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

Teresa Infante¹, Luca Del Viscovo², Maria Luisa De Rimini³, Sergio Padula⁴, Pio Caso⁴ and Claudio Napoli⁵, ⁶

¹ Department of Advanced Clinical and Surgical Sciences, University of Campania “Luigi Vanvitelli”, Naples, Italy
² Department of Precision Medicine, Section of Diagnostic Imaging, University of Campania “Luigi Vanvitelli”, Naples, Italy
³ Unit of Nuclear Medicine, A.O.R.N. Dei Colli, Monaldi Hospital, Naples, Italy
⁴ Department of Cardiology, A.O.R.N. Dei Colli, Monaldi Hospital, Naples, Italy
⁵ Clinical Department of Internal Medicine and Specialistics, Department of Advanced Clinical and Surgical Sciences, University of Campania “Luigi Vanvitelli”, Naples, Italy
⁶ IRCCS SDN, Naples, Italy

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 hereditability”, 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 hereditability”, 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 events¹⁰, 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

Address for correspondence: Teresa Infante, Department of Advanced Clinical and Surgical Sciences, University of Campania “Luigi Vanvitelli”, 80138, Naples, Italy. E-mail: teresa.infante@unicampania.it
Received: August 7, 2019  Accepted for publication: September 24, 2019

Copyright©2019 Japan Atherosclerosis Society
This article is distributed under the terms of the latest version of CC BY-NC-SA defined by the Creative Commons Attribution License.

Advance Publication Journal of Atherosclerosis and Thrombosis
Accepted for publication: September 24, 2019  Published online: November 12, 2019
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. 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 approach. Currently, epigenetics, combined with the innovative system biology approach, is useful for personalized CHD therapy. Furthermore, unlike DNA variations, epigenetic modifications are, potentially, pharmacologically reversible. 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 prevention. Furthermore, emerging hybrid imaging techniques in the field of cardiovascular medicine, such as PET/CT and PET/MR, provide simultaneous morphological and functional parameters.

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. 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 tests. 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. Based on this evidence, the test is a recommended clinical tool to assess risk prediction in a wide population of subjects (Fig. 1A and 1B).

CTCA

CCTA evolved not only to reduce radiation exposure, but also to optimize image acquisition, achieving more spatial and contrast resolution. 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 lesions. Furthermore, radiation doses are lower with wide-area detector scanners because of shorter
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 shift. 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 scanning times and lower redundant radiation from either overlapping sequential axial scans or helical oversampling.

Effectively, “temporal resolution” is one of the objectives. 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 other. 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 shift. 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 cycles\textsuperscript{35}.

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 resolution\textsuperscript{36} (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 perfusion\textsuperscript{37}. In addition, Tonino \textit{et al.} pointed out that 20\% of lesions with $\geq 70\%$ stenosis did not produce ischemia, and 17\% of lesions with 50\% stenosis caused ischemia\textsuperscript{38}.

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\textsubscript{CT}) and the functional impact of stenosis on blood flow all along the vessel; values of FFR\textsubscript{CT} below 0.8 are considered expression of critical hypoperfusion\textsuperscript{39} (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 calcification\textsuperscript{40}. 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 treatment\textsuperscript{41}.

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\textsuperscript{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 detection\textsuperscript{42}. Perfusion is determined by the first pass of a gadolinium-based contrast agent: regions that are hypoperfused 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 tissue\textsuperscript{42} (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 study\textsuperscript{44}. 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 resolution\textsuperscript{45}. 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 CHD\textsuperscript{46}.

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 cardiomyopathies\textsuperscript{47}. PET is more accurate than SPECT for diagnosing obstructive CHD and quantifying global and regional myocardial blood flow (MBF) and myocardial flow reserve (MFR)\textsuperscript{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 plaques\textsuperscript{49}. 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 MBF\textsuperscript{50} (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 plaques (Fig. 1K, 1L and 1M). PET/CT is today well established in clinical routine imaging. PET/MR represents a new integrated protocol for simultaneous evaluation of viability, perfusion, morphological and functional imaging, thus eliminating ionizing radiation and improving tissue contrast. At present, 18F-FDG PET/MRI is potentially a very promising evaluation technique for in vivo imaging of systemic atherosclerosis-related inflammation.

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 effects. 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 plaques. 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 calcification.

Also of growing interest is the use of other radio tracers for functional imaging of the atherosclerotic plaques, such as 11C-PK11195, or tracers of molecular calcification, such as Sodium Fluoride (18F-NaF), and hypoxia radiotracers, such as 18Fluoromisonidazole (FMISO), 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 visualization 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 treatments.

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 medicine.

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-BiImaging 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) was 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 Barabasi1999, who proposed that the biology of living organisms can be studied in a complex of nodes, and edges between nodes, that lead to the formation 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 networks61, 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 SH2B2B3 adapt protein 3 (SH2B3) resulted in a connector gene in the different sub-networks, representing a novel target for prevention or treatment of hypertension55.

Similar to hypertension, hypercholesterolemia is a quantitative trait controlled by the interactions among several quantitative trait loci and environmental factors60. 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

<table>
<thead>
<tr>
<th>Consortium</th>
<th>Description</th>
<th>URL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PRIMARY PREVENTION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>International Childhood Cardiovascular Cohort (i3C)</td>
<td>Cohorts of children and adolescents in follow up for CVD morbidity and mortality</td>
<td><a href="https://www.i3cconsortium.org/index.html">https://www.i3cconsortium.org/index.html</a></td>
</tr>
<tr>
<td>CARDIoGRAMplusC4D</td>
<td>Combination of data from multiple large scale genetic studies to identify risk loci for CHD</td>
<td><a href="http://www.cardiogramplusc4d.org/">http://www.cardiogramplusc4d.org/</a></td>
</tr>
<tr>
<td>Wellcome Trust Case Control (WTCCC)</td>
<td>Collection of GWASs to shed light on the genetic architecture of human diseases</td>
<td><a href="https://www.wtccc.org.uk/">https://www.wtccc.org.uk/</a></td>
</tr>
<tr>
<td>Cardiogenics</td>
<td>Collection of GWASs on cardiovascular disorders</td>
<td><a href="https://www.cardiogenics.org/web/">https://www.cardiogenics.org/web/</a></td>
</tr>
<tr>
<td>CVgenes@target</td>
<td>European collaboration to investigate genomic variants affecting atherosclerosis risk for identification of underlying genes and involved pathways</td>
<td><a href="http://cvgenesattarget.eu/">http://cvgenesattarget.eu/</a></td>
</tr>
<tr>
<td>Uk Biobank</td>
<td>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</td>
<td><a href="https://biobank.ctsu.ox.ac.uk/">https://biobank.ctsu.ox.ac.uk/</a></td>
</tr>
<tr>
<td>Global Lipids Genetics (GLGC)</td>
<td>World-wide collaboration to understand the genetic etiology of quantitative lipid traits</td>
<td><a href="http://lipidgenetics.org/">http://lipidgenetics.org/</a></td>
</tr>
<tr>
<td>Genetic Investigation of ANthropometric Traits (GIANT)</td>
<td>International collaboration seeking to identify genetic loci that modulate human body size and shape, including obesity</td>
<td><a href="https://portals.broadinstitute.org/collaboration/giant/index.php?title=GIANT_consortium&amp;oldid=251">https://portals.broadinstitute.org/collaboration/giant/index.php?title=GIANT_consortium&amp;oldid=251</a></td>
</tr>
<tr>
<td>European Research Infrastructure for Imaging Technologies in Biological and Biomedical Sciences (Euro-BioImaging)</td>
<td>Open access to a broad range of technologies in biomedical imaging such as CT, MR, PET, coupled also to biobank data</td>
<td><a href="http://www.eurobioimaging.eu/">http://www.eurobioimaging.eu/</a></td>
</tr>
<tr>
<td>Advanced Cardiovascular Imaging Consortium (ACIC)</td>
<td>Clinical program collecting CCTA scans and patient characteristics aimed to reduce cost and improve safety and efficiency in patient care</td>
<td><a href="https://clinicaltrials.gov/ct2/show/NCT00640068">https://clinicaltrials.gov/ct2/show/NCT00640068</a></td>
</tr>
<tr>
<td>Cardiac Atlas Project (CAP)</td>
<td>Large-scale database of cardiac MR examinations and associated clinical data structural and functional of the normal heart and cardiac diseases</td>
<td><a href="http://www.cardiacatlas.org/">http://www.cardiacatlas.org/</a></td>
</tr>
<tr>
<td>PowerShare Network</td>
<td>Highly scalable cloud-based platform that enables the secure access and exchange of images and patient data</td>
<td><a href="https://www.nuance.com/healthcare/medical-imaging/powershare-network.html">https://www.nuance.com/healthcare/medical-imaging/powershare-network.html</a></td>
</tr>
<tr>
<td><strong>SECONDARY PREVENTION</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CARDIoGRAMplusC4D</td>
<td>Combination of data from multiple large scale genetic studies to identify risk loci for MI</td>
<td><a href="http://www.cardiogramplusc4d.org/">http://www.cardiogramplusc4d.org/</a></td>
</tr>
<tr>
<td>GENetIcs of sUbSequent Coronary Heart Disease (GENIUS-CHD)</td>
<td>Clinical cohorts of patients with established CHD with biological sample repository and longitudinal follow up for subsequent events</td>
<td><a href="http://www.genius-chd.com/">http://www.genius-chd.com/</a></td>
</tr>
<tr>
<td>Myocardial Infarction Genetics (MIGen)</td>
<td>Combination of data from multiple large scale genetic studies to identify risk loci related to early MI</td>
<td><a href="http://www.kathiresanlab.org/collaborators/myocardial-infarction-genetics-exome-sequencing-consortium/">http://www.kathiresanlab.org/collaborators/myocardial-infarction-genetics-exome-sequencing-consortium/</a></td>
</tr>
</tbody>
</table>

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 membrane.

Beyond environmental and lifestyle factors, obesity is highly influenced by genetics at multiple loci (polygenic), considering this condition is an inherited trait. 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 PTTPRE, IL-6R, and SLC6A5 genes with a putative key role in obesity risk.

**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 strategies. 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.

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 (GDPD) and S100 calcium binding protein A7A (S100A70) as key driver genes, suggesting a potential role for target in CHD.

Furthermore, Mäkinen et al. integrated 14 GWASs, including patients and controls of the CAR-Diogram 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 (GLOI) and peptidylprolyl isomerase I (PPI) were identified as key driver genes. Results were also validated in human aortic endothelial cells. 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 genes.

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. 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, neutrophile hormone activity, peptide YY receptor activity, NOD-like receptor signaling pathway, and RIG-I-like receptor signaling pathway, thus providing new pathogenic mechanisms in CHD. 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 pathogenesis.

Recently, Wang and Loscalzo developed a novel network based algorithm, called the Seed Connector algorithm (SCA), to discover disease modules. 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, 18 of which were associated with the cardiovascular system, confirmed the key functional role of seed connectors.
in CHD characterization. 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 significant association of USF2 and WAS genes with CHD.

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

<table>
<thead>
<tr>
<th>Big data source</th>
<th>Computational method</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHS (SNPs); Microarrays; GO</td>
<td>WGCNA; BN algorithm</td>
<td>Identification of SH2B3 as a key driver gene in blood pressure regulation</td>
<td>65</td>
</tr>
<tr>
<td>Microarrays; DAVID</td>
<td>WGCNA</td>
<td>Identification of FADS3 as a candidate hub gene for FCHL</td>
<td>67</td>
</tr>
<tr>
<td>GEO; DAVID; KEGG</td>
<td>PCC analysis</td>
<td>Identification of dysfunctional network in FH patients involving SNARE proteins, MAPK pathways and endocytosis processes</td>
<td>68</td>
</tr>
<tr>
<td>GEO; TRANSFAC; TRED; HMDD; miR2Disease; starBase; miRecords; TarBase; KEGG</td>
<td>Interaction analysis</td>
<td>Identification of a key role of SPI, STAT1, SFFV and SPII genes in FH pathological mechanisms; significant association of USF2 and WAS genes with FH</td>
<td>90</td>
</tr>
<tr>
<td>SNPs, Microarrays, GOAmiGO2; KEGG</td>
<td>WGCNA</td>
<td>Differential co-expression of IL-6 and IL1B in tissues of obese patients; identification of PTPRE, IL-6R, and SLC6A5 as key genes in insulin-related pathways</td>
<td>70</td>
</tr>
</tbody>
</table>

### Personalized therapy

<table>
<thead>
<tr>
<th>Big data source</th>
<th>Computational method</th>
<th>Results</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000 Genomes project; STAGE study (SNPs); ConsensusPathDB; AmiGO 2; DGIdb</td>
<td>Girvan-Newman algorithm</td>
<td>Identification in CHD module of several kinases and GPCR genes for which drug already existed or target drugs in development</td>
<td>94</td>
</tr>
<tr>
<td>IntAct; MINT; TRANSFAC; GO; DrugBank; PharmGKB; TTD</td>
<td>SCA algorithm</td>
<td>Identification of NRP1 as seed connector targeted by Pegaptanib (DB04895)</td>
<td>78</td>
</tr>
<tr>
<td>HPRD; PhosphositePlus; Phospho.ELM; BioGRID; MINT, IntAct; DisGeNET; GWAS Catalog; GWASdb; DrugBank; PharmGKB; TTD</td>
<td>Reference distance distribution</td>
<td>Hydroxychloroquine was significantly associated with a reduced risk of CHD</td>
<td>21</td>
</tr>
<tr>
<td>OMIM; DisGeNe; GWAS Catalog; HGMD; HPRD; PhosphositePlus; Phospho.ELM; BioGRID; MINT, IntAct; DrugCentral database</td>
<td>Permutation testing</td>
<td>Identification of several approved drugs (fasudil, parecoxib, dexamethasone) or natural products (resveratrol, luteolin, daidzein, caffeic acid) as novel drug targets in CHD</td>
<td>96</td>
</tr>
</tbody>
</table>

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; FHCL: 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: Neurophilin 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; SPI: specificity protein 1; SPII: 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.
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 pathogenesis.

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 network analyses were performed 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 pathogenesis.

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

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

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.
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 intelligence. 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 approach. Recent studies on machine learning approaches exploiting CTCA showed high prognostic accuracy in risk stratification and prediction of mortality in CHD patients. 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 methods.

### 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 unclear. 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 expression. Furthermore, a recent study showed that genetic variants have a regulatory impact on circulating miRNAs, highlighting an interplay between genetic and epigenetic components.

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 burden (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 patients (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 transcription 1 (SP1), and proviral integration oncogene spi1 (SPII) 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 (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. 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, Lempääinen 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 CCR1 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 CHD.

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 remodeling.

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 CHD.

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 approach (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 years. 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, fenofibates, 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 polymorphisms. 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 activity. The protective effect of statins for primary prevention has also been demonstrated in three large clinical studies: the MEGA, the JUPITER and HOPE-3. 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

<table>
<thead>
<tr>
<th>PRIMARY PREVENTION</th>
<th>Epidrug</th>
<th>Conditions</th>
<th>Effects</th>
<th>Phase/Status</th>
<th>NCT</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Monotherapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polyphenols (cocoa)</td>
<td>Hypertension, Hypercholesterolemia</td>
<td>To evaluate the effects of flavonoids 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</td>
<td>Phase 3/ Completed</td>
<td>NCT00511420 87)</td>
<td></td>
</tr>
<tr>
<td>Diet plus pravastatin</td>
<td>Hypercholesterolemia</td>
<td>To evaluate the primary preventive effect of pravastatin against CHD</td>
<td>Phase 4/ Completed</td>
<td>NCT00211705 99, 100)</td>
<td></td>
</tr>
<tr>
<td>Rosuvastatin</td>
<td>Elevated hs-CRP</td>
<td>To determine the safety and effectiveness of long-term therapy with rosuvastatin in reducing the risk of major cardiovascular events</td>
<td>Phase 3/ Completed</td>
<td>NCT00239681 101)</td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>Normotensive, LVH, obesity, CHD</td>
<td>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</td>
<td>Phase 4/ Completed</td>
<td>NCT02226510</td>
<td></td>
</tr>
<tr>
<td><strong>Combined therapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosuvastatin plus candesartan/ hydrochlorothiazide</td>
<td>CV intermediate risk, normal cholesterol and blood pressure</td>
<td>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</td>
<td>Phase 4/ Completed</td>
<td>NCT00468923 102)</td>
<td></td>
</tr>
<tr>
<td>Fenofibrate plus atorvastatin</td>
<td>Dyslipidemia</td>
<td>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</td>
<td>Phase 3/ Completed</td>
<td>NCT00616772</td>
<td></td>
</tr>
<tr>
<td>Metformin Plus statins</td>
<td>CHD, Overweight</td>
<td>To test the beneficial impact of 1year metformin treatment on carotid artery atherosclerosis evaluated by US</td>
<td>Phase 4/ Completed</td>
<td>NCT00723307 107)</td>
<td></td>
</tr>
<tr>
<td><strong>SECONDARY PREVENTION</strong></td>
<td>Epidrug</td>
<td>Conditions</td>
<td>Effects</td>
<td>Phase/Status</td>
<td>NCT</td>
</tr>
<tr>
<td><strong>Monotherapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resveratrol</td>
<td>CHD</td>
<td>To assess the improvement of endothelial function, lipidomic signatures, and cell signaling in patients undergoing CABG</td>
<td>NA/ Recruiting</td>
<td>NCT03762926</td>
<td></td>
</tr>
<tr>
<td>Fluvastatin</td>
<td>ACS</td>
<td>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</td>
<td>Phase 4/ Completed</td>
<td>NCT00171275 101)</td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>SA and UA</td>
<td>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</td>
<td>Phase 4/ Recruiting</td>
<td>NCT01715714</td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>STEMI</td>
<td>To evaluate the effect of metformin therapy following STEMI in terms of LVEF assessed by CMR and MACE occurrence</td>
<td>Phase 2 and 3/ Completed</td>
<td>NCT01217307 108)</td>
<td></td>
</tr>
<tr>
<td>Metformin</td>
<td>MI</td>
<td>To determine if pretreatment with metformin can reduce myocardial injury in patients undergoing elective CABG surgery</td>
<td>Phase 4/ Completed</td>
<td>NCT01438723 109)</td>
<td></td>
</tr>
<tr>
<td><strong>Combined therapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folic acid plus vitamins</td>
<td>CHD</td>
<td>To evaluate the clinical effects of homocysteine lowering treatment with B vitamins during 3-5 years follow-up of patients undergoing PTCA and CABG</td>
<td>Phase 3/ Completed</td>
<td>NCT0035540</td>
<td></td>
</tr>
<tr>
<td>Folic acid plus rosvastatin</td>
<td>CHD</td>
<td>To compare the effects on endothelial function of the forearm resistance vessels in patients with a previous history of PTCA</td>
<td>Phase 2/ Completed</td>
<td>NCT006935</td>
<td></td>
</tr>
<tr>
<td>Folic acid plus simvastatin plus vitamins</td>
<td>MI</td>
<td>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 folate acid plus vitamin B12</td>
<td>Phase 3/ Completed</td>
<td>NCT00124072</td>
<td></td>
</tr>
<tr>
<td>Folic acid plus methotrexate</td>
<td>CHD</td>
<td>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.</td>
<td>Phase 2 and 3/ Completed</td>
<td>NCT00759811</td>
<td></td>
</tr>
<tr>
<td>Ezetimibe plus simvastatin</td>
<td>SA</td>
<td>To evaluate the efficacy of ezetimibe plus simvastatin treatment vs simvastatin alone in patients affected by SA after PTCA</td>
<td>NA/ Recruiting</td>
<td>NCT03771053</td>
<td></td>
</tr>
<tr>
<td>Ezetimibe plus simvastatin</td>
<td>ACS</td>
<td>To compare the of these combined therapy in terms of reduction in the risk of the occurrence of MACEs, cardiac death, and stroke</td>
<td>Phase 3/ Completed</td>
<td>NCT00202878 104)</td>
<td></td>
</tr>
</tbody>
</table>

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.
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 patients. 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 stroke.

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 inhospital MI as well as lower levels of C-reactive protein, malondialdehyde, and N-terminal pro-B-type natriuretic peptide.

Metformin is the most successful oral medication for type 2 diabetes (T2D), lowering cardiovascular mortality and morbidity. Metformin can epigenetically act by inhibiting several histone modifying enzymes and DNA methyltransferases. 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 US. 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 events. 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.

**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 regimen. 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.

**Funding**

This work was supported by “Ricerca Corrente 2018” grant funded by Italian Ministry of Health. T.I. is a postdoc with a specific project “Nuove tecniche in immunologia clinica” granted by “Programma VALERE” of the University of Campania “Luigi Vanvitelli”, Department of Advanced Clinical and Surgical Sciences and awarded ESC Congress 2019.
References


2) Infante T, Forte E, Punzo B, Cademartiri F, Cavaliere C, Salvatore M, Napoli C. Correlation of Circulating miR-765, miR-93-5p and miR-433-3p to Obstructive Coronary Heart Disease Evaluated by Cardiac Computed Tomography. Am J Cardiol, 2019; 124: 176-182


22) Leopold JA, Loscalzo J. Emerging Role of Precision Medicine in Cardiovascular Disease. Circ Res, 2018; 122: 1302-1315


33) Qu TT, Li JY, Xiao XJ, Zhang XL, Song ZF, Guo YX, Guo JX. Contrast dose reduction with shortened injection durations in coronary CT angiography on 16-cm Wide-detector CT scanner. Br J Radiol, 2018; 91: 20180580


49) Sheikine Y, Akram K. FDG-PET imaging of atherosclerosis: Do we know what we see? Atherosclerosis, 2010; 211: 371-380
53) Buscombe JR. Exploring the nature of atheroma and cardiovascular inflammation in vivo using positron emission tomography (PET). Br J Radiol, 2015; 88: 20140648
54) Andrews JPM, Fayad ZA, Dweck MR. New methods to image unstable atherosclerotic plaques. Atherosclerosis, 2018; 272: 118-128
57) Jung KH, Lee KH. Molecular imaging in the era of personalized medicine. J Pathol Transl Med, 2015; 49: 5-12
66) Nardi C, Postiglione A, Triggiani M, Corso G, Palumbo...


73) Liu J, Jing L, Tu X. Weighted gene co-expression network analysis identifies specific modules and hub genes related to coronary artery disease. BMC Cardiovasc Disord, 2016; 16: 54


76) Wang RS, Loscalzo J. Network-Based Disease Module Discovery by a Novel Seed Connector Algorithm with Pathobiological Implications. J Mol Biol, 2018; 430: 2939-2950


[99] Allen SC, Mamotte CDS. Pleiotropic and Adverse Effects of Statins-Do Epigenetics Play a Role? J Pharmacol Exp Ther, 2017; 362: 319-326

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

<table>
<thead>
<tr>
<th>Epigenetic tag</th>
<th>Participants</th>
<th>Samples</th>
<th>Description</th>
<th>Status</th>
<th>NCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA methylation</td>
<td>200</td>
<td>Blood samples, aortic tissues</td>
<td>To investigate the relationship between DNA methylation the degree of coronary atherosclerosis in subjects undergoing carotid ultrasound and coronary angiography</td>
<td>Recruiting</td>
<td>NCT03462277</td>
</tr>
<tr>
<td>microRNAs</td>
<td>80</td>
<td>Plasma</td>
<td>To determine the levels of cardiospecific circulating miRNAs in patients with different stages of atherosclerosis according to 640-slice multispiral CT</td>
<td>Recruiting</td>
<td>NCT03855891</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Plasma</td>
<td>MiR-8059 down-regulation is correlated to coronary calcification detected by CTCA</td>
<td>Completed</td>
<td>NCT01992848</td>
</tr>
<tr>
<td></td>
<td>4,000</td>
<td>Plasma</td>
<td>To determine the expression level of miR-320a as effective biomarker in evaluating the diagnosis of CHD</td>
<td>Recruiting</td>
<td>NCT02751060</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Plasma</td>
<td>To evaluate the potential diagnostic and prognostic value of circulating miRNAs compared with cTnl for suspected ACS</td>
<td>Recruiting</td>
<td>NCT02755207</td>
</tr>
</tbody>
</table>

### SECONDARY PREVENTION

<table>
<thead>
<tr>
<th>Epigenetic tag</th>
<th>Participants</th>
<th>Samples</th>
<th>Description</th>
<th>Status</th>
<th>NCT</th>
</tr>
</thead>
<tbody>
<tr>
<td>microRNAs</td>
<td>460</td>
<td>Plasma</td>
<td>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</td>
<td>Recruiting</td>
<td>NCT03635255</td>
</tr>
<tr>
<td></td>
<td>4,000</td>
<td>Plasma</td>
<td>To determine whether the expression level of miR-320a are effective as biomarker for prognosis and treatment effects in CHD</td>
<td>Recruiting</td>
<td>NCT02751060</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Plasma</td>
<td>To research the vulnerability of ateromatous 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</td>
<td>Recruiting</td>
<td>NCT03391908</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>Plasma</td>
<td>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</td>
<td>Not yet recruiting</td>
<td>NCT03890822</td>
</tr>
</tbody>
</table>

ACS: acute coronary syndrome; cTnl: 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.