Genetic Risk Factors & Cardiovascular Disease

Lead Article

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The genetics of cardiovascular disease: from genotype to phenotype

R. Sanders Williams, MD; Pascal J. Goldschmidt-Clermont, MD

Duke University Medical Center - Durham, NC - USA

Genetics plays only a minor role in the current practice of clinical cardiology, despite a widespread appreciation of the familial nature of common cardiovascular disorders. Recent discoveries have elucidated the molecular basis for several heritable forms of cardiomyopathy, dysrhythmia, accelerated atherosclerosis, and structural malformations of the heart, but patients with these conditions are rarely encountered except in a few specialized referral centers. However, the completion of the Human Genome Project and advances in large-scale genome technologies have raised expectations that the determination of genotypes in individual patients will soon become germane to decisions made every day by busy clinicians. Beyond question, recent advances in cardiovascular genetics have provided powerful new insights into disease mechanisms, and a burgeoning literature attests to the enthusiasm with which cardiovascular investigators are seeking to define relationships between genotypes and phenotypes in common, as well as rare, forms of heart disease. This review provides a critical framework within which the clinical cardiologist can understand and evaluate the specific advances in cardiovascular genetics most likely to have practical value for patient management in the near term.

SELECTED ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>CAD</td>
<td>coronary artery disease</td>
</tr>
<tr>
<td>FH</td>
<td>familial hypercholesterolemia</td>
</tr>
<tr>
<td>LOD</td>
<td>log odds ratio (score)</td>
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<tr>
<td>LQTS</td>
<td>long QT syndrome</td>
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<tr>
<td>MCIP</td>
<td>modulatory calcineurin interacting protein</td>
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<tr>
<td>MLP</td>
<td>muscle LIM protein</td>
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<tr>
<td>QTL</td>
<td>quantitative trait locus</td>
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<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
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<tr>
<td>TLR</td>
<td>toll-like receptor</td>
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Keywords: genome; genotype; phenotype; cardiomyopathy; atherosclerosis; hypertension

Address for correspondence: R. Sanders Williams, Dean, Duke University School of Medicine, Box 2927 DUMC, Durham, NC 27710, USA
(e-mail: willi397@mc.duke.edu)

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In the decade that follows the completion of the Human Genome Project, the primary goals of cardiovascular genomics will be different (Figure 1). The challenge now is to determine the value of genetic diagnosis in the prevention and treatment of the common forms of coronary artery disease, hypertension, heart failure, and arrhythmia that are the leading causes of death and hospitalization in industrialized nations (Figure 2). Any experienced clinician knows that individual patients categorized under the same diagnosis may exhibit a quite different clinical course of disease and may respond variably to the same therapy. Genetic differences among individuals account for much of this variation. The lifestyle habits we as cardiologists recommend on the basis of epidemiologic data to prevent cardiovascular disease have value, but likely are ineffective or unnecessary for many individuals (and perhaps even harmful to some) based on genetic variations we currently cannot discern or interpret correctly. In principle, a comprehensive understanding of the specific genetic variables that influence an individual’s risk for cardiovascular disease, the rate of progression of disease, and the likely responses to specific preventive or therapeutic measures should foster the formulation of individualized health maintenance plans, with profound public health benefits. Moreover, insights gained from a greater understanding of genotype:phenotype relationships in common as well as rare forms of cardiovascular disease should foster drug discovery or biotechnological innovations that will add to our therapeutic armamentarium.

The next era of cardiovascular genomics, therefore, will be dedicated mostly to the study of polygenic disorders, complex traits, and genetic variations that modify responses to environmental stresses or different therapies. Knowledge gained from past studies of rare monogenic disorders has heuristic value in this quest, and will be discussed in the first section of this review. An overview of progress in the more complex task of defining specific genetic variants that increase risk for, or modify the course of, common cardiovascular diseases will follow.

Proponents of a massive investment in cardiovascular genomics argue that genotyping, in conjunction with measurement of traditional clinical variables, will provide to clinicians a much more refined ability to stratify individuals with respect to future risk for morbid events, and to develop individualized prevention and treatment plans (Figure 3). How realistic is this goal? We will examine the assumptions that must prove true if genetic medicine is to fulfill the expectations of its advocates with respect to the daily practice of cardiology.
SINGLE-GENE DISORDERS THAT CAUSE CARDIOVASCULAR DISEASE

Atherosclerosis

Early death from myocardial infarction or stroke, based on accelerated atherosclerotic vascular disease, is a prominent feature of inherited abnormalities of lipoprotein metabolism, of homocysteine and mucopolysaccharide accumulation, and in kindreds with familial forms of diabetes or hypertension.

Familial lipoprotein disorders were well characterized long before the availability of genetic mapping techniques, and precise genetic defects leading to disease have now defined the specific biochemical and clinical phenotypes classified prior to the genomic era (Table I). The prototypic abnormality is familial hypercholesterolemia (FH), which results from mutations in the gene encoding the low-density lipoprotein (LDL) receptor. A large number of different mutations in this gene can produce a similar phenotype, the severity of which correlates with functional properties of the mutant receptor protein and the number of defective alleles (heterozygous and homozygous forms). Mutant alleles are relatively common within the US population (1 in 500) and heterozygotes manifest approximately 2-fold increased risk for coronary heart disease prior to age 60. Individuals who carry 2 mutant alleles have the devastating phenotype of full-blown FH with early death from advanced atherosclerosis. Particular LDL receptor defects that are characteristic of ethnically or geographically isolated populations can sometimes be traced back in time through many generations to a single individual (founder) who transmitted the disease-causing allele to many descendants. In FH, the biochemical phenotype (elevated LDL cholesterol) can be measured easily and individuals homozygous for a defective allele almost invariably manifest the phenotype (high penetrance). A similar phenotype results from familial defects in apolipoprotein B-100, the ligand for the LDL receptor. Genotyping provides a definitive diagnosis and may be useful for genetic counseling, but the incremental value of genetic testing over biochemical measurements of plasma lipoprotein levels for clinical decision-making usually is low. Accelerated forms of atherosclerosis are also encountered with other disruptions of metabolic pathways, such as in homocysteinuric and mucopolysaccharidoses due to α- and β-hydroxysterol 11β-hydroxylase deficiency.

Several heritable abnormalities of blood pressure regulation are attributable to specific gene defects that have been defined over the past decade. The phenotypes can be severe, and include both hypertension and hypotension. Hypertensive individuals manifest affect-

Table I. Mendelian disorders influencing coronary heart disease risk factors.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Disease (gene)</th>
<th>Characteristics</th>
</tr>
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<tbody>
<tr>
<td>Increased LDL-C</td>
<td>FH (LDLR)</td>
<td>Dominant, CHD</td>
</tr>
<tr>
<td></td>
<td>Familial-defective apo B-100</td>
<td>Dominant, CHD</td>
</tr>
<tr>
<td>Decreased HDL-C</td>
<td>Apo A-I defect</td>
<td>Recessive, CHD</td>
</tr>
<tr>
<td></td>
<td>Tangier disease (ABC 1)</td>
<td>Recessive, CHD</td>
</tr>
<tr>
<td>Diabetes (type 2)</td>
<td>MODY 1 to IV (TFs, glucokinase)</td>
<td>Dominant, early-onset type 2 diabetes mellitus</td>
</tr>
<tr>
<td>Hypertension</td>
<td>GRH (11β-OH-AS)</td>
<td>Dominant, HBP, stroke</td>
</tr>
<tr>
<td></td>
<td>Liddle’s syndrome (NaCh)</td>
<td>Dominant, HBP, alkalosis</td>
</tr>
<tr>
<td></td>
<td>Mineralocorticoid receptor defect</td>
<td>Pregnancy-associated HBP</td>
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Abbreviations: apo, apolipoprotein; CHD, coronary heart disease; FH, familial hypercholesterolemia; GRH, glucocorticoid-remediable hypertension; HBP, high blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; LDLR, low-density lipoprotein receptor; MODY, maturity-onset diabetes of the young; NaCh, sodium channel; TF, transcription factor; 11β-OH-AS, 11β-hydroxylase and aldosterone synthase.
teins that, directly or indirectly, act to regulate renal salt reabsorption and therefore net salt balance and vascular volume homeostasis. A series of elegant genetic studies have attributed the genetic basis for Bartter syndrome, Gitelman syndrome, and Liddle syndrome to ion channels or transport proteins in the thick ascending limb, the distal collecting duct, and the cortical collecting tubule of the renal nephron, respectively.8,9 Other mutations map to enzymes involved in synthesis of mineralocorticoids. A particularly interesting and severe form of genetic hypertension is caused by a missense mutation in the gene encoding the mineralocorticoid receptor, which alters its selectivity among different steroid hormones while retaining its signaling properties. In these patients, high concentrations of progesterone during pregnancy act as agonists of the mutant mineralocorticoid receptor and exacerbate the hypertensive phenotype.10

### Cardiomyopathy

Genetic cardiomyopathies lead to sudden death, arrhythmia, angina pectoris, or heart failure, or may be detected in asymptomatic individuals on the basis of an abnormal physical exam, EKG, or imaging study. Defects in approximately 15 specific genes are known to be responsible for Mendelian inheritance of hypertrophic or dilated cardiomyopathies (Table II).11 In a manner similar to the surprisingly narrow functional category into which genes that cause familial hypertension fall, investigators in the cardiomyopathy field were surprised by the observation that the genes responsible for hypertrophic cardiomyopathy uniformly encode proteins of the sarcomere.12 Moreover, almost all of the culprit mutations exert their deleterious effects in a dominant manner not by haploinsufficiency (loss of function of the product mutant allele), but by incorporation of mutant proteins into the contractile apparatus. The presence of a mutant protein within the sarcomere, even when the corresponding normal protein is also present, is sufficient to trigger the hypertrophic response, which takes a variety of anatomic forms in which hypertrophy is symmetric or asymmetric within the left ventricle. Defects in force generation at a molecular level are linked to abnormalities in calcium metabolism that activate signaling cascades leading to hypertrophy.

Hypertrophic cardiomyopathy can progress to a dilated ventricular phenotype at end stage, but some genetic abnormalities result in primary forms of dilated cardiomyopathy that do not pass through a detectable hy-
pertrophic stage, at least at the gross level. Cellular hypertrophy, however, is a common feature of dilated cardiomyopathy. Interestingly, different alleles of the same gene (cardiac troponin T, cardiac actin, or cardiac β-myosin heavy chain) can cause hypertrophic or dilated forms of cardiomyopathy, while mutations within other genes exclusively lead to only one form of cardiomyopathy.

Even though disease phenotypes in kindreds with heritable forms of cardiomyopathy are often severe and lethal at an early age, individuals within the same kindred, or unrelated individuals bearing an identical mutant allele, may exhibit markedly different phenotypes. Such variable penetrance of a monogenetic disorder presents both a diagnostic and a clinical challenge, and is instructive with respect to the future goal of identifying polygenic variations responsible for complex traits. An interesting, and still unanswered, question pertinent to hypertrophic cardiomyopathy is whether clinical management is best guided by phenotypic or genotypic classification. We know that the severity of the hypertrophic phenotype is an unreliable predictor of morbid events, since certain kindreds carrying specific mutated alleles display high rates of sudden death despite only moderate hypertrophy. Knowledge of the specific genotype can have tangible clinical value, since certain mutations have been associated in multiple kindreds with extremely high risk for lethal events. However, most mutant alleles causing hypertrophic cardiomyopathy do not have such clear predictive value. In some kindreds, individuals known to harbor a disease-causing allele remain asymptomatic and without anatomic manifestations of cardiac hypertrophy in middle age. As more cases and kindreds with hypertrophic cardiomyopathy have been identified in recent years, the dire prognosis suggested previously in most of the literature on this subject appears to reflect selection bias, and in many cases an abnormal genotype and even moderate degrees of ventricular hypertrophy may be compatible with a normal lifespan without symptoms attributable to hypertrophic cardiomyopathy.

Close surveillance, forced alterations of lifestyle, implantable defibrillators, or pharmacologic treatment of asymptomatic individuals on the basis of a mutant genotype in one of the genes associated with hypertrophic cardiomyopathy appear to be justified currently only in those bearing one of the particularly malignant alleles, or when family members have suffered sudden cardiac death. Longitudinal follow-up of phenotypically normal patients bearing mutant alleles is fragmentary at present, but will ultimately be required to answer this question.

Lethal arrhythmias complicate all forms of cardiovascular disease, but constitute a primary abnormality in the absence of detectable structural heart disease in several heritable syndromes, for which the genetic basis has been determined. LQTS, Brugada syndrome, and Lenègre disease are now clearly attributed to defects in sodium and potassium channels (HERG, SCN5A, KVLQT1) of the cardiomyocyte. Atrial fibrillation and preexcitation syndromes (Wolff-Parkinson-White) have been attributed to mutations in the \textit{PRKAG2} gene, which encodes a regulatory subunit of AMP-activated protein kinase.

As in hypertrophic cardiomyopathy, the question arises as to whether clinical management of such patients should be best guided by a phenotypic or genotypic classification. Mutations within the same gene that encodes a cardiac sodium channel (SCN5A) are the cause of conditions that would be classified either as LQTS, Brugada syndrome, or Lenègre disease based on morphology of EKG repolarization waves. Such phenotypic diversity among patients harboring defects in a single gene is partly a function of discrete mutations affecting different domains within the protein, but other factors are at work as well, since a single mutant allele can be responsible for LQTS or Brugada syndrome phenotypes in different individuals within the same
kindred. At present, genetic diagnosis and our level of understanding of biophysical properties of cardiac ion channels that are altered by specific mutations are insufficient to predict the clinical phenotype, or to provide confident stratification of risk of sudden death. Certain mutations that produce LQTS phenotypes appear to carry a benign prognosis in some kindreds, but sudden death has been noted in a single individual within large kindreds who otherwise appear to have only electrocardiographic abnormalities. Genetic diagnosis and more extensive clinical outcomes data on individuals and families with LQTS and related disorders suggest that the ominous clinical course seen in many of the kindreds first reported is not a universal feature of patients with mutations in these genes. Yet broader application of genotypic diagnosis and more refined clinical phenotyping, possibly including provocative tests, should lead to more rational selections of patients to receive implantable defibrillators or specific pharmacologic therapies.

Congenital malformations of the heart, valves, and great vessels

In addition to the hypertrophic and dilated cardiomyopathies already discussed, the genetic basis for several heritable disorders that deform cardiovascular structures has been defined. Marfan syndrome, like hypertrophic cardiomyopathy and LQTS, is a well-recognized cause for sudden death during exertion in young persons, including some prominent athletes. A defect in the fibrillin gene, which encodes a protein important for the physical integrity of connective tissues, leads to dilation of the proximal aorta and aortic valve regurgitation. Death results from aortic rupture. Williams syndrome, characterized by supravalvular aortic stenosis and a specific form of mental retardation, is attributed to defects in the gene encoding elastin. Holt-Oram syndrome includes prominent defects of cardiac septal and limb deformities, and is caused by mutations in TBX5, which encodes a transcriptional regulatory protein. Mutations in a transcription factor called Nkx2.5 have been noted in patients with tetralogy of Fallot, atrial septal defect, and congenital atrioventricular conduction block. Defects in mitochondrial DNA can also be associated with cardiomyopathy and conduction abnormalities.

Complexity of genotype:phenotype relationships in monogenic disorders

The identification of each precise genetic defect that can cause a specific cardiovascular disease has been an achievement of considerable importance. These discoveries have brought clarity to our understanding of pathobiology, provided definitive diagnostic tools, and in some cases opened avenues to novel therapeutic measures. This discovery process is ongoing, and we can expect the lists of well-characterized genetic disorders that affect the cardiovascular system to continue to grow. Some common themes emerging from these studies are instructive as we consider the future of genetic medicine.

A large number of different genetic variations in a single gene can produce a similar disease phenotype. Over 150 different mutations in the LDL receptor gene cause familial hypercholesterolemia, and more that 50 different mutations in the β-myosin heavy chain gene cause familial hypertrophic cardiomyopathy. In the case of FH, these mutations result in loss of function of the relevant protein, and can be compensated, at least in part, by the presence of a normal LDL receptor allele on the sister chromosome. In familial hypertrophic cardiomyopathy, mutant proteins act in a dominant manner as poison polypeptides, and many different variants of the protein lead to the pathological consequences of the disorder. An implication of these observations is that future studies of polygenic disease traits and modifier genes will face a daunting level of complexity on the basis of the sheer number of genetic variations in any single gene that may be pertinent to any given phenotype.

Defects in several different genes encoding structurally distinct proteins can produce a similar phenotype. This principle is illustrated both by LQTS, the muscular dystrophies, and familial hypertrophic cardiomyopathy, where individuals who are indistinguishable by clinical criteria may harbor defects in many different genes. Such genetic diversity can be understood when functional relationships between proteins encoded by the different disease gene are apparent, as in the observations that genes causing hypertrophic cardiomyopathy encode sarcomeric proteins, but such functional relationships may not be evident when genetic variants associated with disease phenotypes are first discovered. Defects in a single gene can produce different disease phenotypes, and a mutant allele may cause severe and even lethal disease in some individuals, but not in others. In some cases, an identical mutation can produce different disease phenotypes in different individuals, or produce no apparent disease at all. Such profound phenotypic differences can occur even within closely related individuals of a single kindred. The implication of this principle for future studies of polygenic...
disease traits is that the genetic background in which a specific allele is expressed can have a profound effect on the ultimate phenotype, even when the mutant allele is capable of great mischief. As our search for variant alleles that modify the natural history of cardiovascular disease turns toward alleles that have much less potent capabilities for influencing the phenotype, the difficulty in discerning causal relationships between genotype and phenotype will be even greater.

Thus, recent studies of highly penetrant disease genes in humans have produced invaluable insights, and set the stage for future studies germane to the patients encountered daily by practicing clinicians everywhere. Some cautionary principles are evident, however, and we should not expect easy or rapid success in forging the genetically based medicine to which we aspire.

**Lessons from model organisms**

The field of cardiovascular genetics and genomics provides many examples of how the rapid information flow between clinical and basic investigators can produce new insights of high value. Following the identification of a mutant allele associated with a disease phenotype in humans, the well-developed state of techniques to manipulate the genome of experimental animals often affords the opportunity to introduce the same mutation into an animal model. For cardiovascular disorders, efforts of this nature have focused largely on the mouse, but the value of zebrafish, fruit fly (Drosophila), worm (Caenorhabditis elegans) and even slime molds (Dyctostelium) or yeast (Saccharomyces cerevisiae) also are apparent, and useful models have been created in larger mammals like the rat or rabbit. Such studies help to establish causation in a rigorous manner, and to enhance understanding of pathobiology. In addition, animal models of cardiomyopathy, hypertension, atherosclerosis, and arrhythmias are invaluable for preclinical testing of novel therapeutic measures.

Animal models may confirm hypotheses proposed on the basis of human data, but sometimes produce unanticipated and valuable new insights. An interesting recent example is provided by the observation that a mouse model of muscular dystrophy based on loss of function of the delta-sarcoglycan gene develops cardiomyopathy, not from cardiomyocyte dysfunction per se, but from myocardial ischemia based on coronary vasomotor dysfunction. Cardiomyopathy in this model could be prevented by chronic administration of a calcium channel antagonist.

Models of human disease produced by genetic manipulations also empower the discovery process in human genetics. For example, the gene encoding the homeodomain transcription factor Nkx2.5 became the focus of human studies and was found to be associated with atrial septal defects and conduction abnormalities in humans because its important role in cardiovascular development had been previously defined in animal models. Nkx2.5 proteins in humans and other mammals are closely related to a gene in *Drosophila* called tinman, defects of which prevent formation of the *Drosophila* heart (the muscular dorsal vessel of the insect). Thus, a discovery emerging from fruit fly genetics and developmental biology of the mouse heart led directly to the discovery of a cardiovascular disease gene in humans.

Another advantage of animal models of inherited cardiovascular defects is the opportunity to study such defects within a stabilized (inbred) genetic environment, as opposed to the ever-changing genetic milieu that characterizes inheritance of human traits. Such opportunities will be explored further in the next section.

**POLYGENIC DISORDERS (TRAITS) AND GENETIC VARIANTS THAT MODIFY THE COURSE OF CARDIOVASCULAR DISEASE**

**A challenging opportunity**

Opinion surveys show that the public believes that the greatest opportunity provided by the completion of the sequencing of the human genome resides in the diagnosis and treatment of dreadful conditions like cancer and heart disease. This perceived opportunity was ranked above the discovery of genes that extend lifespan. Eric Lander, a pioneer of modern genetics, was quoted to say: “The Human Genome Project aims to produce biology’s periodic table, the prime organizer of the chemical elements. Not 100 elements, but 100 000 genes.” However, whereas the periodic table of Mendeleev was truly universal, the genetic code appears to contain a multitude of inconsistencies. It is estimated that, out of the three billion base pairs that constitute the human genome, in excess of two million can vary. Such variants, or single nucleotide polymorphisms (SNP), are believed to account not only for the wonderful heterogeneity within the human species, but also for susceptibility, or relative resistance, to common forms of cardiovascular disease. The tracking of the variants that confer susceptibility to heart disease may provide unprecedented diagnostic tools and targets for drugs and devices that could revolutionize...
cardiovascular medicine as we know it. It was said “This is just halftime for genetics, the game started around 1900 and the really interesting second half is about to begin.” This point of view is intriguing, the challenges of linking disease phenotypes with genetic variants may require an effort that is orders of magnitude beyond what it took to get to the current state of genetics.

Lessons from model organisms

We ended our discussion of monogenic disorders by reference to animal models, and we introduce the section on polygenic disease traits using a few examples from recent investigations that have employed model organisms as diverse as mice and yeasts. One principle to be illustrated is that a genetic variation may have a profound influence on the course of disease, without necessarily having any bearing on the events that initiate the disease (Figure 1). Moreover, a genetic variation without any discernable consequence in the absence of a disease-causing stress can mean the difference between life and death once a disease process has been initiated by another cause. Another important principle is that variant alleles in multiple genes may have important consequences that are evident only in combination with each other. In other words, the potentially important role of a genetic variant in a given gene for modifying the course of disease may be discernable only in combination with a genetic background in which specific variant alleles of other genes are present. Furthermore, a potentially dreadful combination of gene variants can become harmless because of the presence of one or multiple variants whose effect is to confer resistance to a given disease process. It is our opinion that the future of cardiovascular genetics and genomics is likely to be dominated by genes that exhibit these principles. It is important to continue the discovery of mutations in single genes that are sufficiently powerful to constitute the proximate cause of disease. However, the genetic information most pertinent to the everyday practice of medicine, and to rational and individualized plans for health maintenance, is likely to come largely from a greater understanding of the combinations of genetic variants, both susceptibility and resistance variants, that influence the course of disease.

The study of cardiomyopathy has been advanced by numerous laboratories that have generated models by manipulating the mouse genome. Several dozen different genes, when overexpressed or disrupted, produce phenotypes that closely resemble hypertrophic or dilated cardiomyopathies in humans by morphologic, biochemical, and clinical criteria. These genes include the murine orthologues of human genes responsible for familiar hypertrophic and dilated cardiomyopathies, but extend to many genes encoding components of signal transduction pathways capable of provoking hypertrophy and heart failure. The degree to which specific conclusions drawn from such studies are directly and strictly pertinent to human disease remains to be established, but principles illustrated by such studies are almost certainly relevant.

A large body of literature implicates abnormal metabolism of calcium as a central feature of most, if not all, forms of cardiac hypertrophy and heart failure. Several groups have presented data that support a mechanistic model that implicates calcium-regulated protein kinases (calmodulin-dependent protein kinases) and phosphatases (calcineurin) that signal through specific transcription factors (NFAT, MEF2, GATA4, PGC-1, and others) to activate target genes, the altered transcription of which drives the hypertrophic process. Sustained and unrelenting activation of these pathways ultimately leads to heart failure and death from ventricular arrhythmias in several murine models. The protein phosphatase calcineurin occupies a nodal point in this mechanistic model, and is subject to regulation by a family of endogenous inhibitors called MCIP proteins (modulatory calcineurin interacting proteins) (Figure 4). One member of this gene family, MCIP1, is itself upregulated when calcineurin is activated by the appropriate changes in intracellular calcium, thereby establishing a negative feedback loop to limit further increases in calcineurin activity. The expression of MCIP1 is increased in animal models of cardiac hypertrophy, but is insufficient to block the hypertrophic process from progressing to heart failure in death. Transgenic animal models in which the level of MCIP1 expression is increased show no apparent consequences in the absence of hypertrophic stimulus, but are protected from hypertrophy, arrhythmia, and death evoked in genetic, pharmacologic, or pressure-overload models of cardiac hypertrophy. Thus, MCIP1 provides an example of a gene, the variant function of which produces no obvious phenotype in unstressed animals, but hyperfunction of which protects against deleterious consequences of cardiac hypertrophy.

Other instructive studies illustrate the ability of genetic variations unrelated to causation of disease to prevent heart failure in murine models. A deficiency of a cytoskeletal protein called MLP (muscle LIM protein) results in a severe form of dilated cardiomyopathy in mice, and MLP protein expression is reduced in failing hearts from humans. The lethal phenotype associated
with MLP deficiency in mice, however, can be prevented by genetic manipulations that reduce the function of phospholamban, a protein of the sarcoplasmic reticulum that regulates the calcium transport protein SERCA2. Likewise, a genetic variant engineered to inhibit the function of the β-adrenergic receptor kinase can prevent heart failure in this model.

These models of cardiovascular disease generated in transgenic mice, and their prevention by additional mutations introduced into mouse genome, provide mechanistic information useful for selection of candidate genes to be interrogated for meaningful polymorphic variations in human genetic analyses. Model organisms including mice can be bred in a manner to allow the phenotypic consequences of specific genetic variations to be discerned against a background where other genes are identical (inbred strains). This measure of control is impossible in human studies. Nevertheless, such studies illustrate principles pertinent to the design of human investigations. “Forward” genetics refers to the process of searching for the culprit gene for a given trait. “Reverse” genetics refers to the process of altering the function of a given gene, by overexpression, constitutive activation, other mutations, or deletion, in an animal model (vertebrate or invertebrate), and then performing functional and structural analyses of the resulting phenotype. With the latter process, discoveries can be made upon altering the genetic milieu of the experiment (switching the type of inbred mice, for example), which in turn can reveal alteration in phenotype. Quantitative trait locus (QTL) analysis can be used to identify genes, and variant(s) thereof, that “modify” the penetrance and/or manifestation of the original gene defect.

Such modifiers have been sought by our colleagues at Duke. Using transgenic mice that develop severe dilated cardiomyopathy due to the cardiac-specific overexpression of calsequestrin, marked strain-specific variation of cardiac function and survival was found, independent of transgene expression. Reciprocal backcross strategy was employed, using two inbred strains showing distinct differences in survival and cardiac function, to map the genes that modified the heart failure phenotype. Genome-wide scan for linkage was used to identify two loci significantly linked to survival with a maximum likelihood ratio statistic of 36.2 (LOD score approximately 7.8) on chromosome 2 and of 26.5 (LOD score approximately 5.7) on chromosome 3. The chromosome 3 locus was also significantly linked to cardiac function with a maximum likelihood ratio statistic of 42.9 (LOD score approximately 9.3). Such an approach should allow for the rapid identification of candidate genes involved in disease susceptibility in human populations and could bring new insights into the pathogenesis of heart failure and other cardiovascular ailments.

Recent studies in yeast models also have direct bearing on the future of cardiovascular genomics. Investigators mapped genes responsible for the quantitative...
ability of yeast to grow at higher temperatures, a determinant of pathogenic potential in human infections. The study revealed an unexpected complexity in the architecture of a QTL. Three different alleles within a single locus were found to be essential for the effect, and no single or double variants were associated with the phenotype. The authors suggest appropriately that, combinations of both common and rare variants are likely to underlie quantitative traits and the number of genes could be far greater than expected. A more comprehensive approach to QTL dissection therefore needs to include one in which multiple genes can be tested individually and in combination.36

Methods for studying the genetics of complex cardiovascular disease in humans

Association studies of candidate genes. Outside of the work on inherited monogenic diseases already described, most of the existing literature that describes relationships between specific genetic variations in the human population and cardiovascular disease has come from association analyses that focus attention on a small number of candidate genes. In this research design, polymorphic variations, either simple nucleotide repeats or single nucleotide polymorphisms, are defined by DNA sequencing or other methods within transcribed, intronic or flanking regions of genes that encode proteins known or hypothesized to contribute to the pathobiology of the disease phenotype in question.

Clinical variables are measured to classify individuals as affected or unaffected, or to subdivide subjects into multiple categories of disease severity. Statistical methods assess the probability that an association between genotype and phenotype observed in this manner is greater than that attributable to chance alone and the predictive value of the genetic test exceeds that provided by clinical variables alone.

We will subsequently describe several examples of interesting and potentially important information that has been garnered by this approach. The ingredients of success include: (i) good guesswork in selecting the candidate genes and the specific polymorphic sites within the genes for interrogation, (ii) the accuracy and detail in which clinical phenotypes are defined, (iii) the economy and scale of the allelotyping technology, and (iv) the number of subjects available for study. This approach is inherently expensive and prone to failure, and promising findings from small studies must be confirmed in larger and different populations.

Whole-genome scanning

The association studies described in the previous section are based on the biased selection of gene(s) believed to contribute to the disease process on the basis of biological and/or clinical data. Such biased selection represents an inherent weakness of the approach, as it is fair to say that for monogenic traits that have been elucidated, the culprit gene that was eventually identified had not, in most instances, been linked to the disease process in prior investigations. Hence, it is usually accepted that a nonbiased and far-reaching approach would be preferable. Technical advances make it feasible now to define allelic variants within all of the ≈40 000 genes of the human genome with a high degree of precision in large numbers of human subjects within a brief period of time. The costs of such efforts are falling to levels (millions but not hundreds of millions of dollars) that are in reach of both industrial and academic investigators.

A recent review37 included a succinct and salient discussion of the requirements for success in whole-genome scanning studies to identify genetic variants that contribute meaningfully to the incidence and progression of cardiovascular disease:

An alternative to dependence on good guesswork would be an unbiased method for scanning the complete genome for polymorphisms that confer increased risk. The extent of linkage disequilibrium in the human genome is a critical determinant of the probability of success for this approach, and current estimates range from around 5-10 kb38 to about 60 kb.39 The number of single nucleotide polymorphisms (SNPs) required for whole-genome scans is unclear, and the estimates range downward from an upper limit of around 500 000.34 For genetic variants that confer low relative risk (eg, 1.5) and are of low-to-intermediate allele frequency (0.1-0.3) in the population, very large numbers of patients and controls (of the order of thousands rather than hundreds) would be necessary to have a good chance of detecting an effect.40 It is evident that genotyping of this magnitude (2×10^9 genotypes for 2000 patients and matched controls at 500 000 SNPs), at present day costs, is technically and financially challenging. Selection of appropriate candidate SNPs would reduce this number substantially, but the basis for choosing the candidates is unclear.37

Other systematic prioritization of genes

In principle, we would like to identify all variants of all genes and assay them for their contribution to some aspects of cardiovascular disease. However, this is unrealistic at the present time due to a lack of knowledge of the complete gene set of the human genome and
considering our limited ability to identify variants on this scale and assay them to generate proof of concept for their contribution. Thus, in practice, one must take an approach that falls somewhere between a study restricted to known candidates vs an investigation of the whole-genome complement of genes. Such an approach should involve prioritization of candidate genes based on programmatic qualification mechanisms. The identification of candidate genes through a multifaceted approach that involves unique expression profiling assays as well as identification of loci through genetic studies is under way. A major challenge for these efforts is the development of functional assays that have the ability to measure the contribution of gene variants to the disease phenotype. If the relative contribution of any one variant is small, then the cohort of studied patients must be extremely large to uncover and reveal the contributions. Such determinations are done by association studies, measuring the contribution of allelic variants to the disease extent. But again, if the contribution of any one variant is small, then the ability to measure an association is dependent on the study of large numbers of patients. Perhaps the most critical and challenging aspect of these efforts will be the mathematical and statistical tools that will be needed to interpret the collected data.

For example, if the effects of gene variants are indeed subtle, the strategies for gene identification that are based on observing major changes in the expression of genes may overlook those genes that play a critical role in the pathway that controls cardiovascular function or any other phenotype subject to subtle, but significant variation. Thus, assays using DNA arrays or other methods to score for major induction of genes, even if genes are scored as coinduced, may not reveal the full extent of the changes of significance. An alternative approach to this problem is to analyze gene expression data obtained from vascular tissue and search for patterns of gene expression that can provide discrimination of the phenotypes of disease progression in the tissue samples. Gene groups can be identified, based on Bayesian regression analyses, which distinguish cardiovascular tissue samples that are otherwise very similar. In our experience, the genes within the groups that provide the discrimination are often expressed at a rather low level, and would not be scored in simple comparisons, but in fact make significant contributions to the discrimination. Presumably, these genes represent components of interacting pathways and it is the sum total of expression of these genes, taken together rather than in isolation, that provide the determining molecular phenotype. Thus, with the help of new computational methodologies, it has become possible to identify patterns and groupings of gene expression that distinguish cellular phenotypes, such as the progression of vascular disease. Understanding of the genes that constitute those discriminatory groupings then provides a rationale for focusing on a new class of candidate genes in which variation, either due to amino acid change or slight alterations in expression, might in effect impact the ultimate phenotype of cardiovascular disease.

**Recent progress in risk stratification using genetic variables**

While the strategy outlined in the previous section is likely to lead to information that will be clinically relevant, to date, there are no genetic tests that can be recommended with confidence as useful to clinicians taking care of cardiac patients. Nevertheless, some important lessons have been learned through research on novel variants. Some examples will be provided to illustrate the current state of the field, and the complexities that have emerged in the process of trying to use gene variants that were selected intuitively as diagnostic tools (biased approach). Our goal is not to provide an exhaustive list of such SNPs and their studies in the cardiovascular field (Web sites where such exhaustive lists can be found are provided, see Table III), but instead, to use instructive examples as a way to review the challenges of the biased approaches.

**Advances in genetic epidemiology**

Atherosclerosis is a paradigm for complex, multifactorial, ailments that affect humans in an age-dependent fashion. Atherosclerosis is a chronic inflammatory process that affects selectively arterial vessels, and is, at least in part, genetically programmed. Early-onset (premature) coronary artery disease (CAD), defined as

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**Table III.** Web sites for listings and further information on single nucleotide polymorphisms (SNPs).

- [http://genecanvas.idef.insERM.fr](http://genecanvas.idef.insERM.fr)
- [http://cardiology.stanford.edu/reynolds>All_projects/project_2.htm](http://cardiology.stanford.edu/reynolds>All_projects/project_2.htm)
age of onset less than 50 years of age, is known to have a particularly strong genetic component. A family history of CAD is one of the most robust risk factors for CAD, even after adjustment for environmental risks that may be shared within families. Several studies have identified that the relative risk (RR) of developing CAD in a first-degree relative (sib) is between 3 and 12, depending upon the age of onset in the proband.\(^1\) The risk of premature CAD in monozygotic and dizygotic twins has been measured.\(^41\) For men, the relative risk of death from CAD before the age of 55 was >8 for monozygotic twins and >3.5 for dizygotic twins.\(^41\) For women, the corresponding relative risks for death from CAD before age 65 were >15.0 for monozygotic twins and >2.5 for dizygotic twins.\(^41\)

Unstable coronary syndromes and adverse events following coronary intervention, well-known consequences of atherosclerosis of the coronary vessels, are typical examples of the interplay between genetic and environmental factors. Platelet activation is a prerequisite for such thromboembolic events, and is dependent upon exposure to disrupted endothelium, circulating cytokines, and other agonists released during plaque rupture or vascular injury during angioplasty. At the paroxysm of platelet activation, the surface receptor glycoprotein (GP) IIb/IIIa (integrin \(\alpha_{IIb}\beta_3\)) changes its conformation, leading to its higher-affinity binding to extracellular ligands such as fibrinogen and von Willebrand factor (engagement of the receptor), and consequent aggregation of activated platelets. Antagonists for GPIIb/IIIa have proven helpful for patients with coronary thromboembolic events. Hence, it was conceived that a number of genetic polymorphisms within the subunits of such platelet receptors may contribute to gain or loss of function, thereby predisposing some individuals to thrombotic events. Although the mechanism of platelet activation and thrombus formation is generally well established, much controversy remains whether individual platelet polymorphisms contribute to an increased likelihood of ischemic events.

Due to the substitution of a cytosine for a thymidine at position 1565 in exon 2 of the \(GP\text{IIIa}\) gene, the platelet antigen 2 (\(\text{PI}^2\)) variant (also referred to as HPA1b) displays a proline instead of a leucine at amino acid 33.\(^42\) The structural change in GPIIb/IIIa that is induced by the \(\text{PI}^2\) variant is the cause for a severe form of neonatal alloimmune thrombocytopenia. It is also responsible for cases of posttransfusion purpura. \(\text{PI}^2\) has been implicated in arterial thrombosis and in the development of unstable coronary syndromes. A higher prevalence of the \(\text{PI}^2\) allele has been reported in patients with unstable angina or myocardial infarction and in siblings of patients with a history of premature ischemic heart disease. Mikkelsen et al reported a higher prevalence of \(\text{PI}^2\) among victims of sudden cardiac death whose coronary arteries contained thrombus.\(^43\) Still other studies have not confirmed the association between \(\text{PI}^2\) and MI, maintaining the controversy over the influence of the \(\text{PI}^2\) polymorphism.\(^44,45\) A significant concern, which is illustrated by studies of \(\text{PI}^2\), relates to the fact that negative studies are less likely to be published, and since negative studies more often involve larger cohorts of patients, the process leads to a bias of the literature on the SNP (a process that has been described as the "funnel" effect).\(^46\) Meta-analyses of the data can be useful, but also carry additional bias due to selection of surveyed studies. Three meta-analyses have been conducted for \(\text{PI}^2\), two showed a weak association of the SNP with CAD and complications thereof, and one did not.\(^47\) Despite the variance in epidemiologic data, most studies examining the molecular effect of the \(\text{PI}^2\) polymorphism on GPIIb/IIIa function have been consistent, showing that the mutation results in increased platelet responsiveness.\(^48\)

Thus, in spite of its molecular effect on the structure of GPIIb/IIIa, illustrated by the immune consequence of the SNP and data on the function of the receptor, the anticipated consequence for \(\text{PI}^2\) on clinical events could not be established unambiguously. There are many other examples involving proteins that are central to CAD, including the insertion/deletion polymorphism of the angiotensin-converting enzyme or the fibrinogen B3 448 polymorphism, which are associated with a change in function of the gene in question, and yet, a clinical consequence of the variant could not be established unambiguously. Many explanations have been advanced to explain the controversies of genetic epidemiology. The first study often suggests a stronger genetic effect than is found by subsequent studies. Both bias and genuine population diversity might explain why early association studies tend to overestimate the disease protection or predisposition conferred by a genetic polymorphism. A frequent caveat of these studies consists of the difficulty of matching accurately the patient cohort with a control group. At times imperceptible differences between case and control lead to overestimation of the contribution of a SNP to a given phenotype.

Another complexity of genetic epidemiology is the evolving treatment of the patients over time. Thus, if a SNP has a real impact on a disease process, it is high-
ly possible that empirical treatments that have been supported by randomized trials may indeed address the effect of the SNP on the disease process. For example, PlA2 has been shown to modify the response of platelets to established antagonists, such as aspirin, and in some instances, the benefit for patients of well-known therapeutic strategies.42,49 Hence, the association of the SNP once measured to be significant for patients developing acute thrombosis following angioplasty may become undetectable with more efficient antithrombotic prevention.

In spite of its growing pains, genetic epidemiology is a science that likely will make substantial contributions to cardiovascular medicine. A recent example illustrates how genetic epidemiology can provide support to novel theories on atherosclerosis and its etiology. Atherosclerosis is known as a unique inflammatory process that affects exclusively the arterial tree. Our ability to mount a successful inflammatory response to bacterial pathogens requires an efficient innate immune defense, however, the high efficacy of such defense may, in turn, contribute an increased risk of atherosclerosis.

Genetic variants of toll-like receptor 4 (TLR4) confer differences in the inflammatory response elicited by bacterial lipopolysaccharide and are related to the development of atherosclerosis.50 Thus, as compared with subjects with wild-type TLR4, subjects with the Asp299Gly TLR4 SNP have lower levels of proinflammatory cytokines, acute-phase reactants, and soluble adhesion molecules, such as interleukin-6 and fibrinogen, all biomarkers that are known to indicate a heightened risk for atherosclerosis and thromboembolic consequences. These individuals are also more susceptible to severe bacterial infections, but appear to display a lower risk of atherosclerosis. Hence, the Asp299Gly TLR4 polymorphism, which attenuates receptor signaling and diminishes the inflammatory response to Gram-negative pathogens, is associated with a decreased risk of atherosclerosis. This finding, if confirmed by future studies, supports the hypothesis that innate immunity may play a part in atherogenesis, and consequently helps advance our understanding of the pathogenesis of atherosclerosis.

CONCLUSION

A concept that has helped us organize our thought process relative to the impact of gene variants on common forms of chronic cardiovascular disease consists of a “bin model” illustrated in Figure 5. Accordingly, many chronic ailments can be reduced to a series of bins, where individual patients can be categorized according to the advancement of their own disease process. Each individual bin corresponds to a significant quantum of advance for the disease process. Such passage can occur with a defined probability for a given individual. We believe that the advance of the genomic field will help measure, once interdigitated with clinical databases, most of the relevant probabilities for various chronic ailments. Concurrent with the discrete passage from one bin to the next, is a continuous biological process that characterizes the cardiovascular tissue and the surrounding organism at each stage of the disease. As patients pass from one clinical bin to the next, it is anticipated that their health care cost would increase.

Abbreviations: ACE-I, angiotensin-converting enzyme inhibitor; CABG, coronary artery bypass grafting; ICD, implantable cardioverter-defibrillator; PCI, percutaneous coronary intervention.
biased approaches described in previous sections, and validated by large-scale genetic epidemiologic studies, it may become possible to individualize the estimation of the risk of passing from one bin to the next. Typical examples of bins for a common trait such as CAD would include the preclinical stage, stable angina, acute coronary syndromes, heart failure, and sudden versus other forms of cardiac deaths. Of course, concurrent with the discrete passage from one bin to the next, is a continuous biological process that characterizes the cardiovascular tissue and surrounding organism at each stage of the disease. The variants, or groupings thereof (metahaplotypes), that validate as contributing significantly to the prediction of passing from one bin to the next, likely belong to genes that control known, or yet to be discovered, pathways that drive the underlying biological process. Further integration of the genetic risk with environmental influence could be accomplished through the collection of nongenetic biomarkers such as inflammatory cytokines, other markers of inflammation and stem cell availability, as well as metabolites that reflect the driving biological pathways. The prioritization of such markers could be done in a fashion quite similar to that used to rank genes and their variants, with ranking based on their contribution to the prediction of probability for passing from one bin to the next. Eventually, it is the interdigation of such a multidimensional data matrix that will provide the tools that clinicians can use to individualize risk assessment for individual patients, and offer them a “health plan” that would be best suited for their individual needs. This type of approach will, in the long run, significantly improve the societal opportunity for life sheltered from the damaging effects of common cardiovascular ailments, and in doing so, optimize the cost-effectiveness and safety of recommended diagnostic, preventive, and therapeutic strategies.

We are grateful to our many colleagues from our laboratories and from around the world who have contributed to the ideas presented here.

THREE KEY QUESTIONS

Knowledge in the field of cardiovascular disease genetics is advancing so rapidly that by the time this issue of Dialogues in Cardiovascular Medicine reached its audience, there was a very real risk that new discoveries, new answers, and new questions would have upstaged part of this issue’s offerings. This is why the Editors took particular care to pose three questions that are likely to have considerable staying power. The main focus, which links the three questions, is on pharmacological prospects. Thus, in the face of the current profusion of existing or impending genetic tests, Ian Day asks: “In what situations is genetic testing currently useful for management of individual patients to prevent or treat cardiovascular disease?” Alistair Hall, reflecting on the fact that the present-day manifestations of cardiovascular disease are grounded in a genetic makeup resulting from the 5-million-year history of human evolution, asks: “In what manner are advances in genetics most likely to alter the clinical practice of cardiology within the next 10 years?” Finally, Hugh Watkins shares his thoughts on the prospects of pharmacogenetics ushering in a more evidence-based approach to cardiovascular pharmacological therapy, and asks: “Should genetic testing become a standard component of clinical trials in order to advance evidence-based medicine cardiology practice?” His answer is a qualified yes: we are not quite there yet, but getting very close.
REFERENCES

1. Breslow JL.
Genetic markers for coronary heart disease.

The human LDL receptor: a cysteine-rich protein with multiple Alu sequences in its mRNA.

3. Soria LF, Ludwig EH, Clarke HR, Vega GL, Grundy SM, McCarthy BJ.
Association between a specific apolipoprotein B mutation and familial defective apolipoprotein B-100.

4. Hu FL, Gu Z, Kozich V, Kraus JP, Ramesh V, Shih VE.
Molecular basis of cystathionine beta-synthase deficiency in pyridoxine responsive and nonresponsive homocystinuria.

5. Scott HS, Litjens T, Hopwood JJ, Morris CP.
A common mutation for mucopolysaccharidosis type I associated with a severe Hunter syndrome phenotype.

Lysosomes and the sclerotic arterial lesion in Hunter's disease.
Hum Pathol. 1975;6:653-657.

7. Lifton RP, Gharavi AG, Geller DS.
Molecular mechanisms of human hypertension.
Cell. 2001;104:545-556.

G\K royalty's variant of Battier's syndrome, inherited hypokalaemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotransporter.

Mechanism by which Liddle's syndrome mutations increase activity of a human epithelial Na+ channel.

Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy.

11. Towbin JA, Bowles NE.
The failing heart.

12. Seidman JG, Seidman C.
The genetic basis for cardiomyopathy: from mutation identification to mechanistic paradigms.

13. Schonberger J, Seidman CE.
Many roads lead to a broken heart: the genetics of dilated cardiomyopathy.

14. Emery AE.
The muscular dystrophies.

15. Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, Keating MT.
A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome.

16. Priori SG.
Long QT and Brugada syndromes: from genetics to clinical management.

Novel PRKAG2 mutation responsible for the genetic syndrome of ventricular preexcitation and conduction system disease with childhood onset and absence of cardiac hypertrophy.

SCN5A mutations associated with an inherited cardiac arrhythmia, long QT syndrome.

Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene.

The elastin gene is disrupted by a translocation associated with supravalvular aortic stenosis.
Cell. 1993;73:159-168.

Mutations in human Tbx5 [corrected] cause limb and cardiac malformation in Holt-Oram syndrome.


44. Ridker PM, Hennekens CH, Schmitz C, Stampfer MJ, Lindpaintner K.
Platelet glycoprotein IIb/IIIa and risks of myocardial infarction, stroke, and venous thrombosis.

45. Goldschmidt-Clermont P, Roos C, Cooke GE.
Platelet polymorphism and thromboembolic events: from inherited risk to pharmacogenetics.
*J Thromb Thrombolysis.* 2000;8:89-103.

46. Kandzari DE, Goldschmidt-Clermont PJ.
Platelet polymorphisms and ischemic heart disease: moving beyond traditional risk factors.

47. Zhu MM, Weedon J, Clark LT.
Meta-analysis of the association of platelet glycoprotein IIb/IIIa PlA1/A2 polymorphism with myocardial infarction.

Increased platelet aggregability associated with platelet GPIIIa PlA2 polymorphism: the Framingham Offspring Study.

49. Cooke GE, Bray PF, Hamlington JD, Pham DM, Goldschmidt-Clermont PJ.
PlA2 polymorphism and efficacy of aspirin.

Toll-like receptor 4 polymorphisms and atherogenesis.
With rapidly increasing knowledge of genetic factors influencing disease, there is the potential to apply genetic tests in clinical practice. Contexts to be considered are those that could benefit the patient or individual either by diagnosis, prognosis, monitoring, screening, guiding genetics counseling, or even from “the right to know.” Some sequence variants are rare (such as the monogenic mutations causing severe disease in a few families); other variants are common polymorphisms with smaller “polygenic” effects on disease traits. The principles of gene testing are considered in this review in the context of situations where it has been claimed that such testing could be of utility. Examples are given from early life, dyslipidemias, aneurysmal disease, arrhythmias, hypertension, and dietary interactions.

**GENERAL ASPECTS OF DNA TESTS**

Laboratory assays are traditionally categorized to have four potential roles, namely diagnosis, prognosis, monitoring, and screening. A fifth category, counseling and reproductive choice, is particularly relevant in clinical genetics. A sixth category, the right to know, should be considered. In contrast with cancer genetics, somatic change of the genome is not believed to have any major role in cardiovascular genetics. Therefore, tests of the human genome for monitoring cardiovascular pathol-
pounds) and test turnaround in days, the latter requires an expensive test (eg, 500 to 1000 UK pounds) and test turnaround can take several months. These factors, and availability, may determine clinical utilization of such tests.

Screening tests add a further layer of complexity. In contrast with diagnosis and prognosis, where the patient or proband has presented to the clinical system, screening seeks to take at-risk groups, often large numbers of individuals, and to identify some with special risk. The implication of “making patients of individuals” and of the mechanisms and politics of dealing with wide catchments of the population are substantial. The criteria of Wilson and Jungner require: (i) a sensitive, specific test capable of identifying those at risk; (ii) an implementable and affordable test; and (iii) that treatment or management is possible and affordable. These general concepts are important in considering the future of gene tests in cardiovascular risk prevention.

TREATING THE POPULATION OR TREATING THE INDIVIDUAL? A CONSIDERATION OF TRADITIONAL CORONARY RISK REDUCTION

Contemporary practice for coronary risk management is based on knowledge of significant risk factors identified from epidemiological studies and (frequently, but not always) from evidence in secondary or primary prevention trials that reduction of the risk factor by lifestyle change, dietary, drug, or other means will reduce the risk of coronary event recurrence or occurrence. However, risk is not certainty for the individual patient. Neither does risk management guarantee risk reduction. Furthermore, although it may be true that risk reduction could benefit all segments of the population, for example, cholesterol-lowering drugs help even those in lower percentiles of the distribution, absolute cost, cost-benefit ratio, or risk of side effects preclude such generalized prescribing. Thus, population-based and risk-based cut points tend to be used in treating the individual patient. Such algorithmic medicine is difficult to practice, except with the use of systematic general guidelines and appropriate computer programs. There remain gaps between the ideal and the reality of such practice. Nevertheless, on a population basis, recognition and management of multiple risk is now far in advance of that prior to the epidemiological studies of the past half century and of the past two decades of drug development. It is into this background that attempts are being made to introduce genetic markers, which could further enhance risk prediction in the population, or stated another way, could further individualize patient risk assessment and management.

CARDIOVASCULAR DISEASE IN EARLY LIFE: RARE SEVERE MUTATIONS

It is beyond the scope of this article to describe in detail the important advances that have been made in clinical and molecular genetics in understanding major gene and cytogenetic defects contributing to congenital heart disease. However, developmental abnormalities affect approximately 1% of births, and causal gene identification not only enables understanding of pathogenesis, but permits gene tests of use for attaining definitive molecular diagnosis and possibly assessing prognosis, and for counseling families about recurrence risk. Examples include NKX2.5 mutation in atrial septal defect; deletions within chromosome 7q11.23 containing the ELN (elastin) gene causing Williams syndrome and specific inactivating mutations of ELN causing supravalvular aortic stenosis; deletions within chromosome 22q11, which cause haploinsufficiency of the T-box transcription factor gene Tbx1, causing 50% of occurrences of interrupted aortic arch or truncus arteriosus. Gene tests are in routine use.

DYSLIPIDEMIAS AND GENETICS: RARE AND COMMON GENOTYPIC EFFECTS

Genetic variation underpins many of the more severe dyslipidemias, and in addition underpins polygenic variation important to setting plasma lipid levels in the general population. Centers with specialized lipid clinics plus specialized laboratory expertise do apply gene tests in some restricted contexts. Three well-known genes are considered here, namely, APOE and APOB which encode, respectively, the apolipoproteins E and B, and the low-density lipoprotein (LDL) receptor gene (LDLR).

APOE: rare and common genotypes

Type III dyslipidemia, vascular disease, and apolipoprotein E defects

Type III dyslipidemia patients characteristically display palmar xanthomata and an increased incidence in early coronary and peripheral arterial disease. Type III dyslipidemia is characterized by lipoprotein particles with properties intermediate between triglyceride-rich very-low-density lipoprotein (VLDL) particles secreted by the liver and the cholesterol-rich LDL derivatives, which are returned to the liver. The intermediate nature of this lipoprotein fraction reflects its pathological
origin as “remnant” particles representing partially delipidated VLDL and chylomicrons. These particles are usually rich in APOE, but although APOE is the usual ligand for specific receptor-mediated clearance, the APOE of patients with type III hyperlipidemia is unable to mediate lipoprotein clearance. The genetic basis is that there are three isoforms of APOE—E2, E3, and E4. Most type III patients have the E2/E2 phenotype and APOE ε2/ε2 genotype. Apolipoprotein E acts as a ligand for the LDL receptor and is important in clearance of cholesterol-rich lipoproteins from plasma. At codons 112 and 158, E2 contains arginines and E3 contains one of each. The absence of the positively charged arginine at these positions appears to cause poor receptor binding. However, the E2/E2 phenotype occurs in an estimated 1% of the population, whereas only a small (fewer than 5%) percent of E2/E2 individuals display a type III dyslipidemia. The dyslipidemia is generally only apparent in adults, unmasked by obesity, diabetes, and aging, all of which increase VLDL secretion, and also unmasked by hypothyroidism or other disease. In hypothyroidism, the conversion of intermediate-density lipoprotein (IDL) to LDL by hepatic lipase is poor. Secondary compromise, genetic or environmental, to IDL clearance, which is critically rate-limited by E2/E2 phenotype, precipitates type III dyslipidemia. Biochemical evaluation is available in many centers and a few back this up with APOE genotyping, by one of numerous polymerase chain reaction (PCR)–based methods. The sophistication and cost of APOE genotyping, which establishes a clear molecular diagnosis to complement clinical and biochemical diagnosis, puts this gene test on the fringes of current practice, helpful, but not essential. In addition to the common alleles of APOE described above, a number of rare variants have also been described. In contrast with the ε2 allele, some are dominant in their effect on plasma lipid phenotype and are associated with type III dyslipidemia (reviewed in reference 9). Characterization of such genetic variants is generally only within the scope of the research laboratory at present, but it is obvious that the approach to evaluation and counseling of a family should be quite different according to whether there is recessive occurrence of the relatively common (1/100) ε2/ε2 genotype or transmission of a rare dominant allele.

**Common polymorphisms of small effect: no clinical role yet**

A much commoner factor influencing cholesterol level is the APOE E4 isoform, which possesses an arginine at codon 112 where isof orm E3 possesses a cysteine. Allele ε4 of APOE is found in approximately 30% of the general population (ε3 being the most common allele). ε3/ε4 heterozygotes display on average 10% higher cholesterol levels than ε3/ε3 homozygotes, due to differential effects of isoforms on lipoprotein particle turnover. This is an example of an epidemiological impact of common genetic polymorphism on the spectrum of population cholesterol levels, but it is not currently relevant to clinical management. If some hypolipidemic drug were found only to be effective in one genetic subgroup, the genotypes would be of more interest.

**LDLR: rare mutations of severe effect**

In familial hypercholesterolemia (FH), caused by codominant mutations in the LDL receptor gene (LDLR), cholesterol levels are typically twice the population average for age and gender. This disorder frequently results in heart attacks in middle age in the 1 in 500 heterozygotes in the population and in childhood coronary disease in the 1 per million homozygotes. The disorder has illustrated several important principles including the value of recognition of a strong familial tendency, the understanding and dietary and drug manipulation of the LDL/receptor/cholesterol pathway and the recognition of multiplicative risk in combination with factors such as smoking. In founder populations such as South African whites and French Canadians, a small number of mutations account for most FH families. In these countries, a small panel of direct tests can be readily applied to establish a molecular and family diagnosis of FH in patients selected from coronary risk or lipid clinics, or from community cholesterol screening. In more complex populations, the entire gene must be scanned because most families will display a different mutation, so most countries have not attempted molecular diagnostics. In over 90% of cases, cholesterol levels alone, with family history and evaluation, are sufficient to establish an inferred genetic diagnosis. Furthermore, it is the biochemical phenotype, not the genotype, which will determine coronary risk. However, it has been argued that an early “genetic” diagnosis by DNA test is as galvanic to lifestyle choice and compliance with therapeutic options, as a coronary event in later life. A counterargument is that a genetic diagnosis may induce fatalism. The MedPed program in the USA and the national FH program in Holland aim to establish molecular diagnosis and to trace relatives of affected individuals. In such programs, there is some element of enhanced diagnostic information, some element of cascade screening, and some element of counseling.
concerning the familial condition. This represents a refinement of the broad brush general cardiovascular risk screening of the population, to take account of a small subset at particular risk.

**APOB**: rare mutation of moderate effect

Some apparent FH is attributable not to **LDLR**, but to a defect in the **APOB** gene that encodes apolipoprotein B, the component of LDL that binds the receptor: this **ligand defect** is called familial defective apolipoprotein B (FDB). A mutation common in Western Europe, “R3500Q” in **APOB**, occurs at 1/700 frequency in some countries. Coronary risk is 7 times the population risk in FDB, although cholesterol levels may vary more between family members and within individuals over time, than for FH: quite a few FDB individuals are normocholesterolemic. Testing for the R3500Q mutation is simple, indeed relatives of known probands may present to their doctor requesting the gene test. However, it is difficult to explain a “gene-positive, but cholesterol-negative” result and occasional cholesterol checks are at present a more accessible clinical option. Nevertheless, with greater public and professional awareness about genetic factors, one might choose to monitor the cholesterol levels of FDB-positive individuals more frequently. Most practice lists will contain a few such individuals, most unrecognized. The subtleties remain beyond current clinical practice.

**VESSEL WALL**: FIBRILLIN-1 GENE (**FBN1**) IN AORTIC ANEURYSMAL DISEASE

Marfan syndrome is an autosomal dominant heritable disorder of connective tissue with prominent manifestations affecting the skeletal, ocular, and cardiovascular systems. The incidence is estimated to be up to 1/5000 with perhaps 25% of cases representing new mutations. Essentially, all classic Marfan families and cases have turned out to display linkage to human chromosome 15q21.1 and/or mutations in the **FBN1** gene encoding fibrillin-1. Mutations in this same gene also cause a series of other related disorders of connective tissue collectively known as type-I fibrillinopathies. While the skeletal abnormalities of Marfan syndrome may be the most obvious, it is the cardiovascular features that are often fatal. In particular, progressive dilatation of the aortic root and aortic dissection and rupture are frequent and mitral and aortic valve insufficiency may also occur early. These features are the predominant causes of death in over 90% of patients. Genotype-phenotype correlations are recognized with particular types of mutation resulting in quite distinct clinical phenotypes, not necessarily involving the vascular system. A distinct set of missense mutations in exons 24-32 predispose to early onset of cardiovascular complications, whereas missense mutations in the proline-rich exons 1-10 predispose to late-onset, mild cardiovascular complications.

“Query Marfan syndrome” is a common referral question to genetics clinics. Evaluation is complex and phenotypic heterogeneity is considerable. Nevertheless, long-term cardiovascular monitoring should benefit a subset. There is a major need for molecular diagnostics and improved genotype-phenotype understanding. However, **FBN1** is large and only now are laboratories starting to offer mutation scanning. This scanning will contribute to the improved diagnosis, prognosis, counseling, and selection for clinical monitoring, for a small number of individuals at high risk of aortic and valvular complications.

**DRUG SIDE EFFECTS: ARRHYTHMIAS**

The investigation of families with long-OT syndrome, a group who are risk from sudden death due to ventricular tachycardia and fibrillation, has revealed mutations within several genes that code for ion channel proteins (eg, **KVLQT1**, **HERG**, **SCN5A**, **minK**, **MiRP1**, and **RyR2**). Although such families are rare, their investigations have provided insight into the cellular mechanisms associated with acquired tachyarrhythmias including drug-associated long-OT. Drug-associated tachyarrhythmias and OT prolongation are now an important issue in drug trials and regulation. The low penetrance of long-OT mutations means that all relatives of an affected patient should be screened because they will be at risk of developing torsade de pointes if exposed to either cardiac or noncardiac drugs that block potassium channels. At present, such gene testing is undertaken diagnostically and prognostically only in a very small number of patients and families, but screening of such genes could become commonplace for the prediction and avoidance of rare, but very severe drug side effects.

**POSSIBLE PANELS OF TESTS IN CORONARY DISEASE AND COMMERCIAL SECTOR DRIVE**

Numerous genetic epidemiological studies have made claims of associations between common polymorphisms and coronary risk traits and events. Several genotypes have resulted in vast literature, including meta-analyses. The angiotensin-converting enzyme gene (**ACE**),
**APOE**, and methylene tetrahydrofolate reductase gene (*MTHFR*) feature prominently. The *ACE* “deletion” allele relative risk for a coronary event is probably in the range 1-1.1.24 Other genotypes that have been claimed to influence coronary risk include apolipoprotein(a), platelet glycoprotein IIIa, coagulation factor VII, cholesteryl-ester transfer protein, stromelysin-1, plasminogen activator inhibitor 1, and connexin 37.25,26.27 Stromelysin-1, a matrix metalloproteinase, has been quite consistently implicated in risk of restenosis. Such genetic epidemiology is certainly advancing our understanding of pathology. Traditional risk factors such as cholesterol level or blood pressure are measured in order to decide on lifestyle advice and on specific drug interventions. At present, no single common polymorphism adds significantly to the existing algorithms for identifying those at high risk, although some future arsenal of markers, taken together, could plausibly do so. However, a couple of genotypes do show some association with dietary or drug response. The clinical tests currently available reflect more drive by commercial organizations than needs drive from the clinical sector. Two illustrative examples are considered here.

**AGT and the concept of common genotype-tailored risk-factor management**

Angiotensinogen (AGT) is a major component of the renin-angiotensin system, which is important in saltwater homeostasis and maintenance of vascular tone, thus influencing blood pressure. It has been shown that common molecular variants in AGT associate with essential hypertension, pregnancy-induced hypertension, angiotensin levels, and blood pressure response to low-salt diet or some drugs. It seems that either a variation in the protein sequence of angiotensinogen, or an associated polymorphism (“G-6A”) in the AGT promoter (on-off switch) cause these small interindividual differences.27 On the basis of granted patents, Myriad Genetics, a genomics company based in Salt Lake City, Utah (better known for its work on the early-onset breast cancer genes), already offers prognostic genotyping tests (claimed to predict likely responders to diet or drugs) for this AGT polymorphism under the trademark CardiaRisk™ (www.myriad.com).

**MTHFR, plasma homocysteine, and dietary folate**

Plasma homocysteine levels associate with risk of ischemic heart disease, and a reduction in homocysteine level by 3 µmol/L has been estimated from meta-analysis to confer 16% risk reduction for ischemic heart disease, 24% for stroke, and 25% for deep venous thrombosis.28 The *MTHFR* C677T mutation causes the enzyme to be thermolabile and confers higher homocysteine levels, approximately 3 µmol/L.29 Comparing TT subjects with wild type CC subjects and also confers a summary odds ratio of 1.21 (confidence interval [CI] 1.06-1.39) for ischemic heart disease. Dietary folate and B vitamin supplementation has been shown to reduce risk of restenosis. Since TT homozygotes will, on average, display higher homocysteine level, such individuals are predicted, on average, to be at higher risk. On this basis, at least one company (Sciona, UK, www.sciona.com) has offered gene testing. However, why not just measure and manage the homocysteine level? On the other hand, why should an individual not be entitled to know about one of the genetic contributors to his biochemical and, ultimately, clinical phenotype? The answers hinge on relative priorities and relative costs.

**PERCEPTIONS OF USEFULNESS: CLINICALLY CONTROLLED OR MARKET DRIVEN?**

Genotypic tests, which steer the use of expensive drugs and clinical follow-up services, and guide management of major health issues for the individual patient, are of course finding more use by the clinical sector than those that do not. A futuristic review of tailored therapy to fit individual profiles in the context of genetics and coronary artery disease has considered various genotypes and responses to high-fat diet, smoking, balloon angioplasty, and lipid-lowering drugs.30 However, the author notes that firm criteria of proof of clinical utility have mostly not yet been met. Personal curiosity and the “right to know” are carrying different genotypic tests of more minor significance into the direct marketing sector. In the UK, direct marketing of genetic tests to the general public (as would be perfectly possible for the AGT test) is permissible, but subject to a (voluntary) code of practice and guidance established (1997) by the Advisory Committee on Genetic Testing, now subsumed by the Human Genetics Commission of the Department of Health. If public knowledge, availability, and test utility all increase substantially and test costs diminish, “high street” or “mail order” tests could abound. Some patients already present to their doctor with internet printouts and a few avail themselves of high street self-testing kits and apparatus for other cardiovascular risk factors.

(see references on next page)
REFERENCES


25. Rosenthal N, Schwartz RS.
In search of perverse polymorphisms.

Prediction of the risk of myocardial infarction from polymorphisms in candidate genes.

27. Lalouel JM, Rohrwasser A, Terreros D, Morgan T, Ward K.
Angiotensinogen in essential hypertension: from genetics to nephrology.

Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis.
_BMJ._ 2002;325:1202.

29. Jukema JW, Kastelein JJ.
Tailored therapy to fit individual profiles.
Genetics and coronary artery disease
In what manner are advances in genetics most likely to alter the clinical practice of cardiology within the next 10 years?

Alistair S. Hall, MB, ChB, PhD, FRCP
Professor of Clinical Cardiology - BHF Heart Research Centre at Leeds - Leeds - UK

The role of gene abnormalities in the causation and modulation of cardiovascular disease is a source of intense and unabated interest. Diseases described as monogenetic—hypertrophic cardiomyopathy, long QT syndrome, familial hypercholesterolemia—are best understood, though they reveal frequent discordance between gene abnormalities and clinical phenotype. This is explained by the presence of multiple gene defects, and by gene-dose, gene-gene, and gene-environment interactions. Common conditions such as coronary artery disease, metabolic syndrome, and hypertension represent the logical conclusion to the spartan lifestyle of mankind throughout millions of years of evolution. The next 10 years will provide important insights into the “purpose” of the genes that we commonly share, which were previously beneficial, but now predispose to modern diseases.

ADVANCING TOWARD OUR BEGINNINGS

As Sir Winston Churchill once stated, “The further back we look the further ahead we can see.” Ten years is an extremely short time in genetic terms—being roughly equal to half a generation. Even 1000 years equates to just 40 generations as compared with the very many evolutionary steps that have brought us to this time and place. For the majority of humanity’s existence on earth (Homo sapiens thought to have first existed just 30,000 years ago), the main challenges faced have been to find sufficient food and to avoid succumbing to trauma and infection. Before the relatively recent advent of farming, man lived a nomadic existence seeking out edible plants and berries and also hunting animals. An efficient metabolism gave a genetic advantage in times of famine, a strong immune system permitted resistance to infection, and efficient thrombus formation reduced the likelihood of death from trauma. There was no smoking, no obesity, no added salt or sugar, lots of exercise, and a life expectancy that made the development of the time-dependent diseases of today most unlikely. It is through such times that the genes that we all share were selected. These genes may not always be ideal when facing new environmental challenges such as plentiful and enriched foods (high in salt, carbohydrate, and unsaturated fats) cigarette smoke, and inadequate levels of exercise (Figure 1).

CLARITY OR COMPLEXITY?

The promise of new genetic technologies and scientific understanding is that greater clarity will be brought to our understanding of health and disease. There are many examples of this being true, as expertly summarized and reviewed in the earlier manuscripts of this publication. Williams and Goldschmidt-Clermont (see Lead Article) tabulate important cardiovascular disease states that result directly from genetic abnormalities that are transmitted from one generation to another according to recognized Mendelian patterns of inheritance. These include uncommon diseases of lipid and glucose metabolism, blood pressure regulation, as well as cardiac myocyte structure and function. They serve as examples of other disease states for which a genetic basis is suspected, but not yet defined. However, even these examples underline the complexity that exists at all levels between gene and clinical disease. What was once simplistically seen as a predictable lin-
ear relationship (abnormal codon → abnormal gene → abnormal protein → abnormal cell → abnormal tissue → abnormal organ → patient with disease) is now known to be much more complex. Let me illustrate this with the famous nursery rhyme:

For want of a nail, the shoe was lost.
For want of a shoe, the horse was lost.
For want of a horse, the rider was lost.
For want of a rider, the battle was lost.
For want of a battle, the kingdom was lost.
(And all for the want of a horseshoe nail.)

As with genetics, a small variation in the initial conditions (single nucleotide polymorphism = nail) can result in huge, dynamic transformations in concluding events (disease phenotype = loss of the kingdom). Each step has a linear logic to it, yet when placed together the relationship between initial cause and eventual effect borders on being utterly random and unpredictable. This is known as the “chaos theory.” However, while the complexity of a system may make it appear random or chaotic—the predictable elements of the system (eg, water crystal) create both infinite uniqueness (eg, snow flake) as well as distinctive patterns (Figure 2). While a relatively new concept to mathematicians and scientists, the consequences of this concept (random biological variability) are very familiar to the practicing clinician. Experience, pattern recognition, clinical judgment, discussion, consultation, caution, and reflection are just some of the skills employed daily in conjunction with the dictates of abnormal clinical tests, therapy guidelines, medical textbooks, randomized controlled trials, and meta-analyses. Doctors used to be taught from an early stage that, “There is no ‘always’ or ‘never’ in medicine.”

The challenge for cardiologists 10 years from now will be to work with scientists to bridge the current divide between the obvious linearity (cause determines effect) of many elements of genetics to the obvious complexity of: (i) gene-gene; (ii) gene-environment; (iii) time-gene; (iv) environment-time; and (v) environment-environment and countless other interactions that result in apparent “chaos” (cause appears to determine multiple effects; eg, single gene defect in a single family resulting in very distinct phenotypes such as hypertrophic and dilated cardiomyopathy OR multiple causes resulting in apparently same effect; eg, multiple different defects in multiple different genes resulting in the same disease state such as hypertrophic cardiomyopathy). The practice of medicine in conjunction with new genetic knowledge is well illustrated by familial breast and ovarian cancer. Causative

Figure 1. Suggested chronology (in years) of human evolution to the present time. On the right, “modern” man (San Bushmen living in Namibia).

Figure 2. Frost formation.
genes have been identified (BRCA-1 and BRCA-2) and can be identified by a patented diagnostic test. However, more than 80% of families with disease will not have evidence of this genetic cause. Furthermore, 15% of those individuals testing positive will NOT go on to develop cancer. The other factors that should influence the decision of an individual woman who has tested positive to make similarly be viewed to be complex.

PERSONS, PEOPLES, AND POPULATIONS

The principle ethic that guides clinical practice, as described in the Hippocratic oath, is one that creates a focus upon an individual patient. The challenge is to do good and not to harm that unique individual. However, while seeking to embrace the importance of understanding each patient’s genetic uniqueness—it is equally essential that a clinician also consider the wider genetic profile shared with other close, and also more distant, relatives. Gender, ethnicity, and family history can each reveal important “complex” information/patterns reflecting the genetic makeup of an individual and also the likely interaction of that broad genetic profile with environment and also time.

AGE AND CORONARY ARTERY DISEASE

Coronary artery disease is viewed by many as occurring as an inevitable result of the degenerative aging process. When describing observed changes that are associated with aging, it is largely assumed that chronological time is the cause and not the marker of these changes. The presence of advanced biological changes of “aging” in young children with the genetic condition of progeria—begins to challenge this strongly held perception. Similarly, data that relate to the length of chromosomal telomeres (the sticky ends) and longevity, strongly suggest the presence of an internal biological clock that is only partially influenced by chronology. The telomeres are compared with a sequentially knotted piece of string, from which one knot is excised each time the chromosome is replicated. Once all have been removed the chromosome becomes unstable and can no longer divide. As a result, tissues degenerate and aging is observed. Oxidative stress (eg, as caused by smoking) would appear to accelerate this process, and reduction of such stress has been shown to prolong survival in mouse models. The next 10 years will further improve our understanding of aging, and so better equip clinicians to combat it’s effects.

GENDER AND CARDIOVASCULAR GENETICS

Men differ from women in their basic genome as a result of males having an X and a Y chromosome and women having two X chromosomes. Furthermore, males as a group are observed to have an earlier age of onset and also an increased incidence of coronary artery disease. Nevertheless, the exact mechanisms by which maleness predisposes to coronary artery disease and also the factors that make femaleness protective are still not understood. Generally speaking, a person’s phenotype denotes them as either male or female, yet this is not always the case. A specific gene on the Y chromosome (SRY, sex-related Y) is responsible for male sex development during embryogenesis. An absent or defective SRY gene results in a female phenotype (including development of ovaries) despite the presence of the male XY chromosomal configuration. When an individual has a single X, but no Y chromosome (Turner syndrome, 1 in 3000 newborn females, may be as high as 2% of all human conceptuses, only a small fraction of whom survive to term) or two X and one Y chromosome (Klinefelter syndrome, 1 in 500 males), incomplete gender development occurs towards the female and male phenotypes, respectively. This suggests that the Y chromosome is an essential prerequisite of maleness—being mediated by the formation of testes and the presence of testosterone and its metabolic derivatives. What then is the mechanism by which the presence of a Y chromosome is associated with accelerated coronary atheroma OR the presence of two X chromosomes is associated with a delay in coronary atheroma formation? Recent trials indicate that replacement of hormones in postmenopausal women does not adequately prevent cardiovascular disease—suggesting an alternate mechanism for gender differences. I believe that the next 10 years will provide the answer to this important genetic question, once again suggesting responsive strategies.

ETHNICITY AND CARDIOVASCULAR GENETICS

Within the UK, the gene pool of the Ancient Britons was added to in the Iron Age by the Celts and then subsequently by the invasions of the Anglo-Saxons, Romans, Norwegian Vikings, Danish Vikings, and Normans. Over the last 1000 years, the UK population has been further extended with a variety of ethnic groups that include many individuals from the Indian Subcontinent and the Caribbean Islands. It is notable that there is an increased in-
Genetics and the future practice of cardiology

The incidence of metabolic disorders and coronary artery disease in the first of these ethnic groups and a decreased incidence in the second, despite broadly similar environmental influences. It is also well recognized that similar to African-Americans, hypertension, stroke, and increased salt sensitivity occur more commonly among the Afro-Caribbean community. A number of evolutionary explanations for these observations have been advanced. For example, it has been suggested that frequent environmentally induced food shortages in the Indian Subcontinent may have resulted in a selective pressure for survival of individuals with efficient metabolisms, whose descendents now develop "metabolic syndrome" in a food-rich environment. The increased salt sensitivity of African-Americans and Afro-Caribbean has also been attributed to a "survival of the fittest" effect in response to heat-induced salt and water loss before, during, and after transport from Africa on environmentally oppressive slave ships. It is to be hoped and expected that a better understanding of the specific and more general genetics of ethnicity will inform and improve the prediction and treatment of disease both within and also beyond individual ethnic groups.

**FAMILY HISTORY—THE IDEAL GENETIC TEST?**

The pedigree depicted in (Figure 3) results from a highly complex array of elemental factors—and yet delivers a very clear and simple clinical message. This is in fact a very sophisticated genetic test that is available for use now. Because of its elegance and simplicity, it is often overlooked, with focus diverted to more challenging technologies. By looking back, members of Generation IV can readily see the genetic susceptibility that they have and also a clear indication of their future risk.

Familial aggregation of premature coronary artery disease has been recognized for many years. There is robust evidence that even after correcting for "traditional" risk factors, a positive family history remains strongly predictive of early disease. Coronary artery disease risk prediction models that have included a positive family history as a variable have found it to be second only to age in its independent predictive value and twice as powerful an indicator as smoking. Furthermore, it is apparent that the adverse effect of factors such as smoking are much magnified when a family history is present. Observations such as these, and also observed interactions between homocysteine levels and family history, have caused some to strongly urge that a positive family history be recognized as a highly modifiable risk factor. This is even more true when families are collectively targeted for preventative interventions—an approach that has many clear merits.

The clinical relevance of a positive family history is known to increase in the following situations: (i) the more events have occurred, (ii) when events occur at a very early age, (iii) when multiple generations are affected, and (iv) when conventional risk factors provide an inadequate explanation (Table I, page 34).

A large number of groups have sought to measure "a positive family history" by using scoring systems that vary in their complexity, but apparently less so in their accuracy. The Family Risk Score (FRS) devised by Hunt in 1986, basically sought to measure familial risk using the following formula (O; observed events – E; expected events)/the square root of E. To establish a baseline estimate of expected events (E), the FRS was used. FRS produces a score that is taken to indicate a positive family history when greater than or equal to 0.5. In 2001, a study published in the American Journal of Cardiology by Williams et al clearly demonstrated the great clinical value and cost effectiveness of eliciting a cardiovascular family history. A total of 130,175 families were...
studied by documenting a self-reported cardiovascular pedigree, calculating a validated and standardized FRS, and then followed each for 14 years to assess occurrence of new events. The prevalence of a positive family history was 14% for the general population, but 72% for the families of those going on to have a premature coronary event (men before 55 y women before 65 y). Given the rapid availability, low cost, ease of conduct of this global test of genetic makeup, and also the likely effects of time and environment, it is greatly to be hoped that in 10 years the FRS will be a routine part of the complete cardiology assessment.

CONCLUDING REMARKS

It is a truism in science that the discovery of the answer to one question generates multiple additional questions. Hence, in the search for greater clarity we often discover significant complexity. After 5 million years on this planet, will the next 10 years permit scientists to unravel this complexity sufficiently to provide the clarity needed to change the routine clinical practice of cardiology? Despite the way of posing this question, I very much hope and believe that important developments will indeed occur. However, I expect the greatest impact of genetics on cardiology to result from an improved understanding of our common genetic heritage and also the inappropriately named “nonmodifiable risk factors” for atherosclerosis. While we may not be able to turn back time, change our parents, our gender, or our ethnic origins—the science of genetics has the very real potential to shed light on these simple markers of complex genetic makeup—thereby refining both individual and population strategies for disease prevention and treatment through better use of currently available technologies.

REFERENCES


5. Hopkins PN, Williams RR, Hunt SC.
Magnified risks from smoking for coronary prone families in Utah.

6. Higgins M.
Epidemiology and prevention of coronary heart disease in families.

7. Khaw KT, Barrett-Connor E.
Family history of heart attack: a modifiable risk factor.
Circulation. 1986;74:239-244.

8. Chesebro JH, Fuster V, Elveback LR, Frye RL.
Strong family history and cigarette smoking as risk factors of coronary artery disease in young adults.

Higher plasma homocysteine and increased susceptibility to adverse effects of low folate in early familial coronary artery disease.

10. Hunt SC, Williams RR, Barlow GK.
A comparison of positive family history definitions for defining risk of future disease.

11. Anderson KM, Odell PM, Wilson PW, Kannel WB.
Cardiovascular risk profiles.

Usefulness of cardiovascular family history data for population-based preventive medicine and medical research (the Health Family Tree Study and the NHLBI Family Heart Study).
Should genetic testing become a standard component of clinical trials in order to advance evidence-based cardiology practice?

Hugh Watkins, MD, PhD, FRCP
Field Marshal Alexander Professor of Cardiovascular Medicine - University of Oxford - Oxford - UK

Medical therapy currently assumes that all individuals with a given condition should be treated in the same way, guided by empirical evidence from clinical trials. This ignores individual differences, which may predict variable therapeutic efficacy or adverse drug reactions. Considerable evidence suggests that genetic makeup determines much of this variability, and major efforts are being made to harness genetics to predict drug responses. Pharmacogenetics can improve the power and informativeness of clinical trials and increase the value of new or existing drugs. However, the precise characteristics of the underlying genetic variants will strongly impact the ease with which pharmacogenetic studies can deliver these high expectations, to the extent that it is not yet efficient to systematically include genetic testing in clinical trials.

THE PROBLEM
Both individual clinical practice and the cumulative experience reported in the literature indicate that conventional pharmacological therapy is a very imperfect science. The proportion of patients in whom the desired goal is achieved is variable, but usually surprisingly low, while adverse effects are a persistent problem. These include both the predictable problems of under- or overachieving the intended pharmacological intervention and unpredictable but stereotyped, “idiosyncratic” adverse drug reactions (ADRs). Monitoring drug levels, or a target response, can be instituted for a subset of drugs where the therapeutic window is narrow. However, in the vast majority of circumstances it is not possible to predict likely responders or those at risk of ADRs and so therapy is conducted on a “trial and error” basis.

The disadvantages of this approach are obvious. At the very least, treatment is inefficient with substantial wastage of resources (both drugs and consultation time) and compliance is low. Even with extensive regulatory mechanisms in place, ADRs constitute a top ten cause of mortality. Because serious unpredictable ADRs tend to be rare, they are often only documented late in the course of development of new therapies, or in postmarketing surveillance. Many otherwise good agents are therefore lost and the cost of bringing a drug to market drastically increased.

THE PROMISED SOLUTION: PHARMACOGENETICS
In keeping with all other biological traits, individual variability in drug response will inevitably share both genetic and environment components. In some instances, the heritability of variable drug response is known to be high, leading to the notion that a population might be partitioned into responders and non-responders by their genetic makeup (Figure 1). Further, because a drug’s action is mediated by a relatively circumscribed number of target and signaling proteins, it is reasonable to hope that the number of genes determining a particular response may be lower, and the size of individual genetic effects may

**Keywords:** pharmacogenetics; gene; polymorphism; drug; clinical trial; prediction; prevention; adverse drug reaction

**Address for correspondence:** Prof Hugh Watkins, Department of Cardiovascular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK (e-mail: hugh.watkins@cardiovascular-medicine.oxford.ac.uk)

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be larger (and hence more tractable), than in the common complex trait diseases. Given this, the obvious clinical need, and the advances arising from the Human Genome Project, major investments are being made toward unraveling such genetic factors, and the field of pharmacogenetics is burgeoning. Thus, the question posed for this article now raises real issues, whereas, even 5 to 10 years ago, it would have made little sense.

**Pharmacokinetic and pharmacodynamic variation**

Many instances of interindividual variation in drug handling have long been known to be genetic, for example, those attributable to variants of the cytochrome P450–metabolizing enzymes. Such pharmacokinetic variation can be attributed to genetic variants impacting on absorption, distribution, metabolism, and excretion with marked effects on the bioavailability of a given agent (Figure 2). Systematic analysis of such genetic variants therefore holds the promise of tailoring individual dosage regimens to achieve the desired levels at the target organ. For example, the cytochrome P450 CYP2C9 enzyme metabolizes many clinically important drugs, including warfarin, the angiotensin II receptor antagonists, and fluvastatin. Uncommon variants in CYP2C9 (affecting ≈1 in 250) reduce its activity and markedly influence dose requirements; life-threatening bleeding episodes have been reported in poor metabolizers of CYP2C9 exposed to warfarin.

Variation that will affect a drug’s action and interactions, that is, that will alter its pharmacodynamic profile (Figure 2), is currently less well understood at the DNA level, but the genetic influence is likely to be just as pervasive. Variation within the target molecule, for example, G-protein–coupled receptors or second messenger systems, will be of significance, but perhaps more important will be the genetic heterogeneity underlying the etiology of most common conditions. A genetic classification of disease may predict biological, hence therapeutic, responses. For example, only in a subset of asthma patients do elevated leukotrienes contribute to the disease; accordingly, stratification by genotype in the 5-lipoxygenase gene predicts response to antiasthma treatment with a specific leukotriene inhibitor. By combining genetic sources of both pharmacokinetic and pharmacodynamic variation, it is reasonable to hope that a genetic classification may allow prediction of responders, nonresponders, and adverse responders to relevant classes of drugs (Figure 3, page 38).
GENETIC APPROACHES

As with analysis of inherited disease, many of the early precedents relate to single gene influences on phenotype. These are likely to provide informative examples, but, as with disease, probably account for a minority of pharmacogenetic variation. Examples include major effect variants in key enzymes in metabolism (see above) and in drug transport, for example, genetic variants in the multidrug resistance (MDR)–1 gene, important for handling a number of chemotherapy agents and also digoxin.\(^5\) Inherited mutations underlying susceptibility to malignant hyperthermia illustrate a monogenic pharmacodynamic adverse response.\(^6\) Single gene examples of disease-causing mutations that define the genetic etiology of a heterogeneous condition, and hence predict response to treatment, include the different genetic etiologies of long QT syndrome.\(^7\) Subtle variants in the same genes illustrate the way in which rare genetic variants can underlie idiosyncratic ADRs; for example, sporadic mutations, as well as relatively prevalent single nucleotide polymorphisms, in potassium channel subunits, have been shown to underlie antibiotic-induced cardiac arrhythmias.\(^8\)\(^9\) For identification of both single gene effects and the presumably more prevalent oligogenic influences on drug response, a choice of genetic strategies exists.

### Candidate gene studies

Candidate gene studies are limited in that they can only test known genes that are already plausibly implicated in the underlying process.

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**Figure 3.** Detailed genetic analysis (either candidate gene or genome-wide single nucleotide polymorphism [SNP] typing) of extended early clinical trials aims to partition the population into responders, nonresponders, and those at risk of adverse drug reactions (ADRs) (A). If genotyping can identify responders, selected entry into subsequent phase 3 trials has the potential to improve power to show efficacy. Thereafter, the drug could be targeted to those it would benefit with reduction in risk of ADRs (B).
However, this limitation may be less of a handicap in investigating drug responses as the downstream pathways are often quite well mapped out. Thus, DNA can be typed for variants affecting proteins thought likely to be involved in drug handling, the target itself, and downstream signaling. A prominent, but as yet unsubstantiated, study of this type is the proposed role of cholesteryl ester transfer protein (CETP) polymorphisms in the response to “statins.” In parallel, the candidate net can be thrown much wider by harnessing the emerging genomic and proteomic tools for identification of novel candidate genes. For example, DNA microarray experiments for expression profiling (comparing normal and pharmacologically manipulated systems) can prioritize genes for screening for DNA variants. Such pharmacogenomic approaches can also provide new data for mechanistic studies and perhaps even new markers for early signs of drug toxicity.

Genetic analysis of variants in candidate genes is conducted by association study, comparing the incidence of a given variant in cases and controls—in this instance, for example, responders versus nonresponders or those with ADR versus those without. In principle, this is a simple study design lending itself easily to collection of the necessary data within the framework of a randomized control trial. In practice, different properties of the underlying genetic polymorphisms (particularly the allele frequency and mode of gene action) have a dramatic impact on the trial sample size needed. In addition, experiences with candidate gene analysis in disease association studies indicate the importance of taking other complexities into consideration. These include analyses of multiple variants in a gene to gain information on haplotype structure, consideration of gene-gene and gene-environment interactions, and awareness of the confounding effects of population admixture.

**Systematic approaches to defining pharmacogenetic variants**

The power of genetics is at its greatest when genome-wide analyses can be performed without the constraints of prior hypotheses. The conventional approach to genome-wide analyses has been to use linkage analysis in families, as has been widely successful in single gene disorders and has had some notable recent successes in complex traits. However, data on drug responses will not often be available in related individuals, and so linkage analysis is generally not an option. Systematic approaches to genome-wide association have been much discussed, and may be now coming of age. Once the consensus sequence of the Human Genome Project was complete, focus shifted to defining the extent of variation in individuals. An extremely large set of single nucleotide polymorphisms (SNPs) is being assembled by public and private efforts, particularly the SNP consortium, with a view to constructing genome-wide high-density SNP maps. Major uncertainty exists, however, as to the extent of linkage disequilibrium across the human genome, and particularly as to whether conserved “blocks” exist in a way that will much reduce the number of SNPs that need to be typed to provide genome-wide coverage. In the meanwhile, there is a premium on intragenic polymorphisms and, particularly, those polymorphisms likely to exert a direct functional effect. The first genome-wide SNP association study to detect a disease-susceptibility allele has just been published, likely heralding a new era of very large-scale association analyses. This study, which implicates a cytokine gene in the etiology of coronary artery disease, involved typing of over 60,000 SNPs in 100 cases and 500 controls as a preliminary experiment, prior to reevaluating the most promising 600 SNPs in more than 2,000 cases and controls.

Optimism exists in some quarters that it will be possible to use “abbreviated SNP maps” to identify categories of drug response by complex pattern recognition rather than by going on to formally define a particular susceptibility variant (or set of variants). Thus, the hope would be that an SNP profile could be established for patients displaying either adverse events or a therapeutic response. If such a profile was sufficient to capture genome-wide susceptibility information, and yet was relatively simple to perform, then it could be used in individual preprescription analyses.

Even if the optimism is well placed, SNP genotyping capacity and prices will need to continue to improve at least as steeply as they have done over the past few years. One potential solution to the cost of SNP typing, which is being explored in complex trait genetic analyses as well as in pharmacogenetic studies, is the pooling of DNA samples among cases and among controls (as distortions in the frequency of particular variant between cases and controls could, in theory, be detectable by typing just the two pools).

**“Proof of principle” studies**

In the meantime, a compromise position will likely be large-scale and systematic analyses of SNPs across each of a large number of candidate genes, thus broadening the scope and allowing the testing of more
than the most obvious genes while still falling short of a truly genome-wide analysis. Those studies that are currently offered as “proof of principle” fit this category. For example, a severe hypersensitivity reaction occurs in approximately 5% of individuals treated with the antiretroviral abacavir. As this side effect had overt immunological features, 114 polymorphisms in 12 candidate gene families were selected for an association analysis, allowing the identification of risk-associated variants in the tumor necrosis factor-α (TNF-α)/HLA B57 locus. 20 In a treatment cohort of 200 individuals, the presence of three human leukocyte antigen (HLA) alleles had a positive predictive value for hypersensitivity of 100% and a negative predictive value of 97%. 21

Limitations

The feasibility of defining clinically useful pharmacogenetic variants will depend on parameters that will vary for each gene/drug interaction. As with all susceptibility alleles, the power of a genetic study and subsequent utility of a genetic marker will critically depend on the size of the genetic effect. Model organism genetics tells us that weak effect variants will far outnumber the occasional major effect variants. The overall contribution to the disease or response will reflect both the size of the effect and the population prevalence of the susceptibility allele. Thus, functionally significant variants in the various CYP genes tend to be present at between 2% to 10% of the population, an appropriate frequency to be optimistic about their utility. Variants that are much rarer will be less tractable and less effective in cost terms (unless the adverse effect is exceptionally severe). It is also likely that genotyping will only be the investigation of choice where there is not another simple functional assay that gives a readout reflecting the cumulative impact of a number of genes. For example, a competing strategy to predict an individual’s pharmacokinetic profile is the administration of a small panel of tracer substances followed by their analysis in blood and urine to give a detailed picture of the full complement of metabolic and excretion pathways variation.

**HOW CAN PHARMACOGENETICS BE USED TO IMPROVE DRUG DEVELOPMENT, TRIALS, AND SURVEILLANCE?**

If it is shown to be feasible to define pharmacogenetic variants with important predictive accuracy, then, in the near future, we should expect to see changes to the way in which drugs are evaluated in clinical trials.

**More information from phase 2 studies**

If possible, the most attractive option would be to perform comprehensive genetic analyses in phase 2 studies, even if this required an enlargement of such studies, in order to yield substantial benefits in the more costly phase 3 studies. Perhaps most plausibly this will first be achieved for ADR pharmacogenetics. DNA from individuals with ADRs would be compared with others enrolled in the trial by either the candidate gene or systematic approach. In pragmatic terms, this would most easily be achieved by archiving blood samples (for example, a stored blood spot much as is used for the Guthrie test) on all enrolled subjects as this would be cheaper and easier to orchestrate than retrospective collection of informative samples. Clearly, however, there are additional ethical considerations (discussed below). Once predictors of an adverse outcome are identified, genotyping could be performed at entry into phase 3 studies with considerable reduction in risk and with increased likelihood of ultimately gaining regulatory approval.

**More efficient phase 3 studies**

Much harder to achieve, but of significant promise, would be to use genetic analysis of larger phase 2 trials to define responder and nonresponder profiles. If feasible this would allow smaller and more economical phase 3 trials restricted to a potentially susceptible population. Exclusion of predicted nonresponders would save such individuals from fruitless participation and would flag (at a much earlier stage) the need for additional therapies. Such genotype criteria have already entered the drug development process in a few instances. A phase 3 trial of desipramine required CYP2D6 genotyping to exclude the 7% to 10% of “poor metabolizers” who could be predicted to not benefit; this removed the need for continuous plasma concentration monitoring. An analogous approach, for example in primary prevention trials, is to use DNA typing for documented susceptibility variants to enrich the study for those most at risk; this would increase the event rate and the power of the study to resolve differences in treatment efficacy at an early stage.

**Can genotyping improve trial power?**

Under some circumstances, genotyping could reduce the trial size needed to show efficacy of a given agent. A large proportion of nonresponders can mask a good response in a subset (see Figure 1) such that genetic stratification could yield a more significant result. By modeling single gene effects, Cardon et al
have shown that the impact on sample size is critically dependent on allele frequency (best when the variant is common) and on mode of action (dominant effects needing smaller sample sizes than codominant or recessive).\textsuperscript{12} Importantly, this means that sample size can be either much smaller, or much larger, than that needed in a nongenetic trial, and so attempts to gain power by genotyping in a large (eg, phase 3) study would only make sense where the details of the genetic factors contributing to outcome are known.

**Surveillance**

Analysis of DNA from the full phase 3 trial population—and indeed from postmarket surveillance—may, however, be needed to document the susceptibility to serious, but rare, ADRs. Similarly, if the power to resolve drug responses or resistance is not adequate in phase 2, or the research not complete (a realistic situation at present), DNA analysis may be needed from selected individuals from the phase 3 study (those informative for a given response). It appears to be the view of most pharmaceutical companies that this might most economically be performed by sample archiving at the point of enrolment rather than by retrospective collection.

**Ethical and legal issues**

Informed consent is often considered to be especially problematic in genetic studies. However, the main reason for this is the potential of genetic analysis to reveal data that have implications for individuals other than the study subject (ie, first-degree relatives) or that have the potential to yield predictive information about future disease liability. These concerns should be much less prominent in pharmacogenetic analyses as the familial impact is low and drug response variants will generally not relate to underlying disease risks. Where they do, for example, in classification of genetic subtypes of a heterogeneous disease, the problem is generally less in clinical trials because the individuals are already either known to be affected or at high risk (in the case of primary prevention trials). One issue that will need exploring and specific explanation during the consent process, is that pharmacogenetic testing has obvious commercial implications and data arising from a study (for example, an academic study) might later lead to exploitable intellectual property. Concerns have also been voiced about the partitioning of populations into those for whom a treatment may not be offered, but, so long as the genetic test is sound, these individuals would not have benefited from the proposed treatment anyway. One can anticipate a move toward reimbursement being restricted for efficacious therapies, or licensing being subject to pairing with a prescription diagnostic test, appropriate regulation will only be feasible if the underlying genetic science can be clearly validated.

**Other postulated benefits**

One justification sometimes offered for routine collection of DNA on all participants in a study is the hope that such a DNA collection could contribute to research into the underlying disease etiology. While genetic analysis into the basis of disease susceptibility holds huge potential in the long term for identification of new targets and the derivation of rational therapies, the power of such studies is dependent upon the selection of extreme phenotypes. Thus, patients with typical age of onset, or severity, of disease (as are usually enrolled into clinical trials) are often of insufficient genetic enrichment to be informative. Similarly, disease-gene identifying studies need to be designed with genetic issues paramount: in particular, this will involve close genetic matching of the control subjects and it may also require collection of affected family members (in order to perform genome-wide linkage studies). Further, a quite different consent process is needed. Thus, such approaches would not support a proposal for DNA collection to become a standard component of routine clinical trials.

**ROUTINE GENETIC TESTING: IF NOT YET, WHEN?**

At present, much of the research into the potential of pharmacogenetics is driven by commercial pressures and is conducted by the pharmaceutical industry.\textsuperscript{18,19} This is not to say that advances in the field will not be of sound benefit for evidence-based cardiology, but rather that commercial considerations may determine the timetable of when and how systematic genetic sampling becomes a standard component of clinical trials. Where it is anticipated that genetic testing could accelerate phase 3 studies, facilitate regulatory approval, or even salvage a drug that had had to be dropped due to an unacceptable ADR rate, we can expect to see imminent applications. Certain drug indications will have greater priority. Pharmacogenetic predictions will be most relevant where treatment is administered long term, where there is a narrow therapeutic window (and no easy alternative way to measure or predict levels), or where there are serious unpredictable idiosyncratic complications. In many other instances, where treatments are well tolerated with fewer serious complications, pharmacogenetics will
move more slowly even though it may yet have much to offer in terms of prioritizing treatments by likely efficacy. Because of the current advantages of relatively simple genetic systems and candidate genes, pharmacogenetic tests are likely to be introduced first in situations where the biology is already quite well understood. Such knowledge will also be necessary to determine whether genotyping could reduce trial size.

While we are not yet at the stage where systematic genetic testing can be considered a cost-effective component of clinical trials, we are already close to the time where systematic collection of such samples can be justified—to allow genotyping in informative subsets thereafter. The field is moving particularly fast and the indications are that pharmacogenetics (particularly in the field of adverse events) will be the first area of complex trait genetic analysis to impact directly on health care. While the advent of truly “personalized” medicine is still a long way off, the days of blind treatment identical for all are numbered.

REFERENCES


18. Roses AD.
Pharmacogenetics.

Mosteller M.
Maximizing the value of medicines by
including pharmacogenetic research in drug
development and surveillance.

20. Lai E, Bowman C, Bansal A,
Hughes A, Mosteller M, Roses AD.
Medical applications of haplotype-based
SNP maps: learning to walk before we run.

Association between presence of HLA-B*5701,
HLA-DR7, and HLA-DQ3 and hypersensitivity
to HIV-1 reverse-transcriptase inhibitor
abacavir.

22. Murphy MP, Beaman ME, Clark
LS, et al.
Prospective CYP2D6 genotyping as an ex-
cclusion criterion for enrollment of a phase III
clinical trial.
It is difficult today to appreciate the extent to which Carl Wiggers dominated cardiovascular physiology during the middle of the 20th century. The impact of his work far exceeded his individual discoveries, which were many, but instead reflected the totality of his research, most of which was carried out in the Department of Physiology at Western Reserve University in Cleveland Ohio. Wiggers provided a lucid description of the cardiac cycle, often referred to as the “Wiggers’ Diagram,” that related changing pressures in the heart and great vessels, ventricular volume, heart sounds, and the electrocardiogram. He was a pioneer in cardiac electrophysiology who described impulse propagation through the ventricles, mechanisms responsible for premature systoles, the vulnerable period of the cardiac cycle when the ventricles are most susceptible to fibrillation, and the role of cardiac compression and electrical countershock in reversing ventricular fibrillation. He studied irreversible shock, pointed out that coronary vessels are end-arteries, and identified the cause of the early pump failure after coronary occlusion as inability of electrical excitation to induce an effective contraction. He provided evidence that cardiac glycosides and epinephrine increase myocardial contractility, and showed that epinephrine abbreviates systole. Wiggers was a great teacher who pioneered in correlating basic physiology with clinical medicine. No fewer than 38 of his pupils and associates became department heads or research directors.

He was also a first-rate clinician whose work on the mechanisms of human disease was seminal for the development of modern cardiology. His contributions to hemodynamics provided the foundation that made cardiac catheterization an indispensable clinical tool in the 1940s and 1950s. Because of his many contributions, Wiggers was widely regarded as the “Dean of Cardiovascular Physiology.”

**Address for correspondence:**
Prof Arnold Katz, MD, 1592 New Boston Road, PO Box 1048, Norwich, VT 05055-1048 (e-mail: arnold.m.katz@dartmouth.edu)

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**BIOGRAPHY**

Carl John Wiggers was born in Davenport, Iowa, on May 28, 1883, to a farming family who, for economic reasons, had emigrated from northern Germany. He was the only member of his grammar school class to complete high school, which, Wiggers noted, was the equivalent of a college education today. He stated that he “drifted” into medicine after intending to be a pharmacist, and chose the University of Michigan because its medical school enjoyed a strong academic reputation and recognized that “clinical work must rest on a sound scientific foundation.”

Wiggers wrote: “a few of my medical classmates accepted the work in medical sciences because of their inherent interest in it; the majority, I fear, endured it as sort of a purgatory preparatory to their entrance into the clinical heavens where the angels are attired in nurses’ uniforms.” After receiving his MD, in 1906, Wiggers accepted an Assistantship in Physiology for “pecuniary reasons”; at the same time he began a medical practice to supplement his income. In 1910, his chief at Michigan, Warren P. Lombard, took a sabbatical leave and appointed Wiggers substitute Chair of Physiology. This led Wiggers to “megalencephalia” so severe that he almost accepted the Chair of Physiology at a second rate school. However, after several of his senior colleagues pointed out that “many a promising career is wrecked by consideration of rank and emolument,” he...
decided instead to spend a year with Otto Frank in Munich. By mistake, however, he resigned from his position at Michigan rather than taking a leave of absence, and when he found himself without a job had to cancel this trip. Luck and a recommendation by a powerful colleague led to an offer from Cornell, where he became Assistant Professor of Physiology in 1911. The following year, he was able to spend several months with Frank, where, in spite of performing only three animal experiments (because of a strong antivissectionist movement), Wiggers became expert in the optical recorders he used in much of his subsequent research. These instruments provided a major advance over Ludwig’s smoked drum kymograph. I recall one such instrument in which a light beam provided a weightless “lever” more than 40 feet long that recorded pressures from a transducer connected to an artery by lead tubing, tiny movements of a mirror attached to a tense rubber diaphragm were amplified to project a moving image of a light source onto a spinning roll of photographic film (Figure 1). This device allowed Wiggers to record simultaneously the pressures in various chambers of the heart, measurements that led to his classic description of the cardiac cycle (Figure 2).

Wiggers again found himself substitute Chair of Physiology in 1917 when the two senior department members at Cornell went to Europe because of World War I. Wiggers’ contribution to the war effort included oversight of electrocardiography at General Hospital No. 9 in Lakewood, New Jersey, and studies on the pathophysiology of hemorrhagic shock, a topic to which he returned during World War II. In 1918, he moved to Cleveland to become Chair of the Department of Physiology at Western Reserve University, a position that he held until his retirement in 1953. He did most of his major work during these 35 years. After retiring, Wiggers became the first editor of...
Circulation Research in spite of having been “shown a cartoon depicting a captive editor surrounded by a ring of cannibals, variously labeled contributor, reader, publisher, and printer. Javelins were being hurled at the editor, presumably to tenderize his flesh preparatory to putting him in the stew pot.”5 Wiggers stated that he took this risk because “[my] pachydermal covering had thickened with the years.” He later described his editorship as a “gratifying experience.”5

WIGGERS AS WRITER AND ADMINISTRATOR

Wiggers’ mastery of writing, which is apparent in the quotations cited above, played an important role in his success as a scientist. He noted, “Readers are greatly influenced in their judgment of a research project by literary style; a poor presentation can easily damage the best investigation.”10 Describing the balance between providing too many or too few data, he wrote: “A good paper, like a good glass of beer, should neither be largely foam nor flat; it should have just the right head of foam to make it palatable.”9

Although Wiggers viewed administration as a “necessary evil, detracting from his primary objectives, research, teaching, and the training of young investigators and teachers,”2 he served many national and international organizations, notably the American Physiological Society and American Heart Association.

WIGGERS AS SCIENTIST

Wiggers’ scientific contributions are best viewed not as individual discoveries, but instead as a totality that provided the foundation for modern cardiology. His greatness reflected an emphasis on data rather than interpretation, as evidenced in his statement: “…it is not what you believe, but what you find, that people are interested in.”2 A story, told to me by a source I cannot recall, illustrates Wiggers’ respect for experiment. In the late 1920s, Corneille Heymans, then a young Belgian pharmacologist, reported that increasing carotid artery pressure slows the heart. However, this response could not be reproduced by Wiggers. In 1927, during a series of visits to American laboratories, Heymans arrived in Cleveland and from his hotel phoned Wiggers who said: “Tomorrow there will be a dog.” The following day Heymans and Wiggers together confirmed this response, which led Wiggers to state: “The dog tells the truth.” One can only speculate why, if this story is not apocryphal, Wiggers had been unable to demonstrate this effect, perhaps he had used an anesthetic that abolished the reflex. Heymans remained in Wiggers’ lab to carry out additional experiments on the carotid sinus reflex11 that contributed to Heymans’ 1938 Nobel Prize for Medicine and Physiology.

CONCLUSION

Wiggers was one of the rare scientists whose research provided the foundation for a major field of medicine. Many of the remarkable achievements of modern cardiology, such as open-heart surgery, treatment of cardiogenic shock, and management of cardiac arrhythmias, can be traced back to work carried out by this remarkable man who believed that “every disease is an experiment that nature performs, and its signs and symptoms are the manifestations of abnormal function” (cited by 4). As noted by Braunwald,3 throughout most of the 20th century “physiology was the unchallenged ‘queen’ of the cardiovascular sciences, and Carl J. Wiggers, the unchallenged ‘king’ of cardiovascular physiology.”

REFERENCES

1. Fenn WO
2. Katz LN.
3. Braunwald E.
4. Feil H, McCubbin JW.
5. Wiggers CJ.
6. Katz AM.
7. Katz AM.
8. Wiggers CJ, Katz LN.
9. Wiggers CJ.
10. Wiggers CJ.
11. Heymans C.
The control of the heart rate consequent to changes in the cephalic blood pressure and in the intracranial pressure. Am J Physiol. 1928;85:498-506.
Clinical recognition that hypertrophic cardiomyopathy (HCM) was genetically determined set the stage for the systematic collection of family data allowing researchers to exploit the rapid developments in what had been termed at the time of this paper, “the new genetics.” The paper by Geisterfer-Lowrance et al is a seminal work not only in the field of HCM, but also in the wider field of cardiovascular genetics, as it utilizes a number of principles in genetic analysis that hold true over a decade later.

Phenotypic classification was central to the authors’ success. To ensure that they had a genetically homogenous population, careful delineation of the HCM disease status in large families had been undertaken, allowing this group to use genetic linkage analysis to map the disease gene to chromosome 14q11-12. The importance of this clinical work should not be underestimated, as without this solid platform linkage analyses are almost certain to fail. The authors also understood that the existence of neutral polymorphisms in their candidate genes could confound their analyses and lead to false assumptions of etiological significance. Great care was taken by the authors to ensure that variants discovered in the DNA coding sequence satisfied a number of criteria, eg, segregation with disease, absence from normal controls and evolutionary conservation, each designed to increase the likelihood that the mutation in question was disease-causing.

In hindsight, we can see also that this group also had some good fortune contributing to their success. The region of linkage contained two of the few mapped genes in the human genome at that time, namely, MYH6 and MYH7. These encode the α and β myosin heavy chains, excellent candidate genes for HCM. Secondly, because of technological constraints the authors used restriction enzyme analyses to determine whether sequence differences existed between normal- and disease-gene-bearing chromosomes. It was fortunate that the mutation in the large family under investigation (Arg403Gln) altered a DdeI restriction site and was therefore detectable by this method.

Anyone interested in HCM genetics should, however, be grateful for this good fortune, as the identification of MYH7 as the first HCM-causing disease gene has led to an extremely fruitful decade. Up to 9 sarcomeric protein-encoding genes have now been shown to cause HCM (as well as other phenotypes, such as dilated and restrictive cardiomyopathies), with over 100 disease-associated mutations having been described. Continued biochemical/biophysical analyses of these mutated proteins have begun to provide insights into how these mutations cause disease and result in such divergent phenotypes.

It may be argued that little direct clinical benefit has yet been delivered by our understanding of the molecular basis of HCM. Indeed, some may say that we have learned more about myocardial biology than we have advanced treatment. It is important, however, to put discoveries in to a proper historical context. HCM had probably generated more debate and confusion than true understanding about its etiology in the preceding 30 years. It is with this in mind that the importance of this paper must be judged.

1990

Gabriela Sabatini beats Steffi Graf and a 19-year-old Pete Sampras beats Andre Agassi to win the US tennis Open Single titles; US soul singer Marvin Gaye gets a star on Hollywood’s walk of fame; and the USA, England, France, USSR, and East and West Germany sign agreements allowing the two Germanys to merge.
Studies have highlighted the proatherogenic effect of intravascular inflammation and the role of infectious agents in atherogenesis. It seems reasonable to hypothesize that genetic variants affecting the immune response could affect susceptibility to cardiovascular disease. The toll-like receptors have been shown to be critical in the control of innate immunity. Activation of these receptors results in the release of proinflammatory molecules. Polymorphisms of toll-like receptor 4 (TLR4) have been characterized (Asp299Gly and Thr399Ile), which impair the efficiency of receptor signaling, providing an opportunity to assess the effects of genetic determinants of immune responsiveness on the risk of atherosclerosis.

The study population was that of the Bruneck study, consisting at recruitment in 1990 of 125 males and females in 4 age ranges (40-49 years, 50-59 years, 60-69 years, and 70-79 years). Follow-up evaluations took place in 1995 and 2000. Of these subjects, 826 had complete data for the first study period, DNA was available on 810, and 716 were available for sonographic evaluation at the final time point. Each subject had a clinical evaluation including whether or not they had a history of bacterial infections and cardiovascular events. Blood samples were analyzed for a number of inflammatory markers including CRP, α1-antitrypsin, interleukins IL-2 and IL-6, among others, and DNA was obtained. Atherosclerosis and its progression were assessed by the measurement of standard intermediate phenotypes following ultrasound scanning of the internal and common carotid arteries.

Of the 810 subjects available for DNA analysis, 53 were heterozygous and 2 homozygous for the Asp299Gly allele. 46 of these subjects also carried the Thr399Ile allele and 9 had an isolated Thr399Ile. Compared with wild type, carriers of the Asp299Gly allele (alone or in combination with Thr399Ile) had lower levels of a number of inflammatory markers, cytokines, and acute-phase reactants, without any association between the polymorphisms and common vascular risk or lifestyle factors. Furthermore, carriers of the TLR4 polymorphisms appeared to be more prone to acute bacterial infection than their wild type counterparts (14.5% versus 6% in wild type). With regard to atherosclerosis, in each of three independent scoring methods the Asp299Gly allele was found to be a significant protective factor, with no evidence for differential effects of the TLR4 polymorphisms in subgroups defined by lifestyle or common cardiovascular risk factors. Similarly, cardiovascular events and cardiovascular deaths were significantly reduced in the Asp299Gly subgroup.

The authors therefore concluded that the presence of the Asp299Gly polymorphism predicted low levels of circulating inflammatory molecules, an increased risk of severe bacterial infection, and a decreased risk of cardiovascular disease. One must be cautious in assessing the significance of the findings of one study; however, this paper provides fascinating support for the role of an efficient innate immune response in predisposing to atherosclerosis and the evolutionary payoff that such a system demands in terms of increased risk of cardiovascular disease.

German goalkeeper Oliver Kahn wins the Golden Ball Award as the World Cup’s most outstanding player; American cyclist Lance Armstrong wins his fourth consecutive Tour de France; and Maxime Brunerie, 25, fires at French president Jacques Chirac on the Champs-Elysées during the Bastille Day parade.
Genetic advances have allowed us to make great progress in determining the molecular substrate for a large number of Mendelian diseases. However, progress in determining the genetic basis for common, genetically complex diseases has been much slower.

Genetic association studies look for differences in frequency of genetic markers in unrelated affected individuals and unaffected controls. It has been common practice to use such techniques in a candidate gene approach, where polymorphisms in a known gene are used in studies of a defined phenotype, eg ACE I/D polymorphism and hypertension. Improved techniques for rapid detection and typing of polymorphisms have suggested that this technique could be used on a genome-wide scale in the near future, allowing researchers to identify genomic regions of interest in the search for disease predisposing genetic variants. Two general strategies for such investigations have been proposed, termed the “direct” and “indirect” methods.

The direct method aims to identify all the common variants in coding and regulatory regions of all genes, in the hope that these would underlie susceptibility to common disease. Frequencies of these variants are then compared in study populations and controls, disease association being indicated by a difference in frequency of a given variant between them. The improved cataloguing of human genes following the human genome project has made this approach more of a reality. However, we still don’t have a complete list of those common variants that could be implicated in disease causation, thus these methods are currently impracticable.

The indirect strategy avoids the needs for complete maps of genes and common variants, by relying instead on association between disease phenotypes and neutral polymorphisms located near a risk-conferring variant. Associations such as these arise because of the existence of linkage disequilibrium (LD) between the neutral polymorphism and the risk-conferring locus. At present, the neutral polymorphisms of choice are single nucleotide polymorphisms (SNPs). Unfortunately, we don’t know how far LD extends from any given marker and, therefore, how many markers we need to provide a sufficiently dense map to find association using indirect strategies.

Kruglyak uses computer population simulations to attempt to answer these important questions. Although these simulations are no replacement for detailed experimental studies, they do allow us to draw some startling and potentially disturbing conclusions. The results suggest that the average distance that useful levels of LD will extend is only 3 kb, suggesting that some 5000 000 SNPs will be required for genome-wide LD mapping strategies to be successful.

Although systematic SNP detection efforts are now under way to generate the maps required for these analyses, technologies will be required that can type 500 000 SNPs in thousands of individuals sufficiently cheaply and rapidly to make the maps useful. At present, such technologies are not widely available. This paper asks important questions of all those interested in disease gene mapping.
his article is a leader to a paper presented by Newton Morton’s group (Lonjou C, Collins A, Morton N. Allelic association between marker loci. Proc Natl Acad Sci U S A. 1999;96:1621-1626). The paper from Lonjou et al is highly mathematical, densely argued, and for the uninitiated a difficult introduction to the use of linkage disequilibrium (LD) in the study of complex disease. However, a number of important conclusions about the use of isolated populations to facilitate such studies are reached. The article by Kruglyak is important in a number of respects. Not only does the author help the casual reader better understand the significance of the findings of Lonjou et al, but he also provides a refresher course on the principles of LD and its use in the study of human disease.

LD refers to the nonrandom association of alleles at two linked loci. In general, the closer together the two loci, the more likely it is that the association of the alleles will remain. LD is of interest in human genetics when one locus is a previously uncharacterized disease locus and the other is a known polymorphic marker, eg, single nucleotide polymorphisms (SNPs). The frequency of the known marker will be higher in patients if it is in LD with an unknown disease allele that confers increased risk of the disease in question. Workers can, in theory, use maps of known polymorphisms to look for association with disease phenotype and infer that a disease susceptibility allele must lie close to, in LD with, the marker.

The extent to which useful LD extends from a given marker has been much debated. However, empirical, experimental evidence to address this question is sparse. Lonjou et al attempt to address this by extracting from the published literature haplotype frequencies at 2 genomic regions in a wide variety of populations and compare levels of LD. The regions chosen were the MNS and Rh blood group systems. The populations examined included a number of subpopulations from 8 major geographic regions as well as 6 populations considered by Lonjou et al to be genetic isolates.

The conclusions of Lonjou et al concerning the levels of LD in these distinct populations, subpopulations, and isolates may be disheartening to an investigator hoping to take advantage of increased LD in such subpopulations and isolates. Little if any increase in LD was found in most of the subgroups they tested when compared with more admixed populations. However, the insights of Kruglyak may still provide some hope for researchers in human genetics seeking to use LD for disease gene mapping, as he uses this commentary to lay out sound principles by which the difficulties highlighted by Lonjou may be overcome.

The paper of Lonjou et al has many strengths to recommend it to the determined reader. The experimental methods are sound, especially the decisions to study many different populations and the use of bi-allelic markers systems, which approximate common disease genes. As such, this paper sets a framework and imperative for more extensive studies of LD in the human genome, with the hope that eventually a human genome LD map will provide a sound base from which studies of human disease may proceed apace.

1999

King Hussein of Jordan dies and is succeeded by his son Abdullah II; the US Senate acquits President Clinton of impeachment charges; and General Olusegun Obasanjo is elected president of Nigeria
One short phrase can sum up the importance of this paper and the message it delivers, “keep an open mind.” Approximately one third of cases of dilated cardiomyopathy (DCM) appear to have a genetic origin. A number of genetic loci have been determined for DCM, and in a few cases the causative gene has been cloned and mutations have been identified. Inherited DCM can be found alone or in combination with skeletal muscle involvement. In the latter setting, the genes implicated are most often those encoding components of the dystrophin-glycoprotein complex (DGC). In cardiac and skeletal muscle, the DGC comprises dystrophin, the syntrophins, α- and β-dystroglycan, the sarcoglycans (α, β, γ, and δ), and sarcospan. The α- and γ-sarcoglycans are restricted in their expression patterns to cardiac and skeletal muscle, whereas other isoforms are additionally expressed in smooth muscle cells.

The experiments in this paper were designed to study phenotypic differences found in mice that were null for either α or δ sarcoglycans (Sgca or Sgcd, respectively). Sgca mice failed to express α-sarcoglycan in cardiac or skeletal muscle cells. This was associated with a loss of other sarcoglycan components in these tissues. The main phenotypic effect of this was a progressive muscular dystrophy. No signs of a cardiomyopathy were observed. Sgcd null mice (lacking expression in skeletal, cardiac, and vascular smooth muscle) had a very different phenotype. These mice developed a skeletal myopathy, characterized histologically by large areas of necrosis. By the age of 3 months, they had also developed a DCM.

The Sgcd mice had no expression of δ-sarcoglycan in vascular smooth muscle, eg, coronary arteries, whereas the Sgca mice had preservation of the DGC in this tissue. Could this be the cause of the myonecrotic phenotype and the development of DCM? In vivo perfusion of coronary arteries in Sgcd mice showed vascular constrictions that could be exacerbated by exercise and diminished by the administration of nicorandil. The timing of the development of these constrictions was consistent with them being the cause of the myonecrosis.

It has often been assumed that DCM caused by mutations of the DGC resulted from increased susceptibility to contraction-induced damage and subsequent cell death. Indeed, this may still be the case for DCM associated with most of the dystrophin-sarcoglycan components as well as defects of the cytoskeleton. However, this paper presents compelling evidence that mutations of δ-sarcoglycan may lead to muscular dystrophy and DCM by a novel mechanism.

The demonstration of a vascular component to DCM in Sgcd mice may also suggest that defining DCM at a molecular level in patients could have significant therapeutic consequences, and that treatment strategies may in the future be tailored to an individual patient’s underlying molecular pathology.

Russian president Boris Yeltsin replaces Prime Minister Sergei Stepashin with Vladimir Putin;
more than 17,000 people die during earthquakes (7.4 on the Richter scale) in Turkey;
and the people of East Timor vote for independence from Indonesia.
The pathways that link appropriate stimuli to re-programming of myocyte gene expression patterns, cell growth, and hypertrophy are poorly understood, but such understanding will be critical if we are to develop new methods of countering the myocardial hypertrophic response and decreasing the associated mortality. Control of intracellular calcium levels is crucial to the normal functioning of the cardiac myocyte. In response to increased stretch or loads, intracellular calcium levels are known to rise in working heart preparations. Many trophic or humoral factors that can induce cardiac hypertrophy, eg, angiotensin II, are also known to increase intracellular calcium levels. Although different trophic factors may use different second messenger systems (eg, the mitogen-activated protein kinase or protein kinase C pathways), activation of these pathways also results in an increase in intracellular calcium. These data implicate a rise in intracellular calcium as having a central role in the development of cardiac hypertrophy.

Study of the immune response has shown that lymphocytes utilize a calcium-dependent phosphatase, calcineurin, in coordinating immune activation. Calcineurin regulates immune response genes by dephosphorylating a family of transcription factors known as NF-ATs (nuclear factors of activated T cells). Dephosphorylated NF-ATs translocate to the nucleus and activate immune response genes. The immunosuppressive drugs cyclosporin A (CsA) and FK 506 inhibit this process by decreasing calcineurin's ability to dephosphorylate NF-AT transcription factors. Therefore, control of intracellular calcium levels is critical to normal control of the immune response. Could the myocardium use a similar system to generate a hypertrophic response following appropriate stimuli?

Molkentin et al describe a number of elegant experiments, the first of which consisted of a yeast two-hybrid screen to find proteins interacting with the transcription factor GATA4, a known effector of cardiac gene expression. They identified a member of the NF-AT family (NF-AT3), suggesting a mechanism of coupling intracellular calcium levels to gene transcription in the heart. The authors also showed that NF-AT3 could bind to and activate promoters of genes up-regulated during the hypertrophic response (acting in a synergistic fashion with GATA4) and that the intracellular hypertrophic response to angiotensin II could be blocked by treatment with CsA and FK 506.

These initial experiments led the investigators to generate transgenic mice expressing a constitutively active calcineurin transgene in the myocardium. All mice expressing this active transgene developed significant myocardial hypertrophy. Many also subsequently developed dilated cardiomyopathy (DCM) and sudden death. The hearts of the transgenic mice also expressed a fetal gene program. Of course, it is possible that the calcineurin-induced hypertrophy was initiated via an NF-AT3-independent pathway. To answer this question, the authors generated transgenic lines expressing a constitutively active, calcineurin-independent NF-AT3 in the myocardium, using wild type NF-AT3 as controls. The controls did not develop hypertrophy, but all the mutants expressing a constitutively active NF-AT3 developed severe myocardial hypertrophy. Thus, activated NF-AT3 alone is sufficient to induce hypertrophy.

These findings are of startling significance, showing not only that altered calcium handling is associated with myocardial hypertrophy, but also providing us with a mechanism whereby these signals can be transferred to the nucleus and gene transcription programs altered. The results also raise the possibility that drugs such as CsA could have a role in the treatment of myocardial hypertrophy.

The state of Israel celebrates its 50th anniversary; Philip Roth wins the Pulitzer prize for his novel “American Pastoral”; and a landmark peace settlement, the “Good Friday Accord,” is reached in Northern Ireland.
A molecular basis for cardiac arrhythmia: \textit{HERG} mutations cause long QT syndrome

M. E. Curran, I. Splawski, K. W. Timothy, G. M. Vincent, E. D. Green, M. T. Keating

\textit{Cell}. 1995;80:795-803

LQTs, the long QT syndrome, is a relatively uncommon disorder (1/10 000), but with terrible consequences for those families segregating the phenotype. It usually presents as palpitation, syncope, or sudden death in healthy young individuals. Increasingly, the familial nature of the condition is being recognized and a significant number of asymptomatic individuals are now ascertained on family screening. LQTS was first reported in association with deafness in 1957 and without deafness in the early 1960s. Many theories of disease pathogenesis arose, most commonly implicating abnormal myocardial sympathetic nervous system balance, or abnormal myocardial ionic homeostasis.

The first report of a successful genetic linkage study in LQTS was that of Keating et al in 1990, but no disease-causing mutation was described in these studies. This locus became known as LQT1. The paper of Curran et al was one of three very important papers published between the first months of 1995 and January 1996. By this time it had become clear that LQTS was a genetically heterogeneous disease, with a number of disease loci resulting in the same or similar clinical phenotypes. These different loci were termed LQT1, LQT2, etc. The disease locus LQT2 had been mapped to chromosome 7q35-36. As was the case for LQT1 on chromosome 11, sequence analysis of plausible candidate genes had not identified a disease-causing mutation.

This paper beautifully demonstrates the value of the candidate gene approach and the power that can be gained from the use of bioinformatic systems. Any human geneticist with a mapped disease locus, but with no identified causative gene, was well advised in the mid-1990s to keep a close watch on the literature, as newly identified genes were rapidly mapped to specific chromosomal regions. In 1994, the human ether-a-go-go-related gene (\textit{HERG}) was mapped to the LQT2 locus. At this time, the function of the protein encoded by \textit{HERG} was not known, but the predicted amino acid sequence had homology to a potassium channel, \textit{HERG} thus became a strong candidate for the LQT2 gene. Curran found mutations in \textit{HERG} in 6 families, and thus concluded that it was the disease-causing gene. As pointed out above, other papers (one by Keating and coworkers in the same edition of \textit{Cell}), confirmed defects of cardiac ion channels as the molecular basis of LQTS.

Many data on the clinical phenotype of LQTS were gathered before the genetic heterogeneity of the disease was known. It is now well recognized that much intragenetic and intergenetic heterogeneity of disease expression exists. Indeed, it has become increasingly recognized that a significant proportion of mutation-carrying individuals may remain free of disease. Other genotype-phenotype correlations have emerged over time and include risk factors for arrhythmia stimulation, specific ECG abnormalities, risk of cardiac events by age, lethality of cardiac events, and mean age at presentation.

Molecular genetic understanding of LQTS has allowed the introduction of presymptomatic diagnosis (by DNA analysis) and treatment. Investigators have also gained a greater understanding of the natural history of the disease, leading to the redefining of LQTS diagnostic criteria. An understanding of secondary LQTS, eg, drug-induced LQTS, has opened up new avenues of investigation in pharmacogenomics and may lead in the future to genotype-specific prescribing of drugs known to prolong the QT interval. Finally, the possibility of therapies targeted at the defective ion channel in an individual patient may offer the prospect of mutation-targeted intervention.

\textbf{1995}

British trader Nick Leeson is arrested for his involvement in the collapse of Barings Bank PLC; the graves of czar Nicholas and his family are found in St Petersburg; and a nerve gas attack by the Aum Shinrikyo (Supreme Truth) cult in the Tokyo subway kills 12 and injures thousands
Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene


Marfan syndrome (MS) is a common inherited single-gene disease characterized by ocular, skeletal, and cardiovascular features. Much of the disease-associated morbidity and mortality is preventable by routine clinical screening, but variability of disease expression makes detailed prognostication difficult in individual cases. MS is inherited in an autosomal dominant fashion with almost complete penetrance. Most specialist MS clinics will have extensive pedigrees for clinical follow-up. MS was therefore an excellent candidate disease for early molecular genetic analysis. Furthermore, as well as elucidating the molecular nature of MS, it was hoped by workers in this field that a greater understanding of MS would result in a greater understanding of related phenotypes, eg, familial ectopia lentis (FEL), Beals syndrome (congenital contractural arachnodactyly [CCA]), and familial abdominal aortic aneurysm (FAAA).

Linkage of MS to a disease locus on chromosome 15 (15q15-21.3) was established in 1990. The extracellular microfibrillar glycoprotein fibrillin had been mapped to this location and abnormalities of fibrillin handling were demonstrated in fibroblast culture from patients with MS. Fibrillin was therefore an excellent candidate gene for MS. At the time of this study, the genomic structure of fibrillin was only partially understood. Indeed, the full cDNA sequence was yet to be published. However, with the available sequence data, the authors set out to determine whether mutations in fibrillin could be demonstrated in MS patients. Using the scant polymorphic marker maps available and by identifying novel restriction fragment length polymorphisms (RFLPs) in the fibrillin gene, linkage to fibrillin with statistically significant LOD (logarithm of the odds) scores was demonstrated in two multiplex families. The first efforts at identifying mutations, based on southern blotting, failed, indicating that gross genomic rearrangements were not common in MS.

The key to the success in identifying mutations in fibrillin lay in the decision to study DNA from de novo patients, ie, those with no previous family history.

Single-strand conformation polymorphism analysis (SSCP) of cDNA from these patients identified a single aberrantly migrating band in 1 patient from an initial panel of 14 patients. Sequencing of the cDNA revealed a nucleotide transversion that resulted in the substitution of proline by arginine at codon 239 (Arg239Pro). This mutation was not present in either phenotypically normal parent, very strong evidence for its role in disease pathogenesis. A second panel of 43 MS patients was then screened for this mutation. Again, the Arg239Pro was found in 1 de novo patient, thus confirming fibrillin as the protein mutated in MS.

Of course we now know much more about the functioning of the wild type fibrillin protein and many mutations in the gene (now called fibrillin-1) have now been described. Identification of fibrillin has not led to new developments in the treatment of MS patients, eg, drug development, however, progress has been made in our understanding of the natural history and genetics of MS and other “fibrillinopathies” such as FEL and FAAA. Genotype-phenotype correlations, other than those relating to neonatal onset, are poor in MS. Hence, the clinical utility of molecular diagnostics is limited and remains largely a research procedure. Prenatal diagnosis based on mutation identification or linkage analysis is an issue frequently faced by the geneticist in the MS clinic, however, difficulties arising from variable disease expression have not been resolved by the identification of fibrillin-1 as the causative gene.

1991

Miguel Indurain of Spain wins the Tour de France bicycle race; the Warsaw Pact is formally dissolved; and Boris Yeltsin is inaugurated as the first freely elected president of the Russian Republic
Low-density lipoprotein (LDL) is now unequivocally causally linked to the development of coronary heart disease (CHD), one of the leading causes of death in the Western world. As in many areas of medicine, study of the role of LDL in CHD has been greatly facilitated by the study of rare metabolic disorders that result in raised plasma concentrations of LDL and increased CHD risk. These studies have resulted in the elucidation of pathogenic mechanisms in four disorders that raise plasma LDL.

The four disorders that result in elevated plasma LDL described in this review include firstly familial hypercholesterolemia (FH). From its initial description in 1938 to the present, our increase in understanding of FH has been phenomenal, principally following the work of Goldstein and Brown. A defect of the LDL receptor (LDLR) in individuals with FH was first demonstrated in 1973. Now, over 600 mutations of the LDLR gene have been described and effective treatments for this condition have resulted directly from this work. The authors rightly received a Nobel Prize for their contributions.

The second genetic disorder of LDL metabolism discussed is familial ligand-defective apoB-100. This disorder was distinguished from FH in 1986 and subsequently shown to be due to mutations in the gene for apoB-100 (the main protein component of LDL) that resulted in proteins with reduced ability to bind the LDLR and subsequent accumulation of LDL in the plasma.

The third disease described is sitosterolemia, an autosomal recessive disorder that results in the accumulation of LDL with increased levels of plant sterols in addition to LDL. Defects of two ABC transporters (ABCG5, ABCG8), which likely form heterodimers, result in increased absorption of dietary cholesterol and plant sterols and defective bile secretion of these sterols, therefore resulting in hypercholesterolemia.

The “cholesterol quartet” was completed by the discovery of the underlying defect in autosomal recessive hypercholesterolemia (ARH). This disorder, differentiated from FH by the presence of normal plasma LDL levels in the parents of affected children, causes a severe phenotype similar to homozygous FH. However, LDLR functioning in cultured fibroblasts is almost normal and no mutations in the LDLR gene have ever been described in this group. The defect in ARH has now been shown by Hobbs and coworkers to result from mutations in a gene encoding a previously unrecognized cytosolic LDLR “adaptor” protein, essential for LDLR localization to clathrin-coated pits and subsequent endocytosis and receptor recycling. Interestingly, the normal fibroblast phenotype seen in ARH patients suggests a hepatocyte-specific role for this adaptor molecule.

Of course, each of these diseases is individually rare when compared with the incidence of CHD in the general population. Also, we now understand that CHD in most individuals results from a complex interaction of genetic and environmental factors rather than simple monogenic defects. However, these studies (along with subsequent discoveries, eg, cholesterol 7α-hydroxylase deficiency) have placed the LDLR at the center of research into the pathogenesis of CHD and resulted in the introduction of β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase–inhibitor therapy. No finer example of the translation of basic biochemical and molecular biological research into patient management comes easily to mind.

Pope John Paul II visits the Middle East; media magnate Silvio Berlusconi wins his second, nonconsecutive term as Italian prime minister; and up to 125 people are killed and hundreds injured in a stampede at a football stadium in Accra, Ghana.
Myocyte-enriched calcineurin-interacting protein, MCIP1, inhibits cardiac hypertrophy in vivo


Proc Natl Acad Sci U S A. 2001;98:3328-3333

Blairolkent et al (see page 55) beautifully described a calcineurin-dependent pathway that led to the induction of a cardiac hypertrophic gene transcriptional program and subsequent myocardial hypertrophy. This led to hopes that calcineurin inhibitors, eg, cyclosporin A (CsA), would be potent antihypertrophic agents. Previous investigations had yielded conflicting results, with some studies reporting an exaggerated hypertrophic response following exposure to CsA. Rothermel et al decided on a different experimental approach to address this issue.

Myocyte-enriched calcineurin-interacting protein (MCIP1) is expressed in cardiac and skeletal striated muscle. MCIP1 binds to the catalytic subunit of calcineurin and directly inhibits its effect on nuclear factor of activated T cells (NF-AT), thereby inhibiting calcineurin-mediated effects on target genes. MCIP1 would therefore be expected to have a potent antihypertrophic effect, if calcineurin was indeed central to the hypertrophic program. Interestingly, MCIP1 expression is induced by calcineurin, establishing a potential negative feedback loop on unrestrained calcineurin activity.

Rothermel et al generated transgenic mice overexpressing a human cDNA-encoding MCIP1, under control of the α-MHC (myosin heavy chain) promoter. Expression of the cDNA would therefore be restricted to cardiomyocytes. Unexpectedly the promoter (which contained 3 noncoding exons) produced aberrant splicing between the second noncoding exon of α-MHC and acceptor sequences within the MCIP1 cDNA, thus removing the authentic MCIP1 initiation codon. A shorter than expected MCIP1 product (beginning at amino acid 81) was generated. The truncated protein was shown to accumulate stably in transgenic hearts and retain the ability to inhibit calcineurin.

Previous work had shown massive cardiac hypertrophy in mice expressing a transgene encoding a constitutively active form of calcineurin (CnA*). MCIP1 transgenic mice were crossed with the CnA* mice to produce doubly heterozygous offspring. In addition, mice overexpressing MCIP1 were exposed to two stimuli known to induce hypertrophy, adrenergic stimulation via isoproterenol infusion and a program of forced exercise.

The MCIP1 overexpressing animals had no obvious myocardial phenotype other than a reduced cardiac mass when compared to wild type littermates (5% to 10% reduction). In each of the three experimental setups (double heterozygotes, adrenergic stimulation, forced exercise), overexpression of MCIP1 was found to result in significant reduction in hypertrophy when compared with appropriate controls. In addition, cardiac hypertrophy in CnA* mice often progresses to a dilated phase and predisposes to sudden death, both seen in many forms of human hypertrophic cardiomyopathy. This progression and reinduction of the fetal gene program were inhibited by the concomitant overexpression of the MCIP1 transgene in the doubly heterozygous animals. Some important conclusions were drawn from these studies. MCIP1 was confirmed as an in vivo antagonist of calcineurin signaling. It was shown that concentrations of MCIP1 required to produce these effects had little effect on cardiac structure, other than a small reduction in cardiac mass, and that the effects of MCIP1–induced calcineurin inhibition reduced the hypertrophic response to genetic (CnA*), environmental, and pharmacologic stimuli.

These findings strongly implicate calcineurin as an important downstream mediator of the hypertrophic response and when one considers the morbidity and mortality associated with myocardial hypertrophy (sudden death, heart failure), they should provide a strong stimulus to future drug development. In addition, MCIP1 is highlighted as a potentially novel target for such therapeutic intervention.

2001

Animator William Hanna (Yogi Bear, the Flintstones, Tom and Jerry), dies, aged 90; the film “Gladiator” wins five Oscars; and the world’s largest offshore oil rig sinks in Brazil, killing 10 persons
## Bibliography of One Hundred Key Papers

selected by R. Sanders Williams, MD; Pascal J. Goldschmidt-Clermont, MD

Duke University Medical Center - Durham, NC - USA

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### Bibliography of One Hundred Key Papers

Association between CYP2C9 genetic variants and anticoagulation-related outcomes during warfarin therapy.  
*JAMA.* 2002;287:1690-1698.

Hill JA, Rothermel B, Yoo KD, et al.  
Targeted inhibition of calcineurin in pressure-overload cardiac hypertrophy. Preservation of systolic function.  

Hixson JE, Blangero J.  
Genomic searches for genes that influence atherosclerosis and its risk factors.  

Hobbs HH, Leitersdorf E, Goldstein JL, Brown MS, Russell DW.  
Multiple crm-mutations in familial hypercholesterolemia. Evidence for 13 alleles, including four deletions.  

Hobbs HH, Russell DW, Brown MS, Goldstein JL.  
The LDL receptor locus in familial hypercholesterolemia: mutational analysis of a membrane protein.  

Hu FL, Gu Z, Kozich V, Kraus JP, Ramesh V, Shih VE.  
Molecular basis of cystathionine beta-synthase deficiency in pyridoxine responsive and nonresponsive homocystinuria.  

Kandzari DE, Goldschmidt-Clermont PJ.  
Platelet polymorphisms and ischemic heart disease: moving beyond traditional risk factors.  

PIA polymorphism of glycoprotein IIIa and risk of adverse events after coronary stent placement.  

PIA polymorphism of platelet glycoprotein IIIa and risk of restenosis after coronary stent placement.  

Keating MT, Sanguinetti MC.  
Molecular and cellular mechanisms of cardiac arrhythmias.  
*Cell.* 2001;104:569-589.

Khogali SS, Mayosi BM, Beattie JM, McKenna WJ, Watkins H, Poulton J.  
A common mitochondrial DNA variant associated with susceptibility to dilated cardiomyopathy in two different populations.  

Toll-like receptor 4 polymorphisms and atherogenesis.  

Kruglyak L.  
Genetic isolates: separate but equal?  

Kruglyak L.  
Prospects for whole-genome linkage disequilibrium mapping of common disease genes.  

Laule M, Cascorbi I, Stangl V, et al.  
A1/A2 polymorphism of glycoprotein IIIa and association with excess procedural risk for coronary catheter interventions: a case-controlled study.  
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<td>Olson EN, Williams RS.</td>
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<tr>
<td>Rothermel B, Vega RB, Yang J, Wu H, Bassel-Duby R, Williams RS.</td>
<td>A protein encoded within the Down syndrome critical region is enriched in striated muscles and inhibits calcineurin signaling.</td>
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