

Cardiovascular Disease: Genes and Public Health

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Abstract

Introduction: The advances in molecular biology hold great promise for complex conditions such as cardiovascular disease. Early accurate diagnosis and new preventive and treatment strategies are among the potential benefits of genetic understanding. Many genes and mutations have been discovered that contribute to rare Mendelian forms of cardiovascular disease. However, there has been little tangible success in defining specific mutations that explain the more common forms of cardiovascular disease. Of the numerous genes tested, inconsistent results are a recurring theme. **Methods:** This review addresses broader issues that touch on the clinical and epidemiological context in which the genetics of cardiovascular disease might develop. How do genes and environment interact in cardiovascular disease? What characteristics of a marker might make it useful? How will genetic understanding be used? What is the place of physiology in molecular biology? Is the future of genetics in patient management or public health? **Results:** It is concluded that individuals and communities are unlikely to accept widespread DNA screening, and less likely to tolerate genetic manipulation. Genetic complexity will make the identification of specific mutations an expensive and potentially thankless task. **Conclusions:** Genetics may reveal new pathophysiological mechanisms against which simple, safe and effective public health measures can bring benefit to the greatest number.

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Introduction

The excitement surrounding the Human Genome Project and its impending completion has raised expectations of the possibilities for genetics in common disease. It is assumed that the identification of mutations that confer susceptibility will provide the basis for new means of detection, prevention and treatment.

Cardiovascular disease remains one of the commonest causes of death, despite the fact that the epidemic of cardiovascular disease has peaked in many industrialised nations. In Australia, 1 in 3 people die from coronary heart disease and 1 in 5 succumb to stroke.¹ Populations in Asia and Central and Eastern Europe are experiencing an "epidemiological transition" that delivers reduced infant mortality and increased life expectancy, but seemingly at the cost of higher prevalence of cardiovascular disease.^{1,2} An understanding of the basic mechanisms that predispose to cardiovascular risk has considerable public health potential.

Genes or Environment?

Within populations there exist social, cultural, economic, biological and psychological influences that exert important

effects on the prevalence of cardiovascular disease. The evidence supporting this assertion comes from significant changes in population cardiovascular risk. A pertinent example is the change over time in cardiovascular risk in geographically stable populations, such as the decline in cardiovascular disease in Western Europe and the United States since the 1950s.³ Migration studies also provide incontrovertible evidence of the importance of lifestyle and behavioural characteristics.⁴ Where measured, changes in environment appear to affect cardiovascular risk factor levels of all members of the community, resulting in alterations in the population average level of risk.

Attempts to identify environmental causes of cardiovascular risk have focused on nutrition and smoking.³ However, specific elements of the complex fabric of behaviours and lifestyles are difficult to separate and largely determined by community-wide fashions and trends. It is not simple to ascribe individual variation in risk to individual environment.

Largely genes determine individuality. Genes also explain in part familial patterns of disease. Examples come from families that exhibit Mendelian diseases resulting from major DNA mutation such as familial hyper-

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cholesterolaemia resulting from mutations in the LDL receptor gene and hypertension in Liddle's syndrome resulting from mutations of the genes encoding the β - or γ -subunits of the epithelial sodium channel.^{5,6}

However, the common familial patterns of cardiovascular disease or associated risk factors are not Mendelian in nature.⁷ Typically, there is increased predisposition to cardiovascular disease when a first degree relative is affected⁸—in clinical terms, a positive family history. However, familial aggregation is not only seen when a relative is affected. Quantitative cardiovascular risk factors such as blood pressure, weight and cholesterol correlate between family members across the entire range. Offspring of parents with low blood pressure are just as likely to have low blood pressure, as offspring of parents with high blood pressure might have high blood pressure.⁷

One implication of the correlations of cardiovascular risk factors between relatives is the existence of polygenes, each exerting a quantitative effect on the risk factor. In contrast to Mendelian disease where one gene is affected by mutation (often in the coding region), polygenes are likely to be affected by mutations in gene regulatory regions that modulate gene expression in a graded manner.⁹

Using mathematical modelling, it is possible to estimate the components of variance in risk factors that can be ascribed to genes or environment.^{10,11} However, mathematical analyses cannot provide a definitive answer to the question “Are genes or environment more important in determining cardiovascular risk?”. In fact, the question is spurious. Both genes and environment are important, and most simply conceived in terms of environmental factors affecting the population mean and genes affecting one's rank within the population.¹²

Populations or Patients?

The alignments of public health with populations and clinical medicine with diseased individuals have influenced strategies used to study the genetics of cardiovascular disease. Much research has been predicated on the medical model, i.e. studies of hypertensive, obese or hypercholesterolaemic patients. Case-control association analyses and affected sibling-pair linkage studies are typical examples. However, a population perspective may be more valuable. Rose¹³ emphasised that more cases of disease can result from a large number of people at moderate risk, than from a small number of subjects at high risk. Blood pressure, cholesterol and weight show a linear relationship with cardiovascular risk, such that each increment in risk factor level above the population minimum is associated with increased personal risk. The MRFIT study demonstrated¹⁴ that 32% of the deaths that could be attributed to the effects of blood pressure occurred in

people with systolic blood pressures of less than 140 mmHg.

Although likely, it cannot be assumed that the genetic factors that determine clinically high levels of risk factors are identical to those that determine population average levels. The most informative and comprehensive genetic analyses will not only address the highest phenotypic levels, but also the genetic explanations of population variation in risk factor levels.

Case-Control Studies

Case-control comparisons have been the mainstay of clinical cardiovascular research. It is not surprising that they also account for the majority of genetic analyses. However, there are special problems that have limited the impact of the many studies published to date. One of the most studied candidates is the ACE gene and it serves as an example of the pitfalls that abound.

One of the first papers to draw attention to the ACE gene in human cardiovascular disease described the deletion (D) allele as a “potent” risk factor for myocardial infarction.¹⁵ The basis for such a claim was the small difference in prevalence of the DD genotype between cases (32%) and controls (27%). The difference may have achieved statistical significance, but the relatively high rate of the DD genotype in controls suggests that the DD genotype would not be useful in predicting risk of myocardial infarction.

The predictive value of a particular genotype depends not only on its relative distribution between cases and controls, but also on the prevalence of the condition. In Australia, controls outnumber cases by 2:1. Therefore, if the case frequency of a particular genetic marker is twice that of controls, there will be in the community an equal number of controls and cases with the genotype (Fig. 1a). The predictive value of such a test would be no better than flipping a coin.

In attempts to define genetic tests that might more clearly separate cases and controls, subgrouping is often used. In the case of the ACE gene, subgroups were defined as those cases and controls at low phenotypic risk (based on lipids and weight) and the presence of the CC genotype of the angiotensin type 1 (AT1) receptor gene.¹⁶ In this subgroup, the frequency of the DD genotype was 83% in cases versus 27% in controls. The differential (if confirmed) is clearly sufficient to have predictive power. However, it is a case of diminishing returns (Fig. 1b). Of the original 610 cases, only 6 fulfilled the subgroup criteria of ACE DD genotype, AT1 CC genotype and low phenotypic risk.

A recurring problem with case-control studies has been inconsistency. Recent meta-analyses have attempted to reconcile the discrepant results of association studies of the ACE DD genotype and cardiovascular disease.¹⁷⁻¹⁹ The

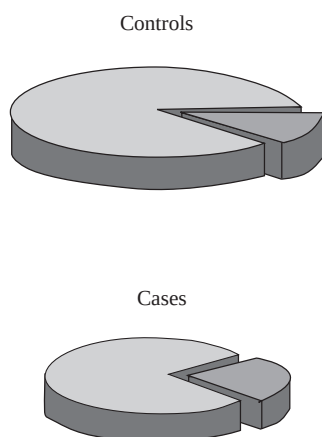


Fig. 1. Panel (a)

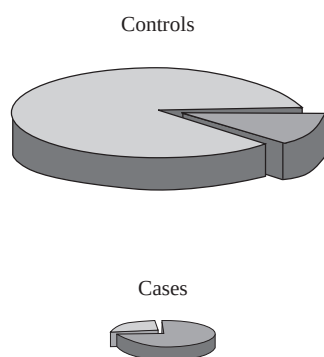


Fig. 1. Panel (b)

Fig. 1. Panel (a) depicts a population such as Australia in which cardiovascular disease accounts for one third of deaths (cases:controls = 1:2). Let us presume a genetic marker of cardiovascular disease (depicted as slice of the pie) is twice as frequent in cases as it is controls. Then, in a random sample of the population, the chance of being a case or control based on the presence of the marker is 50:50.

Panel (b) depicts a subgroup of cases defined on the basis of several coincident phenotypes and/or genotypes. Although the relative frequency of the marker is far greater in the subgroup of cases, they represent such a small proportion of the population that their relevance is very limited.

discrepancies between studies may have a number of explanations. Among these are the possibility that there is no important biological consequence of the DD genotype and that the positive studies represent false positives published by eager editors. In contrast to this uncharitable view is the possibility that the DD genotype is important in some populations, but not others. Differences in the environment or the genetic make-up of a population may highlight or obscure effects of the ACE DD genotype. Indeed, the population prevalence of the genotype varies dramatically across the continents (Fig. 2). Such variation is greater than many of the reported differences between cases and controls. This fact emphasises the possible bias that might result in studies in which cases and controls are not carefully matched to avoid population or racial stratification.

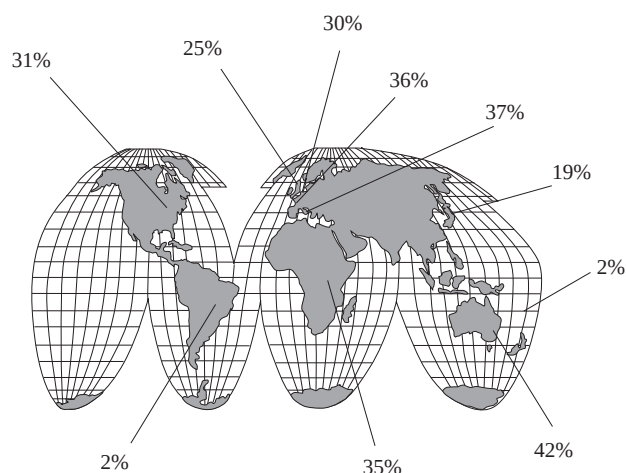


Fig. 2. Comparison of the population prevalence of the ACE DD genotype in healthy subjects in various global regions. The variation internationally is greater than that observed between cases and controls that have reported association between the ACE DD genotype and cardiovascular disease.

Nevertheless, the absence of readily replicable findings indicates that the marker in question does not exert a robust effect on predisposition to cardiovascular disease. Markers that have small and inconsistent differences between cases and controls are never likely to find a place as useful predictors of individual risk.

Mutation Identity or Physiological Effect?

One important unresolved issue is the nature of the polygenic determinants of cardiovascular risk. Studies of Mendelian disease have revealed the molecular complexity of relatively simple phenotypes. For example, hypertrophic obstructive cardiomyopathy may be the result of a single mutation in one of four or more genes.²⁰ For each of these genes, tens of specific causative mutations have been identified in different families. In the case of lipid disorders, 14 variants of the gene encoding apolipoprotein E have been associated with familial dysbetalipoproteinaemia, a genetic lipid disorder characterised by elevated plasma cholesterol and triglyceride levels and an increased risk for atherosclerosis.²¹ Seven apoE variants were associated with other forms of hyperlipoproteinaemia. Similar complexity has emerged in relation to familial hypercholesterolaemia which may be the result of any one of more than 150 reported mutations in the gene encoding the LDL receptor.^{22,23} This genetic heterogeneity is a sobering example for those involved in the genetics of common multifactorial disease. In this regard, a pessimistic view might be that 5 major cardiovascular risk factors are each determined by 10 polygenes and that for each polygene there might exist 20 different mutations. Assuming a simple additive model, each polygene would account for one fifth of the genetically-determined component of cardiovascular risk. If predictive genetic testing were the

goal, then a battery of 1000 genetic markers would be required to detect the possible contributory mutations. Such analyses are possible with the new technologies such as hybrid arrays, but the value of detecting markers of such small individual effect would be doubtful. The optimistic scenario is one in which a limited number of key mutations might affect a small number of “master genes” that could explain the concordance of related cardiovascular risk factors manifest as the “metabolic syndrome”.

Whether overwhelmingly complex or relatively simple, the identification of genetic mutations is not an end in itself. The lesson from hypertrophic obstructive cardiomyopathy is that the various mutations in different genes have effects on RNA and protein expression and integrated cell function that converge at a higher physiological level. In the case of cardiomyopathy, the relevant level is at the function of the sarcomere,²⁰ fundamental changes to which produce a similar clinical picture. Once mutations are known, then the downstream core physiological effects can be defined.

Molecular Biology and Physiology

The productive union of molecular and physiological sciences is essential to place genetic discoveries in context. The path is neither simple nor rapid. For example, recent demonstration of linkage between systolic blood pressure and the genes encoding the β - and γ -subunits of the epithelial sodium channel²⁴ will lead to further analyses in attempt to identify DNA variants of potential functional importance in or around these genes. Once identified, the physiological consequences of mutations will need to be studied in relation to the levels of relevant mRNA and proteins. Cellular physiology will be required to analyse the changes in channel characteristics *in vitro*, followed by studies to define the *in vivo* cellular correlates. This will inform functional analyses of the relevant organ system, such as the kidneys, intestines and even the baroreflex.²⁵ The definition of each of the steps between DNA mutation and disease provides greater insight into fundamental effects of genetically heterogeneous mutations, thereby, increasing the opportunities to devise effective means of prevention and treatment.

The Genetic Paradox—Private Genes, Public Health

Although the vision of genetic discovery often includes the possibility of improved diagnosis and therapy including genetic engineering, the reality may be less technological. The cost-effectiveness of genetic diagnosis of cardiovascular susceptibility is far from proven. Potentially expensive molecular tests will have to prove themselves a worthwhile addition to simple clinical measures such as blood pressure or cholesterol. The benefits will also need to be sufficient to counter-balance the threat to privacy associated with individual diagnostic DNA analysis. Given

the concern surrounding genetic engineering of crops, the likelihood is low that the community will accept gene therapy, especially when it is of unproven long-term benefit and undefined risk.

Instead, molecular analyses are likely to provide a fundamental starting point for an understanding of pathophysiology. Among the methods discovered and devised to prevent the downstream effects of mutant DNA, it must be hoped that some will be simple, cheap and efficacious advice regarding behaviour and lifestyle. One exciting prospect is that the genome-wide search for genes associated with cardiovascular risk may reveal new genes and unprecedented genetic mechanisms that may be modulated by harmless dietary measures. It may be an ironic outcome of the massive molecular investment in cardiovascular disease that the dividend might be returned in the currency of public health prevention.

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