice.

3.6.7. Data sharing

With a vast amount of cardiovascular multiomic data present in the existing biobanks and public dataspace, a progressive program can be envisioned for systematic discovery, refinement and validation of various biomarker and drug targets. Capabilities for systematic, computational ASCVD simulation are also rapidly changing, based on the accelerated implementation of AI/ML assisted tools. Such full-scale usage of multi-modal data per patient will be essential in deploying effective personalized approaches for atherosclerosis risk prediction. These efforts can lead to significant societal benefits in terms of efficacy and patient follow-up but are currently dependent on the considerable efforts invested by the research community to develop safe federated tools for health-care data sharing and integrated analyses across borders. Ongoing efforts in this area include large transatlantic consortia, such as the NextGen project (https://www.nextgentools.eu), funded by the European Union's HORIZON-HLTH-TOOL program. This initiative involves over 20 academic and clinical institutions, along with non-profit organizations and small-to-medium enterprises. These consortia bring together multiple biobanks containing cardiovascular disease (CVD) omics data with the goal of: 1) Developing tools for the

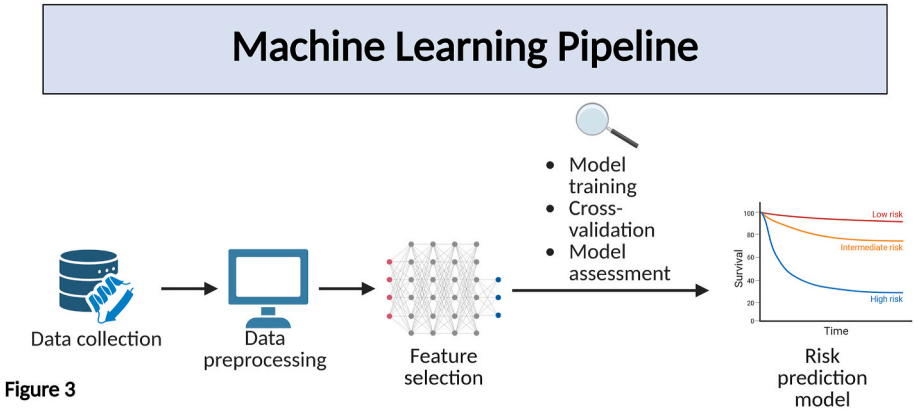


Fig. 3. Machine learning pipeline
Typical steps of a machine learning pipeline. First step is data collection. This could for example be multiomics data. Next step is preprocessing, then feature selection (selecting a subset of relevant features for use in model construction). This is followed by model training, cross-validation, and model assessment. Finally, this will result in a new risk prediction model.

prediction, prevention, diagnosis, monitoring, and treatment of patients; 2) Enhancing the use of genomic data through advanced integration and workflow solutions; 3) Creating data analytics platforms and harmonized infrastructure across sites to support federated data demonstration. A key objective of these initiatives is to foster trust among stakeholders—both partners and the public—by adopting a "human-in-the-loop" approach and proactively addressing ethical, legal, and societal challenges. Specifically, the NextGen governance framework and its robust regulatory processes are designed to enable secure, multi-jurisdictional access to phenotypic and genomic data, in alignment with broader European initiatives such as "1+ Million Genomes" and the European Health Data Space.

3.7. Integration of machine learning with multiomic data for the clinical management of ASCVD

Omics technologies, including genomics, transcriptomics, epigenomics, proteomics, and metabolomics, provide complementary insights into the molecular mechanisms underlying the development and progression of ASCVD. When analyzed together, these data layers form the foundation of multiomics, a comprehensive framework that enables the identification of complex biological interactions and regulatory pathways that would be missed by single-omics approaches. Integrated with clinical information, multiomic data can support precise risk stratification and the discovery of novel therapeutic targets [100]. ML, a class of algorithms capable of identifying patterns and generating predictions from large and complex datasets, offers powerful tools for extracting meaningful insights from multiomic data. Its application to ASCVD is particularly relevant, given the multifactorial nature of the condition, which reflects interactions between genetic, environmental, and lifestyle factors.

ML methods are well-suited to managing the complexity and volume of multiomic data. These algorithms are particularly effective in deciphering biological systems by capturing non-linear interactions within molecular networks and identifying subtle patterns across diverse data types [60,101,102]; patterns that may not be detectable using traditional statistical approaches. ML techniques also support the discovery of complex relationships between molecular markers and individual-level factors such as sociodemographic, clinical, and pharmacological characteristics [103]. Commonly used ML algorithms include random forests, decision tree, gradient boosting, and support vector machines (SVM) [104]. Deep learning models have shown promise for both feature extraction and predictive modeling [105].

3.7.1. ML pipelines

A typical ML pipeline begins with data collection followed by data preprocessing, which includes normalization, correction for batch effects, and imputation of missing values. Feature selection is then applied to avoid overfitting and improve model interpretability (Fig. 3). Methods such as Boruta and clustering of variables (CoV) are commonly used. Dimensionality reduction methods such as multiple coinertia analysis (MCIA), sparse generalized canonical correlation analysis (sGCCA) and STATIS (structuration des Tableaux á Trois Indices de la Statistique) help represent multiple high-dimensional datasets in lower-dimensional spaces while preserving critical information [106].

Once preprocessing is complete, ML models are trained to perform specific tasks such as classification, regression, clustering, or risk prediction. Model performance is typically assessed through cross-validation and, when possible, validated on independent external cohorts. To improve clinical utility and transparency, interpretability tools such as SHAP (Shapley Additive Explanations) values and attention mechanisms are increasingly used [107], allowing to better understand which features contribute most to the model predictions.

3.7.2. Strengths

A key strength of ML approaches lies in their ability to integrate heterogeneous data sources and accommodate the inherent variability seen in ASCVD. Specific ML-based multiomics tools, such as MOFA (Multiomics Factor Analysis), DeepMO or DIABLO, facilitate the simultaneous analysis of multiple omics layers [108,109]. Widely used platforms for implementing these methods include general-purpose ML libraries such as scikit-learn and TensorFlow, as well as domain-specific tools like Bioconductor (R), which offer accessible frameworks for model development, evaluation, and visualization.

3.7.3. Challenges

Despite these promising advances, the translation of ML-based multiomic approaches into clinical practice still faces several technical and ethical challenges including: A) High dimensionality vs. limited sample size: Omics datasets often contain thousands of features (e.g., genes, proteins) but relatively few samples, increasing the risk of overfitting and reducing model generalizability; B) Data heterogeneity: Combining different omics layers (genomics, transcriptomics, proteomics, etc.) involves harmonizing diverse data types, scales, and distributions, which is non-trivial for standard ML algorithms [104]; C) Noise and missing data: Omics measurements can be noisy or incomplete, requiring robust preprocessing, imputation methods, and feature selection strategies to ensure model stability; D) Interpretability: Many ML models, especially deep learning architectures, function as black boxes. This makes it challenging to extract biologically meaningful insights from predictions; E) Computational cost: Training complex models on large-scale, multiomic data demands significant computational resources, including memory, processing power, and storage.

Reproducibility and transparency are also ongoing concerns, particularly in clinical settings. Ethical considerations must not be overlooked, including issues related to data privacy, informed consent, and algorithmic fairness [110]. Biases may arise when underrepresented ethnic or socioeconomic groups are excluded from training data, potentially leading to disparities in model performance and clinical outcomes. Strategies to mitigate these risks include data augmentation, oversampling of minority classes, and the implementation of fairness-aware ML algorithms. Additionally, federated learning approaches have been developed to enable decentralized model training, thereby protecting patient privacy, and promoting equitable access to ML-driven tools. The development of robust governance frameworks and the integration of explainable ML techniques will be critical to ensure responsible, trustworthy adoption in real-world healthcare settings.

3.7.4. Clinical application

Overall, the integration of multiomic analyses with ML methods has shown promising potential in the development of clinical prediction models, the elucidation of disease mechanisms, and the identification of therapeutic targets [111,112]. In the cardiovascular field, Reel et al. [113] applied ML to classify subtypes of endocrine hypertension in hypertensive patients using multidimensional multiomic data. Employing eight ML classifiers, they developed models based on 409 features, including plasma miRNAs, metabolites, and steroids, and demonstrated that the multiomic approach outperformed single-omics analyses by accurately identifying diverse forms of endocrine hypertension with high sensitivity and specificity. Several additional studies further underscore the clinical and mechanistic value of multiomics. Lau et al. [114] showed that integrating transcript abundance, protein abundance, and protein turnover in a mouse model of pathological cardiac hypertrophy led to a 75 % increase in the discovery of disease-associated genes across multiple genetic backgrounds. This study also highlighted the ability of protein turnover data to reveal post-transcriptional regulation and uncover novel disease pathways. In patients with advanced heart failure supported by left ventricular assist devices (LVADs), transcriptomic and phosphoproteomic profiling of cardiac tissue identified a distinct molecular signature capable of distinguishing patients with potential for myocardial recovery [115]. The integrated approach

revealed key regulatory pathways, including cell cycle control and extracellular matrix remodeling, with implications for patient stratification and therapeutic targeting. Similarly, in end-stage nonischemic dilated cardiomyopathy, the combination of metabolomics, transcriptomic, and proteomics from human cardiac tissue and plasma revealed extensive metabolic reprogramming [116]. The study identified critical alterations in energy metabolism, such as ATP depletion, impaired fatty acid import, and suppression of the tricarboxylic acid cycle, providing new insights into the metabolic underpinnings of heart failure. This research underscores the transformative potential of ML-powered multiomics to drive both mechanistic understanding and precision medicine in ASCVD.

In summary, the integration of ML with multiomic data represents a promising strategy to improve our understanding and clinical management of ASCVD. These approaches enable the development of highly personalized risk prediction models, offering the potential for earlier diagnosis, targeted prevention, and improved patient outcomes. While important technical and ethical challenges persist, ongoing innovation in ML techniques and data integration frameworks will be key to unlocking the full potential of multiomics in cardiovascular research and care.

3.8. Integration of environmental and lifestyle factors in multiomics models

The integration of environmental and lifestyle factors in predictive multiomics models of ASCVD has, to our knowledge, not yet been tried. However, lifestyle factors such as diet along with data on microbiome, anthropometric measures, and blood tests has been incorporated in models using AI/ML to predict glycemic responses to a meal [117]. It is likely that a similar model could be used to include diet and other lifestyle factors in multiomic models for prediction of ASCVD.

Likewise, efforts have been made to measure the exposome (the environmental exposures that an individual encounters throughout life) [118]. Although methods for integrating this data is still in very early phases there would be future possibilities for incorporating exposome data in multiomics models used for ASCVD prediction. Further, social factors has been shown to affect epigenetic markers such as DNA methylation [119], and incorporation of these in multiomic models of ASCVD could be another step towards personalized medicine.

3.9. Perspectives

3.9.1. ML/AI, imaging- and omics data in ASCVD

While AI and ML methods are currently mostly used in research settings, it is becoming increasingly clear that they have great potential for clinical application, also outside of omics analysis, as these are still rarely applied in clinical settings. A diagnostic field with a great potential for ML/AI approaches are various forms of image analysis. Diagnosis of atherosclerotic disease states primarily relies on various imaging techniques, starting from ultrasound-based analysis of the carotid artery (often used as a low-cost method to assess the general state of atherosclerosis) - continuing with coronary computed tomography angiography (CCTA) and magnetic resonance imaging (MRI). More specific diagnostics include coronary artery calcium (CAC) scans or invasive techniques such as optical coherence tomography (OCT) or intravascular ultrasound (IVUS) analysis. AI/ML-based image analysis has already proven to outperform clinicians in other fields such as the diagnosis of skin cancer [120]. Similar progress is being made in CVD [121], and also specifically for imaging of atherosclerosis [122,123], where AI approaches are used to better detect subtle changes in all types of image data. The application of AI/ML can even be extended to the analysis of ECG [124] or pulse-wave-velocity data for evaluation of arterial stiffness [125].

Omics and imaging offer distinct but complementary insights into cardiovascular (CV) risk, reflecting molecular predisposition and structural disease burden, respectively. Omics approaches—including genomics, transcriptomics, proteomics, and metabolomics—enable deep profiling of the biological processes underlying atherosclerosis and other CVDs. Tools such as polygenic risk scores allow for early, lifelong risk stratification, capturing predisposition long before clinical symptoms or detectable lesions emerge. These molecular signatures are particularly valuable for personalized prevention and identifying individuals who may benefit from intensified surveillance.

In contrast, imaging modalities such as coronary artery calcium (CAC) scoring, carotid intima-media thickness (cIMT), and coronary CT angiography provide direct visualization of subclinical disease, making them powerful tools for refining short-to medium-term risk, especially in individuals with uncertain profiles. While omics offers biological granularity and upstream insight, imaging delivers real-time assessment of disease expression. Their integration holds promise for enhancing predictive accuracy and informing tailored interventions. Importantly, omics may serve as a filter to identify individuals at elevated molecular risk who could benefit from targeted imaging to detect subclinical disease—thus bridging early risk detection with actionable therapeutic decision-making.

3.9.2. Multiomics in single cell analyses

Various multiomics analyses of plasma and circulating cells, in combination with advanced computational analysis techniques, have shown great potential for diagnostics and personalized therapy decisions [126]. On the one hand, plasma can be analyzed by proteomics or lipidomics [127,128] using mass spectrometry or NMR, providing quantitative information for thousands of analytes at a cost comparable to standard MRI (while routine blood analysis covers only a handful of parameters). On the other hand, DNA, RNA, and proteins can also be analyzed and quantified from circulating cells (leukocytes, platelets, or erythrocytes), including even epigenetic analysis of leukocyte chromatin accessibility [129]. While these cells are readily accessible in blood samples, cells of the vasculature such as endothelial or smooth muscle cells (as well as plaque leukocytes) are not directly present in collected blood. However, their signatures can often be detected with sufficient precision by analyzing the contents of extracellular vesicles released into the circulation [130]. Based on single cell analyses [131] of atherosclerotic lesions or material from patients with CVD, we know that multiple cellular transitions and changes occur that are not monitored in current routine analysis, but which may be of central importance in the progression of pre-disease states and the sudden transition from a sub-clinical phenotype to a clinically manifested life-threatening state such as myocardial infarction. Computational analysis is required to find the specific patterns in these rich data sets, and this is where machine learning approaches can be helpful. In the future, multiomics-based analyses could be routinely used to enable precision diagnostics and personalized precision medicine including professional prevention and therapy decisions.

4. Discussion

The summarized evidence in this review suggests that approaching ASCVD from different molecular angles using multiomics data may help us take a step ahead for accurate diagnostics and treatment of subjects/patients with ASCVD/ACS or risk of these pathological conditions (Fig. 2). While much data is available from experimental and clinical research, a major gap that still needs filling is the lack of robust and standardized clinical applications of multiomics data. A major effort is thus necessary to bring these powerful new data and technologies to the clinical field. Further support will be offered by the generation of clinically applicable/approved AI/ML algorithms able to translate large datasets into valuable information for accurate precision medicine approaches.

CRediT authorship contribution statement

Liv Tybjærg Nordestgaard: Conceptualization, Writing – original draft, Visualization, Writing – review & editing. **Brooke N. Wolford:** Writing – original draft, Writing – review & editing. **David de Gonzalo-Calvo:** Writing – original draft, Writing – review & editing. **Miron Sopić:** Writing – original draft, Writing – review & editing. **Yvan Devaux:** Writing – original draft, Writing – review & editing. **Ljubica Matic:** Writing – original draft, Writing – review & editing. **Stephanie Bezzina Wettinger:** Writing – original draft, Writing – review & editing. **Johannes A. Schmid:** Writing – original draft, Writing – review & editing. **Núria Amigó:** Writing – original draft, Writing – review & editing. **Lluís Masana:** Visualization, Writing – review & editing. **Alberico L. Catapano:** Writing – review & editing. **Dimitris Kardassis:** Writing – original draft, Writing – review & editing. **Paolo Magni:** Writing – original draft, Writing – review & editing.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) used ChatGPT in order to get an overview of sequencing technologies used in genomics and epigenomics. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Financial support

LTN was supported by the Research Council at the Capital Region of Denmark and Independent Research Fund Denmark grant ID: 10.46540/3100-00007B. BNW has received funding from the European Union's Horizon Europe research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101110878." DdGC has received financial support from Instituto de Salud Carlos III (Miguel Servet 2020: CP20/00041) co-funded by the European Union. CIBERES (CB07/06/2008) is an initiative of the Instituto de Salud Carlos III. MS is funded by the European Union (HORIZON-MSCA-2021-PF- MAACS 101064175; HORIZON-MSCA-2021-SE-01-01 - MSCA Staff Exchanges 2021 CardioSCOPE 101086397) and the Ministry of Science, Technological Development, and Innovation, Republic of Serbia through Grant Agreement with University of Belgrade-Faculty of Pharmacy No: 451-03-47/2024-01/200161. Y.D. is funded by the EU Horizon 2020 project COVIRNA (grant agreement # 101016072), the National Research Fund (grants #C14/BM/8225223, C17/BM/11613033 and COVID-19/2020-1/14719577/miRCOVID), the Ministry of Higher Education and Research, and the Heart Foundation-Daniel Wagner of Luxembourg. LM is the recipient of fellowships and awards from the Swedish Research Council [VR, 2023–02724, 2019–02027], Karolinska Institute Consolidator program, Swedish Heart-Lung Foundation [HLF, 20240094, 20230357, 20210466, 20200621, 20200520, 20180244, 20180247, 201602877], Swedish Society for Medical Research [SSMF, P13-0171]. SBW is supported by HORIZON EIC 2022 PATHFINDER CHALLENGE CARDIOGENOMICS Project 101114924 TargetMI and HORIZON-WIDERA-2022-TALENTS-01 Project 101086768 BioGeMT both being funded by the European Union. JAS is funded by the Austrian Science Fund (FWF, project I-5215) and the Austrian Research Promotion Agency (FFG, project 895307). DK is supported by the Hellenic Foundation for Research and Innovation (HFRI) Research Program "Funding Projects in Leading-Edge Sectors – RRFQ: Basic Research Financing (Horizontal support for all Sciences) grant No 15529 and MSCA Staff Exchanges program CardioSCOPE Grant No 101086397. PM is supported in part by the European Union (AtheroNET COST Action CA21153; HORIZON-MSCA-2021-SE-01-01 - MSCA Staff Exchanges 2021 CardioSCOPE 101086397) and by the Italian Space Agency (ASI; N. 2023-7-HH.0 CUP F13C23000050005 MicroFunExpo).

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Y.D. holds patents and licensing agreements related to the use of RNAs for diagnostic and therapeutic purposes and is SAB member of Firalis SA. None of the other authors have any conflicts of interests.

Acknowledgements

This article is based upon work from AtheroNET COST Action CA21153 (www.atheronet.eu) funded by COST (European Cooperation in Science and Technology). Figures were created with Biorender.com.

References

[1] Abbafati C, Abbas KM, Abbasi-Kangevari M, Abd-Allah F, Abdelalim A, Abdollahi M, et al. Global burden of 87 risk factors in 204 countries and territories, 1990–2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020;396:1223–49.
[2] Visseren F, Mach F, Smulders YM, Carballo D, Koskinas KC, Bäck M, et al. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice. Eur Heart J 2021;42:3227–337.
[3] Nayor M, Brown KJ, Vasan RS. The molecular basis of predicting atherosclerotic cardiovascular disease risk. Circ Res 2021;128:287–303.
[4] Richardson TG, Leyden GM, Wang Q, Bell JA, Elsworth B, Smith GD, et al. Characterising metabolomic signatures of lipid-modifying therapies through drug target mendelian randomisation. PLoS Biol 2022;20:1–17. https://doi.org/10.1371/journal.pbio.3001547.
[5] Trinder M, Uddin MM, Finneran P, Aragam KG, Natarajan P. Clinical utility of lipoprotein(a) and LPA genetic risk score in risk prediction of incident atherosclerotic cardiovascular disease. JAMA Cardiol 2021;6:287–95.
[6] Lee S, Akioyamen LE, Aljenedil S, Rivière JB, Ruel I, Genest J. Genetic testing for familial hypercholesterolemia: impact on diagnosis, treatment and cardiovascular risk. Eur J Prev Cardiol 2019;26:1262–70.
[7] Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG, Tybjærg-Hansen A. Loss-of-Function mutations in APOC3 and risk of ischemic vascular disease. N Engl J Med 2014;371:32–41.
[8] Kersten S. ANGPTL3 as therapeutic target. Curr Opin Lipidol 2021;32:335–41.
[9] Kersten S. Role and mechanism of the action of angiopoietin-like protein ANGPTL4 in plasma lipid metabolism. J Lipid Res 2021;62:100150. https://doi.org/10.1016/j.jlr.2021.100150.
[10] Sturm AC, Truty R, Callis TE, Aguilar S, Esplin ED, Garcia S, et al. Limited-variant screening vs comprehensive genetic testing for familial hypercholesterolemia diagnosis. JAMA Cardiol 2021;6:902–9.
[11] Mega JL, Stitziel NO, Smith JG, Chasman DI, Caulfield MJ, Devlin JJ, et al. Genetic risk, coronary heart disease events, and the clinical benefit of statin therapy: an analysis of primary and secondary prevention trials. Lancet 2015;385:2264–71.
[12] Marston NA, Kamanu FK, Nordio F, Gurmu Y, Roselli C, Sever PS, et al. Predicting benefit from evolocumab therapy in patients with atherosclerotic disease using a genetic risk score results from the FOURIER trial. Circulation 2020;141:616–23.
[13] Khera AV, Chaffin M, Aragam KG, Haas ME, Choi SH, Natarajan P, et al. Genome-wide polygenic scores for common diseases identify individuals. Nat Genet 2019;50:1219–24.
[14] Inouye M, Abraham G, Nelson CP, Wood AM, Sweeting MJ, Dudbridge F, et al. Genomic risk prediction of coronary artery disease in 480,000 adults: implications for primary prevention. J Am Coll Cardiol 2018;72:1883–93.
[15] O'Sullivan JW, Raghavan S, Marquez-Luna C, Luzum JA, Damrauer SM, Ashley EA, et al. Polygenic risk scores for cardiovascular disease: a scientific statement from the American heart association. Circulation 2022;146:E93–118.
[16] Vassy JL, Posner DC, Ho YL, Gagnon DR, Galloway A, Tanukonda V, et al. Cardiovascular disease risk assessment using traditional risk factors and polygenic risk scores in the million veteran program. JAMA Cardiol 2023;8:564–74.
[17] Mars N, Koskela JT, Ripatti P, Kiiskinen TTJ, Havulinna AS, Lindbohm JV, et al. Polygenic and clinical risk scores and their impact on age at onset and prediction of cardiometabolic diseases and common cancers. Nat Med 2020;26:549–57.
[18] Wald NJ, Old R. The illusion of polygenic disease risk prediction. Genet Med 2019;21:1705–7. https://doi.org/10.1038/s41436-018-0418-5.
[19] Marquez-Luna C, Loh P-R, , Consortium S asian type 2 diabetes, Consortium TST 2 D, Price AL. Multi-ethnic polygenic risk scores improve risk prediction in diverse populations. Genet Epidemiol 2017;48:811–23.
[20] Marnetto D, Pärna K, Läll K, Molinaro L, Montinaro F, Haller T, et al. Ancestry deconvolution and partial polygenic score can improve susceptibility predictions in recently admixed individuals. Nat Commun 2020;11:1–9. https://doi.org/10.1038/s41467-020-15464-w.
[21] Surakka I, Wolford BN, Ritchie SC, Hornsby WE, Sutton NR, Elvenstad Gabrielsen M, et al. Sex-specific survival bias and interaction modeling in coronary artery disease risk prediction. Circ Genomic Precis Med. 2023;16:E003542.

[22] Kullo IJ, Jouni H, Austin EE, Brown SA, Kruisselbrink TM, Isseh IN, et al. Incorporating a genetic risk score into coronary heart disease risk estimates: effect on low-density lipoprotein cholesterol levels (the MI-GENES Clinical Trial). Circulation 2016;133:1181–8.
[23] Goldstein BA, Knowles JW, Salfati E, Ioannidis JPA, Assimes TL. Simple, standardized incorporation of genetic risk into non-genetic risk prediction tools for complex traits: coronary heart disease as an example. Front Genet 2014;5:1–9.
[24] Aragam KG, Dobbyn A, Judy R, Chaffin M, Chaudhary K, Hindy G, et al. Limitations of contemporary guidelines for managing patients at high genetic risk of coronary artery disease. J Am Coll Cardiol 2020;75:2769–80.
[25] Schunkert H, Angelantonio E Di, Inouye M, Patel RS, Ripatti S, Mcevoy JW, et al. Clinical utility and implementation of polygenic risk scores for predicting cardiovascular disease A clinical consensus statement of the ESC Council on Cardiovascular Genomics , the ESC Cardiovascular Risk Collaboration , and the European Association of Preventive Cardiology. Eur Heart J 2025;46:1372–83. https://doi.org/10.1093/eurheartj/ehae649.
[26] Zhao LY, Song J, Liu Y, Song CX, Yi C. Mapping the epigenetic modifications of DNA and RNA. Protein Cell 2020;11:792–808. https://doi.org/10.1007/s13238-020-00733-7.
[27] Tang H, Zeng Z, Shang C, Li Q, Liu J. Epigenetic regulation in pathology of atherosclerosis: a novel perspective. Front Genet 2021;12:1–7.
[28] Schiano C, Trama U, Coscioni E, Infante T, Coppola A, de Nigris F, et al. Epitranscriptome: a novel regulatory layer during atherosclerosis progression. Curr Med Chem 2024;31.
[29] Miroshnikova VV, Panteleeva AA, Pobozheva IA, Razgildina ND, Polyakova EA, Markov AV, et al. ABCA1 and ABCG1 DNA methylation in epicardial adipose tissue of patients with coronary artery disease. BMC Cardiovasc Disord 2021;21:1–12.
[30] Ghaznavi H, Mahmoodi K, Soltanpour MS. A preliminary study of the association between the ABCA1 gene promoter DNA methylation and coronary artery disease risk. Mol Biol Res Commun 2018;7:59–65.
[31] Mohammadpanah M, Heidari MM, Khatami M, Hadadzadeh M. Relationship of hypomethylation CpG islands in interleukin-6 gene promoter with IL-6 mRNA levels in patients with coronary atherosclerosis. J Cardiovasc Thorac Res 2020;12:221–8. https://doi.org/10.34172/jcvtr.2020.37.
[32] Willmer T, Mabasa L, Sharma J, Muller CJF, Johnson R. Blood-based DNA methylation biomarkers to identify risk and progression of cardiovascular disease. Int J Mol Sci 2025;26:1–34.
[33] Zaina S, Heyn H, Carmona FJ, Varol N, Sayols S, Condom E, et al. DNA methylation map of human atherosclerosis. Circ Cardiovasc Genet 2014;7:692–700.
[34] Fu Y, Timp W, Sedlazeck FJ. Computational analysis of DNA methylation from long-read sequencing. Nat Rev Genet 2025:1–15. https://doi.org/10.1038/s41576-025-00822-5.
[35] Yan Y, Chai X, Liu J, Wang S, Li W, Huang T. DeepMethyGene : a deep - learning model to predict gene expression using DNA methylations. BMC Bioinf 2025. https://doi.org/10.1186/s12859-025-06115-2.
[36] Yan F, Telonis AG, Yang Q, Jiang L, Garrett FE, Sekeres MA, et al. Genome- - wide methylome modeling via generative AI incorporating long- - and short- - range interactions. 2025.
[37] Greißel A, Culmes M, Burgkart R, Zimmermann A, Eckstein HH, Zernecke A, et al. Histone acetylation and methylation significantly change with severity of atherosclerosis in human carotid plaques. Cardiovasc Pathol 2016;25:79–86. https://doi.org/10.1016/j.carpath.2015.11.001.
[38] Shi H, Ossip DJ, Mayo NL, Lopez DA, Block RC, Post WS, et al. Role of DNA methylation on the association between physical activity and cardiovascular diseases: results from the longitudinal multi-ethnic study of atherosclerosis (MESA) cohort. BMC Genom 2021;22:1–13.
[39] Lu AT, Quach A, Wilson JG, Reiner AP, Aviv A, Raj K, et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. Aging (Albany NY) 2019;11:303–27.
[40] Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. Nat Rev Genet 2018;19:371–84. https://doi.org/10.1038/s41576-018-0004-3.
[41] Baccarelli A, Ghosh S. Environmental exposures, epigenetics and cardiovascular disease. Curr Opin Clin Nutr Metab Care 2012;15:323–9.
[42] Cech TR, Steitz JA. The noncoding RNA revolution - trashing old rules to forge new ones. Cell 2014;157:77–94. https://doi.org/10.1016/j.cell.2014.03.008.
[43] Nossent AY, Binder CJ. Noncoding RNAs in atherosclerosis. Atherosclerosis 2023;374:21–3.
[44] Sopić M, Karaduzovic-Hadziabdic K, Kardassis D, Maegdefessel L, Martelli F, Meerson A, et al. Transcriptomic research in atherosclerosis: unravelling plaque phenotype and overcoming methodological challenges. J Mol Cell Cardiol 2023;6:100048.
[45] Steenman M, Espitia O, Maurel B, Guyomarch B, Heymann MF, Pistorius MA, et al. Identification of genomic differences among peripheral arterial beds in atherosclerotic and healthy arteries. Sci Rep 2018;8:1–12.
[46] Perisic L, Aldi S, Sun Y, Folkersen L, Razuvaev A, Roy J, et al. Gene expression signatures, pathways and networks in carotid atherosclerosis. J Intern Med 2016;279:293–308.
[47] Jin H, Mees BME, Biessen EAL, Sluimer JC. Transcriptional sex dimorphism in human atherosclerosis relates to plaque type. Circ Res 2021;129:1175–7.
[48] Mokry M, Boltjes A, Slenders L, Bel-bordes G, Cui K, Mekke JM, et al. and clinical presentation. 2023;1:1140–1155.
[49] Rykaczewska U, Zhao Q, Saliba-Gustafsson P, Lengquist M, Kronqvist M, Bergman O, et al. Plaque evaluation by ultrasound and transcriptomics reveals BCLAF1 as a regulator of smooth muscle cell lipid transdifferentiation in atherosclerosis. Arterioscler Thromb Vasc Biol 2022;42:659–76.
[50] Gillies RJ, Kinahan PE, Hricak H. Radiomics: images are more than pictures, they are data. Radiology 2016;278:563–77.
[51] Oikonomou EK, Williams MC, Kotanidis CP, Desai MY, Marwan M, Antonopoulos AS, et al. A novel machine learning-derived radiotranscriptomic signature of perivascular fat improves cardiac risk prediction using coronary CTangiography. Eur Heart J 2019;40:3529–43.
[52] De Winther MPJ, Bäck M, Evans P, Gomez D, Goncalves I, Jørgensen HF, et al. Translational opportunities of single-cell biology in atherosclerosis. Eur Heart J 2023;44:1216–30. https://doi.org/10.1093/eurheartj/ehac686.
[53] Li Y, Ren P, Dawson A, Vasquez HG, Ageedi W, Zhang C, et al. Single-cell transcriptome analysis reveals dynamic cell populations and differential gene expression patterns in control and aneurysmal human aortic tissue. Circulation 2020;142:1374–88.
[54] Hu Z, Liu W, Hua X, Chen X, Chang Y, Hu Y, et al. Single-cell transcriptomic atlas of different human cardiac arteries identifies cell types associated with vascular physiology. Arterioscler Thromb Vasc Biol 2021;41:1408–27.
[55] Dobnikar L, Taylor AL, Chappell J, Oldach P, Harman JL, Oerton E, et al. Disease-relevant transcriptional signatures identified in individual smooth muscle cells from healthy mouse vessels. Nat Commun 2018;9. https://doi.org/10.1038/s41467-018-06891-x.
[56] Alencar GF, Owsiany KM, Karnewar S, Sukhavasi K, Mocci G, Nguyen AT, et al. Stem cell pluripotency genes Klf4 and Oct4 regulate complex SMC phenotypic changes critical in late-stage atherosclerotic lesion pathogenesis. Circulation 2020;142:2045–59.
[57] Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med 2017;377:1119–31.
[58] Park I, Goddard ME, Cole JE, Zanin N, Lyytikäinen LP, Lehtimäki T, et al. C-type lectin receptor CLEC4A2 promotes tissue adaptation of macrophages and protects against atherosclerosis. Nat Commun 2022;13:1–17.
[59] Fernandez DM, Rahman AH, Fernandez NF, Chudnovskiy A, Amir E ad D, Amadori L, et al. Single-cell immune landscape of human atherosclerotic plaques. Nat Med. 2019;25:1576–88.
[60] Devaux Y, Zhang L, Lumley AI, Karaduzovic-Hadziabdic K, Mooser V, Rousseau S, et al. Development of a long noncoding RNA-based machine learning model to predict COVID-19 in-hospital mortality. Nat Commun 2024;15.
[61] Chauviere L, Hoffbeck L, Shoaib M, Tessier F, Firat H, Satagopam V, et al. Firalink: a bioinformatics pipeline for long non-coding RNA data analysis. Non-coding RNA Res 2023;8:602–4.
[62] Ferkingstad E, Sulem P, Atlason BA, Sveinbjornsson G, Magnusson MI, Styrmisdottir EL, et al. Large-scale integration of the plasma proteome with genetics and disease. Nat Genet 2021;53:1712–21.
[63] Ganz P, Heidecker B, Hveem K, Jonasson C, Kato S, Segal MR, et al. Development and validation of a protein-based risk score for cardiovascular outcomes among patients with stable coronary heart disease. JAMA, J Am Med Assoc 2016;315:2532–41.
[64] Lambert SA, Gil L, Jupp S, Ritchie SC, Xu Y, Buniello A, et al. The Polygenic Score Catalog as an open database for reproducibility and systematic evaluation. Nat Genet 2021;53:420–5.
[65] Williams SA, Ganz P. A proteomic surrogate for cardiovascular outcomes that is sensitive to multiple mechanisms of change in risk. Sci Transl Med 2022;14.
[66] Helgason H, Eiriksdottir T, Ulfarsson MO, Choudhary A, Lund SH, Ivarsdottir EV. Evaluation of large-scale proteomics for prediction of cardiovascular events. JAMA 2023:725–35.
[67] Nurmohamed NS, Pereira JPB, Hoogeveen RM, Kroon J, Kraaijenhof JM, Waissi F. Targeted proteomics improves cardiovascular risk prediction in secondary prevention: the impact of statin treatment? Eur Heart J 2022;43:3811.
[68] Royer P, Björnson E, Adiels M, Álvez MB, Fagerberg L, Bäckhed F, et al. Plasma proteomics for prediction of subclinical coronary artery calcifications in primary prevention. Am Heart J 2024;271:55–67. https://doi.org/10.1016/j.ahj.2024.01.011.
[69] Sun BB, Chiou J, Traylor M, Benner C, Hsu YH, Richardson TG, et al. Plasma proteomic associations with genetics and health in the UK Biobank. Nature 2023;622:329–38.
[70] Carrasco-Zanini J, Pietzner M, Davitte J, Surendran P, Croteau-Chonka DC, Robins C, et al. Proteomic signatures improve risk prediction for common and rare diseases. Nat Med 2024;30.
[71] Grundy SM, Feingold KR. Guidelines for the management of high blood cholesterol. Endotext; 2000. http://www.ncbi.nlm.nih.gov/pubmed/26247090.
[72] Carrasco-zanini J, Pietzner M, Davitte J, Croteau-chonka DC, Robins C, Torralbo A, et al. Proteomic predic , on of common and rare diseases precision healthcare university research Institute, vols. 1–21. Queen Mary University of London , Computational Medicine , Berlin Institute of Health at Charité-Universitätsmedizin Berlin , National Institute for Hea; 2023.
[73] Mahajan VS, Jarolim P. How to interpret elevated cardiac troponin levels. Circulation 2011;124:2350–4.
[74] Hinton J, Mariathas MN, Gabara L, Allan R, Nicholas Z, Kwok CS. Association between troponin level and medium-term mortality in 20 000 hospital patients. Heart 2023;109:1772–7.
[75] Ciffone N, McNeal CJ, McGowan MP, Ferdinand KC. Lipoprotein(a): an important piece of the ASCVD risk factor puzzle across diverse populations. Am Hear J Plus Cardiol Res Pract 2024;38:100350. https://doi.org/10.1016/j.ahjo.2023.100350.
[76] McGarrah RW, Crown SB, Zhang GF, Shah SH, Newgard CB. Cardiovascular metabolomics. Circ Res 2018;122:1238–58.

[77] Nagana Gowda GA, Raftery D. NMR metabolomics methods for investigating disease. Anal Chem. 2023;95:83–99.
[78] Ruiz-Canela M, Hruby A, Clish CB, Liang L, Martínez-González MA, Hu FB. Comprehensive metabolomic profiling and incident cardiovascular disease: a systematic review. J Am Heart Assoc 2017;6:1–22.
[79] Berkowitz L, Cabrera-Reyes F, Salazar C, Ryff CD, Coe C, Rigotti A. Sphingolipid profiling: a promising tool for stratifying the metabolic syndrome-associated risk. Front Cardiovasc Med 2021;8:1–12.
[80] Zhu Q, Scherer PE. Ceramides and atherosclerotic cardiovascular disease: a current perspective. Circulation 2024;149:1624–6.
[81] Boffa MB, Koschinsky ML. Oxidized phospholipids as a unifying theory for lipoprotein(a) and cardiovascular disease. Nat Rev Cardiol 2019;16:305–18.
[82] Ozcariz E, Guardiola M, Amigó N, Rojo-Martínez G, Valdés S, Rehues P, et al. NMR-based metabolomic profiling identifies inflammation and muscle-related metabolites as predictors of incident type 2 diabetes mellitus beyond glucose: the Di@bet.es study. Diabetes Res Clin Pract 2023;202.
[83] Lind L, Fall T, Ärnlöv J, Elmståhl S, Sundström J. Large-scale metabolomics and the incidence of cardiovascular disease. J Am Heart Assoc 2023;12.
[84] Lv J, Pan C, Cai Y, Han X, Wang C, Ma J, et al. Plasma metabolomics reveals the shared and distinct metabolic disturbances associated with cardiovascular events in coronary artery disease. Nat Commun 2024;15. https://doi.org/10.1038/s41467-024-50125-2.
[85] Di Minno A, Gelzo M, Caterino M, Costanzo M, Ruoppolo M, Castaldo G. Challenges in metabolomics-based tests, biomarkers revealed by metabolomic analysis, and the promise of the application of metabolomics in precision medicine. Int J Mol Sci 2022;23.
[86] Armitage J, Baigent C, Barnes E, Betteridge DJ, Blackwell L, Blazing M, et al. Efficacy and safety of statin therapy in older people: a meta-analysis of individual participant data from 28 randomised controlled trials. Lancet 2019;393:407–15.
[87] Cook D, Brown D, Alexander R, March R, Morgan P, Satterthwaite G, et al. Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework. Nat Rev Drug Discov 2014;13:419–31.
[88] Tsimikas S, Nj V, Sg H, W S, Mj G, Bf B, et al. Antisense therapy targeting apolipoprotein(a): a randomised, double-blind, placebo-controlled phase 1 study. Lancet 2015;10:1472–83.
[89] Libby P, Buring JE, Badimon L, Hansson GK, Deanfield J, Bittencourt MS, et al. Atherosclerosis, vol. 5. Nat Rev Dis Prim; 2019. p. 1–18.
[90] Suur BE, Chemaly M, Lindquist Liljeqvist M, Djordjevic D, Stenemo M, Bergman O, et al. Therapeutic potential of the Proprotein Convertase Subtilisin/Kexin family in vascular disease. Front Pharmacol 2022;13:1–19.
[91] Ivanisevic T, Sewduth RN. Multi-omics integration for the design of novel therapies and the identification of novel biomarkers. Proteomes 2023;11.
[92] Finotello F, Eduati F. Multi-omics profiling of the tumor microenvironment: paving the way to precision immuno-oncology. Front Oncol 2018;8:1–9.
[93] Peyvandipour A, Saberian N, Shafi A, Donato M, Draghici S. Systems biology: a novel computational approach for drug repurposing using systems biology. Bioinformatics 2018;34:2817–25.
[94] Alimohammadi M, Pichardo-Almarza C, Agu O, Díaz-Zuccarini V. Development of a patient-specific multi-scale model to understand atherosclerosis and calcification locations: comparison with in vivo data in an aortic dissection. Front Physiol 2016;7:1–15.
[95] Buckler AJ, Karlöf E, Lengquist M, Christian Gasser T, Maegdefessel L, Matic LP, et al. Virtual transcriptomics. Arterioscler Thromb Vasc Biol 2021;41:1738–50.
[96] Karlöf E, Buckler A, Liljeqvist ML, Lengquist M, Kronqvist M, Toonsi MA, et al. Carotid plaque phenotyping by correlating plaque morphology from computed tomography angiography with transcriptional profiling. Eur J Vasc Endovasc Surg 2021;62:716–26. https://doi.org/10.1016/j.ejvs.2021.07.011.
[97] Amrute JM, Luo X, Penna V, Yang S, Yamawaki T, Hayat S, et al. Targeting immune-fibroblast cell communication in heart failure. Nature. Springer US; 2024. http://www.ncbi.nlm.nih.gov/pubmed/39443792.
[98] O'Sullivan JW, Raghavan S, Marquez-Luna C, Luzum JA, Damrauer SM, Ashley EA, et al. Polygenic risk scores for cardiovascular disease: a scientific statement from the American heart association. Circulation 2022.
[99] Chen Q Sen, Bergman O, Ziegler L, Baldassarre D, Veglia F, Tremoli E, et al. A machine learning based approach to identify carotid subclinical atherosclerosis endotypes. Cardiovasc Res 2023;119:2594–606. https://doi.org/10.1093/cvr/cvad106.
[100] Belmonte T, Benitez ID, García-Hidalgo MC, Molinero M, Pinilla L, Mínguez O, et al. Synergic integration of the miRNome, machine learning and bioinformatics for the identification of potential disease-modifying agents in obstructive sleep apnea. Arch Bronconeumol 2024. https://doi.org/10.1016/j.arbres.2024.11.011.
[101] Liu Z, Liu L, Weng S, Guo C, Dang Q, Xu H, et al. Machine learning-based integration develops an immune-derived lncRNA signature for improving outcomes in colorectal cancer. Nat Commun 2022;13:1–14.
[102] Perez-Pons M, Molinero M, Benítez ID, García-Hidalgo MC, Chatterjee S, Bär C, et al. MicroRNA-centered theranostics for pulmoprotection in critical COVID-19. Mol Ther Nucleic Acids 2024;35:102118. https://doi.org/10.1016/j.omtn.2024.102118.
[103] de Gonzalo-Calvo D, Martínez-Camblor P, Bär C, Duarte K, Girerd N, Fellström B, et al. Improved cardiovascular risk prediction in patients with end-stage renal disease on hemodialysis using machine learning modeling and circulating microribonucleic acids. Theranostics 2020;10:8665–76.
[104] de Gonzalo-Calvo D, Karaduzovic-Hadziabdic K, Dalgaard LT, Dieterich C, Perez-Pons M, Hatzigeorgiou A, et al. Machine learning for catalysing the integration of noncoding RNA in research and clinical practice. EBioMedicine 2024;106:105247. https://doi.org/10.1016/j.ebiom.2024.105247.
[105] Abbasi EY, Deng Z, Ali Q, Khan A, Shaikh A, Al Reshan MS, et al. A machine learning and deep learning-based integrated multi-omics technique for leukemia prediction. Heliyon 2024;10:e25369. https://doi.org/10.1016/j.heliyon.2024.e25369.
[106] Meng C, Zeleznik OA, Thallinger GG, Kuster B, Gholami AM, Culhane AC. Dimension reduction techniques for the integrative analysis of multi-omics data. Briefings Bioinf 2016;17:628–41.
[107] Tang X, Zhang J, He Y, Zhang X, Lin Z, Partarrieu S, et al. Explainable multi-task learning for multi-modality biological data analysis. Nat Commun 2023;14.
[108] Lin Y, Zhang W, Cao H, Li G, Du W. Classifying breast cancer subtypes using deep neural networks based on multi-omics data. Genes 2020;11:1–18.
[109] Picard M, Scott-Boyer MP, Bodein A, Perin olivier, Droit A. Integration strategies of multi-omics data for machine learning analysis. Comput Struct Biotechnol J 2021;19:3735–47.
[110] Tat E, Bhatt DL, Rabbat MG. Addressing bias: artificial intelligence in cardiovascular medicine. Lancet Digit Heal 2020;2:e635–6. https://doi.org/10.1016/S2589-7500(20)30249-1.
[111] García-Hidalgo MC, Benítez ID, Perez-Pons M, Molinero M, Belmonte T, Rodríguez-Muñoz C, et al. MicroRNA-guided drug discovery for mitigating persistent pulmonary complications in critical COVID-19 survivors: a longitudinal pilot study. Br J Pharmacol 2024:1–16.
[112] Chu G, Ji X, Wang Y, Niu H. Integrated multiomics analysis and machine learning refine molecular subtypes and prognosis for muscle-invasive urothelial cancer. Mol Ther Nucleic Acids 2023;33:110–26. https://doi.org/10.1016/j.omtn.2023.06.001.
[113] Reel PS, Reel S, van Kralingen JC, Langton K, Lang K, Erlic Z, et al. Machine learning for classification of hypertension subtypes using multi-omics: a multi-centre, retrospective, data-driven study. EBioMedicine 2022;84:1–15.
[114] Lau E, Cao Q, Lam MPY, Wang J, Ng DCM, Bleakley BJ, et al. Integrated omics dissection of proteome dynamics during cardiac remodeling. Nat Commun 2018;9. https://doi.org/10.1038/s41467-017-02467-3.
[115] Drakos SG, Badolia R, Makaju A, Kyriakopoulos CP, Wever-Pinzon O, Tracy CM, et al. Distinct transcriptomic and proteomic profile specifies patients who have heart failure with potential of myocardial recovery on mechanical unloading and circulatory support. Circulation 2023;147:409–24.
[116] Flam E, Jang C, Murashige D, Yang Y, Morley MP, Jung S, et al. Integrated landscape of cardiac metabolism in end-stage human nonischemic dilated cardiomyopathy. Nat Cardiovasc Res 2022;1:817–29.
[117] Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, et al. Personalized nutrition by prediction of glycemic responses. Cell 2015;163:1079–94.
[118] Jiang C, Wang X, Li X, Inlora J, Wang T, Liu Q, et al. Dynamic human environmental exposome revealed by longitudinal personal monitoring. Cell 2018;175:277–291.e31. https://doi.org/10.1016/j.cell.2018.08.060.
[119] Suglia SF, Hidalgo B, Baccarelli AA, Cardenas A, Damrauer S, Johnson A, et al. Improving cardiovascular health through the genomics research : a scientific statement from the American heart association. 2025.
[120] Salinas MP, Sepúlveda J, Hidalgo L, Peirano D, Morel M, Uribe P, et al. A systematic review and meta-analysis of artificial intelligence versus clinicians for skin cancer diagnosis. npj Digit Med 2024;7.
[121] Barison A, Timoteo AT, Liga R, Borodzicz-Jazdzyk S, El Messaoudi S, Luong C, et al. Cardiovascular imaging research and innovation in 2023. Eur Hear J - Imaging Methods Pract 2024;2:1–8. https://doi.org/10.1093/ehjimp/qyae029.
[122] Chen SW, Wang SL, Ng TF, Ibrahim H. Artificial intelligence in cardiovascular imaging. Cardiovasc Coron Artery Imaging 2022;2:51–72.
[123] Covas P, De Guzman E, Barrows I, Bradley AJ, Choi BG, Krepp JM, et al. Artificial intelligence advancements in the cardiovascular imaging of coronary atherosclerosis. Front Cardiovasc Med 2022;9.
[124] Yao X, Rushlow DR, Inselman JW, McCoy RG, Thacher TD, Behnken EM, et al. Artificial intelligence–enabled electrocardiograms for identification of patients with low ejection fraction: a pragmatic, randomized clinical trial. Nat Med 2021;27:815–9. https://doi.org/10.1038/s41591-021-01335-4.
[125] Marshall AG, Neikirk K, Afolabi J, Mwesigwa N, Shao B, Kirabo A, et al. Update on the use of pulse wave velocity to measure age-related vascular changes. Curr Hypertens Rep 2024;26:131–40. https://doi.org/10.1007/s11906-023-01285-x.
[126] Sopic M, Vilne B, Gerdts E, Trindade F, Uchida S, Khatib S, et al. Multiomics tools for improved atherosclerotic cardiovascular disease management. Trends Mol Med 2023;29:983–95. https://doi.org/10.1016/j.molmed.2023.09.004.

[127] Abrahams T, Nicholls SJ. Perspectives on the success of plasma lipidomics in cardiovascular drug discovery and future challenges. Expert Opin Drug Discov 2024;19:281–90. https://doi.org/10.1080/17460441.2023.2292039.
[128] Nieddu G, Formato M, Lepedda AJ. Searching for atherosclerosis biomarkers by proteomics: a focus on lesion pathogenesis and vulnerability. Int J Mol Sci 2023; 24.
[129] Khan AW, Paneni F, Jandeleit-Dahm KAM. Cell-specific epigenetic changes in atherosclerosis. Clin Sci. 2021;135:1165–87.
[130] Brown PA, Brown PD. Extracellular vesicles and atherosclerotic peripheral arterial disease. Cardiovasc Pathol 2023;63:107510. https://doi.org/10.1016/j.carpath.2022.107510.
[131] Lin H, Zhang M, Hu M, Zhang Y, Jiang WW, Tang W, et al. Emerging applications of single-cell profiling in precision medicine of atherosclerosis. J Transl Med 2024;22:1–14.
[132] Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. U.S.A 1977;74:5463–7.
[133] McGinn S, Gut IG. DNA sequencing - spanning the generations. N Biotechnol 2013;30:366–72. https://doi.org/10.1016/j.nbt.2012.11.012.
[134] Goodwin S, McPherson JD, McCombie WR. Coming of age: ten years of next-generation sequencing technologies. Nat Rev Genet 2016;17:333–51.
[135] Hu T, Chitnis N, Monos D, Dinh A. Next-generation sequencing technologies: an overview. Hum Immunol 2021;82:801–11. https://doi.org/10.1016/j.humimm.2021.02.012.
[136] Logsdon GA, Vollger MR, Eichler EE. Long-read human genome sequencing and its applications. Nat Rev Genet 2020;21:597–614. https://doi.org/10.1038/s41576-020-0236-x.