Clinical review

Science, medicine, and the future

Single gene disorders or complex traits: lessons from the thalassaemias and other monogenic diseases

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As a result of the revolution in the biological sciences following the development of recombinant DNA technology and the sequencing of most of the human genome, the role of genetics in the pathogenesis of human disease now dominates biomedical research. There is every sign that the rapidly evolving technology of the post genome era will unravel the function of the human genome and explain how the 50 000 to 100 000 genes interact with one another and the environment to make us what we are.

The central question for the medical sciences is the extent to which it will be possible to relate events at the molecular level with the clinical findings or phenotypes of patients with particular diseases. This problem will permeate every aspect of medical research and practice in the future. It will dominate predictive genetics and genetic counselling. It will also be of major importance for clinical decision making as new and novel approaches to the treatment of disease become available, particularly those involving genetic manipulation. Further exploration of the genome may also provide information on some of the common killers of Western society, such as heart disease, stroke, diabetes, and psychiatric disease, leading to a new form of pharmacology in which drugs are tailored to an individual's genetic make up. Even more important, and certainly more complex, will be relating genotype to phenotype. Many of our most important diseases almost certainly reflect varying susceptibility, due to the action of many different genes and a wide variety of environmental factors and to the ill understood biology of ageing.

Is there any way of guessing the likely levels of complexity that will be encountered as the genetic basis of disease is explored with the new technology? Theoretically, monogenic diseases should be the simplest models for asking to what extent it is possible to predict phenotypes from genotypes. The commonest diseases caused by a single gene (monogenic diseases) in humans are the genetic disorders of haemoglobin, and these were the first to be explored at the molecular level. This article briefly outlines the progress that has been made in relating the molecular pathology of such diseases to their clinical diversity and describes the same problem for other families of monogenic disorders. Finally, I will summarise the lessons that have been learnt from studies of these conditions and what they illustrate about the kinds of difficulties that are likely to be encountered with the move towards the analysis of common multigenic disorders.

Summary points

- The challenge for medical science is to relate events at molecular level with clinical findings in patients with particular diseases
- Lessons learnt from the study of monogenic disorders such as the thalassaemias suggest that the clinical application of new knowledge about the genome to common multigenic disorders may be slow
- Patients with the same genotype have very different clinical conditions because, even in monogenic disorders, other genes are also involved and environmental circumstances affect the clinical manifestations
- Relating genotype to phenotype is thus the challenge for genetic medicine over the next century

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Inherited disorders of haemoglobin

The inherited disorders of haemoglobin are by far the commonest monogenic diseases; it is estimated that about 7% of the world's population are carriers. The disorders fall into two groups: the structural variants of haemoglobin and the thalassaemias.

The structure of haemoglobin changes during embryonic, fetal, and adult life. Adult and fetal haemoglobins have α chains combined with β (haemoglobin A, $\alpha_2\beta_2$), δ (haemoglobin A_2, $\alpha_2\delta_2$), or γ chains (fetal haemoglobin, $\alpha_2\gamma_2$). The inherited disorders of haemoglobin result from many different mutations involving either the α or β globin genes.

Although over 400 variants of structural haemoglobin have been identified, only three, haemoglobins S, C, and E, reach high frequencies in certain populations. The thalassaemias, on the other hand, which are classified into the α and β thalassaemias depending on

which globin chain is ineffectively produced, are widely dispersed throughout the tropical world. I will focus on the β thalassaemias, because they are producing an increasingly serious public health problem throughout the Mediterranean region, the Middle East, the Indian subcontinent, and South East Asia.

The β thalassaemias

Pathophysiology and clinical diversity

The hallmark of all the β thalassaemias is defective β globin synthesis, which leads to imbalanced globin chain production and an excess of α globin chains. The globin chains aggregate in red cell precursors and result in their abnormal maturation and premature destruction in the bone marrow. Abundant evidence shows that the severity of the β thalassaemias is related to the degree of imbalance of the globin chains. The excess of α globin chains precipitate and cause both mechanical damage to the red cell precursors and their products and also lead to oxidative destruction of their membranes. Imbalanced synthesis of the globin chains results in a variable degree of anaemia, which stimulates erythropoietin production, leading to intense proliferation and expansion of the bone marrow with resulting skeletal deformities. Because the red cells are abnormal they are destroyed in the spleen, which may become massively enlarged. Although the production of fetal haemoglobin is almost switched off by birth, in most normal adults some red cell precursors continue to produce a few γ chains and hence a small amount of fetal haemoglobin is produced. In the face of imbalanced synthesis of the globin chain in β thalassaemia these cells come under intense selection because part of the excess of α chains are bound to γ chains to produce fetal haemoglobin, thus reducing the magnitude of precipitation of the α chains. Hence in all the severe forms of β thalassaemia there is a relatively high level of fetal haemoglobin in the red cells but never enough to compensate for the overall deficit of β chains.

Despite these well defined consequences of defective production of β globin the clinical picture of the disease is remarkably diverse (box). At their worst, homozygotes are profoundly anaemic from the second or third month of life and if not treated with regular blood transfusion die within the first two years, a condition known as β thalassaemia major. On the other hand, some patients with apparently the same genotype have a much milder illness, ranging from a condition that is only a little less severe than the major form, through a spectrum of increasing haemoglobin concentrations, to one in which there are no symptoms and which is often ascertained on routine blood examination. This rather diverse collection of β thalassaemias are called the β thalassaemia intermedias. Even more remarkably, the heterozygous carrier states for β thalassaemia show equal phenotypic diversity. Typically, the β thalassaemia trait—that is, the inheritance of a single β thalassaemia allele—is associated with extremely mild anaemia and morphological changes of the red cells. In some cases, however, the disorder may be completely silent, with no anaemia or haematological abnormality. Yet in others it may be almost as severe as the major form of the disease. In other words, although β thalassaemia is usually a recessive condition, there is also a dominantly inherited form.

Phenotypic heterogeneity of β thalassaemia

Homozygous or compound heterozygous states

β thalassaemia major; profound anaemia requiring lifelong blood transfusion

β thalassaemia intermedia; moderate to mild anaemia (not dependent on transfusion)

Heterozygous states

β thalassaemia trait; mild anaemia

'Silent' β thalassaemia; phenotypically normal

Dominant β thalassaemia; moderate to severe anaemia

Diverse clinical phenotypes

Progress in our understanding of the clinical diversity of β thalassaemia has resulted largely from an appreciation of its pathophysiology. As mentioned, the basic defect in this disease is an inability to make β globin chains, which results in an excess of α chains that precipitate and damage the red cell precursors and their progeny. Therefore anything that tends to reduce the excess of α chains ought to ameliorate the disease, and this is indeed the case. Perhaps the most surprising outcome of this work is that although heterogeneity of the mutations at the β globin locus that underlie the β thalassaemias account for some of its clinical variability, a great deal cannot be explained in this way, and it is now clear that several other gene loci are involved.

Variable severity the β thalassaemia alleles

Over 180 different mutations have been identified in the β globin genes of patients with β thalassaemia. With the exception of a few deletions, the bulk is made up of single base changes or loss of one or two bases, all of which interfere with gene action and usually cause a drastic reduction in the output of β chains. Some of the mutations, however, involve regions of DNA (promotors) close to the β globin genes that are involved in their regulation or cause more subtle defects of gene expression during the stage when the primary product of the β globin genes is processed. Some of these mutations cause only a mild reduction in the level of β globin production. These observations account for part of the clinical diversity of β thalassaemia. Patients may be homozygous or compound heterozygous for severe mutations, in which case they have the clinical picture of thalassaemia major, or they may be similarly affected with milder mutations, which results in the intermediate forms of the disease. To complicate matters further some patients inherit a severe mutation from one parent and a mild mutation from the other, giving rise to a wide spectrum of clinical phenotypes. The silent β thalassaemias result from particularly mild mutations whereas the dominant β thalassaemias, which cause severe disease in heterozygotes, result from a particular class of mutations at one end of the β globin gene.

Although this diversity of mutations can give rise to a wide spectrum of disorders, it is now apparent the position is not as simple as this. Many families are encountered in which siblings may have inherited identical β thalassaemia mutations, yet one has severe disease and the other is much more mildly affected. Clearly other genes must be involved.

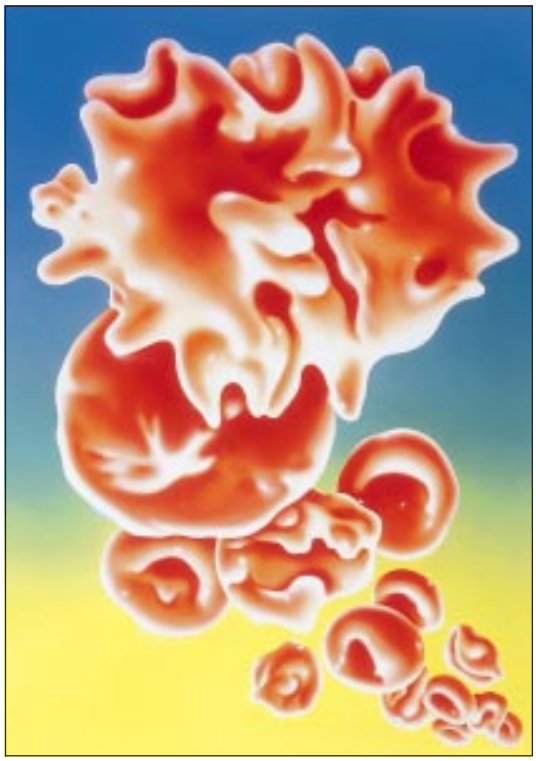
Primary genetic modifiers

The excess of α chains that are produced in response to defective β chain synthesis may be modified by genetic heterogeneity at at least two other loci. In many populations in which β thalassaemia is common, α thalassaemia (a genetic defect in the production of α chains) also occurs at a high frequency. Remarkably, homozygotes or compound heterozygotes for β thalassaemia who also inherit α thalassaemia tend to have a much milder illness because of the lower concentration of excess α globin chains. Because, as with the β thalassaemias, many different mutations of varying severity underlie the α thalassaemias, this interaction alone provides the basis for considerable clinical diversity.

Surprisingly, the small amount of fetal haemoglobin that is produced in normal adults is under strong genetic control. Thus patients with potentially severe forms of β thalassaemia may have a milder illness if they inherit a genetic determinant that allows them to produce more γ chains than usual in adult life. Again the mechanism is clear; the increased numbers of γ chains bind excess α chains to form fetal haemoglobin and hence there is less globin chain imbalance. Work over recent years has shown that the genetic determinants for fetal haemoglobin production in adult life are themselves heterogeneous, some being encoded near the γ globin genes, others on different chromosomes. Hence the various interactions of these modifiers can also cause considerable clinical diversity of the β thalassaemias.

Secondary modifiers

As well as the effect of variability at the α and γ chain loci, it is now apparent that the complications of β thalassaemia may be genetically modified by variability at many different loci. For example, there is the potential for variability at at least three different loci, which can modify the severity of the bone disease that is particularly common in patients with severe β thalassaemia. Similarly, loci have been identified that modify the rate of iron loading or the level of bilirubin in response to haemolysis and ineffective erythropoiesis, and, incidentally, the frequency of gallstone formation. There are several other examples of secondary modifiers of this type.



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In thalassaemia, affected erythrocytes are variously shaped and fragile

Phenotypic variability due to co-evolution

There is increasing evidence that the high frequency of the thalassaemias reflects heterozygote advantage against *Plasmodium falciparum* malaria. That every population has a different set of β thalassaemia mutations suggests that this selective force is fairly recent, otherwise the same mutations would appear throughout the tropical world. But genetic protection against *P falciparum* malaria is not restricted to the haemoglobin disorders. Rather, during the relatively short period (at least in evolutionary terms) that we have been exposed to this parasite many other genetic systems have been modified, including red cell enzymes and membranes, but, more importantly, the immune system and cytokines and other effectors that are involved in response to infection. These polymorphisms also vary widely among different races, again reflecting recent selection by malaria. Hence children with β thalassaemia who come from different parts of the world may have totally different responses to infection.

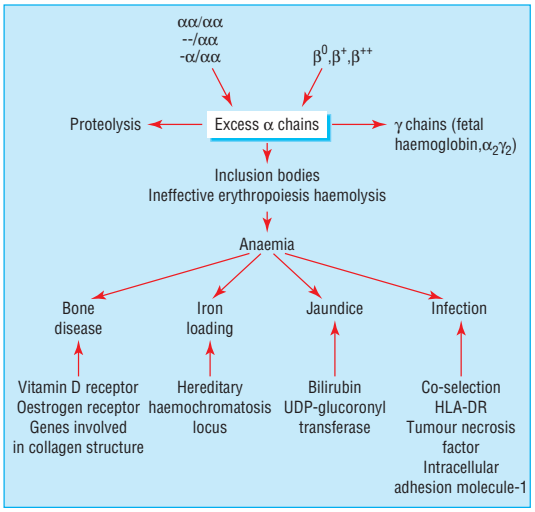
Ecological and ethological factors

The study of the role of the environment in modifying monogenic disease has been neglected. Increasing evidence from analyses of patients with the same mutations who live in different countries shows that environmental factors may be extremely important in determining the course of their illness. Apart from obvious factors such as socioeconomic status, availability of medical care, state of nutrition, and exposure to infection, it is apparent that more subtle effects, including climatic, may be of considerable importance.

It is also becoming clear that there are major differences between races in the response and acceptance of chronic illness. Differences in religious belief are of prime importance, not only in shaping patients' response to illness but also in affecting the pattern of medical care. There are complex interactions of social and cultural factors; in some populations children with chronic diseases are virtually excluded from society, for example.

Lessons for the further exploration of genetic disease

Recent studies of the reasons for the clinical diversity of the β thalassaemias suggest that they reflect layer upon layer of genetic complexity, with a strong environmental component (figure). As well as remarkable heterogeneity of the primary mutations at the β globin locus, there are at least two major modifying loci, and the complications of the disease may also be fine tuned by variability at loci that have nothing to do with haemoglobin production. Each of these modifying loci may themselves show considerable heterogeneity, and the frequency of different alleles may vary widely in particular populations. The number of potential combinations, and hence



Factors modifying phenotypes of β thalassaemia

phenotypes, is extremely large. What at first sight seemed to be a relatively simple monogenic disorder is, in effect, an extremely complex syndrome in which many different genes are involved together with equally numerous ill understood effects of the environment.

All monogenic diseases show considerable clinical variability, and although the defective genes have been identified and the mutations related to the phenotype in some cases, it is clear that wherever adequate studies have been done there is clinical variability among patients with the same genetic defect. No doubt the kind of modifiers that have been discovered for the thalassaemias will be identified for these other diseases. In one particular case—mutations that involve transcription factors, which may be involved in the regulation of many different genes, each with their own modifiers—we would expect to see widely differing phenotypes, and this is the case.

Where does this story of remarkable genetic complexity leave us when we come to consider some of the common disorders of Western society? In many cases these conditions do not show a high level of heritability. For example, concordance rates in twin studies for insulin dependent diabetes have ranged from 0.2 to 0.6 and for coronary artery disease in much the same range. This shows that these diseases, which are not inherited in a Mendelian fashion anyway, are probably the result of varying susceptibility to a wide range of environmental factors mediated by many different genes. Since each of these genes will themselves have their own modifiers, just as occurs in thalassaemia, it is clear that when we try to dissect the major loci involved in susceptibility to these diseases we are entering into another order of complexity. The situation will not be much easier in the specialty of cancer, where it is becoming apparent that there are multiple routes through oncogene mutations to many of the common cancers. The application of recently developed microchip and gene expression array technology suggests that there may be some overall pattern to the activation of different genes in the same type of cancer, and it is beginning to look as if therapy will have to be tailor made for almost every individual cancer.

These observations suggest that progress towards the clinical application of our increasing knowledge of the human genome may be slow. At the moment we cannot predict for certain the clinical course of any simple monogenic disease. The idea that we may be able to predict the occurrence of a heart attack or the onset of diabetes from examining a child's genotype at birth is even further away. Even for the monogenic diseases we still do not know what is just "noise" in the system and what is clinically important. Clearly, relating genotype to phenotype will be one of the great challenges for genetic medicine over the next century.

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Further reading

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One hundred years ago

A proposed international counterblast to tobacco

Not long before Field Marshal the Duke of Wellington retired from the office of head of the British Army, he issued the famous "General Order No. 577," in which he declared in a tone of horrified surprise that "the Commander-in-Chief has been informed that the practice of smoking, by the use of pipes, cigars, or cheroots [cigarettes as yet were not], has become prevalent among the officers of the Army, which is not only in itself a species of intoxication occasioned by the fumes of tobacco, but undoubtedly occasions drinking and tippling by those who acquire the habit; and he entreats the officers commanding several regiments and in the adjoining apartments, and to discourage the practice among the officers of junior rank in their own regiments."

One vainly tries to imagine how the Iron Duke's feelings would have been shocked had he lived to see the *matériel* for "the practice of smoking" figure most prominently among the comforts sent by a grateful and admiring nation to the soldiers fighting its battles among the kopjes of South Africa. But he would have found a soothing unction for his sentimental bruises in the programme of the Congrès International Antitabacique, which is to be held in Paris this year during the great Exhibition. No fewer than 106 questions are proposed for the consideration of the Congress; and tobacco is to be discussed and doubtless solemnly damned from the historical, chemical, physiological, pathological, hygienic, social, criminological, and several other points of view. (*BMJ* 1900;i:531)