Published in final edited form as:

J Mol Cell Cardiol. 2018 February ; 115: 142–157. doi:10.1016/j.yjmcc.2018.01.008.

Implementing Genome-Driven Personalized Cardiology in Clinical Practice

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Abstract

Genomics designates the coordinated investigation of a large number of genes in the context of a biological process or disease. It may be long before we attain comprehensive understanding of the genomics of common complex cardiovascular diseases (CVDs) such as inherited cardiomyopathies, valvular diseases, primary arrhythmogenic conditions, congenital heart syndromes, hypercholesterolemia and atherosclerotic heart disease, hypertensive syndromes, and heart failure with preserved/reduced ejection fraction. Nonetheless, as genomics is evolving rapidly, it is constructive to survey now pertinent concepts and breakthroughs. Today, clinical multimodal electronic medical/health records (EMRs/EHRs) incorporating genomic information establish a continuously-learning, vast knowledge-network with seamless cycling between clinical application and research. It can inform insights into specific pathogenetic pathways, guide biomarker-assisted precise diagnoses and individualized treatments, and stratify prognoses.

Complex CVDs blend multiple interacting genomic variants, epigenetics, and environmental risk-factors, engendering progressions of multifaceted disease-manifestations, including clinical symptoms and signs. There is no straight-line linkage between genetic cause(s) or causal gene-variant(s) and disease phenotype(s). Because of interactions involving modifier-gene influences, (micro)-environmental, and epigenetic effects, the same variant may actually produce dissimilar abnormalities in different individuals. Implementing genome-driven personalized cardiology in clinical practice reveals that the study of CVDs at the level of molecules and cells can yield crucial clinical benefits.

Complementing evidence-based medicine guidelines from large (“one-size fits all”) randomized controlled trials, genomics-based personalized or precision cardiology is a most-creditable paradigm: It provides customizable approaches to prevent, diagnose, and manage CVDs with treatments directly/precisely aimed at causal defects identified by high-throughput genomic technologies. They encompass stem cell and gene therapies exploiting CRISPR-Cas9-gene-editing, and metabolomic-pharmacogenomic therapeutic modalities, precisely fine-tuned for the individual patient.

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Following the Human Genome Project, many expected genomics technology to provide imminent solutions to intractable medical problems, including CVDs. This eagerness has reaped some disappointment that advances have not yet materialized to the degree anticipated. Undoubtedly, personalized genetic/genomics testing is an emergent technology that should not be applied without supplementary phenotypic/clinical information: Genotype Phenotype. However, forthcoming advances in genomics will naturally build on prior attainments and, combined with insights into relevant epigenetics and environmental factors, can plausibly eradicate intractable CVDs, improving human health and well-being.

Keywords

Personalized or precision medicine; Genome sequencing; Hemodynamics & myocardial mechanics phenome; Genome- & phenome-wide association studies (GWAS/ PheWAS); Genomic decoding of phenotypic diversity; Biomarkers

1. Introduction

The genie is out of the bottle and large amounts of information about the genome will become part of the medical care most of us receive in the not very distant future.

Francis Collins, in his book, “**The Language of Life**,” Harper Perennial, 2011

The function of organismal proteins ultimately derives from the precise sequencing of their amino acids. Similarly, genetic information is encoded by the structure of DNA; the sum total of this information for any organism is its *genome* and its study is *genomics*. Like proteins, DNA has the same sequence-specificity that human codes and languages have: the nucleotide sequence forms the message. The DNA information-molecule confers genetic continuity between generations but also a capacity for genetic variation within-and-between generations.

Genetics' clinical usefulness rests on the notion of gene causal-variants resulting in or predisposing to disease. Inheritable form-and-function variations may accrue from new genetic combinations through meiosis, viable flaws during cellular DNA replication, or mutations elicited by miscellaneous factors. Robustness describes the insensitivity of some functionality or trait against genetic and environmental challenges; notably, there is some mutational and environmental robustness notwithstanding variations. Mutations at one level, e.g., in the DNA nucleotides may not all be expressed at other levels; i.e., as protein alterations or observable changes in phenotype. “Environmental,” here, denotes the Bernardian *microenvironment* of each cell, or the surrounding 3-D extracellular tissue-permeating matrix in which cells are embedded [1,2]. Consequently it includes not only outside influences but also modifying factors originating within the body, such as hormones.

The term *genomics* is generally understood to mean the coordinated investigation of a large number of genes in the context of a biological process or disease. It may be long before we attain comprehensive data and a composite understanding of the genomics of common complex cardiological disorders, but as genomics is nowadays advancing most rapidly, it is

beneficial to have available for a cardiology audience a broad survey of pertinent concepts and findings. Accordingly, I offer here an overview of important genetic and genomics approaches and assessments pertaining to cardiology. We are progressing toward a new *personalized/precision cardiology* where clinical multimodal data/traits, including genomic features, become part of a vast disease knowledge-network that can inform precise diagnoses and individualized treatments [2]. In such a setting, a diagnosis itself will usually provide insight into a specific pathogenetic pathway.

The contemporary focus on genetics in medical research and practice does not reflect a genetic deterministic view. We are what we are as the result of a complex *interplay* between our genetic make-up, our environment, and the cultural milieu in which we are raised and live. Our genes, carrying the instructions for the intricate biochemical processes underpinning all our vital activities, are but one – albeit crucial – part of this complex interplay.

Genotype Phenotype

Accordingly, my underlying theme is that cardiological disorders arise from a blend of multiple interacting genomic/genetic, epigenetic, and environmental risk factors, which lead to a progression in time of multifaceted disease-manifestations, including clinical symptoms and signs, as indicated in Figure 1. As this figure shows, there is not a straight-line linkage between a genetic cause or causal-variant and a particular phenotype. Because of interactions involving *modifier*-gene influences, (micro)-environmental, and epigenetic effects, the same variant may actually produce dissimilar abnormalities in different individuals. This should be born in mind.

2. Genome, genotype, genetics and genetic diversity

Genome can denote the complete set of nuclear DNA (organized into 22 autosomes + 2 sex chromosomes, X and Y) but can also pertain to the maternally inherited mitochondrial genome that contains its own double-stranded small circular DNA molecules (mtDNA) within mitochondria [3]. disorders due to mtDNA mutations are rare. A genotype is a locatable functional region of a genomic sequence, typically denoting how an individual differs from others. An individual's genotype refers to the combination of alleles, alternative forms of a gene, carried by the individual—e.g., the ABO blood types concern the *ABO* gene with an allelic series involving 3 alleles. In the multifactorial genotype–environment interaction, the inherited genotype influences the potential and the confines of the phenotype, which encompasses the entirety of recognizable/measurable traits. Because genes encode information required for every biological function, understanding how this is achieved is equally germane to the study of disease as of normal function. We now have technology to identify genetic/genomic differences and, in certain cases, infer their consequences for disease-risk and treatment choices; hence, they inform treatment decisions [2].

Cardiology patient-care improvements ensue from genomic studies. Some patients with high cholesterol are heterozygous for a non-functional variant of the low-density-lipoprotein-receptor gene (*LDLR*). Lifestyle interventions alone are ineffective in these individuals at

reducing the likelihood of early-onset cardiovascular disease (CVD) [4]. Consequently, identification of carriers of the non-functional receptor informs statin therapy at an early age, rather than first trying diet and exercise control of cholesterol levels. The prompt use of statins in these patients confers therapeutic benefit [4].

Genetics encompasses the study of inherited biological variation, genes, and heredity. It explains phenotypic continuity and the relatedness between structural (cytoskeletal and sarcomeric proteins for movement and organelle transport), or functional (signal-transducing proteins and metabolic enzymes) macromolecules and subcellular structures and cells in different individuals, as well as between generations. Biological variation patterns change, reflecting genetic modifications – e.g., mutations, random chromosomes' assortment into gametes, breakage and recombination (Figure 2A), etc. Genes are enormously versatile, and all changes are heritable.

Humans, plants and bacteria share a common genetic code, revealing common primordial ancestry. Thus, gene therapy can introduce transgenic cells into host somatic tissue to correct defective function or into the germ-line for transmission to descendants. Genetic engineering of recombinant/transgenic organisms can insert synthesized DNA (genes); recombinant human antithrombin III (tgATIII) is obtained from transgenic goat milk.

3. Gene expression: flow of genetic information to phenotypic traits

Gene expression allows information from interacting genes to be used in the orchestrated biosynthesis of functional products; it is a tightly regulated genotype-to-phenotype progression in response to (micro)-environmental changes. It comprises both ON/OFF switches controlling when products are made and regulation adjusting their fluxes. Genes can be viewed as nodes in a network, with input being proteins, the *transcription factors* (TFs) – key regulators of gene expression – and output being gene expression intensities. Regulatory feedback loops subserve adaptive changes. TFs can alter patterns and timing of gene expression, yielding *cytodifferentiation* producing in humans > 200 cell types, organized into tissues and organs. The majority of the genes in any cell type is repressed, leaving a small subset of expressed genes, distinct from those in other cell types.

Cell communication during development can lead to cyto-histological pattern formation and morphogenesis/organogenesis, based on chemical diffusion patterns and regulated by the *Homeobox* subset represented by *Hox* genes [5]. *Hox* genes encode homeodomain proteins that are TFs serving as master-switches ensuring that different bodily-parts/organs, including the heart, form in the appropriate places and assume the right shapes. Targeted disruption of the *HoxA-3* gene, also known as *Hox-1.5*, leads to multiple defects [6,7]. Mice carrying 2 mutated copies of the gene die at birth from defective development of the heart and great vessels.

Steps in gene expression may be modulated, including DNA transcription, RNA alternative splicing, translation, and post-translational protein modifications [8]. And one gene may produce several functionally different proteins in a variety of ways: alternative splicing allows 1 gene to make diverse messenger RNAs (mRNAs) and, hence, different proteins; a

protein may be modified chemically after its production, acquiring atypical function; and, proteins interact in complex pathways and networks, undergoing morphomechanical alterations. As protein-protein interactions adjust cell activities, spotting them can elucidate cellular processes including signaling pathways.

Many biochemical processes act synergistically to regulate when genes are transcribed into RNA [1,2,9]. Likewise, translational regulation determines when and where mRNAs are decoded into protein [10]. Underlying mechanisms are known, but not how they interact in gene and protein networks to regulate complex morphomechanical traits/processes in heart disease. At the genomic level, TFs (themselves products of genes) affect gene activities involved in synthesizing mRNA during transcription of protein-coding genes. Polygenic inheritance can be explained by additive effects of many loci modulated by *epistasis*, where mutations at loci controlling earlier steps (gene 1 or 2) can be epistatic on (sanctioning) gene expression farther along the pathway [11] or by background modifier-gene influences. Genes can also be *pleiotropic* if they affect more than 1 trait.

At the cellular level, cardiomyocytes are stippled with mechano-sensitive, voltage-gated ion-channels creating a versatile electrophysiological profile. At the proteomic level, proteins interact with other proteins forming protein-complexes and enter pathways leading to various normal or abnormal effects pertaining to signaling, metabolic, or cytoskeletal-and-contractile functions. Cardiomyocytes are packed full of long chains of myofibrils supplying the contractile forces needed for cardiac function. The tubular cardiomyocytes are interconnected by porous intercalated disks enabling electromechanical connections and communication. Cardiomyocytes are aligned in bundles with 3-D organization and gene expression adapted to location and function. Metabolic reactions can likewise be integrated into networks whose molecular fluxes are controlled by catalyzing enzymes.

In disease, chains of metabolic interactions evolve along paths which can transform local perturbations associated with susceptibility-conferring gene-variants into recognizable pathogenetic pathways culminating in abnormal phenotypes (Figure 1), with extensive clinical implications. How the information in the human genome is utilized is one central question in personalized cardiology [2,9]. Understanding of associations between genetic information and corresponding disease(s) manifestation(s) is gained through gene expression studies [10].

4. 3-D genomic architecture and mapping

Genomic architecture and 3-D organization of gene networks are highly intricate [12]. Chromosomes, being nucleotide chains, are linear entities; accordingly, through most of the 20th century, the genome was envisaged as a linear chromosomal text mapped and sequenced end-to-end in successive chromosomes [13]. Figure 2B depicts genetic marker – trait associations in the human genome based on such a linear approach [14]. Proteins too are linear molecules, but it has long been known that their spatial configuration is what confers their structural and functional properties.

Chromosomes are likewise not linear stacks but, rather, gene associations arranged in adaptively advantageous ways. Chromosomal gene expression is itself explicable as an adaptive process: a classic example are the above-mentioned *Hox* gene-clusters, which are preserved as 4-8 distinct regions among vertebrates. The Hox TF proteins regulate/micromanage organismal and cardiac development [15], specifically contributing to the patterning of cardiac progenitor-cells and great-artery formation, and it is their 3-D stereotactic configuration that determines pertinent gene expression timings. While recent work has uncovered the role of Hox TFs in heart development, less is known about downstream cell fate-determining genes they activate-or-inhibit, and mechanisms underlying related congenital heart defects (CHDs) [15].

The discovery of enhancers, which enhance/facilitate the initiation of gene transcription, was initially perplexing since they can act at great linear distances from the genes they regulate. However, ensuing studies revealed that enhancers and the TFs bound to them, loop around in 3-D to the gene promoter to influence gene expression [16]. The 3-D genome is also highly dynamic, with chromatin structural winding-unwinding being intimately linked to gene activity (Figure 3).

4.1. 3-D chromatin configuration epigenetically modulates dynamic gene activity

Chromatin is the complex of DNA and histones, specialized proteins disposed in a chain of histone-octamers encircled by DNA to form *nucleosomes* (Figure 3); the nucleosome chain is folded repeatedly to enable DNA compaction into the resultant nuclear chromosomes. Post-translational epigenetic histone modifications affecting 3-D chromatin configuration allow chromatin to respond to (micro)-environmental cues subserving gene regulation. Gene transcription can be contingent on epigenetic regulatory factors like the number of acetyl groups linked to the histone proteins around which DNA winds, or the amount of DNA methylation. Highly basic histone amino (N)-terminal tails can protrude from their own nucleosome and make contact with adjacent nucleosomes; tails modifications affect inter-nucleosomal interactions and thus overall chromatin configuration [17]. Histone modifications are vital in gene-processing and expression. The adjustable histone-scaffolding thus provides for potentially heritable phenotype modifications not involving base-sequence changes.

Current methodologies allow investigations of interactions between different genomic DNA-segments and TFs. They use chemicals to cross-link to DNA the TF-proteins that bind to it, so that proteins and DNA remain attached. Then massively parallel, next generation sequencing (NGS) [2,9] identifies the DNA sequences to which these TF-proteins are attached. All genomic gene-cluster interactions can be studied concurrently in large-scale population studies encompassing genome-wide and phenome-wide association studies (GWAS and PheWAS), as epitomized in Figure 4.

5. Gene interactions and gene regulation

Genome annotation is the analysis of the NGS-produced raw DNA sequence to determine its biological significance. It involves multiple studies, including the nucleotide sequence itself, the structure of protein products, and protein–gene regulatory interactions [18,19]. In

functional genomics, regulation of gene expression refers to the control of amount-and-timing of appearance of gene products [20]. Cells regulate their genes in response to heterogeneous signals; (micro)-environmental effects and epigenetic modifications play key roles and are crucial moderators impinging on mechanisms of complex diseases, such as atherosclerotic heart disease (AHD) which is highly correlated with the lifestyle.

The main purpose of most genes in the human genome is to appropriately regulate the expression (ON/OFF switching) of other genes, and our genome appears to be explicitly organized for gene regulation [1,2,9]. Regulation genes encode specialized protein-cofactors, the TFs that help control the expression of genes encoding other proteins. Binding of TFs to particular sites on the DNA [9] can affect the target-genes in an activating-or-repressing mode. Transcription regulation by repressors/activators greatly enriches gene expression control and modulates myocardial responses to hormones and growth factors.

Specialized proteins interact with histones to remodel chromatin into the “open,” receptive state, so that available TFs can bind readily to the DNA [9]. TFs binding to a specific DNA sequence promotes recruitment of primary-transcript-producing RNA polymerase to *promoter* DNA-regions just ahead of the coding sequence of genes; this commences contiguous gene expression (Figure 3). Normally, multiple different transcription-regulating proteins respond to pertinent biological signals and change gene transcription rates, allowing cells to make proteins only as needed. Transcription inhibition by repressors binding to specific DNA sequences, or simply interfering with the binding of activating TFs to DNA, considerably extends the range of regulatory mechanisms. In recent years, a versatile type of gene regulation has emerged, involving small non-coding RNAs [21].

5.1. Gene networks

Normal/disease phenotypic traits may accrue not simply from any specific genes or even collections of genes, but from the dynamic interactions of interconnected gene networks exhibiting feedback control, feedforward amplification, robustness, hierarchy, and self-organization [9]. Network thinking localizes biological function in the “wiring” and organization of component subdivisions, and this can be readily described in terms of pathways. The networked genome is multiply connected in interlaced fashion and the interconnections are dynamic, shifting over time. The proximity of genes or specific sequence regions along a linear chain is less important than their proximity gauged in terms of number of nodes along the shortest interposed path. Regulatory gene networks can be seen as time-dependent maps of gene interactions, with activation or inactivation by various factors [9].

6. Iconic representations of the genome

An iconic representation of the genome is the circular visualization generated by Circos™. This software arranges tabular genomic data in a circular form. Successive arcs/sectors represent the chromosomes lined-up end-to-end, from chromosome 1 to 22, then Y and X; ribbons crisscrossing the center show various connections between different parts of the genome (Figure 5). As its creators explain, “Data which represent connections between objects or between positions are very difficult to organize when the underlying layout is

linear” [22]. Such connections are just what genomic data consist of: alignments between different genomic regions, relationships between them, genes implicated in the same disease, and interacting gene products. Circos images draw the eye toward the ring center, where the thickets of ribbon-lines underscore the *interconnectedness* of the genome (Figure 5).

7. The genomic basis of CVD and pharmacogenetics applications

Genotype Phenotype (Figure 1). Common causes of chronic ill-health, heart disease, stroke, diabetes, and so on, include environmental factors, lifestyles, and ageing, modulated by our genetic make-up. Identifying genes shaping susceptibility to different maladies can elucidate divergent vulnerabilities to various CVDs, and improve diagnosis, prevention and treatment.

Until relatively recently, genetic investigations focused on individual genes or proteins related to certain phenotypes. Today, genome-wide high-throughput examinations let us explore multifactorial disease-related biomolecular networks bridging genotypes and disease phenotypes to ascertain complex CVD mechanisms, allowing for variable expressivity and penetrance due to gene–gene and gene–environment interactions [2,9,23,24]. Obviously, gene mutations must reside in an accommodating genetic background for a disease-phenotype to manifest. Time- and tissue-specific gene expression data embody causative and disease-progression mechanisms, modulated by background modifier-gene interactions in shaping the detailed phenotypes and disease-course. By experiments on genetically related species, we seek homologous modifiers of the human CVD [25].

Powerful new genomic testing technologies are also facilitating identification of normal/abnormal cellular functions, metabolic processes, and conditions including inherited cardiomyopathies and other CVDs resulting from causal/susceptibility variants in nuclear and mitochondrial DNA [26]. New genomic/genetic testing can help diagnose diseases with atypical manifestations, or otherwise requiring extensive-and-costly evaluation. Pharmacogenetic testing is another application (Table 1). It explains individual variations in pharmacodynamics (effects on drug-receptors) and pharmacokinetics (drug uptake-distribution-metabolism) [2]. It identifies patients at-risk for adverse effects or non-responders, and drugs and dosages best for particular individuals [1,2,9,27].

8. Targeted microarrays and genome-wide NGS

Microarray chips evaluate the expression of thousands of genes simultaneously. DNA microarrays exploit selective single-stranded DNA–DNA or DNA–RNA hybridization through probe–target complementary base-pairing [2,9]. The ability to simultaneously characterize tens-of-thousands of transcripts has allowed recognition of genes/gene-clusters expressed differentially in diseased and healthy tissues. DNA microarrays encompass previously sequenced genes covalently attached as microscopic single-stranded DNA probe-spots on glass, nylon-membrane, or silicon-chip support; a million different probes can query a DNA sample. RNA is sequenced by first converting it to complementary DNA (cDNA) using reverse-transcriptase [2,9].

A microarray can contain multiple probes for different parts of a gene, while subunits of a protein may be encoded by different genes, necessitating multiple probes [28]. To study gene expression under various conditions, mRNA specimens are converted into fluorophore-labeled cDNA—fluorophore-dyes reemit light upon light-excitation. This cDNA is then washed over a microarray with probes representing all the genes that could possibly be expressed in the samples; if hybridization occurs to a probe, the gene is expressed and signal intensity indicates how strongly.

One can thus study global gene expression in a specimen-biopsy; e.g., a microarray panel for gene-variants of hypertrophic cardiomyopathy (HCM) would suit a patient with episodes of syncope and ECG findings of left ventricular (LV) hypertrophy in absence of pressure overload. Other panels (e.g., Brugada syndrome and long QT syndrome) are targeted for patients with characteristic prolonged QT interval and associated family histories, or in standard CHD evaluations [29].

Massively parallel NGS methods rely on reading small DNA-fragments and subsequently reconstructing the data to infer the original DNA target-strand: Conceive a linear genomic target aggregate and a systematic process where smaller fragments are released onto random locations along it [2]. The target is deemed sequenced when reasonable coverage by sequenced fragments (“reads”) accumulates with no gaps (every sidewalk-parking-space gets occupied). Bioinformatics [30] links high-throughput NGS data with techniques for information storage, distribution, and analysis to identify correlations between gene-DNA sequences and CVD susceptibilities [2].

NGS has boosted large-scale population sequencing; the 1000 Genomes Project (1KGP) provides comprehensive descriptions of common human genetic variations [31]. RNA-Seq [2], the direct sequencing of transcripts by NGS technologies, can potentially deliver myocardial whole-genome-transcriptome expression profiling. NGS has already led to disease targeted gene panels, and more recently to whole genome and exome sequencing, whose cost will soon trail that of widely-ordered specific genetic tests and cardiological diagnostic procedures—\$6,000 for a targeted therapy compared to \$9,000 for a PET-scan is “money-well-spent.”

9. Transcription factor mutations impact cardiogenesis

Transcriptional regulation during cardiomyocyte differentiation and cardiogenesis relies on precise quantitative choreography of thousands of genes. Gene expression programs of specific myocardial cell-types include mRNA transcripts from “housekeeping” genes active in most cells and cell-type-specific genes active largely in 1-or-few cell type(s). During heart development, TFs regulate an intricate chamber- and stage-specific gene expression program. Extensive and interdependent genomic occupancy by TF-proteins TBX5, NKX2-5, and GATA4 controls coordinated cardiac gene expression, differentiation, and morphogenesis. Complex gene expression patterns in single and double knock-out (KO) mouse embryos suggest complex interrelationships between TBX5, NKX2-5, and GATA4 [32]. Such regulatory-network control applies to many TF interactions that result in tissue-specific gene expression. CHD have strong genetic etiologies, including disruptions of

developmentally regulated TFs, whose mutations induce disorders in cardiogenesis [33-35]. Likewise, GATA5 loss-of-function mutations underlying tetralogy of Fallot inform allele-specific therapies [36].

10. Genomics-based “precision” and “evidence-based” medicine in cardiology

Proponents of evidence-based Medicine (EBM) guidelines urge treatment decisions based on outcomes of large randomized controlled trials [37], rather than the individualized mechanistic understanding of disease and therapeutics provided by genomics [2,9,38]. There is still much unexplained inter-patient variability in clinical cardiology and electronic medical/health record (EMR/EHR) systems are helping address this. EMRs are frequently updated, large digital datasets linking diverse sources, with the capacity to communicate unique and powerful insights from the linked datasets. They provide clinical detailed multimodality information about large numbers of *individual* patients. EBM emphasizes “one-size fits all” treatments based on well-designed, conducted and preferably *replicated* investigations. Precision medicine is a paradigm centered on the idea that heterogeneous medical treatments should be tailored to the *individualities* of subgroups of patients - essentially, better treatment is dictated by genomic/epigenetic disease signatures [1,2,9].

Common complex multifactorial CVDs are potentially affected by disparate modifier-genes. NGS testing has certainly revealed this and the more we sequence, the more we appreciate that no 2 disease-cases are alike [2]. This is why EBM is flawed in the complex multifactorial-disease space: you need to pretend that blanket-named maladies/syndromes are the *same* entity in order to have statistical power [39], which entails ignoring genomic facts exposed by sequencing.

In cardiology, the 2 paradigms - evidence-based and precision - can be beneficial in a *complementary* relationship. Cardiology is increasingly informed by new technologies promoting granularized-diagnosis, and this imposes major demands for individualized bioinformatics data accessed from online-portals through EMRs and dedicated analytical software. Nevertheless, molecular genetics and the outcomes of genome projects will not replace longstanding patterns of cardiological practice and research. Adept clinical care will still entail history taking, comprehensive physical examination, and well-established multimodal catheterization and noninvasive-imaging findings, along with other supplemental laboratory investigations, backed-up by EBM guidelines. Cardiological therapeutic decision-making will continue to rely heavily on objective epidemiology and randomized studies, clinical trials, and experimental whole-animal disease models. Nonetheless, the developing front-line tools of molecular genetics undoubtedly represent a powerful new acquisition to our arsenal, and will help manage inter-patient variability when fused into clinical practice.

10. The EMR can aid multimodal phenotype-genotype insights

Cardiology practice presently entails fast-tracked adoption of the EMR, along with electronic-data-interchange (EDI) capability for easy, secure data-exchange by computer. EMRs are digital versions of patients' charts built to share information [40]. Migration of

genomic data, in particular, to a cloud-infrastructure – e.g., Amazon Web Services, <https://aws.amazon.com/> – is attractive because of scalability, sharing attributes, and data-compression condensing storage needs. With a single uncompressed human whole-genome involving $\approx 250\text{-}300\text{GB}$ of data, lossless data-compression, cutting file-sizes by $\approx 50\text{-}100\text{-fold}$ without quality loss, is critical; when opened/decompressed, the original data-file is retrieved.

Certainly, without an approach aiming at *granularizing* diagnostics (identifying distinct subentities of blanket-named diseases), the full potential of emerging phenotype-genotype insights for *precision* CVD-management cannot be attained. Availability of NGS genomic and epigenomic testing-results in EMRs, alongside phenotypic trait-collections and medical-histories, is auguring *precision* CVD-management in cardiology. In-depth pluridisciplinary bioinformatics studies correlating underlying genomic/epigenomic variations (including mutations and polymorphisms) with hemodynamics, fluid-dynamics, and myocardial mechanics phenotypic manifestations (Figure 6) are now feasible [41,42]. They can make for high-level insightful coupling of genomic/epigenomic variations with findings from multimodal, multiscale technologies encompassing micromanometric/velocimetric catheterization hemodynamics [1,43-51], systolic-diastolic cardiac/myocardial mechanics [1,52-64], and digital cardiovascular imaging [1,65-67]. In upcoming years they should inform individualized schemes for prevention and management of complex CVDs through timely detection of multifaceted abnormalities, reflected in genomic/epigenomic and proteomic/metabolomic results, and in subtle hemodynamic signs/traits signaling cardiac dysfunction, prior to maladaptive remodeling and failure [68].

Although each of the multimodal platform studies enumerated above may be analyzed individually, the central objective should be the construction of disease models incorporating all (or most) genomic variants and expressed phenotypic traits, with their dynamic peculiarities. In the not-too-distant future, the analysis of fused multiple-technology-platform data can contribute information for detection of the earliest onset of a particular CVD, its refined classification, and stratification of risk. Additionally, it could give a genomic context for developing novel therapeutic targets (Table 1), or for intervening early enough to provide maximum benefit. The efficacious implementation of such a multifactorial plan is likely to be an iterative process. Quantitative genotypic-phenotypic trait correlations, typical of GWAS and involving state-of-the-art high-resolution measurements, are certainly practicable today. Thus, using EMR-data, HCM genomic-variants could be correlated to specific traits displayed in high-fidelity hemodynamic tracings [1,26,43,46], as those illustrated in Figure 6.

Statistically, hypothesis-free GWAS can assess associations between a hemodynamic phenotype, or hemodynamic traits, and any of 100K to $> 1\text{M}$ single-nucleotide polymorphisms (SNPs – “snips”) across the entire genome (Figure 4). A polymorphism at a given locus is linked to a hemodynamic trait if it occurs at a significantly higher frequency among probands compared to controls. Commercial GWAS panels allow near-comprehensive assessment of notable hemodynamic trait-influencing variants across the genome (Figure 4), using standard regression or categorical data statistics. This should enable us to “genotype” common genomic variants with linked phenotypic traits, allowing

new insights into disease diagnosis and pathophysiology [1,2,9,26], and providing *hemodynamic markers* for particular polymorphisms and causal-variants of CVD.

11. Genomic variants linked to hemodynamic traits

GWAS test whether a hemodynamic trait and an allele/variant are correlated, but when there are many interacting trait-causing polymorphisms in the population, association with any particular allele can be weak. Furthermore, the effects of single loci need to be considered in the context of their genetic and environmental backgrounds. The genetic variance underlying a trait can include epistatic loci [11]. Explaining quantitative- or categorical-trait variation requires comprehensive accounting for genetic and nongenetic factors and their intricate interactions. Cardiovascular health/disease is defined by interrelated molecular pathways (specified by the genome, epigenome, transcriptome, proteome, and metabolome; see Figure 1), expressed in features/measurements of the hemodynamic and cardiovascular mechanic phenome. Notwithstanding these complications, predictable relationships between genotypic causal-variants and phenotypic disease-expression are needed before applying genetic testing to clinical decision-making [1,2,9,26].

Multimodal, high-technology-studies data available in EMRs offer the prospect of eventually identifying, predicting and treating complex phenotypic disease-changes in systolic and diastolic cardiac function, from genotypic and epigenetic testing-data combined with environmental factor/life-style history. With multisensor micromanometric/velocimetric catheterization and digital cardiac imaging modalities [43-54,56,58,59,62-67], novel extensive genotype-phenotype hemodynamic correlations through GWAS of CVDs are feasible. A predictable relation between genotype and detailed hemodynamic disease-signs is needed to apply genetic testing to clinical decision-making [2,9,26,69]. High-fidelity and high-resolution multimodal studies as are exemplified in Figure 6 offer nowadays the prospect of identifying detailed, multifaceted phenotypic pattern changes in systolic and diastolic dynamics and correlating them with causative genomic variants [1,2,9,26]. There are currently about 50 known CVDs directly caused by mutations in genes encoding cardiac proteins; these diseases include the inherited cardiomyopathies [1,2,9,26], valvular heart disease [70,71], primary arrhythmogenic conditions, metabolic disorders, and the congenital heart syndromes [32-36]. Identification of genetic causes of CVD has allowed better and earlier diagnosis of at-risk individuals, and is helping guide stratification and prognosis and inform therapies; genetic predictive and carrier testing is indispensable in screening asymptomatic relatives.

12. Whole genome and whole exome sequencing

NGS allows detailed analyses of novel heterogeneous transcripts totally free of any a-priori-knowledge/hypothesis, quite an advantage over arrays (Figure 4). NGS can interrogate whole genomes (WGS), or exomes (WES), or specific gene-clusters to discover entirely novel mutations and CVD-causing genes [72]. It conveys quantitative measurements based on fluorophore-marker signal intensity. WES/WGS are increasingly applied to disorders for which standard genetic tests cannot identify cause(s). The hypothesis-free approach of WES/WGS is especially valuable in diagnosing genetic conditions in children and patients

with severe and/or multisystemic conditions, encompassing CHD [73]. It is especially advantageous in detecting de novo pathogenic variants using a *trio*-based study design, analyzing *both* biological parents *and* the proband [74].

EMR data obtained by diagnostic WES/WGS can be used to refine these methods further and to fuel genetic discovery. Whole-genome NGS can identify causal/susceptibility variants linked to complex CVDs and traits, leading to further studies to characterize disease-mechanisms, suggest diagnoses, and develop treatments for multifactorial maladies. Obviously, DNA variations outside the exons can affect gene activity and protein production and therefore can lead to genetic disorders – variations that WES would miss. In contrast, WGS can identify virtually all genomic variants.

The American College of Medical Genetics and Genomics has policies regarding WES and WGS, including their use and interpretation [75]. Notably, a set of genes commonly affected by base-sequence uncertainty resulting from low sequencing coverage [30] irrespective of WES protocol, tissue samples, and commercial platform biases, involves genes associated with heart failure [76]. Cardiologists should be aware of these limitations in WES. Projects such as the Medical Exome bridge the gap between gene panels and WES/WGS by curating only genes of clinical relevance and improving disease-targeted sequencing [77]. One direct outcome of WES/WGS is the potential to produce abundant valuable information not directly relevant to the clinical question(s) addressed [78]. Thus, DNA sequencing to evaluate a cardiomyopathy could also generate information about inherited arrhythmias and susceptibilities to other genetic conditions. As its cost plummeted sequencing spread, helping create DNA-databases in the US and overseas, with mounting data on mutations/variants, epigenetic switches, and lifestyle factors impacting susceptibilities to common diseases, such as diabetes and CVD [2,9,26,79].

13. Phenotype-genotype pairing entails epigenomic/environmental effects

Phenotypes are complex and dynamic, with varying timescales of change. Phenomics, the systematic study of normal/disease phenotypes on a genome-wide scale, complements genomics with NGS. Genome-wide scale implies that analyses of specific phenotypes incorporate interacting contributions from the entire genome. Phenomics typically encompasses multiple phenotypic assays on a large set of genetic variants. It is rapidly evolving into a discipline differentiating phenotypes and linking their traits to the correlated gene-variants [80].

In tracing phenotype-genotype connections, always allow for epigenomic control and *backup*-pathways cropping-up when a main molecular route to creating a specific protein is blocked/disabled. Through epigenomic control turning-off one gene may prod another on, such that the absent protein gets made. As a plethora of DNA sequence data is now available, the challenge is to quantify individual phenotypes in a manner that can be specifically matched to correlated genotypes, as I emphasized already in discussing hemodynamic disease traits. This requires mapping the human epigenome (Figure 4) too, to enrich appreciation of how gene functions are regulated, offering deeper insights into complex trait components of CVD syndromes. Examples of resulting phenotypic attributes

include morphological measures such as cellular size (cardiomyocyte hypertrophy), metabolic rates of nutrient uptake or breakdown, hemodynamic features on high-fidelity tracings, and molecular measures such as transcript profiles and mass-spectrometry fingerprints. Comprehensive trait-profiling through metabolomics, complementing genetic profiling with the effects of diet, lifestyle, drug treatment, the microbiome, etc., can accurately identify heterogeneous conditions encompassing diverse pathobiologies, such as heart failure (HF) with preserved (HFpEF) or reduced (HFrEF) ejection fraction [81,82].

Surely, a genetic system's ability to manifest additive genetic-phenotypic variations presupposes its capacity to withstand/buffer the deleterious effects of preceding/successive mutations. Genomes that curtail mutation-lethality will be better able to engender phenotypic variations from mutational events, effectively “reconnoitering” a greater proportion of genotypic–phenotypic space without encountering lethal restraints. Phenotypic variation is produced through multifaceted, intricate, dynamic interactions between genotype, the epigenome, and the environment [1,2,9,83,84]. The epigenome is a heritable layer of information encoded not in the genomic DNA sequence but in chemical modifications of DNA or histones; these modifications, together with TFs, operate as spatiotemporal regulators of genomic activity.

Detailed, comprehensive phenotypic dynamic data allow the formative, intricate genotype–phenotype interactions to be studied. Acquisition of such *phenomics* data (encompassing all conceivable categories of phenotypes and variances) on an organism-wide scale is progressing significantly [80]. The hurdle is that, unlike the genome, which is identical in every cell, the epigenome varies from tissue to tissue, between individuals, and over time; and the phenotype can be described at many levels, from specific molecules to hemodynamic/cardiac-mechanic (Figure 6) functional attributes [1,2,9,26,43,46,56,70,71,83–85], and so on. Acquisition and dissemination of phenomics data requires availability and cross-compatibility of cost-effective information management systems, namely, EMR with EDI capability.

14. Genome-wide and phenome-wide association studies in cardiology

GWAS reveal genetic markers suggesting causes/risks for traits and diseases, and may guide patient-specific diagnosis, prevention, and therapy. They are exemplified by unbiased studies comparing the SNPs between patient and control populations; they can define correlation between a grouping of tag-SNPs and disease risk. A tag-SNP is a representative SNP of a genomic region with a nonrandom collection of SNPs defining a *haplotype*. In an antithetical approach to GWAS, PheWAS determine which phenotypes, diagnoses, or traits (outcome) are linked with given genotypes/genetic variants [86]. Consequently, both GWAS and PheWAS appraise the extent to which phenotypes are caused by genetic variation.

Some SNP alleles are the actual susceptibility variants, or *causative* SNPs, that contribute to the risk of acquiring a particular disease. Rather than functional/causative variants, most SNPs are *markers* for linkage-or-association studies exploring the genetic susceptibility to a particular disease. SNPs occur in linked sets of SNP alleles along a region of a chromosome (non-random association, or *linkage disequilibrium*), forming haplotypes that differ among

individuals. Haplotype maps are valuable for identifying genes/alleles related to health and disease. However, the SNP alleles identified through GWAS are typically not causative but rather in linkage disequilibrium with the true causal-variants. To find the genomic regions with genes implicated in a disorder, the frequencies of many SNP alleles are compared in individuals with-and-without it. Such haplotype associations with a particular disease imply that there may be causative genes in the region. Breakage and recombination (Figure 2A) is the major process untying associations between SNPs.

Keeping in mind that *Genotype* $\rightarrow$ *Phenotype*, using SNPs as DNA linkage-markers we can trace disease gene-variants through families and determine their approximate locations on different chromosomes. By contrast, GWAS and PheWAS use unbiased methodologies, such as genome-wide SNP arrays, to identify disease-associated genetic variants. They employ an agnostic approach in the search for unknown polygenic disease variants, for which the ability to interrogate a large number of SNPs covering the *entire* genome is critical [87]. With polygenic/complex diseases GWAS/PheWAS demand large sample numbers, to attain sufficient statistical power for spotting associations of the typically small additive effect-size of common variants while avoiding high rates of false-positives and lack of reproducibility. Moreover, gene–environment/epigenetic interactions entailing lifestyle factors, as the personal choice of food, create additional complexity. WGS of several thousand individuals are performed in large consortia, such as the 1000 Genomes Project [31]. The large sample-sizes needed in studies of complex diseases, such as AHD [88], may be achieved by pooling several GWAS through meta-analysis.

In a reversal of the GWAS paradigm, PheWAS exploit extensive, detailed EMR systems to select among numerous phenotypes those associated with one-or-more genetic variants. Thus, whereas GWAS consider only one disease/trait at a time, PheWAS look at multiple diseases and traits simultaneously and can identify genetic causal-variants with pleiotropic effects [89]. Pleiotropy occurs when 1 gene influences 2 seemingly unrelated phenotypic traits; consequently, a mutation in a pleiotropic gene may affect several traits [2,9,26,90]. The advantage of PheWAS over GWAS is the capacity to identify miscellaneous diseases sharing *common* genetic etiology. Thus, genetic variants near genes *CDKN2A/CDKN2B* are associated with both type 2 diabetes and AHD, suggesting a shared mechanism or pathway [89,91].

15. Synteny of mouse and human genomes

An optimal way for identifying coding sequences along with regulatory regions of human genes is genomic investigation of non-human species, easy to maintain and breed in a laboratory; the lab-mouse is of particular import. Valuable information is also obtained using other expeditiously breedable species with evolutionarily conserved genes shared with mammals: the nematode worm (*Caenorhabditis elegans*), fruit-fly (*Drosophila melanogaster*), zebrafish (*Danio rerio*), and yeast (*Saccharomyces cerevisiae*) [92].

Genetically and genomically, human and mouse are very similar, having about the same number of coding-genes with similar sequence, and with many disease-genes nearly identical [92]. Just within the past few years, low-cost NGS analyses and genome-

engineering using CRISPR-Cas9 technology [1] have revolutionized application of mouse and larger-animal models for study and precise treatment of human disease. *Synten*y is the mapping between positions on one genome and those with the same sequence in another genome; it indicates how extensively a genome was rearranged during evolution. Synteny-blocks, genomic regions evolutionary conserved among multiple species, are pivotal in molecular and comparative genomics. Syntenic relationships among chosen species are lucidly visualized as interactive Circos plots (Figure 5): ribbons then graphically connect in different species homologous segments belonging to the same synteny-block. The ability directly to relate human patient-data with mouse-models at the nucleotide-level is opening new research chapters with enormous clinical potential.

Mice are essential for learning more about CVD genomics and testing drug therapies not just because mice are remarkably similar genomically to humans, but also because their disease-pathophysiology is comparable. Mice have a fast-tracked lifespan, with 1 mouse year $\approx$ 30 human; their entire life-cycle can be studied within just 2-3 years. Mice can be genetically manipulated to mimic virtually any human disease. Reverse genetics seeks to discover what traits/phenotypes result from a particular genetic sequence; tweaking individual genes/base-sequences allows assessment of phenotypic results. Interventions encompass induced gene-mutagenesis to create human disease-models (“knock-out” mice), or introduction of therapeutic sequences into genomic target sites to correct genetic defects (“knock-in” mice). Mouse models exist for many diseases, including β -thalassemia, atherosclerosis, and Duchenne muscular dystrophy; they allow molecular studies and testing of therapies.

Human and murine chromosomes with particularly conserved synteny (Figure 5) can be studied. In both species, the myosin genes, α - and β -MHC, are regulated/expressed in an antithetical manner during development and during stress in the adult myocardium. Down-regulation of the adult myosin heavy chain, α -MHC, with up-regulation of embryonic β -MHC, occurring during hypertrophy and failure, reduce myofibrillar ATPase activity and systolic function [93]. A murine study showed that miR-208, a microRNA (miR) encoded by an intron of the α -MHC gene, effectuates the stress-dependent switch in MHC composition. Non-coding miRs regulate gene expression post-transcriptionally, by destabilizing the mRNA and causing translational-silencing. They partake in the re-activation of the myocardial fetal gene program during pathological remodeling; knocking-out miR-208 sequences blunted adult expression of β -MHC in response to hypertrophic stress stimuli [93].

16. Stem cell therapy for cardiac diseases

Cardiomyocytes are produced during embryonic development, differentiate, and endure throughout post-embryonic life; if damaged, they cannot usually [94,95] be replaced. Other differentiated cells, including blood and epithelial cells, have short life-spans. They are replaced by stem cells (SCs) [96], which divide yielding daughter cells that can either differentiate or remain as SCs, a source for production of differentiated cells throughout life. Treatment using SCs, capable of growing into different heart-cell types, could potentially repair/regenerate damaged myocardium [97]. SCs expand in number during development and exhibit open-ended self-renewal capacity throughout life, maintaining a *multipotent*

differentiation-potential by inhibiting expression of lineage-specification genes [98]. Upon transplantation they can repopulate tissues; this prompts cardiac transplantation of SCs to replace failing/damaged myocardium and blood vessels, restoring cardiac function; however, important problems remain [99,100,101].

Many combined SC varieties, such as myoblast cardiac progenitor-cells, bone marrow SCs, angiopoietic progenitor-cells, mesenchymal SCs, and fat-derived SCs, are investigated as sources [99]. They are delivered to the diseased heart by various methods [102], including: *intracoronary infusion* via catheter, with-or-without balloon occlusion; *intravenous injection*, least invasive; *transepicardial injection*, delivering cells directly to the infarcted/scarred myocardium during open-heart procedures/routine bypass-surgery; and *transendocardial injection* into the ventricular wall by transaortic injection-needle catheter, positioned using sophisticated imaging. SCs are also being used as a vehicle for gene therapy. Gene therapy with genetically modified cells (GMCs) offers advantages over direct gene transfer and therapy with non-GMCs. GMCs can be programmed to regulate their product: the therapeutic transgene(s) may be controlled by compounds administered to turn them on-and-off, as needed.

17. Gene therapy for cardiac diseases

Gene therapy replaces defective, down-regulated, or missing genes with normal, enabling normal function or, alternatively, adds a new gene to restore or improve well-being. After extensive research to redesign/identify safer recombinant DNA vectors to deliver particular genes to target-cells, recent viral-vector trials delivered promising results for cardiac cells [103]. Viruses have either RNA or DNA as their single- or double-stranded genetic material. If *virion* particles (consisting of a *capsid*, outer protein-shell, and an inner core of nucleic acid) contain genomic RNA, upon entering the host-cell this RNA is reverse-transcribed into a complementary DNA (cDNA) that is integrated into the host chromosomal-DNA. Viral-vector assembly entails replacing viral genome-segments with desired gene(s); structural proteins needed for viral capsid and host-genome integration are included. Currently, efforts are directed toward complex, polygenic, chronic cardiac diseases, such as AHD and HF [1,2,9,26,104].

Gene delivery systems entail analyses of route, dose, and frequency of administration [105]. Viruses act as “Trojan horses,” to genetically modify target-cells. Unlike traditional medications administered repeatedly, gene therapy resembles surgery where the objective is *single-intervention* with *life-long* cure. Viral-vector-mediated gene transfer employs replication-deficient viruses such as retrovirus, adenovirus, and adeno-associated virus, and exploits viruses' ability to infect mammalian cells and use host-machinery to produce viral-vector proteins. Suitably altered viruses convey gene(s) appropriate for direct correction of causative variant(s) in target-cells and tissues [106]. Delivery is imprecise, with potential for insertional mutagenesis, and limited to cells that the virion can infect. Some viruses used as vehicles (e.g., simple retroviruses) can only infect dividing cells because their DNA cannot pass the nuclear membrane; its integration into the recipient genome can only occur during mitosis when the nuclear membrane is dissolved. This restricts their usefulness in cardiology, since cardiomyocytes are non-dividing. Contrariwise, the lentivirus genome

encodes proteins permitting nuclear-passage of viral nucleic acid without mitosis, with permanent integration of therapeutic DNA into non-dividing cells. After integration, RNA polymerase II transcribes the viral genes making mRNA, and viral-vector proteins are produced [107].

Recombinant adeno-associated virus (rAAV) vectors are well-suited for gene transfer to cardiomyocytes, because after entering host-cells they form *episomes*, i.e., DNA-strings existing autonomously in the cytoplasm thus minimizing insertional mutagenesis-risk. The episomal rAAV genomes concatemerize (coil) into stable circular configurations maintained extra-chromosomally, allowing for long-term normal gene expression in the myocardium [108].

18. CRISPR-CAS9 technology and genome engineering

Genome-engineering addresses problems in producing made-to-order genome modifications to correct variants predisposing to disease(s). Homology directed repair (HDR), a natural nucleic acid repair mechanism, is initiated by the presence of random DNA double-strand breaks (DSBs), as made by nucleases, reactive oxygen species, or ionizing radiation, and can implement genome modification in many organisms, including humans [109]. However, insertion of normal/non-mutated gene(s) at an off-target genomic locus might inadvertently inactivate some adjacent essential gene or turn-on a gene inappropriately [110]. The CRISPR (clustered regularly interspaced short palindromic repeat)-Cas9 (CRISPR-associated nuclease 9) method uses a guide RNA (gRNA) to target Cas9 to a specific nucleotide sequence (Figure 7); it has emerged as a simple, precise and most rapid genome editing technology [1] and is revolutionizing medical genetics and medicine.

Composed of sequence-specific DNA-binding domains fused to a non-specific DNA-cleavage module, CRISPR-CAS9 can create targeted DSBs thus controlling the specificity of genome-editing, and acts as a programmable *cut-and-paste-gene* tool to correct disease-causing genetic mutations. The developed technology can be applied to functional elucidation of causal genetic variants and genomic screening, transcriptional modulation, and gene therapy [111]. The CRISPR system can induce precisely targeted DNA DSBs, which can subsequently be repaired with an exogenous DNA donor-sequence, to replace defective gene(s) with normal (Figure 7). Thus, the natural DNA-repair mechanisms can insert the desired gene(s) with precision. Such genome-modification techniques, used to knock-out unwanted genes and/or knock-in needed genes, can treat precisely genetic/genomic cause(s) of CVD. The efficiency of the genetic exchange would be expected to be low, however, yielding relatively limited corrective gene-replacements amid multitudes of affected cardiac cells. Development of liposomes and polymeric nanoparticles as DNA-carriers with comparatively high transfection levels and efficiency, may prove invaluable in applying human artificial chromosomes and synthetic oligonucleotides for gene therapy [112].

19. Genomics-informed precision medicine in atherosclerosis

The prominent Russian pathologist Anichkov discovered the significance and role of cholesterol in atherosclerosis pathogenesis [113]. Today's technology has brought us much closer to exquisite precision in its diagnosis and treatment. Cholesterol is an essential component of plasma membranes and of numerous cellular processes. Nearly all human cells can synthesize it, but the majority of cholesterol is made in steroid-producing cells and the liver for transfer to other cells; humans do not require cholesterol in the diet. It is transported through the blood as part of lipoprotein particles. To preserve normal cholesterol homeostasis, cholesterol synthesis uptake and efflux are tightly regulated in our cells [114]. Atherosclerotic cardiovascular disease is multifaceted and generally not attributable to single-gene mutations. The regulation of large coronary arteries in coronary artery disease (CAD) is complicated by the fact that these vessels are responsive to changes in myocardial metabolic demands and coronary blood flow [115]. Interestingly, calcific aortic valve disease (CAVD), prevalent in the elderly, has similar risk factors to atherosclerosis, and is characterized by high lipoprotein(a) and low density lipoprotein cholesterol levels, abnormal hydrodynamic shear stress patterns, endothelial basement membrane disruption, oxidative stress, inflammation, cell infiltration, lipid deposition, and calcification [1,2,9,41,70,71,116]. In such complex, multifactorial disorders the role of causative genes is assessed by *integrating* genetic mechanisms with environmental risk factors responsible for the whole spectrum of the atherosclerotic clinical phenotypes. The search for individual gene variants involved in complex clinical entities such as atherosclerosis is replaced by investigation of multifaceted, *multifactorial* causality.

19.1. The mevalonate cholesterol biosynthetic pathway

Cholesterol synthesis variations are closely linked to cholesterol *uptake*, via receptors such as the low-density-lipoprotein receptor (LDLR), and *export* from the cell, via transporters such as the ATP-binding cassette transporter A1 (ABCA1) [117]. ABCA1 is an integral cell membrane protein which exports excess cholesterol from cells and is encoded by the *ABCA1* gene. Discovery of the LDLR gene, *LDLR*, and of its impaired variant protein product in patients with familial hypercholesterolemia (FH) exposed the pivotal role of the repression by intracellular cholesterol of the 3-hydroxy-3-methyl-glutaryl coenzyme A reductase (HMG CoA reductase), the rate-controlling enzyme of the mevalonate metabolic pathway that produces cholesterol (Figure 8). The pathway is regulated such that cholesterol synthesis ensues in response to a lower-than-normal concentration of cholesterol in the endoplasmic reticulum.

Key to understanding the importance of mevalonate biosynthesis in atherosclerosis was the seminal work by Goldstein and Brown on HMG CoA reductase [114], which paved the way for the introduction of therapy involving HMG CoA reductase inhibitors, the *statins*, now one of the most widely prescribed medications in the world for decreasing hypercholesterolemia and preventing atherosclerosis and its sequelae. As shown in Figure 8, statins inhibit HMG CoA reductase and thus the cellular synthesis of cholesterol; this triggers activity of the SREBP transcription factor, which upregulates transcription of the

LDLR receptor and enhances uptake of LDL from the plasma, thus depressing plasma LDL cholesterol levels.

Later studies of persons with very low cholesterol levels identified another key gene in the cholesterol pathway, coding Proprotein Convertase Subtilisin/Kexin-Type 9 (PCSK9; the initials SK9 relate to bacterial subtilisin and yeast kexin and the presence of 9 secretory serine proteases)[118]. The SREBP transcription factor also upregulates transcription of *PCSK9*, which leads to posttranslational degradation of the LDLR because binding of PCSK9 to it targets the receptor for lysosomal degradation. Gain-of-function mutations of *PCSK9* are associated with hypercholesterolemia and amplified risk of cardiovascular events. Contrariwise, loss-of-function mutations of *PCSK9* produce lifelong low plasma cholesterol levels, resistance to coronary disease, and seemingly no ill effects.

19.2. Genetic testing underpinnings for the management of atherosclerosis

Genetic testing plays a major role in the current management of atherosclerosis. Technological advances and the development of affordable high-throughput methods have led to the identification of genetic risk factors in research and clinical practice. Numerous genetic loci ascertained to date embody variants that result in atherosclerosis risk, with build-up of atherosclerotic plaque in the vessels that supply O₂ and nutrients to the heart and brain, producing CAD and/or cerebral ischemia or hemorrhage.

Specifically, approximately 20% of the loci are located near genes with known functions in the metabolism of low-density lipoproteins (LDLs), triglyceride-rich lipoproteins (TRLs), or lipoprotein(a) (a modified LDL particle encoded by *LPA*), supporting key roles for these pathways and metabolites in the disease; this gives internal validity to findings of GWAS – sometimes labeled common variant association studies (CVAS) [119]. An additional 5–10% of the loci relate to hypertension, a known and modifiable causal risk factor. Thus, guanylate cyclase 1, soluble, alpha 3 (*GUCY1A3*) and nitric oxide synthase 3 (*NOS3*) are important regulators of vascular tone and platelet aggregation; common DNA sequence variants at the *GUCY1A3* and *NOS3* loci have been linked to both hypertension and CAD [120,121].

The fusion of genetic and molecular epidemiology – correlating genetic variations in genes and pathways with intermediate biomarkers of disease – affords a powerful means for detecting underlying disease processes linked to atherosclerosis. Thus, high serum levels of C-reactive protein (CRP), which rise in response to inflammation, have been shown to represent a risk factor for coronary heart disease (CHD) in prospective studies [122]. However, genetic variations and haplotypes in the *CRP* gene are correlated with differences in CRP levels, but not with risk of CHD [123]. This suggests that CRP levels are not causally related to CHD but likely co-vary with (a) causal factor(s). Great care is necessary in evaluating individual risk and in studies aiming to elucidate relationships between genetic variants, biomarkers of disease, and disease risk. In this context, it should be born in mind that many complex cardiological diseases, such as atherosclerosis, are probably the result of numerous rare genetic variations present within a single genome. Thus, one person might not carry the same set of variants as another, even if both have the *same* disease.

19.3. True genomic precision medicine applications in atherosclerosis on the horizon

Identifying genetic/genomic underpinnings of atherosclerotic disease has motivated screening of patients and individuals that are deemed at risk. In rare monogenic syndromes, the diagnostics, risk stratification, and, in some cases, individualized treatment decisions become unambiguous. But for common, polygenic and multifactorial diseases, such as atherosclerosis, the situation is more complex owing to interaction of multiple factors, including the underlying genetic predisposition and modifiable external risks. Epigenetics challenge the notion that the genome is the only source of pertinent information: *Genotype Phenotype*. There are significant gene-environment interrelations and interactions contributing to the great variance in atherosclerosis phenotypes, and the exposure to environmental risks can escalate the levels of complexity in practice.

Statin drugs inhibit HMG CoA reductase, the enzyme that controls the rate of de novo cholesterol synthesis. This leads to an amplified LDL receptor expression and, therefore, a lowering of plasma LDL cholesterol. These therapies, albeit impressive and highly efficacious, do not in fact represent application of an individual's genomic information in his/her own clinical care, since the applied remedies are used independently of a patient's *LDLR* or *PCSK9* variant status [124,125]. Likewise, PCSK9 inhibitors increase the LDLR number and thus lower the plasma concentration of LDL cholesterol. Treatment with PCSK9 inhibitors, such as the monoclonal antibody REGN727 (alirocumab) [118], will lead to increased cellular LDL receptors, enhanced uptake of LDL, and lowering of LDL cholesterol levels, and represents a true genomic medicine application in cardiology.

Such Instances of true genomic medicine applications in cardiovascular disease are less common than in fields such as cancer, and are even less common for *atherosclerotic* cardiovascular disease. Relatively few Mendelian syndromes have been described for atherosclerosis, and there are few other hereditary atherosclerotic syndromes included in the American College of Medical Genetics and Genomics (ACMG) recommendations for reporting and clinical investigation [126], aside from inactivating mutations in the *LDLR* and *PCSK9* genes.

As noted above, loss of function mutations in *PCSK9* produce lifelong low cholesterol levels, resistance to coronary disease, and seemingly no ill effects [118]; a gene editing-based therapeutic approach that introduced such mutations via a one-time injection could extend these protective effects into large segments of the population at risk. Gene editing to diminish the expression/function of a gene product is tractable, as shown in Figure 7, with current CRISPR-Cas9 RNA-guided endonuclease technology [109-111]. Similarly, for genes (e.g., *LDLR*) in which injurious mutations confer increased risk of CAD, future developments may enable upregulation or potentiation of gene activity.

At present, our ability to collect genomic data outpaces our ability to understand and act on it in the management of atherosclerosis. As our understanding advances, we should see more effective clinical trials based on disease genetics; furthermore, we may forecast that some hitherto unrewarding medications will be accepted as safe and effective for subgroups of patients with specific genetic markers. The research and dissemination initiatives of the National Human Genome Research Institute and other groups should speed the evaluation

and incorporation of genomic findings and technologies into routine clinical care, where and as appropriate. Such research programs, in conjunction with future projects now in early planning phases, will address many of the questions and hurdles associated with genomic medicine implementation. These efforts have significant potential for truly personalizing medical evaluations and treatments and enhancing the efficacy of health care. Integration with other -omics technologies, involving epigenomics and transcriptomics and novel bioinformatics and systems medicine approaches. They may hasten recognition and exploitation of presently unknowable advances.

20. Conclusions and future directions

Assessing the likely cardiological applications of ongoing genome projects is not easy, although enough was known before modern-day successes to suggest that the study of disease at the level of molecules and cells would bestow major clinical benefits. Personalized or *precision* cardiology is a medical paradigm based on one's genome and providing customizable approaches to prevent, diagnose, and treat disease with the right-treatment, *fine-tuned* for the individual patient at the appropriate dosage. As exemplified in the Epigram by the pioneer Francis Collins, in the fervor generated by the HGP, many proclaimed that genome technology will provide imminent solutions to intractable medical problems, including CVDs. This eagerness has reaped some disappointment that such advances have not *yet* materialized to the degree anticipated. At present, personalized genetic testing is an emergent technology and one should be cautious applying it without accompanying phenotypic information. *Genotype Phenotype*.

Future advances in genomics will undoubtedly build on what has been attained and, combined with insights into how epigenetics and environmental factors affect cardiovascular health, have the potential to eradicate intractable CVDs - a tantalizing prospect. Personalized medicine promises a future in which all individuals will have their full genomic information linked to their EMR/EHR. Such data, with overlaid clinical interpretation, will allow cardiologists to develop a strategy based on the patient's susceptibility to different diseases and anticipated responses to different types of treatment. It appears that currently we are on the tipping point of a whole new game in how we develop CVD treatments and comprehensive approaches to management aiming at improving quality of life.

Acknowledgments

Funding: Research support, for work from my Laboratory surveyed here, was provided by: National Heart, Lung, and Blood Institute, Grant R01 HL 050446; National Science Foundation, Grant CDR 8622201; and North Carolina Supercomputing Center and Cray Research.

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Highlights

- Today, clinical multimodal electronic medical/health records include genomics data
- Personalized cardiology (PC) exploits clinical benefits of molecular/cellular CVD study
- PC aims to prevent, diagnose, and manage CVD with treatments pointed at genomic defects
- PC uses stem cell & gene therapies, CRISPR-Cas9-gene-editing, & other personal cures
- Genomics, epigenetics & environmental factors study, may help eradicate complex CVD

Fig 1. Genotype Phenotype

Cardiological disorders arise from a combination of multiple genomic/genetic, epigenetic, and environmental risk factors interacting in pathogenetic trajectories and leading to a progression in time of multifaceted manifestations of disease, including clinical symptoms and signs. As is shown in the right lower corner panel, there is not a straight-line linkage present between a genetic cause/causal-variant and a particular phenotype. Indeed, because of interactions involving different *modifier-gene* influences as well as environmental and epigenetic effects, the same etiologic cause may actually produce dissimilar phenotypes/abnormalities in different individuals.

Fig 2.

A Breakage, crossover, and recombination of parental chromosomes (M, F) during meiosis results in the generation of chromosomes (C_1 , C_2) that share DNA from both parents. The frequency of recombination between alleles of 2 genes depends on the distance between them: genes located close together will have linked (rarely separated) alleles. Accordingly, one nearby allele can be used as a marker for another, and we can utilize SNP *markers* linked to genetic disease/risk alleles without pinpointing the precise culpable mutation. **B** Diagram showing all single nucleotide polymorphism (SNP)-trait associations with P-value 5.0×10^{-8} , mapped onto the human genome by chromosomal locations and displayed on the human karyotype. The Genome-wide Association Studies (GWAS) Catalog is supplied jointly by the National Human Genome Research Institute (NHGRI) and the European Bioinformatics Institute (EMBL-EBI). The diagram is released nightly and available on the NHGRI-EBI Catalog website. Associations are displayed by chromosome and color-coded by class of phenotype. Currently, there are well over 10,000 published genome-wide significant SNP associations, across hundreds of diseases and quantitative traits. The number or letter below each chromosome is its name (1-22, X, Y). Reproduced from Pasipoularides [26], by permission of the Revista Española de Cardiología and the Spanish Society of Cardiology.

Fig 3.

A nucleosome is a section of DNA that is wrapped around a core of positively charged proteins called *histones* that bind the negatively charged DNA and aid in its packaging. Double-stranded DNA loops around 8 histones twice, forming the *nucleosome*, which is the building block of chromatin packaging. Chromatin forms chromosomes within the nucleus and exists in two forms: euchromatin, is less condensed and can be transcribed; heterochromatin, is highly condensed and is typically not transcribed. There are many ways that gene expression is controlled epigenetically. Adding or removing chemical groups to or from histones can alter gene expression; acetylation and phosphorylation make the histones less positively charged, because acetyl and phosphoryl groups are negative, and their tight hold on DNA becomes looser. Conversely, extensive methylation of cytosine in DNA is correlated with reduced DNA transcription. Adapted from Pasipoularides [83], by permission of the Journal of Cardiovascular Translational Research and Springer US.

Fig 4.

Genome-wide association studies (GWAS) involve an examination of a genome-wide set of genetic variants in different individuals to determine if any variant is associated with a particular phenotypic trait; they reveal genetic markers that expand understanding of risks and causes for many diseases, and may guide diagnosis and therapy on a patient-specific basis. Phenome-wide association studies (PheWAS), or “reverse GWAS,” ascertain what clinical disease associations/susceptibilities can be attributed to a single genetic causal variant (a particular genotype). When studied in combination, PheWAS and GWAS can provide new valuable gene–disease pathogenetic insights.

Fig 5.

The CircosTM graphics circular ideogram layout facilitates the display of genomic relationships between pairs of positions on the circumference, by the use of linking ribbons, which encode the position, size, and orientation of related genomic elements. In Circos plots, genomic chromosomes are circularly arranged and relationships between genomic regions can be clearly demonstrated by links within the circle, and can reflect any type of correspondence—e.g., defined on the basis of similarity (base sequence or protein), or by category (functional or structural component). If the relationship has an associated quantity, this quantity can be represented by the thickness of the link; by coloring the linking ribbons, tracking relationships to/from an element becomes easier. In this particular CircosTM plot are depicted, clockwise from top right, the genomes of a human, a chimpanzee, a mouse, and a zebrafish, arranged in a circle with each color square corresponding to a pair of chromosomes. The links within the circle connect similar DNA sequences, visually emphasizing just how much DNA humans share with the other 3 species shown. After Krzywinski et al. [22], reproduced with Dr. Krzywinski's permission.

Fig 6.

A Deep left ventricular (LVP) and aortic root (AOP) pressures in hypertrophic cardiomyopathy at rest and during supine bicycle exercise, which elicits an abnormal LVP diastolic decay, suggesting impaired ventricular relaxation; LVP decays throughout diastole, in sharp contrast to the normal pattern shown in panel **C**. **B** Pressure-flow relationship with large early and enormous mid- and late-systolic dynamic gradients in hypertrophic cardiomyopathy. From top downward: aortic velocity signal, and deep left ventricular (LV), left ventricular outflow tract (LVOT), and aortic root (AO) micromanometric signals, measured by retrograde triple-tip pressure plus velocity multisensor left-heart catheter. Left atrial (LA) micromanometric signal was measured simultaneously by trans-septal catheter. The vertical straight line identifies the onset of SAM-septal contact, determined from a simultaneous M-mode mitral valve echocardiogram (not shown); the majority of aortic ejection flow is already completed by this time. The huge mid- and late- systolic gradient (hatched area) is maintained in the face of minuscule remaining forward or even negative aortic velocities. AO, aortic; AOP, aortic root pressures; LA, left atrial; LV, left ventricular; LVOT, left ventricular outflow tract; LVP, left ventricular pressure; SAM, systolic anterior motion of the mitral valve. Adapted from Pasipoularides [1], with permission of PMPH-USA.

Fig 7.

In essence, the DNA editing technique CRISPR/Cas9 works like a biological version of a word-processing program's find-and-replace function. A cell with a defective chromosomal DNA portion is transfected with an enzyme complex containing: a guide-RNA molecule, a DNA-cutting enzyme, and a healthy DNA strand. The specially designed synthetic guide-RNA molecule finds the defective target-DNA strand and a DNA-cutting enzyme (nuclease) then cuts it off, so that it can be subsequently *replaced* with the healthy DNA strand.

Fig 8.

The cholesterol biosynthesis pathway. Cholesterol biosynthesis is a complex process involving more than 30 enzymes. A simplified version is shown here, highlighting the step of mevalonate synthesis, which is inhibited by statins so as to reduce intracellular cholesterol levels; through several interposed steps, these lead to upregulated expression of the *LDLR* gene. Enhanced *LDLR* expression increases receptor-mediated endocytosis of LDL and thus lowers serum LDL; the liver produces/contains the most LDL receptors and therefore it removes most LDL from blood. Notable is the adverse side-effect of the attendant impaired synthesis and lowered body's levels of coenzyme Q10, which is important in cellular energy generation processes and as a powerful antioxidant, reducing free radicals that can damage cells and DNA. Taking CoQ10 supplements can help increase CoQ10 levels and may reduce statin side effects.

Table 1

Genotyping in Pharmacogenomics. Implementation of Genetic Data for a Better Prediction of Response to Medications and Adverse Drug Reactions

<p>Individual Differences: Phenotypic differences between subjects can affect drug absorption, metabolism, or activity. Pharmacogenomics testing assesses how patients may respond to prescribed drugs based on their individual genetic makeup and is performed by massively parallel Next Generation Sequencing (NGS) [1,2,9,26].</p>	<p>Metabolizer Phenotypes: Individuals with variants associated with elevated drug metabolism (ultra-fast metabolizers) can benefit from higher doses in order to achieve therapeutic effects. Other variants can bring about poor drug metabolism and are associated with higher risk for toxicity.</p> <p>Drug Absorption: Biological membrane transporters are crucial determinants of drug absorption. Genetic variants in the genes encoding these transporters has been linked to major differences in the pharmacokinetics of drug absorption.</p> <p>Drug Activity: Drug activity can also be affected by genetic variability associated with the biological drug targets.</p>	<p>Genes Assayed in Pharmacogenomics*</p> <table><tr><td><i>ABCB1</i></td><td><i>ACE</i></td><td><i>ADRA2A</i></td><td><i>AGTR1</i></td><td><i>ANKK1</i></td></tr><tr><td><i>APOE</i></td><td><i>ATM</i></td><td><i>CDA</i></td><td><i>CES1</i></td><td><i>CNR1</i></td></tr><tr><td><i>COMT</i></td><td><i>CYP1A2</i></td><td><i>CYP2B6</i></td><td><i>CYP2C19</i></td><td><i>CYP2C8</i></td></tr><tr><td><i>CYP2C9</i></td><td><i>CYP2D6</i></td><td><i>CYP3A4</i></td><td><i>CYP3A5</i></td><td><i>CYP4F2</i></td></tr><tr><td><i>DPYD</i></td><td><i>DRD1</i></td><td><i>DRD2</i></td><td><i>ERCC1</i></td><td><i>F2</i></td></tr><tr><td><i>F5</i></td><td><i>FAAH</i></td><td><i>G6PD</i></td><td><i>GRIK4</i></td><td><i>GSTP1</i></td></tr><tr><td><i>HLA-B</i></td><td><i>HTR1A</i></td><td><i>HTR2A</i></td><td><i>HTR2C</i></td><td><i>IFNL3</i></td></tr><tr><td><i>ITPA</i></td><td><i>KIF6</i></td><td><i>MTHFR</i></td><td><i>NAT2</i></td><td><i>NOS1AP</i></td></tr><tr><td><i>NQO1</i></td><td><i>OPRM1</i></td><td><i>SCN2A</i></td><td><i>SLC6A4</i></td><td><i>SLCO1B1</i></td></tr><tr><td><i>TPMT</i></td><td><i>UGT1A1</i></td><td><i>UGT2B15</i></td><td><i>VKORC1</i></td><td><i>XRCC1</i></td></tr></table> <p>* As examples [26], consider the <i>CYP2C19</i> genotype, which impacts pharmacodynamics and platelet response to clopidogrel, and the <i>CYP2C9</i> and <i>VKORC1</i> genotypes whose polymorphisms influence the pharmacodynamic response to oral coumarin anticoagulants.</p>	<i>ABCB1</i>	<i>ACE</i>	<i>ADRA2A</i>	<i>AGTR1</i>	<i>ANKK1</i>	<i>APOE</i>	<i>ATM</i>	<i>CDA</i>	<i>CES1</i>	<i>CNR1</i>	<i>COMT</i>	<i>CYP1A2</i>	<i>CYP2B6</i>	<i>CYP2C19</i>	<i>CYP2C8</i>	<i>CYP2C9</i>	<i>CYP2D6</i>	<i>CYP3A4</i>	<i>CYP3A5</i>	<i>CYP4F2</i>	<i>DPYD</i>	<i>DRD1</i>	<i>DRD2</i>	<i>ERCC1</i>	<i>F2</i>	<i>F5</i>	<i>FAAH</i>	<i>G6PD</i>	<i>GRIK4</i>	<i>GSTP1</i>	<i>HLA-B</i>	<i>HTR1A</i>	<i>HTR2A</i>	<i>HTR2C</i>	<i>IFNL3</i>	<i>ITPA</i>	<i>KIF6</i>	<i>MTHFR</i>	<i>NAT2</i>	<i>NOS1AP</i>	<i>NQO1</i>	<i>OPRM1</i>	<i>SCN2A</i>	<i>SLC6A4</i>	<i>SLCO1B1</i>	<i>TPMT</i>	<i>UGT1A1</i>	<i>UGT2B15</i>	<i>VKORC1</i>	<i>XRCC1</i>
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