Conferences and Reviews

Molecular Genetics of Retinitis Pigmentosa

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This is an edited summary of an Interdepartmental Conference arranged by the Department of Medicine of the UCLA School of Medicine, Los Angeles. The Director of Conferences is Anthony T. Campagnoni, PhD, Professor of Biobehavioral Sciences.

Retinitis pigmentosa is a model for the study of genetic diseases. Its genetic heterogeneity is reflected in the different forms of inheritance (autosomal dominant, autosomal recessive, or X-linked) and, in a few families, in the presence of mutations in the visual pigment rhodopsin. Clinical and molecular genetic studies of these disorders are discussed. Animal models of retinal degeneration have been investigated for many years with the hope of gaining insight into the cause of photoreceptor cell death. Recently, the genes responsible for two of these animal disorders, the *rds* and *rd* mouse genes, have been isolated and characterized. The retinal degeneration of the *rd* mouse is presented in detail. The possible involvement of human analogues of these mouse genes in human retinal diseases is being investigated.

(Farber DB, Heckenlively JR, Sparkes RS, Bateman JB: Molecular genetics of retinitis pigmentosa. West J Med 1991 Oct; 155:388-399)

DEBORA B. FARBER, PhD*: Recent advances in molecular genetics have made it possible to identify and isolate genes responsible for human diseases. One of these diseases, retinitis pigmentosa, will be the focus of our discussion. The methodologic approaches used in the study of this disease have been applied to the study of many other inherited disorders and, recently, for the isolation of the gene responsible for cystic fibrosis, an autosomal recessive disease. Some examples of gene defects identified using similar techniques are listed in Table 1.

Retinitis pigmentosa can be considered a model for the study of genetic disorders. It affects thousands of persons in the United States and many more all over the world. Patients and families affected with the disease and animals with inherited retinal degenerations similar to retinitis pigmentosa have been investigated for a number of years. Animal models have special advantages for the exploration of the aberrant mechanisms associated with the disease, such as the easier availability of tissues of defined age, sex, and genetic background.

What is the primary lesion in retinitis pigmentosa that causes the retinal photoreceptor cells to die and leads to eventual blindness? If the origin of the disease can be elucidated, it may be possible to design treatments that will arrest or cure it. Unfortunately, retinitis pigmentosa is not one disease but the name given to an umbrella of disorders that have blindness as a common end. It has therefore been necessary to classify the different types of retinitis pigmentosa and its related syndromes to group patients and families. Only in this way can the genetic background be appropriately correlated with biochemical, cell and molecular biologic, and physiologic findings. Classification systems based only on clinical features, however, may not always correlate with the genetic defects.

Our understanding of the mechanisms that may be abnormal in specific types of retinitis pigmentosa has been substantially advanced during the past five years, due largely to the application of the techniques of molecular biology. Indeed, during 1989, a gene involved in the disease of a family with autosomal dominant retinitis pigmentosa was localized to the long arm of human chromosome 3,[1] and the genes responsible for the retinal degeneration of two different animal models, the *rds* mouse[2] and the *rd* mouse,[3] were isolated and characterized.

The heterogeneity of retinitis pigmentosa and the classification of its different types are discussed first. We then describe the general approaches that have been used to study these diseases using specific examples. We also summarize the results of our work on the *rd* mouse, including the isolation and characterization of the gene responsible for the degeneration of the *rd* photoreceptor cells, and show that biochemical abnormalities similar to those in the *rd* mouse were present in a patient with dominant retinitis pigmentosa whom we have studied.

TABLE 1.—*Genetic Disorders for Which the Candidate Gene Approach Has Proved or Is Proving Useful in Identifying (Isolating) the Responsible Gene(s)*

Atherosclerosis	Duchenne muscular dystrophy
Colon cancer	Alcoholism
Retinoblastoma	Schizophrenia
Neurofibromatosis	Diabetes mellitus
Cystic fibrosis	Spinocerebellar atrophy

*The Jules Stein Eye Institute and the Department of Ophthalmology, UCLA School of Medicine.

This study was supported by National Institutes of Health grants EY02651 and EY08285 (Dr Farber), EY07758 (Dr Heckenlively), and EY08082 (Dr Bateman); Research to Prevent Blindness, Inc, William and Mary Greve International Scholar Award (Dr Bateman); The National Retinitis Pigmentosa Foundation (Dr Farber); and The George Gund/National Retinitis Pigmentosa Foundation Grant (Drs Sparkes, Bateman, and Farber). Dr Farber is a recipient of a Research to Prevent Blindness Senior Scientific Investigators Award.

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ABBREVIATIONS USED IN TEXT

ATP = adenosine triphosphate
cDNA = complementary DNA
cGMP = cyclic guanosine monophosphate
mRNA = messenger RNA
OAT = ornithine aminotransferase
RFLP = restriction-fragment-length polymorphism

Definition and Clinical Findings in Retinitis Pigmentosa

JOHN R. HECKENLIVELY, MD*: Retinitis pigmentosa is the name given to a set of diseases that have in common a progressive retinal degeneration characterized by progressive visual field loss, a pigmentary retinopathy, and, at some point in the disease, night blindness.[4] This broad definition encompasses a large number of ocular-only forms and secondary types in which the pigmentary retinopathy is part of a syndrome or a systemic disease. Most forms of retinitis pigmentosa begin in adult life, but less commonly it may be congenital or manifest itself in childhood.[5] The term "retinitis" is a misnomer because there is little evidence that inflammatory processes are involved in any important way in these diseases.

The estimated incidence of retinitis pigmentosa (all types) in the United States is 1 in 3,500, and the carrier state may be as frequent as 1 in 80 persons.[6] The most common mendelian inheritance pattern in the United States is autosomal recessive at 31%; autosomal dominant represents 17%, and X-linked recessive is the least common at 10% (Table 2).[7-10] Many patients with the disease (42%) have no family history and are often said to have "simplex" inheritance. Retinitis pigmentosa is found in all races; the distribution in racial subgroups closely follows that found for the normal population (Table 3).[11,12]

X-Linked recessive inheritance is the least common of the retinitis pigmentosa inheritance types, but it often is the best remembered. Many male patients with the disease, without having a family pedigree taken, are told inappropriately by their physicians that they inherited it from their mother. This may cause feelings of guilt in the mother and resentment toward the mother by the son. Similarly, many of these patients are told not to have children as "they will inherit the disease." In fact, only patients with the autosomal dominant form—less than 20% of affected patients—are at immediate risk of passing the disease to their children.

The diagnosis is typically made by finding that a patient has a visual field loss in a pattern characteristic of retinitis pigmentosa and seeing a pigmentary retinopathy on fundus examination. An electroretinogram is an important confirmatory test, and the results will be abnormal to nonrecordable by standard single-flash techniques.[8]

*The Jules Stein Eye Institute and the Department of Ophthalmology, UCLA School of Medicine.

TABLE 3.—*Retinitis Pigmentosa (RP) Population: Distribution by Race*

Race or Ethnicity	UCLA RP Registry,* %	Normal California,† %	Normal United States,† %
White	81	76	83
African American	4	8	12
Hispanic‡	9	19	6.5
Asian	5	5	1.5
American Indian	1	1	0.5

*Data on 500 patients.
†From Statistical Abstracts of the United States 1984[11]; reproduced by permission of the US Department of Commerce.
‡Includes all persons of Spanish origin and overlaps with other categories (from Heckenlively et al[12]).

At least 14 forms of ocular-only retinitis pigmentosa can be identified by clinical criteria of inheritance pattern, changes on the electroretinogram, the measurement of rod function, and retinal appearance (Table 4).[5,13-26] Why are there so many types of retinitis pigmentosa? Most of these disorders arise from dysfunction or maldevelopment of the retinal pigment epithelium or the photoreceptors (Figure 1).[27(p43)] Multiple pathways are known to be essential; if they are disrupted, the photoreceptors will degenerate. Each of these pathways is presumably under the control of separate genes.

Several systemic syndromes include a progressive pigmentary retinopathy. The most common, the Usher syndrome, is characterized by retinitis pigmentosa and congenital hearing loss that may be complete or partial. Clinical evidence suggests that these two forms of the Usher syndrome are caused by different genes. Type II was recently linked to the long arm of chromosome 1.[28] The Usher syndrome constitutes about 10% of patients with retinitis pigmentosa seen at UCLA. Surveys for this disease at schools for the deaf often find a 3% to 6% prevalence in the students.[25]

The next most common retinitis pigmentosa syndrome (slightly less than a 1% prevalence in the UCLA RP Registry) is the Bardet-Biedl syndrome, characterized by polydactyly, obesity, short stature, mild mental retardation, and hypogonadism. In this syndrome, the retinal degeneration usually includes a macular degeneration.[29]

Most of the more common types of retinitis pigmentosa tend to have a similar appearance of the fundus: diffuse retinal atrophy and retinal vessel attenuation, with either release of pigment or migration of retinal pigment epithelial cells that aggregate on retinal vessels, particularly at bifurcations. This gives a characteristic bone spicule-like pigmentary pattern (Figure 2).[30] Degeneration typically starts in the equatorial retina and can be measured on the visual field test. The affected retina in this area becomes less sensitive to stimula-

TABLE 2.—*Distribution of Retinitis Pigmentosa by Mendelian Inheritance*

Study	Population	Patients, No.	Simplex, %	Autosomal Recessive, %	Autosomal Dominant, %	X-Linked, %
Boughman and co-workers, 1980[7]*	United States*	670	15*	69	10	6
Heckenlively, 1987[8]	California	609	42	31	17	10
Boughman and Fishman, 1981[9]	Illinois	300	50	16	22	9
Jay, 1982[10]	England	426	42	16	24	18

*Represents proportion of cases not accounted for by probability alone.

TABLE 4.—*Ocular-only Types of Retinitis Pigmentosa (RP) Due to Single Gene Site Abnormalities and Identified Clinically or Genetically*

Type	*Source*
Autosomal Dominant	
Rhodopsin mutations (chromosome 3)	Dryja et al, 1991[13]
Chromosome 8 RP1 gene	Blanton et al, in press[14]
Infantile onset (rare)	Heckenlively, 1988[15]
Irish family chromosome 3 (not rhodopsin)	McWilliams et al, 1989[16]
Others (rod-cone and cone-rod degenerations)	Heckenlively, 1987[17]
Autosomal Recessive	
Type I (rod-cone)	Heckenlively, 1987[17]
Type II (cone-rod)	Heckenlively, 1987[17]
Preserved para-arteriolar retinal pigment epithelial RP	Heckenlively, 1981[18]
Leber's amaurosis congenita (typical)	Foxman et al, 1985[5]
Congenital RP with macular colobomata	Heckenlively et al, 1988[19]
Juvenile RP	Foxman et al[5]
Retinitis punctata albescens	Ellis and Heckenlively, 1983[20]
Goldmann-Favre disease	Favre, 1958[21]
X-Linked Recessive	
Choroideremia (Xq21)	Cremers et al, 1989[22]
Retinitis pigmentosa*	Bird, 1975[24]
Common RP Syndromes (autosomal recessive)	
Usher type I (congenital profound deafness and RP)	Fishman et al, 1983[25]
Usher type II (congenital partial deafness and RP)	Fishman et al, 1983[25]
Bardet-Biedl (polydactyly, obesity, mild mental retardation, hypogonadism, pigmentary retinopathy)	Schachat and Maumenee, 1982[26]

*Two gene sites have been identified on the X chromosome for retinitis pigmentosa (from Musarella et al, 1988[23]).

tion, and often a "ring scotoma" will be found where there is intact functioning central and peripheral retina, but the mid-peripheral retina does not "see" the test target, and a donut of vision loss can be mapped (Figure 3).[31] At this stage of the disease, patients begin to bump into objects in their path because they do not see them.

Many of the patients with retinitis pigmentosa have a typical pattern of visual field loss; it starts in the equatorial region, then involves the anterior retina and may leave a peripheral ring with a central island of intact vision, similar to that shown in Figure 3. With further progression, the peripheral ring and central area diminish in size, and eventually in some patients the central vision desaturates and fails.[31] Some patients will maintain good central vision into advanced states of the disease, even though they will have a severe disability because they cannot see anything outside the central tubular field.

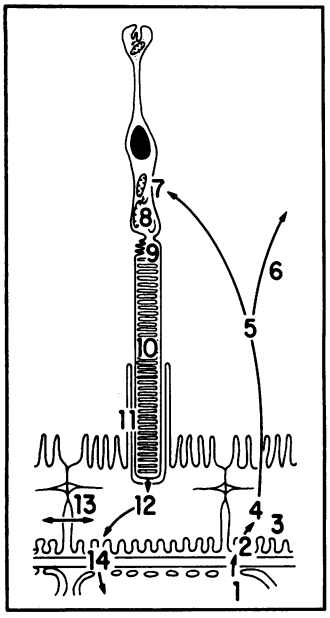

Figure 1.—A diagram is shown of mechanisms that may be involved in the degeneration of photoreceptor cells; the figure shows interaction of photoreceptor and retinal pigment epithelial (RPE) cells: **1,** uptake or transport defect; **2,** abnormal membrane receptor sites in RPE; **3,** intracellular RPE metabolic defects; **4,** transepithelial transport defect; **5,** RPE to photoreceptor donor defect; **6,** RPE to other retinal cell defects (such as Mueller cells); **7,** abnormal sites in photoreceptor cell; **8,** micrometabolism malfunctions; **9,** faulty membranogenesis; **10,** inability to stabilize membranes; **11,** malfunction in phagocytosis; **12,** malfunction in lysis; **13,** defective RPE lateral transport and communication; **14,** inability to void lytic products (from Heckenlively[27]; reproduced with permission from JB Lippincott Co).

Natural History of Retinitis Pigmentosa

Patients with retinitis pigmentosa have been reported to lose on the average about 4.6% of remaining visual field each year.[32] Studies at UCLA looking at visual field loss of this disease found a mean deterioration of 7.7% per year (mea-

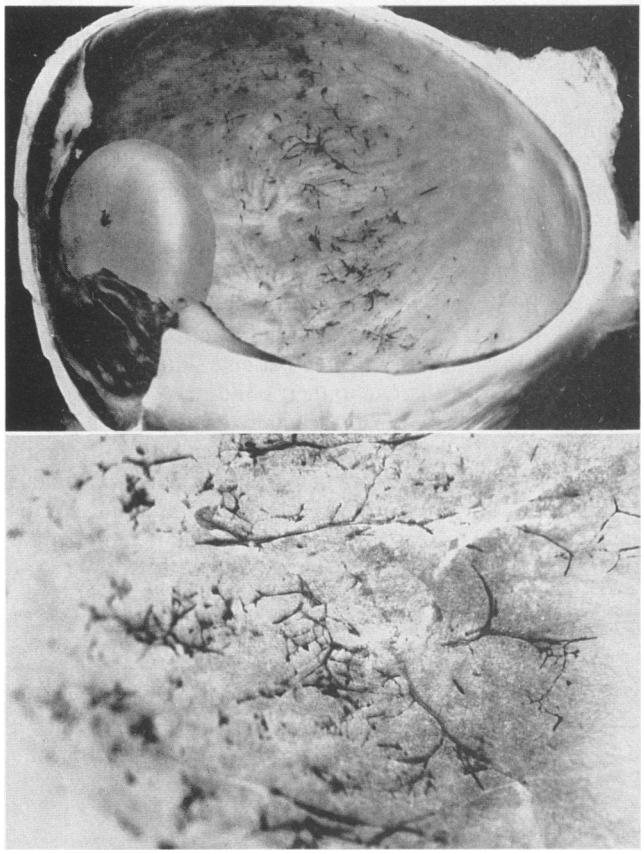

Figure 2.—Top, A gross specimen of eye taken at autopsy from a man with advanced retinitis pigmentosa shows distribution of pigmentation in equatorial regions. **Bottom,** A magnified view of the retina shows pigment clumping on retinal vessels and in the retina. Full report available (from Meyer et al[30]).

suring visual field loss over time). This rate dropped to a mean of 3.5% in a group of 39 study patients with retinitis pigmentosa placed on a regimen of vitamin E and β-carotene and observed for an average of eight years.[33] Statistically significant levels of improvement were not reached due in part to the large variance in the visual field measurements.[34,35] The use of antioxidants has been suggested to be of benefit in several ocular conditions.[36]

Retinitis pigmentosa does not result in sudden blindness. The disease typically progresses slowly over three to five decades after its usual first manifestation from age 20 to 30 years. Most patients do not go blind from the disease therefore, but many will have a severe disability in more advanced stages and may be legally blind (visual field 10 degrees or less with a 3-mm white target).

In almost half of patients with retinitis pigmentosa, posterior subcapsular cataracts also develop, some of which will cause enough visual loss to necessitate surgical correction. After cataract operations, patients do well, and there is no evidence that retinal degeneration is exacerbated, but there is an average delay of two months in achieving best visual acuity when compared with regular patients with cataracts.[37]

The use of molecular biologic techniques promises to be of great help to clinicians in sorting out the many forms of retinitis pigmentosa. There is also the potential of more rational treatment and more accurate genetic counseling. Linkage studies have already shown at least two gene sites on the X chromosome, possibly with different forms of the disease.[23] In an important recent study, 17 of 148 (12%) patients from different pedigrees with autosomal dominant retinitis pigmentosa were found to have a base-pair substitution in the rhodopsin gene.[1]

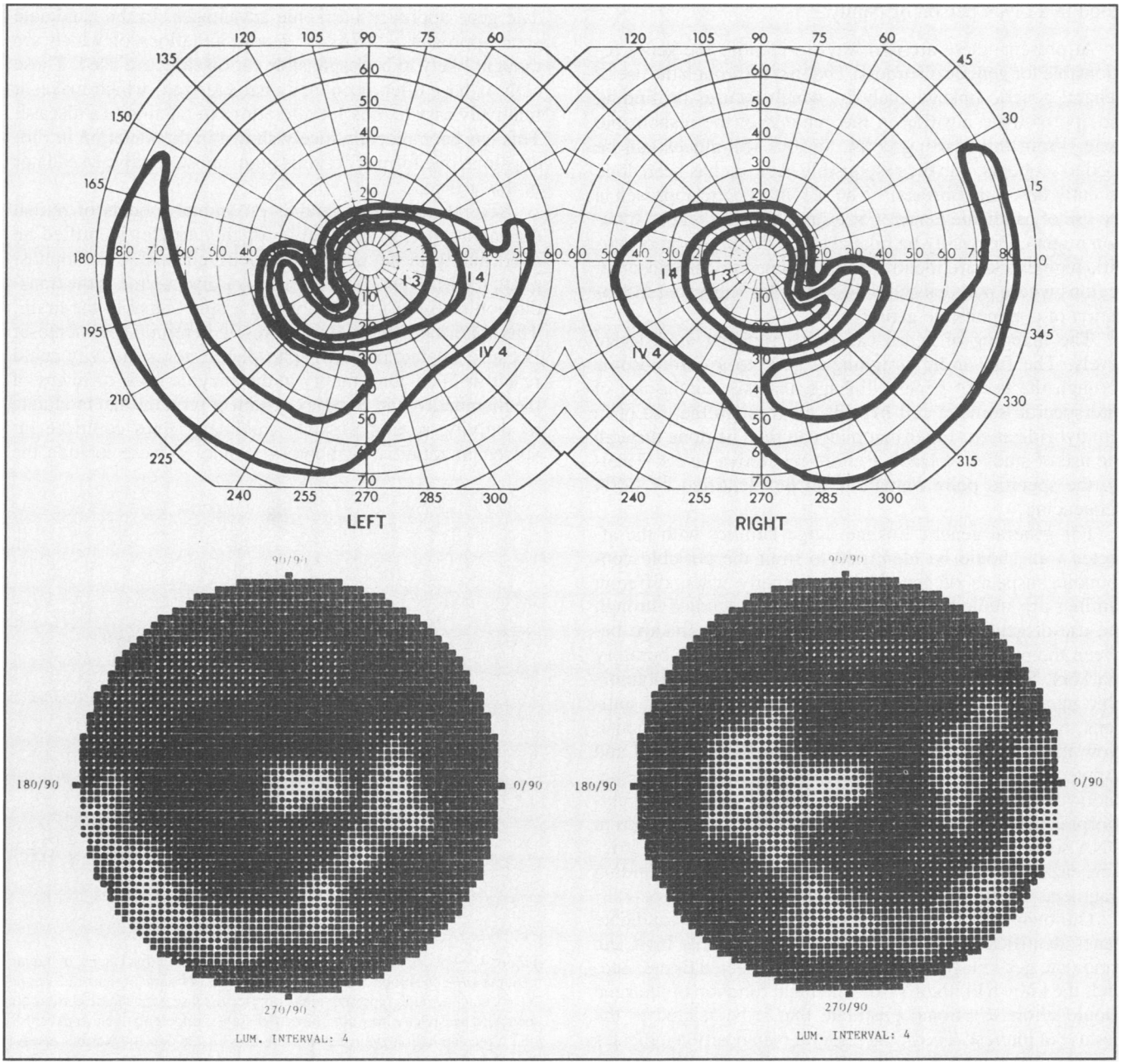

Figure 3.—Goldmann visual field **(top)** and octopus visual field **(bottom left and right)** from a 33-year-old man with autosomal dominant rod-cone degeneration show superior visual field depression and midperipheral ring scotoma formation (from Heckenlively[31]; reproduced with permission from JB Lippincott Co).

Genetic Approaches to Retinitis Pigmentosa

ROBERT S. SPARKES, MD*: Family studies have indicated that many types of retinitis pigmentosa have a genetic basis. This in turn suggests the possibility of considerable genetic heterogeneity as the basis for retinitis pigmentosa. It is important to identify the genes responsible for these disorders:

- Our understanding of their normal and abnormal functions would be improved.
- Rational forms of treatment might be developed at the physiologic or biochemical level.
- Gene therapy may become available sometime in the distant future.
- This information can be used to improve genetic counseling, probably at the prenatal and postnatal levels, by facilitating early detection of persons at risk. This may also lead to preventive measures once the basis of the disorder is understood in a given person or family.

At present, there are two ways to identify the genes responsible for genetic disorders. The first is through the use of general genetic linkage analysis, which focuses on finding the approximate position in the genome of the responsible gene. From this starting point, various techniques can be used to sequence into the responsible gene, as has been done recently for cystic fibrosis.[38] The second genetic approach is the use of candidate genes. For example, genes whose function might conceivably be related to the development of retinitis pigmentosa are identified and mapped to chromosomal regions where perhaps general linkage has suggested the location of a responsible gene.

The mapping of genes can be carried out at different levels. The first is by assigning a gene to a chromosome through the use of general linkage analysis, or the use of interspecific somatic cell hybrids, or through the use of in situ hybridization. Finer mapping can then be done through the use of studies of DNA at more restrictive levels. Finally, the specific point mutation can be identified by DNA sequencing.

For general genetic linkage, large families with the affected trait should be identified to limit the possible confounding aspects of genetic heterogeneity when different families are studied. Such families are then studied through the use of genetic markers to try to determine linkage between the disease locus and one of a number of laboratory markers. For many years, only a few phenotypic polymorphic markers were available, such as the erythrocyte antigens, erythrocyte isoenzymes, and serum protein variants. Now these markers can be extensively complemented and supplemented through the study of DNA variations, especially through the use of restriction-fragment-length polymorphisms (RFLP). In this sensitive technique, variations in DNA sequence can be identified through the use of restriction enzymes that cut the DNA at specific sites, yielding fragments of characteristic length.

Our own work has largely focused on the use of candidate genes identified according to the following criteria: First, the candidate gene must be expressed in the affected tissue. Second, the known biologic or biochemical function of the gene should allow a rational postulate that it be related to the disease of interest. Third, the relevant map position indicates that the gene could be involved in the disease. (This can be further evaluated by showing linkage cosegregation of the disease gene with the test gene in families.) Finally, molecular changes may be able to be shown in the gene by several techniques.

Animal homologies as discussed later may also often give clues as to which genes may be candidate genes. For several reasons, the study of candidate genes seems appropriate at this time. Several tissue-specific complementary DNA (cDNA) libraries are available. Easily sampled tissues, such as blood cells, can be obtained for the analysis of tissue-specific diseases, even though the gene of interest may not lead to blood disease but be expressed in some other tissues, such as the retina. Several mapping techniques are available to assign the gene to a specific chromosome and chromosomal region. Several technologies have been developed to study DNA variation that can be useful for genetic linkage analysis. Compared with general linkage analysis, the candidate gene approach has some advantages. In the candidate gene approach, cDNAs of genes, mutations of which are thought likely to be responsible for disease, are used. These cDNAs have often been well characterized, which makes it relatively easy to look for alterations in relation to a disease. This has been recently nicely shown in the mutation in rhodopsin in one form of retinitis pigmentosa, as discussed later in this article.[1]

Several years ago, studies on animal models of retinal degeneration (see last section of this article) identified an abnormality in the activity of cyclic guanosine monophosphate (cGMP)-phosphodiesterase, a key enzyme in the transduction pathway for transmitting a light signal to the brain. This abnormality may also be present in retinitis pigmentosa. Because a defect in cGMP-phosphodiesterase activity could result from an abnormality in the enzyme itself or in any of the members of the complex system of proteins that modulate its activity, several possible candidate genes could be involved in retinitis pigmentosa. Some of these include the

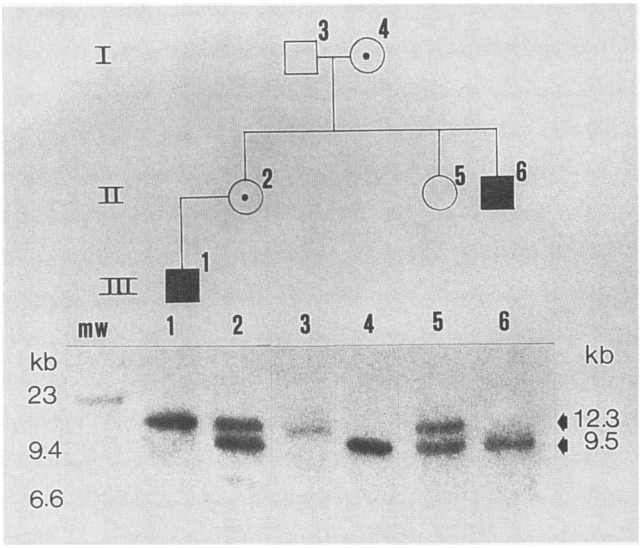

Figure 4.—A pedigree and autoradiograph are given. In a *Taq* I digest of human genomic DNA, the probe L1.28 detects two alleles of fragment sizes 12.3 kb and 9.5 kb. Black symbols represent men with Norrie's disease; obligate carriers are identified by circles with a dot. The carrier state is undeterminable in patient 5, and a recombination event betwen the DXS7 locus and the Norrie's disease locus has occurred in patient 2. The lane marked "mw" shows molecular weight markers using *Hin*dIII-digested λ (from Ngo et al[55]; reproduced with permission from *Clinical Genetics*).

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TABLE 5.—*Lod Scores Between the Norrie's Disease and the DXS7 Loci**

Family (Locus)‡	Lod Scores at Recombination Fractions (θ)†						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
Bleeker-Wagemakers et al (Ja)[51]	0.81	0,80	0.74	0.67	0.52	0.35	0.17
Bleeker-Wagemakers et al (P 2248)[51]	2.18	1.24	1.98	1.77	1.35	0.90	0.45
Bleeker-Wagemakers et al (P 2785)[51]	0.90	0.89	0.84	0.77	0.61	0.44	0.24
De la Chapelle et al[52]	2.41	2.37	2.21	2.00	1.56	1.09	0.57
Kivlin et al[53]	1.61	1.58	1.46	1.29	0.94	0.56	0.21
Hill et al[54]	1.41	1.38	1.28	1.15	0.86	0.54	0.24
Total	9.32	9.16	8.51	7.65	5.84	3.88	1.87
Present family	−∞	−1.70	−1.00	−0.70	−0.40	−0.22	−0.10
Total (present study)	−∞	7.46	7.51	6.95	5.44	3.66	1.77

*Not shown are 3 families reported by Bleeker-Wagemakers et al[51] and identified as P, P 9500, and "unpublished"; all are uninformative for the marker and give lod scores of 0.00 at all recombination fractions.
†The recombination fractions for women and men were equal.
‡Lod scores from the 6 families are recalculated on the basis of their published pedigrees (from Ngo et al[55]).

different subunits of G proteins (rod and cone transducins), rhodopsin, S antigen, the subunits of rod and cone cGMP-phosphodiesterases, and even the interphotoreceptor retinol-binding protein.

As noted, an abnormality of rhodopsin occurs in a form of autosomal dominant retinitis pigmentosa, a point of special interest in the candidate gene approach. The gene for human rhodopsin has been mapped to the long arm of chromosome 3.[39,40] The gene for autosomal dominant retinitis pigmentosa has been linked in a large Irish family to an anonymous probe (C17) that is located on the long arm of chromosome 3.[16] Dryja and colleagues picked up on this finding and examined the rhodopsin gene directly in other families with autosomal dominant retinitis pigmentosa.[1] They found that about 10% of the families with this type of condition had a mutation within codon 23 (CCC to CAC) of the first exon. They were then able to screen relatively quickly using specific oligonucleotide probes to identify persons who may or may not have this change. They found this change in 17 of 148 unrelated patients but not in any of 102 unaffected persons. This strongly supports the postulate that this point mutation is responsible for the retinitis pigmentosa in the families studied.

Thus, through the use of genetic techniques, especially the candidate gene approach, a gene responsible for a disease can sometimes be quickly identified, and the genetic basis for a disease can begin to be understood.

Hereditary Retinal Disease— Molecular Studies on Human Families

J. BRONWYN BATEMAN, MD*: The candidate gene approach has been used in our laboratory to study two retinal diseases that have a genetic basis: Norrie's disease and retinitis pigmentosa. Norrie's disease is an X-linked recessive disorder characterized by retinal detachment early in life.[41-46] As the retina is an extension of the brain and its function is to transform light energy to nervous impulses, both mechanical and biochemical abnormalities can cause profound loss of vision. The retina is nourished from the layer of pigment epithelial cells with which it is in close contact; if it becomes detached from this layer, atrophy, disorganization, and loss of function ensue. Complete blindness eventually occurs. In Norrie's disease, the retina detaches from the retinal pigment epithelium, and this causes profound and usually complete loss of vision. Additional features include variable mental retardation and hearing loss.

The gene for Norrie's disease was localized to the X chromosome by the characteristic inheritance pattern. As DNA probes for the X chromosome became available, close linkage between an RFLP identified by the DNA probe L1.28 (DXS7) and the disease was described in the mid-1980s. The L1.28 probe is an "anonymous" probe of unknown purpose that had been assigned to the region p11.3-p11.4 of the X chromosome[47,48]; therefore, the gene for Norrie's disease was assigned to the same area. The cumulative lod score, which reflects the statistical likelihood of linkage (or proximity) between the two genes, was 9.32 at a recombination fraction of 0.00 by 1987.[49-54] A lod score of this magnitude indicates that many meioses (informative matings) had been studied without a single recombinational event between the L1.28 probe and Norrie's disease loci. In other words, the genes for L1.28 and Norrie's disease had to be in close proximity on the X chromosome, or, alternatively, the Norrie's disease gene was within the L1.28 sequence.

We studied a family affected with X-linked recessive Norrie's disease and identified the first recombinational event between the disease locus and the DXS7 locus identified by the probe L1.28 (Figure 4).[55] We recalculated the lod score for the previously published cases and, with the addition of the UCLA family, changed the maximum lod score to 7.58 at a recombination frequency of 0.038 ± 0.036 (Table 5).[51-55] This finding indicated that the L1.28 probe is useful but may not be completely reliable for prenatal diagnosis and that the gene for Norrie's disease is not within the DNA sequence identified by the L1.28 probe.

We then looked for an alternative candidate gene in an effort to identify other probes for prenatal or perinatal diagnosis. Ornithine aminotransferase (OAT; L-ornithine:2-oxo-acid aminotransferase, EC 2.6.1.13) is a mitochondrial matrix enzyme catalyzing the major catabolic pathway of ornithine in human tissues. It has been mapped to the same region of the X chromosome as L1.28. A cDNA clone for the messenger RNA (mRNA) encoding human OAT (pHOAT) has been isolated and characterized.[56] Our group used somatic cell hybrids and in situ hybridization to map OAT and OAT-like gene sequences to two human chromosomes in regions 10q26 and Xp11.2[57] and used somatic cell hybrids to localize the genes to regions 10q23 and Xp11-Xp21.[58] This

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enzyme is present in many mammalian tissues,[59] including the retina, retinal pigment epithelium, and ciliary body of the eye.[60] An absence or severe deficiency of OAT is thought to be the primary defect in patients with gyrate atrophy,[61-64] an autosomal recessive ocular disease marked by myopia, cataracts, and a severe progressive degeneration of the choroid and retina.[61,65]

The X chromosome-associated OAT-like gene sequences (Xp11.2)[57] share the same general chromosomal regional assignment as the L1.28 probe (Xp11.2-Xp11.3). Although abnormalities of OAT activity (chromosome 10) had not been reported in patients with Norrie's disease, the proximity of OAT-like gene sequences to the L1.28 probe on the X chromosome made it a good candidate gene.

In this study, we used a human OAT cDNA to investigate the UCLA family with Norrie's disease that had shown the recombinational event. When genomic DNA from this family was digested with the *Pvu* II restriction endonuclease, a complex pattern of at least 14 bands was found. Only one of these bands (11.2 kb) is specifically localized to chromosome 10; the remaining identifiable 12 bands map to the X chromosome. We detected an RFLP of 4.2 kb. The 4.2-kb fragment was absent in the affected men and cosegregated with the disease locus (Figure 5; Table 6)[66]; we calculated a lod score of 0.602, at $\theta = 0.000$ (Table 7). No deletion could

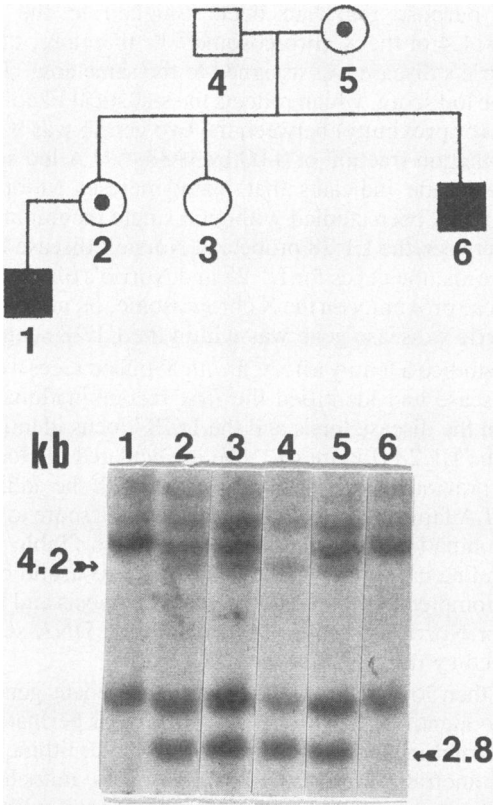

Figure 5.—A pedigree and Southern blot hybridization pattern show the distribution of the 4.2-kb restriction-fragment-length polymorphism (RFLP) from a 6-member family affected with Norrie's disease. The number of each lane corresponds to the number assigned to the persons in the pedigree. Genomic DNA was digested with *Pvu* II endonuclease and hybridized to ornithine aminotranferase (OAT) complementary DNA probe. A circle with a dot indicates the carrier state as determined by the L1.28 probe, and the arrow marks the RFLP. The status of person 3 is indeterminable using the L1.28 probe and is heterozygous based on the 4.2-kb band identified by the OAT cDNA probe (from Ngo et al[66]; reproduced with permission from *Genomics*).

TABLE 6.—*Mendelian Inheritance of the 4.2-kb Fragment: Copy Number Determined by Laser Densitometry**

Status	Sex	Density of 11.2-kb Band (area in AU/mm)	Density of 4.2-kb Band (area in AU/mm)	Ratio of Density	Copy Number (Person)†
Carrier	F	6.79	0.21	0.031	1(#2)
Carrier	F	6.04	0.34	0.056	1(#5)
Normal	M	1.27	0.06	0.049	1(#4)
Normal	F	2.22	0.22	0.099	2
Unknown ...	F	6.53	0.29	0.045	1(#3)

AU = absorption unit

*Genomic DNA from each of these persons was digested with *Pvu* II endonuclease and subjected to agarose gel electrophoresis to separate the fragments. The bands that contained ornithine aminotransferase (OAT) or OAT-related sequences were detected by autoradiography using a human OAT complementary DNA labeled with phosphorus 32. Using the LKB 2400 GelScan XL densitometer, we measured the density of the OAT hybridizable bands on the autoradiogram, which were reported as area (AU/mm). The OAT band representing the functional OAT gene on chromosome 10 (11.2-kb fragment) was used as an internal standard. The ratio of the 4.2- and the 11.2-kb band was determined to calculate the copy number of the 4.2-kb fragment. The normal female used in this study is from a family without Norrie's disease. It is assumed that the normal persons tested are homozygous for the allele represented by the 11.2-kb fragment. The resulting inference regarding the number of alleles at the OAT-related locus is confirmed by the distribution of the marker locus phenotype in this family (from Ngo et al[66]).

†The number in parentheses corresponds to the persons in the pedigree (see Figure 5).

be detected by chromosomal analysis of Southern blots with other enzymes. These results suggested that one of the OAT-related sequences on the X chromosome could be in close proximity to the Norrie's disease locus and represented the first report that the OAT cDNA may be useful for identifying the carrier state or for prenatal diagnosis, or both.[66]

Because the lod score obtained with the human OAT probe was not statistically significant to establish linkage, other families with this disease were sought. In collaboration with Jane Kivlin, MD, of the Medical College of Wisconsin (Milwaukee), we used the same probe to study a multigeneration family from Utah. A different RFLP of 7.5 kb was identified after digestion with *Pvu* II restriction endonuclease; this RFLP mapped to the X chromosome and was inherited in an X-linked manner. A recombinational event between the OAT locus represented by this RFLP and the disease locus was observed (Figures 6 and 7). Linkage analysis of these two loci in this family revealed a maximum lod score of 1.88 at a recombination fraction of 0.10 (Table 8). Although both families have members affected with the same disease, the lod scores were reported separately because the 4.2- and 7.5-kb RFLPs may represent two different loci for the X-linked OAT sequences.[67]

The recombinational event found with the 7.5-kb RFLP indicates that the molecular defect of Norrie's disease is not within the sequence detected by this polymorphism. At present, however, we cannot exclude the possibility of a relationship between some of the OAT-related sequences represented by the 4.2-kb RFLP[66] and the disorder, as no recombinations have been identified. In view of the recombinations between the DXS7 locus represented by the L1.28 probe, the OAT locus represented by the 7.5-kb RFLP, and the Norrie's disease locus, the use of either or both the 4.2- and 7.5-kb RFLPs in addition to the L1.28 probe may increase the reliability and feasibility of identifying carriers and affected fetuses for prenatal diagnosis.

The identification of a common RFLP between the two families will allow the scores to be additive. A cumulative lod score of 3 or greater would establish the OAT as a new and useful DNA marker for Norrie's disease. These studies show the complexity that can be encountered when using candidate genes to identify cosegregation or linkage with a disease.

TABLE 7.—*Lod Scores for Norrie's Disease and the Human Ornithine Aminotransferase Complementary DNA**

	θ Values							
	0.000	*0.010*	*0.050*	*0.100*	*0.200*	*0.300*	*0.400*	*0.500*
Lod scores.....	0.602	0.589	0.536	0.470	0.338	0.211	0.096	0.000

*These are the recombination fractions for female members of the UCLA family studied. Because the genotypes identified by the 4.2-kb restriction-fragment-length polymorphism (RFLP) are known for each of the persons in this family, the estimate of the frequency of the RFLP was not required for the analysis. The frequency of Norrie's disease was assumed to be 0.0001 (from Ngo et al[66]).

TABLE 8.—*Lod Scores for Linkage Between Norrie's Disease and the Human Ornithine Aminotransferase (OAT) X-Linked Locus Detected by the 7.5-kb Restriction-Fragment-Length Polymorphism (RFLP)**

	θ Values						
	0.001	*0.010*	*0.050*	*0.100*	*0.200*	*0.300*	*0.400*
Lod scores.....	0.423	1.376	1.860	1.879	1.562	1.060	0.465

*The frequency of Norrie's disease in this Utah family was assumed to be 0.001. The frequency of the OAT allele represented by the 7.5-kb RFLP was calculated to be 0.345 based on female control subjects (from Ngo et al[67]).

We have also used the candidate gene approach to analyze large families with retinitis pigmentosa to identify cosegregation, linkage, or both. Biochemical studies described later by Dr Farber have suggested that the defect(s) in some of these disorders could be in the phototransduction pathway. Indeed, a mutation of rhodopsin has been shown in some patients with autosomal dominant retinitis pigmentosa.[1] We investigated a large kindred of three generations with more than 75 members affected with an autosomal dominant form of retinitis pigmentosa for RFLPs identified for the following candidate retinal specific genes: rhodopsin, transducin α_1 (rod specific), S antigen (48 kd), and interphotoreceptor retinol-binding protein. For each of the probes, an RFLP was identified in the family and mendelian inheritance shown. An RFLP of 1.2 kb was detected after digestion with *Rsa* I restriction endonuclease using the rhodopsin probe. Hybridization with the transducin α_1 probe after *Pvu* II restriction endonuclease digestion showed a pattern of four distinct bands of sizes 2.0, 1.8, 0.6, and 0.2 kb, with the 1.8-kb band being the RFLP. The interphotoreceptor retinol-binding protein probe detected 4.3- and 6.3-kb RFLPs with the *Bgl* II restriction endonuclease.[68] The S-antigen probe identified seven fragments with 3.9-kb and 4.3-kb polymorphisms.

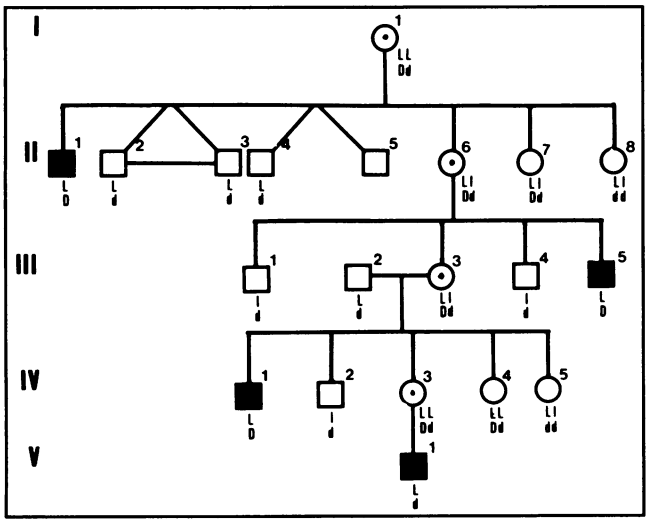

Figure 6.—The pedigree is from a 5-generation family affected with Norrie's disease. Members from whom DNA was obtained are identified with a number. Alleles identified with the L1.28 probe are designated as "L" or "l", allele "L" representing the 12.3-kb restriction-fragment-length polymorphism (RFLP) and allele "l" the 9.5-kb RFLP. Typing of this pedigree with the L1.28 has been reported by Kivlin et al.[53] Alleles identified with the human ornithine aminotransferase complementary DNA probe are designated as "D" for the presence of the 7.5-kb RFLP and "d" for the absence of the same fragment. Allele D cosegregates with the presence of Norrie's disease except for person V1, who is a recombinant affected offspring expressing the "d" allele; a recombinational event has occurred in person IV3 (from Ngo et al[67]; reproduced with permission from *Genomics*).

Lod scores were calculated, and cosegregation with the disease could be excluded for each of the candidate genes as the cause of the retinitis pigmentosa in this family. Linkage between the candidate gene and the disease was excluded for S antigen, transducin α_1, and interphotoreceptor retinol-binding protein. Although the lod score was low, linkage between the rhodopsin and the disease loci was suggestive. Because of the single crossover between rhodopsin and the disease loci, a mutation of the rhodopsin gene is unlikely in this family.

Although inconclusive, these studies show the practical value of the candidate gene approach for human studies and support the genetic heterogeneity of retinal degenerations. The retina is a specialized structure, and the spectrum of the response to insults is limited. The biochemical pathway for phototransduction is complicated, and defects in any of the proteins or enzymes involved in this pathway could lead to retinal degeneration. The future is promising for the identification and treatment of retinal diseases and other disorders of genetic origin.

Inherited Retinal Degeneration of the *rd* Mouse

Morphologic and Biochemical Findings

DR FARBER: The disease affecting the *rd* mouse retina begins early and progresses rapidly. At the light-microscopy level, no morphologic differences are found at 10 postnatal days between normal and *rd* mouse retinas, but at 13 days of age, the *rd* photoreceptors are degenerating rapidly, and by 20 postnatal days, most *rd* visual cells have died.[69] All other retinal cells, including the pigment epithelium, survive the disease.

Many years ago, we discovered a biochemical abnormality in the *rd* retina that is present before any morphologic abnormality is observed. As early as postnatal day 6, the

level of cGMP begins to rise above normal[70] due to a deficiency in the activity of cGMP-phosphodiesterase.[71] This reduced activity of the degradative enzyme of cGMP is restricted to the photoreceptor cells of the *rd* retina.[72] Other tissues of the *rd* mouse, such as blood—plasma, serum, lymphocytes, and erythrocytes—brain, muscle, and skin, have cGMP-phosphodiesterase activities comparable to those of control mouse tissues.

Cyclic GMP-phosphodiesterase is composed of four subunits, two with catalytic activity, α and β, and two identical γ subunits with inhibitory activity.[73,74] This enzyme is fundamentally important for phototransduction in rod photoreceptors. Its activation by light is mediated by components of the visual cell, including rhodopsin, transducin, and guanosine triphosphate.[75] Cyclic GMP-phosphodiesterase deactivation is also a complex process that requires the participation of other components of the photoreceptors, such as rhodopsin kinase, adenosine triphosphate (ATP), and a 4.8-kd protein also known as S antigen.[76]

We showed that cGMP-phosphodiesterase is synthesized normally by the *rd* photoreceptor cells and that there is no difference in the time of the onset of expression nor in the amount produced when compared with synthesis by normal mouse photoreceptors.[77] From the first time that cGMP-phosphodiesterase molecules are detected in the *rd* retina, however, and throughout the developmental period before visual cell degeneration, the cGMP-phosphodiesterase level is lower than in control retina. This suggests that the enzyme is more labile and becomes degraded soon after it is made. This could be due to one (or more) mutation occurring at the level of the DNA encoding the enzyme, which results in changes in the protein itself, or to the presence of a more active proteolytic system in the *rd* retina than in control retina.[77] Preliminary studies seem to indicate that the latter is not the case. Furthermore, cGMP-phosphodiesterase from *rd* retina is minimally activated by light,[78] which strengthens the possibility of a lesion in the enzyme itself or in any of the components of the photoreceptor cell involved in its activation or deactivation.

Studies on the mRNAs encoding rhodopsin, the α, β, and γ subunits of transducin, the γ subunit of cGMP-phosphodiesterase and 48-kd protein, showed that these mRNAs are qualitatively normal in the *rd* retina and that they appear at the same time and are found in the same concentration as in control retina.[79-81] In addition, each of the proteins translated from these mRNAs has the same time of appearance in developing normal and *rd* retinas.[80] We concluded, therefore, that a delay in the expression of any of these six proteins involved in the activation or deactivation of cGMP-phosphodiesterase does not seem to be associated with the cause of the *rd* disease. Another abnormality of the *rd* retina was found, however, during the investigation of the functionality of rhodopsin: the visual pigment cannot be phosphorylated in vitro at any age, although it is present in concentrations comparable with normal until the photoreceptors start to degenerate.[82] Thus, we studied in normal and *rd* mouse retinas the activities of rhodopsin kinase, the enzyme that catalyzes the transfer of phosphate from ATP to rhodopsin, and of protein phosphatase 2A, the enzyme that dephosphorylates rhodopsin, because an excess of this activity could prevent the detection of phosphorylated rhodopsin. Our results confirmed the latter possibility.[83]

In previous investigations, we had examined the effect of several cations and nucleotides on the phosphorylation of rhodopsin in the outer segments of rod photoreceptors from normal retinas and showed that high levels of cGMP inhibited rhodopsin phosphorylation.[84] Our recent findings with the *rd* retina are consistent with the notion that the high cGMP concentrations present from early development in the *rd* photoreceptors may activate protein phosphatase 2A, a hypothesis that we are currently testing. This abnormality in the *rd* retina would then be secondary to the deficiency in cGMP-phosphodiesterase activity, which generates the elevated levels of cGMP.

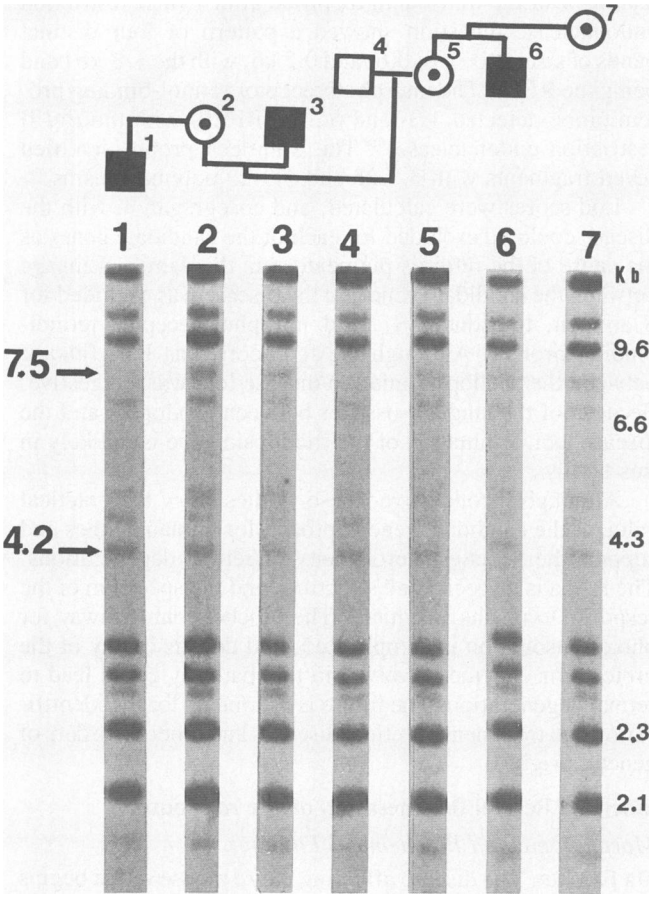

Figure 7.—A Southern blot hybridization pattern shows the distribution of the 7.5-kb restriction-fragment-length polymorphism (RFLP)-selected members of the 5 generations affected with Norrie's disease. Genomic DNA was digested with *Pvu* II endonuclease and hybridized to the human ornithine aminotransferase (OAT) complementary DNA probe. The 7.5-kb RFLP is identified by an arrow. Alleles identified with the human OAT cDNA probe are designated as "D" for the presence of the 7.5-kb RFLP and "d" for the absence of the same fragment. Allele "D" cosegregates with the presence of Norrie's disease except for person 1 (V1 of Figure 6) who is a recombinant affected offspring expressing the "d" allele; a recombinational event has occurred in person 2 (IV3 of Figure 6) (from Ngo et al[67]; reproduced with permission from *Genomics*).

Location on Mouse Chromosomes of Candidate Genes for the rd *Mutation*

The *rd* gene has been localized on mouse chromosome 5.[85] To confirm that none of the proteins involved in the activation or deactivation of cGMP-phosphodiesterase are the site of the *rd* locus, we determined the chromosomal location of the corresponding cDNAs and of all other retinal cDNAs that became available to us. These studies were carried out using hamster-mouse somatic cell hybrids and

TABLE 9.—Assignment of Some Photoreceptor Genes to Mouse Chromosomes

Gene	Mouse Chromosome
Rhodopsin	6
Transducin α_1	9
Transducin β_1	4
S antigen[4]	1
cGMP-PDEαS	18
cGMP-PDEγ[1]	11

cGMP-PDE = cyclic guanosine monophosphate-phosphodiesterase

[1]From Danciger et al.[88] [2]From Danciger et al.[88] [3]From Danciger et al.[87] [4]From Danciger et al.[89]

mouse interspecific backcrosses. Table 9 summarizes our results.[86-90] Indeed, we did not assign any of the other genes investigated to mouse chromosome 5. This indicates that none of the proteins encoded by these genes is the primary lesion of the *rd* disease.

Identification and Cloning of a Candidate cDNA for the rd *Gene*

At the same time that these studies were carried out, we began to search for the *rd* gene with a totally different approach. Our strategy was based on the assumption that the mRNA encoded by the *rd* gene would be either absent or expressed in greater or lesser amounts in normal than in affected *rd* photoreceptors.

We took advantage of the fact that the retina of the adult *rd* mouse has no photoreceptor cells and is constituted solely by the inner retinal layers, to subtract the cDNAs of these inner retinal layers from the cDNAs of a normal retina. This allowed us to obtain a pool of 588 photoreceptor-specific cDNAs. These clones were screened for the *rd* gene by differential hybridization, using single-stranded retinal cDNAs from 9- to 11-day-old, morphologically normal, *rd*/$^+$ mice and single-stranded retinal cDNAs from their affected *rd/rd* littermates. The greatest amount of age-matched retinal tissue can be obtained at this age, before full degeneration of the *rd/rd* visual cells. We found three clones that hybridized differently with the two probes. When the DNA of these three clones was used, however, to probe Northern blots containing normal and *rd/rd* mouse retinal RNAs, only one clone, zr.408, hybridized more faintly to the *rd/rd* RNA than to the age-matched normal RNA.[3]

We characterized the zr.408 cDNA, and from its properties we inferred that it was a strong candidate for the *rd* gene. The zr.408 clone hybridized a 3.3-kb mRNA from normal retina and only weakly a larger 3.6 kb from *rd/rd* retina. This size difference could result from alternative splicing, polyadenylation site differences, or the presence of a structural alteration in the corresponding gene of the *rd/rd* mouse. In addition, zr.408 hybridized to the DNAs from somatic cell hybrids, permitting us to map the corresponding gene to mouse chromosome 5. The zr.408 clone hybridized different-sized restriction fragments from genomic normal ($^+/^+$) and *rd/rd* DNAs, suggesting a structural difference between the $^+/^+$ and *rd/rd* gene sequences at sites homologous to zr.408. This was further confirmed by the fact that $^+/^+$ and *rd/rd* polymorphisms were present in the *rd*/$^+$ DNA at about half the intensity found for each in the DNAs from the homozygous mice. Finally, the mRNA hybridized by zr.408 was detectable on postnatal day 1 in normal and *rd/rd* retinas; its concentration increased steadily during development but was always lower in the *rd/rd* retina. Following the degeneration pattern of the *rd* retina, the levels of zr.408 mRNA decreased sharply by 14 postnatal days and were no longer detectable at 31 days of age. Thus, the expression of the candidate *rd* cDNA is abnormal in the *rd/rd* retina several days before cGMP levels become elevated in the visual cells. This is the earliest molecular lesion that has been shown in the *rd* mouse disease.[3]

To further confirm that zr.408 does in fact correspond to the *rd* gene, we undertook two molecular genetic studies. The aim of the first was to verify that the gene corresponding to zr.408 and the *rd* gene map to the same location on mouse chromosome 5. The second project was designed to show cosegregation of the candidate *rd* gene with the expression of the *rd* disease, photoreceptor cell degeneration. We carried out these studies using interspecies backcrosses, which have been shown to give RFLPs more frequently than mouse inbred crosses.

In an interspecies backcross, a wild mouse and an inbred mouse are mated. All the resulting F1 offspring are heterozygous for every gene. The F1 animals are then backcrossed either with wild or inbred mice, and the progeny consist of animals homozygous or heterozygous for specific genes. If two genes being investigated are close together on a chromosome, few recombinants will be produced. From the rate of recombination, it is possible to estimate the distance in centimorgans at which the two genes are located.

The DNAs from the NFS/N and *Mus mus musculus* mice were analyzed for RFLPs that hybridized to the zr.408 probe. These RFLPs were then identified in DNAs from 62 backcrossed progeny of the cross (NFS/N × *M m musculus*)F1 × *M m musculus*. The inheritance pattern of the gene corresponding to zr.408 was compared with the inheritance patterns of two other genes that lie on opposite sides of the *rd* gene on mouse chromosome 5, *Afp* and *Gus*. By determining the number of backcrossed progeny heterozygous for zr.408 and homozygous for *Afp* or *Gus*, or vice versa, we could establish the number of recombinants present.[91] This permitted us to position zr.408 between *Afp* and *Gus* at distances similar to those established for the *rd* gene with respect to *Afp* and *Gus* on standard chromosome maps (V. M. Chapman, W. K. Noell, D. Adler, *Mouse Newsletter,* 1975, volume 53, p 61).

For our cosegregation study, DNAs from C57BL/6J *rd/rd* and *Mus spretus* mice were analyzed for RFLPs that hybridized to the zr.408 probe. These RFLPs were then examined in DNAs from 72 backcrossed progeny of the cross (C57BL/6J *rd/rd* × *M spretus*)F1 × C57BL/6J *rd/rd*. The animals homozygous and heterozygous for zr.408 were identified. These results were then compared with the histologic results showing the presence or absence of retinal degeneration obtained by examining the retinas of the 72 backcrossed mice. We found that zr.408, the candidate *rd* gene, cosegregated with the *rd* disease with no recombinants obtained, that is, each of the assignments to *rd/rd* or *rd*/$^+$ determined by blot hybridization with zr.408 corresponded exactly to the histologic identification.[91]

The results described offer strong evidence for zr.408 corresponding to the *rd* gene. We have recently determined the nature of its expressed product. This gene encodes the β

subunit of cGMP-phosphodiesterase,[92] which explains our previous findings.

Histopathologic and Biochemical Findings on Human Eyes Affected With Autosomal Dominant Retinitis Pigmentosa

We studied the eyes of a 17-year-old boy affected with autosomal dominant retinitis pigmentosa who died in a motorcycle accident. He had been examined by an ophthalmologist when he was 15, at which time he had a loss of night vision. Although his visual acuity was normal, his fundi had the typical appearance of retinitis pigmentosa. His electroretinogram showed that the scotopic and photopic responses were barely recordable.

The eyes of this donor were enucleated within an hour after death. The right eye was fixed for morphologic[93] and immunocytochemical studies, and the left eye was frozen at −80°C and used for biochemical studies.[94]

Light-microscopic analysis of the retina showed photoreceptor cell degeneration increasing from the fovea to the equatorial area and then decreasing toward the periphery. We counted the number of rods and cones throughout the retina and compared the results with those obtained from a normal human retina of similar age used as reference. The superior temporal quadrant was the least affected, its periphery retaining about 40% of the rods present in the same quadrant of the normal retina. The periphery of the other three quadrants had pronounced reductions in the number of rod cells, the superior nasal quadrant retaining 19% rods, the inferior nasal 11%, and the inferior temporal practically none. In addition, no rods were present in the equatorial region of the retina. Although the cone population suffered less drastic reductions, with a loss of about 25% of the foveal cones, there were no cones left in the equatorial region of the retina. The retinal pigment epithelium showed no unusual accumulation of lipofuscin or debris, and the cells of the inner retina remained unchanged.

For the biochemical studies, the frozen eye was cut into four quadrants, which were dissected in an ultramicrotome maintained at −30°C. After removing the vitreous, we took sequential 3-mm retinal punches from each quadrant, ensuring the analysis of every piece of retina. The features that we measured in each punch of the retina were all those investigated previously in the *rd* mouse. We found that cGMP-phosphodiesterase activity was lower than normal throughout the retina and that there was minimal or no detectable activity in the most central and equatorial regions. Except for a portion of the periphery of the superior temporal quadrant where the level of cGMP-phosphodiesterase was reduced by 10%, all the rest of the peripheral retina had 25% to 45% of the activity present in comparable regions of the age-matched normal retina.

We studied the phosphorylation of rhodopsin in punches of retinitis pigmentosa retina that contained rod photoreceptors and rhodopsin. As with the *rd* mouse, we could not detect the incorporation of phosphorus 32 (from ^{32}P-ATP) into rhodopsin.

The greatest difference between the retina with retinitis pigmentosa and the normal one was found in the pattern of distribution of cGMP, which concentration was much reduced in all areas. Cyclic GMP is localized mostly in rods,[95] and the cells of the inner retina do not contribute substantially to cGMP levels.[96] Taking advantage of this specific localization and of our measurements of the number of rods in every square millimeter of the normal and affected retinas, we estimated the cGMP concentration per photoreceptor cell in all areas of each quadrant. Normal visual cells contained similar amounts of cGMP throughout the retina, which ranged between 2 and 4×10^{-18} mol per rod. An exception to this was the fovea, which had minimal or no cGMP. In contrast, the photoreceptors of the affected retina contained high levels of cGMP in specific areas: about 40-fold to 100-fold higher than normal levels in the few outer segment-devoid rods of the postequatorial region and in the periphery of the inferior temporal quadrant and 15-fold to 20-fold higher than normal levels in the rods from the area close to the periphery of the superior temporal quadrant. The rest of the peripheral retina had twofold to threefold higher than normal levels of cGMP per visual cell. No other retinal punches contained measurable cGMP, correlating with the loss of rod photoreceptors.

Therefore, the same biochemical abnormalities found in the *rd* mouse retina were present in this autosomal dominant case of retinitis pigmentosa. These findings leave open the possibility that a similar mechanism may be responsible for the degeneration of the visual cells in the diseases affecting the *rd* mouse and the dominant type of human retinitis pigmentosa. We are currently carrying out molecular biologic studies that will confirm or rule out such a hypothesis.

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