Precision Cardiovascular Medicine in the Post-Genomic Era

Nikhil V. Munshi, M.D., Ph.D.

University of Texas Southwestern Medical Center

Internal Medicine Grand Rounds

January 3, 2020

This is to acknowledge that Dr. Munshi, M.D., Ph.D. has disclosed that he does not have any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr. Munshi will not be discussing off-label uses in his presentation.

Nikhil V. Munshi, M.D., Ph.D., Associate Professor of Medicine, Cardiology Division

Research and Clinical Interests

My laboratory focuses on understanding the molecular underpinnings of cardiac conduction system formation and how these mechanisms impact normal and pathological cardiac rhythms. My clinical interests include management of coronary care unit (CCU) patients and the genetics of cardiovascular disease.

Purpose and Overview

The notion of precision medicine has entered the public consciousness and promises to impact how we practice medicine in the future. In this regard, precision medicine has already made significant inroads within specific sub-specialties, such as Oncology. Here, I will define the scope of precision medicine and describe the historical basis for its current importance. Focusing on cardiovascular medicine, I will highlight several specific examples of how precision medicine is poised to impact clinical care. Finally, I will outline several emerging areas of rich patient information content that are likely to influence clinical medicine in the near future.

Educational Objectives

1) To define the scope of precision medicine
2) To highlight specific examples where precision medicine has begun to impact clinical care
3) To provide an overview of emerging sources of patient data that are likely to be incorporated into the precision medicine framework in the future

SCOPE OF PRECISION MEDICINE

Definition. Healthcare delivery is undergoing a major transformation resulting from ongoing technological advancements. Innovations ranging from the electronic health record (EHR) to next-generation DNA sequencing to wearable technology are revolutionizing patient care. Although these innovations appear broad and unrelated, many of them can be unified under the umbrella of "Precision Medicine," a term that entered the public consciousness following Barack Obama's State-of-the-Union speech in January of 2015.

During his speech, President Obama remarked "Doctors have always recognized that every patient is unique, and doctors have always tried to tailor their treatments as best they can to individuals. You can match a blood transfusion to a blood type – that was an important discovery. What if matching a cancer cure to our genetic code was just as easy, just as standard? What if figuring out the right dose of medicine was as simple as taking our temperature?" With his speech, the President introduced the Precision Medicine Initiative (PMI) to all Americans.

One definition of precision medicine is "…a health-care model that facilitates efficient and accurate identification of the optimal course of care for an individual patient."[1] However, the following terms are related to and often confused with precision medicine[2]:

Stratified medicine: the use of demographic or clinical characteristics to cluster similar patients.

Personalized medicine: individualized patient care for diagnosis, prognosis, and treatment.

Genomic medicine: the incorporation of genomic data to guide patient care.

Digital medicine: the use of smart phones and wearable sensors to report medical information.

Rationale and Motivating Factors. The motivation for progressing towards a precision medicine model for clinical care stems in large part from the many flaws inherent to our current "one-size-fits-all" approach[1-3]. For example, treatment plans are currently synthesized based on a limited set of information obtained from the patient, including the history and physical exam, vital signs, and laboratory and imaging data. Furthermore, the heterogeneity amongst different patients is largely ignored, especially in terms of disease diagnosis and pharmacological treatment efficacy. In fact, many pieces of patient-specific information vary with time, and currently very little attention is paid to how

these changes influence the diagnosis and treatment of individual patients. Finally, we know from many post hoc analyses conducted as part of clinical trials that specific subgroups often respond particularly well to a given treatment.

To further illustrate this point, let us consider the top 10 prescribed medications in 2015[4]. The number needed to treat (NNT) is a popular method for comparing the effectiveness of various treatments in a standardized manner. For the top 10 medications, the NNT ranges from 1 in 4 (adalimumab for arthritis, etanercept for psoriasis, and infliximab for Crohn's disease) to 1 in 25 (esomeprazole for heartburn). Also included on this list are several general internal medicine drugs, including rosuvastatin (1 in 20), fluticasone (1 in 20), and duloxetine (1 in 9). If we now conceptualize the NNT as the inverse, or the number who fail treatment (NFT), then we see that the NFT for esomeprazole, for example, is 24. Taken together, this example serves to illustrate the point that the vast majority of patients that we treat every day do not in fact respond to our treatment, supporting the notion that more precise patient selection may improve outcomes.

One potential solution to this conundrum is precision medicine. In a given population of individuals with a specific disease, for example, only a small subset will respond to treatment due to a variety of genetic and environmental influences. In many cases, randomized control trials will identify specific patient subgroups that respond particularly well in post hoc analyses. In turn, these groupings stratify patients who are candidates for a given treatment to predict increased efficacy, although this must be formally established in subsequent randomized clinical trials. This type of patient selection for a given treatment has been called "stratified medicine." The precision medicine construct takes this patient selection model one step further by incorporating additional patient-specific information, such as genomics, biomarkers, the EHR, and many others that will be detailed below. In theory, precision medicine allows utilization of a comprehensive set of variables to more reliably predict who will respond to a particular treatment.

Although a strong desire to practice precision medicine has emerged recently, several factors have contributed importantly to the increasing momentum[1-3]. First, disruptive changes in sequencing technology have made feasible the generation of various "omic" datasets that are required to fuel precision medicine. Second, with the explosion in datasets being generated, there have been concomitant improvements in computational infrastructure to allow efficient data storage and analysis. Third, the parallel uptake of EHR into clinical practice and the rise of Bioinformatics as an academic discipline have further contributed to the precision medicine movement. Fourth, contemporary clinical trials are beginning to utilize internet and social media networks for patient enrollment and data sharing. In turn, this can lead to clinical trials that are both personalized and capable of testing multiple questions in parallel. Finally, the "quantified self"

movement has popularized wearable technology, which reports on an individual's environmental exposures.

Precision medicine frameworks. In one popular vision of precision health[5], several interlocking feedback loops inform patient care. Focusing on the patient, disease risk is calculated across the lifespan by utilizing information from family history, genomics, and socioeconomic factors. Throughout the lifetime, customized monitoring, such as wearable technology, smart appliances, imaging, and biomarkers, can report on environmental exposures and identify sudden changes in disease risk. In turn, these variable are fed back to the patient to re-calculate risk in real time. Finally, patients engage in an integrated health portal that consists of a data analytic framework to provide real time risk assessment and a hospital system for diagnostic testing and treatment. The health portal loop feeds back onto both the monitoring and patient loops through learning and adaptation to complete the network.

In the complementary but non-overlapping "high-definition medicine" framework[6], precision medicine is envisioned as a series of four ascending steps for optimal patient care. "High-definition medicine" is defined as "…the dynamic assessment, management, and understanding of an individual's health measured at … its most basic units." The first step is "Defining a Personal Baseline of Health" and incorporates both genomics and polygenic risk scores. The second step is "High-Definition Prevention" and involves the use of patient-derived "omic" datasets and data from wearable technology and the Internet of Things. The third step is "High-Precision Treatment," which utilizes genome engineering, cell-based therapy, pharmacogenetics, and precision medicines. The fourth and final step is "Billions of High-Resolution People," which incorporates dynamic data warehouses, artificial intelligence (AI), and digital twins to learn, adapt, and improve disease risk prediction.

A BRIEF HISTORY OF PRECISION MEDICINE

Achievements in Human Genomics. Completion of the Human Genome Project (HGP) marked the end of a ten-year investment to obtain a roadmap for human genomics[7]. Needless to say, there were several achievements that led up to the HGP, beginning with Gregor Mandel's proposed Laws of Genetics in 1865. Watson and Crick's discovery of the DNA double-helix in 1953 preceded the determination of the genetic code in 1966. Sanger sequencing was described in 1977, and the HGP was launched in 1990. Following the complete sequencing of the yeast (1996), E.coli (1997), roundworm (1998), and fruit fly (2000) genomes, the human genome sequence was initially published in 2001 with a final draft completed in 2004.

After a complete human genome sequence became available, the era of functional genomics began with the first publication of a genome wide association study (GWAS) in 2005. GWAS can be performed using a case-control design or on an unselected population for a continuously variable trait (e.g. height)[8]. Then, genomic DNA is obtained from each individual in the study, and single nucleotide polymorphisms (SNPs) are genotyped typically by using a SNP microarray. SNP variations in individual patients are compared between groups or across a continuous trait to identify associations between specific SNPs and the disease or trait under study. Following multiple-testing (Bonferroni) correction, statistically significant SNP associations are nominated and can be visualized in the form of a Manhattan plot. Since microarray SNPs are "tag" SNPs, they may or may not be causative for the associated trait, and theoretically all SNPs in linkage disequilibrium can be considered plausible candidates. From many published GWAS to date, we can make the following conclusions: 1) the effect sizes are relatively modest (typical odds ratio: 1.0-1.5) and 2) associated SNPs most often land in non-coding regions of the genome.

Arguably the most important technological advance fueling the precision medicine imperative was the advent of next-generation sequencing (NGS). Traditional Sanger sequencing, which was used for the HGP, excels at sequencing specific areas of the genome but is too slow and expensive for clinical use. In contrast, NGS functions by sequencing all sequences derived from the cell or tissue of interest followed by bioinformatics mapping to a reference genome to provide comprehensive sequence data for an individual. In comparison to traditional sequencing, NGS is rapid and relatively inexpensive, although occasional lapses in sequencing coverage can occur. Nevertheless, NGS has not only revolutionized the use of clinical genome sequencing, but it has allowed rapid generation of many additional "omics" datasets that can inform the practice of precision medicine.

From a clinical standpoint, entire genomes can be sequenced (whole genome sequencing, WGS) from individual patients for a reasonable cost. Alternatively, there are methods to enrich for the protein-coding genome (whole exome sequencing, WES), which further reduces the complexity and cost of clinical sequencing. The popularity of clinical sequencing has led to a cottage industry of direct-to-consumer genetic testing. Incredibly, the number of commercial genetic tests ordered has risen exponentially since 2017. Unfettered growth in the commercial clinical sequencing industry has motivated continued refinements in sequencing capabilities. Along with improved handling of genomic samples, sequencing hardware has also undergone revisions such that the cost of genome sequencing now stands at $600 with occasional offers as low as $200 per genome. Thus, the remarkable decreases in sequencing costs have far outpaced expectations based on Moore's Law.

Imatinib for Chronic Myelogenous Leukemia (CML). The BCR-ABL translocation is a common genetic aberration in chronic myelogenous leukemia[9]. As a result of the translocation, BCR-ABL is a constitutive tyrosine kinase that can phosphorylate multiple growth-related substrates in the absence of counter-regulation. Thus, unrestrained growth signaling contributes to the pathogenesis of CML. Imatinib is a small molecule that specifically blocks the ATP-binding site of the BCR-ABL fusion protein, thus preventing unchecked downstream phosphorylation. Remarkably, imatinib appears to be relatively specific for the BCR-ABL fusion protein, which minimizes off-target toxicities. Therefore, the BCR-ABL status of CML, or any other tumor, can be considered a genomic biomarker of imatinib efficacy[10]. Overall, this example represents one of many success stories in Oncology for the development of precision medicines based on disease mechanism.

Ivacaftor for Cystic Fibrosis (CF). Cystic Fibrosis is another disease that represents a success for precision medicine. Although it has been known for many years that mutations in the CFTR gene cause CF, it has remained difficult to design directed treatments due, in part, to the heterogeneity of causative mutations. However, it subsequently became clear that individual mutations could be grouped into six classes based on their biochemical defects. For example, class I mutations lead to a lack of functional CFTR as a result of nonsense, frameshift, or splice site mutations. In contrast, class II and III mutations lead to trafficking defects and defective channel regulation, respectively, due to missense mutations or deletions that maintain reading frame. Thus, small molecules have been designed to target these individual defects in a very precise manner. In the first of many clinical trials, ivacaftor was shown to be efficacious in CF caused by the Gly551Asp CFTR mutation[11]. Subsequently, clever combinations of treatments have been devised to target more complex CFTR mutations[12].

The Precision Medicine Initiative (PMI). With the announcement of the Precision Medicine Initiative in 2015, President Obama laid out a vision for both short- and long-term goals. In the near term, the plan was to expand efforts in cancer genomics to prevent and treat malignancy based on the many successes already realized in Oncology. In terms of long-term goals, the PMI aimed to advance precision medicine in all other realms of health care. Specifically, he proposed a $130 million NIH investment in the "All of Us" research program, which began recruiting participants in 2017. The goal of the program is to enroll 1,000,000 participants across America to create a comprehensive database containing information from the EHR, physical measurements, genomics, wearable sensors, and other sources. Aside from the clear utility of this database for research purposes, participants are given free access to their own health information.

Taken together, the vision for the “All of Us” program is to create a centralized repository of longitudinal, patient-derived information in a heterogeneous cohort that can provide a rich resource to answer a variety of clinical questions.

PRECISION MEDICINE IN CARDIOVASCULAR DISEASE

The African American Heart Failure Trial (A-HeFT). In one of the first examples of precision medicine, the African-American Heart Failure Trial (A-HeFT) established the efficacy of isordil and hydralazine in addition to optimal medical treatment for African-American patients with heart failure due to left ventricular dysfunction. The rationale for this trial emerged from a previous negative study in which isordil and hydralazine were compared to prazosin and placebo for heart failure treatment. In subgroup analysis, the authors found that African-American patients in the isordil-hydralazine arm had reduced mortality compared to the other two groups. Based on this post hoc observation, A-HeFT randomized 1050 African-American patients with heart failure to isordil-hydralazine (N=518) or placebo (N=532) arms. As hypothesized, patients in the isordil-hydralazine treatment group demonstrated reductions in the primary composite endpoint, including all-cause mortality. Based on the results of this trial, isordil-hydralazine was approved by the FDA for add-on heart failure therapy in African-American patients, thus demonstrating one of the first examples of stratified medicine.

Pharmacogenomics. Pharmacogenomics refers to the practice of utilizing a patient’s genetic information to guide treatment decisions. The concept of pharmacogenomics is attractive because it is well-established that the beneficial and toxic effects of a given drug must be weighed against one another and that each patient has a unique therapeutic window depending on their ability to absorb, metabolize, or otherwise utilize a drug. In a heterogeneous group of patients with the same diagnosis and the same prescription, for example, there exist four therapy-related outcomes: 1) the drug is toxic and beneficial, 2) the drug is toxic and not beneficial, 3) the drug is not toxic or beneficial, and 4) the drug is beneficial and not toxic. To distinguish these possibilities, pharmacogenomics has been implemented for clopidogrel and warfarin dosing within the cardiovascular disease realm.

Oral clopidogrel is a prodrug absorbed in the intestine via the ABCB1 transporter. 85% of clopidogrel is inactivated by esterases, while the remaining 15% undergoes hepatic metabolism by a variety of cytochrome P450 enzymes into active metabolites, which then go on to bind to P2Y12 receptors on platelets in the bloodstream. Theoretically, polymorphisms in any of the genes responsible for clopidogrel absorption and/or metabolism could ultimately influence the efficacy of a given clopidogrel dose.

Since certain CYP2C19 alleles cause functional defects in clopidogrel metabolism, the TIMI group tested the hypothesis that genetic variation in CYP2C19 influences platelet inhibition and clinical outcomes following an acute coronary syndrome (ACS)[13]. In the first stage of this trial, the investigators demonstrated that carriers of at least one CYP2C19 reduced-functional allele had reduced levels of the active clopidogrel metabolite in their bloodstream along with a reduced response to platelet aggregation. In the second stage of the trial, the investigators demonstrated a 53% increase in the primary outcome (risk of death from cardiovascular causes, myocardial infarction, or stroke) in CYP2C19 reduced-function allele carriers versus non-carriers, and a three-fold increase in the rate of stent thrombosis. Taken together, these data strongly suggested that CYP2C19 reduced-function alleles directly contribute to levels of active clopidogrel metabolites and clinical outcomes.

To further explore the association between specific CYP2C19 genotypes and clinical outcomes, Mega and colleagues performed a meta-analysis of nine randomized control trials that evaluated the use of clopidogrel following acute coronary syndrome[14]. For the composite endpoint of cardiovascular death, myocardial infarction, or ischemic cerebrovascular accident, the investigators found a 55% increase in patients with one reduced-function CYP2C19 allele and a 76% increase in those with two CYP2C19 alleles. In terms of stent thrombosis, patients with one allele had a 2.7-fold increase, and those with two alleles had a 4-fold increase. Altogether, this meta-analysis confirmed the strong association between CYP2C19 genetic status and clinical outcomes following clopidogrel treatment for acute coronary syndromes.

Similar to clopidogrel, warfarin undergoes metabolism by cytochrome P450 enzymes in the liver and depends upon the function of vitamin K metabolic pathways. Based on prior knowledge that specific CYP2C19 and VKORC1 alleles influence the dose of warfarin needed to achieve therapeutic anticoagulation, Klein and colleagues conducted a study to estimate the required warfarin dose based on clinical and pharmacogenomic information[15]. They found that a pharmacogenetic algorithm outperformed both a clinical algorithm and a fixed-dose approach as measured by the mean absolute error between the predicted and actual warfarin dose. In addition, the authors observed that the pharmacogenetic approach was particularly effective at estimating dosing regimens outside the fixed range – less than 21 mg per week or greater than 49 mg per week. Thus, an algorithm incorporating genetic information improved warfarin dosing prediction, although both the derivation and validation groups were derived from the same retrospective cohort.

To definitively address whether pharmacogenetic information improves warfarin dosing, two prospective randomized control studies of a genotype-guided approach versus a clinical algorithm were performed[16, 17]. However, the results were mixed. In the first study by Kimmel and colleagues, genotype-guided dosing of warfarin did not improve anticoagulation control as assessed by the percentage of time that the INR was in

the therapeutic range during the first 28 days of therapy. In the second study by Pirmohamed and colleagues, pharmacogenetic dosing was associated with a higher percentage of time in the therapeutic range for INR during the first 12 weeks after initiation. Taken together, these studies could not definitively answer the question of whether genotype-based dosing improves the anticoagulation response to warfarin, and additional studies will be needed to settle this issue.

Many factors have precluded rapid uptake of genotype-based dosing for both clopidogrel and warfarin, including the emergence of alternative treatments (e.g. prasugrel, NOACs, etc.). Nevertheless, a recently published trial attempted to resurrect genotype based dosing to determine whether patients should receive new P2Y12 inhibitors, such as prasugrel and ticagrelor, or the less-potent clopidogrel post-PCI to minimize bleeding complications[18]. The authors found that a genotype-guided strategy was non-inferior to the standard treatment group for the primary combined outcome of net adverse clinical events, including death, myocardial infarction, stent thrombosis, CVA, or major bleeding. Interestingly, genotype-guided treatment appeared to decrease major or minor bleeding in subgroup analysis. Based on the non-inferiority of a genotype-guided approach, it appears reasonable to implement in clinical practice to minimize the cost of treatment with a P2Y12 inhibitor.

Polygenic Risk Scores. As mentioned previously, GWAS have identified many SNPs associated with cardiovascular traits, yet their individual effect sizes are relatively modest. To overcome this issue, investigators have developed polygenic risk scores involving numerous SNPs in aggregate to improve predictive capability. Using coronary artery disease as an example, well-established risk factors, such as elevated cholesterol, smoking, and high blood pressure, make a substantial impact on the risk of developing disease. While these risk factors integrate environmental exposures, a polygenic risk score would incorporate genetics to establish an overall risk. Thus, a polygenic risk score could stratify patients with a given traditional risk factor profile, and, in some cases, persuade the clinician to take action in those individuals that surpass a certain threshold of combined CAD risk.

Two major studies evaluated the role of CAD polygenic risk scores in various aspects of clinical care. In the first study[19], the investigators performed a retrospective analysis to confirm that individuals with elevated risk scores are at increased risk of incident and recurrent coronary events. Furthermore, they analyzed four trials that had evaluated statin use in primary and secondary CAD prevention to demonstrate that individuals at the highest genetic risk of CAD derived the most clinical benefit from statin therapy. In the second study[20], Khera and colleagues retrospectively assessed the influence of genetic risk and adherence to a healthy lifestyle on incident coronary events. Aside from showing that both independently contribute to clinical CAD, perhaps the most interesting finding across cohorts was that a healthy lifestyle can compensate for

high genetic risk, and poor adherence to a healthy lifestyle can compensate for low genetic risk of incident coronary events.

Personal Genomics. In a landmark case study, Ashley and colleagues described the incorporation of personal genomics into clinical decision-making in a 40 year old man that presented to Stanford's Center for Inherited Cardiovascular Disease[21]. The patient was a young otherwise healthy man with a significant family history for sudden cardiac death (SCD), CAD, abdominal aortic aneurysm, and cerebral aneurysm. Given his family history, whole genome sequencing was performed, and he was evaluated by a cardiologist and genetic counselor. Focusing on protein-coding variants, he was found to have three potential candidate gene mutations to explain his family history of SCD. Serendipitously, he was also found to have variants associated with CAD, hemochromatosis, and parathyroid tumors for which he received genetic counseling. Using a polygenic risk score, he was found to have an elevated risk of obesity, CAD, and diabetes. From a known pharmacogenomics panel, several polymorphisms were identified that modulate the response to statins, clopidogrel, warfarin, and beta blockers. In addition to genetic counseling, a statin was initiated based on his increased polygenic risk score for CAD and his genetic predisposition to a good statin response without myopathy. Aspirin initiation was also discussed due to the presence of a specific LPA risk allele. Finally, the need for high-potency P2Y12 inhibitors and reduced warfarin doses was discussed if these medications were deemed necessary in the future.

EMERGING SOURCES OF PATIENT GENOMIC DATA

Integrated Multi-Omics. Aside from the EMR, several additional sources of patient data can be obtained to inform precision medicine. For example, we have discussed how personal genomics can yield information regarding treatment response and disease risk. In addition, unbiased information regarding gene expression (transcriptome), protein translation (proteome), and products of intermediary metabolism (metabolome) can be obtained from patient blood samples. While the genome provides the blueprint, the transcriptome represents what part of the genome is being actively utilized, and the proteome encompasses the transcriptome that has been translated into proteins. Similarly, the metabolome reflects the flux of substrates flowing through the individual as well as the net conversion rates of individual enzymes. All of these data sources (multi-omics) can then be integrated with the use of bioinformatics to provide additional patient-derived information to improve clinical care.

Epigenome. With the publication of the Human Genome in 2001, only 30,000 protein-coding genes were identified comprising only 2% of the genome. Consequently, the

Encyclopedia of DNA Elements (ENCODE) consortium was launched to annotate the remaining 98%. Based on the findings of the ENCODE consortium in 2012, it became clear that DNA and DNA-associated histones are enzymatically modified to provide an additional layer of information above the genome (the "epigenome"). Therefore, DNA methylation and histone modifications can be mapped across the genome from patient blood samples. Several examples exist in the literature linking patient-derived epigenome information to disease risk in large cohorts – so-called epigenome wide association studies (EWAS).

Microbiome. In recent years, it has become appreciated that the gut microbiome plays an important role in homeostasis and disease[22]. In general, disease states are marked by gut dysbiosis in which the composition of the gut microbiota is altered. Interestingly, the interaction between diet and microbiome composition can also profoundly influence specific disease states. For example, certain metabolites found in the gut of non-vegetarians can be metabolized by gut bacteria into TMAO, which has profound effects on macrophages, endothelial cells, and platelets to increase the risk of cardiovascular diseases, such as atherosclerosis, heart failure, and acute thrombotic events. Thus, baseline and subsequent microbiome analysis will soon emerge as a critical components of precision medicine to predict antecedent disease, monitor treatment efficacy, and guide recommendations for lifestyle modifications.

Exposome. Aside from genetics, environment clearly plays a major role in disease development. To date, however, measurements of environmental exposure, such as history of tobacco use, cholesterol levels, and elevated blood pressure, are crude by today's standards. The emergence of wearable technology has revolutionized the yield of information on exposure variables in individual patients[5]. Examples of such technology include wristbands (vital signs, movement, and glucose levels), shoes (cadence, impact, force, and balance), contact lenses (intraocular pressure and glucose), and breath analysis (organic compounds and lung cancer detection). This information can be integrated using the Internet of Things with data obtained from a patient's car and home. Smart devices in the bathroom (toothbrushes, toilets, mirrors, and scales), the bedroom (bed, smartphone, pill case), and the refrigerator (dietary habits and food quality) can report rich datasets that inform precision medicine.

Temporal Pan-Omics. Returning to patient-derived information content, we will now consider the role of temporal resolution in these measurements. In this regard, a landmark study was performed on a single patient in which numerous personalized omic datasets were generated over a 14-month period[23]. All of these rich data sources were combined into a single temporal framework named the integrative personal omics profile

(iPOP). During the course of the study, the subject experienced sequential human rhinoviral and respiratory syncytial viral infections. Interestingly, he developed sustained hyperglycemia following the second viral infection, which subsequently responded to intensive lifestyle changes. Over the 401 study days, blood was obtained at 20 time points. Blood samples were split into peripheral blood mononuclear cell (PBMC) and serum fractions, which were extensively analyzed. From PBMCs, whole genome and transcriptome sequencing was performed in addition to proteome profiling. From serum, untargeted and target proteome profiling, metabolomics, antibody profiling, and clinical lab testing were done.

Based on personal genomics for the study subject, polygenic risk scores were utilized to determine a high overall risk (>50%) for several diseases, including open angle glaucoma (~90%), dyslipidemia (~70%), CAD (~60%), and basal cell carcinoma (~60%). Interestingly, risk for basal cell carcinoma, type 2 diabetes, and age related macular degeneration were most significantly increased by adding polygenic risk scores, while the risk of obesity and prostate cancer were most significant decreased by incorporating genetics.

Given the temporal information obtained during the study and the onset of incipient hyperglycemia, the authors had the unique opportunity to explore the contribution of genetics and environment to diabetes pathogenesis. The pretest probability of developing diabetes in this patient was calculated at ~30%, but incorporation of the polygenic risk score increased this probability to 50%. Approximately one month after recovering from a respiratory syncytial viral (RSV) infection, the subject demonstrated fasting glucose levels in the diabetic range (>120 mg/dL) with a concomitant increase in HbA1c levels up to 6.7. The temporal cytokine profile demonstrated a distinct signature following RSV infection, suggesting that these may contribute to the patient's viral response and subsequent development of diabetes. Based on the observed clinical findings, the patient incorporated substantial lifestyle changes to return his glucose levels and HgbA1c to the normal range.

THE FUTURE OF CARDIOVASCULAR PRECISION MEDICINE

Comprehensive Integrative Personal Omics Profile (iPOP). As a natural extension of the N-of-1 iPOP study outlined above, the same authors recently reported on a more comprehensive iPOP in a cohort of healthy patients enriched for the risk of diabetes[24]. Cohort phenotyping included standard elements, such as personal and family history, clinical exam, and social behaviors. Additionally, results from enhanced tests (imaging and exposome) and emerging tests (genome, transcriptome, proteome, immunome, metabolome, and microbiome) were collected for each patient periodically over a median follow up time of 2.8 years. Several actionable health discoveries were identified

during the course of the study. For metabolic disorders, gene mutations were discovered in two patients, and diabetes and pre-diabetes were identified by lab testing in 69 patients. For cardiovascular disease, one patient had a cardiomyopathy gene mutation, two patients had arrhythmias by wearable technology, three patients had actionable pharmacogenomics variants, nine patients were noted to have early stage disease by serial imaging, and 18 patients were identified with hypertension by blood pressure measurement. Lyme disease and obstructive sleep apnea were diagnosed by wearable technology in two patients, elevated cystinuria risk was detected by personal genomics in one patient, and macroalbuminuria was observed in two patients by clinical testing. For oncological disease, five patients were observed to have early stage disease by clinical testing and eight patients harbored gene mutations that predispose to malignancy, one of whom was found to have early-stage thyroid cancer that was subsequently resected. Aside from actionable health discoveries, an equally important result of the cohort study was the efficacy of making lifestyle changes based on structured feedback with the acquired data.

Disease Reclassification. Disease heterogeneity is a major contributor to the overall lack of treatment response. In congestive heart failure, investigators have attempted to stratify patients with reduced and preserved ejection fractions using cluster analysis with a set of clinical phenotypes[25-27]. Interestingly, these phenomapping studies separated patients into distinct clusters that differed with respect to demographics, clinical trajectory, and disease severity. From these studies, it appears logical to infer that each of these patient clusters may respond differently to evidence-based treatments, which would in turn help to target treatments more precisely in the future. Collectively, these studies clearly demonstrate proof-of-concept that heart failure can be further stratified. However, a major drawback to these studies is that the clinical parameters used for training the clusters were pre-specified, so the analysis is not entirely agnostic to preconceived notions of the disease and thus introduces some level of bias.

To overcome this hurdle, my lab has recently performed ATAC-seq, an assay that measures accessible chromatin across the genome, in human heart tissue. Chromatin accessibility is thought to identify genomic elements (distal enhancers) that orchestrate gene expression. Furthermore, chromatin accessibility profiles provide a mark of accumulated environmental effects and are thus more stable than gene expression, which varies on a much shorter time scale. Thus, we profiled explanted heart tissue from patients with ischemic, non-ischemic, and hypertrophic cardiomyopathy as compared to normal controls. Remarkably, we identified specific chromatin accessibility signatures that separated patient samples by disease etiology. Interestingly, we found two patients that were originally classified as NICM and ICM by clinical criteria, but who we found subsequently to possess mixed signatures, and we are currently going back through the patient charts to obtain relevant collateral history.

Plasma Cell-Free DNA. Recent years have witnessed yet further advances in clinical genomics. When cells die by apoptosis or necrosis, their DNA ends up in the blood stream as short fragments (cell-free DNA; cfDNA)[28]. In cancer, for example, this property has been exploited to perform "liquid biopsies," since the DNA sequence of the tumor differs from the un-mutated patient genome. Similarly, these assays have extended their utility to prenatal testing and transplant rejection, two additional scenarios in which "foreign" DNA can be identified as distinct from "host" DNA. To expand the utility of cfDNA assays, it would be ideal to detect tissue-specific DNA even if the sequence were indistinguishable from the "host" genome. Taking advantage of the fact that DNA can be chemically modified by the addition of methyl groups to cytosine in a tissue-specific manner, it has now become possible to determine the tissue origin of cfDNA obtained in the bloodstream.

A recent study demonstrated the feasibility of determining patient tissue damage profiles[29]. In control patients, for example, it was observed that the majority of cfDNA originates from blood cell types, such as erythrocytes, granulocytes, lymphocytes, and monocytes with a minor contribution from endothelial cells, neurons, and hepatocytes. In contrast, blood collected from patients that recently underwent an Islet cell transplant demonstrated the emergence of cfDNA originating from pancreatic cell types, such as acinar and beta cells. In liver failure patients, hepatocyte cfDNA emerged in the bloodstream, while granulocytes became the overwhelming source of cfDNA in patients with bacterial sepsis. Collectively, this study demonstrates the potential power of measuring cfDNA from the blood as an indicator of tissue-specific damage elsewhere in the body.

REFERENCES

1. Antman EM and Loscalzo J. Precision medicine in cardiology. *Nature reviews Cardiology*. 2016;13:591-602.
2. Topol EJ. Individualized medicine from prewomb to tomb. *Cell*. 2014;157:241-53.
3. Ashley EA. Towards precision medicine. *Nat Rev Genet*. 2016;17:507-22.
4. Schork NJ. Personalized medicine: Time for one-person trials. *Nature*. 2015;520:609-11.
5. Gambhir SS, Ge TJ, Vermesh O and Spitler R. Toward achieving precision health. *Science translational medicine*. 2018;10.
6. Torkamani A, Andersen KG, Steinhubl SR and Topol EJ. High-Definition Medicine. *Cell*. 2017;170:828-843.
7. Green ED, Guyer MS and National Human Genome Research I. Charting a course for genomic medicine from base pairs to bedside. *Nature*. 2011;470:204-13.
8. Tam V, Patel N, Turcotte M, Bosse Y, Pare G and Meyre D. Benefits and limitations of genome-wide association studies. *Nat Rev Genet*. 2019;20:467-484.
9. Savage DG and Antman KH. Imatinib mesylate--a new oral targeted therapy. *N Engl J Med*. 2002;346:683-93.
10. Druker BJ, Talpaz M, Resta DJ, Peng B, Buchdunger E, Ford JM, Lydon NB, Kantarjian H, Capdeville R, Ohno-Jones S and Sawyers CL. Efficacy and safety of a specific inhibitor of the BCR-ABL tyrosine kinase in chronic myeloid leukemia. *N Engl J Med*. 2001;344:1031-7.
11. Ramsey BW, Davies J, McElvaney NG, Tullis E, Bell SC, Drevinek P, Griese M, McKone EF, Wainwright CE, Konstan MW, Moss R, Ratjen F, Sermet-Gaudelus I, Rowe SM, Dong Q, Rodriguez S, Yen K, Ordonez C, Elborn JS and Group VXS. A CFTR potentiator in patients with cystic fibrosis and the G551D mutation. *N Engl J Med*. 2011;365:1663-72.
12. Grasemann H. CFTR Modulator Therapy for Cystic Fibrosis. *N Engl J Med*. 2017;377:2085-2088.
13. Mega JL, Close SL, Wiviott SD, Shen L, Hockett RD, Brandt JT, Walker JR, Antman EM, Macias W, Braunwald E and Sabatine MS. Cytochrome p-450 polymorphisms and response to clopidogrel. *N Engl J Med*. 2009;360:354-62.
14. Mega JL, Hochholzer W, Frelinger AL, 3rd, Kluk MJ, Angiolillo DJ, Kereiakes DJ, Isserman S, Rogers WJ, Ruff CT, Contant C, Pencina MJ, Scirica BM, Longtine JA, Michelson AD and Sabatine MS. Dosing clopidogrel based on CYP2C19 genotype and the effect on platelet reactivity in patients with stable cardiovascular disease. *JAMA : the journal of the American Medical Association*. 2011;306:2221-8.
15. International Warfarin Pharmacogenetics C, Klein TE, Altman RB, Eriksson N, Gage BF, Kimmel SE, Lee MT, Limdi NA, Page D, Roden DM, Wagner MJ, Caldwell MD and Johnson JA. Estimation of the warfarin dose with clinical and pharmacogenetic data. *N Engl J Med*. 2009;360:753-64.
16. Kimmel SE, French B, Kasner SE, Johnson JA, Anderson JL, Gage BF, Rosenberg YD, Eby CS, Madigan RA, McBane RB, Abdel-Rahman SZ, Stevens SM, Yale S, Mohler ER, 3rd, Fang MC, Shah V, Horenstein RB, Limdi NA, Muldowney JA, 3rd, Gujral J, Delafontaine P, Desnick RJ, Ortel TL, Billett HH, Pendleton RC, Geller NL, Halperin JL, Goldhaber SZ, Caldwell MD, Califf RM, Ellenberg JH and Investigators C. A

pharmacogenetic versus a clinical algorithm for warfarin dosing. *N Engl J Med*. 2013;369:2283-93.
17. Pirmohamed M, Burnside G, Eriksson N, Jorgensen AL, Toh CH, Nicholson T, Kesteven P, Christersson C, Wahlstrom B, Stafberg C, Zhang JE, Leathart JB, Kohnke H, Maitland-van der Zee AH, Williamson PR, Daly AK, Avery P, Kamali F, Wadelius M and Group E-P. A randomized trial of genotype-guided dosing of warfarin. *N Engl J Med*. 2013;369:2294-303.
18. Claassens DMF, Vos GJA, Bergmeijer TO, Hermanides RS, van 't Hof AWJ, van der Harst P, Barbato E, Morisco C, Tjon Joe Gin RM, Asselbergs FW, Mosterd A, Herrman JR, Dewilde WJM, Janssen PWA, Kelder JC, Postma MJ, de Boer A, Boersma C, Deneer VHM and Ten Berg JM. A Genotype-Guided Strategy for Oral P2Y12 Inhibitors in Primary PCI. *N Engl J Med*. 2019;381:1621-1631.
19. Mega JL, Stitziel NO, Smith JG, Chasman DI, Caulfield M, Devlin JJ, Nordio F, Hyde C, Cannon CP, Sacks F, Poulter N, Sever P, Ridker PM, Braunwald E, Melander O, Kathiresan S and Sabatine MS. Genetic risk, coronary heart disease events, and the clinical benefit of statin therapy: an analysis of primary and secondary prevention trials. *Lancet*. 2015;385:2264-2271.
20. Khera AV, Emdin CA, Drake I, Natarajan P, Bick AG, Cook NR, Chasman DI, Baber U, Mehran R, Rader DJ, Fuster V, Boerwinkle E, Melander O, Orho-Melander M, Ridker PM and Kathiresan S. Genetic Risk, Adherence to a Healthy Lifestyle, and Coronary Disease. *N Engl J Med*. 2016;375:2349-2358.
21. Ashley EA, Butte AJ, Wheeler MT, Chen R, Klein TE, Dewey FE, Dudley JT, Ormond KE, Pavlovic A, Morgan AA, Pushkarev D, Neff NF, Hudgins L, Gong L, Hodges LM, Berlin DS, Thorn CF, Sangkuhl K, Hebert JM, Woon M, Sagreiya H, Whaley R, Knowles JW, Chou MF, Thakuria JV, Rosenbaum AM, Zaranek AW, Church GM, Greely HT, Quake SR and Altman RB. Clinical assessment incorporating a personal genome. *Lancet*. 2010;375:1525-35.
22. Tang WH and Hazen SL. The Gut Microbiome and Its Role in Cardiovascular Diseases. *Circulation*. 2017;135:1008-1010.
23. Chen R, Mias GI, Li-Pook-Than J, Jiang L, Lam HY, Chen R, Miriami E, Karczewski KJ, Hariharan M, Dewey FE, Cheng Y, Clark MJ, Im H, Habegger L, Balasubramanian S, O'Huallachain M, Dudley JT, Hillenmeyer S, Haraksingh R, Sharon D, Euskirchen G, Lacroute P, Bettinger K, Boyle AP, Kasowski M, Grubert F, Seki S, Garcia M, Whirl-Carrillo M, Gallardo M, Blasco MA, Greenberg PL, Snyder P, Klein TE, Altman RB, Butte AJ, Ashley EA, Gerstein M, Nadeau KC, Tang H and Snyder M. Personal omics profiling reveals dynamic molecular and medical phenotypes. *Cell*. 2012;148:1293-307.
24. Schussler-Fiorenza Rose SM, Contrepois K, Moneghetti KJ, Zhou W, Mishra T, Mataraso S, Dagan-Rosenfeld O, Ganz AB, Dunn J, Hornburg D, Rego S, Perelman D, Ahadi S, Sailani MR, Zhou Y, Leopold SR, Chen J, Ashland M, Christle JW, Avina M, Limcaoco P, Ruiz C, Tan M, Butte AJ, Weinstock GM, Slavich GM, Sodergren E, McLaughlin TL, Haddad F and Snyder MP. A longitudinal big data approach for precision health. *Nat Med*. 2019;25:792-804.
25. Ahmad T, Pencina MJ, Schulte PJ, O'Brien E, Whellan DJ, Pina IL, Kitzman DW, Lee KL, O'Connor CM and Felker GM. Clinical implications of chronic heart failure phenotypes defined by cluster analysis. *J Am Coll Cardiol*. 2014;64:1765-74.

26. Shah SJ, Katz DH, Selvaraj S, Burke MA, Yancy CW, Gheorghiade M, Bonow RO, Huang CC and Deo RC. Phenomapping for novel classification of heart failure with preserved ejection fraction. *Circulation*. 2015;131:269-79.
27. Segar MW, Patel KV, Ayers C, Basit M, Tang WHW, Willett D, Berry J, Grodin JL and Pandey A. Phenomapping of patients with heart failure with preserved ejection fraction using machine learning-based unsupervised cluster analysis. *European journal of heart failure*. 2019.
28. Dor Y and Cedar H. Principles of DNA methylation and their implications for biology and medicine. *Lancet*. 2018;392:777-786.
29. Moss J, Magenheim J, Neiman D, Zemmour H, Loyfer N, Korach A, Samet Y, Maoz M, Druid H, Arner P, Fu KY, Kiss E, Spalding KL, Landesberg G, Zick A, Grinshpun A, Shapiro AMJ, Grompe M, Wittenberg AD, Glaser B, Shemer R, Kaplan T and Dor Y. Comprehensive human cell-type methylation atlas reveals origins of circulating cell-free DNA in health and disease. *Nat Commun*. 2018;9:5068.