

STATE-OF-THE-ART REVIEW

Cardiovascular Precision Medicine in the Genomics Era



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SUMMARY

Precision medicine strives to delineate disease using multiple data sources—from genomics to digital health metrics—in order to be more precise and accurate in our diagnoses, definitions, and treatments of disease subtypes. By defining disease at a deeper level, we can treat patients based on an understanding of the molecular underpinnings of their presentations, rather than grouping patients into broad categories with one-size-fits-all treatments. In this review, the authors examine how precision medicine, specifically that surrounding genetic testing and genetic therapeutics, has begun to make strides in both common and rare cardiovascular diseases in the clinic and the laboratory, and how these advances are beginning to enable us to more effectively define risk, diagnose disease, and deliver therapeutics for each individual patient. (J Am Coll Cardiol Basic Trans Science 2018;3:313-26) © 2018 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

In launching the Precision Medicine Initiative in early 2015, President Obama defined the effort as “delivering the right treatments, at the right time, every time to the right person.” This new era of precision medicine strives to redefine diseases using multiple sources of data, including lifestyle, medical history, imaging, genomics, proteomics, metabolomics, sensor data, and more. In being both more precise and more accurate in our diagnosis and in our definitions of subtypes of disease, we can treat patients based on the true underpinnings of their specific presentations, rather than grouping patients into broad categories with one-size-fits-all treatment. The goal of this strategy is not only to give patients more efficient and effective care, but also to reduce patient harm and limit health care costs arising from unnecessary or inappropriate treatment.

In this review, we discuss existing successes of precision medicine in multiple clinical areas and their implications in cardiovascular medicine. We then examine how precision medicine, most specifically that surrounding genetic testing and genetic therapeutics, has begun to make strides in both common and rare cardiovascular diseases (CVDs). In addition, we examine how basic research, including the use of induced pluripotent stem cells (iPSCs), is advancing precision cardiovascular medicine.

LESSONS FROM PRECISION MEDICINE IN ONCOLOGY AND CYSTIC FIBROSIS

Precision medicine has already made significant strides in patient care, bolstering support for its potential in cardiovascular medicine. Precision

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**ABBREVIATIONS
AND ACRONYMS****CAD** = coronary artery disease**CaM** = calmodulin**CF** = cystic fibrosis**CHD** = coronary heart disease**CML** = chronic myelogenous leukemia**CRS** = conventional risk score**CVD** = cardiovascular disease**DCM** = dilated cardiomyopathy**DMD** = Duchenne muscular dystrophy**FH** = familial hypercholesterolemia**GRS** = genomic risk score**HCM** = hypertrophic cardiomyopathy**HDR** = homology directed repair**iPSC** = induced pluripotent stem cells**IVF** = in vitro fertilization**LDL-C** = low-density lipoprotein cholesterol**LQTS** = long QT syndrome**NGS** = next-generation sequencing**PGD** = preimplantation genetic diagnosis**SNP** = single nucleotide polymorphism**ssODN** = single-stranded oligodeoxynucleotide

medicine first emerged as a viable therapeutic path in oncology. Two of the earliest examples of therapeutics targeted to precise genetic mutations appeared in chronic myelogenous leukemia (CML) and *HER2*-positive breast cancers (Figure 1).

Almost all patients with CML have a genetic mutation known as the Philadelphia chromosome, where a translocation of chromosomes 9 and 22 creates a fusion protein known as Bcr-Abl (1). The fusion of these 2 proteins can induce elevated tyrosine kinase activity that promotes dysregulated cell growth and cancer. Because oncogenic Bcr-Abl kinase activity is elevated compared with that of the unaltered Abl protein, a specific inhibitor of the fusion protein proved to be 1 of the first precision therapeutics not only in oncology but in all of medicine (1). This inhibitor, known as imatinib, received Food and Drug Administration approval in 2001 and engendered early enthusiasm for precision medicine, especially after it showed additional efficacy in another difficult-to-treat cancer, gastrointestinal stromal carcinoma (2).

A second early precision medicine advance arose in the fight against breast cancer. After the discovery that 20% to 25% of breast cancers show an overexpression of *HER2*, the human epidermal growth factor receptor, Genentech (South San Francisco, California) developed an antibody-based strategy to target and bind the extracellular portion of

the protein (3). Called trastuzumab, the drug launched in 1998 and became a leading option for patients with *HER2*-positive breast cancer. Coupled with a diagnostic kit to determine *HER2* status (4), trastuzumab became one of the first of many precision medicine treatments that focused on genetic diagnosis followed by a gene-specific medication.

Another clear example of precision medicine success is in cystic fibrosis (CF), an autosomal recessive disorder caused by mutations in *CFTR* (the CF transmembrane regulator conductance gene) (5). Pathogenic mutations in this gene cause abnormally viscous respiratory and gastrointestinal secretions, leading to bronchiectasis, multiple drug-resistant pneumonias, pancreatitis and pancreatic insufficiency, as well as malabsorption, among other complications. Before modern medical intervention, most patients with CF died in early childhood.

Although there are >100 disease-associated *CFTR* mutations, they can be grouped into classes based on

their effects on the protein (5). Molecular therapeutics can now be used to target the defects representative of 2 of these classes: *CFTR* regulation (Class III) and *CFTR* processing (Class II).

Ivacaftor, a drug that targeted a specific class III mutation, showed benefits in preclinical and clinical trials due to careful patient selection. Participants were selected based on the presence of the target mutation and showed dramatic improvements in CF symptoms, including a reversal of chronic sinusitis and improvement in lung function as measured by nasal potential difference, as well as unforeseen benefits in overall health, such as positive weight gain (5). However, had clinical trial drugs been administered to all CF patients, overall benefits would likely have been low, and the clinical trial might have failed, despite positive benefits in the true target participants (5).

This lesson of appropriate participant and patient selection in precision medicine trials is a lesson that should be carried into cardiovascular precision medicine. A recent report suggested that many failed chronic heart failure trials might have instead been successes if patients were more carefully chosen based on precise biomarkers linked to action of each therapeutic (6). Defining disease at a molecular level to treat it more precisely would improve not only clinical trial outcomes but also patient care and will become increasingly possible as we learn more about genetic associations with disease through continued adoption of and research studying clinical genetic sequencing. Increased investigation of sequencing data will allow us to better link the genotype of a patient, that is, the collection of genetic variants they possess that influence their condition, with their disease phenotype, its observable characteristics, and its presentation.

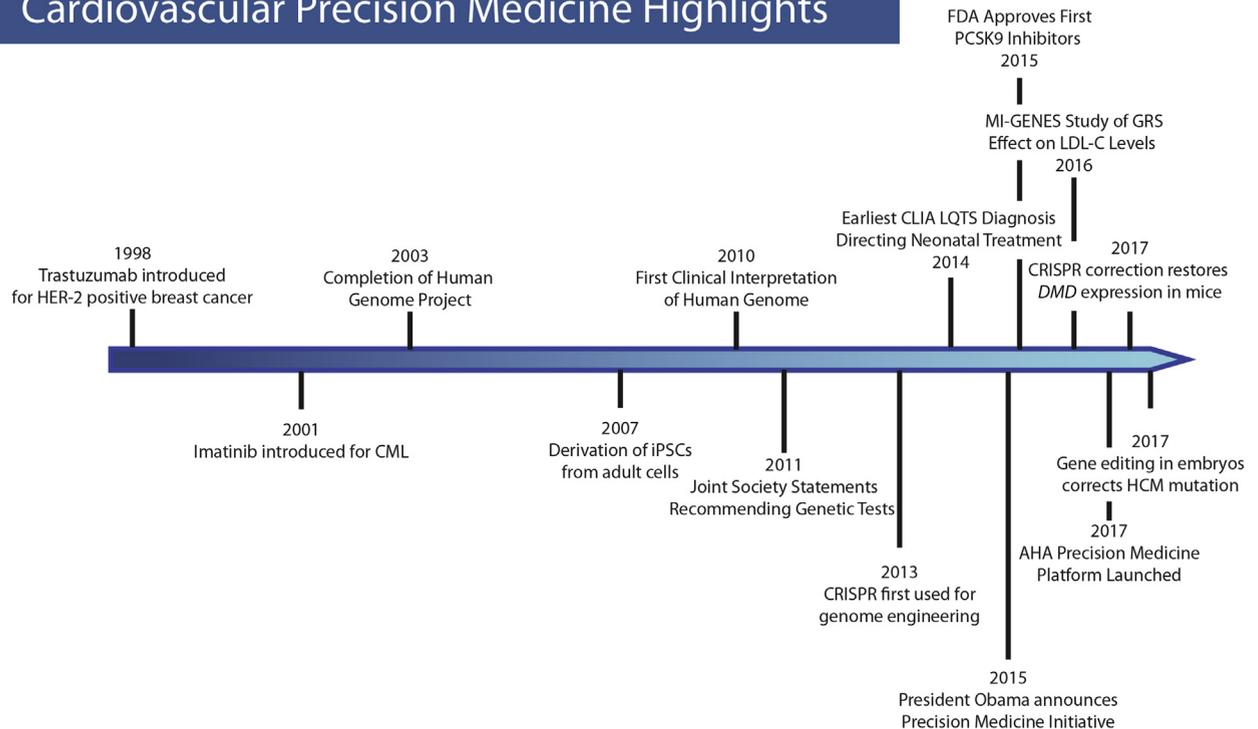
CLINICAL APPLICABILITY OF GENETIC TESTING IN CVD AND PRECISION MEDICINE

The cost of genetic testing has fallen dramatically over the past decade due to major advances in sequencing technology, in particular, the advent of next-generation sequencing (NGS). As the cost of NGS continues to fall and more potential disease-associated and disease-causing variants are identified, clinical genetic testing is becoming more common and more informative. Although genetic therapy and testing are not the only routes toward precision medicine, their increasing presence and usefulness have positioned them at the forefront of many discussions surrounding precision medicine, including this review.

In 2010, we introduced an approach to the evaluation of a personal genome in a clinical context (7). A patient with a family history of coronary artery

FIGURE 1 Cardiovascular Precision Medicine Timeline

Cardiovascular Precision Medicine Highlights



This timeline highlights key events in the history of precision medicine, such as the introduction of the first precision medicine drugs and the announcement of the Precision Medicine Initiative, as well as specific case studies cited in this review. Notable in this presentation is the explosion of activity in the past 5 years as advances in genome sequencing and genome editing technologies have rapidly increased the speed of basic research and clinical application of precision medicine technologies in cardiovascular medicine. AHA = American Heart Association; CLIA = Clinical Laboratory Improvement Amendments; CML = chronic myelogenous leukemia; FDA = Food and Drug Administration; HCM = hypertrophic cardiomyopathy; iPSCs = induced pluripotent stem cells; LDL-C = low-density lipoprotein cholesterol; LQTS = long QT syndrome.

disease (CAD) and sudden death was evaluated by a cardiac clinical team in conjunction with whole genome sequencing and interpretation. The genomic analysis revealed an increased genetic risk for myocardial infarction and type 2 diabetes. In addition, a pharmacogenomics analysis was performed to assess how the genetics of the patient might influence response to certain drugs, including lipid-lowering therapies and warfarin (7). This clinical assessment, which focused heavily on cardiovascular risk, suggested that whole genome sequencing might provide clinically relevant information for patients.

A 2011 joint statement from the Heart Rhythm Society and the European Heart Rhythm association recommended genetic testing as a class I indication for patients with a number of channelopathies and cardiomyopathies, including long QT syndrome (LQTS), arrhythmogenic right ventricular

cardiomyopathy, familial dilated cardiomyopathy (DCM), and hypertrophic cardiomyopathy (HCM) (8). Similarly, a statement from the American Heart Association and the American College of Cardiology recommended genetic testing for HCM, DCM, and thoracic aortic aneurysms to facilitate familial cascade screening and deduce causative mutations (9,10).

The diagnostic power of genetic testing is significant across the spectrum of CVDs, ranging from cardiomyopathies to life-threatening arrhythmias (10-12). In the clinic, genetic testing can:

1. clarify disease diagnoses: genetic testing can help to clarify the diagnosis of diseases that cause similar clinical presentation (e.g., cardiac hypertrophy could be TTR amyloidosis, Fabry disease, or sarcomeric HCM);

2. facilitate cascade screening: genetic testing can help to identify relatives at risk for CVD before disease symptoms manifest if a disease-associated variant is found in a proband and then screened for in relatives;
3. direct more precise therapy: genetic testing can help physicians choose appropriate treatments and plan appropriate timing of those treatments. For example, inherited connective tissue disease due to variants in *ACTA2*, *MYH11*, or *TGFBR2* might prompt consideration of surgical intervention at a smaller aortic aneurysm diameter (13); and
4. identify patients for targeted therapies: targeted medical therapies, including antibody-based therapeutics, gene editing, and silencing technologies, are available or under development for several genetic diseases, including LQTS, Duchenne muscular dystrophy (DMD), TTR cardiac amyloidosis (14), and Fabry disease (13,15).

Cascade screening can be a powerful application of genetics in the cardiology clinic. In cascade screening, a proband is first identified to have a variant associated with disease. From there, each first-degree relative, including parents, children, and siblings, are also screened for the variant. When the variant is found in a relative, they become the proband for another round of first-degree relative screening. Although the cascade screening discussed here refers to genetic screening, the same technique is used to screen for other indicators of disease, such as a combination of genetic and cholesterol screening used in familial hypercholesterolemia (FH) (16).

Cascade genetic screening is particularly useful in scenarios when disease symptoms may develop slowly over time and may not yet present in the healthy but genetically affected family member. This can allow for enhanced clinical follow-up for these family members and peace of mind for those who do not carry the mutation (13). An analysis of the UK National Health Service FH cascade screening services showed the screening to be highly cost-effective (17), something that was also demonstrated for HCM (18,19) and LQTS (20). Genetic testing may also be useful across a range of inherited CVDs as a post-mortem “molecular autopsy” in cases of sudden cardiac death, where it can be used to identify additional at-risk family members (21,22).

As the use of genetic testing in cardiovascular medicine becomes more accessible in the community, it is important to consider its usefulness in a broad range of disease states. We will next discuss 3 case studies of both rare and common CVDs in which

genetic testing is currently of clinical benefit: LQTS, CAD, and FH.

SPOTLIGHT ON PRECISION MEDICINE IN A RARE CVD: LQTS

A clear benefit of precision medicine is the ability to better diagnose and treat rare disease, especially in cases in which we have therapeutics with specific molecular targets. LQTS is one such instance in which designation of a genetic mutation can inform and direct clinical care, as well as help assess the risk of sudden cardiac death (23). Approximately 70% of genotype-positive LQTS cases are accounted for by variants in sodium and potassium channels, most notably those encoded by 3 main genes: *KCNQ1*, *KCNH2*, and *SCN5A* (23). These genotypes designate subtypes LQT1, LQT2, and LQT3, respectively. These genotyping designations can suggest the most appropriate and effective medical interventions. For example, mexiletine, a voltage-gated sodium channel blocker, has been shown to reduce arrhythmic events in LQT3, whereas β -blockers may actually be proarrhythmic (24). Alternatively, β -blockers can reduce the risk of cardiac events in both LQT1 and LQT2, and genotype can help suggest the most effective β -blockers for each (25).

Advances in the speed of clinical genome sequencing can lead to more rapid diagnosis and improved case management in neonatal CVD, including LQTS. Our group conducted Clinical Laboratory Improvement Amendments-certified whole genome sequencing on a newborn infant who presented with 2:1 atrioventricular block and ventricular arrhythmias. This patient was one of the youngest to receive an implantable cardioverter-defibrillator due to the aggressive treatment needed to manage the arrhythmias. Our rapid whole genome sequencing detected a variant in *KCNH2* previously associated with LQTS and a previously unknown variant in *RNF207* (26). The discovery of these variants allowed molecular confirmation of disease within 10 days of birth, before discharge from the hospital, and allowed tailoring of pharmacotherapy to the affected ion channel. Normally, this kind of genetic sequencing and screening has a 4- to 8-week turnaround. In this case, precision medicine in the form of rapid, neonatal sequencing allowed for early diagnosis and precision treatment of disease in a particularly vulnerable patient.

Another potential avenue in precisely targeting LQTS therapy lies in reduced expression of mutant calmodulin (CaM) RNA. In instances of calmodulinopathies, including LQTS, in which a dominant negative mutation occurs in 1 of 3 genes encoding for

identical CaM proteins (*CALM1*, *CALM2*, or *CALM3*), silencing or reducing expression of the gene could relieve the disease phenotype (27). In almost all cases in which CaM mutations cause disease, they do so by disrupting proper binding of calcium to the protein (28). This can lead to LQTS by interfering with calcium channel inactivation in the cell, which predisposes the cell to arrhythmogenicity and promotes action potential prolongation (28-30). Limpitikul et al. (31) used a CRISPR interference (or CRISPRi) system to knock down *CALM2* expression by binding to the mutant gene and physically blocking RNA transcription in cardiomyocytes derived from patient iPSCs. These iPSC cardiomyocytes serve as an advantageous system for testing molecular and genetic therapies of CVD, as discussed later in this review. In this study, partial suppression of both the healthy and mutant *CALM2* alleles allowed for relief of the mutant allele-influenced disease phenotype as assessed by the shortening action potential duration and accelerating intracellular calcium inactivation in these iPSC cardiomyocytes (27). With similar therapeutic suppression systems for *CALM1* and *CALM3*, CRISPR interference could become a viable precision medicine strategy in calmodulinopathies. Patients could easily be sequenced to determine which *CALM* gene requires suppression and then be treated with the appropriate therapy.

SPOTLIGHT ON PRECISION MEDICINE IN A COMMON CVD: CAD AND CARDIOVASCULAR RISK SCORES

Although the earliest precision medicine strategies were developed for rare diseases, attention to common genetic variation with small but additive phenotypic effects revealed an additional and potentially broadly applicable arm of precision medicine. Assessing the effect of genetic variants in Mendelian diseases, in which mutations in a single gene control disease and a single, rare, disease-causing variant can often be traced through families, is relatively straightforward compared with complex diseases. In complex diseases, in which a constellation of many common variants spread across many genomic loci each have a small, additive effect on disease, it can be far harder to tease apart the effect of each individual variant. However, now, with the release of population-level genomic datasets like the UK Biobank and analyses that combine information from >6 million genetic variants (32), we may finally be poised to assess the impact of each variant and develop and implement genetic risk scores for more widespread conditions like CAD.

CAD can lead to significant symptomatic burden, heart failure, arrhythmias, and sudden death. For years, familial and lifestyle risk factors for CAD, including age, smoking, obesity, activity levels, and more have been identified and used to predict the risk of a patient for developing CAD (33). Yet the ability to predict risk based on genetic variation, and the potential effect of that knowledge on patient behavior and outcome, was not available until recently.

The MI-GENES (Myocardial Infarction Genes) Study researched whether informing patients of their genomic risk score (GRS) in addition to a conventional risk score (CRS) would affect low-density lipoprotein cholesterol (LDL-C) levels or lifestyle behaviors, including initiation of statin therapy (34). The GRS was calculated from 11 single nucleotide polymorphisms (SNPs) reported to be associated with CAD in previous genome-wide association studies (35). The study randomized participants without CAD to 1 of 2 groups. The first received a 10-year estimation of risk of CAD calculated from a CRS alone, whereas the second group received a CRS plus a CAD-specific GRS. The GRS group received risk information from a genetic counselor and also met with a physician for shared decision-making regarding statin therapy (34).

At the end of the study period, the group that had received GRS information had a significantly lowered LDL-C level than the plain CRS group. They were also more likely to be using statins at this final checkup, although this effect was likely due to increased prescription recommendation from the doctors (39.2% vs. 21.9%; $p < 0.01$). The study found no effect of GRS on lifestyle behaviors (e.g., dietary fat intake or physical activity levels), but it also found no increase in patient anxiety (34,36). The research group reported in separate studies that patients who received the GRS had slightly higher perceived personal control and genetic counseling satisfaction (37) and that patients who received a GRS were more likely at 6 months post-disclosure to have sought out additional information about coronary heart disease (CHD) and genetic risk factors of CHD than patients who received a CRS (38). Although the study was limited by lack of physician blinding and included genetic information from only 11 SNPs, it provided an important example of a GRS affecting clinical measures, underscoring the potential of such information to change practitioner prescribing behavior.

Although the MI-GENES study could not differentiate between effect on the patient versus effect on the health care provider, a more recent study by Knowles et al. (39) looked at the usefulness of GRS as a motivational tool for reducing CAD risk factors. Patients who received standard-of-care plus GRS

showed no significant changes, including medication use, blood pressure, physical activity, weight, and high-density lipoprotein concentration compared with standard-of-care patients (39). Although modest beneficial effects were seen in physical activity and weight loss in a subgroup of patients with a high GRS, and the study showed no negative psychological effects of adding GRS to standard-of-care treatment, the investigators noted that a larger study is needed to refute or confirm their findings (39).

In the MI-GENES study, a GRS was used to integrate multiple genetic variants to give a risk of disease rather than focusing on a single variant as a target for a therapy or drug. Additional studies investigated whether this kind of multivariant GRS could predict the clinical response of a patient to statin treatment. A 2015 study published in *The Lancet* found that patients who fell into the highest quintile of genetic risk, assessed from 27 SNPs, derived the greatest benefit from statin therapy (40). Another more recent study that analyzed at-risk scores derived from up to 57 SNPs also found similar results (41).

Although the effect of each individual variant on complex disease risk may be small, the influence that a pool of disease-associated genetic variants exerts on complex disease risk has the potential to be as significant as traditional lifestyle risk factors. A recent study examined data from thousands of individuals to investigate the association of both genetic and lifestyle risk factors with CAD (42). Individual patient genetic risk scores were determined by analysis of 50 genetic variants previously associated with CAD (42). The researchers found that those with a high GRS had a 91% higher relative risk of incident coronary events than those with a low GRS (42).

To assess the association of lifestyle behaviors and risk of coronary events, the investigators also scored each individual for adherence to 4 healthy lifestyle behaviors described by the American Heart Association: no smoking, no obesity, weekly physical activity, and a healthy diet. On the basis of the number of behaviors exhibited, participants were classified as having a favorable, intermediate, or unfavorable lifestyle (42). The study found that within each of the GRS categories, having a favorable lifestyle was associated with a 45% to 47% decrease in risk for coronary events compared with an unfavorable lifestyle (42). However, the finding that a high genetic risk correlated with a 91% increase in coronary events independent of lifestyle behaviors indicated the strong effects that heritable genetic factors could have on cardiovascular health, even in common complex diseases.

FH: HOW OUR UNDERSTANDING OF RARE VARIANTS CAN IMPROVE TREATMENT PRECISION IN COMMON DISEASE

FH affects approximately 1 in 250 people in the population and is characterized by elevated LDL-C levels. Left untreated, this disease can lead to atherosclerosis and premature CVD (43). Because of its high prevalence and risk of severe complications, it is the only CVD recommended for universal population-based screening by the World Health Organization (43). Although the current recommendation endorses lipid screening, genetic testing is encouraged for family-based cascade screening. Genetic testing is also useful to separate heterozygous and homozygous cases, as well as to uncover potential precision medicine targets (43).

FH can be caused by mutations in genes, including *LDLR*, *PCSK9*, and *APOB*. *PCSK9* provides a sentinel example of disease understanding that led to therapeutic development. The *PCSK9* protein typically binds to the LDL receptor, resulting in its breakdown. However, gain-of-function mutations in *PCSK9* can enhance its affinity for the LDL receptor, causing an increase in its breakdown. This reduces the amount of the available LDL receptor, and more LDL builds up in the blood, leading to atherosclerosis and premature heart disease (44). Research investigating loss-of-function mutations in *PCSK9* revealed that nonsense mutations were frequent in some populations and were correlated with lower LDL levels (45,46). This lifelong decrease in LDL levels due to decreased function of *PCSK9* reduced the risk of CHD by up to 88%, a greater benefit than that conferred by short-term statin treatment (45,47,48).

This finding that decreased *PCSK9* levels could not only have direct effects on LDL levels but also broader effects on CHD risk suggested that reducing levels of *PCSK9* activity might provide therapeutic benefits. This reduction could be induced in patients using *PCSK9* therapeutic inhibitors, monoclonal antibody-based drugs that target *PCSK9* and attempt to reduce its activity. These antibody inhibitors bind to *PCSK9* with high affinity and disrupt its ability to bind to the LDL receptor. This leaves more of the receptor available on the cell surface, which is then able to pull more LDL out of the blood, thus lowering overall levels and reducing the risk of CVD. In a review of long-term studies, *PCSK9* inhibitors versus placebo showed a >50% decrease in LDL-C levels at 24 weeks and 30% to 40% decrease compared with current medical treatments, together with a decreased risk of CVD, although they showed little to no effect on all-cause mortality (49).

PCSK9 inhibition at the level of RNA rather than protein has also shown promise in clinical trials. Inclisiran, a long-acting siRNA that targets *PCSK9* transcripts for degradation by the intrinsic RNAi pathway, reduced LDL-C levels in clinical trials after both single and multiple dose treatments, with effects that persisted for up to at least 6 months (50). The reductions in LDL levels were comparable to those seen in anti-*PCSK9* antibody treatments, with the potential for fewer, more long-lasting treatment doses. Although the study had limitations, including being only single-blinded and containing mainly healthy patients, it provided encouraging and powerful preliminary results that RNA silencing therapeutics might be a viable precision medicine option for lowering LDL-C.

Although there remain barriers to access for these treatments, most notably high cost and low approval from insurance companies (51), *PCSK9* inhibitors are an example of cardiovascular medicine driven by genomics, with research suggesting a potential therapeutic target that could lead to effective, precise treatment.

These 3 examples highlight the current state of precision medicine in both rare and common CVDs (Central Illustration). In the following, we examine some cutting-edge technologies that may herald the next wave of precision medicine, including correcting disease-associated genetic mutations rather than targeting their products.

TACKLING CVD BY CORRECTING DISEASE-ASSOCIATED MUTATIONS: CRISPR AND THE FUTURE OF GENETIC PRECISION MEDICINE

As a precision medicine guide, the genome can provide targets for precise therapeutics and metrics of disease risk. Yet precision medicine could also include changing the genome itself using new editing technologies like CRISPR systems. Recent work has looked at how this type of editing could be beneficial in DMD, in which disease-driven cardiomyopathy commonly results in death.

Adapted from bacterial immune defenses, CRISPR systems target and cut DNA with the aid of small RNA guides. Scientists took advantage of this biological system to create molecular “scissors” with the potential to cut, edit, and correct disease-causing mutations in the genome. DMD, an X-linked recessive disease caused by mutations in the gene coding for dystrophin, is a prime target for this sort of specific editing and correction. Mutations in dystrophin, a critical structural protein that connects the cytoskeleton to the

CENTRAL ILLUSTRATION Cardiovascular Precision Medicine Integrates Basic Science Techniques With Genomic Information

Clinical Genome Sequencing

- Identify disease-causing mutations in patients and family members (e.g. cascade screening in FH)
- Direct disease treatment (e.g. LQTS subtype-informed drug selection)
- Clarify disease diagnoses

Targeted Therapeutics

- Tackle molecular underpinnings of specific disease subtypes (e.g. *PCSK9* inhibition via antibodies in FH)
- Sequencing can aid placement of patients into appropriate clinical trials

Genetic Risk Scores

- Risk of complex disease calculated from influence of many variants (e.g. in coronary artery disease, risk score discussion can affect statin usage)
- Association of genetic risk with disease outcomes can be as strong as lifestyle risk

Induced Pluripotent Stem Cells

- Model disease and test new therapies in vitro (e.g. testing calmodulin knockdown in LQTS)
- Potential source of autologous cells for transplantation (e.g. iPSC-erythroblasts to treat beta thalassemia)

CRISPR Genome Editing

- Potential to stop disease before it starts (e.g. editing in HCM embryos)
- Target disease at the DNA level (e.g. editing of *DMD* in mice can alleviate disease symptoms)

State-of-the-art genetic technologies are revolutionizing cardiovascular precision medicine.

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As clinical genome sequencing becomes more common and basic science techniques like CRISPR and induced pluripotent stem cells begin to transition toward clinical applicability, the world of cardiovascular medicine moves toward one where we can be more precise and accurate with our diagnoses and treatment. Using knowledge gained from population sequencing projects, such as genome-wide association studies, and disease models in the laboratory, we can begin to develop deeper understandings of disease at a molecular level to predict the risk of a patient for developing a disease and present them with the right treatment when they do—or the right preventative strategy in advance. This review focuses on how precision medicine, genomics, and new genetic tools intertwine to create a new era of cardiovascular precision medicine. FH = familial hypercholesterolemia; HC = hypertrophic cardiomyopathy; iPSC = induced pluripotent stem cells; LQTS = long QT syndrome.

extracellular matrix, lead to progressive muscular weakness and wasting. Fatality from DMD is caused by progressive muscle weakness, so a sustained, genome-level change that would permanently correct dystrophin expression and function could lead to a lasting change in the lives of DMD patients and potentially avoid lethal cardiac phenotypes.

Deletions of exons before exon 51 in the *DMD* gene disrupt the proper DMD reading frame, introduce a premature stop codon, and represent a significant proportion of DMD mutations. Correcting these reading frames by skipping exon 51 could theoretically restore the proper open reading frame and recover some level of dystrophin function (52). A group led by Dr. Eric Olson used a recently described CRISPR system, Cpf1 (CRISPR from *Prevotella* and *Francisella* 1) to either create “reframing” indels in exon 51 or disrupt exon splice sites and skip exon 51 entirely.

In both mouse models of DMD and iPSCs from a DMD patient, Cpf1 editing restored dystrophin expression and rescued phenotypes of disease, including metabolic abnormalities in iPSC-derived cardiomyocytes and rescue of fibrosis and inflammatory infiltration in Cpf1-treated mouse hearts (52). This provides a promising outlook for the future of CRISPR editing in cardiovascular precision medicine to change disease phenotype by editing the underlying genome.

However, many challenges remain before cardiovascular genome editing can become a viable therapeutic option. Most prominent in recent discussions surrounding human genome editing have been off-target effects, the potential for the CRISPR system to incorrectly cut nontarget positions in the genome, creating small insertions and deletions that could alter gene function in unanticipated ways.

Another potential roadblock to therapeutic CRISPR systems is delivery. In the DMD study (52), mice were injected with the Cpf1 system as zygotes. This allows for editing while the organism is still a single or only a few cells. This should allow a correct edit to propagate through the many rounds of cell division that go on to create an entire organism. Although this strategy is effective in the laboratory, we cannot plan to treat all patients at the moment of conception. Rather, therapeutic genome editing strategies will have to find efficient delivery strategies to fully developed organs. Current studies are testing myriad delivery methods for genetic therapeutics, including in vivo electroporation (53), viral delivery (54), direct injection to the heart (55), polymer-based gene delivery (56), and so on. As these delivery methods become more efficient and reliable, the promise of safe, targeted, therapeutic gene editing will become closer to being a reality.

Finally, the efficiency of editing is a concern in genome editing therapeutics. In the DMD study, 12 of the 24 CRISPR-treated mouse pups showed signs of editing, and only 5 carried appropriately corrected DMD alleles. Of those 5, they showed varying levels of

correction in tissue, from 8% to 50% (52). These varying levels at all stages of the study illustrated the various steps at which efficiency might stand between CRISPR and an effective therapeutic. First, all parts of the CRISPR system must effectively reach the target cells, as discussed in the previously described delivery concerns. Once there, it must then reliably perform editing in the cells. Once the genome has been cut, there is a percentage of the time when the cell corrects the cut back to the unedited, mutant state, rather than creating an insertion or deletion. This means that even if a cut occurs, it may not create the intended frameshift, knockout, insertion, or deletion. Finally, even when introduction of the CRISPR system happens at an early stage, such as with the mouse zygotes in this study, the correction may only happen in a subset of cells, leading to chimeric expression through the adult organism once cell division proceeds.

Although genome editing in an entire organism involves many concerns around delivery and efficiency, therapeutic editing strategies may not need to happen at the level of the entire organism to enact positive changes in disease. A study by Ding et al. (57) used CRISPR-Cas9 packaged in adenoviruses to target and mutagenize *PCSK9* in mouse livers as a potential therapy for FH, much like the previously discussed inhibitors and siRNA treatments. They found that by targeting the first exon of *PCSK9* in 5-week-old mice using a virally delivered CRISPR construct, they could achieve approximately 50% mutagenesis of *PCSK9* in the liver, which could lead to substantially lowered plasma levels of *PCSK9* and decreased plasma cholesterol levels (57). This study provided evidence that genome editing might still be a beneficial treatment in some diseases in which it is not necessary to alter every copy of the gene and in which gene alterations in only specific organs could still have dramatic effects on disease phenotypes. This type of treatment would also provide a potential benefit to patients who already have established disease, rather than requiring neonatal editing before the disease has developed.

Recent efforts have tried to circumvent delivery and efficiency issues by editing the human genome at the moment of fertilization. A paper published in August 2017 described CRISPR editing of an HCM-associated *MYBPC3* (cardiac myosin binding protein C) allele by co-injecting a CRISPR construct with sperm from a patient with a heterozygous *MYBPC3* deletion into healthy donor eggs (58). The proposed genome editing would be a potential method of increasing healthy embryos for in vitro fertilization (IVF) in conjunction with

pre-implantation genetic diagnosis (PGD). Because the patient carried a heterozygous mutation, without intervention, 50% of the embryos created from fertilization of the donor eggs with the patient sperm should contain 2 wild-type or healthy alleles, whereas 50% should be heterozygous for the disease allele. To increase the percentage of embryos with 2 healthy alleles, the group designed a CRISPR-Cas9 construct, complete with the Cas9 nuclease protein and single-guide RNA targeting a disease-causing deletion in *MYBPC3* that would cut the mutant allele near the site of the deleterious deletion. They also introduced wild-type exogenous single-stranded oligodeoxynucleotide (ssODN) templates to encourage homology-directed repair (HDR) at this site, in which the cut allele would repair itself using this wild-type template.

When they injected their CRISPR construct, ssODNs, and sperm at the same time, they found that the percentage of homozygous wild-type embryos increased to 72.4%, a marked increase over the expected 50%. In addition, they investigated the presence of off-target cutting in their embryos, examining 23 likely off-target cutting sites that they further investigated by sequencing in a number of their edited embryos. The group found no evidence of off-target cutting by their CRISPR construct at their likely off-target locations in the genome. They performed whole genome sequencing in selected embryos, and concluded that there were no off-target effects of their CRISPR construct (58). However, they did not perform whole genome sequencing on each edited embryo, which might have revealed rare off-target events.

Unexpectedly, the group found that the HDR often used the maternal wild-type allele as a template for correcting the cut mutant allele rather than their co-injected ssODN. This implied that genomic repair in these early-stage embryos used different mechanisms from HDR seen in other cell types, perhaps due to the evolutionary importance of genome integrity at these early stages (58). Although their work marks a significant advance toward human genome editing, this finding contradicts the previously imagined path toward human genome editing in embryos. It may be that correction of deleterious mutations using the healthy allele of the alternate parent may be possible while introduction of a desired sequence by ssODN may be quite difficult. This poses a technical roadblock to the oft-discussed “designer baby” future in which traits are inserted into embryos and may also pose limitations on correction in scenarios in which both parents harbor a heterozygous disease allele.

Interest in these findings prompted global discussions and the proposal of alternate explanations for the observed allele ratios. In a response, Egli et al. (59) remarked that these included an inability to detect large genetic deletions that might have been caused by the CRISPR-Cas9 cutting using the methods of the original study (59). They also noted that additional fertilization abnormalities might have resulted in an appearance of 2 wild-type alleles without appropriate CRISPR-Cas9 cutting and repair, including parthenogenesis, when 2 copies of the maternal genome would appear due to failure to extrude a polar body. These 2 maternal copies could appear to be a wild-type maternal and a “corrected” paternal copy in the original assay. More strikingly, they noted the physical separation of the maternal and paternal genomes during the developmental stage when the CRISPR cutting and repair were assumed to be happening, calling into question the availability of the maternal allele as a repair template for the paternal allele (59). These concerns make a strong case for further investigation into the mechanisms of DNA repair at these early developmental timepoints.

In addition, this technique would only work in conjunction with IVF and PGD, which are already established methods of screening for embryos without deleterious mutations, without the associated risks of off-target gene editing. IVF and PGD alone have been used with great success for selection of embryos without a genetic predisposition to some CVDs, including Marfan syndrome, myotonic dystrophy, and DiGeorge syndrome, in at-risk families (60-62). In these cases, autosomal dominant or X-linked mutations can be identified before IVF. Because the previously described CRISPR editing would still require PGD and would create only a projected approximate 20% increase in the number of available nonaffected embryos for IVF, it is worth questioning whether the potential deleterious effects of off-target CRISPR mutations are outweighed by the increase in embryo numbers. Other discussions surrounding the paper have raised the alternate strategy of intervening and editing germline stem cells before sperm are formed, reducing risk to embryos and circumventing ethical implications surrounding embryo editing (63). Although there are still technical hurdles in such alternate paths, and ethical considerations surrounding what kinds of edits we as a society will decide are appropriate or inappropriate to make, it highlights the fact that there is still much discussion to be had and scientific exploration to be done surrounding this topic before moving forward.

However, it is notable that one of the first demonstrations of genome editing in embryos occurred in HCM. The research group argued that their first trial focused on *MYBPC3* because approximately 40% of identified genetic variants that cause HCM appear in *MYBPC3* and current treatment options mostly focus on symptom management rather than treating the genetic cause of the disease. The proposed genome editing could prevent transmission of a disease-associated allele to the next generation but is still far from being ready to approach clinical trials. In addition, such a strategy would only have potential to be effective in situations when there is a single disease-associated allele definitively linked to disease in only 1 parent. Such strategies would have less potential in disease in which multiple variants all have small, additive effects in disease, such as CAD, as described previously.

Although there are many roadblocks to therapeutic genome editing described here, the science surrounding CRISPR systems is progressing rapidly in academic and industrial settings, much of which focuses on its potential as a therapeutic treatment. These tools are also being used in conjunction with other technologies (e.g., iPSCs) to further develop basic cardiovascular research and test new therapies in laboratory settings.

iPSCs AND PRECISION MEDICINE: BASIC RESEARCH AT THE INDIVIDUAL LEVEL

iPSCs have provided a unique model for in vitro testing of human genetics. Created from differentiated adult cells, iPSCs are de-differentiated using a cocktail of genes that revert the cells to an embryonic-like, undifferentiated stem cell state (64). These cells can then be differentiated into many different cell types, including cardiomyocytes, endothelial cells, and fibroblasts, using small molecules and growth factors. This allows us to take cells from a patient and turn them into an unlimited number of cardiomyocytes in the laboratory, creating a system to test personalized therapeutics on a patient's own cells without having to take and culture cardiac biopsies. iPSC-derived cardiomyocytes have been used to characterize cellular disease phenotypes (65), assist high-throughput drug discovery (66), and investigate cardiovascular metabolism (67), among myriad other applications (68), thus solidifying their critical role in current cardiovascular investigation.

In addition, the combination of iPSCs and current genome editing technologies like CRISPR-Cas9 allows us to create and investigate a multitude of genetic variants in the laboratory. By editing the genomes of

healthy, control iPSCs to contain putative disease-causing mutations and then differentiating those cells to cardiomyocytes, we can investigate whether these mutations cause cellular phenotypes of disease. Conversely, we can also use gene editing strategies to correct suspected disease-associated mutations in patient-derived iPSCs to see if the correction relieves phenotypes. Both strategies allow us to create additional evidence to support these putative mutations and identify them as potential precision medicine targets in affected patients (69).

One of the first studies to demonstrate the therapeutic potential of CRISPR editing in iPSCs corrected disease-associated mutations in iPSCs derived from patients with β -thalassemia. In these patients, mutations in human hemoglobin beta (*HBB*) can lead to severe anemia due to a decrease in production of β -globin. However, the CRISPR-corrected cells, when differentiated with erythroblasts, showed a return to normal levels of *HBB* expression and could provide a source of cells for autologous transplantation back into the affected patients (70). Although this kind of therapeutic, autologous transplantation carries risks, including the potential for residual undifferentiated cells to form tumors, it demonstrates the tremendous power that we now have to correct patient-specific mutations in their own cells.

GENOTYPE-GUIDED WARFARIN DOSING: A NOTE ON THE IMPORTANCE OF CARDIOVASCULAR PRECISION MEDICINE STUDIES ACROSS POPULATIONS

Although genetic testing can have diagnostic and therapeutic value in many cardiovascular care situations, its use must be evaluated for efficacy, cost, and benefit above and beyond typical diagnostics and therapies, and across multiple populations. One example of an area in which proposed genetic testing currently shows the potential for added value but a need for study across diverse populations is in warfarin dosing. Warfarin is an anticoagulant drug that shows a wide range of effectiveness and effective doses across the population. Typically, patients are given an estimated first dose based on international normalized ratio testing, and then dosing is carefully adjusted based on additional measurements over time.

However, known variants in genes such as *CYP2C9* and *VKORC1* affect warfarin metabolism, which gave rise to the idea that including genetic testing in warfarin dosing decisions might provide a clinical benefit (71). The EU-PACT (European Pharmacogenetics of Anticoagulant Therapy) warfarin trial, which

focused on a population of mostly European ancestry, found that adding genetic testing significantly increased the amount of time patients spent in a therapeutic indicator range (72). However, another clinical trial in the United States, the COAG (Clarification of Optimal Anticoagulation through Genetics) trial, found no benefit from using a pharmacogenomic approach in an ethnically diverse population (73).

Although the discrepancies in these results were likely due to differences in the effects of the genetic variants assessed in populations of different ethnic backgrounds, inconsistencies among these early trials led many to question the usefulness of pharmacogenomics in warfarin dosing (71). More recently, the GIFT trial (Genetic Informatics Trial of Warfarin to Prevent Deep Vein Thrombosis) found that perioperative, genotype-guided warfarin administration in the case of some elective surgeries reduced the risk of a composite negative outcome that included major bleeding, venous thromboembolism, and death compared with clinically guided dosing (74). However, this trial again focused on a mostly homogeneous population (91.0% white) (74), and none of the trials to date have taken into account the *CYP2C9* variants that are more frequent in populations of African ancestry (75).

Not only have warfarin-related variants been less studied among populations of African and Hispanic ancestry, but warfarin dose variability appears higher in these populations than in European populations (76). Despite standing to gain potential benefit from genotype-guided dosing to overcome this wide variability, warfarin-dosing studies still focus largely on European populations, and algorithms developed based on these populations may miss or misinterpret variants important for others (76). The potential for genotype-guided warfarin dosing to reduce adverse events is present, but studies must focus on a wider range of ancestries before this precision medicine strategy can be considered a wide success.

CONCLUSIONS

Technological advances in our capacity to sequence and interpret the genome, as well as our ability to turn that information into effective treatments, are rapidly increasing. Precision medicine treatments in oncology and CF have now been successful and long-lasting enough to provide precedent for the coming wave of new treatments aided by genome sequencing. Not only are we now better able to find and target disease-causing variants, but we are now building toolkits to change these variants at the

genome level, editing them out before they have the opportunity to manifest disease.

In addition to genomics, advances in phenotyping disease will add to our ability to correctly diagnose and treat patients. Deep phenotyping, defined as “the precise and comprehensive analysis of phenotypic abnormalities in which the individual components of the phenotype are observed and described” (77), can allow for computational analysis of patient phenotypes. Combined with genomic data, this reveals connections between previously unrelated phenotypes and between phenotypes and potential genes and molecules of interest (78). By shifting our view of phenotype from a binary variable of presence or absence of disease to a view that describes phenotype as the complex combination of many diverse measurements, we will be better equipped to examine underlying genetic and molecular causes of disease (78). This shift will require advances in phenotyping conducted in the cardiovascular clinic, where the phenotypic measurements themselves must be precise to derive meaningful insights from them. Advances in cardiac imaging technologies and the incorporation of informatics and imaging biomarkers may lead the way in redefining precise phenotypes of CVD (79).

Although precision medicine is accelerating diagnosis and treatments across fields, there remain current limitations that the field must strive to tackle in the coming years. One of the most pressing is the need for precision genomics studies across diverse ethnic populations. Many of the genetics studies conducted to date have been performed on populations of mostly European ancestry. Variants that correlate with disease phenotype or pharmacogenomic response in these populations may not similarly correlate across other populations or may be weighted differently in their contributions to disease phenotype, as is the case for the variants implicated in warfarin pharmacogenomics discussed earlier. Conversely, variants that may be common in less studied populations may be falsely classified as pathogenic variants, invalidating cascade screening in family members and leading to misdiagnoses (80). Although more studies have begun to investigate variant effects across diverse populations, we should strive for even greater adoption of this necessary inclusion across study designs. Recent papers addressing these concerns, including the study by Hindorff et al. (81), highlight the need for community engagement to overcome study enrollment obstacles and further stress that diverse study populations would allow for more equitable results. They also point out that support for these diverse

studies must come from multiple levels across the scientific community, from individual researchers to funding agencies, journal editors, and governments (81,82).

In addition, despite the multitude of cardiovascular genetic studies conducted each year, many genetic variants that appear in the clinic are still categorized as variants of unknown significance, which leave doctors and patients without clear answers about disease causes or risks for future generations. We must put a concerted effort behind taking these variants of unknown significance out of genetic data sets and into laboratory settings where we can test their effects both *in vitro* and *in vivo* to understand their relationship to disease. This will require high-throughput testing in model systems using new genetic technologies, including the previously discussed iPSC systems and CRISPR tools. These technologies can simultaneously be harnessed as tools to identify the effects of all possible variants in cardiovascular genes using techniques like saturation mutagenesis. Saturation mutagenesis, which strives to create each possible mutation in a gene to study its effect on function, has now been used to investigate

genes involved in multiple diseases, including lipodystrophies (83). New tools like these can help us to map the effects of genetic variants on disease even before we see them in patients.

Advances in genomics and phenomics, together with technological innovations in the laboratory, propel us forward into a new era of cardiovascular care. Although work to this point has led to breakthroughs for patients and their families, new tools and datasets give us more power than ever to make headway in the fight against CVDs. Together, basic and translational scientific progress strive toward the goal of giving each individual the right treatment at the right time.

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