Genetic mapping of retinitis pigmentosa — implications for South African patients

A review

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The term 'retinitis pigmentosa' (RP) encompasses a group of hereditary degenerative disorders of the retina, which are both genetically and clinically heterogeneous. The finding of molecular markers for certain forms of RP potentially allows for presymptomatic and prenatal diagnosis of a proportion of RP families. These developments and their implications for affected South African families are highlighted in this article.

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Retinitis pigmentosa (RP) is a heterogeneous group of inherited degenerative retinal disorders with an incidence of about 1:3 500 in the USA;[1] it is one of the more common causes of genetic blindness. The condition may be transmitted as an autosomal dominant, an autosomal recessive or an X-linked trait; it may also occur sporadically and can be a component of a number of genetic syndromes such as Usher syndrome. Molecular studies of each genetic type of RP have revealed that there are both allelic and non-allelic forms of heterogeneity. However, the phenotypic and pathological changes in all three genetic forms of RP are essentially identical. Classically the disorder presents with constricted visual fields early in its course, and later with abnormal accumulation of pigmentation in the retina. Variability in the age of onset of symptoms and, eventually, blindness would appear to depend, at least in part, on the mode of inheritance. Affected individuals have abnormal electroretinograms in the early stages, and significant visual handicap is invariably present in middle age.

X-linked recessive RP (XLRP)

XLRP is regarded as the most severe form of RP with an incidence of about 1:20 000.[2] To date, 3 distinct loci for XLRP have been reported, mapped to the short arm of the X-chromosome. The first of these was mapped near to the marker, DXS7, at Xp11.3,[3] a second at Xp21.1, close to the ornithine transcarbamylase gene,[4] and a third in the region of Xp21.2-3, near the Duchenne muscular dystrophy gene.[2]

Autosomal recessive RP (ARRP)

Usher syndrome comprises classic ARRP and associated congenital deafness.[5] One form of Usher syndrome, classified type I, characterised by profound congenital deafness, vestibular areflexia and progressive RP from early childhood, has been mapped to chromosome 14q (USH1a (French)).[6] More recently 2 additional loci for this same form of Usher syndrome, USH1b (non-Acadian) and USH1c (French-Acadian), were mapped to chromosomes 11q and 11p respectively.[5,7] Usher syndrome, classified type II, with moderate hearing loss, normal vestibular response and progressive RP from adolescence, has been mapped to 1q32-41 (USH2a).[8] However, in 20% of type II families investigated, the condition is not linked to this chromosome 1 locus indicating that there must be another locus (USH2b).[9] There are therefore at least five different genes responsible for these two closely related phenotypes of ARRP.

Moreover, a recent study of 126 unrelated ARRP patients[1] revealed a single affected individual who is homozygous for a nonsense base substitution in the rhodopsin gene on chromosome 3q. This would appear to represent a null mutation in the rhodopsin gene which causes rod photoreceptor dysfunction and ARRP.

Autosomal dominant RP (ADRP)

To date, four loci associated with ADRP have been mapped to four different chromosomes, emphasising non-allelic heterogeneity in ADRP. In 1989, an ADRP locus was found to be linked to the rod photoreceptor gene, rhodopsin, on chromosome 3q21-q24[10] and extensive studies of this candidate gene have revealed over 75 different mutations, all associated with ADRP and thus demonstrating allelic heterogeneity.

Linkage to a second candidate gene has been demonstrated, a transmembrane protein, peripherin/RDS, on chromosome 6p21.1-cen;[11] a number of different mutations within this gene have been shown to cause ADRP. A third locus at the pericentric region of chromosome 8[12] has been inferred by linkage; however no causative gene has yet been identified. The existence of a fourth locus associated with ADRP, located on chromosome 7, was recently announced at the International Retinitis Pigmentosa Association (IRPA) meeting in Johannesburg by Shomi Bhattacharya of London University.

Finally, exclusion of linkage to these mapped loci in some ADRP families provides conclusive evidence for the existence of yet another ADRP locus.[13,14]

The South African approach

Following a nationwide survey in 1985, Oswald *et al.*[15] published an overview of RP in South Africa. The proportions of the different types of RP, according to the

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mode of inheritance, were found to be in general accordance with those published in international studies. Although the figures reported at that stage were recognised as incomplete, the relative frequencies of the various forms of RP found provided a perspective of the situation in South Africa.

DNA banking

In 1990, with support from the RP Foundation of South Africa, a DNA Banking Centre was established in the molecular laboratory of the Department of Human Genetics, University of Cape Town. To date, blood specimens for DNA extraction and banking have been received from 75 RP families, comprising 440 individuals (Table I).

Table I. Genetic categorisation of the 75 RP families from the UCT DNA banking centre (Dec 1992)

Inheritance pattern		No. of families	No. of affected individuals
Undifferentiated RP	(69%)		
ADRP		21 (28%)	82
ARRP		8 (11%)	11
XLRP		3 (5%)	3
Sporadic		9 (12%)	7
Indeterminate		10 (14%)	11
Syndromic RP	(27%)		
Usher (types I and II)		19	28
RP-deafness-renal dysfunction syndrome		2	3
Genetic retinal disease	(4%)		
Staargaardt			
XL		1	1
AD		2	7
		75	153

These figures represent a mere fraction of individuals thought to be affected in this country. According to the RP Foundation of South Africa, which estimates that there are some 600 RP families throughout the country, fear of stigmatisation often prevents RP sufferers from seeking medical assistance (C. Medefindt — personal communication). In Table I, the term 'sporadic' has been used to define a single RP patient with no other family history of RP. The term 'indeterminate' refers to those families with more than one affected individual but where there is insufficient information to indicate typical Mendelian patterns of inheritance.

In collaboration with the Department of National Health and Population Development, genetics nursing sisters throughout the country conduct home visits in order to obtain blood specimens and collect pedigree data. Arrangements are made for RP family members to be examined at the Department of Ophthalmology and/or Groote Schuur Hospital's Department of Otolaryngology (for audiometric assessment of Usher syndrome patients) or by other ophthalmological colleagues. A proforma has been compiled which clearly indicates the clinical information required for laboratory studies, given that it recently emerged that precise ophthalmological information on retinal change in RP is crucial for direction of molecular research.[16,17]

One investigation, directed at correlating the molecular genotype with the clinical phenotype, involved a study of 20 ADRP patients with different rhodopsin mutations from 6 families, where rod and cone function and rhodopsin levels were examined.[18] In this study the functional phenotypes showed some degree of intrafamilial consistency and in some patients with different rhodopsin mutations there were discernible differences in the pattern of retinal dysfunction.

Molecular genetic research

In the absence of a defined biochemical defect, molecular genetic practice is to identify and define the subchromosomal position of a gene responsible for a particular genetic disorder. This approach may then provide a basis for cloning strategies and lead to the identification of the basic mutation at the DNA level. Initially the chromosomal positioning involves genetic linkage and cytogenetic analysis which is then followed by physical mapping with available molecular DNA markers and, finally, identification of the gene product, the nature of expression of the mutated gene and the mechanism by which it causes the phenotype.

In 1989, molecular linkage studies of two large British RP families revealed that one form of early onset ADRP (type I) is associated with a marker, C17, at the D3S47 locus, which in turn is linked to the rhodopsin gene on the long arm of chromosome 3.[10] In South Africa, our initial focus was on one large family with a history of early-onset ADRP. Ancestors of this family had emigrated from England in the early 20th century and there are now many descendants living in the Transvaal and Natal. Molecular linkage analysis that focused on the marker, C17, was undertaken in this ADRP family and it was established that the disorder was not linked to the D3S47 locus on chromosome 3 in this particular family.[13]

The next phase in the programme therefore entailed a candidate gene approach in the search for the RP gene. This involves testing for linkage with candidate markers from elsewhere on the human genome, chosen because of their role in the biochemical pathways that govern vision. Such a candidate gene approach is expensive, time-consuming and labour-intensive, but more cost-effective than the alternative of a sweeping search through the genome with random markers along each chromosome.

In the current South African RP project, candidate and anonymous markers have been used from chromosomes 2, 3, 4, 6, 8 and 10. Despite extensive studies, tight linkage to any recognised locus has not yet been established, but some of the markers were uninformative in this family and additional markers, in the same region of the chromosomes in question, will now be used. Several dinucleotide repeat polymorphic markers have also been obtained to render the marker loci more informative, given the high polymorphic information content of such markers.

An additional six smaller South African families with ADRP are currently under investigation and have been typed with the rhodopsin-linked markers on chromosome 3. All of these

families have been uninformative or have shown no clear association with the markers used to date and are now being studied with additional markers. As C17 is not a suitable probe for predictive testing in all chromosome 3-linked ADRP families, direct testing for rhodopsin mutations is being undertaken in families where the lod score is not less than –2,0 at $\theta = 0,05$.[19] Once linkage has been established in a RP family, intragenic molecular studies will be undertaken to establish the precise mutation in that family.

In a collaborative project involving families with the Usher syndromes initiated in 1991, Professor Bill Kimberling of Omaha, Nebraska, studied a total of 27 families with Usher syndrome, type II, including 4 families from South Africa. Linkage was found between the disease locus and a marker on the long arm of chromosome 11 (11q13) in two South African families with the Usher syndrome, type II (USH1b).[5]

Discussion

In the light of international experience to date, it is anticipated that each South African RP family is likely to have a different mutation at the molecular level. Our initial aim is to identify the defect in ADRP families with a view to offering a service for predictive testing for the presymptomatic gene carrier and the option of antenatal diagnosis for RP families on request. An immediate consequence of this would be that some forms of ADRP could be diagnosed rapidly, even in the absence of a family history. In addition, accurate and timely counselling could be offered to affected patients if specific phenotypes were found to be associated with certain mutations.

In the long term a better knowledge of the molecular defects that cause RP may also contribute to the understanding of the pathogenic mechanisms of the disorder. In turn, this information may eventually facilitate the development of an effective therapeutic approach.

The momentum of research in RP internationally was highlighted at the recent International Retinitis Pigmentosa Association (IRPA) congress held in Johannesburg. The meeting provided a forum for interaction between scientists (including cell biologists, molecular biologists, biochemists and geneticists), ophthalmologists, nursing sisters and the lay RP groups. It was evident from this and the previous IRPA meeting in Dublin in 1990, that a highly motivated and organised lay support group can indeed provide the impetus for strongly directed research.

Given the high level of genetic heterogeneity evident in ADRP and the fact that the disorder in most ADRP families is not associated with the three candidate regions on 3q, 6p and the pericentric region on 8, the most important point to emerge from the meeting was the announcement of a fourth candidate region for ADRP on chromosome 7 (Shomi Bhattacharya, London University). However, the issue of heterogeneity is still evident since some families, in which ADRP was not linked to 3, 6 or 8, were also not linked to chromosome 7 (Peter Humphries, Dublin). The likelihood of a fifth locus for ADRP underlines the extensive genetic heterogeneity in this group of hereditary degenerative disorders of the retina.

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