Systems Medicine as an Emerging Tool for Cardiovascular Genetics

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Cardiovascular disease (CVD) is a major contributor to morbidity and mortality worldwide. However, the pathogenesis of CVD is complex and remains elusive. Within the last years, systems medicine has emerged as a novel tool to study the complex genetic, molecular, and physiological interactions leading to diseases. In this review, we provide an overview about the current approaches for systems medicine in CVD. They include bioinformatical and experimental tools such as cell and animal models, omics technologies, network, and pathway analyses. Additionally, we discuss challenges and current literature examples where systems medicine has been successfully applied for the study of CVD.

Keywords: systems medicine, cardiovascular disease, omics, networks, functional characterization

INTRODUCTION

Cardiovascular disease (CVD) morbidity and mortality pose a major public health burden worldwide, and the prevalence of CVD is rapidly increasing. CVD is a heritable condition (1) and has a complex and heterogeneous etiology involving numerous environmental and genetic factors of disease risk (2). Increasing our understanding of the multifactorial, complex underpinnings of CVD promises to have a global impact on the promotion of health. The invention of arrays and analysis of multiple case–control samples have led to the identification of numerous genetic variants associated with coronary artery disease (CAD) risk. In 2007, the first genome-wide association studies (GWAS) for CAD were published, identifying a locus on chromosome 9p21 (3–5). To date, 56 loci have been identified due to denser genotyping and a higher number of individuals (6). Almost all of them are located in non-coding parts of the genome (6, 7). From these analyses, the locus on chromosome 9p21 is the locus with the highest population-attributable risk. It influences different isoforms of the non-coding RNA ANRIL (8). Besides GWASs, the first exome-wide association study on CAD was published in 2016, not only confirming known variants for CAD such as ANGPTL4 and LPL but also identifying novel variants such as SVEP1 (9). Results from the GWAS area have also led to the development of novel therapeutics, most prominently targeting lipid metabolism through inhibitors for PCSK9 (6). So, despite large success of GWASs (10–12) and sequencing approaches (13, 14) in identifying genetic loci associated with CVD, the underlying pathophysiological mechanisms involve different genotypes and changes in a systems level – for instance, at the transcriptome, proteome, and metabolite levels (Figure 1). To provide a more comprehensive picture, the systematic integration of multidimensional “omics” datasets evolves as the next challenge for the future, including molecular findings of interactions between proteins, metabolites, regulatory RNAs, and DNA as well as knowledge from cell biology, animal experiments, and human phenotypic and clinical data. In this systems approach, all known (patho-)physiological components of CVD are integrated appropriately in order to create a dense modular network incorporating information from various disciplines and novel effective computational models. These approaches will guide the next steps in cardiovascular research, enhance our understanding.
of disease susceptibility, treatment, and monitoring, and might influence preventive actions (15).

In this review, we discuss state-of-the-art systems medicine approaches in CVD. We provide an overview of existing bioinformatical and experimental methods commonly used in systems medicine and illustrate selected exemplary CVD studies from the current literature.

**METHODOLOGY – OMICS APPROACHES**

Omics is the study of different biological entities, including genomics, proteomics, transcriptomics, or metabolomics, describing different aspects (components and interactions) of the cell (16).

In systems medicine, such omics data types are integrated to better understand the global relationship among genotype, environment, and phenotype and to reveal the underlying molecular systems and their function (17). Consequently, a more comprehensive picture is taken by the combination of multidimensional omics data covering different levels of knowledge about the cell, genome, or environment.

Subsequent systems medicine approaches, e.g., network and pathway analysis, reveal novel insight into the disease or condition of interest. Here, the relationship among genes, proteins, transcripts, or other biological entities is analyzed and interpreted on a genome-wide scale (e.g., network) or a more detailed scale (e.g., pathway, sub-network).

In the following, we first review selected omics data types and methods for data integration and subsequent data analysis. **Table 1** provides additional information along with selected resources (databases, tools) for each presented data type.

**Genomics**

Genomic studies investigate the sequence, structure, and function of the entire genome in a cell. DNA sequencing and assembling is one of the most prominent techniques in genomics. Established techniques and methods involve Shotgun sequencing (e.g., Sanger method), Next-generation sequencing (NGS), and
| Name                          | Description                                                                                                                                                                                                                             | Webpage                                      | Reference |
|-------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------|-----------|
| **Genomics**                  |                                                                                                                                                                                                                                        |                                              |           |
| Ensembl                       | System for genome annotation, analysis, storage, and dissemination designed to facilitate the access of genomic annotation from chordates and key model organisms                                                                                  | http://www.ensembl.org (18)                 |           |
| 1000 Genomes project          | The goal of the 1000 genomes project was to find most genetic variants with frequencies of at least 1% in the populations studied                                                                                                | http://www.1000genomes.org (19)             |           |
| UCSC Genome browser           | Web tool for rapid and reliable display of any requested portion of the genome at any scale, together with several dozen aligned annotation tracks                                                                                       | https://genome.ucsc.edu (20)                |           |
| **Transcriptomics**           |                                                                                                                                                                                                                                        |                                              |           |
| GEO gene expression omnibus   | Public functional genomics data repository supporting MIAME-compliant data submissions. Tools are provided to help users query and download experiments and curated gene expression profiles                                                               | http://www.ncbi.nlm.nih.gov/geo (21)        |           |
| ArrayExpress                  | Archive of functional genomics data stores data from high-throughput functional genomics experiments                                                                                                                                     | https://www.ebi.ac.uk/arrayexpress (21)    |           |
| Expression atlas              | Provides information on gene expression patterns under different biological conditions. Gene expression data are re-analyzed in-house to detect genes showing interesting baseline and differential expression patterns                               | https://www.ebi.ac.uk/gxa (22)              |           |
| GXD the mouse gene expression database | Collects and integrates the gene expression information in MGI, focusing on endogenous gene expression during mouse development                                               | http://www.informatics.jax.org/ expression.shtml (23) |           |
| **Proteomics**               |                                                                                                                                                                                                                                        |                                              |           |
| PRIDE – PRoteomics IDENTifications | Data repository for proteomics data, including protein and peptide identifications, posttranslational modifications, and supporting spectral evidence                                                                           | https://www.ebi.ac.uk/pride/archive (24)    |           |
| ProteomicsDB                  | Database for the identification of the human proteome                                                                                                                  | https://www.proteomicsdb.org (25)           |           |
| PeptideAtlas                  | Compendium of peptides identified in a large set of tandem mass spectrometry proteomics experiments                                                                      | http://www.peptideatlas.org (26)           |           |
| NIST libraries of peptide tandem mass spectra | Comprehensive, annotated mass spectral reference collections from various organisms and proteins                                                                          | http://peptide.nist.gov                     |           |
| COPaKB                        | Proteome biology platform specifically for cardiovascular research                                                                                                          | http://www.heartproteome.org (27)           |           |
| MitoCarta                      | Inventory of human and mouse genes encoding proteins with mitochondrial localization                                                                               | http://www.broadinstitute.org/ scientific-community/science/ programs/metabolic-disease-program/ publications/mitocarta/mitocarta-in-0 (28) |           |
| **Metabolomics**             |                                                                                                                                                                                                                                        |                                              |           |
| HMDB – the human metabolome database | Database containing detailed information about small molecule metabolites in human, containing chemical data, clinical data, and molecular biology/ biochemistry data                                          | http://www.hmdb.ca (29)                     |           |
| MetaboLights                  | Database for metabolomics experiments and derived information. The database is cross-species, cross-technique and covers metabolite structures and their reference spectra, their biological roles, locations and concentrations, and experimental data from metabolic experiments | http://www.ebi.ac.uk/metabolights (30)     |           |
| BIGG models                  | Knowledgebase of genome-scale metabolic network reconstructions, integrating multiple published genome-scale metabolic networks into a single database | http://bigg.ucsd.edu (31)                   |           |
| MetaCyc                      | Database of non-redundant, experimentally elucidated metabolic pathways. It is curated from the scientific experimental literature and contains pathways involved in both primary and secondary metabolism, as well as associated compounds, enzymes, and genes | http://metacyc.org (32)                     |           |
| ConceptMetab                 | Compound set network tool                                                                                                                                                                                                 | http://conceptmetab.med.umich.edu/ (33)    |           |
| MetDisease                   | Metabolic network app for Cytoscape                                                                                                                                                                                                     | http://metdisease.ncbi.org/ (34)            |           |
| **Interactomics**            |                                                                                                                                                                                                                                        |                                              |           |
| IntAct – molecular interaction database | Database system and analysis tools for molecular interaction data derived from literature curation or direct user submissions                                                                 | http://www.ebi.ac.uk/intact (35)           |           |
| BioGRID – biological general repository for interaction datasets | Interaction repository with data compiled through comprehensive curation, containing protein and genetic interactions, chemical associations and posttranslational modifications | http://thebiogrid.org (36)                 |           |
| STRING – protein–protein interaction networks | Database of known and predicted protein–protein interactions, including direct (physical) and indirect (functional) associations                                                                  | http://string-db.org (37)                   |           |
Illumina sequencing. They mainly differ in the length of sequence reads (number of base pairs), coverage, sequencing/processing time, and costs. Whole-genome sequencing analyzes all coding and non-coding regions of the genome. While it has the highest coverage producing large amount of data, it is so far the most expensive method. By contrast, whole exome sequencing covers just the coding sequences of all genes at a high coverage but with cheaper costs. Other techniques include the identification of common single nucleotide polymorphisms (SNPs) by GWASs and CpG methylation sites by epigenome-wide association studies (EWASs), both covering the whole genome (6).

Subsequently, sequenced DNA fragments are assembled and annotated using reference genomes [e.g., RefSeq (38), Genome Reference Consortium]. Such obtained DNA sequences can then be used to study certain mutations, including genetic variants (SNPs), copy number variations (CNVs), and deletions or insertions of gene fragments (39).

Transcriptomics

The transcriptome can be defined as “the complete set of transcripts in a cell, and their quantity, for a specific developmental stage or physiological condition” (40). Often, it is used to determine expression patterns of transcripts and how they change under certain conditions, such as disease status and drug treatment. A transcriptome analysis aims, depending on the technique in use, to catalog and annotate RNA, including coding and non-coding transcripts, to query gene structures, and to support constructing and mapping interaction networks.

The most popular technique in transcriptomics analysis is the use of microarrays. They allow us to study tens of thousands of transcripts (RNA) on a genome-wide scale with different conditions in parallel (e.g., disease and healthy). Additionally, they have an extensive coverage, high-throughput applicability, uncomplicated data analysis, and are relatively inexpensive (41). However, microarrays still suffer from several technical limitations (42, 43). They are limited by the amount of RNA required, the dynamic range, the semi-quantitative approach, and the detection of predefined transcripts (41). These limitations have been widely solved by a new technique, RNA-Seq (RNA-Seq), which sequences all transcripts in a sample multiple times, obtaining a high-resolution and high-quality genome-wide transcriptome scan (40). RNA-Seq provides absolute quantification of transcripts and includes splice variants, unknown RNAs, and RNAs too short to be captured by microarrays. However, RNA-Seq is a more expensive technology than microarrays, while it requires large data storage and powerful computation resources. Additionally, analysis of RNA-Seq data involves complex bioinformatical approaches (41, 44).

After performing such genome-wide transcript scans, a well-established method for verifying individual promising targets is the use of a quantitative real-time polymerase chain reaction (qPCR). In contrary to microarrays or a genome-wide RNA-Seq analysis, it monitors the amplification of a single target DNA molecule.

In the research of CVD, transcriptomics approaches have already led to the discovery of novel biomarkers, e.g., GDF15 for acute coronary syndromes, angina pectoris, and heart failure (45–47), as well as several circulating microRNAs for coronary heart disease and myocardial infarction (MI) (48–50).

Proteomics

Proteins have individual interconnected properties that contribute to the phenotype of a cell. Together, all proteins form a complex and dynamic system, the proteome, comprising all interconnected and dynamic properties of the proteins, including their abundance, isoform expression, subcellular localization, interactions, turnover rate, and posttranslational modifications (PTMs) (51).

Proteome studies are still rare in relation to CVD, due to complex methodology involved. A comprehensive review on proteomics in combination with systems biology approaches in CVD is given by Langley et al. (52). In proteomic studies, it is recommended to use plasma rather than serum due to its more stable protein suspension. To avoid masking of low-abundance proteins by high-abundance proteins, such as albumin and immunoglobulins, immunodepletion techniques or protein enrichment tools can be used. Even though protein enrichment tools allow to reduce the dynamic range of protein concentrations, they maintain representatives of all proteins. For initial protein separation, two-dimensional (2-D) gel electrophoresis is well established. The introduction of differential in-gel electrophoresis (DIGE) allows separation of two sets of protein mixtures by pre-labeling with fluorescent dyes and gives higher reproducibility than 2-D gels. After separation, protein spots are picked and digested with proteolytic enzymes and subsequently analyzed by tandem mass spectrometry (MS/MS) (53). Gel-free shotgun proteomics include stable isotope labeling with amino acids in cell culture (SILAC), which allows higher protein resolution but is only operable in cell culture experiments and makes quantification more difficult (52).

Although recent advances in mass spectrometry (MS)-based proteomics have resulted in a quantitative system-wide analysis of the proteome, including PTMs, protein–protein interactions (PPIs), and cellular localization, researchers see a next-generation proteomics integrating novel approaches to gain further insight into the proteome by improving sensitivity, robustness, and high-throughput of MS-based proteomics. This allows us to discover novel disease-related biomarkers and screen molecular targets of drugs (54).

Metabolomics

Metabolites are chemical entities transformed during metabolism that can serve as signatures of biochemical activity. Most metabolites are lipids (phospholipids, glycerophospholipids, and sphingolipids), acylcarnitines, amino acids, biogenic amines, hormones, bile acids, or fatty acids. They are detected quantitatively from body fluids (e.g., serum) or tissues and are measured by nuclear magnetic resonance (NMR) spectroscopy and MS. The following factors should be considered when choosing a method: cost-effectiveness, coverage of metabolic content, accuracy, and throughput. NMR is a quantitative, non-destructive technique, allowing the detection of a wide range of diverse metabolites simultaneously, while sample storage and preparation are very simple. By contrast, MS coupled with ultra-performance liquid
chromatography (UPLC-MS) has better resolution power and higher sensitivity than NMR. However, the data quality highly depends on the sample quality. Mainly, two different chromatographic techniques are used for the detection of metabolites: hydrophilic interaction liquid chromatography (HILIC) and reversed phase liquid chromatography (RP-LC). RP-LC would be the primary choice for the analysis of biofluids due to its unpolar stationary phase. HILIC can be complimentary to RP-LC by targeting the resolution of polar metabolites (55).

Metabolomics is the study of metabolite profiling to link cellular pathways to biomolecular mechanisms, and recent advances in NMR and MS technology will provide further applications in disease diagnosis, altered metabolic pathways in diseases and under drug treatment. Although metabolomics suffers from some limitations, including potential confounding effects (i.e., gender, age, diet, and environment) or limited reproducibility, it has a promising potential in providing both supplementary and complementary data important for biomarker identification and validation (56, 57), as shown, e.g., by Huang et al. (58).

**Interactomics**

Genes, proteins, or other biological entities should not be seen in isolation, rather than dynamically interacting in molecular pathways. Such interactions mostly occur on a protein level and, thus, are called PPIs. The so-called interactome is highly dynamic and can vary between different cell and tissue types (59–61), biological or cellular contexts (62, 63), time points, and disease conditions (60, 64, 65). Additionally, proteins can interact with different partners within different pathways based on their function: they can interact on a physical level or within a protein complex (66), through regulation (67), or PTMs (62), which makes it challenging to understand the underlying biology and its molecular mechanism. For accomplishing this, researchers suggest to systematically map gene and protein interactions (68, 69), and there have already been the effort of linking the interactome to human diseases (65, 70).

**Integration of Heterogeneous Omics Data**

As omics data are becoming more easily available for different data types, they can be combined (i.e., integrated) for a better understanding of their relationship and the underlying molecular systems. Data integration has been reported as the combination of data discovery and data exploitation (71), and recent computational advances provide methods for addressing the challenges of integrating heterogeneous data types. These include the identification of network scaffolds by delineating existing interactions between cellular components, the decomposition of such network scaffolds into constituent parts, and the development of system models to simulate and predict the network behavior, expressing as a particular phenotype. Existing studies have been conducted to map cellular pathways on a genome scale to gain insight into cellular responses to environmental perturbations, to develop biomarkers or disease-associated patterns (16). Established methods for analyzing the data include Bayesian Networks (63, 71–73), self-organizing maps (74), or unsupervised network reconstruction (75). A recent review describes how visualization tools can be used to analyze and interpret integrated protein interactions, gene expression, and metabolic profile data (76). Additionally, a recent approach suggested a downstream workflow for integrating heterogeneous data types and procedures for functional analysis that focus on biological pathways by emphasizing the use of curated knowledge resources coupled with expert-guided examination and interpretation of omics data for the selection of potential molecular targets (77).

In the context of CVDs, only few studies have been conducted that use integrated omics data to understand the mechanisms and to identify novel biomarkers. Hou et al. used an omics toolbox with proteomics and transcriptomics to identify novel biomarkers and drug targets in heart failure (78), while Barallobre-Barreiro et al. integrated proteomics and metabolomics to gain mechanistic insights and identify novel biomarkers for CVD (79).

**Network Biology**

The analysis of integrated omics data includes the reconstruction, understanding, and modeling of networks that control the behavior of the cell. Summarizing, biological networks are represented as graphs in which components (e.g., genes, proteins, metabolites, reactors, or regulators) are modeled as nodes and their interactions (e.g., regulations, enzymatic reactions, or physical interactions) as edges. These networks can be derived from different types of molecular interactions or on different levels, including PPIs, metabolic interactions, signaling, and transcription–regulatory interactions. In particular, the group of Barabási studied and reviewed the large-scale structure and system-scale function of cellular networks (80–82), as well as the evolutionary mechanisms that might have shaped their development (83).

In systems medicine, we seek to identify the components of complex systems and to model their dynamic interactions (84). Following Luis and Weiss systems-based approaches involve four logical steps: (a) definition of the system under study (e.g., cardiomyocytes), (b) identification of system components (e.g., proteins regulating a property of interest), (c) determination of component interactions (e.g., network), and (d) modeling of the network dynamics (e.g., how it changes over time or responds to various perturbations) (85). In the context of CVDs, systems-based approaches are becoming more important as the cardiovascular system is highly complex and involves multiple omics data types to be integrated and analyzed. Recent advances in this field could demonstrate the usefulness of reconstructed networks based on integrated omics data. For example, Zhao and Huang reconstructed and analyzed a human heart-specific metabolic network based on transcriptome and proteome data. The tissue specificity of the underlying data was already previously shown to be essential for studying diseases and phenotypes (59, 60), and, based on heart-specific omics data, Zhao and Huang could detect epistatic interactions in the human heart, as well as potential biomarkers for CVD (86). A related study reconstructed a human metabolic network from human cardiomyocytes to accomplish metabolic functions required for maintaining the structural and functional integrity of the cell (87). In contrary, Ryall et al. reconstructed a signaling network specific to cardiac myocyte hypertrophy to identify the most influential species.
in the cardiac hypertrophy signaling network and demonstrate how different levels of network organization affect myocyte size, transcription factors, and gene expression (88).

Pathway Analysis

Along with network analysis, a pathway analysis has become a useful method for gaining a detailed insight into biomolecular mechanisms among genes, proteins, metabolites, etc., as it reduces complexity, has increased explanatory power (89) and investigates biological pathways rather than genome-scale networks. Methods and tools for pathway analysis have been developed and studied, such as Pathway-Express, an impact analysis including classical statistics along with crucial factors such as the magnitude of each gene's expression change, their type and position in the given pathways, and their interactions (90). Other pathway analysis methods and tools include KEGG, KegArray, PathVisio, Ingenuity, and others [for a detailed review, please refer to Wheelock et al. (91)]. These methods integrate different data types and pathway information and perform subsequent pathway analyses, for example, whether certain pathways are enriched for genes differentially expressed under certain conditions (75, 91). These analyses will provide more information on molecular characteristics in the cell under disease conditions or perturbations.

METHODOLOGY – EPIDEMIOLOGICAL STUDIES AND EXPERIMENTAL SETTINGS

One of the main challenges of systems medicine is the investigation of complex changes and interactions in the human body. In particular, epidemiological studies bear an enormous potential for studying the underlying CVD pathophysiology and evaluating environmental and genetic factors. Population-based and disease cohorts become increasingly available, e.g., the first being the Framingham Heart Study that started in 1948 (92).

Human Samples

When collecting human samples, their suitability and availability for future applications should be considered. Gene expression and physiology can vary greatly between tissues and cell types, depending on time and function. Secretion samples, including saliva, tear fluid, urine, or feces, are easily accessible but none of them is suitable for all research questions.

In cardiovascular research, blood samples offer a broader range for analyses, such as serum, plasma, or blood cells. Red blood cells are commonly removed by density gradient centrifugation, resulting in the collection of peripheral blood mononuclear cells (PBMCs), including lymphocytes, monocytes, and dendritic cells. Blood cells are often used for functional analyses, e.g., gene expression analyses, while serum and plasma are classical sources for biomarker measurements as they include molecules, such as miRNAs, from cells and tissues from the whole body (93). By contrast, obtaining tissue samples is more challenging, in particular from tissues that are difficult to biopsy (e.g., heart, brain). Especially, cardiac biopsies are extremely invasive and only available when surgery or heart catheter procedures are performed. Major cardiac cell types are fibroblasts, myocytes, endothelial cells, and vascular smooth muscle cells. Additionally, transient cells, such as immune cells, are present in cardiac tissue and, thus, influence gene expression as well (94, 95).

Taken together, epidemiological studies provide the opportunity to study cardiovascular phenotypes over time while obtaining various human samples. Sampling should be carefully considered to take differences between sampling material and possible future applications into account.

Cell Models

A common way to study molecular functions and interactions is the use of cell cultures. Although living cells can be isolated from fresh biopsies or blood, their life span is limited, which is particularly true for cardiac myocytes (94). Various immortalized or modified cell lines are available, such as THP-1 and HL-1 cells, allowing an easier handling. However, immortalization can change physiology and gene expression of the cells. To study the effect of differential gene expression, various knockdown and overexpression tools are being used, such as RNA interference and transcription activator-like effector nucleases (TALENs). The recently discovered CRISPR/Cas-9 system revolutionizes gene editing by making it faster, easier, available for many species, and allowing multiplexing (96). Additionally, invasive tissue biopsies could be avoided by differentiating induced pluripotent stem cells from skin or PBMCs into cardiac-like cells (97). Collecting these cells during sampling will provide numerous options for future research.

Animal Models

Animal models are broadly used to study the pathogenesis of complex human diseases. They easily enable standardization of the environment, genetic background, age, and diet. Careful selection of the model has to be done to answer the respective question, taking physiology, biochemistry, and pathophysiology into account. There are various small animal models to study CVD, such as mice, rats, rabbits, and guinea pigs. Mice are by far the most frequently used animal models due to their small size, fast breeding, cost-effectiveness, and the possibility for genetic modifications. However, their cardiovascular system is different from humans (98, 99), as their hearts are smaller, their blood volume is lower, their heart rate is faster, they have no pericardial fat, and a different heart physiology and blood cell composition than humans (99, 100). Most importantly, mice have high antiatherogenic high-density lipoproteins (HDL) and low proatherogenic low-density lipoproteins (LDL), which makes wild-type mice almost resistant to atherosclerosis. To model the pathophysiology of atherosclerosis, mainly two genetically modified mice are being used: ApoE and LDL receptor (Ldlr) knockout mice. ApoE knockout mice lack apolipoprotein E, leading to hypercholesteremia, and spontaneous atherosclerosis development. Even though the lesions resemble human lesions, plasma cholesterol levels are considerably higher and the predominant circulating lipoprotein is VLDL instead of LDL. Additionally, apolipoprotein E influences atherosclerosis development in many ways, e.g., via its immunomodulatory properties. Ldlr knockout mice slowly develop atherosclerotic lesions, similar to humans. High-fat diet increases plasma
cholesterol levels and leads to a more rapid lesion development. Even though lesion morphology is similar to ApoE knockout mice, Ldr knockout mice are more similar to the human pathophysiology as hyperlipidemia is milder and plasma lipoprotein profile resembles humans (98). Additionally, various heart failure disease models exist, such as transverse aortic constriction (TAC) and left anterior descending artery-lication mice (101, 102).

Rats are not as frequently used as mice as they are bigger and genetic modifications were not possible until the introduction of targeted gene disruption using engineered zinc-finger nucleases (ZFNs) in 2009 (103). Similar to mice, wild-type rats do not develop spontaneous atherosclerosis. However, stress can lead to myocardial lesions (100). To date, various genetically modified rat models as well as models for hypertension, MI, and heart failure exist (104–106). Rabbits are well-established models for diet-induced atherosclerosis as their lipoprotein metabolism is similar to humans but they are lacking hepatic lipase. Additionally, there are rabbit models for dyslipidemia, heart failure, and transgenic rabbits (104, 107, 108). Guinea pigs are not so commonly used for the study of CVD, mainly to study arrhythmias and cellular electrophysiology, e.g., with isolated hearts (109, 110). Furthermore, guinea pig models of cardiac hypertrophy and heart failure exist (111). Besides rodents, zebrafish are another comparably new emerging small animal model for CVD. Their short reproductive cycles, easy handling, and the possibility for genetic modifications made them a widely used model for cardiovascular development, regeneration, and currently also arrhythmias (112–114).

Not only small but also large animal models are available for the study of CVD, and they are more similar to human in terms of anatomy, physiology, and size. Pigs have been used as models for CVD for years, particularly to study valvular heart disease and develop surgical procedures. They are similar to human in terms of spontaneous atherosclerotic lesion development, blood composition, heart anatomy, lipid profiles, lipoprotein metabolism, genome, and RNA (99). Besides from pigs, large animal models for CVD include dogs, sheep, goats, and non-human primates (104, 115). However, large animal models are more expensive, need more space and have longer reproductive cycles. Consequently, they are not being used so often and transgenic models, assays, and antibodies are only limited available. Additionally, the number of dogs and non-human primates for cardiovascular research is declining due to ethical aspects, even though their heart physiology is very similar to humans.

SYSTEMS MEDICINE APPROACHES IN CARDIOVASCULAR DISEASE – EXAMPLES

The SH2B3 Locus in Relation to Blood Pressure

High blood pressure (BP) is a leading cause of CVD worldwide with a high prevalence in the general population (116). Using large-scale GWAS meta-analyses with up to 200,000 individuals, more than 40 BP SNPs were identified so far. However, those variants explain only 2–3% of BP variation (117), and the molecular mechanisms that lead to increased BP are largely unknown. To identify novel candidate genes involved in BP regulation, Huan et al. (118, 119) applied systems approaches by computationally combining genetic, transcriptomic, and phenotype data. Screening for associations between BP traits and overall gene expression from whole blood of 7,017 individuals, 34 distinct genes were significantly related to BP, which in aggregate explained 5–9% of BP variation.

Furthermore, in order to seek for molecular key drivers of BP regulation, Huan et al. (119) and Rotival et al. (120) combined SNP data of known genetic BP variants with gene coexpression networks. In both studies, BP-related variants (121–123) around the gene encoding SH2B adaptor protein 3 (SH2B3) were shown to dysregulate highly coexpressed sub-networks. In monocytes, this sub-network contained five BP genes (CRIP1, RAB11FIP1, MYADM, TIPARP, and TREMI) (120), whereas the sub-network identified in whole blood comprised six trans-regulated BP genes (ARHGEF40, TAGAP, MYADM, FOS, PPP1R15A, and S100A10) (119). In order to validate their findings from network analysis, transcriptome profiling was performed in whole blood of Sh2b3−/− and wild-type mice (119). A significant overlap of dysregulated genes in networks driven by SH2B3 variants and Sh2b3−/− was shown. Further examination of the role of SH2B3 in the development of hypertension was performed by Saleh et al., who investigated BP in Sh2b3−/− mice (124) in response to low-dose angiotensin II supplementation. In untreated mice, kidneys of transgenic mice showed greater levels of inflammation, oxidative stress, and glomerular injury in relation to wild-type mice. Such effects were further increased after angiotensin II infusion. In addition, aortas from Sh2b3-deficient mice exhibited stronger inflammation. Bone marrow transplantation of SH2b3−/− into wild-type animals reproduced the hypertensive phenotype, strongly indicating that the predominant effect of SH2B3 on BP is mediated by hematopoietic cells.

GUCY and CCT7 in Myocardial Infarction

Myocardial infarction is a major cause of death in developed countries, and it is best predicted for middle-aged adults by a positive family history of MI (125), underlining the genetic component of MI. Erdmann et al. (13) recruited a family of 32 members diagnosed with CAD and DNA available in 15 members and performed exome sequencing in 3 affected distant family members diagnosed with MI and a positive family history of MI (125), underlining the genetic component of MI. They identified 35 novel variants with minor allele frequency below 0.5% were identified in 15 members and performed exome sequencing in 3 affected distant family members and subsequent two-locus linkage analysis. Four rare variants with minor allele frequency below 0.5% were identified and validated in the remaining family members. A loss-of-function mutation in the gene encoding guanylate cyclase 1, soluble, alpha 3 (GUCY1A3) and a missense mutation in the chaperonin containing TCP1 subunit 7 (CCT7) encoding gene were identified. In subsequent experimental settings, the functional implications of these variants were characterized. Transfection of the GUCY1A3 mutation into human embryonic kidney (HEK 293) cells and downregulation of CCT7 by siRNA led to a strong reduction of soluble guanylyl cyclase (α1-sGC) α1 levels. Next, the authors investigated α1-sGC levels and NO-dependent cGMP formation in platelets extracted from family members carrying single GUCY1A3 or CCT7 mutations, a double mutation, or none of the rare alleles. Carriers of the digenic mutation exhibited a significant reduction of α1-sGC levels and cGMP formation.
Since cGMP was known to inhibit platelet activation, representing an important feature of thrombus formation in MI, loss of Gucy1A3 was tested in mice. Gucy1A3-deficient mice showed an increased thrombus formation, indicating an increased risk of MI via dysfunctional nitric oxide signaling in rare allele carrying family members.

In summary, both examples used OMICs data to screen for putative disease-causing genes, which were further characterized by in vitro experiments or translation into an animal model.

**Cardiac Proteomics and Metabolomics**

In a few studies, systems medicine approaches have also been used to assess the interplay between cellular proteins as well as metabolites and oxidative stress in the context of CVD. Mayr et al. combined proteomics and metabolomics approaches to study protein kinase C-mediated cardioprotection by modulation of glucose metabolism (126). A recent study by Chouchani et al. used comparative metabolomics analysis to identify conserved metabolic pathways during ischemia reperfusion, specifically succinate as a metabolic signature of ischemia and subsequently a potential therapeutic target for ischemia–reperfusion injury (127).

**CHALLENGES**

As systems medicine is a new, rapidly emerging approach with extensive tools and strategies being developed, researchers are facing multiple challenges. Figure 2 gives an overview about the most prominent challenges to be considered.

**OUTLOOK AND APPLICATIONS**

Systems medicine emerged as a powerful tool to study complex diseases by the integration of multidimensional “omics” datasets with data from human studies and experimental laboratory data. CVD is a multifactorial disease and by applying systems medicine approaches, clinical translation might be promoted. Consequently, risk assessment, prevention, and treatment as well

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**FIGURE 2** | Challenges for systems medicine approaches.
as biomarker and drug development can be improved. Due to the interdisciplinary nature, many challenges have to be faced. However, first examples have shown success in the promotion of translational projects in CVD. In a recent study, Kang et al. studied tissue repair based on regenerating tissue from zebrafish fins and cardiac ventricle (128) in order to identify potential novel therapeutic strategies targeting injured human tissue. RNA-Seq from regenerated compared with uninjured tissue revealed strong upregulation of the gene encoding leptin β (lepb, which is paralog to mammalian leptin. ChIP sequencing of histone marks revealed an enhancer element 7 kb upstream of lepb (lepb linked enhancer LEN), responsible for directing lepb gene expression in regenerating tissue, and indicating that LEN can interact with the mammalian transcriptional machinery. Subsequently, in transgenic zebrafish, targeted proliferation of cardiomyocytes could be achieved in resected ventricular apex by inducing the cardiomyocyte mitogen neuregulin 1 (nrg1) via LEN fusion to the nrg1 promoter, showing that transgenic application of LEN was capable to boost injury induced cardiomyocyte proliferation, suggesting novel ways for dynamic therapy upon occurrence of using tissue regeneration enhancer elements.

**AUTHOR CONTRIBUTIONS**

TH, DB, CM, and TZ contributed substantially to the conception, drafting, and revision of the manuscript and approved the final version.

**ACKNOWLEDGMENTS**

The authors thank Simone Schnella for the editorial assistance.

**FUNDING**

The work has been supported by the German Center of Cardiovascular Research (DZHK e.V.; grant no. FKZ 812/Z1710101), the BMBF (German Federal Ministry of Education and Research) grant no. 01ZX1408A (symAtrial), and the Else Kröner-Fresenius-Stiftung (grant no. 2014_A92).

**REFERENCES**

1. Banerjee A, Silver LE, Heneghan C, Welch SJ, Mehta Z, Banning AP, et al. Relative familial clustering of cerebral versus coronary ischemic events. *Circ Cardiovasc Genet* (2011) 4(4):390–6. doi:10.1161/CIRCGENETICS.110.995114

2. Lloyd-Jones D, Adams R, Carnethon M, De Simone G, Ferguson TB, Flex K, et al. Heart disease and stroke statistics – 2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* (2009) 119(3):e21–181. doi:10.1161/CIRCULATIONAHA.108.191261

3. Helgadottir A, Thorleifsson G, Manolescu A, Gretarsdottir S, Blondal T, Jonasdottr A, et al. A common variant on chromosome 9p21 affects the risk of myocardial infarction. *Science* (2007) 316(5830):1491–3. doi:10.1126/science.1142842

4. McPherson R, Pertsomilis A, Kavaslar N, Stewart A, Roberts R, Cox DR, et al. A common allele on chromosome 9p21 associated with coronary heart disease. *Science* (2007) 316(5830):1488–91. doi:10.1126/science.1142447

5. Samani NJ, Erdmann J, Hall AS, Hengstenberg C, Mangino M, Mayer B, et al. Genomewide association analysis of coronary artery disease. *N Engl J Med* (2007) 357(5):443–53. doi:10.1056/NEJMoa072366

6. Kessler T, Vilne B, Schunkert H. The impact of genome-wide association studies on the pathophysiology and therapy of cardiovascular disease. *EMBO Mol Med* (2016) 8(7):688–701. doi:10.15252/emmm.201506174

7. McPherson R, Tybjaerg-Hansen A. Genetics of coronary artery disease. *Circ Res* (2016) 118(4):564–78. doi:10.1161/CIRCRESAHA.115.306566

8. Holdt LM, Beutner F, Scholz M, Gabel G, Bercht H, et al. ANRIL expression is associated with atherosclerosis risk at chromosome 9p21. *Arterioscler Thromb Vasc Biol* (2010) 30(3):620–7. doi:10.1161/ATVBAHA.109.196832

9. Myocardial Infarction, Investigators CAEC, Stribiziel NO, Strümpf KE, Masca NG, Erdmann J, et al. Coding variation in ANGPTL4, LPL, and SVEP1 and the risk of coronary artery disease. *N Engl J Med* (2016) 374(12):1134–44. doi:10.1056/NEJMoa1507652

10. Nikipay M, Goea A, Won HH, Hall LM, Willenborg C, Kanoni S, et al. A comprehensive 1.000 genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet* (2015) 47(10):1211–30. doi:10.1038/ng.3396

11. Kessler T, Erdmann J, Schunkert H. Genetics of coronary artery disease and myocardial infarction – 2013. *Curr Cardiol Rep* (2013) 15(6):368. doi:10.1007/s11886-013-0368-0

12. Schunkert H, Konig IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. *Nat Genet* (2011) 43(4):333–8. doi:10.1038/ng.784

13. Erdmann J, Stark K, Esslinger UB, Rumpf PM, Koesling D, de Wit C, et al. Dysfunctional nitric oxide signalling increases risk of myocardial infarction. *Nature* (2013) 504(7480):432–6. doi:10.1038/nature12722

14. Do R, Stribziel NO, Won HH, Jorgensen AB, Duga S, Angelica Merlmi P, et al. Exome sequencing identifies rare LDLR and APOA5 alleles conferring risk for myocardial infarction. *Nature* (2015) 518(7537):102–6. doi:10.1038/nature13917

15. Schnabel RB, Baccarelli A, Lin H, Ellnor PT, Benjamin EJ. Next steps in cardiovascular disease genomic research – sequencing, epigenetics, and transcriptomics. *Clin Chem* (2012) 58(1):113–26. doi:10.1373/clinchem.2011.170423

16. Joyce AR, Palsson BO. The model organism as a system: integrating ‘omics’ data sets. *Nat Rev Mol Cell Biol* (2006) 7(3):198–210. doi:10.1038/nrm1857

17. Vidal MF, Furlong EEM. From OMICS to systems biology. *Nat Genet* Rev (2004) 5:10. Available at: http://www.nature.com/nrg/posters/omics/index.html

18. Yates A, Akanni W, Amode MR, Barrett DL, Bills K, Carvalho-Silva D, et al. Ensembl 2016. *Nucleic Acids Res* (2016) 44(D1):D710–6. doi:10.1093/nar/gkv1157

19. Genomes Project C, Abecasis GR, Altshuler D, Auton A, Brooks LD, Durbin RM, et al. A map of human genome variation from population-scale sequencing. *Nature* (2010) 467(7319):1061–73. doi:10.1038/nature09534

20. Kent WJ, Sugnet CW, Furey TS, Roskin KM, Pringle TH, Zahler AM, et al. The human genome browser at UCSC. *Genome Res* (2002) 12(6):996–1006. doi:10.1101/gr.229102

21. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, et al. NCBI GEO: archive for functional genomics data sets – update. *Nucleic Acids Res* (2013) 41(Database issue):D991–5. doi:10.1093/nar/gks1193

22. Petryszak R, Keays M, Tang YA, Fonseca NA, Barrera E, Burdett T, et al. Expression atlas update – an integrated database of gene and protein expression in humans, animals and plants. *Nucleic Acids Res* (2016) 44(D1):D746–52. doi:10.1093/nar/gkv1045

23. Hill DP, Begley DA, Finger JH, Hayamizu TF, McCright IJ, Smith CM, et al. The mouse gene expression database (GXD): updates and enhancements. *Nucleic Acids Res* (2004) 32(Database issue):D368–71. doi:10.1093/nar/gkh069
24. Vinczai JA, Cordas A, del-Toro N, Dianes JA, Griss J, Lavida I, et al. 2016 update of the PRIDE database and its related tools. Nucleic Acids Res (2016) 44(Database issue):D447–D453. doi:10.1093/nar/gkw1145

25. Wilhelm M, Schlegl J, Hahne H, Moghaddas Golhami A, Lieberenz M, Savitski MM, et al. Mass-spectrometry-based draft of the human proteome. Nature (2014) 509(7502):582–7. doi:10.1038/nature13319

26. Desiere F, Deutsch EW, King NL, Nesvizhskii AI, Mallick P, Eng J, et al. The PeptideAtlas project. Nucleic Acids Res (2006) 34(Database issue):D655–6. doi:10.1093/nar/gkj1003

27. Zong NC, Li H, Li H, Lam MP, Jimenez RC, Kim CS, et al. Integration of cardiac proteome biology and medicine by a specialized knowledgebase. Circ Res (2013) 113(9):1034–53. doi:10.1161/CIRCRESAHA.113.310151

28. Calvo SE, Clauser KR, Mootha VK. MitoCarta2.0: an updated inventory of mammalian mitochondrial proteins. Nucleic Acids Res (2016) 44(Database issue):D1251–7. doi:10.1093/nar/gkv1003

29. Wishart DS, Jewison T, Guo AC, Wilson M, Knox C, Liu Y, et al. HMDB 3.0 – the human metabolome database in 2013. Nucleic Acids Res (2013) 41(Database issue):D801–7. doi:10.1093/nkj/dgs1065

30. Haug K, Salek RM, Conesa P, Hastings J, de Matos P, Rijnbeek M, et al. MetaBioLights – an open-access general-purpose repository for metabolomics studies and associated meta-data. Nucleic Acids Res (2013) 41(Database issue):D1381–8. doi:10.1093/nkj/dgs1063

31. Bonaca MP, Morrow DA, Braunwald E, Cannon CP, Jiang S, Breher S, et al. Prognostic utility of growth differentiation factor-15 in patients with chronic heart failure. J Am Coll Cardiol (2007) 50(11):1054–60. doi:10.1016/j.jacc.2007.04.091

32. Krieger CJ, Zhang P, Mueller LA, Wang A, Paley S, Arnaud M, et al. MetaCyc: a multiorganism database of metabolic pathways and enzymes. Nucleic Acids Res (2004) 32(Database issue):D438–42. doi:10.1093/nkj/dgh100

33. Cavalcante RG, Patil S, Weymouth TE, Bendinskas KG, Karnovsky A, Sartor MA. ConceptMeta: exploring relationships among metabolite sets to identify links among biomedical concepts. Bioinformatics (2016) 32(19):10536–43. doi:10.1093/bioinformatics/btw016

34. Duren W, Weymouth T, Hull T, Omenn GS, Athey R, Burant C, et al. BiGG models: a platform for integrating, standardizing and sharing genome-scale metabolic and transcriptional network models: a platform for integrating, standardizing and sharing genome-scale metabolic and transcriptional network models. Nucleic Acids Res (2014) 44(Database issue):D515–22. doi:10.1093/nkj/dgw049

35. Orchard S, Ammari M, Aranda B, Breuza L, Briganti L, Broackes-Carter F, et al. Database 11.0: the MIntAct project – IntAct as a common curation platform for 11 molecular interaction databases. Bioinformatics (2014) 30(15):2239–41. doi:10.1093/bioinformatics/btu179

36. Stark C, Breitkreutz BJ, Chatr-Aryamontri A, Boucher L, Oughtred R, et al. STRING database: protein-protein association networks constructed from integrated genome-scale datasets. Nucleic Acids Res (2006) 34(Database issue):D422–9. doi:10.1093/nkj/dgl038

37. Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, et al. The string database: in-depth analysis and new functionality. Nucleic Acids Res (2013) 41(Database issue):D610–615. doi:10.1093/nkj/dgs356

38. Rual JF, Veney C, Hao T, Huet TL, Dricot A, Dricot A, et al. Metabolic and molecular profile of human primary cells. Nucleic Acids Res (2004) 32(Database issue):D438–42. doi:10.1093/nkj/dgh100

39. Langley SR, Dwyer J, Drozdov I, Yin X, Mayr M. Proteomics: from single molecules to biological pathways. Cardiovasc Res (2013) 97(4):612–22. doi:10.1093/cvr/cvs346

40. Bylund D, Henriksson AE. Proteomic approaches to identify circulating biomarkers in patients with abdominal aortic aneurysm. Am J Cardiovasc Dis (2015) 5(3):140–5.

41. Afetala AF, Munoz J, Heck AJ. Next-generation proteomics: towards an integrative view of proteome dynamics. Nat Rev Genet (2013) 14(1):35–48. doi:10.1038/nrg3356

42. Lage K, Carlton EO, Klühs M, Ehrhardt MJ, Astashkin S, Saifer D, et al. Metabolomics-based methods for early disease diagnostics. Expert Rev Mol Diagn (2008) 8(5):617–33. doi:10.1586/14737159.8.5.617

43. Huang L, Li T, Liu YW, Zhang L, Dong ZH, Liu SY, et al. Plasma metabolic profile determination in young ST-segment elevation myocardial infarction patients with ischemia and reperfusion: ultra-performance liquid chromatography and mass spectrometry for pathway analysis. Chin Med J (Engl) (2016) 129(12):1078–86. doi:10.3391/cjm.20160852

44. Bengtsson I, Daveji S, Stensvold PO. A meta-analysis of the mouse and human protein-encoding transcriptomes. Proc Natl Acad Sci U S A (2004) 101(16):5607–12. doi:10.1073/pnas.0400772101

45. Goh KI, Cusick ME, Valle D, Childs B, Vidal M, Barabási AL. The human disease network: the potential diagnostic value of circulating microRNAs. EMBO Mol Med (2012) (11):1176–85. doi:10.1002/emmm.201201749

46. Kempp T, von Haelting S, Peter T, Alhoff T, Cicoira M, Doehner W, et al. Pronostic utility of growth differentiation factor-15 in patients with chronic heart failure. J Am Coll Cardiol (2007) 50(11):1054–60. doi:10.1016/j.jacc.2007.04.091

47. Kuwabara Y, Ono K, Horie T, Nishi H, Nagao K, Kinoshita M, et al. Increased microRNA-1 and microRNA-133a levels in serum of patients with cardiovascular disease indicate myocardial damage. Circ Cardiovasc Genet (2011) 4(4):446–54. doi:10.1016/j.circgr.2011.05.003

48. Bylund D, Henriksson AE. Proteomic approaches to identify circulating biomarkers in patients with abdominal aortic aneurysm. Am J Cardiovasc Dis (2015) 5(3):140–5.

49. Afetala AF, Munoz J, Heck AJ. Next-generation proteomics: towards an integrative view of proteome dynamics. Nat Rev Genet (2013) 14(1):35–48. doi:10.1038/nrg3356

50. Lage K, Carlton EO, Klühs M, Ehrhardt MJ, Astashkin S, Saifer D, et al. Metabolomics-based methods for early disease diagnostics. Expert Rev Mol Diagn (2008) 8(5):617–33. doi:10.1586/14737159.8.5.617

51. Huang L, Li T, Liu YW, Zhang L, Dong ZH, Liu SY, et al. Plasma metabolic profile determination in young ST-segment elevation myocardial infarction patients with ischemia and reperfusion: ultra-performance liquid chromatography and mass spectrometry for pathway analysis. Chin Med J (Engl) (2016) 129(12):1078–86. doi:10.3391/cjm.20160852
110. Kui P, Orosz S, Takacs H, Sarusi A, Csik N, Boross F, et al. New in vitro model for proarrhythmia safety screening: IKs inhibition potentiates the QTc prolonging effect of IKr inhibitors in isolated guinea pig hearts. J Pharmacol Toxicol Methods (2016) 80:26–34. doi:10.1016/j.vascn.2016.04.005

111. Foster DB, Liu T, Kammers K, O'Meally R, Yang N, Papanicolaou KN, et al. Integrated omic analysis of a guinea pig model of heart failure and sudden cardiac death. J Proteome Res (2016). doi:10.1021/acs.jproteome.6b00149

112. Haase et al. Systems Medicine in Cardiovascular Genetics

113. Seto SW, Kiat H, Lee SM, Bensoussan A, Sun YT, Hoi MP, et al. Zebrafish models of cardiovascular diseases and their applications in herbal medicine research. Eur J Pharmacol (2015) 768:77–86. doi:10.1016/j.ejphar.2015.10.031

114. Zhang Y, Shimizu H, Sui KL, Mahajan A, Chen JN, Cai H. NADPH oxidase 4 induces cardiac arrhythmic phenotype in zebrafish. J Biol Chem (2014) 289(33):23200–8. doi:10.1074/jbc.M114.587196

115. Conceicao G, Heinonen I, Lourenco AP, Duncker DJ, Falcao-Pires I. Animal models of heart failure with preserved ejection fraction. Neth Heart J (2016) 24(4):275–86. doi:10.1007/s12471-016-0815-9

116. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. Lancet (2005) 365(9455):220–6. doi:10.1016/S0140-6736(05)70151-3

117. Munroe PB, Barnes MR, Caulfield MJ. Advances in blood pressure genomics. Circ Res (2013) 112(10):1365–79. doi:10.1161/CIRCRESAHA.112.300387

118. Huan T, Esko T, Peters MJ, Pilling LC, Schramm K, Schurmanc C, et al. A meta-analysis of gene expression signatures of blood pressure and hypertension. PLoS Genet (2015) 11(3):e1005035. doi:10.1371/journal.pgen.1005035

119. Huan T, Meng Q, Saleh MA, Norlander AE, Joehanes R, Zhu J, et al. Integrative network analysis reveals molecular mechanisms of blood pressure regulation. Mol Syst Biol (2015) 11(1):799. doi:10.15252/msb.20145399

120. Rotival M, Zeller T, Wild PS, Maouche S, Szymczak S, Schilli D, et al. Integrating genome-wide genetic variations and monocyte expression data reveals trans-regulated gene modules in humans. PLoS Genet (2011) 7(12):e1002367. doi:10.1371/journal.pgen.1002367

121. International Consortium for Blood Pressure Genome-Wide Association Studies, Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, et al. Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature (2011) 478(7367):103–9. doi:10.1038/nature10405

122. Levy D, Ehret GB, Rice K, Verwoert GC, Launer LJ, Dehghan A, et al. Genome-wide association study of blood pressure and hypertension. Nat Genet (2009) 41(6):677–87. doi:10.1038/ng.384

123. Wain LV, Verwoert GC, O’Reilly PF, Shi G, Johnson T, Johnson AD, et al. Genome-wide association study identifies six new loci influencing pulse pressure and mean arterial pressure. Nat Genet (2011) 43(10):1005–11. doi:10.1038/ng.922

124. Saleh MA, McMaster WG, Wu J, Norlander AE, Funt SA, Thabet SR, et al. Lymphocyte adaptor protein LNK deficiency exacerbates hypertension and end-organ inflammation. J Clin Invest (2015) 125(3):1189–202. doi:10.1172/JCI76327

125. Lloyd-Jones DM, Nam BH, D’Agostino RB Sr, Levy D, Murabito JM, Wang TJ, et al. Parental cardiovascular disease as a risk factor for cardiovascular disease in middle-aged adults: a prospective study of parents and offspring. JAMA (2004) 291(18):2204–11. doi:10.1001/jama.291.18.2204

126. Mayr M, Liem D, Zhang J, Xiong JI, Avliyakulov NK, Yang JI, et al. Proteomic and metabolomic analysis of cardioprotection: interplay between protein kinase C epsilon and delta in regulating glucose metabolism of murine hearts. J Mol Cell Cardiol (2009) 46(2):268–77. doi:10.1016/j.yjmcc.2008.10.008

127. Chouchani ET, Pell VR, Gaude E, Aksentijevic D, Sundier SY, Robb EL, et al. Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. Nature (2014) 515(7527):431–5. doi:10.1038/nature13909

128. Kang J, Hu J, Karra R, Dickson AL, Tornini VA, Nachtrab G, et al. Modulation of tissue repair by regeneration enhancer elements. Nature (2016) 532(7598):201–6. doi:10.1038/nature17644

**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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