Trends in
Molecular Medicine

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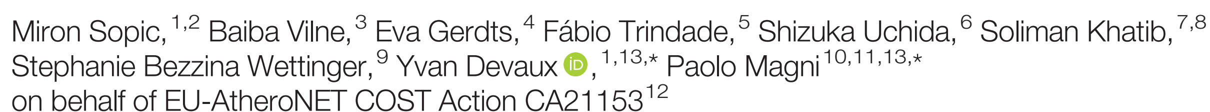
Opinion

Multiomics tools for improved atherosclerotic cardiovascular disease management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on behalf of EU-AtheroNET COST Action CA21153[12]

Multiomics studies offer accurate preventive and therapeutic strategies for atherosclerotic cardiovascular disease (ASCVD) beyond traditional risk factors. By using artificial intelligence (AI) and machine learning (ML) approaches, it is possible to integrate multiple 'omics and clinical data sets into tools that can be utilized for the development of personalized diagnostic and therapeutic approaches. However, currently multiple challenges in data quality, integration, and privacy still need to be addressed. In this opinion, we emphasize that joined efforts, exemplified by the AtheroNET COST Action, have a pivotal role in overcoming the challenges to advance multiomics approaches in ASCVD research, with the aim to foster more precise and effective patient care.

Highlights

Multiomics approaches are pivotal in understanding atherosclerotic cardiovascular disease (ASCVD) and offer promising preventive and therapeutic strategies beyond traditional risk factors.

Integrating genomics, epigenomics, transcriptomics, proteomics, and metabolomics data enhances risk stratification quality.

Artificial intelligence (AI) and machine learning (ML) models provide advanced tools for accurate ASCVD risk prediction by integrating multiomics and clinical data.

Large-scale collaborative efforts are essential for gathering comprehensive data sets to train AI/ML models effectively.

Standardized data, interdisciplinary collaboration, and regulatory approval are crucial for successful implementation.

The need for multiomics approaches to improve management of ASCVD

ASCVD (see Glossary) remains a persistent global health challenge, contributing significantly to premature mortality and reduced quality of life. It arises from a complex interplay of modifiable (e.g., lifestyle, smoking, alcohol intake, uncontrolled hypertension, hypercholesterolemia, obesity, and type 2 diabetes mellitus) and non-modifiable (i.e., genetic background, age, and sex) risk factors [1]. The current paradigm for managing ASCVD largely relies on clinical risk algorithms, such as the **Systematic Coronary Risk Evaluation 2 (SCORE2)** system in Europe [2]. However, risk scores primarily rely on a limited set of conventional risk factors, potentially neglecting underrepresented subpopulations [3–5] (Box 1). This context highlights the need for a more comprehensive and holistic approach to ASCVD management. To achieve this goal, multimodal research initiatives are needed to first unravel the complex pathological mechanisms beyond ASCVD, which will then lead to the discovery of novel biomarkers and therapeutic targets to improve ASCVD management in a personalized manner. By combining data spanning from genotypes to phenotypes and the myriad molecular intermediates spanning the entire spectrum of 'omics disciplines, we can gain deeper insights into the complexity of atherosclerosis and reduce its societal burden. A comprehensive understanding of various molecular and pathophysiological aspects of atherosclerosis, crucial for clinical application, is facilitated by different 'omics, including genomics [6,7], epigenetics [8], transcriptomics [9], proteomics [10], and metabolomics [11–13].

Multiomics and systems biology use mathematical modeling as a critical step in predicting and comprehending the intricate behaviors of complex biological systems, which allows for better understanding of complex diseases, such as atherosclerosis [14]. The integration of different 'omics methodologies, collectively referred to as 'multiomics', provides a potent means to virtually quantify cellular behavior and offers a formidable tool for uncovering the genetic variations and pathways that contribute to the disease, representing a major step toward precision medicine in ASCVD [15–19]. For instance, integration of epigenetic, transcriptomic, and proteomic data

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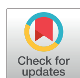

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Box 1. Background information for the clinical risk algorithms and risk factors

Clinical risk algorithms are developed to improve risk stratification and prediction of future cardiovascular events, but they are based upon only a few traditional risk factors [systolic blood pressure, sex, tobacco smoking, and serum non-high-density lipoprotein (non-HDL) cholesterol level] and result from studies that are biased toward certain populations. Therefore, applying SCORE2 is less sensitive for the identification of high-risk subjects among under-represented subgroups in epidemiological studies, such as young adults, women, and people with obesity. Sex-based differences in the association between clinical risk factors and ASCVD are well documented [3–5]. For example, in the Tromso Study, higher serum cholesterol level was a stronger risk factor for myocardial infarction (MI) in men, while higher blood pressure was a stronger risk factor in women [71]. Several studies have documented that the blood pressure-attributed risk for MI starts at a lower level of blood pressure in women [72,73]. While controlling traditional risk factors can improve treatment outcomes and prognosis, a more in-depth characterization of the molecular mechanisms leading to atherosclerotic plaque development and rupture is needed for effective risk management in specific subpopulations, possibly highlighting novel pathogenetic features particularly relevant to these groups. Considering the multifactorial pathophysiology of ASCVD, it is evident that a more systemic view of the disease is required. In this regard, multiomic approaches can improve our understanding of the relationships between disease components and help with better risk stratification.

provided valuable insights into the association between subclinical atherosclerosis, accelerated Grim epigenetic age, and proinflammatory profiles, shedding light on the potential role of chronic inflammation in mediating the adverse effects of subclinical atherosclerosis on health [20].

While the potential of multiomics studies in advancing ASCVD management is undeniable, it remains a nascent concept. Numerous attempts are under way to deploy this approach; however, the lack of established guidelines for study design, methodologies, preanalytical considerations, and data integration poses a substantial barrier to its broader and more clinically relevant implementation. In this opinion, we emphasize key considerations in the design of ASCVD multiomics studies, provide a concise overview of the technologies used with their respective advantages and limitations, and highlight ongoing initiatives leveraging ML for robust data integration (Figure 1). It is our opinion that, to realize the full potential of multiomics studies in the field of ASCVD management, interdisciplinary dialog and meticulous planning are paramount. Thorough considerations encompassing inclusion/exclusion criteria, sample types and collection timing, technology selection, data and sample handling, data integration algorithms, and, perhaps most crucially, data interpretation, need standardization to maximize clinical applicability of multiomics research initiatives.

Methodological requirements and challenges of multiomics studies

To maximize the usefulness of multiomics studies, they should involve the integration of vast amounts of data not only from genomics, transcriptomics, proteomics, and metabolomics, but also including lifestyle (e.g., data collected through different questionnaires) and clinical information. Moreover, a thorough knowledge of the advantages and pitfalls of ‘omics technologies, together with appropriate study design and study planning, is essential in multiomics studies because it determines the accuracy and reliability of the results. Elements, such as sample size, sample source and type, selection of ‘omics platforms, end-points, and statistical analysis methods, must be carefully considered when designing studies. Here, we discuss the various techniques [**whole-exome sequencing (WES)**, **short read sequencing (SRS),** bulk RNA sequencing, **single cell RNA sequencing (scRNA-seq)**, **mass spectrometry (MS)**, and **nuclear magnetic resonance (NMR)**] developed for ‘omics investigations in the context of ASCVD research, their advantages and disadvantages, and recommendations for a more homogenous use and increased reproducibility of findings among independent labs (Table 1).

Whole-exome and short read sequencing

The genomic sequence is largely independent of pre-analytical variables and is relatively stable throughout life, except in cancerous tissues. WES is commonly conducted using SRS, in which

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the genome is read in short (150-base pair) fragments, which are mapped to the reference sequence bioinformatically. The costs of analysis and interpretation, storage, and processing power are still significant. The recent pangenome reference will improve some issues related to the reference genome [21]. Approaches to flag rare variants in the reference sequence have been developed [22,23]. Moreover, there is increasing interest in noncoding genes as well as intergenic and regulatory regions of the genome. However, the mitochondrial genome is often not properly assessed [24] and neither is mosaicism, which is becoming evident in atherosclerosis using single cell WES [25].

SRS is unable to distinguish identical or highly similar reads from repetitive DNA sequences or parts of pseudogenes and their functioning counterparts. Long read sequencing can resolve this problem once error rate and cost of sequencing decrease [26]. Although various software analysis packages exist to identify structural variants, which are estimated to constitute 17.2% of rare deleterious alleles [27], from SRS data [28], few studies on atherosclerosis take structural variants into account. Ultimately, multiomics approaches may prove critical to understanding the functionality of variants, which remains challenging. Allele frequency in relevant ethnic groups is also important for this aspect.

Bulk and single cell RNA sequencing

Bulk RNA sequencing has enabled unbiased exploration of the RNA landscape. However, challenges arise in library preparation, including target enrichment and optimization of sequencing depth depending on the abundance of the targets [e.g., long noncoding RNAs (lncRNAs) are known to be expressed at a very low levels]. In addition, protocols enabling simultaneous analysis of long and short transcripts remain sparse. Unlike standard RNA-sequencing methods, the Oxford Nanopore platform relies on direct RNA sequencing, which provides information beyond differential expression, including the detection of specific RNA modifications [29,30]. Currently, the major limitations of this methodology include the relatively large amount of starting material (may be limiting in clinical studies), library preparation protocols (especially when focusing on lncRNAs), and the availability of bioinformatic tools for adequate and reliable detection of modified bases. The emergence of single cell profiling, such as scRNA-seq, has highlighted the presence of cellular heterogeneity in tissues and diseases, making it an important topic of discussion [31]. Since these methods enable the simultaneous acquisition of tens of thousands of molecular signatures, the data acquisition process is variable, depending on the specific reagents used to isolate the target materials as well as the sequencing depth, especially for those transcripts expressed at low levels, such as lncRNAs [32,33]. Moreover, the quantification of these signatures is heavily reliant on the equipment and reagents used during sample conversion, such as the preparation of sequencing library from total RNA in the case of RNA-sequencing techniques.

Additionally, the bioinformatic computational analysis significantly influences the results, because even slight changes in parameter settings can lead to dramatic differences in outcomes. Consequently, most scientific journals now require researchers to disclose the catalog and batch numbers of each reagent used, as well as the parameters for each bioinformatic tool. Furthermore, the generated raw and processed data should be publicly uploaded to repositories [e.g., **Gene Expression Omnibus (GEO)**]. To ensure transparency and reproducibility, it is recommended that all computational codes and programs utilized be made available to the public through online platforms (e.g., **GitHub**[i]).

Mass spectrometry and untargeted proteomics

MS remains the preferred approach for untargeted proteomics. Antibody- and aptamer-based technologies are gaining momentum as targeted strategies and can complement MS-based

Glossary

Artificial intelligence (AI): simulation of human intelligence in computer systems to perform tasks such as learning, problem-solving, and decision-making.
Atherosclerotic cardiovascular disease (ASCVD): chronic pathological condition characterized by the build-up of fatty deposits on the inner walls of arteries.
Gas chromatography (GC): analytical technique for separating/analyzing volatile compounds in a mixture.
Gene Expression Omnibus (GEO): publicly accessible database hosted by the National Center for Biotechnology Information.
GitHub: web-based platform that facilitates collaborative development of software projects.
High-resolution mass spectrometry (HRMS): analytical technique used to accurately determine the mass-to-charge ratio of ions in a sample with exceptional precision and accuracy.
Liquid chromatography (LC): analytical technique widely used to separate/analyze compounds in a liquid sample.
Low-density lipoprotein (LDL): lipoprotein that carries cholesterol and other lipids from the liver to various tissues in the body.
Machine learning (ML): subfield of AI that focuses on the development of algorithms and statistical models that enable computer systems to learn from, and make predictions or decisions based on, data without being explicitly programmed.
Mass spectrometry (MS): analytical technique used to determine the molecular composition of a sample by measuring the mass-to-charge ratio of ions.
Non-high-density lipoprotein (non-HDL): low-density, very-low-density, and intermediate-density lipoproteins.
Nuclear magnetic resonance (NMR): analytical technique used to study the structure, dynamics, and interactions of molecules.
Parallel reaction monitoring (PRM): targeted MS technique used for comprehensive and sensitive quantification of specific analytes.
Selected reaction monitoring (SRM): a targeted MS technique used to quantify specific analytes.

methods, such as **selected reaction monitoring (SRM)** and **parallel reaction monitoring (PRM)**. While the discovery potential is lost from untargeted to targeted MS methods or protein arrays, the gains in sensitivity (allowing the measurement of signaling proteins in tissue or products released from atherosclerotic plaques in blood), reproducibility, and quantification accuracy support a faster integration of surrogate biomarkers in clinical practice [14,18]. Nonetheless, untargeted MS remains fundamental for mechanistic and drug development studies, particularly to unveil obscure proteoforms and protein variants as well as to discover post-translational modifications. Regardless, the output of untargeted MS can be optimized if we accelerate the implementation of more reproducible and quantitative data-independent acquisition approaches (as opposed to classic data-dependent ones), eliminating the stochasticity in the selection of proteins/peptides for fragmentation, resulting in more complete data, and generating spectral libraries that can be shared among laboratories to harmonize protein quantification [34]. The development, sharing, and strict adherence to **standard operating procedures (SOPs)** is recommended for both non-MS or MS-based methods. This is especially important when using blood-derived fluids, where new sources of bias are introduced with prefractionation or depletion approaches required to unveil low-abundance species, hindering multiomics data integration [35,36].

Analytical platforms for metabolomics

Metabolomics offers a novel approach to the discovery of disease biomarkers that could be used to assess the risk of developing a disease and to diagnose diseases, including ASCVD, before clinical symptoms appear [37–40]. Targeted metabolomics focuses on the analysis of a specific set of molecules of interest, whereas untargeted metabolomics focuses on all of the metabolites present in a biological sample in a particular physiological state, with no prior knowledge of what to expect [40,41].

Due to advances in analytical instrumentation and bioinformatic tools, the field of metabolomics has grown exponentially over the past decade. The two main analytical platforms in metabolomics research are NMR spectroscopy and **high-resolution mass spectrometry (HRMS)** coupled with **liquid or gas chromatography (LC or GC**, respectively). NMR has the capacity to provide structural information on lipoprotein distribution of lipids but lacks sensitivity, besides requiring high purchasing and operating costs. Due to their high sensitivity and selectivity, LC-MS/MS-based techniques are most commonly used in metabolomics with the wide dynamic range of MS systems [42]. The choice between these platforms depends on the specific structural information required, with NMR focusing on lipoprotein distribution and LC-MS/MS offering high resolution of lipid species. The typical workflow for untargeted metabolomics includes the following steps, each of which requires careful attention to protocol implementation: sample preparation and metabolite extraction; data acquisition; data processing by bio/chemoinformatic tools; data analysis using univariate and multivariate statistics; metabolite identification; and data interpretation [43].

Pre-analytical considerations

To ensure the coherence, accuracy, and reproducibility of ‘omics data, careful consideration of various factors is essential throughout the study process (Box 2). For instance, when focusing on liquid biopsy and biomarker development, the specific type of blood derivative (e.g., whole blood, serum, plasma, or platelet-poor plasma), the time from collection to processing, and the processing conditions used are critical. Transcriptomics and proteomics signatures are different in plasma and serum (due to the coagulation process and the activity of platelets), and different types of plasma show significant variations in their transcriptomic signature. Platelets carry RNAs, and the differences in the number of platelets from sample to sample can introduce

Short read sequencing (SRS): high-throughput DNA-sequencing technique that generates short fragments of DNA sequences (50–300 base pairs in length).
Single cell RNA sequencing (scRNA-seq): technique used to analyze the transcriptome of individual cells at a single cell resolution.
Standard operating procedure (SOP): document that outlines a step-by-step process to be followed in experiments within a specific research setting.
Systematic Coronary Risk Evaluation 2 (SCORE2): cardiovascular risk evaluation system that considers age, sex, total cholesterol levels, systolic blood pressure, and regional genetic risk.
Systematic Coronary Risk Evaluation 2 Older People (SCORE2OP): version of SCORE2 for people over 70 years of age.
Very low-density lipoprotein (VLDL): lipoprotein synthesized in the liver that transport triglycerides, cholesterol.
Whole-exome sequencing (WES): a comprehensive procedure used to determine the complete, or nearly complete, DNA sequence of the exome of an organism simultaneously.

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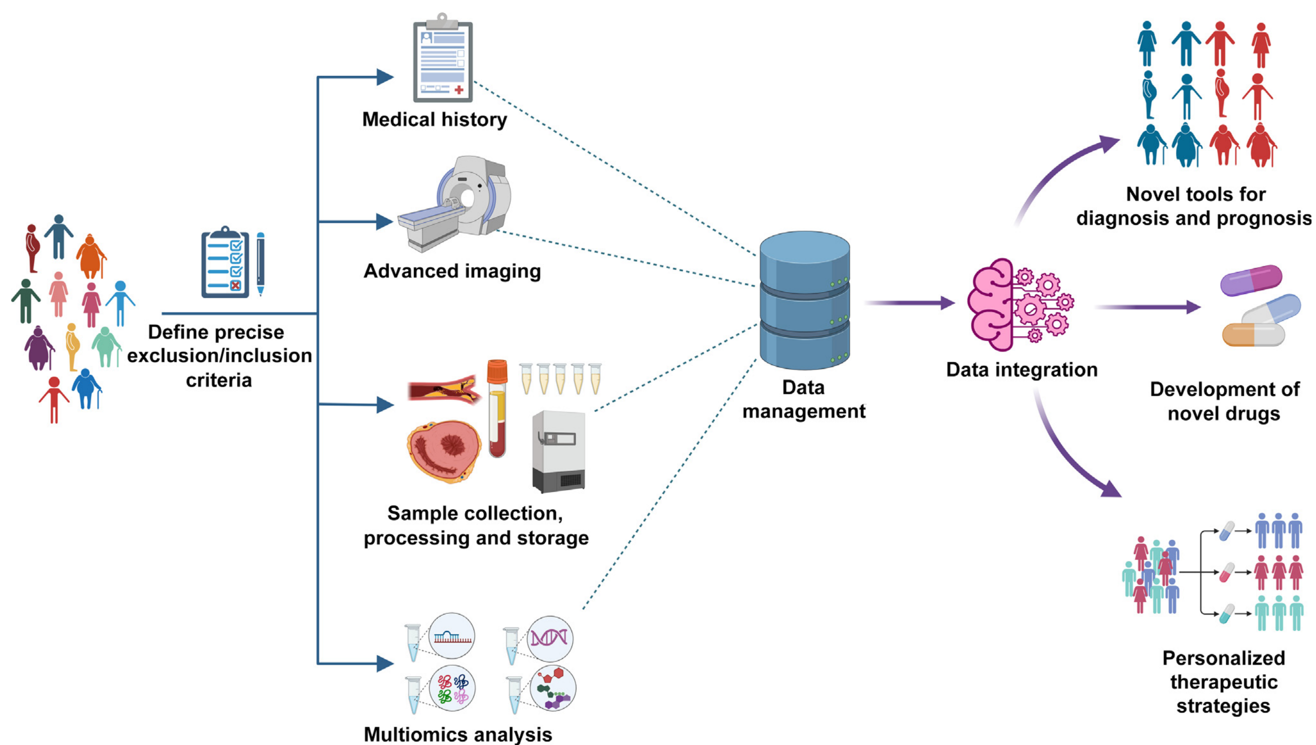

Figure 1. Overall workflow of multiomics studies in atherosclerotic cardiovascular disease (ASCVD) research. It is important to emphasize the significance of defining precise inclusion and exclusion criteria based on specific research objectives and the risk populations under consideration. The crucial data inputs include comprehensive medical histories, imaging results, and detailed information on sample collection, processing, storage, and quality. It is also important to specify the methodologies used for genomics, transcriptomics, proteomics, and metabolomics analyses. The available data are then integrated through machine learning (ML) algorithms, enabling the development of: (1) innovative diagnostic and prognostic tools; (2) novel drug targets to enhance drug development; and (3) personalized therapeutic strategies. Figure created with BioRender (biorender.com).

significant bias toward RNA quantification. To mitigate this issue, the use of platelet-free plasma is recommended, unless platelet-related effects are important for the study [44,45]. In addition, for transcriptomics studies, it is recommended to use EDTA to prepare plasma, and not heparin, which inhibits PCR [44]. Regular and platelet-free plasma have distinct proteomic signatures, underscoring the importance of platelet management in proteomic analysis [46]. By contrast, platelets do not appear to introduce bias in metabolomics studies [47].

Hemolysis is a common preanalytical issue that can occur either *in vivo* due to a disease or *ex vivo* during blood collection or handling [48]. In both daily clinical practice and research studies, *ex vivo* hemolysis represents one of the most frequent pre-analytical errors [48]. Hemolysis can significantly impact transcriptomic [44], proteomic [49], and metabolomic profiles [48] and should be actively prevented. Thus, it is crucial to document any signs of hemolysis for accurate analysis.

Lipemic samples are relatively common in ASCVD due to underling dyslipidemia. Therefore, it is crucial to investigate the impact of lipemia on sample preparation and analysis, ensuring the integrity of the metabolomics data. Furthermore, recent guidelines highlighted the importance of measuring **non-high-density lipoprotein (non-HDL)**-cholesterol and lipid markers in postprandial samples to provide insights into the dynamic changes of proatherogenic **very low-density lipoprotein (VLDL)** and chylomicron remnants [50]. This recommendation necessitates careful

Table 1. Summary of genomic, transcriptomic, proteomic, and metabolomic methodological approaches

Type	Approach	Principle	Strengths	Limitations	Refs
Genomics					
WES	SRS	Sequences are read in short fragments (typically 150 base pairs) and then aligned to reference genome	Cheaper than long read sequencing Generally high accuracy in variant calling Very small quantity of DNA required Laboratory methods and bioinformatic pipelines well established Considerable experience working with WES	Errors in alignment can result in missed variants or false positives, especially in regions of high similarity, such as pseudogenes Not well suited for structural variation Reference genome may have its own variations, which then are not recognized	[21,22,63]
	Long read sequencing	Pacbio Single Molecule Real-Time (SMRT) involves real-time sequencing by synthesis of fragments typically of ~10–20 kb In Oxford Nanopore sequencing, nucleotides are identified in real time using changes in electrical current as single strand of DNA is passed through molecular nanopore with many sequenced in parallel; allows long reads of 10–100 kb or ultra-long reads of 100–300 kb	Best approach for structural variants Shows potential to replace SRS in future	Bioinformatic pipelines not easily available Error rate, although greatly improved, can be higher than with SRS Cost remains high Mainly used to resequence a targeted region that indicates structural variation	[26,64]
Transcriptomics					
Bulk RNA sequencing	Illumina based	Sequencing by synthesis approach during which fluorescently labeled nucleotides are incorporated into growing DNA strands, and fluorescence signals are detected and recorded to determine sequence of DNA molecules	High-throughput technology Cheaper than long read sequencing Laboratory methods and bioinformatic pipelines are well established	Since RNA is converted to cDNA before sequencing, information about RNA modifications is lost Absence of protocols for simulations miRNA and mRNA/lncRNA analysis No information on cell-specific transcriptomes	[65,66]
	Direct Oxford Nanopore	Sequencing involves passing RNA molecules through nanopores embedded in a membrane. As RNA passes through nanopore, it disrupts ionic current, generating unique electrical signals characteristic of RNA sequence. These signals are recorded and analyzed in real time to decode RNA sequence	Generating long reads (even spanning thousands of bases) Detection of RNA modifications	Low-throughput technology Currently more expensive than sequencing-by-synthesis approach Laboratory methods and bioinformatic pipelines are not well established Protocols for small RNA sequencing are not established No information on cell-specific transcriptomes	[29]
Single cell RNA seq	10x Genomics Chromium Fluidigm C1 Clontech iCell8	Sequencing enables analysis of gene expression in individual cells to uncover cellular heterogeneity in a sample (e.g., tissues)	Identify distinct cell types/populations, characterize cell states, and explore cellular dynamics within population of cells	Large batch effects and noise leading to loss of data Limited sensitivity and miss low-abundance transcripts (e.g., lncRNAs) Cost is high; thus, number of cells analyzed is limited for deeper sequencing depth	[22]
Proteomics					
Untargeted proteomics	Discovery MS	Intact proteins (top-down) or peptides (bottom-up) resulting from enzymatic digestion (e.g., trypsin) are separated	Highly customizable separation, ionization, and detection techniques	Acquisition and maintenance costs Limited sensitivity	[14,67]

Table 1. (continued)

Type	Approach	Principle	Strengths	Limitations	Refs
		by liquid chromatography, ionized, and injected into mass spectrometer. Protein or peptide fragments are selected and analyzed according to mass-to-charge ratio (*m/z*). Resulting spectra are matched against databases of known sequences for identification. Peptides can also be sequenced *de novo* Quantification can be performed label-free or with isotope tags/labeling	Discovery of new protein species (including different splicing products, post-translational modifications and protein variants) Complete coverage of proteome is theoretically possible Minute amounts of sample required (~1 μg) Absolute quantification is possible	Bias for higher-abundance species Batch effects can be appreciable Data sparsity[a] Throughput can still preclude application in large cohort studies Absolute quantification can be expensive and technically demanding (label based) or has low accuracy (label free)	
Targeted proteomics	SRM[b] – MS	Set of precursor ions (peptides) is selected in first quadrupole for fragmentation in second quadrupole. Only a set of specific fragment ions are selected for detection using third quadrupole Typically performed in triple quadrupole mass spectrometers Quantification can be performed label free, by spiking with labeled reference peptide or even using heavy peptides	More affordable than high-resolution spectrometers, facilitating clinical implementation Higher specificity and sensitivity than discovery MS due to peptide/fragment filtering and higher dwell time[c] Higher signal/noise ratio Better performance in absolute quantification than untargeted MS Full customization of targets to monitor	Selection of optimal peptide targets and transitions can be time-consuming Requires pre-selection of product ions to measure (preventing post-acquisition method refinement) Increasing number of species to quantify comes at expense of instrument performance (lower signal/noise ratio)	[34,68]
	PRM – MS	Set of precursor ions (peptides) selected for fragmentation, and all resulting product ions are measured Typically performed in quadrupole-Orbitrap or QqTOF systems Quantification can be performed label free, by spiking with a labeled reference peptide, or even using heavy peptides	Higher mass accuracy than in SRM Higher specificity and sensitivity than discovery MS due to peptide filtering and higher dwell time[c] Higher signal/noise ratio Better performance in absolute quantification than untargeted MS Full customization of targets to monitor	Acquisition and maintenance cost Increasing number of species to quantify comes at expense of instrument performance (lower signal/noise ratio)	
	Antibody-based microarray (e.g., Olink assay)	Proximity extension assay Dual recognition immunoassay in which two antibodies binding to target protein are labeled with DNA oligonucleotides, which hybridize when in proximity. DNA double chain is then used as template for PCR amplification Quantification can be performed by quantitative PCR or next-generation sequencing	High specificity without antibody cross-reactivity High sensitivity and dynamic range High stability, reproducibility, and throughput	Limited proteome coverage (3072 proteins) No discovery potential Relative quantification[d] Biased toward plasma proteome	[14,18,69]
	Aptamer-based microarray (e.g., SomaScan assay)	Protein affinity-based approach with modified aptamers Aptamers (single-stranded oligonucleotides) are modified to endow protein-like functional groups, allowing their binding to specific proteins in multiplex Modified aptamers are then amplified and quantified by quantitative PCR	High specificity High sensitivity and dynamic range High stability, reproducibility, and throughput	Limited proteome coverage (7000 proteins) No discovery potential Relative quantification Biased toward plasma proteome	[14,18,70]

(continued on next page)

Table 1. (continued)

Type	Approach	Principle	Strengths	Limitations	Refs
Metabolomics					
Targeted metabolomics	Analyses of known metabolites	Absolute qualitative and quantitative analysis of test substance using standard substances Isotope internal standards could improve sensitivity and accuracy of substance qualitative and quantitative analysis	High sensitivity and accuracy Qualitative and quantitative analysis of metabolites	Limited coverage of metabolites	[41]
Untargeted metabolomics	Analysis of unknown metabolites	Analysis of all of metabolites present in biological sample in particular physiological state, with no prior knowledge of what to expect	Extensive coverage of metabolites. A higher amount of chemical and biological knowledge could be obtained from sample data set	Lack of standard substances, false-positive signals, lacks absolute qualitative and quantitative data on metabolites	[41]
		Untargeted metabolomics using NMR	Provide structural information on lipoprotein distribution of lipids Fundamentally quantitative Nondestructive	Lacks sensitivity Suffers high purchasing and operating costs	[34]
		Untargeted metabolomics using high-resolution GC or LC-MS/MS	High sensitivity and selectivity High resolution of lipid species Wide dynamic range of MS systems	Suffers matrix effects Difficulty in distinguishing isomers	[34]

[a]Can be greatly improved when using data-independent acquisition approaches.
[b]Also known as multiple reaction monitoring (MRM).
[c]Dwell time is time spent by the spectrometer to collect target peptide ions (higher in targeted approaches because other peptides are ignored).
[d]Absolute quantification is already possible but in smaller Olink panels (<50 proteins).

consideration during sample collection and preparation to ensure that the timing of sample collection aligns with the postprandial state.

Artificial intelligence and machine learning as solutions for multiomics data integration

As mentioned previously, high-throughput 'omics technologies offer promise for personalized treatments and improved outcomes in ASCVD risk prediction beyond conventional factors through integration with **AI** and **ML**. However, to fully realize the benefits of AI/ML-based approaches for ASCVD risk prediction, several challenges need to be addressed. First, large-scale studies collecting different types of data from the same patients are crucial for accurate patient stratification and risk prediction. Collaborative efforts across multiple institutions and healthcare providers are also necessary to gather such comprehensive data sets. However, integrating data from different studies is challenging due to inconsistencies in experimental design, sample preparation, 'omics profiling methodologies, and data analysis workflows [51]. Data quality poses another issue because certain 'omics data (genomics and transcriptomics) are more reliable and consistent compared with others (epigenomics, proteomics, and metabolomics). This discrepancy makes it difficult to develop AI/ML models that can accurately account for differences in data quality [51,52]. Integrating AI/ML with clinical data presents additional challenges due to variations in data formats, structures, and descriptions, as well as complexities associated with processing 'omics data. Additionally, incomplete information, inconsistencies, and noise in clinical data can compromise analysis outcomes. Biases related to ethnicity, gender, and socio-economic status must also be carefully considered to avoid inflated AI/ML models. Safeguarding

Box 2. Considerations before starting an ASCVD multiomics study

Relevant inclusion/exclusion criteria: should be clearly defined before both hypothesis-driven or hypothesis-generating types of study. Factors to take into account include age, sex, ethnicity, clinical end-point and how it is defined, and, if the clinical end-point is MI, whether before or how long after the MI. Frequency matching by sex and defined age groups should also be specified.

Variables to be included in the study: anthropometric data: (height, body weight, body mass index, waist circumference, etc.), and lifestyle data (smoking status, alcohol consumption, physical activity, diet, etc.).

Number of samples: dependent on the number of ʻomics used, hypothesis and funds available. Currently there are no recommendations for power analysis.

Costs: consider available funds and costs of different ʻomics platforms.

Questionnaire: should be in line with the hypothesis and include demographic, anthropometric, and lifestyle data. Several standardized questionnaires are available. Consider whether data will be used for further studies and include additional details. Back-translate if questionnaire is in more than one language.

SOPs: should be developed for every step of the study (pre-analytical, analytical, and post-analytical).

Sample type: whole blood, serum (clotting activator, gel separator, etc.), plasma (EDTA, heparin, citrate, etc.), platelet-poor plasma, peripheral blood mononuclear cells, neutrophils, platelets, tissue, and so on.

Pre-analytical considerations: data collection; sample collection (type of tubes), treatment (centrifugation speed, etc.), transport (temperature, timing), coding, aliquoting (number of aliquots) and storage (+4, –20, –80°C). Consider timing of sample (after fasting, post prandial, during MI, after MI). Consider storing some standards at time of sample collection to see effect of long-term storage. Records of sampling time and duration of processing. In case of blood samples, take note of hemolysis or lipemia. Consider using Standard Preanalytical Code Version 3.0.

Clinical data: results of coronary angiography, percutaneous coronary intervention, heart echocardiography, and other imaging techniques, blood tests (glucose, hemoglobin A1c, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, non-HDL-cholesterol, troponin, N-terminal pro B-type natriuretic peptide, high sensitive C-reactive protein, etc.). Presence of other co-morbidities or diseases (obesity, dyslipidemia, DM, nonalcoholic fat liver diseases, chronic kidney disease, thyroid disease, cancer), medication and doses.

Ethics and informed consent: consult ethical experts and/or undergo training in ethics and informed consent design; consider using data/samples for future studies; include in the informed consent the possibility of using samples/data outside of study institution/country and specify types of project; ensure compliance with national and European General Data Protection Regulation.

patient privacy and confidentiality is crucial when handling sensitive information, necessitating appropriate data de-identification and secure transfer protocols. Furthermore, regulatory approval by authorities, such as the European Medicines Agency, is essential, but still does not rely on established procedures, for implementing AI/ML models into medical products [53].

Optimizing AI/ML models to overcome overfitting or underfitting issues is critical. High dimensionality and complex interactions in multilayered information, coupled with relatively small sample sizes, require careful feature selections to enhance disease prediction. Understanding the relationships between different ʻomics layers and their relevance is crucial for accurate modeling [51,52]. Selecting appropriate algorithms tailored to specific circumstances is essential, especially when dealing with complex data or generating large volumes of high-throughput data [54]. Model complexity, interpretability, and clinical relevance are also factors to consider. While complex models may provide accurate predictions, they can be challenging to implement and computationally demanding. Prioritizing clinical relevance over predictive accuracy is vital for actionable insights and improved patient outcomes. Ensuring model generalizability across different patient populations and seamless integration with clinical systems requires significant investments in computational infrastructure, personnel training, and maintenance [51,54].

Clinician's corner

The current guideline-recommended ASCVD risk charts, such as SCORE2 and **Systematic Coronary Risk Evaluation 2 Older People (SCORE2OP)**, are based on risk over a 5–10-year period. Although important in the daily clinical practice, these risk scores are not very accurate in the prediction of longer term individual ASCVD risk. Furthermore, they underestimate the risk in women and in young individuals or those with obesity.

As we approach precision medicine strategies in different clinical areas, novel approaches should also be implemented in this field by inclusion of biomarkers of key causal mechanisms in the development of ASCVD.

A particularly promising research area is the integration of multiomics data with lifestyle and clinical data and use of AI and network analyses for exploring such complex data sets.

More complete clinical characterization is needed during patient recruitment. Missing clinical data are still a major caveat in any ʻomics study.

Health workers in clinical and laboratory medicine should be aware of the pre-analytical factors resulting in analytical variance not explained by biological factors, and adhere to rigorous SOPs for sample collection and processing.

Multiomics studies has the potential to improve elucidation of pathophysiology, which can aid identification of novel drug targets.

To address these challenges, researchers can share data and codes, use standardized protocols, review AI/ML research, optimize models, and validate with diverse data sets for reliability [40,41], thus enabling AI/ML to effectively integrate multiple layers of information and advance our understanding of complex diseases, such as ASCVD.

Translational perspectives

To facilitate the successful translation of findings from bench to bedside and advance the management of ASCVD, the establishment of a clear translational roadmap is imperative. The application of novel multiomics-based tools in clinical practice depends on not only their transparency and evidence-based benefits, but also fostering trust among end-users (i.e., healthcare providers and patients). Trust in these innovative tools is a cornerstone of their adoption and effective utilization to improve ASCVD management.

The translation of ‘omics tools in ASCVD management necessitates adherence to evidence-based medicine principles. The road to widespread clinical use for any novel ‘omics tool is fraught with multiple criteria that must be met to ensure its reliability and clinical utility [55,56]. As discussed in the preceding text, pre-analytical and analytical standardization is crucial during the discovery process to ensure reproducibility of findings [56]. Extensive independent validation steps are required to assess sensitivity, specificity, positive and negative predictive values, and overall diagnostic accuracy of multiomics biomarkers and models in clinical settings [55].

The wealth of information provided by multiomics studies enables the development of theranostic approaches, where diagnosis and therapy are seamlessly intertwined. For instance, Mendelian randomization studies can help unravel causality by exploiting genetic variants as natural experiments to determine whether specific biomarkers are implicated in disease pathogenesis [57,58].

Only when a new multiomics marker demonstrates high analytical precision and accuracy, coupled with diagnostic robustness in terms of specificity, sensitivity, predictive values, and likelihood ratios, can it be considered for approval and reimbursement by health authorities. This stringent process, requiring unwavering commitment to precision and reliability, is essential for obtaining the necessary regulatory clearance.

An additional critical aspect of this translation process is the inclusion of patients and patient organizations in the planning and implementation of multiomics studies [59]. Patient compliance with new tools and sophisticated approaches can be challenging, and their active engagement is key to addressing this issue. Patient organizations can have a pivotal role in disseminating information, fostering awareness, and soliciting feedback.

Concluding remarks

Multiomics studies hold the promise to discover new preventive, diagnostic, and therapeutic strategies for ASCVD. Recent studies showed that it is useful to go beyond **low-density lipoprotein (LDL)** cholesterol, and other traditional risk factors, and that anti-inflammatory drugs are beneficial in the treatment of ASCVD [60,61]. New clinical tools should be able to provide more information to better stratify patients with ASCVD and replace the old one-size-fits-all approach with a new one that is tailored to each patient. Ideally, this so-called ‘deep phenotyping’, followed by appropriate data interpretation, would provide a unique molecular identity of each patient that can complement the clinical data and then be used for a specific therapeutic approach or ‘fine-tuning’ of current therapy, as is now being done in some therapies for cancer [62]. In the evolving landscape of ASCVD precision medicine, defining the ideal combination of ‘omics

Outstanding questions

How can we enhance the accuracy of individual ASCVD risk assessment using multiomics data and AI/ML approaches in the context of precision medicine?

What are the critical challenges in implementing a successful multiomics strategy for ASCVD research?

Should resources be focused on well-designed smaller multiomics studies or investing in AI/ML methods for integrating larger single-omics studies?

What is the optimal approach for integrating ‘omics layers to extract meaningful insights from multiomics data?

How can we effectively validate clinical models developed using AI/ML in the context of ASCVD risk prediction?

What methods should be used to assess statistical power when designing multiomics studies for ASCVD?

Can deep phenotyping through multiomics approaches improve patient stratification and personalized therapeutic strategies for ASCVD management?

How can we overcome biases related to ethnicity, gender, and socioeconomic status when using AI/ML in ASCVD research?

What are the potential long-term risks and ethical considerations of integrating AI/ML in ASCVD management?

What are the most effective ways to share multiomics data and AI/ML algorithms across institutions to facilitate collaborative research in ASCVD?

Can multiomics approaches be used to identify early molecular signatures of ASCVD before clinical symptoms appear?

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platforms and bioinformatic algorithms is challenging due to rapid technological advancements. Notably, ongoing development of explainable ML and AI approaches is crucial for trust and usability among patients and clinicians. The future likely entails merging 'omics data with digital markers to enhance prediction and risk stratification, promising improved patient care.

However, in the case of ASCVD, we are still a long way from translating this concept from bench to bedside. The high cost of high-throughput 'omics technologies still prevents their widespread use in large-scale studies. It is important to discuss and resolve some of the fundamental challenges (see Outstanding questions) and then develop and implement appropriate multiomics approaches for cardiovascular research first. Given the multidimensional nature of the challenges, ranging from study design and methodological challenges to data processing and data integration, it is obvious that joint interdisciplinary efforts are needed to successfully overcome the current obstacles. Bringing together researchers, analytic specialists, clinicians, and data scientists ensures accuracy and generalizability of results and accelerates the implementation of multiomics approaches in the clinic (see Clinician's corner). This interdisciplinary concept is the underlying basis of the CA21153 Network for implementing multiomics approaches in atherosclerotic cardiovascular disease prevention and research (AtheroNET COST).

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Declaration of interests

The authors declare no conflict of interests.

Resources

[i]https://github.com

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