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GENETICS (CHRISTOPHER NEWTON-CHEH AND J. GUSTAV SMITH, SECTION EDITORS)

Personalized Medicine and Cardiovascular Disease: From Genome to Bedside

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Abstract Cardiovascular diseases are among the leading causes of morbidity and mortality in the developed world despite advances in cardiac care over the last few decades. Although most of these advances in cardiovascular medicine have been made through trials of therapies on a population scale, the success of such therapies in a patient often is dependent on particular aspects of each individual. In this review, we present the evidence for the impact that the use of personalized medicine and genetics can have in the cardiac care of a patient, first through risk stratification based on inherent genetic risk inferred from the results of genome-wide association studies, to the use of pharmacogenomics to guide clinical management. Finally, we discuss the coming flood of human genome sequencing and the potential impact it will have on the care of patients from the standpoint of cardiovascular disease.

Keywords Cardiovascular disease · Genome · Personalized medicine

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Introduction

Cardiovascular diseases (CVD) are the leading causes of death in the United States, with heart disease and stroke ranking as the first and third leading causes of death, respectively [1]. Past studies have shown that a significant proportion of CVD risk is familial. For example, patients with a first-degree family member with a history of cardiac arrest are at a 50% higher risk of having myocardial infarction (MI) or cardiac arrest themselves [2]. Twin studies have not only shown co-inheritance of CVD, but also of the risk factors for CVD as well [3]. This contribution of family history has been well-accepted as a prominent risk factor now incorporated into several risk scoring systems for MI, such as the Reynolds Risk Score [4].

Large-scale genotyping and sequencing technologies have made it possible now to not only define the components of this heritable CVD risk but also to use it to make meaningful contributions to patient care. The attractive idea of using this genetic information to evaluate patients for disease risk and to optimize medical management tailored to the individual has come to be known as *personalized medicine*.

Genetic information can be divided into three categories: 1) common genetic variation associated with disease risk, 2) pharmacogenomic information in which gene variants are associated with drug response, and 3) rare genetic variation that requires deeper sequencing of individuals. In this review, we discuss aspects of each of these categories.

Common Variations and Cardiovascular Disease Risk

With the advent of DNA genotyping platforms, it has become increasingly easy and cost-effective to genotype

many hundreds of thousands of single nucleotide polymorphisms (SNPs) in large numbers of cases and controls in what has become widely known as a genome-wide association study (GWAS). These experiments have made it possible to catalog a large component of common genetic variation (i.e., variation in the genetic code that is present in a significant proportion of the population, generally defined as 5% or more) and to correlate the presence of these variants with disease. Here we present an overview of the stronger GWAS findings in relation to CVD.

Coronary Artery Disease

One of the most well-studied phenotypes using GWAS continues to be coronary artery disease (CAD). Multiple GWAS have at this point identified over 20 loci that have been associated with CAD in various populations (Table 1), including loci in the genes encoding lipoprotein A [5], the metalloproteinase ADAMTS7, and even the gene that encodes ABO blood type, with increased risk conferred by the allele that results in blood group O [6, 7]. However the most reproduced risk variants associated with CAD continue to be a group of SNPs clustered in the 9p21 locus.

This locus was uncovered quite early in the history of GWAS, with the almost simultaneous discovery of its association with CAD by several groups, including the deCODE group in an Icelandic cohort [8], the Wellcome Trust Case Control Consortium in white cohorts in England [9], and the Ottawa Heart Study group in Canada, also in a white cohort [10]. Since then, the 9p21 locus has also been

Table 1 Select replicated SNP associations with cardiovascular phenotypes

Disease	Region	SNP[a] - allele	Nearest gene(s)	Odds ratio	Population(s)	Context	References
Coronary artery disease	1p13.3	rs599839-A	*CELSR2*	1.29	White, African-American, South Asian	Near Gene 3′	[9, 14, 69, 70]
	1p32.3	rs11206510-T	*PCSK9*	1.15	White	Intergenic	[7, 69]
	1q41	rs17465637-C	*MIA3*	1.20	White	Intron	[7, 9, 69]
	2q33.2	rs6725887-C	*WDR12*	1.17	White	Intron	[7, 69]
	3q22.3	rs9818870-T	*MRAS*	1.15	White	UTR 3′	[7, 71]
	6p24.1	rs12526453-C	*PHACTR1*	1.12	White, South Asian	Intron	[7, 69, 70]
	6q25.3	rs3798220-C	*LPA*	1.51	White	Missense	[7, 72]
	9p21.3	rs1333049-C	*CDKN2A, CDKN2B*	1.47	White, East Asian, African American	Intergenic	[8, 9, 12, 14, 73]
	9q34.2	rs514659-C	*ABO*	1.21	White	Intron	[6, 7]
	10q11.21	rs501120-T	*CXCL12*	1.33	White	Intergenic	[7, 9, 69]
	10q23.31	rs1412444-T	*LIPA*	1.11	White, South Asian	Intron	[70, 74]
	10q24.32	rs12413409-G	*CNNM2*	1.12	White	Intron	[7, 70]
	11q22.3	rs974819-T	*PDGFD*	1.07	White, South Asian, African American	Intergenic	[14, 70]
	15q25.1	rs1994016-C	*ADAMTS7*	1.19	White	Intron	[6, 7, 70]
	19p13.2	rs1122608-G	*SMARCA4*	1.15	White, African American	Intron	[7, 14, 69]
	21q22.11	rs9982601-T	*KCNE2*	1.20	White	Intergenic	[7, 14, 69]
Atrial fibrillation	1q21.3	rs13376333-T	*KCNN3*	1.52	White	intron	[75]
	4q25	rs6843082-G	*PITX*	2.03	White, Chinese	Intergenic	[22, 75–77]
	16q22.3	rs2106261-T	*ZFHX3*	1.25	White	Intron	[76, 77]
Hypertension	16q23.3	rs11646213-T	*CDH13*	1.28	White	Intergenic	[28, 29]
QT interval	1p36.31	rs846111-C	*RNF207*	n/a	White	Missense	[34, 78]
	1q23.3	rs12143842-T	*NOS1AP*	n/a	White	Intergenic	[34, 78]
	3p22.2	rs11129795-A	*SCN5A*	n/a	White	Near Gene 3′	[34, 78]
	6q22.31	rs12210810-C	*PLN*	n/a	White	Intergenic	[34, 78]
	7q36.1	rs4725982-T	*KCNH2*	n/a	White	Intergenic	[34, 78]
	11p15.5	rs2074238-T	*KCNQ1*	n/a	White	Intron	[34, 78]
	16p13.13	rs8049607-T	*LITAF*	n/a	White	Intergenic	[34, 78]
	16q21	rs37062-G	*CNOT1*	n/a	White	Intron	[34, 78]

[a] In cases where multiple SNPs in the same region are associated, the SNP with the strongest effect size was chosen

SNP single nucleotide polymorphism

replicated in other ethnic groups as well, including East Asians and African Americans [11–14]. Interestingly enough, this region has also been found to be associated with intracranial aneurysms, abdominal aortic aneurysms [15], and gliomas [16], implicating a possible unifying vascular phenotype. The mechanism behind how variants at 9p21 specifically impact the risk of CAD remains unclear. Current research has implicated these variants in changes in the expression of the tumor suppression genes *CDKN2A* and *CDKN2B* via changes in putative binding sites of *STAT1* [17•]. *STAT1* itself lies downstream of interferon-gamma signaling, thus providing a connection between CAD and vascular inflammation. This is despite the fact that these genes are over 100 kilobases away from the 9p21 SNP sites. Another intriguing hypothesis involves the expression of the non-coding RNA *ANRIL*, which also lies nearby the 9p21 locus and may itself modulate the expression of *CDKN2A* and *CDKN2B* [18, 19].

The effect size of the 9p21 variant, although being quite reproducible, has been noted to be modest in terms of predicting coronary risk. Pooled odds ratios in a meta-analysis of several GWAS were 1.29 for one disease risk allele and 1.67 for two disease risk alleles [20]. This effect, however, has been found to be independent of all other previously known environmental risk factors, and thus does have potential to further guide risk stratification beyond our current risk prediction guidelines.

Other SNP Associations in CVD

Common variation affecting other cardiovascular diseases has also been extensively studied. Three loci have now been reproducibly associated with the development of atrial fibrillation (Table 1). The most consistent variant appears to be in 4q25, which has now been confirmed independently in at least two studies [21, 22]. Like the 9p21 locus, the variants involved are in a gene desert. The nearest gene is *PITX*, which is involved in the development of the sidedness of the heart and in the differentiation of the left atrium, thus establishing a possible link with the studied phenotype. Other cardiovascular phenotypes for which strong associations have been discovered through GWAS include hypertension [23–29], left ventricular hypertrophy [30, 31], lipid levels [32], and quantitative electrophysiologic characteristics such as the PR and QT intervals [33–35].

Clinical Utility of GWAS Results

What do we do with all the data from these GWAS studies? Although these studies have highlighted many new gene targets and pathways for research, using these data at the bedside for risk prediction for patients has not entered into widespread adoption as of yet. This is partly due to questions in the applicability of the data. Will an SNP associated with CAD in whites be of utility in an African-American patient? Although some of these loci (namely 9p21) seem to cross ethnicities, likely due to being causative themselves rather than being a tag for a causative mutation elsewhere, other loci have not been reproduced as easily in more diverse cohorts, and some have not been reproduced at all, even in cohorts of the same ethnicity.

Another challenging aspect of these GWAS results is that although many of these variants have been found to be statistically significant (with *P* values reaching genome-wide significance on the order of 10^{-6} to 10^{-8}), the effect size of these variants individually has generally been found to be small, with odds ratios generally less than 2. In fact, whereas most variants associated with disease risk have been published with their accompanying odds ratios, this measure of effect size can actually give an overestimation of the inferred increased risk. In terms of clinical utility, it is likely that testing for one variant alone may not be as useful as testing for panels of variants, with the cumulative information more likely to result in risk assessment that is actionable in a clinical setting. In order to more easily estimate cumulative genetic risk, Morgan et al. [36] have suggested the use of likelihood ratios of disease risk instead of odds ratios, which can be utilized easily when combining the effects of multiple variants. This type of estimate can take advantage of the genotypes at several SNPs using Bayes' rule to arrive at a post-test odds of disease (Fig. 1). However, at this time many if not most publications do not provide enough data to calculate likelihood ratios for each variant, which hopefully will change as standards for publishing GWAS data improve.

A few studies have already evaluated the use of SNP panels and the additional information they provide to clinicians regarding disease risk. One such study evaluated the use of a 13 SNP genotyping panel to develop a genetic risk score for CAD [37••]. This score was found to be significantly predictive of CAD, with those in the top quintile having 1.7 times increased risk of CAD as compared to those in the lowest quintile, an effect that was comparable to traditional risk factors such as LDL cholesterol or systolic blood pressure. This effect did not change significantly with adjustment for family history. However, the authors were unable to demonstrate significant reclassification improvement using the genetic score over the use of traditional risk factors and family history by a c-index statistic. Notably, many more variants associated with CAD have since been uncovered, and the use of these additional variants could increase the power of genetic testing.

Despite the challenges, many genotyping services, including those offered by several direct-to-consumer services such as 23andme, Navigenics, and deCODEme,

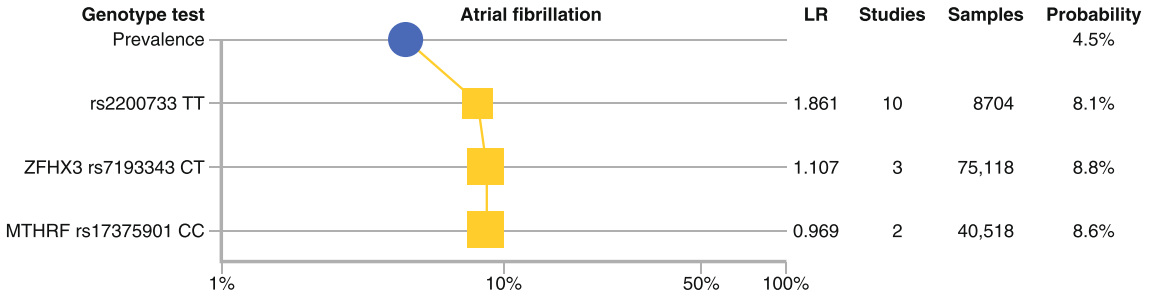

Fig. 1 Representative disease patient "riskogram" for developing atrial fibrillation incorporating genetic information, using likelihood ratios (LR) for three highly reproduced disease-associated single nucleotide polymorphisms (SNPs). The *blue circle* represents the patient's initial pre-test probability and the *yellow squares* represent the patient's post-test probabilities after accounting for genotype at each successive SNP. The probability of developing atrial fibrillation over a 10-year period is displayed on the x-axis in a logarithmic scale

have incorporated GWAS results for many cardiovascular phenotypes such as CAD and hypertension into their reports. There continues to be a need however to study the impact genotyping tests can have on actual patient care in a prospective fashion. One study by Bloss et al. [38•] has shown that the use of direct-to-consumer genotyping did not seem to increase any disease or test-related anxiety to a significant degree, although the results of these tests did not seem to positively affect patient behavior to counteract any additional disease risk inferred from these tests. There are now pilot projects at several centers around the country evaluating clinical outcomes and patient satisfaction after receiving genotyping information. These genotyping tests are quickly becoming widely available, and physicians will need to be well equipped to understand how to use this genetic data in clinical practice.

Pharmacogenomics and Cardiovascular Disease Management

Although the field of genetics informing disease risk is still under development, pharmacogenomics (genomic information relating to how a medication will affect a patient) has already seen active use in the clinical management of patients. While the field of oncology has paved the way with pharmacogenomics-guided chemotherapy regimens, cardiovascular pharmacogenomics is beginning to have an impact on the care of both inpatients and outpatients. We review some of the data behind cardiovascular pharmacogenomics here.

Warfarin

Perhaps the most well proven use of pharmacogenomics relating to cardiovascular disease management is in the use of anticoagulants, specifically warfarin. In 2004, there were more than 30 million prescriptions for warfarin in the United States alone [39], and atrial fibrillation continues to be a leading indication for its use. Warfarin traditionally has been a difficult medication to manage, given the wide variety of factors that can affect dosing. In the past, physician prescribing of warfarin has generally been empiric, evaluating responses to a starting dose and adjusting accordingly, based on a flowchart or sometimes on an individual practitioner's clinical intuition. Errors in dosing clearly can have disastrous results, with life-threatening clots in patients who are subtherapeutic and significant bleeding in those who are supratherapeutic. This has been improved somewhat with the introduction of oral anticoagulation clinics nationwide that have attempted to standardize the management of warfarin dosing.

Over the past decade it has become clear that a considerable amount of the variability of warfarin dosing is due to genetic factors. In 2002, Higashi et al. [40] evaluated the effect of variants in *CYP2C9*, known to metabolize warfarin in the liver, on outcomes from warfarin therapy. They found that patients with at least 1 variant allele had an increased risk of above-range INRs (HR=1.40) and serious or life-threatening bleeding (HR=2.39) [40]. However, variants in *CYP2C9* alone could not explain a significant portion of the genetic heritability of warfarin response. In 2004, another group using linkage studies in rats, mice, and humans identified gene variants in *VKORC1*, which seemed to be the etiology of both warfarin resistance and combined deficiency of vitamin-K–dependent clotting factors type 2 (VKCFD2)[41]. The impact of variation in this gene was confirmed by a study in 2005 in which the *VKORC1* exons were sequenced in 147 patients on warfarin, identifying a common polymorphism in *VKORC1* (1173C>T) that was significantly associated with a higher mean adjusted dose of warfarin [42]. The authors of this study calculated that approximately one third of the genetic variability of warfarin response could be attributed to the known variants in *CYP2C9* and *VKORC1*.

The results of these and other research efforts led an international consortium to develop an algorithm to predict warfarin dosing using both clinical data complemented by genetic information about variants in *CYP2C9* and *VKORC1* based on the characteristics of 4,043 patients [43••]. This group then tested the performance of their algorithm in predicting the correct warfarin dosing in a validation cohort of 1,009 subjects. Their results showed that the algorithm incorporating genetic information outperformed an algorithm based on clinical data only in identifying patients who required dosing of warfarin at the extremes. They further predicted that they would need to genotype only 13.2 patients to avoid one patient being predicted an incorrect dose, defined as 20% larger or smaller than their actual dose. Members of that consortium have since made the algorithm freely available online at http://www.warfarindosing.org.

The US Food and Drug Administration (FDA) has since incorporated references to genetic factors in the prescribing information for warfarin. One prospective study has already found that genotype-guided dose initiation of warfarin reduced hospitalization rates in outpatients [44]. To further validate the use of pharmacogenomics in warfarin dosing on clinical outcomes, the National Heart, Lung, and Blood Institute (NHLBI) has since launched the COAG study, aimed at prospectively evaluating the efficacy of a pharmacogenomic-based dosing algorithm as compared to a clinical-based algorithm with the primary outcome of percentage of time within goal INR [45]. The study is currently ongoing with completion expected in 2012.

Clopidogrel and *CYP2C19*

Another development in the utility of pharmacogenomic information for the management of patients with CVD has centered on the effect of variants in *CYP2C19* (another hepatic cytochrome P450 gene) on the metabolism of clopidogrel and its clinical impact on patients on this medication after coronary stenting. It was first noted in 2004 that patients on clopidogrel after stenting for an ST-elevation myocardial infarction (STEMI) who showed resistance as measured by reduction in ADP-induced platelet aggregation had higher rates of recurrent cardiac events [46]. In this small study, 40% of patients in the highest quartile of resistance to clopidogrel experienced a recurrent event within 6 months, whereas patients in the other quartiles experienced recurrent events only rarely. It was estimated that 25% of all patients showed some element of clopidogrel resistance. The danger of a failure of anti-platelet therapy in these patients is high, as the resulting in-stent thrombosis is associated with a high degree of morbidity and mortality.

Clopidogrel is known to be a pro-drug that requires further activation in vivo by hepatic cytochrome P450 enzymes. Hulot et al. [47] investigated the impact of several candidate variants in the genes for these enzymes, and found that the variant *CYP2C19* 681G>A, since denoted as the *2 variant, which encoded a cryptic splicing site that caused a loss of function mutation, was associated with clopidogrel resistance.

The clinical impact of *CYP2C19*, however, was not fully known until 2009, when Simon et al. [48] evaluated the association of variants in this gene and several others known to be associated with the absorption, metabolism, and biological activity of clopidogrel in a large French registry of acute MI patients receiving clopidogrel. They found that in 1,535 patients who underwent PCI during hospitalization for acute MI, the adjusted risk of death, MI, or stroke in patients with two *CYP2C19* deficiency alleles was 3.58 times that of those with wild-type alleles [48]. Interestingly there was no increased risk seen in those with only one *CYP2C19*-deficiency allele.

This finding was confirmed again in the genetics substudy of the Platelet Inhibition and Patient Outcomes (PLATO) trial, which compared the efficacy of ticagrelor versus clopidogrel for the treatment of acute coronary syndrome. Patients in the clopidogrel arm who had any loss-of-function variant of *CYP2C19* had an event rate of the primary outcome (cardiovascular death, MI, or stroke) of 5.7% versus 3.8% in those without [49]. The effect of the *CYP2C19* variants was not seen in patients in the ticagrelor arm, which the authors put forth as an argument to use ticagrelor in patients with *CYP2C19*-deficiency mutations. However, another study that genotyped patients for variants in CYP2C19 from the Clopidogrel in Unstable Angina to Prevent Recurrent Events (CURE) trial and the Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events (ACTIVE) did not find a significant difference in the efficacy of clopidogrel in patients with and without loss-of-function mutations, although in these cohorts patients with gain-of-function mutations did seem to benefit more from clopidogrel, with an HR of 0.55 in patients with gain-of-function mutations compared to 0.85 in wild-type patients on clopidogrel [50].

In March of 2010, the FDA acted on these study results and added to clopidogrel's drug label information a warning that patients with reduced *CYP2C19* function as identified by genetic testing are at a higher risk for cardiovascular events than those with normal enzyme function while taking clopidogrel. However, the optimal course of action in patients who are found to be resistant to clopidogrel is still unclear. One suggested strategy has been to double the dose of clopidogrel in these patients, although one clinical trial of this strategy did not find any improvement in outcomes [51]. Many advise instead prescribing anti-platelet agents that do not seem to be affected by *CYP2C19* variation such as ticagrelor [49] and prasugrel [52]. Several

commercialized assays for *CYP2C19* genotype are now available, and several health centers including Vanderbilt and the Scripps Clinic, have started to routinely genotype patients for *CYP2C19* variants before initiating dual antiplatelet therapy.

Statin Use and Myopathy

Although the evidence for using pharmacogenomics to guide the use of warfarin and clopidogrel has developed to a critical mass, there is also increasing evidence for the use of genomic information in guiding the use of other drugs to prevent and treat CVD. For example, whereas HMG CoA reductase inhibitors, also known as statins, have been shown to be effective in preventing cardiovascular events, they can induce myopathy and, in severe cases, rhabdomyolysis in a small number of patients. In an effort to try to predict in whom these adverse effects of statins may occur, the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) included a GWAS of 85 cases of statin-associated myopathy along with 90 matched controls, all of European ancestry, and identified a missense SNP rs4149056 in *SLC01B1* that was highly associated with myopathy after statin use [53]. The authors estimated that homozygotes of this mutation had an 18% cumulative risk of myopathy associated with statins within the first year of use, as opposed to 3% in heterozygotes and 0.6% in wild-type. This finding was replicated in a second cohort consisting of the Heart Protection Study. Of note, *SLCO1B1* encodes an organic anion transporter that has a role in hepatic uptake of many drugs, including statins, presenting a biological mechanism behind this association.

PharmGKB Database

As more evidence builds for the use of pharmacogenomics and more genes are implicated in the response to drugs, there arises the issue of how to collect and access this data. One database freely available online is the PharmGKB database, which now contains curated data for more than 2,000 genes that have been implicated in drug response. This database aims to be a comprehensive storehouse of pharmacogenomic data both to assist in further research in pharmacogenomics and to hopefully soon be used as a basis for making effective prescribing decisions using genetic information [54].

Arguably, the pharmacogenomics aspect of personalized medicine is progressing fastest towards clinical application given the larger effect sizes and direct clinical application. Although other studies continue to investigate the effects of gene variants in the management of several other cardiovascular conditions, such as heart failure [55] and hypertension [56–58], many clinical centers are already incorporating genetic data into their prescribing practices, especially for warfarin and clopidogrel. Several commercial genotyping tests have been introduced to the market for many of the variants mentioned in this section as well, and most direct-to-consumer genotyping services include full pharmacogenomic reports. Finally, many pharmaceutical companies are beginning to incorporate genetic studies into their research on candidate drugs in order to develop possible accompanying diagnostics for these drugs, as well as to rescue drugs that did not show efficacy in study populations as a whole but may very well be effective in subpopulations with the right genetic background.

Moving Beyond GWAS: The Missing Heritability Problem, Rare Variants, and Other Sources of Genetic Variation

As evidenced by Table 1, the list of associations between genetic loci and various cardiovascular diseases is extensive and continues to grow through the completion of more GWAS studies along with the combining of past GWAS in large meta-analyses. At the time of writing, the NHGRI catalog of GWAS contained 4,523 SNPs with statistically significant associations with some disease or trait [59].

However, it is quite clear at this point that the common variants as discovered through GWAS have fallen short of explaining the large proportion of heritability of most diseases. For example, a recently published study estimated that common SNPs in total likely explain only 21% of the variance of the QT interval seen in the population at large whereas the total heritability of the QT interval is estimated to be somewhere between 37% and 60% [60]. The percent of heritability of early-onset MI explained by the nine genetic loci of common variation that had been discovered by 2009 was only 2.8% [61]. This is partially explained by the small effect sizes of the disease associations seen thus far, with the majority of GWAS associations having odds ratios ranging from 1.2 to 2.0. And even more concerning is the unclear biological significance of these polymorphisms, given that thus far more than 80% of disease or traits associated SNPs have been located in non-coding regions of the human genome [59], making it difficult to ascertain the mechanisms behind these associations.

These findings beg a question that continues to raise controversy in the genetics community: where is the missing heritability? Many theories abound, including the possibility of SNP–SNP interactions that could amplify the effects of multiple risk alleles. Others have argued that perhaps our estimates of heritability are erroneous due to confounding environmental factors. One theory gaining momentum is that perhaps much more of the genetic basis

of disease than previously thought is a result of rare variants, defined generally as those having a minor allele frequency of <1%. These rare variants have not been included on common genotyping platforms and are only now being discovered at a rapid rate through the development of next-generation sequencing (NGS) technologies that are able to perform massively parallel sequencing of millions of short reads (75–150 base pairs) at a time, enabling the sequencing of entire genomes in days, at costs that are dropping at a shockingly rapid rate. Currently, the 1000 Genomes Project is attempting to catalog the extent of both common and rare variation in human genomes through a coordinated multi-center sequencing project that is now moving past the pilot phase, releasing increasingly larger amounts of data to the public domain about human genetic variation [62]. Armed with these data and NGS technology, a new era of genomic discovery is here that allows us to evaluate the impact of rare variation on disease.

One specific recent study has already provided some credence to the idea that rare variation may indeed be a significant driving force behind genetic disease risk. Holm et al. [63•], using a GWAS in an Icelandic cohort, initially found an association between sick sinus syndrome and a locus of common SNPs at 14q11. Using whole genome sequencing to further interrogate the locus, they uncovered a rare variant in *MYH6*, c.2161C>T, coding for a p. Arg721Typ mutation in linkage with the previously found SNPs that fully explained the entirety of the disease association. This mutation had a frequency of 0.38% in the population studied, but had an odds ratio of 12.53 with a P value of 1.5×10^{-29}, representing one of the most impressive associations of a variant with a complex disease uncovered thus far. The authors estimated that patients with this variant had a lifetime risk of sick sinus syndrome of 50% as compared to 6% in non-carriers. This study and others like it have led the NHLBI to call for the use of NGS to further investigate the impact of rare variation on complex disease.

In addition, whereas the majority of studies thus far have focused on the impact of SNPs, other types of genetic variation exist but remain less well studied. These sources of variation include small insertions and deletions (1–20 base pairs, generally), larger insertions and deletions (often referred to copy number variants), as well as copy-neutral variants such as inversions. Investigation into the impact of these variants has only been made possible on a large scale recently through the use of NGS technologies. Finally, epigenetics, the study of heritable variation that is not encoded by DNA (such as histone modification), is a rising field whose impact on disease is only now beginning to be explored. Only time and further research efforts will tell if the solution to the missing heritability problem lies in one or many of these sources of genetic variation.

Bringing the Whole Genome to the Clinic

Although the pace of research in finding disease association with genetic variants continues unabated, bringing these discoveries to the clinic has been slower. In an effort to demonstrate the utility of genomic information in the clinic, our group performed a clinical assessment of a white patient with a family history of vascular disease and sudden death incorporating information from their whole genome [64••]. Through an analysis of 2.6 million SNPs and 752 copy number variants found in the patient's genome, the patient's risk of complex diseases such as MI, type 2 diabetes, prostate cancer, and others was estimated using approaches incorporating both common and rare variants. The patient's response to drugs was also evaluated, finding that the patient would likely need reduced dosing of warfarin, and that he was likely a poor metabolizer of clopidogrel. The patient was also found to have rare variants in three genes associated with sudden cardiac death, one of which was predicted to be damaging, which was an intriguing finding given the patient's family history. The patient was noted to be at high risk for premature CAD due to the presence of several common variants such as one in the Lp(a) locus mentioned previously, and was noted to have the wild-type version of the *SLCO1B1* variant, denoting a beneficial response to statins without an increased risk of myopathy. The clinical team was thus able to incorporate this genomic information to better assess the patient's disease risk, resulting in a decision to start a statin in a patient with borderline indication by classical risk assessment. Interestingly, the patient's genome also revealed an increased risk of hemochromatosis and pituitary tumors that would not have been known from his family history. This type of analysis of a patient's genome will become more and more common as the price of genome sequencing drops. As of last year, there were more than 2,700 human genomes sequenced, and current trends indicate that by the end of 2011 that number will explode to greater than 30,000 [65]. Physicians will need to become familiar with interpreting this genetic information in order to provide optimal management of patients.

Future Challenges for Personalized Medicine

Clearly, challenges remain in applying genomic data to patients. The majority of GWAS studies have been performed in white cohorts, and due to differences in linkage disequilibrium patterns between ethnicities, applying these results to other ethnicities may be questionable. The effect sizes of the currently known GWAS associations have tended to be small, and the potential of interactions between variants causing disease is as of yet undetermined.

In terms of rare variants, it can be difficult to determine the significance of a rare variant that has not been seen previously. Several algorithms currently exist to attempt to predict the pathogenicity of a particular variant, using different aspects such as evolutionary conservation and the effects of predicted amino acid changes, the more commonly used ones being SIFT [66], PolyPhen [67], and GERP [68]. However it is clear that the predictions from these algorithms do not correlate well (Ashley, unpublished data), and their optimal use in estimating the pathogenicity of rare variants remains unclear.

From a technological standpoint, as the cost of sequencing continues to drop dramatically, the costs of computing, interpretation, and data storage will likely become the biggest hurdle towards effective use of whole genomes. And the ethical issues surrounding whole genome sequencing are not trivial. At this time it is still debatable as to whether true informed consent for whole genome testing is actually possible. Regulatory issues also exist, as the FDA will have to decide as to how to certify laboratories performing genotyping and sequencing for clinical purposes.

Conclusions

Despite the challenges, the tools now exist to study the impact of the genome on human disease and to take advantage of this information to improve the clinical management of patients. A revolution in medicine is occurring, allowing us to take the genome from the bench to the bedside for the first time in human history. As research efforts in genomic medicine progress, physicians must be poised to adapt quickly to this new era and to develop the skills and background needed to use this information to make decisions for the optimal management of patients with cardiovascular disease, both for risk stratification and the tailoring of medical interventions. This new era of personalized medicine has the promise to finally bring the focus of modern medicine on to the individual patient.

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