Molecular genetics has contributed greatly to our understanding of inherited ocular disease. Prior to the development of recombinant DNA technology, basic and clinical scientists were limited to a description and classification of phenotypes based on morphology, biochemistry, and physiology. Progress was severely hampered by the dearth of genetic information. The pace of progress accelerated in the 1990s after the first disease-causing allele for retinitis pigmentosa was reported. The years 1990 through 2000 featured the identification and characterization of multiple gene alleles underlying retinitis pigmentosa and allied monogenic diseases. A second leap in our understanding occurred in the past year. Age-related macular degeneration—which was, until now, refractory to the identification of genes involving significant segments of the patient population—is finally yielding its secrets. However, some genes have no known function. Indeed this is the case for the majority of genes putatively identified by the Human Genome Project. Answers to these questions will come through an amalgamation of genetics, cell biology, physiology, and other disciplines. Collaboration among investigators in these disciplines is already occurring out of sheer fascination over this interesting and important topic. In the end, patients with inherited ocular disease will be the final and highly deserving beneficiaries.

Prior to the advent of modern ocular genetics, clinicians and scientists were limited to the observation and classification of ocular phenotypes that result from gene mutations in human patients or in animals harboring spontaneous mutations. Molecular genetics has provided a powerful tool that has led not only to the identification of many disease-causing mutations but has also allowed the production of additional animal models in which disease mechanisms and therapeutic intervention can be studied. Although genetics has played an important role in ocular diseases in general, initial insights were gained in the field of inherited retinal disease and, because this is my expertise, specific examples will be drawn from this area of investigation.

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The floodgate of gene discovery in the field of retinitis pigmentosa (RP), a much more common, primarily monogenic family of diseases (worldwide prevalence of 1 in 3500 individuals), was opened in 1989 when McWilliam et al., taking advantage of polymorphic genetic markers that significantly improved the resolution of linkage analysis, directed our attention to the long arm of chromosome 3, the repository of the rhodopsin gene. Shortly thereafter, Dryja et al. using the candidate gene approach, published their seminal paper on the prolino-histidine change at amino acid position 23 in rhodopsin. These authors took advantage of a well-characterized clinical population, information concerning the gene structure of rhodopsin, and a relatively new technique called polymerase chain reaction, which allows for easy reproduction and sequencing of amino acid coding segments for a gene of interest.

The candidate gene pathway has not always followed the course described for rhodopsin. In some cases, important clues have been gained from animal models of human disease in which gene mutations causing an ocular phenotype arose spontaneously. The retinal degeneration slow mouse was the first such example in which the discovery of a disease-causing gene in an animal model led directly to the discovery of mutations in its human ortholog. Indeed, the most common causes of dominant RP reported in a recent survey were found in the retinal degeneration slow and rhodopsin genes. The yield from this marriage of the candidate gene approach—animal models and well-characterized human patients—has been high. It has subsequently led to the discovery of many genes underlying monogenic diseases, including at least 100 putative disease-related variants in rhodopsin itself. About 110 of an estimated 160 genes related variants in rhodopsin itself. About 110 of an estimated 160 genes related variants in rhodopsin itself. About 110 of an estimated 160 genes related variants in rhodopsin itself. About 110 of an estimated 160 genes thought to be responsible for inherited retinal disease have now been analyzed using this and other molecular genetic methods.

Molecular genetics has also provided access to highly informative animal models of RP and related genetic diseases through the selective disruption of genes, a process known colloquially as gene knockout. Two high-yield examples are given here. Both involve instances in which the precise biological role of the disease-causing gene was unknown beforehand.

Following several abortive attempts by several groups to determine the function of its protein product, Redmond et al. disrupted the RPE65 gene in mice in 1998. Based on biochemical evidence and the fact that human mutations caused early-onset severe retinal dystrophy, the protein, known variously as P63 and RPE65, was thought by some to be involved in retinol metabolism. The protein’s specific role began to emerge when it was observed that the retinal pigment epithelium (RPE) of the knockout mouse was incapable of synthesizing 11-cis-retinal, the chromophore of the rod and cone photopigments. Moreover, substrates for the isomerohydrolase, the retinyl esters, accumulated in oil droplets in the RPE. Mutations were quickly discovered in the dog ortholog of RPE65; dogs were thereafter successfully treated for their disease with replacement gene therapy. In a remarkably rapid application of these discoveries to human disease, patients with Leber congenital amaurosis due to RPE65 mutations will be enrolled in 2 phase 1 gene therapy trials in 2006. It is now believed that RPE65 is indeed the isomerohydrolase, the enzyme that hydrolyzes retinyl esters and isomerizes the retinol product into 11-cis retinal, prior to its reduction to 11-cis- retinal dehydrogenase.

The ABCA4 gene was also experimentally disrupted in mice, again following genetic evidence for its involvement in a human disease, namely recessive Stargardt macular dystrophy. Studying the retinoid cycle of this genetically engineered animal generated considerable clarity regarding the function of its protein product. It was determined that this protein—rim protein, which is located in the hairpin-like loops at the perimeter of the rod and cone photoreceptor outer segment discs—serves as a phospholipid translocator. This translocation occurs when rim protein “flips” a naturally occurring product of phototransduction—N-retinylidene phosphatidylethanolamine—from the luminal leaflet of the disc phospholipid bilayer to the cytoplasmic leaflet, thereby translocating both phosphatidylethanolamine and all-trans-retinal. Following translocation, the all-trans-retinal is hydrolyzed from its covalent linkage to phosphatidylethanolamine and subsequently reduced to all-trans-retinol before making its way from the photoreceptor outer segment to the RPE for another round of 11-cis-retinal production. When the ABCA4 gene is mutated, this flipping process does not occur efficiently and, because of this delay, 2 all-trans-retinal molecules eventually become covalently linked to phosphatidylethanolamine, forming a bisretinoid. When this complex is ingested by the RPE during the natural phagocytic process, the bisretinoid is converted to a toxic, detergent-like product called A2E in the acidic environment of RPE lysosomes. A2E is further converted to an oxidized species called epoxides that are even more toxic. These insights into the function of rim protein have inspired investigations into methods with which small molecule therapy might be employed to slow the process of retinal degeneration in recessive Stargardt macular dystrophy. The central concept involves reduction of the retinoid load on the retina, either through reduced delivery of retinol to the RPE or inhibition of enzymes in the RPE visual cycle pathway. Thus, in only 16 years, beginning with the discovery of the first RP mutation, a host of dedicated and talented investigators, taking advantage of cutting-edge technologies, well-characterized patient cohorts, and excellent animal models, has made extraordinary progress toward an understanding of the genetics and molecular mechanisms underlying monogenic retinal diseases. We now stand at the doorstep for treatments and cures for some of these diseases.
AGE-RELATED MACULAR DEGENERATION: A MULTIGENIC COMPLEX TRAIT DISEASE

Age-related macular degeneration (AMD) is a multifactorial disease with genetic, dietary, environmental, and age-related components. It has represented an enigma to investigators for many years, partly because of a paucity of genetic information, even though it has been clear for some time, particularly from monozygotic twin studies, that there is a genetic component to AMD.28,30 The feature that caught the attention of these investigators was the composition of drusen, collections of extracellular material between the basal lamina of the RPE and the outer collagenous layer of Bruch membrane. Extensive and elegant immunocytochemical analysis of these structures revealed components of the inflammatory process, particularly molecules associated with the alternative pathway of the complement cascade and its regulators.29,30 The interpretation of these observations met with initial skepticism. It is worth noting in hindsight, however, that potential corroborating evidence was available from a concurrent genome-wide screen of an AMD pedigree, which provided linkage data indicating a disease locus in chromosomal regions 1q25 through 1q31.31 This region contains the regulation of complement activation gene cluster, which includes complement factor H and some of the complement regulators. Subsequent genome-wide scans were in accord with this observation and other chromosome loci were mapped as well.32,34 Completion of the Human Genome Project significantly simplified the process of linkage analysis by providing high-density single nucleotide polymorphisms. In 2005, a true breakthrough occurred in the field of AMD genetics. Four papers published in the spring of that year provided strong evidence for involvement of complement factor H in causing a broad spectrum of AMD phenotypes, including the exudative (“wet”) or neovascular and atrophic (“dry”) forms.35-38 Whereas previous genetic studies had identified gene mutations in small numbers of individuals, this breakthrough discovery included many individuals in medium to large patient cohorts.

Complement factor H is a fluid-phase regulator of the alternative complement pathway.39-40 It is a plasma protein that inhibits complement activation, protecting the host cell from attack (in this case the RPE and choriocapillaris). Factor H exclusively regulates the alternative pathway of complement activation. The protein is primarily synthesized by the liver for systemic use, but it is also synthesized locally in the eye by the RPE. Its structural motif features 20 short consensus repeats or complement control protein modules. Factor H binds to complement fragment C3b, which opsonizes host cells and pathogens and serves as a cofactor for complement factor I in the inactivation (proteolytic cleavage) of C3b on host cell surfaces. All 4 of these definitive papers emphasized the role of a tyrosine 402–to–histidine (Y402H) variant as a predisposing allele for AMD. In one of the studies, homozygotes for this variant accounted for 24% of cases compared with 8% of controls.38 This particular variant attracted considerable attention because the Y402H change occurs in the seventh short consensus repeat, which is a C-reactive protein inhibitory– and C-reactive protein heparin–binding site. C-reactive protein, unless bound by factor H, is an activator of the complement cascade. Moreover, a Y402H change substitutes a positively charged histidine for an uncharged tyrosine and could change the binding properties of complement factor H for the host cell surface, which is coregulated along with C3b during factor H regulation. The consequence of the Y402H variation could be uncontrolled activation of complement on the host cell surface, leading to bystander damage to the RPE and choriocapillaris.

It must be stated emphatically however that Y402H is not the only important variant implicated in factor H involvement. Indeed, it is a predisposing haplotype of variants that should be considered in this case. I62V and D936D variants, in concert with Y402H, are considered important.39 Significantly, there are also protective haplotypes that diminish the chances of developing AMD, an encouraging prospect indeed. Also, because the RPE and choriocapillaris are involved in local secretion of many if not all of the components and regulators of the complement cascade, there is hope for local treatment with the aid of complement alternative pathway inhibitors that could circumvent the risks of global inhibition of the complement cascade. This local therapy would significantly diminish the chances of systemic bacterial infections.

Subsequent to the exciting developments regarding complement factor H, other components have been implicated, namely factor B, an activator of the pathway,41 and LOC387715,42,43 a hypothetical gene without a predicted function at this time. The next 5 years for AMD research will be as exciting as the period from 1990 to 2000 and beyond, when new RP genes were discovered on a regular basis.

OTHER INHERITED OCULAR DISEASES

Other relatively common inherited ocular diseases have not been ignored in the quest for an understanding of underlying molecular mechanisms. In addition to RP and AMD, glaucoma, anterior segment dysgenesis (including corneal dystrophies), and high myopia have received considerable attention; genes for glaucoma and corneal dystrophies have been identified. Recent reviews provide an excellent point of study for current summaries of progress on these fronts.44-47 Like AMD, some of these diseases present a significant challenge because they are multifactorial. For example, keratoconus exhibits ge-
nomic heterogeneity with possible environmental influences. Primary open-angle glaucoma maps to at least 15 genetic loci, but only a few genes have been identified, the best of which (characterized in terms of function) is the gene that codes for myocilin. Again, like AMD, risk factors including hypertension and smoking appear to play a role.

WHERE DO WE GO FROM HERE?

A rational approach to the treatment or cure of a genetic disease incorporates knowledge of the responsible gene(s) and an understanding of the contribution of its protein product in its normal and variant form(s) to the biology and physiology of the cell(s) in which it carries out its function. In the context of monogenic diseases, considerable progress has already been made in the preclinical (animal model) and clinical setting; examples of this have already been provided with respect to RPE65-based Leber congenital amaurosis and recessive Stargardt macular dystrophy. With respect to gene therapy, the challenge is more easily met for recessive diseases because the remedy “simply” calls for gene replacement; although this can still represent a problem when the absolute amount of gene product is an issue (e.g., stoichiometry with other enzyme subunits). For dominant diseases, the problems are more challenging owing to the need to reduce or repress expression of the mutant gene product. Investigators have demonstrated that mutant messenger RNA can be reduced through the use of messenger RNA–specific ribozymes, but this field is far behind that of more straightforward gene replacement. There may also be generic methods for the treatment of inherited diseases, including RP and AMD, namely the use of neurotrophic factors, even in cases of dominant disease. A recently completed phase I clinical trial employing encapsulated cells that constitutively secrete ciliary neurotrophic factor intravascularly suggested that the procedure is not only safe but beneficial for patients with RP near the end stage of their disease. There is reason to believe that this approach might work for atrophic AMD as well, and a small clinical trial to that effect is in progress.

It should be emphasized, despite translational efforts currently under way, that we must not stop our quest for new, fundamental information with respect to gene discovery. Perhaps as many as one third of the RP genes await identification, and we do not know the extent to which the culprits have been exposed for AMD. Fundamental research is the mother’s milk of a rational approach to the treatment of human disease, and this should never be forgotten.

WHAT CAN’T WE LEARN FROM GENETICS?

Genetics is a powerful tool; it is essential for our understanding and rational treatment of inherited disease. However, like all other disciplines, it has its limitations. Genetics cannot predict the role of diet and environmental factors in multifactorial disease causes. Fortunately, epidemiology has played a critical role in this regard. It was epidemiologic studies that demonstrated a highly positive association between smoking and increased risk for AMD. Epidemiologists have also taught us that the intake of ω-3 fatty acids reduces the risk for AMD. Moreover, biostatistics is an essential player in any credible epidemiologic or genetic study.

A gene’s identity is not necessarily informative of the function of its product. Even when a gene, by virtue of its primary structure, can be assigned to a family of characterized genes, knowledge of its function is not guaranteed. For example, pigment epithelium–derived factor, a protein currently employed in a clinical trial for exudative AMD because of its antiangiogenic properties, is a member of the serine protease inhibitor protein family. Nonetheless, it has no serine protease inhibitor protein activity. The gene responsible for dominant Stargardt macular dystrophy, belongs to a family of proteins involved in fatty acid elongation. However, to date it has not been possible to demonstrate this function for its protein product. An extreme example is LOC387715, a hypothetical gene whose structure cannot be assigned to a known family at this time. Nonetheless, recent evidence suggests a very high association between smoking and AMD in individuals who carry a variant of this gene. What is the solution to the characterization of genes with unknown function? Multidisciplinary application of the tools of cell biology, gene disruption, and other powerful adjuncts to genetics will ultimately provide the answers. It is the multidisciplinary approach that makes this field of endeavor so interesting. There is no longer a need to prod collaboration among investigators from diverse disciplines. It is now commonplace for scientists with expertise in genetics, biophysics, chemistry, molecular biology, epidemiology, or ophthalmology to work together toward an understanding of the cause of inherited diseases. Combined, these disciplines provide a broader perspective on diagnosis, prognosis, treatment, and prevention. The next decade will be exciting from many perspectives, most importantly from that of the patients who stand to gain the most from new discoveries and treatments.

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