Hereditary retinopathies: insights into a complex genetic aetiology

Retinitis pigmentosa (RP), a disease currently affecting an estimated 1.5 million people throughout the world, is the most prevalent human retinopathy displaying clear-cut hereditary tendencies – that is, the disease may segregate in an autosomal dominant, in a recessive, or in an X linked fashion. Until recently the cause of the condition in humans remained obscure. However, the fact that the disease segregates according to strict Mendelian ratios indicates that in any given pedigree a single defective gene is involved, a major ongoing challenge of human molecular genetics lying in the isolation and characterisation of such genes. With a genome consisting of some three billion base pairs of DNA and probably between 50,000 and 100,000 genes, the task of locating a single disease-causing gene, particularly when the biochemical defect is unknown, would seem, to say the least, daunting. Nevertheless for RP (and indeed a growing number of other prevalent Mendelian disorders) much progress has been made over the last few years. Work on localising disease genes has been facilitated by an exponentially expanding human genetic map and, in the case of RP, the availability of many large families with the disease. The technique of genetic linkage analysis has been widely employed in the search for such genes. Using this technique, DNA isolated from family members is screened with a large bank of genetic markers. Eventually, through a process of trial and error, a linkage between the disease phenotype and a particular genetic marker is established.

This methodology was first used successfully back in the 1950s for the localisation of the gene which causes myotonic dystrophy and while, in principle, the method was highly effective an insufficient number of informative genetic markers was available to make the methodology routinely feasible. This changed in the mid to late 1970s when genetic markers based on polymorphism at the level of the DNA sequence itself (so-called restriction fragment length polymorphisms or RFLPs) were developed. More recently, a new generation of DNA markers has been developed through the use of the polymerase chain reaction. Such markers, often referred to as 'microsatellites' are based on polymorphism in the numbers of short tandemly repeated DNA sequences (STRs) at a given locus. DNA across the variable region is amplified, and length variation can usually be detected. With such markers the task of localising disease-causing genes has become much more readily achievable.

The first progress to be made in localising an RP-causing gene came from the work of Bhattacharya and colleagues in 1984.1 These workers were able to localise an X linked RP gene by establishing a linkage between an RP locus and the marker L1.28 on chromosome X. As a result of extensive studies undertaken by research teams throughout the world we now know that two X linked RP genes exist on the short arm of the X chromosome.2 However, at the time of writing neither of these genes has been functionally characterised. A great deal of progress has, however, been made over the past five to six years into the aetiology of autosomal dominant forms of RP (adRP). The first breakthrough came in 1989 with the establishment of an adRP locus on the long arm of chromosome 3 as a result of a systematic linkage study in a very large pedigree of Irish origin.3 The gene encoding rhodopsin, the light sensitive pigment of the rod photoreceptors, was found to map in very close proximity to the disease locus.4 Shortly after these initial observations were made a mutation within the rhodopsin gene (a pro→his substitution at codon 23) was identified by Dryja and colleagues.5 This result prompted a massive search for further mutations within the rhodopsin gene in cases of adRP. To date approximately 60 different mutations have been reported (reviewed by Farrar et al6). The majority of these are point substitutions although a number of small deletions have also been encountered.

Rhodopsin-based adRP, however, accounts for only about 20% of all dominant cases of the disease. Defects in other genes must therefore be involved in the majority of other families. Continued genetic linkage studies soon resulted in the establishment of a second locus for adRP. This time a gene was found on the short arm of chromosome 6 close to the locus for peripherin RDS.6 The RDS protein is a structural component of the rod outer segment disc membranes and, moreover, has been implicated in a retinopathy of mice called 'retinal degeneration slow'.7 The linkage data indicated that the RDS protein might also be involved in a retinopathy of humans and soon several mutations within the RDS gene were rapidly identified.8,9 These observations precipitated an intensive search for further mutations within the RDS gene. To date, approximately 20 mutations have been encountered in various retinopathies.10 Most mutations are associated with adRP. However a 2 base pair deletion has recently been encountered in a case of RP punctata albecens11 and, in addition, a number of amino acid substitutions have been encountered in various forms of hereditary macular degeneration.12,13

Taken together, rhodopsin and RDS mutations probably account for up to 30% of all dominant cases of RP and as yet an undetermined proportion of cases of hereditary macular dystrophies. Hence, the search for additional genes has continued, and further linkage studies have resulted in the establishment of other dominant RP loci at the centromeric region of chromosome 814 and, more recently, on both the
short and long arms of chromosome 7. While these genes remain to be isolated, additional pedigrees exist showing no evidence for linkage at any of the five known loci. Thus, at least six genes are implicated in the aetiology of various autosomal dominant forms of RP or macular degeneration. An exciting prospect will be the characterisation of such genes over the next few years.

Progress in gene defect detection in autosomal recessive RP, accounting for up to 50% of all cases, has been much slower. However, it has recently been reported that a small proportion of patients have defects in the gene encoding cyclic GMP phosphodiesterase. Defects in this gene were already known to cause the hereditary retinopathy of mice termed retinal degeneration, or RD. Hunting for the genes for recessive RP, however, using the technique of linkage analysis is not so easy, primarily because fewer large families suitable for linkage work are available. However, using a combination of linkage and candidate gene studies an increasing number of recessive retinopathy genes is likely to be identified in the next few years.

What does all that tell us about the cause of RP in humans? So far two proteins have been implicated in autosomal dominant forms of the disease. The first is rhodopsin, the primary component of the visual transduction cycle and the second is peripherin RD. The fact that defects in rhodopsin have been identified in many cases of the disease renders other components of the visual transduction cycle strong contenders in disease aetiology, and indeed defects in cGMP phosphodiesterase have now been implicated in a proportion of recessive cases as outlined above. Other components of the cycle, including transducin, guanylate cyclase the cGMP-gated channel protein itself, arrestin, and rhodopsin kinase must be strong contenders. In addition, because of the fact that the RDS protein, a structural component of the photoreceptor outer segment disc membranes, has also been implicated in some cases, this would imply that other membrane proteins might also be involved. With regard to how such proteins might exert their pathological effects, Sung and others have recently obtained evidence that a proportion of mutant proteins may not be effectively transported from the endoplasmic reticulum following synthesis. Such defective translocation could compromise the viability of the rod photoreceptors. Alternatively, those mutant proteins that do become incorporated into the outer segment disc membranes may result in some form of structural destabilisation of the membranes themselves. A much more complete picture will emerge following the isolation and characterisation of the additional genes which have recently been located.

Research in this field continues apace. In this issue of the journal, Apfelstedt-Sylla et al describe yet another mutation in rhodopsin, this time affecting the carboxyl terminal sequence. In a second paper in this issue, Moore and colleagues provide both clinical and molecular genetic data on patients and families with forms of adRP which appear to display incomplete penetrance. Lack of penetrance occurs with varying frequencies in a variety of hereditary conditions. It is an intriguing phenomenon. An individual with early onset dominant disease usually has a 50% probability of passing the disease allele on to any of his or her children. Occasionally however, non-affected individuals of affected parents may themselves go on to have children who manifest symptoms of the disease. Just why the non-affected individual escapes the development of disease symptoms when he or she carries a dominant retinopathy gene is, at present, unknown. However, the individual's own genetic background may well influence the activity or expression of the RP-causing gene. Alternatively, environmental factors (dietary intake or metabolism of vitamin A to give but one possible example) could have a significant influence on the expression, or otherwise, of mutated genes. Identification of genetic and/or environmental components which might be involved in the suppression of a disease phenotype is a major and exciting challenge. The work reported in Moore et al's paper indicates that, in a number of families with adRP, 'asymptomatic' carriers of disease genes do in fact manifest mild abnormalities on detailed ophthalmic examination. Identification of the mutations involved in such families (work in progress is reported) will represent an initial step towards an understanding of a phenomenon which might eventually provide major clues on modifying the phenotypic effects of some disease-causing mutations.

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