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Genetic testing in cardiovascular diseases

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Abstract

Purpose of review—The review is designed to outline the major developments in genetic testing in the cardiovascular arena in the past year or so. This is an exciting time in genetic testing as whole exome and whole genome approaches finally reach the clinic. These new approaches offer insight into disease causation in families in which this might previously have been inaccessible, and also bring a wide range of interpretative challenges.

Recent findings—Among the most significant recent findings has been the extent of physiologic rare coding variation in the human genome. New disease genes have been identified through whole exome studies in neonatal arrhythmia, congenital heart disease and coronary artery disease that were simply inaccessible with other techniques. This has not only shed light on the challenges of genetic testing at this scale, but has also sharply defined the limits of prior gene-panel focused testing. As novel therapies targeting specific genetic subsets of disease become available, genetic testing will become a part of routine clinical care.

Summary—The pace of change in sequencing technologies has begun to transform clinical medicine, and cardiovascular disease is no exception. The complexity of such studies emphasizes the importance of real-time communication between the genetics laboratory and genetically informed clinicians. New efforts in data and knowledge management will be central to the continued advancement of genetic testing.

Keywords

cardiovascular disease; congenital heart disease; gene testing; genetics; genomics

INTRODUCTION

In the past 12 months or so, clinical gene testing has begun to grow exponentially as next-generation sequencing technologies penetrate the market. Cardiovascular disease has long been at the forefront of gene testing in the clinic, and this trend is likely to continue. This review will outline the current state of the art in clinical genetic testing, highlight recent advances in addressing several of the core clinical questions in the field, touch on some of

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Conflicts of interest

Dr. MacRae receives royalty payments for gene testing in the cardiomyopathies.

the most interesting new disease genes identified in the past year and discuss the emerging role of whole exome and whole genome sequencing in the clinic.

PRACTICAL GENETICS IN CARDIOVASCULAR DISEASE IN 2014

Germline genetic testing (as opposed to the somatic mutation detection now driving therapeutic choices in many cancers) is often criticized for the low likelihood that a given genotype will change management in most clinical contexts [1,2]. This is to some extent a function of the context in which genetic testing is most commonly used [3]. Cardiovascular disease is one setting in which genetic testing has a maturing clinical role. The utility of genetic testing in cardiovascular disease primarily derives from the high risk of sudden death in many of the Mendelian syndromes and the effectiveness of the implantable defibrillator as a preventive strategy for this risk, irrespective of the underlying biology.

Perhaps because of the impetus for therapy in a potentially lethal set of disorders, it is also in cardiology that the perils of genetic testing may be most apparent [2]. Great care must be taken to avoid too rapid an attribution of causality to a specific DNA variant, which then might assume the status of a diagnostic test. Without rigorous evidence of pathogenicity, which in the case of a known disease gene would minimally require co-segregation with disease in multiple affected family members, genetic test results are essentially inconclusive almost irrespective of the predicted effects on protein structure [4]. Circular reasoning is all too often deployed in small families in which imperfect clinical data and imperfect genetic data appear to bolster each other. If detailed phenotyping is not completed on the entire kindred, and if subtle nondiagnostic, but clearly abnormal, phenotypes are dismissed, then the interpretation of uncertain genotypes is not likely to be feasible. Notably, many clinical approaches to risk estimation in inherited cardiac disease also suffer from the failure to account for familial confounding [5]. Indeed, one of the challenges for modern genetic testing is to demonstrate incremental value for risk prediction over a simple set of clinical tools and quantitative estimates of family risk. Recent work from population cohort studies has identified variants that were previously definitively classified as pathogenic, but demonstrated that in some circumstances these do not result in any pathological phenotype [6]. This emerging recognition that genotype is far from deterministic even in disorders in which the effect sizes are large is likely to color the debate on genetic testing in many conditions.

Nevertheless, in dedicated Cardiovascular Genetics centers where there is informed interaction between the clinician and the laboratory, consistent interpretative standards can be attained and definitive genotypes can be used to drive cascade screening [7]. Such ongoing bidirectional information flow is an important paradigm for the clinical application of genetics in other fields and highlights a need for more extensive infrastructure to support this type of family-based translational medicine. Rigorous criteria will need to be established for the classification of variants, and new approaches to family-based diagnostics using clinical tools and genetic tools will have to emerge to make this approach a reality [7].

Genetic testing focused on traditional panels of known genes can also contribute to cardiovascular disease understanding in many other ways. In many inherited cardiovascular

disorders, investigators have attempted to refine diagnostic categories for decades, almost always employing combinations of simple morphologic criteria from electrocardiography and echocardiography or tomographic imaging. There is accruing evidence that the traditional cardiomyopathy or arrhythmia nosologies do not describe uniform clinical entities. Current efforts to reclassify diseases based on their molecular underpinnings will require consensus standards for variant classification and the collection of large and unselected cohorts [8]. It remains to be seen whether a molecular nosology will be any more successful than traditional nosology at defining distinctive prognostic or therapeutic groups.

Finally, the holy grail of molecular diagnostics would be the identification of subsets of disease where there are pathway-specific therapies available that might reverse the disorder or even prevent the disease phenotype ever from being expressed. Although, at present, there are few specific therapies for inherited heart disorders other than enzyme replacement therapy for some enzyme deficiencies or phlebotomy for hemochromatosis [9], there are a number of imminent clinical trials tailored to very specific genotypic groups within inherited heart disease syndromes. As novel disease genes are identified, so drug development programs or repurposing will continue and this rationale for genetic testing is likely to dominate in the foreseeable future [10].

Misconceptions in the clinical community

The complexity of modern genomics will require considerable investment in the education of frontline practitioners. Given the breadth of clinical decision-making that will require genotypic insight, it is unlikely that this will devolve to increasingly subspecialized physicians, but, rather, all physicians will need to become familiar with the interpretation and practical application of genetic data [1]. The realization that genetic test results are not deterministic is only now permeating guidelines for the management of inherited heart disease [11]. Many of the perceived uncertainties around genetic testing are a consequence of this misunderstanding and the failure to appreciate the importance of pretest probabilities in the interpretation of genetic test results [1].

MAJOR ADVANCES IN GENETIC TESTING IN 2013

For the majority of cardiovascular disease syndromes, current gene panels identify causal variants only in a proportion of individuals, and so, not surprisingly, novel disease genes continue to be identified. Perhaps, the most important single advance in the past year has been the availability of next-generation sequencing technologies to allow gene identification of de-novo mutations underlying severe pediatric-onset phenotypes [12, 13, 14]. For many of these conditions, the morbidity and mortality associated with the phenotype would previously have precluded successful reproduction, so traditional family-based gene identification would not be feasible.

New disease genes for Mendelian disease

Using whole exome sequencing, George and colleagues were able to identify mutations in two of four human calmodulin genes that cause severe neonatal arrhythmias [13]. Independent work from a second group identified mutation in calmodulin1 in a large family

with catecholaminergic polymorphic ventricular tachycardia like syndrome and sudden death [14]. Subsequent work has suggested markedly reduced affinity for calcium as a shared mechanism, though the specific downstream processes that contribute to the proarrhythmia are not yet understood [13].

Similar approaches have begun to unravel the genetic basis of severe forms of congenital heart disease (CHD). Using exome sequencing in proband–parent trios in a series of severe CHD cases and controls, a consortium of investigators observed a substantial excess of damaging de-novo variants in genes that encode proteins involved in histone modification, including *SMAD2* [15]. These data suggest that mutations in such genes may be a cause of as much as 10% of all CHD, though much larger cohorts will be required to characterize the specific casual variants and their phenotypic correlates. Other groups are focusing on identifying subclinical phenotypes in parents that might facilitate the definition of extended kindreds and accelerate the attribution of pathogenicity [16].

As genomic databases are populated, so in-silico gene identification is becoming possible. Using extant deletion boundaries and phenotypic descriptions in the 1p36 syndrome, it proved possible to establish a list of potential cardiomyopathy genes within the deletion [17]. Subsequent work in other cohorts with left-ventricular noncompaction and dilated cardiomyopathy established the gene encoding the transcriptional co-activator *PRDM16* as a cause of these diverse myocardial abnormalities. Rapid modeling of some of the disease-causing mutations in the zebrafish defined disordered cardiomyocyte proliferation and coupling [17].

Another disease gene identified using exome sequencing is *PRKG1*. Mutations in this cGMP-dependent protein kinase were identified in multiple families with thoracic aortic aneurysms and dissections [18]. It is speculated that the mechanism likely involves smooth muscle contractile function, and further implicates this cell type in these forms of vasculopathy.

New phenotypes for specific genes

Perhaps one of the major insights from human molecular genetics in recent years has been the limited specificity of traditional clinical phenotypes. Nowhere is this more obvious than in cardiovascular disease, in which syndromes previously characterized as discrete have proven to be manifestations of the same underlying pathophysiologic mechanisms. While the dissemination of genetic testing has led to increased recognition of so-called overlap syndromes, with features of multiple different cardiac and vascular disorders, there are still insufficient data of adequate rigor to establish a molecular classification of myocardial or vascular disease. Further expansion to include extracardiac phenotypes will only add to the complexity of the genotype–phenotype correlation matrix.

As more extensive studies are undertaken, the breadth of phenotypes associated with a specific phenotype becomes more obvious. *SMAD3* mutations were first described in 2011 as a significant cause of thoracic aortic aneurysms and aortic dissections, in the setting of quite marked premature osteoarthritis [19]. Additional studies of *SMAD3*-associated kindreds have revealed a quite aggressive form of vasculopathy with common

cerebrovascular involvement and infantile aortic disease in some cases [20–22]. These findings extend the recent observations of complex TGF β signaling and possibly oligogenic interactions in regional vascular disease [23]. The availability of TGF β pathway functional assays may help to clarify the clinical management of this diverse set of disorders [24].

This year also saw the description by several independent groups of yet another distinct syndrome associated with mutations in the SCN5A gene, encoding the major subunit of the cardiac sodium channel. In a subset of individuals, mutations in SCN5A that would be predicted to generate increased Purkinje cell automaticity are associated with high-grade ventricular ectopy and reversible dilated cardiomyopathy [25, 26]. This addition of yet another syndrome to the pantheon of SCN5A-associated disorders may reflect improving phenotypic resolution as much as any new biology [27].

Together these overlap phenotypes argue for less gene panel-focused genotyping in cardiovascular disease. However, until the impact of prior probability on the specificity of genetic test results is better understood, a graded approach to the analysis of gene panels is likely to be more rigorous. Ultimately, the resolution of genotype–phenotype relationships will also be impacted by phenome-wide interrogation of genetic space, which has been pioneered by the Vanderbilt group [28].

Multiple variants

As the use of genetic testing and the number of genes being tested in cardiovascular disease expand, so the detection of multiple rare variants in an individual becomes more likely. Several studies have made a case for an individual's burden of variation as a potential risk factor for more penetrant or more severe disease [1,29–31]. Although certainly plausible, the issues with attribution of causality that attend even a single potentially pathogenic variant in a known gene serve to illustrate just how difficult it may be to prove such an association [2,4]. The majority of such reports fail to reconcile the data with the known variable penetrance and pleiotropy in most inherited heart diseases, far less the patterns of segregation in the kindreds reported. These issues are only compounded when the extent of missense variation in the human genome revealed by next-generation sequencing is considered [32].

The extent to which biological validation is necessary is illustrated by the interesting case of a single family in which two private heterozygous mutations in two distinct genes in the nitric oxide signaling pathway, GUCY1A3 and CCT7, were implicated in the susceptibility to coronary artery disease [33]. In-vitro assays suggested synergistic effects to diminish soluble guanyl cyclase activity, while platelets from digenic mutation carriers also exhibited less soluble guanylyl cyclase protein and reduced nitric oxide-inducible cGMP. Finally, mice deficient in a 1-sGC protein had rapid thrombus formation in the microcirculation after local trauma. Despite extensive efforts, it remains difficult to extend these observations beyond a single family, but it may be that, as next-generation sequencing and more robust pathway-specific functional assays are deployed more widely, these findings will be found to be more generalizable.

Modifiers

A sound framework within which to consider multiple variants is that of genetic modifiers of the primary genotype. The high prevalence of variation in penetrance and pleiotropy in most inherited heart disease is a testament to not only the selection pressures at play in disorders with prominent premature sudden death, but also the likely genetic architecture of the underlying modifiers. Despite the inference that common variation is a major contributor of modifier loci, to date, studies have been confounded by the allelic heterogeneity of the primary causal mutations. In elegant work exploiting a KCNQ1 founder mutation in the long QT syndrome in South Africa, it has proven possible to identify at least one modifier of this potassium channel disorder, NOS1AP genetic variation [34]. This successful study also highlights the scale of investigation in human studies and in animal models that will likely be necessary to dissect modifier genes for disorders with the genetic and allelic heterogeneity seen in cardiovascular disease. The collection of such large multiethnic kin-cohort studies with quantitative phenotyping, definitive identification of causal mutations and genome-wide testing for common polymorphism, including copy number variation, has barely begun [35].

Testing of common loci

One final area of development in genetic testing has been the slow emergence of the clinical use of common alleles for disease risk or pharmacological response identified through genome-wide association studies. While the risk estimates for such alleles are by their very nature more precise than those of rare variants, to date, it has proven difficult to implement testing of these loci in medical care. This is a function of the lack of prospectively collected lifetime risk estimates, the absence of objective evidence of added value over existing risk factors and the perceived need for randomized controlled trials to guide implementation. It is notable that to date the major trials of testing for drug response variants (e.g. for Coumadin) have failed to demonstrate clear benefit [36, 37]. Interestingly, the magnitude of the effects observed is close to that of many existing clinical risk factors that are in widespread use in situations of clinical equipoise. The recent edict from the US Food and Drug Administration (FDA) to restrict the claims of direct to consumer genetic testing of common variants suggests that this will be an active area of debate in 2014 [38].

CLINICAL APPLICATION OF NEXT GENERATION SEQUENCING

Next-generation sequencing is currently the primary technology employed by the majority of commercial providers used for cardiovascular gene testing in North America. Typically, these providers will employ capture reagents that cover the entire exome, though for analytic purposes only prespecified panels of genes are studied. In order to generate CLIA-certified results, laboratories have until now had to confirm these data using gold standard Sanger sequencing [39]. The recent CLIA certification of the Illumina next-generation platform promises to change the scope of testing and the scope of reporting, in particular, for consensus actionable variants [40].

Caution must be taken in this new era as large-scale exome and genome-wide sequencing projects have demonstrated large numbers of de-novo null alleles and missense mutations in

each individual, in addition to the much more substantial numbers of previously unidentified segregating rare variants. These findings place immense importance on the development of robust approaches to defining pathogenicity, which at present is poorly understood for the vast majority of disease genes [4,41]. The hurdles to be overcome are best exemplified by the finding in population cohorts of established bona-fide pathogenic variants in known cardiomyopathy disease genes, but in the absence of any detectable phenotype [6]. These data suggest that a much more sophisticated understanding of genetic and environmental modifiers will be necessary for the apriori interpretation even of large effect size variants. As noted above, the study of human disease modifiers is currently in its infancy, and, with one or two notable exceptions, the modifiers described are family-specific. Continued interaction between the laboratory and the clinician, as well as a systematic change in focus from the individual patient to the extended family, will be vital to the practical application of genomic cardiology.

CHANGING RATIONALE FOR GENETIC TESTING

As genetic testing in cardiovascular disease continues to mature, so the rationale for such testing continues to evolve. New disease genes add to our mechanistic understanding of the biology of the disorders. Building a picture of the comprehensive genetic architecture of each syndrome will eventually aid in diagnosis or prognostication, as well as drive the basic science that generates novel therapies. While clinical gene identification is currently of utility mainly in the restriction of cascade screening efforts, this will change as pathway-focused therapies become available [2]. Genetic testing in hypercholesterolemia, which previously had been of no obvious clinical importance given the precision of risk prediction with plasma cholesterol measurements and the empiric nature of subsequent management [42], is likely to become much more prevalent if PCSK9 inhibitors demonstrate efficacy [43]. Similarly, imminent clinical trials in subsets of hypertrophic cardiomyopathy (e.g. genotype positive–phenotype negative individuals or those with RAS-MAP kinase pathway mutations), dilated cardiomyopathy (e.g. lamin A/C mutations) or even allele-specific silencing may soon mandate genetic testing in these disorders.

CHALLENGES THAT REMAIN

The rapid growth of genetic testing in cardiovascular disease is an important paradigm for the broader application of genetic testing across medicine. The core challenges that stand in the way of a vision for genomic medicine are nowhere more obvious than in heart or vascular disease, where the initial presentation may often be an avoidable sudden death.

Chief among these challenges is the need for objective criteria for pathogenicity of individual variants, for without precise risk estimates decision-making on subsequent interventions (each of which has its own risks) cannot be rational. Integrating rare and common variation to generate prospective data across large numbers of incident cases will require a transformation of the scale of human investigation. Unless such studies are incorporated into the fabric of medical care, it is hard to see how the relevant data can ever be collected at adequate scale. Cardiovascular genetics and medicine in general must move beyond individual investigator-driven efforts to ‘learning’ health systems and the era of big

data. Parallel efforts in biological assay development will also be vital, and in many instances it will be important to redefine the clinical syndromes themselves to favor quantitative objective endpoints. Ultimately, integrating clinical data, comprehensive genotypes and pathway-focused functional assays is likely to be essential for genomic medicine to be fully realized [44]. This systems-level understanding of cardiovascular biology will also require transformation of the information flow to and from the bedside, an area in which the impact of decades of resistance to information technology by healthcare providers is beginning to become all too apparent.

CONCLUSION

Genetic testing is emerging from tertiary care centers and is now beginning to reach routine clinical use. Patients are already ordering direct to consumer testing, and as data accrue so these tests will assume greater importance in clinical cardiology. The pace of new gene discovery and translation to the clinic is accelerating. The impact on the cardiovascular practitioner is still uncertain, but is closely aligned with major changes in other areas of clinical practice including care redesign, the medical home and digital health.

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