

Network Medicine: A Clinical Approach for Precision Medicine and Personalized Therapy in Coronary Heart Disease

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Early identification of coronary atherosclerotic pathogenic mechanisms is useful for predicting the risk of coronary heart disease (CHD) and future cardiac events. Epigenome changes may clarify a significant fraction of this “missing heritability”, thus offering novel potential biomarkers for prevention and care of CHD. The rapidly growing disciplines of systems biology and network science are now poised to meet the fields of precision medicine and personalized therapy. Network medicine integrates standard clinical recording and non-invasive, advanced cardiac imaging tools with epigenetics into deep learning for in-depth CHD molecular phenotyping. This approach could potentially explore developing novel drugs from natural compounds (i.e. polyphenols, folic acid) and repurposing current drugs, such as statins and metformin. Several clinical trials have exploited epigenetic tags and epigenetic sensitive drugs both in primary and secondary prevention. Due to their stability in plasma and easiness of detection, many ongoing clinical trials are focused on the evaluation of circulating miRNAs (e.g. miR-8059 and miR-320a) in blood, in association with imaging parameters such as coronary calcifications and stenosis degree detected by coronary computed tomography angiography (CCTA), or functional parameters provided by FFR/CT and PET/CT. Although epigenetic modifications have also been prioritized through network based approaches, the whole set of molecular interactions (interactome) in CHD is still under investigation for primary prevention strategies.

Key words: Coronary heart disease, Cardiac imaging, Network medicine, Primary and secondary prevention, Precision medicine

Introduction

Coronary heart disease (CHD) represents a relevant issue for the public health system¹. In spite of, substantial progress in elucidating genetic and environmental risk factors for CHD, these determinants can explain a part of overall risk, whereas changes in the epigenome may clarify a significant fraction of this “missing heritability”, thus offering novel potential biomarkers for prevention of CHD²⁻⁶. Atherogenic process begins early in human life and it is influenced by maternal risk factors⁷⁻⁹ which include epigenetic

events^{10, 11}. In the era of next generation sequencing, the development of a plethora of high-throughput platforms has provided significant impact concerning the knowledge of CHD molecular characterization generating big “omics” data sets¹²⁻¹⁴. Furthermore, several non-invasive imaging techniques have been developed over the last few years to detect CHD with the aim to guide optimal patient management, such as coronary computed tomography angiography (CCTA), cardiac magnetic resonance (CMR), and nuclear medicine technique positron emission tomography (PET)¹⁵. These advances have raised challenges

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to individualize/focus treatments and develop new diagnostic tools, useful in primary prevention, and in subjects in early stages of disease, as well as for secondary prevention^{6, 16}).

Complex diseases and traits, such as cardiovascular risk factor susceptibility and CHD, are caused by perturbations of biological networks. The network medicine approach, integrating big omics, imaging data, and clinical information, aims to identify pathological interacting genes and proteins, revolutionizing disease knowledge and shifting the understanding of pathogenic phenomena from a reductionist to a holistic approach¹⁷⁻¹⁹). Currently, epigenetics, combined with the innovative system biology approach, is useful for personalized CHD therapy²⁰⁻²²). Furthermore, unlike DNA variations, epigenetic modifications are, potentially, pharmacologically reversible^{23, 24}). The aim of this review is to discuss the network medicine tools that could reveal new candidate genes to translate into clinical practice as diagnostic, prognostic, and predictive biomarkers, which could shed new light into CHD clinical settings and susceptibility to cardiovascular risk factors.

We performed electronic searches in three different electronic databases, including PubMed, Google Scholar, and Web of Science, selecting studies in English between 2013 and 2019 and reporting the association between CHD and its risk factors into network based models, as well as epigenetic-sensitive CHD mechanisms. The search syntax considered terms related to CHD combined with the terms cardiac imaging, system biology, epigenetics, gene expression, network medicine, network pharmacology, drug repurposing, primary and secondary prevention, precision medicine, and personalized therapy. Concerning network-based studies, the search provided five original genetic studies associated to cardiovascular risk factors, ten network based genomic studies for primary prevention of CHD, and four network pharmacology analyses associated with CHD with a possible impact for personalized therapy. Two epigenetic network models identified regulatory pathways and key epigenetic and genetic regulators in CHD and familial hypercholesterolemia.

Cardiac Imaging for CHD Diagnosis and Risk Prediction

The first step in primary prevention of CHD represents non-invasive anatomical evaluation of the coronary tree. Then, the concept of primary prevention should be revisited in regard to recent scientific achievements and the best advances in diagnostic imaging techniques. Minimizing the incidence of

major cardiac events, in the absence of clinically evident underlying obstructive CHD, should be the goal. Several non-invasive imaging techniques have been developed to rule out and/or detect CHD for optimal patient management. Among them, CTCA, which has a higher spatial resolution, provides a direct evaluation and quantification of coronary artery alteration, while CMR and nuclear medicine technique positron emission tomography (PET) provide indirect CHD information, estimating myocardial perfusion and metabolism alterations, thus representing useful tools for evaluating CHD in secondary prevention¹⁵). Furthermore, emerging hybrid imaging techniques in the field of cardiovascular medicine, such as PET/CT and PET/MR, provide simultaneous morphological and functional parameters¹⁵).

Carotid Ultrasound Imaging

Carotid artery ultrasound remains a reliable tool in the current armamentarium of diagnostic modalities used to assess atherosclerosis and quantify the degree of arterial injury at an early stage. The procedure has undergone considerable technological upgrades thanks to the introduction of more powerful probes, new color flow, and 3D imaging techniques^{16, 25}). Its great advantage is that it can be used in a broad range of populations, such as children and adolescents, where the use of ionizing radiation is not recommended or there may be a low compliance to perform lengthy tests²⁶). Carotid intima-media thickness (IMT) is strongly associated with risk factors for atherosclerotic vascular disease and is an independent predictor of future cardiovascular events, thus providing a better risk reclassification^{26, 27}). Based on this evidence, the test is a recommended clinical tool to assess risk prediction in a wide population of subjects²⁷) (**Fig. 1A and 1B**).

CTCA

CCTA evolved not only to reduce radiation exposure, but also to optimize image acquisition, achieving more spatial and contrast resolution²⁸⁻³¹). The real revolution for non-invasive anatomical evaluation of the coronary tree has been achieved only recently, with the introduction of “fourth generation” wide-volume scanners, with up to 16 cm of cranial-caudal coverage in a single rotation, enabling whole-heart coverage at one acquisition time point (within one heartbeat or volume). It eliminates “stair-step” or misalignment artifacts and provides uniform attenuation when evaluating the coronary arteries or measuring contrast attenuation gradients across atherosclerotic lesions²⁹). Furthermore, radiation doses are lower with wide-area detector scanners because of shorter

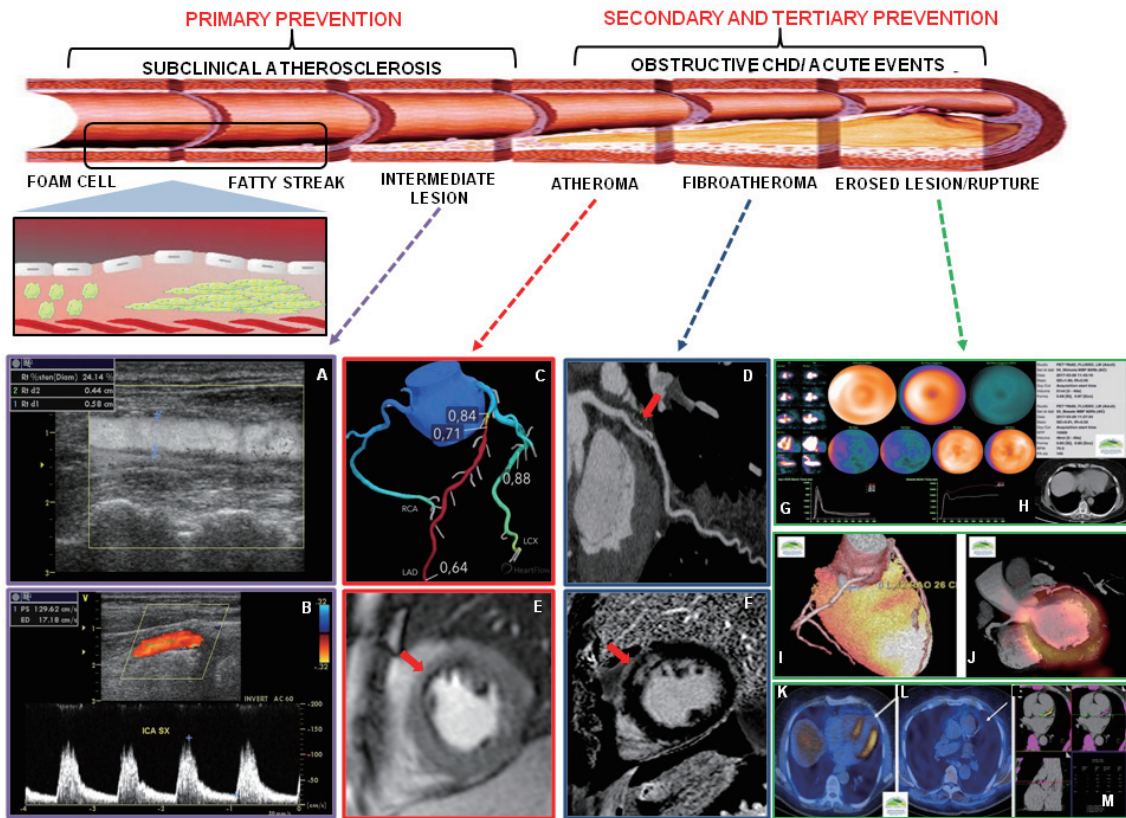


Fig. 1. Cardiovascular disease prevention

Cardiovascular risk factors begin to exert their detrimental effects in the fetal stage. Cardiovascular risk factors accumulate during childhood and adolescence due to unhealthy lifestyles causing subclinical atherosclerosis. Once initiated, atherogenesis continues during adulthood, enhanced by additional risk factors, predisposing to the clinical manifestation of CHD as well as cardiac acute events. The early identification of atherosclerosis determinants and mechanisms from the stage of subclinical pathology (primary prevention) is essential to plan lifestyle interventions that reduce risk factor susceptibility. From adolescence to adulthood, primary prevention aims to control the magnitude of risk factors, which is also supported by imaging screening, such as carotid ultrasound. Secondary prevention and tertiary prevention plans to perform appropriate preventive interventions aiming to reduce the onset of obstructive CHD and the incidence or recurrence of adverse events are also supported by imaging techniques such as: carotid ultrasound A. assessment of subclinical carotid atherosclerosis of left internal carotid artery (ICA); B. spectral doppler waveforms of ICA; C. FFR-CT; D. CCTA (curved multiplanar reconstruction of a stenosis of 50% characterized by a mixed plaque on left marginal artery); E. CMR first pass perfusion acquisition; F. CMR late gadolinium enhancement acquisition; G. ⁸²Rb PET/CT Preserved MBF-MFR (>2); H. Low Dose Gated-CT showing calcified plaque on circumflex coronary artery; I. Off-line Reconstruction. ⁸²Rb PET: Preserved Perfusion on LDA territory/CCTA: Normal LDA vessel; J. Co-registered studies. ⁸²Rb PET: Transient and chronic ischemia on LDA territory/CCTA: Occlusive stenosis of LDA; K. ⁸²Rb PET MPI Stressor Dipyridamole: Transient Perfusion Defect on antero-apical myocardial segments; L. Multimodality MPI ⁸²Rb PET/CT-CAC score showing extensive calcified plaque on LDA; M. Multimodality MPI ⁸²Rb PET/CT-CAC score showing a CAC score ≥ 400 , consistent with ischemia with high risk of future coronary events.

scanning times and lower redundant radiation from either overlapping sequential axial scans or helical oversampling.

Effectively, “temporal resolution” is one of the objectives^{32, 33}. One solution proposed to improve data sampling time, and then temporal resolution, during CCTA acquisition is based on using two x-ray tubes, shifted 90° toward each other³³. Theoretically, this technology, based on acquisition of two different heart views at the same time, could lead to a halving of temporal resolution, but the true limit of this technology lies in detector size. It is so small that it would

require at least three, or even four, cardiac cycles to image the whole coronary tree, which could lead to “misalignment” artifacts deriving from the frequent, even minimal, variability of the RR interval. Unless we assume a perfectly constant RR interval (which is not the rule), it is essential to program the data acquisition during a sufficiently extended diastolic period, with a consequent increase in exposure, in order to post-process each coronary artery position shift³⁴. Since almost cardiac CT systems have now settled down, with rotation speeds ranging from 250 to 280 ms, detector width became discriminant. and it would

be as large as possible to allow the study of the entire cardiac area in the smallest number of heart cycles³⁵).

In addition, the total iodinated contrast material volume can be decreased to as low as 40 mL at 5 mL/sec in patients with normal cardiac output. This new generation of scanners combines the almost complete absence of beam hardening artifacts or from slices misalignment, with a high spatial and contrastographic resolution³⁶ (**Fig. 1C, 1D and Supplementary Fig. 1A**).

Several studies have investigated the relationship between coronary artery stenosis severity and ischemia. The COURAGE sub-study showed that, among patients with coronary artery stenosis $\geq 70\%$, only 32% of patients had severe ischemia, and 40% had negligible ischemia by myocardial perfusion³⁷. In addition, Tonino *et al.* pointed out that 20% of lesions with $\geq 70\%$ stenosis did not produce ischemia, and 17% of lesions with 50% stenosis caused ischemia³⁸. Recently, software for computing DICOM data, obtained with the latest generation of CT scanners, has been developed to evaluate, in a non-invasive way, the fractional flow reserve (FFR_{ct}) and the functional impact of stenosis on blood flow all along the vessel; values of FFR_{ct} below 0.8 are considered expression of critical hypoperfusion³⁹ (**Fig. 1C**). The next goals are characterizing atherosclerotic plaque and predicting plaque instability. The latest generation CT scanner can acquire images at two different energies, 80 and 140 kVp, and perform dual energy computed tomography to characterize plaque elements by their CT attenuation profiles, which allows differentiation between lipids, fibrous and calcification⁴⁰. In primary prevention settings, an important parameter detected by CTCA recently gained importance. Indeed, in the 2018 update of ACC/AHA cholesterol guidelines, coronary artery calcium (CAC) scoring was assumed to be a valuable strategy to reclassify CV risk and begin statin treatment⁴¹.

CMR

CMR is considered the gold standard imaging modality for evaluating myocardial function and perfusion and detecting myocardial scars without exposing the patient to ionizing radiation^{42, 43}. Indeed, CMR provides accurate mapping of myocardial edema, fibrosis, and necrosis in follow up and prognostic assessment. With recent hardware and software improvements, first-pass perfusion imaging, using CMR, has emerged as an attractive alternative to single photon emission computed tomography myocardial perfusion imaging (MPI) for CHD detection⁴². Perfusion is determined by the first pass of a gadolinium-based contrast agent: regions that are hypoper-

fused have a slower uptake and lower gadolinium concentration, appearing as hypointense perfusion defects. In a later evaluation, rapid cellular metabolism of cardiomyocytes produces an early dismissal of intracellular gadolinium highlighting as strongly hyperintense, metabolically slowed down, fibrotic/necrotic tissue⁴² (**Fig. 1E and 1F**).

Perfusion CMR superior diagnostic performance, versus single photon emission computed tomography MPI, has been studied in multiple clinical trials, such as the CE-MARC study⁴⁴. Furthermore, perfusion CMR imaging can resolve the differences in myocardial perfusion between subepicardial and subendocardial layers for its high spatial resolution ranging from 1.2 to 3 mm. This information is clinically relevant because the subendocardium is the most susceptible layer to ischemia and typically the earliest affected. In addition to myocardial perfusion, CMR global and regional systolic LV function can also be obtained, overcoming objective difficulties of a correct and reproducible ultrasound approach, which is strongly affected by the patient habitus. CMR has been also applied to anatomical coronary visualization. Combined with the development of three dimensional acquisition techniques, superior visualization of coronary anatomy was achieved. Nevertheless, CMR still remains limited in the visualization of distal coronary segments due to inferior spatial resolution⁴⁵. Research in this field could focus on plaque composition assessment as well as coronary flow to provide a comprehensive evaluation of the presence, extent, and functional significance of CHD⁴⁶.

Cardiac PET

Cardiac PET is useful for perfusion, functional evaluation, and assessment of myocardial viability with high sensitivity and high negative predictive value in the diagnosis of CHD and cardiomyopathies⁴⁷. PET is more accurate than SPECT for diagnosing obstructive CHD and quantifying global and regional myocardial blood flow (MBF) and myocardial flow reserve (MFR)⁴⁸. ¹⁸F-FDG is mostly used for metabolic evaluation of myocardial viability (**Supplementary Fig. 1B**) and is considered the most sensitive modality for predicting left ventricular functional recovery post-coronary revascularization. Moreover, it may help assess plaque metabolism in atherosclerosis, allowing prediction of vascular risk inflammation (**Supplementary Fig. 1B**) and unstable plaques⁴⁹. PET has high spatial and temporal resolution, allowing the acquisition of dynamic sequences obtained on first-pass extraction at the time of radiotracer infusion to quantify resting and hyperemic regional MBF⁵⁰ (**Fig. 1G and 1H**).

The advent of hybrid imaging overcomes the limitations and drawbacks of standalone modalities, providing both functional and morphological information. Thus, integrated PET/CT and PET/MR systems have huge potential for cardiac imaging assessment. The incremental value of multimodalities imaging lies in accurate spatial co-localization of myocardial perfusion defects and anatomic coronary arteries (**Fig. 1I and 1J**). This combined technology allows detection and quantification of the burden of calcified and non-calcified plaques, quantification of vascular activity and endothelial function, identification of flow-limiting coronary stenosis, and, potentially, identification of high-risk plaques⁵¹(**Fig. 1K, 1L and 1M**). PET/CT is today well established in clinical routine imaging⁵¹. PET/MR represents a new integrated protocol for simultaneous evaluation of viability, perfusion, morphological and functional imaging, thus eliminating ionizing radiation and improving tissue contrast⁵². At present, ¹⁸F-FDG PET/MRI is potentially a very promising evaluation technique for *in vivo* imaging of systemic atherosclerosis-related inflammation⁵².

The selective reorganization of vulnerable atherosclerotic lesions represents a major task in cardiovascular research. ¹⁸F-FDG could be used as an inflammation radiotracer in atheroma to identify sites of active inflammation and to predict future thrombotic effects⁵³. ¹⁸F-FDG PET is a marker of vascular inflammation in large vessels, which is helpful for selecting patients who can benefit from new therapies. Similarly, Gallium-68, labelled DOTATATE, targets the somatostatin receptor subtype 2 (SSTR2) found on the surface of macrophages. Pre-clinical studies have confirmed ⁶⁸Ga-DOTATATE's superiority over FDG in terms of macrophage specificity in atherosclerotic inflammation and in discriminating between unstable and stable coronary plaques⁵⁴. ¹⁸F-fluorocholine (¹⁸F-FCH), a PET tracer with potential anti-inflammatory affinity, shows a strong association between large vessel uptake and atherosclerotic changes in the arterial walls, with an apparent inverse relationship with calcification⁵⁴.

Also of growing interest is the use of other radio tracers for functional imaging of the atherosclerotic plaques, such as ¹¹C-PK11195⁵⁵, or tracers of molecular calcification, such as Sodium Fluoride (¹⁸F-NaF), and hypoxia radiotracers, such as ¹⁸Fluoromisonidazole (FMISO)⁵⁶, both to evaluate intra-plaque calcification and to discriminate between stable and unstable plaques.

The evolution of atherosclerosis and the emergence of new technologies to measure the vulnerability of atherosclerotic plaque allow non-invasive visualiza-

tion of biochemical processes and provide a new molecular imaging pattern in atherosclerosis pathophysiology. At present, the results appear encouraging and may have a wide impact on personalized medicine, allowing an early diagnosis and foreseeing the response to new targeted treatments⁵⁷.

Next, we discuss the most important consortia and clinical networks, including imaging data extracted from the aforementioned techniques.

Cardiovascular Consortia for Precision Medicine Implementation

Since 1948, beginning with the Framingham Heart Study, several consortia have been set up to improve and harmonize clinical studies for primary and secondary prevention. Integral parts of consortia are represented by biobanks storing biological samples or completed patient genotyping and clinical information, thus providing new possibilities in the context of personalized medicine⁵⁸.

By 2002, the possibility of linking data collected in childhood through adulthood to future cardiac events became a realistic focus with the organization of the International Childhood Cardiovascular Cohort (i3C) Consortium, collecting data from over 40,000 children and adolescents and providing three key research areas: 1) follow-up of CV morbidity and mortality; 2) genetic data collection and analysis; and 3) repeated assessment of non-invasive vascular measures (e.g. carotid IMT). Furthermore, several consortia for risk prediction in adulthood have been organized. CARDIoGRAMplusC4D (Coronary ARtery Disease Genome wide Replication and Meta-analysis (CARDIoGRAM) plus The Coronary Artery Disease (C4D) Genetics) consortium combines omics data from multiple large studies to identify risk loci for CHD and MI.

Wellcome Trust Case Control Consortium (WTCCC), Cardiogenics, and CVgenes@target consortia collected adult individuals with CHD and, in association with risk factors, performed case-control genome wide association studies (GWASs) or identified several genetic loci affecting the risk of CHD development, thus representing attractive targets for therapeutic intervention.

The UK Biobank is a prospective cohort study with deep genetic, imaging, and clinical data, along with serum metabolic profiles and biospecimens collected from about 500,000 individuals (40-69 years) in the UK during 2006-2010. One of the most promising aims of both the UK Biobank and the CVgenes@target Consortium is the application of network approach and machine learning.

Multicentre consortia were also instituted for the genetic determination of cardiovascular risk factors. The Global Lipids Genetics Consortium covers several cohort studies aimed at understanding the genetic etiology of quantitative lipid traits in age groups, starting from young adults up to the elderly. Furthermore, the International Consortium for Blood Pressure (ICBP) focuses on the genetics of blood pressure dysfunction, thus providing potential novel therapeutic pathways for CVD prevention. The Genetic Investigation of ANthropometric Traits consortium ultimately contributes to understanding the genetics of obesity onset.

The European Research Infrastructure for Imaging Technologies in Biological and Biomedical Sciences (Euro-BioImaging or EuBI) provides physical user access to a wide range of technologies in biological and biomedical imaging. A biobank service is also available, providing different types of biological materials (DNA, RNA, proteins). The Advanced Cardiovascular Imaging Consortium is a program involving the collection of data related to CCTA scans patient and clinical features. The Cardiac Atlas Project is an open source, freely available, large-scale database of CMR examinations associated with clinical data in order to develop a structural and functional atlas for clinical research. In particular, the MESA (Multi-Ethnic Study of Atherosclerosis) is a large-scale, cardiovascular study population aiming to investigate the manifestation of subclinical to clinical CHD before symptoms onset. The PowerShare Network shares medical images and patient data for artificial intelligence applications.

Cohort studies investigating cardiovascular phenotypes over time provides the opportunity to implement secondary prevention strategies. Indeed, the GENetIcs of sUbsequent Coronary Heart Disease (GENIUS-CHD) is an international consortium collecting patients with known CHD, at baseline and prospective follow up, for cardiac acute events as well as biological samples/DNA or completed genotyping. Furthermore, the Myocardial Infarction Genetics Consortium (MIGen) was instituted to study the genetics of early onset MI (men <50 and women <60) (Table 1).

Network Medicine in CHD Characterization and Cardiovascular Risk Factor Susceptibility

Network Medicine Workflow

The evolution of precision medicine is now the concept of “network medicine”. The term was coined in 1999 by Barabasi⁵⁹, who proposed that the biology of living organisms can be studied in a complex of *nodes*, and *edges* between *nodes*, that lead to the for-

mation of the interactome, or the whole set of molecular interactions in cells⁶⁰. The network approach central focus is on exploiting the complexity of gene regulation, metabolic reactions, and protein-protein interactions (PPIs), which synergistically interact each other⁶⁰. By integrating electronic health records, omics data, and imaging techniques, network medicine aims to provide specific roadmaps for understanding CHD causes and mechanisms and establishing tailored treatments and preventive strategies. Indeed, the main goal is to establish a precision medicine approach by focusing on the intermediate phenotypes (endophenotypes), which may identify risk factors, provide early detection, predict clinical outcomes, and provide innovative drug targets (Fig. 2 and Fig. 3). Clustering and machine learning algorithms are used to integrate data in a multi-level analysis, aiming to build complex molecular networks^{61, 62}. Networks are composed of *nodes*, represented by biological factors such as proteins (protein-protein networks), genes (co-expression networks), metabolites, and epigenetic hallmarks (regulatory networks), and *edges* depicting their relationship such as physical interactions, common metabolic pathways, and shared genes⁶³.

Network Based Approach and Cardiovascular Risk Factors

Hypertension pathophysiology is characterized by a complex interplay between genes and environmental factors⁶⁴. Network approaches were also applied to detect novel candidate genes involved in blood pressure (BP) regulation and hypertension⁵⁵. Huan *et al.* performed a co-expression network analysis, identifying 34 genes responsible of BP variation. To further assess molecular key driver genes in BP regulation, weighted correlation network analysis (WCGNA) was used to build co-expression networks and sub-networks, in which the SH2B adaptor protein 3 (*SH2B3*) resulted in a connector gene in the different sub-networks, representing a novel target for prevention or treatment of hypertension⁶⁵.

Similar to hypertension, hypercholesterolemia is a quantitative trait controlled by the interactions among several quantitative trait loci and environmental factors⁶⁶. Network studies were focused on Familial Hypercholesterolaemia (FH). WCGNA identified a triglyceride-associated co-expression module enriched for genes involved in lipid metabolic processes where an *hub* gene was fatty acid desaturase 3 (*FADS3*), classified as a new gene for FCHL disease risk⁶⁷. Another study investigated co-expressed gene networks that are potentially involved in FH development by integrating GEO microarray datasets and

Table 1. Consortia collecting clinical information, omics and imaging data for primary and secondary prevention in CHD

Consortium	Description	URL
PRIMARY PREVENTION		
International Childhood Cardiovascular Cohort (i3C)	Cohorts of children and adolescents in follow up for CVD morbidity and mortality	https://www.i3ccconsortium.org/index.html
CARDIoGRAMplusC4D	Combination of data from multiple large scale genetic studies to identify risk loci for CHD	http://www.cardiogramplusc4d.org/
Wellcome Trust Case Control (WTCCC)	Collection of GWASs to shed light on the genetic architecture of human diseases	https://www.wtccc.org.uk/
Cardiogenics	Collection of GWASs on cardiovascular disorders	https://www.cardiogenics.org/web/
CVgenes@target	European collaboration to investigate genomic variants affecting atherosclerosis risk for identification of underlying genes and involved pathways	http://cvgenesatarget.eu/
Uk Biobank	Large prospective study with deep genetic, imaging and health related data with the aim of improving the prevention, diagnosis and treatment of a wide range of illness such as CHD	https://biobank.ctsu.ox.ac.uk/
Global Lipids Genetics (GLGC)	World-wide collaboration to understand the genetic etiology of quantitative lipid traits	http://lipidgenetics.org/
International Consortium for Blood Pressure (ICBP)	Collection of GWASs focused on the genetics blood pressure dysfunction	http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000585.v1.p1
Genetic Investigation of ANthropometric Traits (GIANT)	International collaboration seeking to identify genetic loci that modulate human body size and shape, including obesity	https://portals.broadinstitute.org/collaboration/giant/index.php?title=GIANT_consortium&oldid=251
European Research Infrastructure for Imaging Technologies in Biological and Biomedical Sciences (Euro-BioImaging)	Open access to a broad range of technologies in biomedical imaging such as CT, MR, PET, coupled also to biobank data	http://www.eurobioimaging.eu/
Advanced Cardiovascular Imaging Consortium (ACIC)	Clinical program collecting CCTA scans and patient characteristics aimed to reduce cost and improve safety and efficiency in patient care	https://clinicaltrials.gov/ct2/show/NCT00640068
Cardiac Atlas Project (CAP)	Large-scale database of cardiac MR examinations and associated clinical data structural and functional of the normal heart and cardiac diseases	http://www.cardiacatlas.org/
PowerShare Network	Highly scalable cloud-based platform that enables the secure access and exchange of images and patient data	https://www.nuance.com/healthcare/medical-imaging/powershare-network.html
SECONDARY PREVENTION		
CARDIoGRAMplusC4D	Combination of data from multiple large scale genetic studies to identify risk loci for MI	http://www.cardiogramplusc4d.org/
GENetIcs of sUbsequent Coronary Heart Disease (GENIUS-CHD)	Clinical cohorts of patients with established CHD with biological sample repository and longitudinal follow up for subsequent events	http://www.genius-chd.com/
Myocardial Infarction Genetics (MIGen)	Combination of data from multiple large scale genetic studies to identify risk loci related to early MI	http://www.kathiresanlab.org/collaborators/myocardial-infarction-genetics-exome-sequencing-consortium/

CCTA: coronary computed tomography angiography; CHD: coronary heart disease; CT: computed tomography; CVD: cardiovascular disease; GWAS: genome-wide association study; MI: myocardial infarction; MR: magnetic resonance; PET: positron emission tomography.

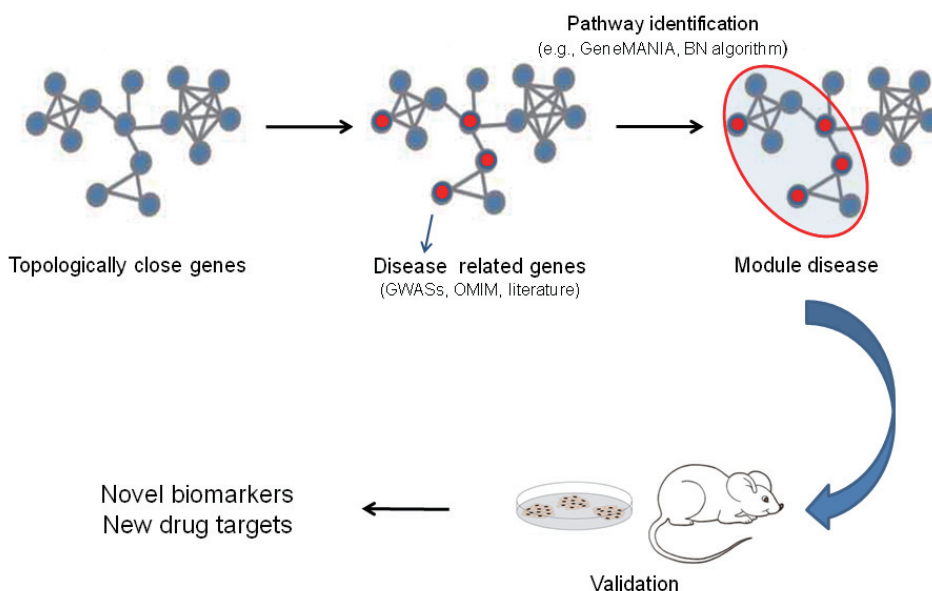


Fig. 2. Nodes, edges and molecular network analysis

The picture shows basic network analysis principles. The blue circles represent genes or their products (nodes) that are topologically related in the human interactome. Each pair of nodes is linked (edges) when a physical or functional interaction occurs between them. The red circles represent the seed genes arising from GWASs, OMIM, and literature, which are clustered in the interactome, as a starting point for a molecular network analysis. By using network-oriented algorithms, it is possible to identify a specific disease module, revealing novel molecular pathways underlying disease. The validation of these bioinformatic findings in animal and cell-based models could provide novel useful biomarkers and/or drug targets to improve precision medicine and personalized therapy.

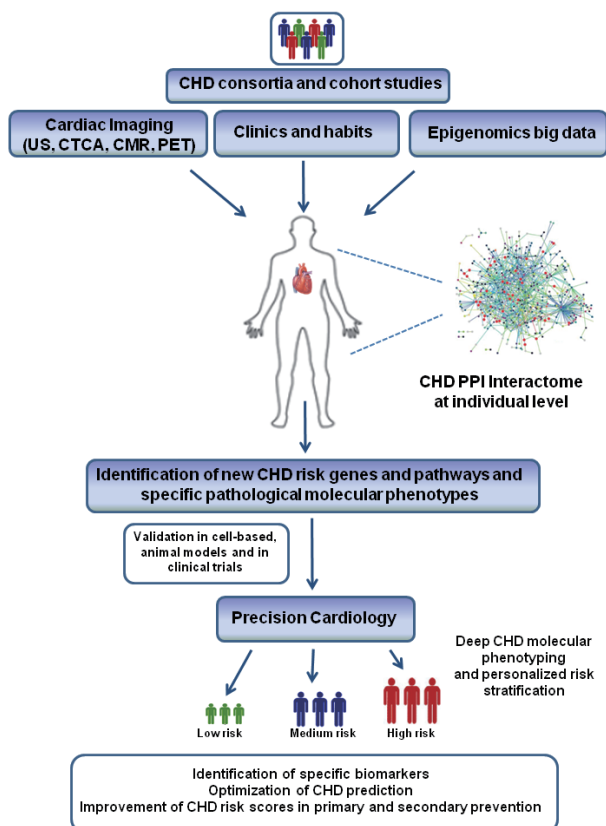


Fig. 3. Workflow of network medicine approach in CHD characterization and cardiovascular risk factor susceptibility

Individuals may have a similar clinical cardiovascular phenotype but a distinct molecular disease profile. Using a network approach, patients undergo deep phenotyping with data analysis, thus improving precision cardiology. The first step is collecting multi-omics and imaging big data on CHD and cardiovascular risk factor studies from available repositories, consortia, and cohort studies, and integrating them to build different types of networks. Machine learning methods and statistical approaches are then applied to obtain the interactome by identifying disease modules and hub nodes at individual level. Functional analysis leads to identification of new disease genes and pathways. The next step is validation of network findings in cell-based models, small and large animal models and in clinical trials. This analytic strategy clusters individuals into groups that are different from those based on clinical phenotypes, allowing a deep CHD phenotyping and personalized risk stratification and improving the identification of disease biomarkers, the prediction and prognosis, and optimize primary prevention.

functional pathways of leukocyte subsets from FH patients and healthy individuals. A total of 4,232 co-expressed relationships were identified in patients showing dysregulation in lipoprotein and cholesterol metabolism as well as neurotrophin signaling (SNARE proteins), MAPK pathways, and endocytosis processes, suggesting an altered transport of low-density lipoprotein receptor (LDLR) across membrane⁶⁸.

Beyond environmental and lifestyle factors, obesity is highly influenced by genetics at multiple loci (polygenic), considering this condition is an inherited trait⁶⁹. Kogelman and colleagues performed a network-based approach study on obesity, building inter-tissue gene co-expression networks between healthy and obese adult subjects from the whole genome expression profiles of four tissues (liver, muscle, subcutaneous and visceral adipose tissues). Weighted gene co-expression network analysis (WGCNA) showed that *IL-6* and *IL1B* genes were differentially co-expressed across tissues in obese patients compared to non-obese individuals. Furthermore, an eQTL mapping approach, used to detect genes influencing mRNA levels, led to the identification of *PTPRE*, *IL-6R*, and *SLC6A5* genes with a putative key role in obesity risk⁷⁰ (Table 2).

Network Based Approach for Primary Prevention of CHD

The network approach modularity has also been applied for CHD, opening new perspectives in understanding pathogenic mechanisms aimed at promoting predictive disease models and primary prevention strategies⁷¹. Furthermore, several investigations of network medicine have been used as a starting point for obtaining clinical and biological information from consortia and cohort studies. Oguz *et al.* identified a molecular network predictive of advanced coronary calcium in CHD, using genotype data from middle-aged male patients of the ClinSeq[®] and Framingham Heart studies. The GeneMANIA algorithm was used to create a functional network associated to “vascular aging” functional pathways and significantly related to high levels of calcium score, thus providing a robust predictive model^{15, 72}.

Several co-expression network analyses were performed to identify key modules and *hub* genes in CHD. Microarray data from patients and controls were downloaded from GEO database. WGCNA identified 21 modules and 30 *hub* genes in CHD associated to membrane biological processes cellular components, and pathway included in hypertrophic cardiomyopathy. Moreover, *hub* genes, which provided glucose-6-phosphate dehydrogenase (*G6PD*) and S100 calcium binding protein A7A (*S100A70*) as

key driver genes, suggesting a potential role for target in CHD⁷³.

Furthermore, Mäkinen *et al.* integrated 14 GWASs, including patients and controls of the CARDIoGRAM Consortium, with tissue gene expression studies of CHD. WGCNA and Bayesian network models revealed specific CHD-associated networks for lipid metabolism, coagulation and immunity. A gene network involved in antigen processing was strongly associated with CHD in which glyoxalase I (*GLO1*) and peptidylprolyl isomerase I (*PP1L1*) were identified as key driver genes. Results were also validated in human aortic endothelial cells⁷⁴. Huan *et al.* performed an integrated system biology analysis by constructing co-expression networks in whole blood gene expression profiles from 188 CHD cases and 188 controls, leading to the identification of 24 co-expression modules. Computational analysis using Key Driver Analysis algorithm indicated *TNFRSF13C* and *EBF1* as key driver genes⁷⁵.

PPI networks were also investigated in the CHD setting. Li *et al.* selected a set of 266 CHD disease genes as initial seed genes and protein data (Fig. 2). PPI network analysis provided 10 separate networks, and a CHD specific network was distributed into 14 modules and 114 *hub* genes. The GO enrichment and KEGG pathway revealed novel functional pathways, such as the androgen receptor signaling pathway, neuropeptide hormone activity, peptide YY receptor activity, NOD-like receptor signaling pathway, and RIG-I-like receptor signaling pathway, thus providing new pathogenic mechanisms in CHD⁷⁶. Duan *et al.* proposed an integrated CHD network analysis combining GWAS genotyping dataset from WTCCC, PPI database and pathway annotation information. Using GIN software, molecular data were organized into specific networks characterized by four modules. Mitogen-Activated Protein Kinase 10 (*MAPK10*) and Collagen Type IV Alpha 2 Chain (*COL4A2*) emerged as significant genes, providing novel clues for clarifying CHD pathogenesis⁷⁷.

Recently, Wang and Loscalzo developed a novel network based algorithm, called the Seed Connector algorithm (SCA), to discover disease modules⁷⁸. Computational analysis identified a CHD network module with 88 proteins and 111 interactions comprising physiological pathways related to genetic loci associated with CHD such as LDL cholesterol and lipoproteins, triglyceride-rich lipoproteins, inflammation molecules, cellular proliferation, and vascular and vascular tone and nitric oxide signaling. GO annotation evidenced 28 seed connectors (Fig. 2), 18 of which were associated with the cardiovascular system, confirmed the key functional role of seed connectors

Table 2. Network based studies associated to cardiovascular risk factors and network pharmacology studies for primary prevention of CHD

Big data source	Computational method	Results	Reference
FHS (SNPs); Microarrays; GO	WCGNA; BN algorithm	Identification of <i>SH2B3</i> as a key driver gene in blood pressure regulation	65
Microarrays; DAVID	WCGNA	Identification of <i>FADS3</i> as a candidate <i>hub</i> gene for FCHL	67
GEO; DAVID; KEGG	PCC analysis	Identification of dysfunctional network in FH patients involving SNARE proteins, MAPK pathways and endocytosis processes	68
GEO; TRANSFAC; TRED; HMDD; miR2Disease; starBase; miRecords; TarBase; KEGG	Interaction analysis	Identification of a key role of <i>SPI1</i> , <i>STAT1</i> , <i>SFFV</i> and <i>SPI1</i> genes in FH pathological mechanisms; significant association of <i>USF2</i> and <i>WAS</i> genes with FH	90
SNPs, Microarrays, GOAmiGO2; KEGG	WGCNA	Differential co-expression of <i>IL-6</i> and <i>IL1B</i> in tissues of obese patients; identification of <i>PTPRE</i> , <i>IL-6R</i> , and <i>SLC6A5</i> as key genes in insulin-related pathways	70
Personalized therapy			
Big data source	Computational method	Results	Reference
1000 Genomes project; STAGE study (SNPs); ConsensusPathDB; AmiGO 2; DGIdb	Girvan-Newman algorithm	Identification in CHD module of several kinases and GPCR genes for which drug already existed or target drugs in development	94
IntAct; MINT, TRANSFAC; GO; DrugBank; PharmGKB; TTD	SCA algorithm	Identification of NRP1 as seed connector targeted by Pegaptanib (DB04895)	78
HPRD; PhosphositePlus; Phospho.ELM; BioGRID; MINT, IntAct; DisGeNET; GWAS Catalog; GWASdb; DrugBank; PharmGKB; TTD	Reference distance distribution	Hydroxychloroquine was significantly associated with a reduced risk of CHD	21
OMIM; DisGeNe; GWAS Catalog; HGMD; HPRD; PhosphositePlus; Phospho.ELM; BioGRID; MINT, IntAct; DrugCentral database	Permutation testing	Identification of several approved drugs (fasudil, parecoxib, dexamethasone) or natural products (resveratrol, luteolin, daidzein, caffeic acid) as novel drug targets in CHD	96

BioGRID: Biological General Repository for Interaction Datasets; BN: Bayesian network; BP: blood pressure; FADS3: fatty acid desaturase 3; CHD: Coronary heart disease; DGIdb: Drug Gene Interaction Database; FH: Familial Hypercholesterolemia; FCHL: Familial Combined Hyperlipidemia; FHS: Framingham Heart Study; GEO: Gene Expression Omnibus; GO: Gene ontology; GPCR: G protein-coupled receptors; GWAS: genome wide association study; HMDD: Human MicroRNA and Disease Database; HPRD: Protein Reference Database; IL1B: Interleukin 1 beta; IL-6: Interleukin-6; IL-6R: Interleukin-6 receptor; IntAct: protein Interaction database; KEGG: Kyoto Encyclopedia of Genes and Genomes; MAPK: mitogen-activated protein kinase; MINT: Molecular Interaction database; NRP1: Neuropilin 1; OMIM: Online Mendelian Inheritance in Man; PCC: Pearson correlation coefficient; PharmGKB: Pharmacogenomics Knowledge Base; PTPRE: Receptor-type tyrosine-protein phosphatase epsilon; SFFV: spleen focus forming virus; SH2B3: SH2B adapter protein 3; SLC6A5: Solute Carrier Family 6 Member 5; SNARE: Soluble NSF Attachment; SNP: Single Nucleotide Polymorphism; SP1: specificity protein 1; SPI1: proviral integration oncogene spi1; STAGE: Stockholm Atherosclerosis Gene Expression; STAT1: signal transducer and activator of transcription 1; TRANSFAC: TRANScriptioN FACTor; TRED: Transcriptional Regulatory Element Database; TTD: Therapeutic Target Database; USF2: upstream transcription factor 2; WAS: Wiskott-Aldrich Syndrome.

in CHD characterization⁷⁸).

Through multidimensional big data (GWASs, SNPs and gene expression) from the Framingham Heart Study, Yao *et al.* built an integrated network by iterated Transfer-Fusion (TFit) algorithm, identifying

13 CVD-related modules. Authors identified several *hub* SNPs related to CHD and the presence of cardiovascular risk factors, such as BP, body mass index, and HDL-C LDL-C, and triglycerides. The integration of gene expression data to the SNP modules showed a

Table 3. Network based genomic studies for primary prevention of CHD

Big data source	Computational method	Results	Ref.
ClinSeq study and FHS (GWAS); GEO; BioGRID	GeneMANIA	Identification of a SNP predictive network of advanced coronary calcium	72
GEO; DAVID; KEGG	WGCNA	Identification of <i>G6PD</i> and <i>S100A7</i> as CHD disease genes	73
CARDIoGRAM Consortium; Ottawa Heart Genomics Study; KEGG	WGCNA; BN algorithm	<i>GLO1</i> and <i>PPIL1</i> were identified as key driver genes in CHD	74
FHS (GWAS); GO	WGCNA; KDA algorithm	Association of <i>TNFRSF13C</i> and <i>EBF1</i> as key driver genes in CHD	75
CADgene; HPRD; DAVID; KEGG	Newman's algorithm	Evidence of AR, NLR and RLR signaling pathways, and neuropeptide and PYY receptor activity as new pathogenic mechanisms in CHD	76
WTCCC; MiMI software	GIN software	Identification of <i>MAPK10</i> and <i>COL4A2</i> as CHD susceptibility genes	77
IntAct; MINT; TRANSFAC; GO	SCA algorithm	Identification of 18 network seed connector genes involved in CHD	78
dbGaP; GWAS catalog; ENCODE	TFit algorithm	Identification of a 3-way relationships between genetic variants, gene expression, and phenotypes in CHD and risk factors	79
CARDIoGRAMplusC4D 1000 GWAS; iRefIndex database	dmGWAS software	Identification of key driver genes (<i>UBC</i> , <i>CAND</i> , and <i>SMARCA4</i>) underlying CHD pathogenesis	80
GEO; TRANSFAC; TransmiR; miRTarBase; miRecords; TarBase; HMDD; KEGG	BFS algorithm	By prioritized core sub network analysis <i>PTEN</i> resulted to be a key regulator gene in CHD	81

AR: Androgen receptor; BFS: breadth-first search; BioGRID: Biological General Repository for Interaction Datasets; BN: Bayesian network; CARDIoGRAM: Coronary ARtery DIsease Genome-wide Replication And Meta-Analysis; CHD: coronary heart disease; COL4A2: Collagen Type IV Alpha 2 Chain; dbGaP: database of Genotypes and Phenotypes; EBF1: Early B-Cell Factor 1; ENCODE: Encyclopedia of DNA Elements; FHS: Framingham Heart; G6PD: G6PD glucose-6-phosphate dehydrogenase; GEO: Gene Expression Omnibus; GLO1: glyoxalase I; GO: Gene ontology; GWAS: Genome-wide association study; HMDD: Human MicroRNA and Disease Database; HPRD: Human Protein Reference Database; IntAct: protein Interaction database; KDA: Key Driver Analysis; Study; KEGG: Kyoto Encyclopedia of Genes and Genomes; MAPK10: Mitogen-activated protein kinase 10; MiMI: Michigan Molecular Interactions; MINT: Molecular INTERaction database; NLR: NOD-like receptor; PPIL1: peptidylprolyl isomerase I; PTEN: Phosphatase and tensin homolog; PYY: peptide YY; RLR: RIG-I-like receptor; S100A7: S100 calcium binding protein A7A; SNP: Single Nucleotide Polymorphism; TFit: iterated Transfer-Fusion; TRANSFAC: TRANSCRIPTION FACTOR database; TNFRSF13C: TNF Receptor Superfamily Member 13C; UBC: polyubiquitin; WGCNA: Weighted correlation network analysis; WTCCC: Wellcome Trust Case Control Consortium.

large number of eQTL-associated genes already associated to CVD phenotypes such as fatty acid desaturase 1 (*FADS1*), 3-Hydroxy-3-Methylglutaryl-CoA Reductase (*HMGCR*), lipoprotein Lipase (*LPL*), LDLR, and sterol Regulatory Element Binding Transcription Factor 2 (*SREBF2*), suggesting the existence of 3-way relationships between genetic variants, gene expression, and phenotypes⁷⁹).

In a recent study, Miao *et al.* performed a tissue-specific network analysis of CHD. Biological inputs of CHD genetic variants were obtained from the CARDIoGRAMplusC4D 1000 GWAS. Co-expression

networks were elaborated, using dmGWAS software, and a CHD interaction network was built using experimentally validated gene interactions and gene co-expression in coronary samples. The network contained 53 genes significantly associated with CHD. A pathway enrichment analysis revealed an overlap in the network with genes involved in regulating peripheral arteries with a set of key driver genes, such as *UBC*, *CAND*, and *SMARCA4*, suggesting that abnormalities in the peripheral vasculature might be also important pathways underlying CHD pathogenesis⁸⁰ (Table 3).

Recently, in medical imaging, we have assisted with a shift from qualitative imaging to quantitative assessment through the extraction of imaging biomarkers by applying artificial intelligence⁸¹). Machine and deep learning approaches involve using raw imaging data to extrapolate imaging features and, together with clinical information and big data, are processed into algorithms to build disease models and neural networks in order to improve diagnosis, make clinical decisions, and predict outcomes towards a personalized medical approach⁸¹). Recent studies on machine learning approaches exploiting CTCA showed high prognostic accuracy in risk stratification and prediction of mortality in CHD patients⁸²⁻⁸⁴). Furthermore, in a recent study, a novel algorithm for T1 mapping and extracellular volume fraction in CMR was tested, providing a better computational efficiency than other well-consolidated methods⁸⁵).

Pathogenic Epigenetic-Sensitive Nodes in Primary and Secondary Prevention of CHD

Genomic variants identified through GWASs account for 9% of CHD risk; therefore, a large proportion of the underlying genetic risk remains unclear⁸⁶). The phenomenon of missing heritability is attributed to the notion that much of a complex trait's heritability lies in SNPs that do not reach genome-wide significance association, but rather in epigenetic sites associated with transcriptionally activity, thus influencing gene expression⁵). Furthermore, a recent study showed that genetic variants have a regulatory impact on circulating miRNAs, highlighting an interplay between genetic and epigenetic components⁸⁷).

In **Supplementary Table 1**, we report a brief description of ongoing or completed clinical trials from the website <https://clinicaltrials.gov/> started between 2013 and 2018 and aimed to test epigenetic changes as useful tags to diagnose, prevent, and treat CHD. Concerning primary prevention, a recent clinical trial aims to evaluate the relationship between DNA methylation and the degree of coronary atherosclerosis detected by coronary angiography. Due to their stability in plasma and easiness of detection, many ongoing clinical trials are focused on the evaluation of circulating miRNAs in blood, in association with imaging parameters such as coronary calcifications and stenosis degree, detected by CCTA to characterize the CHD burden⁸⁸) (**Supplementary Table 1**). Regarding clinical trials for secondary prevention evaluation, most analyzed epigenetic tags are still circulating miRNAs coupled with imaging tools, such as PET/CT, to evaluate infarct-related vessels. In the Multiomics and Imaging-based Assessment of Vulnerable Coronary Plaques in Acute Coronary Syndromes

(MultiPlaque) Study, the outcomes will be to assess the vulnerability degree of the atheromatous plaques, before and after a MI, based on miRNA analysis, associated with invasive and non-invasive data, such as coronary angiography, intravascular ultrasound, optical coherence tomography, and FFR/CT. A multi-parametric model for risk prediction will be developed to evaluate the risk associated with the vulnerable coronary plaques in patients who have suffered acute coronary syndrome, as well as the determination of the rate of adverse events and MACE rates.

A further advancement in the field of epigenetic knowledge could represent the employment of epigenetic big data in network models. Zhang *et al.* developed an integrated systems approach to identify regulatory pathways along with key epigenetic and genetic regulators in CHD. In particular, multiple resources, such as differentially expressed genes and miRNAs, known disease genes, and KEGG analysis were integrated using a breadth-first search algorithm providing a prioritized core sub-network in which *PTEN* was found to be a key regulator gene. This data was also validated on peripheral blood samples of CHD patients⁸⁹) (**Table 3**). Furthermore, to identify additional genes related to FH and their interactions, Chen *et al.* performed a transcriptome and miRNA network analysis integrating the transcription profiles, miRNA datasets, and functional analysis of CHD patients, heterozygous FH patients and healthy subjects. In the regulatory network obtained, specificity protein 1 (*SPI1*), signal transducer and activator of transcription 1 (*STAT1*), spleen focus forming virus and proviral integration oncogene sp1 (*SPI1*) formed local networks with a higher node degree, suggesting an important role of these genes in FH. In addition, the transcription factor, upstream transcription factor 2, c-fos interacting (*USF2*) and the gene, Wiskott-Aldrich syndrome, were identified for the first time to be associated with FH^{90, 91}) (**Table 2**).

Personalized Therapy for Primary Prevention and Secondary Prevention of CHD

Due to network medicine ability to reveal hidden connections, one field of application could be identifying new drug targets as well as drug repurposing^{19, 24, 92-94}). These improvements open a new scenario for the personalized therapy, in which therapeutic strategies are customized to a specific genetic background (**Supplementary Fig. 2**). Network pharmacology exploits sources in drug-target databases and connect therapeutic and targeted proteins and disease-target databases (**Table 2**).

Experimental studies have investigated a net-

work-based approach in novel drug target identification and drug repurposing for primary prevention and treatment of CHD. Recently, Lempiäinen *et al.* prioritized the 68 most likely causal genes for CHD and examined their regulatory roles in 286 metabolic and vascular tissue gene-protein modules. Co-expression networks were constructed by integrating GWAS with PPI datasets, and modules were identified with the Girvan-Newman algorithm. Module analysis was also performed to find novel target for the currently used cardiometabolic drugs. The top identified modules included several kinases and GPCR genes for which drugs already existed (e.g. *CXCR4* and *CCRI* in cellular signaling module), or drugs in development, or for which drug compounds were not reported (e.g. *TRIB3* in the module enriched for extracellular matrix organization and regulation, blood coagulation and platelet activation pathways). These interesting results provided new opportunities both for drug repurposing and for new development in CHD⁹⁵.

A novel algorithm, called SCA, identified 14 seed connector sensitive drug targets not previously reported for CHD (Fig. 2). An interesting seed connector was Neuropilin-1 (*NRP1*), the pharmacological target of pegaptanib (DB04895) an anti-angiogenic drug to treat neovascular age-related macular degeneration. *NRP1* is a membrane receptor involved in physiological and pathological neoangiogenesis and arteriogenesis. In the identified CHD module, *NRP1* was linked to the transcription factor REST, FLT1, and PDGFD, all of which were involved in cellular proliferation and vascular remodeling⁷⁸.

In a recent study, Cheng *et al.* developed a system pharmacology-based platform to assess the interplay between disease proteins and drug targets in the protein-protein interactome to predict CV outcome. Computational characterization was evaluated by reference distance distribution, resulting in four drug network-based predictions. From this analysis, hydroxychloroquine was significantly associated with a 24% reduced risk of CHD²¹.

A recent, network-based analysis of novel drug pleiotropy and drug repurposing in CHD treatment was performed through in silico models by integrating known drug-target interactions, CHD genes, and PPI data. In particular, two drug-target networks for both approved drugs and natural products were assembled considering experimentally validated drug-target interactions. Permutation testing identified significant associations of 51 approved drugs and 27 natural products with CHD, including several well-known anti-CHD natural products (quercetin and luteolin) and known cardiovascular drugs. Furthermore, computational elaboration provided significant links with

other approved drugs, such as fasudil, parecoxib, and dexamethasone, and natural compounds, such as resveratrol, luteolin, daidzein and caffeic acid, thus suggesting a new mechanism of action in CHD intervention and drug repurposing with a network approach⁹⁶ (Table 2).

Epidrugs in CHD Personalized Therapy: Focus on Clinical Trials

Looking for novel therapies to treat CHD, the possibility of exploiting the reversibility of epigenetic changes has been entertained. Indeed, a great variety of small molecules with epigenetic and anti-atherogenic activity have been described in recent years²⁴. Epidrugs can be classified according to their epigenetic effects, in particular, acting on DNA methylation or histone modification, thus providing strategies to ameliorate CHD care and prevent complications. Interestingly, some natural compounds, including polyphenols, fenofibrates, cocoa, and folic acid, can also modulate DNA methylation status, while resveratrol can influence chromatin modifications by inhibiting histone modifying enzymes. Furthermore, statins and ezetimibe may promote epigenetic-based control in CHD prevention through inhibiting HDAC activity.

We reported clinical trials on the above-mentioned epigenetic modulating compounds in CHD, and its risk factors, both in monotherapy and combined therapy, in primary and secondary prevention using the website <https://clinicaltrials.gov/> (Table 4). Cocoa polyphenols were tested as methylation targeting drugs in a clinical trial in both hypertensive and hypercholesterolemic patients, and resulted in a significant reduction of DNA methylation levels in patient blood cells, with an association between DNA methylation and three polymorphisms located in the *MTHFR*, *MTRR*, and *DNMT3B*, thus suggesting a functional effect of these polymorphisms⁹⁷. Statins represent the gold standard cholesterol-lowering agent to prevent CHD. Furthermore, the pleiotropic effects of these therapeutic compounds may depend, in part, on their epigenetic effects, since they can inhibit HDAC activity⁹⁸. The protective effect of statins for primary prevention has also been demonstrated in three large clinical studies: the MEGA, the JUPITER and HOPE-3⁹⁹⁻¹⁰². Another clinical trial for primary prevention investigated fenofibrate treatment, in combination with atorvastatin, to test the efficacy and safety of once daily fenofibrate assumption on cIMT detected by US in dyslipidemic patients with optimal levels of low density lipoprotein cholesterol after atorvastatin treatment. In the setting of secondary prevention, resveratrol, considered a DNA methylation tar-

Table 4. Ongoing and completed clinical trials update on epidrug-based therapy in CHD and cardiac events in primary and secondary prevention of non diabetic subjects

PRIMARY PREVENTION				
Epidrug	Conditions	Effects	Phase/Status	NCT
Monotherapy				
Polyphenols (cocoa)	Hypertension Hypercholesterolemia	To evaluate the effects of flavanols on coronary endothelial function in response to cold pressor testing and the combined cholesterol lowering effect of treatment with cocoa to reduce plasma LDL-c concentrations	Phase 3/ Completed	NCT00511420 ⁹⁷⁾
Diet plus pravastatin	Hypercholesterolemia	To evaluate the primary preventive effect of pravastatin against CHD	Phase 4/ Completed	NCT00211705 ^{99, 100)}
Rosuvastatin	Elevated hs-CRP	To determine the safety and effectiveness of long-term therapy with rosuvastatin in reducing the risk of major cardiovascular events	Phase 3/ Terminated	NCT00239681 ¹⁰¹⁾
Metformin	Normotensive, LVH, obesity, CHD	To investigate the benefit of 12 month metformin XL 2,000 mg/day treatment on LVH assessed by CMR, as well as on fasting insulin resistance index, obesity and improvement in endothelial function	Phase 4/ Completed	NCT02226510
Combined therapy				
Rosuvastatin plus candesartan/hydrochlorothiazide	CV intermediate risk, normal cholesterol and blood pressure	To evaluate rosuvastatin and a combination blood pressure lowering candesartan/hydrochlorothiazide, used alone or together can reduce the risk of heart attacks, stroke and their sequelae in people without known heart disease and at average risk	Phase 4/ Completed	NCT00468923 ¹⁰²⁾
Fenofibrate plus atorvastatin	Dyslipidemia	To test the effect and safety of once daily fenofibrate assumption on cIMT detected by US in dyslipidemic patients who have optimal levels of low density lipoprotein cholesterol atorvastatin treatment	Phase 3/ Completed	NCT00616772
Metformin Plus statins	CHD Overweight	To test the beneficial impact of 1year metformin treatment on carotid artery atherosclerosis evaluated by US	Phase 4/ Completed	NCT00723307 ¹⁰⁷⁾
SECONDARY PREVENTION				
Epidrug	Conditions	Effects	Phase/Status	NCT
Monotherapy				
Resveratrol	CHD	To assess the improvement of endothelial function, lipidomic signatures, and cell signaling in patients undergoing CABG	NA/ Recruiting	NCT03762096
Fluvastatin	ACS	To evaluate the safety and efficacy of fluvastatin, dosed shortly after or immediately when the coronary event occurs as well as cardiac recurrence and MACEs	Phase 4/ Completed	NCT00171275 ¹⁰³⁾
Statin	SA and UA	To investigate whether an additional treatment with statins in high doses before CABG surgery can reduce the incidence of major post-surgery complications including death, MI and stroke	Phase 4/ Recruiting	NCT01715714
Metformin	STEMI	To evaluate the effect of metformin therapy following STEMI in terms of LVEF assessed by CMR and MACE occurrence	Phase 2 and 3/ Completed	NCT01217307 ¹⁰⁸⁾
Metformin	MI	To determine if pretreatment with metformin can reduce myocardial injury in patients undergoing elective CABG surgery	Phase 4/ Completed	NCT01438723 ¹⁰⁹⁾
Combined therapy				
Folic acid plus vitamins	CHD	To evaluate the clinical effects of homocysteine lowering treatment with B vitamins during 3-5 years follow-up of patients undergoing PTCA and CABG	Phase 3/ Completed	NCT003540
Folic acid plus rosuvastatin	CHD	To compare the effects on endothelial function of the forearm resistance vessels in patients with a previous history of PTCA	Phase 2/ Completed	NCT006935
Folic acid plus simvastatin plus vitamins	MI	To evaluate the effectiveness and safety of more intensive cholesterol-lowering for the reduction of major vascular events in a high-risk population, and of the effects of homocysteine-lowering with folic acid plus vitamin B12	Phase 3/ Completed	NCT00124072
Folic acid plus methotrexate	CHD	To evaluate the efficacy of methotrexate to improve health in patients with symptomatic CHD and previous history of PTCA as well as to monitor the incidence of acute cardiac events.	Phase 2 and 3/ Completed	NCT00759811
Ezetimibe plus simvastatin	SA	To evaluate the efficacy of ezetimibe plus simvastatin treatment vs simvastatin alone in patients affected by SA after PTCA	NA/ Recruiting	NCT03771053
Ezetimibe plus simvastatin	ACS	To compare the of these combined therapy in terms of reduction in the risk of the occurrence of MACEs, cardiac death, and stroke	Phase 3/ Completed	NCT00202878 ¹⁰⁴⁾

ACS: acute coronary syndrome; CABG: coronary artery bypass graft surgery; CMR: cardiac magnetic resonance; CT: computed tomography; CTCA: computed tomography coronary angiography; CHD: coronary heart disease; Hs-CRP: high sensitivity C-reactive protein; LVEF: left ventricular ejection fraction; LVH: left ventricular hypertrophy; MACE: major cardiovascular events; MI: myocardial infarction; PET: positron emission tomography; PTCA: percutaneous transluminal coronary angioplasty; STEMI: ST elevation myocardial infarction; UA: unstable angina; SA: stable angina; US: ultrasound.

get, was used to assess the improvement of endothelial function, lipidomic signatures, and cell signaling in patients undergoing CABG.

The efficacy of statins in secondary prevention was tested in several clinical trials, both in monotherapy and in combined therapy. In particular, in the FACS-trial, fluvastatin therapy significantly reduced the one-year occurrence of major adverse cardiovascular events in ACS patients¹⁰³. Another ongoing trial investigates whether additional treatment with high doses of statins in patients with stable angina and unstable angina, before CABG, can reduce the incidence of major post-surgery complications including death, MI, and stroke.

Folic acid, a well-known methyl donor, was also tested in secondary prevention clinical studies, in combination with other natural compounds, such as vitamins, or drugs, such as statins or methotrexate, in the follow up of patients with obstructive CHD or previous MI undergoing PTCA or CABG, aimed at reducing the incidence of major cardiovascular events. Simvastatin was also tested in combination with ezetimibe. Results from the IMPROVE-IT Trial showed that lipid-lowering therapy with ezetimibe, plus simvastatin, improved clinical outcomes in terms of cardiac death, MI recurrence, and stroke¹⁰⁴.

Curcumin, a component of the golden spice *Curcuma longa* characterized by antioxidant and anti-inflammatory properties, induce epigenetic changes both in terms of DNA methylation, inhibition of histone modifying enzymes, and modulation of miRNA expression. No large clinical studies are ongoing, but a study reported its putative beneficial effects in patients undergoing CABG with a decreased incidence of in-hospital MI as well as lower levels of C-reactive protein, malondialdehyde, and N-terminal pro-B-type natriuretic¹⁰⁵.

Metformin is the most successful oral medication for type 2 diabetes (T2D), lowering cardiovascular mortality and morbidity¹⁰⁶. Metformin can epigenetically act by inhibiting several histone modifying enzymes and DNA methyltransferases¹⁰⁶. Although patients who have cardiac disease with T2D comorbidity are likely to benefit most from metformin, indications of cardiovascular benefit have attracted interest in repurposing metformin independently from diabetes. To this aim, a clinical trial tested the putative beneficial effects of metformin repurposing in primary and secondary prevention in non-diabetic patients. The CAMERA trial (Carotid Atherosclerosis: Metformin for Insulin Resistance Study) on overweight CHD patients with CHD already taking statins, showed no effect on carotid artery IMT measured by US¹⁰⁷. The just completed MET-REMODEL trial

investigated putative beneficial effects of one year metformin XL (2000mg/day) treatment in patients with left ventricular hypertrophy, CHD, and obesity. Clinical outcomes were assessed for LVH evaluation by CMR, fasting insulin resistance index, obesity, and improved endothelial function.

In secondary prevention, the GIPS-III trial (Metformin to Reduce Heart Failure After Myocardial Infarction) tested metformin in patients who have ST-segment-elevation MI and reported few or no beneficial effects in terms of left ventricular ejection fraction improvement, detected by CMR, and the incidence of major adverse cardiac events¹⁰⁸. Another trial evaluated the potential efficacy of metformin pretreatment in limiting cardiac injury, assessed by troponin concentrations, during CABG surgery in patients, reporting no significant reduction of periprocedural myocardial injury^{109, 110}.

Conclusions

The possibility of integrating big epigenetic data, non-invasive imaging parameters, and clinical information in network-based models could provide a revolution for detecting novel specific markers of cardiovascular risk, thus improving primary prevention regimens. Furthermore, network approaches could open a new scenario for personalized therapy, in which therapeutic strategies are customized to a specific genetic background. Indeed, a promising challenge could be applying network modeling approaches to large amount of data from consortia, particularly in a cohort of children and adolescents, in order to characterize the molecular alterations at the base of individual susceptibility to develop CHD in the future and the incidence of acute cardiac events. Furthermore, implementing network studies in patients with known CHD could help improve secondary prevention strategies.

Disclosure

There are no conflicts of interest to disclose.

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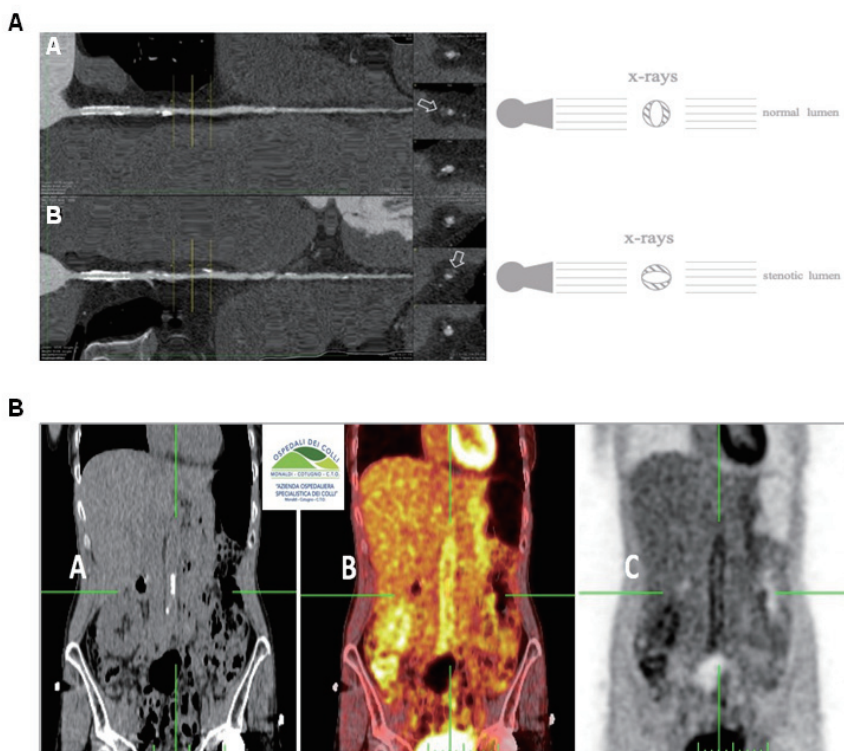
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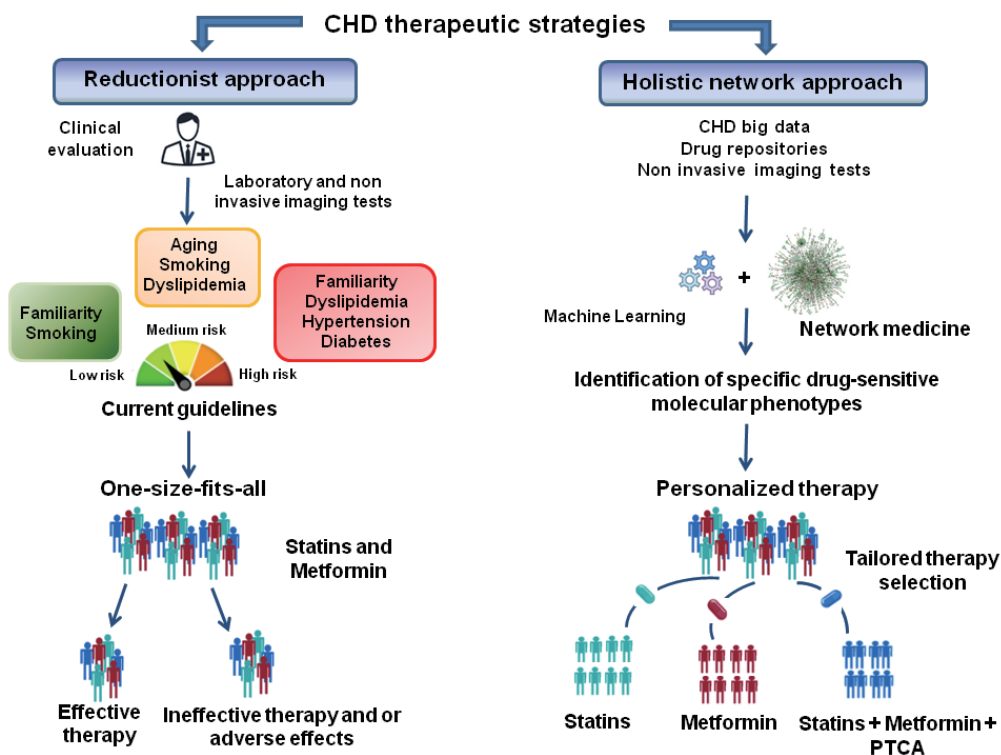
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Supplementary Fig. 1. A. Single wrong projection of a coronary artery could lead to a mistake in the evaluation of stenosis

(A) X-ray beam impact straightened right coronary artery perpendicular to major axis of wide-appearing patent lumen; (B) a 90°-shifted projection shows a stenotic lumen. The right evaluation of stenosis could only be derived by directly measuring area of patent lumen, only achievable in a computer assisted three-dimensional study like CCTA. On the right side of the re-elaborations (A and B) are shown three cross sections of the coronary artery conducted at the point of maximum stenosis, as well as in the immediately preceding and following tract: the width of the lumen is clearly demonstrated, but also the atheromatous plaque can also be seen in its peripheral development (open arrows). Note the optimal representation of stent in the proximal tract of coronary artery, with absolute lack of artifacts projecting in intrastent lumen. B. ¹⁸F-FDG PET/CT: ¹⁸F-FDG increased uptake on Abdominal Aorta plaque. A) CT calcific plaque; B) ¹⁸F-FDG PET/CT Fusion Imaging; C) ¹⁸F-FDG PET.



Supplementary Fig. 2. Workflow of network pharmacology approach in CHD

In the current used reductionist approach, after patient clinical evaluation using laboratory and non invasive imaging tests, the risk to develop clinical manifestations of CHD and the clinical decision making is estimated on the basis of the current guidelines and risk scores where classical cardiovascular risk factors are the predominant indicators leading to the “one-size-fits-all” therapeutic strategies. In the network approach, big data and clinical variables are collected as well as information about drugs and drug targets and integrated through machine learning into network models to create individual molecular/pathway signatures and to identify specific drug sensitive-clinical phenotypes matched to previously defined disease profiles that can guide the selection of tailored treatments. The appropriate tailored drug or medical procedure (i.e. PTCA) is then selected based on this match, to improve the efficacy of treatment and reduce the probability of side effects and recurrence.

Supplementary Table 1. Ongoing clinical trials investigating useful epigenetic tags in primary and secondary prevention of CHD

PRIMARY PREVENTION					
Epigenetic tag	Participants	Samples	Description	Status	NCT
DNA methylation	200	Blood samples, aortic tissues	To investigate the relationship between DNA methylation the degree of coronary atherosclerosis in subjects undergoing carotid ultrasound and coronary angiography	Recruiting	NCT03462277
microRNAs	80	Plasma	To determine the levels of cardiospecific circulating miRNAs in patients with different stages of atherosclerosis according to 640-slice multispiral CT	Recruiting	NCT03855891
	24	Plasma	MiR-8059 down-regulation is correlated to coronary calcification detected by CTCA	Completed	NCT01992848 ⁸⁸⁾
	4,000	Plasma	To determine the expression level of miR-320a as effective biomarker in evaluating the diagnosis of CHD	Recruiting	NCT02751060
	100	Plasma	To evaluate the potential diagnostic and prognostic value of circulating miRNAs compared with cTnI for suspected ACS	Recruiting	NCT02755207
SECONDARY PREVENTION					
Epigenetic tag	Participants	Samples	Description	Status	NCT
microRNAs	460	Plasma	To identify circulating miRNA expression profiles that may serve as a sensitive and reliable biomarker to predict future adverse CV outcomes and death in patients with established CHD	Recruiting	NCT03635255
	4,000	Plasma	To determine whether the expression level of miR-320a are effective as biomarker for prognosis and treatment effects in CHD	Recruiting	NCT02751060
	100	Plasma	To research the vulnerability of atheromatous plaque, based on multiomics data (miRNA signature) and imaging data, in patients with previous ACS (UA or MI) as well as the evaluation of MACEs	Recruiting	NCT03391908
	100	Plasma	To evaluate CHD progression in ACS patients, and to investigate the predictive value of metabolic profiles, patterns of circulating miRNAs and inflammatory mediators on CHD progression evaluated by PET/CT. To evaluate the progression of disease within the infarct-related vessel treated with the use of bioresorbable stent/bioresorbable polymer stents	Not yet recruiting	NCT03890822

ACS: acute coronary syndrome; cTnI: cardiac Troponin I; CT: computed tomography; CTCA: computed tomography coronary angiography; CHD: coronary heart disease; MACE: major cardiovascular events; MI: myocardial infarction; PET: positron emission tomography; UA: unstable angina.