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MEDICINE IN THE 21ST CENTURY

Molecular medicine in the 21st century

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Abstract

When Watson and Crick proposed the double helix model for DNA structure in a 2 page *Nature* article in 1953, no one could have predicted the enormous impact this finding would have on the study of human disease. Over the last decade in particular, major advances have been made in our understanding of both normal biological processes and basic molecular mechanisms underlying a variety of medical diseases. Knowledge obtained from basic cellular, molecular and genetic studies has enabled the development of strategies for the modification, prevention and potential cure of human diseases. This brief overview focuses on the enormous impact molecular studies have had on various aspects of medicine. The inherited cardiac disorder hypertrophic cardiomyopathy is used here as a model to illustrate how molecular studies have not only redefined 'gold standards' for diagnosis, but have also influenced management approaches, increased our understanding of fundamental disease-causing mechanisms and identified potential targets for therapeutic intervention. The near-completion of the Human Genome Project, which identifies the 3.2 billion base pairs that comprise the human genome (the so-called 'Book of Life'), has exponentially heightened the focus on the importance of molecular studies and how such studies will impact on various aspects of medicine in the 21st century. (Intern Med J 2001; 31: 53–59)

Key words: DNA, genetics, Human Genome Project, medicine, molecular.

INTRODUCTION

Over the last decade, major advances have been made in defining the molecular basis of many genetically transmitted medical diseases. Such advances have not only allowed us to gain a better understanding of the primary defect and basic molecular pathogenesis of disease, but have redefined the diagnostic 'gold standards' of many disorders. Improved understanding of the molecular basis of disease will probably allow targeting of pharmacological strategies, as well as providing the cornerstone for gene therapy approaches.

The recent announcement that the Human Genome Project has now sequenced over 90% of the 3.2 billion base pairs that comprise the human genome[1] has heightened the focus and raised expectations of the impact that molecular studies will have on various aspects of medicine in the 21st century. Many new and exciting frontiers are opening in medicine, both to identify the thousands of genes in our genome and to understand their functions and interactions in human disease. It is therefore timely and appropriate to look briefly at how molecular studies affect the practice of medicine today, and what the future may hold.

The field of cardiovascular medicine has been one of the leaders in demonstrating the importance of molecular studies in elucidating disease mechanisms, influencing clinical diagnosis and affecting management. Disease-causing gene defects have now been identified in several cardiovascular diseases, including cardiomyopathies, dysrhythmias, congenital heart malformations, vascular disorders and some forms of hypertension.[2–6] Increasingly, molecular studies are

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defining potential risk factors for atherosclerotic heart disease, giving insights that are informing gene therapy strategies for preventing coronary artery restenosis after angioplasty and promoting angiogenesis in severe coronary artery disease.[7–10]

Hypertrophic cardiomyopathy was the first of these cardiovascular diseases in which a genetic basis was identified and, as such, has acted as a paradigm for the study of a cardiovascular genetic disorder. Hypertrophic cardiomyopathy will be used here as a model of how a genetic disorder is studied and how such studies have impacted on understanding disease mechanisms, identifying high risk subgroup populations, clinical diagnosis and treatment.

HYPERTROPHIC CARDIOMYOPATHY: A MODEL FOR THE STUDY OF AN INHERITED CARDIAC DISEASE

Background

Since the modern description of hypertrophic cardiomyopathy,[11] much interest has been generated in this clinically diverse cardiac disorder. Hypertrophic cardiomyopathy is a primary cardiac disorder characterized by hypertrophy, usually of the left ventricle, in the absence of other loading conditions such as aortic stenosis or hypertension.[12] The disorder has a wide clinical spectrum, from a benign, asymptomatic course to symptoms of heart failure.[13] The most serious complication is sudden cardiac death, with hypertrophic cardiomyopathy being the commonest cause of sudden cardiac death in individuals aged less than 35 years and in competitive athletes.[14–17] Over the last 10 years, hypertrophic cardiomyopathy has been further defined as a 'disease of the sarcomere', with several disease-causing gene mutations being identified that encode sarcomeric proteins.[2,18] Although previously thought of as a rare disorder, recent population-based studies suggest the prevalence of the condition to be as high as 0.2% (or one in 500) in the general population.[19]

Genetic basis of hypertrophic cardiomyopathy

Hypertrophic cardiomyopathy was the first cardiovascular disorder for which the genetic basis was identified (genetic aetiologies for hypercholesterolaemia and Ehlers–Danlos syndrome, both potentially leading to a cardiac phenotype, preceded this). It is a heritable disorder that is transmitted as an autosomal dominant trait (i.e. affected individuals are heterozygous: they have one normal and one mutant copy of the gene). Offspring of affected individuals will therefore have a one-in-two risk of inheriting the mutation (gene defect).

Since 1989, major advances have been made in understanding the molecular basis for hypertrophic cardiomyopathy. Hypertrophic cardiomyopathy is a genetically heterogeneous disease with several causative genes now identified. All of these genes encode sarcomere proteins and include the cardiac β-myosin heavy chain (*β-MHC*), cardiac troponin T gene, α-tropomyosin, myosin-binding protein C (*MyBP-c*), cardiac troponin I, essential and regulatory myosin light chain and, more recently, titin and actin genes. A further form of hypertrophic cardiomyopathy, co-inherited with the Wolff–Parkinson–White syndrome, has been localized to chromosome 7q3, but no gene has yet been identified.[3,18,20]

The relative frequency of causative genes in hypertrophic cardiomyopathy is summarized in Table 1. Over 100 mutations have now been identified in these genes, with most being of the missense type (i.e. a single base change resulting in an amino acid substitution). The identification of these sarcomere protein genes, coupled with basic functional studies, has shed new light on this disease both in understanding the underlying deficit and in clinical diagnosis and treatment.

Impact of genetic studies on understanding pathogenesis

The pathophysiology of hypertrophic cardiomyopathy is complex and this is reflected in the diversity of symptoms. These symptoms include chest pain, which may be typical of angina, symptoms related to pulmonary congestion (i.e. dyspnoea, fatigue, orthopnea and paroxysmal nocturnal dyspnoea), impaired consciousness (i.e. syncopal and presyncopal episodes) and palpitations. The precise mechanisms involved in these symptoms are not always clear in individual patients. However, some pathophysiological

Table 1 Frequency of gene mutations in HCM

HCM gene	Percentage of all HCM
β-Myosin heavy chain	30–35%
Cardiac myosin-binding protein C	15–20%
Troponin T	10–15%
α-Tropomyosin	< 5%
Troponin I	< 1%
Myosin light chains	< 1%
Actin	< 0.5%
Titin	< 0.5%

HCM, hypertrophic cardiomyopathy.

factors recognized to be associated with a clinical course involving such symptoms include left ventricular diastolic dysfunction, severe left ventricular outflow tract obstruction, impaired coronary vasodilator reserve and myocardial ischaemia and supraventricular/ventricular tachydysrhythmias.[13,21]

Identifying genes that cause hypertrophic cardiomyopathy has allowed research to be directed to the key area of sarcomere function and how these mutations result in cardiac dysfunction. There are at least two potential ways in which the mutant genes could exert their dominant effect. A mutant sarcomere protein can act via a dominant negative mechanism, in which incorporation into the multimeric sarcomere structure disrupts function. The mutant protein acts as a 'poison peptide'. Missense mutations that alter only a single amino acid of a contractile protein are likely to cause hypertrophic cardiomyopathy through this mechanism. Alternatively, a dominant mutation may functionally inactivate a gene, producing a null allele (non-functioning gene) and potentially reducing peptide concentrations by 50%. This situation, in which only one allele is operative, is also known as 'haploinsufficiency'. Studies expressing troponin T splice site hypertrophic cardiomyopathy mutations in a cultured avian myotube system have demonstrated the incorporation of mutated peptides into the sarcomere, accompanied by attenuated force development.[22] Such data oppose the null allele hypothesis and imply that hypertrophic cardiomyopathy mutations produce their effects by a dominant negative mechanism.

Impact of genetic studies on diagnosis

Until recently, the diagnosis of hypertrophic cardiomyopathy largely involved electrocardiography and echocardiography. Clinical diagnosis can be made from the character of the pulse and left ventricular impulse and from the characteristic apical systolic murmur that increases with the Valsalva manoeuvre. There is often a fourth heart sound. The echocardiogram is the investigation that most reliably confirms the diagnosis of hypertrophic cardiomyopathy and that provides detailed information about the distribution and severity of hypertrophy, the left ventricular cavity size, assessment of left ventricular systolic and diastolic function, left ventricular outflow tract obstruction and mitral regurgitation. The electrocardiogram has higher sensitivity than the echocardiogram in the detection of affected individuals in family studies, especially among children and adolescents, but has lower specificity when used for screening outpatient populations.[23] Other investigations that may be helpful in confirming the diagnosis or in establishing a 'risk of sudden death' profile include exercise testing (with or without echocardiography), ambulatory Holter monitoring, marked hypertrophy (>35 mm) and a history of cardiac events in other family members.[13]

With the advent of genetic screening, molecular diagnosis has become the 'gold standard'. Several important aspects of molecular diagnosis have evolved subsequently. Many individuals have been identified who carry the disease-causing mutation but who have not developed hypertrophy (genotype positive/phenotype negative). These individuals, who would previously have been classified as unaffected, can now have a diagnosis made in the absence of cardiac hypertrophy (preclinical diagnosis). While definition of such individuals has potential deleterious psychosocial implications, preclinical diagnosis allows potential treatment to be initiated early, with prevention of cardiac events a priority. Genetic diagnosis has also led to the recognition that particular gene mutations cause late-onset disease. For example, *MyBP-c* gene defects produce hypertrophy in the fourth and fifth decades of life, while *β-MHC* gene mutations cause hypertrophy by age 20 years in over 90% of individuals with these defects.[24–26] Thus, the absence of hypertrophy does not exclude an individual in a family with known hypertrophic cardiomyopathy from carrying the gene defect. This has revolutionized screening protocols, which traditionally classified at-risk individuals over age 20 years without hypertrophy as unaffected. The findings from molecular diagnoses clearly indicate that, without genetic data, at-risk individuals should be screened until at least age 40 years.

A second, often overlooked, impact of molecular diagnosis in hypertrophic cardiomyopathy has been the 'negative result'. By identifying a mutation in a family, one is now able to screen any individual within that family. This means that paediatric testing can preempt longitudinal clinical evaluations; if a child does not inherit the mutation (50% of the offspring of affected parents), the child need not be screened every 1–2 years and should be encouraged to participate fully in routine school sporting activities and extracurricular athletics. Importantly, such information provides relief to parents from worrying for that child and the child's offspring. It is pleasing to be able to tell an individual definitively that they do *not* carry the gene defect, rather than to say that their echocardiogram is normal at the moment but it will need to be checked again in 12 months.

Clearly, making a genetic diagnosis in a medical disease has numerous advantages. In hypertrophic cardiomyopathy, a genetic diagnosis can also help in resolving ambiguous diagnoses, such as individuals with a borderline or modest increase in left ventricular wall thickness, including some trained athletes with hypertrophy, patients with systemic hypertension who are suspected of having hypertrophic cardiomyopathy, patients who have hypertrophy in less common sites (e.g. apical hypertrophic cardiomyopathy, where diagnosis can be difficult)[27] and in children. Furthermore, one can now make antenatal diagnoses in families in which a mutation is known. While this clearly raises many ethical issues, prenatal diagnosis may be warranted in a family with a clearly documented 'malignant' phenotype.

Current limitations in laboratory equipment availability and the labour-intensive nature of this work mean that the greatest chance for an individual to have a genetic diagnosis is if they are part of a family with a history of the disease. This allows alternative genetic techniques, such as linkage analysis, to identify which gene is involved. Therefore, an important consideration in all inherited medical disorders is an accurate assessment by the clinician of the family history, which is considered to be the cornerstone of management of patients with genetic disease. Particular emphasis should be placed on a history of the ages at death and causes of death, which may give some estimation of the risk of death within a specific family. Furthermore, because relatives of an affected individual have a risk that is several hundred times higher than the general population, clinical screening of such individuals is justified.

Impact of genetic studies on treatment in hypertrophic cardiomyopathy

Many treatment options are currently available for hypertrophic cardiomyopathy patients. This ranges from no treatment; use of pharmacological agents (e.g. calcium channel blockers, beta-blockers and diuretics); to dual chamber pacing, septal myotomy/myectomy and transeptal ablation of septal myocardium (i.e. the creation of a limited septal infarct by direct injection of alcohol into the septal perforator artery)[28] for individuals with significant left ventricular outflow tract obstruction with symptoms unresponsive to drug therapy, for example.[13]

Molecular studies have allowed significant advances to be made both in terms of targeting treatment and in identifying potential sites of intervention. There now appears to be a clear association of some mutations with severe disease (i.e. 'malignant' mutations). For example, the Arg719Trp or Arg403Gln mutations in the *β-MHC* gene have now been shown in several families worldwide to be associated with poor prognosis and early sudden death.[24,25] Approximately 50% of individuals with these mutations die by age 40 years. This is in contrast to other mutations, for example *MyBP-c* mutations in which most have a normal life expectancy and minimal symptoms.[26] Identification of such individuals with malignant mutations, who are therefore at high risk of sudden cardiac death, will enable clinicians to consider preventative measures. Currently, treatment options include long-term therapy with amiodarone or sotalol or an implantable cardioverter-defibrillator, the latter probably being the most definitive in hypertrophic cardiomyopathy patients at high risk of sudden death.[29]

By understanding how sarcomere mutations perturb biophysical events of muscle contraction and cell signalling pathways within the myocyte, future interventions may involve correcting such defects. Furthermore, with increased understanding of the genetic mechanisms, it may be possible to target therapy to mitigate the genetic defect or, conceivably, to correct the molecular abnormality; that is, to correct the single base abnormality in the mutant allele and effectively cure the disease. Alternatively, the dominant negative mechanism by which these gene mutations act has an impact on the potential for somatic gene therapy for hypertrophic cardiomyopathy, which theoretically would have to be directed toward specific inactivation of the mutant allele.

FROM GENE MUTATION TO CLINICAL DISEASE: UNRAVELLING THE MYSTERY

In a disease such as hypertrophic cardiomyopathy, we now know of at least nine genes that are causative. We have some insight as to how mutations in these genes affect sarcomere function, but we know very little about how the dysfunctional sarcomere leads to the observed phenotype. Current and future efforts will focus on the signalling pathways that lead from the gene defect to disease and what factors influence this pathway to modify the end phenotype (Fig. 1). Recent interest has implied that a Ca^{2+}–calcineurin pathway, shown to have a role in some forms of cardiac[30,31] and skeletal muscle[32] hypertrophy, may also be important in hypertrophic cardiomyopathy. However, emerging data suggest that this is not the case and other potential pathways are therefore the focus of intense study.[33]

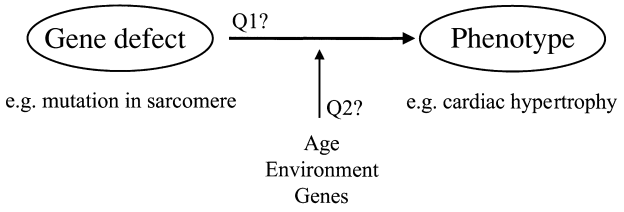

Figure 1 From mutation to disease in hypertrophic cardiomyopathy. Gene mutations cause clinical disease. However, at least two important questions remain the focus of research now and in the future: what is the signalling pathway leading from a gene defect to the clinical phenotype (Q1) and how is this process modified by either genetic and/or environmental factors, such as exercise, gender, pharmaceutical agents etc. (Q2)?

In terms of modifying the expression of the mutant gene, it has been widely reported that within a family with the same mutation there is marked variation in the severity of left ventricular hypertrophy and in the clinical manifestations in affected individuals.[24–26,34,35] Why is it that affected siblings can have such diverse phenotypes (i.e. asymptomatic versus sudden death) if they carry the same gene mutation? This phenomenon of clinical diversity, or 'phenotypic heterogeneity', is a hallmark of hypertrophic cardiomyopathy as well as other medical genetic conditions spanning numerous subspecialties (e.g. muscular dystrophies, Huntington's disease, familial haemochromatosis and familial myeloproliferative diseases).[36–39]

Such an observation implies that factors other than the underlying gene defect modify the expression of the mutant gene. These modifying factors may be genetic, for example a second gene that regulates the expression of the primary defect, or environmental factors, for example age, diet, exercise, gender and pharmaceutical agents. Clearly, the identification of such modifying factors has major implications in being able to alter the natural history of diseases such as hypertrophic cardiomyopathy. One potential genetic modifying factor that has been studied in human hypertrophic cardiomyopathy is the angiotensin-converting enzyme (ACE) gene. Some studies have indicated that the D allele of the *ACE* gene is associated with increased left ventricular hypertrophy and sudden death, implying that this potentially may be a modifying factor in the phenotypic expression of the mutant gene.[40,41] However, to define accurately such modifying factors, rodent models, whereby by genetic and environmental backgrounds can be controlled, will probably play a key role.

IMPACT OF THE HUMAN GENOME PROJECT ON MEDICINE

When Watson and Crick first described their observations regarding the double-helical structure of DNA in their 1953 *Nature* paper, they noted the structure's 'novel features, which are of considerable biological interest'.[42] What an understatement! Nearly 50 years later, one of the greatest of achievements was recently announced at a ceremony in the White House hosted by President Bill Clinton, the sequencing of the human genome, which spans some 3.2 billion base pairs.[1,43] While the sequence is not totally complete or aligned, it is only a matter of months before it will be, when we will have the so-called 'Book of Life'. The code will be useful and used as long as humans exist. With the world's spotlight focused on the unravelling of the human genome, the question arises of how the human genome data will impact on current molecular studies and ultimately on the practice of medicine.

By sequencing the human genome, thousands of genes have been and will be identified. Currently, at least 38 000 genes have been identified in the existing 'rough draft' of the human genome sequence. The total remains a mystery, with many genome scientists predicting close to 50 000 genes.[43,44] The outstanding issues directly resulting from this are: (i) the function of these genes, (ii) what defects in these genes can cause and (iii) how these genes interact. In most cases, such studies would involve both human and animal studies. There are currently many existing families with inherited disorders that span many areas of medicine (e.g. macular degeneration, diabetes, hearing loss, asthma and Alzheimer's disease). Many studies of these inherited disorders have been impeded because of areas in the genome that were unknown. Human genome data will close such 'gaps' and enable identification of novel genes that may or may not be disease causing. Animal studies, as well as *in vitro*/cellular studies, will also unravel many of the mysteries, with newly identified genes being knocked out or overexpressed, and subsequent analysis of the resulting phenotype giving preliminary clues as to the possible function of such genes in human disease.

THE FUTURE IS HERE

The near completion of the Human Genome Project has ensured an explosive start to the 21st century in the area of molecular medicine. The last decade has seen major advances in our understanding of the primary basis of many disorders across all subspecialties of medicine, based on fundamental, basic science

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