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What can we learn about AMD from other retinal diseases?

Donald J. Zack,[1]Michael Dean,[2]Robert S. Molday,[3]Jeremy Nathans,[1]T. Michael Redmond,[4]Edwin M. Stone,[5]Anand Swaroop,[6]David Valle,[1]Bernhard H. F. Weber[7]

[1]Departments of Ophthalmology, Molecular Biology and Genetics, and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, 21287; [2]Laboratory of Genomic Diversity, NCI-FCRDC, Building 560, Room 21-18, Frederick, MD, 21702; [3]Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC V6T 1Z3; [4]NEI-LRCMB, NIH Building 6, Room 339, 6 Center Drive MSC 2740, Bethesda, MD, 20892; [5]Department of Ophthalmology, University of Iowa, Iowa City, IA; [6]Departments of Ophthalmology and Human Genetics, University of Michigan, Ann Arbor, MI; [7]Institute of Human Genetics, Biocenter Am Hubland, 97074 Wuerzburg, Germany

Over the last 10 years there has been an explosion of new information about the genetics of retinal disease. Over 104 genetic loci have been mapped and more than 45 disease causing genes have been identified (see RetNet). The biology of some of these genes is beginning to be understood and animal models that express some of these mutated human genes have been developed. It is likely that the increasing knowledge derived from these studies will have implications for understanding the genetics and pathogenesis of age related macular degeneration (AMD). Selected aspects of the genetics and biology of photoreceptor degeneration are presented, including the role of ABCR in Stargardt disease and its possible relationship to AMD, the role of rom-1, rds-peripherin, and CRX in various forms of photoreceptor degeneration, and the development and initial characterization of mice carrying targeted deletions of the OAT, TIMP-3, and RPE65 genes. The relevance and limitations of these studies for helping to understand AMD are discussed.

Age-related macula degeneration (AMD) can be thought of as a sub-type of retinal degeneration. Although the primary site of injury in AMD is unknown, it is ultimately the loss of photoreceptor function that leads to visual loss. As described in the accompanying article [1], one approach to gaining a better understanding of the disease process is to directly study the genetics of AMD. A second and complementary approach is to take advantage of the increasing body of knowledge about the genetics and molecular basis of other forms of photoreceptor degeneration (see RetNet). Although the specific genes involved, in many cases, may turn out to be different, knowledge about the genetics and biology of photoreceptor cell death in general is likely to provide insights that will be helpful in understanding the pathogenesis of AMD. This latter approach was the unifying theme of the session entitled “What can we learn about AMD from other retinal diseases?”

As noted in the accompanying article [1], some of the difficulties in studying AMD are its late onset (which limits the availability of large pedigrees), complex genetics, and probable strong environmental component. In such situations, it can be useful to study related clinical diseases that demonstrate earlier clinical presentation and simpler Mendelian inheritance patterns. As one example of the successful use of this approach, analysis of a rare juvenile form of glaucoma led to the identification and cloning of a gene that, at least in some populations, is responsible for 2-4% of the far more common adult onset primary open angle glaucoma [2]. Based on a similar rationale, a number of groups have been studying early onset forms of macular degeneration that show Mendelian inheritance patterns, such as Stargardt disease and Best disease. Several talks in the session were devoted to ABCR/RIM, the gene that is responsible for most, and perhaps all, cases of Stargardt disease.

Jeremy Nathans reviewed the positional cloning of the ABCR/RIM [3]. He also pointed out that ABCR/RIM was independently cloned using a protein purification and sequencing approach [4]. ABCR/RIM encodes a 220 kd retina-specific member of the ABC transporter superfamily, which includes such proteins as CFTR, P-glycoprotein and TAP1 and TAP2. Immunocytochemical studies have demonstrated that it is localized to disc membranes within rod outer segments [5]. This finding of rod-specific expression was somewhat surprising in that, based on the clinical characteristics of Stargardt disease, many investigators suspected that the primary gene product would be expressed in RPE cells and/or perhaps cones. Furthermore, the absence of ABCR/RIM in cones suggests either that cones express a different transporter that functions analogously, or that the continuity between cone outer segment discs and the plasma membrane facilitates transport so that an ABCR/RIM homologue is not required.

A major unresolved question is what is the function of ABCR/RIM; more specifically, what does it transport? If, like rhodopsin, some fraction of the ABCR/RIM protein resides in the rod outer segment plasma membrane, then ABCR/RIM may transport a small molecule or ion into or out of the outer segment. An alternative and intriguing possibility is that ABCR/RIM facilitates the movement of a small molecule within the rod outer segment, specifically between the luminal and cytosolic faces of the disc membrane. Such a function could be analogous to the lipid “flippase” activity seen in the mdr class of ABC transporters. In this regard, it is interesting that a major constituent of human RPE lipofuscin, which is associated with normal aging, has been identified as N-retinyl-

Correspondence to: Donald J. Zack, Johns Hopkins University School of Medicine, 809 Maumenee, 600 North Wolfe Street, Baltimore, MD, 21287; Phone: (410) 502-5230; FAX: (410) 502-5382; email: dzack@bs.jhmi.edu

N-retinylethanolamine. N-retinyl-N-retinylethanolamine has been proposed to form in the disc membrane by reaction of retinal and phosphatidylethanolamine. If it or a related derivative does indeed constitute the RPE lipofuscin that accumulates in Stargardt disease, then this model suggests that it may have formed as a result of defective ABCR-mediated transport of retinoids within the rod outer segment.

In an effort to experimentally identify ABCR/RIM's transport substrate, Dr. Nathans described his lab's ongoing efforts at development of a reconsituted transport assay system. The systen is based on the finding that some ABC transporters show an increase in ATPase activity upon addition of their substrates. Using immunoaffinity purified ABCR/RIM from bovine rod outer segments, various compounds are being tested for their ability to increase, or decrease, baseline ATPase activity. Preliminary studies demonstrate that retinal can significantly stimulate ABCR ATPase activity, but vectoral transport has not yet been demonstrated [6].

Michael Dean discussed the genetics of ABCR/RIM [3]. Classical Stargardt disease, which maps to chromosome lp22, is inherited in an autosomal recessive manner. Over 150 unrelated Stargardt patients have been analyzed and over 80 different ABCR mutations have been identified. Many of the mutations are missense alterations and all compound heterozygotes identified to date contain at least one missense or in frame deletion allele. This suggests that expression of an aberrant ABCR protein is required to produce a Stargardt phenotype. Mutations in ABCR/RIM have also been identified with other retinal phenotypes. For example, patients homozygous for a frame-shift mutation in the N-terminal region of ABCR present with retinitis pigmentosa (RP19) [7]. Additional RP and cone-rod dystrophy families with ABCR mutations have also been described [8,9].

The similarity between STGD and some forms of age-related macular degeneration (AMD), and the presence of AMD in some obligate carriers for ABCR mutations, led to the examination of a potential role of ABCR in AMD [10]. A cohort of 167 AMD patients was screened for alterations in the ABCR gene. A total of 19 alterations affecting the coding potential or processing of the gene were identified. The presence of each alteration was also examined in at least 220 population controls. Three common polymorphisms were found that were nearly equally distributed between patients and controls. However, 12 rare variants were found exclusively or nearly exclusively in AMD subjects. These AMD-associated variants included frame-shift and splice site mutations as well as 6 alleles also found in STGD patients. No single variant is significantly associated with AMD, if a conservative Bonferroni correction is applied. However, tests that combine alleles (i.e. CLUMP) demonstrate that ABCR variants are disproportionally found in our AMD subjects. While 40/167 of the AMD subjects had neo-vascularization, only 1/26 patients with ABCR alterations had "wet" AMD. Based on these results, it was hypothesized that some ABCR alleles can predispose an individual to AMD, particularly the "dry" form. Ongoing studies are analyzing the ABCR gene in other groups of patients with AMD, including patients from the Age-Related Eye Disease Study (AREDS).

It should be noted that the relationship between sequence changes in ABCR/RIM and AMD is controversal [11,12]. Edwin M. Stone presented some of the arguments that suggest that at least some of the reported AMD "mutations" in ABCR/RIM are more likely to be polymorphisms rather than disease causing mutations [12]. He first discussed the differences between "sequence variant," "mutation," and "disease-causing mutation," and the difficulties in functionally distinguishing between these terms. A base change identified in a particular gene, even if it co-segregates with a disease, is not necessarily the cause of that disease. Assigning causality can be complicated by incomplete penetrance, as well as variable environmental effects and modifier genes. As one studies more prevalent diseases (which tend to be the ones with greater public health importance), such as AMD, and genes that are highly polymorphic, such as ABCR, establishing causality becomes even more difficult.

The choice of an appropriate control population can also be very important. The outbred human population is very diverse and there have now been identified a number of examples of subpopulations of individuals that harbor common polymorphisms that are rare in other seemingly similar populations. Subtle differences in control and experimental populations can lead to unappreciated differences in allele frequencies and thereby result in the mistaken identification of a polymorphism as a disease-causing mutation.

With these issues in mind, Dr. Stone and colleagues analyzed their available patient populations for base changes in their ABCR genes. One hundred eighty two individuals with AMD, 215 Stargardt families, and 96 control "normal" patients were studied. The AMD patients were from the University of Iowa retina clinic, and the majority of them had evidence of neovascular disease. The entire ABCR gene was screened for both the disease and control populations.

Thirteen hundred twenty one sequence variants were identified, of which 147 were distinct. Using the Bonferroni correction, none of the identified sequence changes were calculated to be significantly more common in the AMD population than in the control population.

In contrast to the situation with AMD, in confirmation of the prior work indicating that ABCR mutations are responsible for Stargardt disease, a number of sequence changes were found to be present statistically significantly more commonly in the Stargardt population than in the control population. It should also be pointed out that for the Stargardt patients, it was estimated that as many as 50% of the disease causing mutations were not identified.

Following Dr. Stone's presentation, there was lively debate about the various issues raised. The fact that the AMD patients studied by Dr. Dean and colleagues had primarily non-neovascular AMD, whereas those studied by Dr. Stone and colleagues had primarily neovascular AMD, was discussed as one possible partial explanation for the differences in results between the groups. An important point that was clear from the discussion is that additional studies will be needed to resolve the controversies surrounding the role of ABCR mutations in AMD.

Bob Molday briefly commented upon the cloning and characterization of ABCR/RIM and its involvement in Stargardt disease [4,13], but concentrated his presentation of the biochemical interaction of peripherin/rds and rom-1. A large number of mutations in peripherin/rds, a membrane protein required for rod and cone outer segment morphogenesis, have been associated with autosomal dominant RP and a variety of pattern (e.g. butterfly-shaped pigment dystrophy, Bull's Eye and adult vitelliform macular dystrophy, cone-rod pattern dystrophy, and fundus flavimaculatus). In addition, an L185P mutation in peripherin/rds, together with a null mutation in its homologous subunit rom-1, have been identified in a novel digenic form of autosomal dominant RP [14].

In an effort to understand the molecular basis for retinal degenerative diseases linked to mutations in peripherin/rds and rom-1, a heterologous (COS-1) cell system for expressing wild-type and mutant peripherin/rds singly or together with rom-1 was developed [15-18]. In this system mmunofluorescence microscopy and western blotting is used to monitor protein expression. The protein complex is then extracted from the membranes with a mild detergent and subunit assembly is analyzed by immunoprecipitation and velocity sedimentation. Using this experimental approach, it has been shown that heterologously expressed wild-type peripherin/rds and rom-1 co-assemble into a heterotetramer that has similar structural properties to the peripherin/rds-rom-1 complex derived from native photoreceptor membranes. In the absence of rom-1, heterologously expressed peripherin/rds self-assembles into a homotetramer. In contrast, when the C214S peripherin/rds mutant implicated in monogenic autosomal dominant RP is expressed in COS-1 cells, the protein is misfolded and incapable of interacting with rom-1 or with itself. One hypothesis to account for the observed photoreceptor degeneration is that insufficient amounts of normal peripherin/rds tetramers and/or a negative effect of the misfolded C214S mutant inhibits normal outer segment morphogenesis in these patients.

The expression system has also been used to analyze the digenic form of autosomal dominant RP linked to an L185P mutation in peripherin/rds and a frameshift mutation in rom-1 that results in a null allele. The expression studies indicate that defective subunit assembly is responsible for this novel form of autosomal dominant RP. In particular, L185P mutant peripherin/rds forms a native-like complex with rom-1, but it is unable to self-assemble into a homotetramer in the absence of rom-1. On the basis of these studies, a subunit assembly model has been developed that emphasizes the dominant role of peripherin/rds-containing oligomers in outer segment disk formation and provides a molecular rationale for phenotypes of individuals who inherit one or both digenic mutations. One prediction of this model is that in the absence of rom-1, peripherin/rds homotetramers can effectively replace peripherin/rds-rom-1 heterotetramers and promote outer segment formation. This prediction has now been tested and confirmed in ROM-1 knockout mice produced by G. Clark and Rod McInnes (University of Toronto).

Donald J. Zack summarized the cloning and characterization of the Cone Rod Homeobox (CRX) transcription factor and its emerging role in retinal disease [19-21]. CRX is a member of the Otx/Otd subfamily of paired-like homeodomain transcription factors. As its name implies, it is specifically expressed in rod and cone cells. (It is also expressed in pinealocytes, which in some species are light sensitive [22].) Crx was cloned by three different approaches: based on it's ability to bind to the rhodopsin promoter [19], based on its sequence similarity to Chx10 [20], and based on its homology to "typical" homeodomain containing genes [21]. Crx protein binds to the promoters of a number of photoreceptor-specific genes. In a cell culture transient transfection assay, Crx transactivates the expression of a several photoreceptor-specific genes, including rhodopsin, IRBP, b-phosphodiesterse, and arrestin. In addition, it acts synergistically with the neural retina leucine zipper (Nrl) protein [23] in activating rhodopsin expression.

Human chromosomal mapping revealed that CRX is located at 19q13.3, which is at or near the site of a previously mapped cone-rod dystrophy (CORDII). Analysis of the CRX genes from several hundred patients has revealed that approximately 5% of CORD patients carry a mutation in one of their CRX genes. Amongst the mutations that have been identified are Arg41Trp, Arg41Gln, E80A, Val242Met, and a 4 bp deletion in codons 196/7 [20,24,25]. Some of the mutations are in the DNA binding homeodomain, whereas others are in the more C-terminal part of the molecule, possibly in the activation domain.

CRX mutations have also been identified in a number of cases of Leber's Congenital Amaurosis (LCA), a condition in which children have significant loss of photoreceptor function at or near the time of birth [26]. Surprisingly, since most cases of LCA are thought to be recessive, in the cases published to date only a single mutated CRX allele was identified. Furthermore, in each case the mutated allele was not found in either of the parents. This suggests that the identified mutations arose de novo and that the disease phenotype in these cases may be inherited in an autosomal dominant manner. This finding, if confirmed, may have implications for genetic counseling of LCA patients.

To increase the complexity, CRX mutations have also been reported in patients with retinitis pigmentosa [25]. How different, yet sometimes very similar, mutations in CRX can cause different clinical phenotypes is unclear, although it is reminiscent of the clinical diversity observed with mutations in other genes such as rds/peripherin and ABCR. Since CRX appears to regulate the expression of a number of photoreceptor genes, it is possible that different CRX mutations differentially affect the expression of CRX's array of target genes and thereby cause different clinical pictures. Alternatively, or in addition, modifier genes or environmental factors may influence the phenotypes associated with CRX mutations. Efforts to resolve these issues utilizing in vitro assays of the function of the various mutant CRX alleles are underway.

At least one case of congenital blindness caused by autosomal recessive CRX mutations has also been identified. Anand Swaroop reported on a family with LCA in which the affected individual is homozygous for a R90W CRX mutation [27]. The parents of the LCA patient were heterozygous for the R90W mutation and, although clinically asymptomatic, revealed a subtle abnormality of cone function upon ophthalmic examination and electroretinography. In addition, the

CRX gene was analyzed in 30 patients with AMD, but no mutations were identified. In a related set of studies, which were not reported at the meeting, autosomal dominant retinitis pigmentosa phenotype appears to be associated with a mutation in the NRL gene [28]. This is particularly interesting from a mechanistic point of view because, as noted above, NRL can act synergistically with CRX in regulating photoreceptor gene expression.

The CRX studies demonstrate that mutations in transcription factors can, at least in some cases, cause photoreceptor cell loss with a clinical phenotype that predominantly involves the macular region. This raises the possibility that sequence variations in CRX and/or other photoreceptor transcription factors could be risk factors for some forms of AMD.

The diseases discussed so far are caused by genes that are expressed preferentially, or exclusively, in photoreceptor cells. However, genes that are expressed preferentially in RPE cells, as well as ubiquitously expressed genes (see gyrate atrophy below), can also cause specific photoreceptor cell loss. T. Michael Redmond summarized the cloning [29,30] and current knowledge about one such gene, RPE65. RPE65, a protein preferentially and abundantly expressed in the RPE is essential to the maintenance of normal vision. Mutations in the human RPE65 gene result in rapidly progressive forms of early-onset retinal dystrophy called autosomal recessive childhood-onset severe retinal dystrophy (arCSRD), ranging in age of onset from more severe forms classified as Leber's congenital amaurosis (LCAII; OMIM) to later onset juvenile retinitis pigmentosa [31,32]. RPE65 has been localized to human chromosome 1p31 and its mouse homolog (Rpe65) to distal mouse chromosome 3 [33]. By linkage analysis, certain arCSRDs were linked to the interval occupied by RPE65 and were shown to be associated with mutations in RPE65. In addition, candidate gene analysis of RPE65 identified different mutations in patients with Leber's congenital amaurosis, providing a second locus for this heterogeneous disorder. Common features of these patients include loss of vision from birth or early childhood, associated with complete night-blindness, extinguished rod electroretinography, and severely compromised cone responses. These symptoms are suggestive of a crucial role for RPE65 in retinal function.

Though its function is not certain, a role in vitamin A metabolism is strongly suspected. Evidence for this is provided by its association with the retinol-binding protein (RBP) and 11-cis-retinol dehydrogenase. RPE65, also known as p63, has been proposed to be the RPE RBP receptor, though this protein does not enhance RBP uptake in transfected cells. Association with the 11-cis-retinol dehydrogenase suggests that it is part of the visual cycle pathway, the process by which the 11-cis-retinal chromophore of visual pigments is photo-isomerized to the all-trans-isomer, and then regenerated in the dark. 11-cis-retinal, the chromophore of all known opsins, vertebrate and invertebrate, is restricted to the eye. In the vertebrate eye, the major site of production of 11-cis-retinoids is the RPE. Though the phenotype of the human RPE65 null mutation is compatible with severe disruption of the visual cycle, resulting in absence of 11-cis-retinal and, ultimately, blindness, a clearer understanding of the biochemical basis for this phenotype is required.

In view of the potentially important role of RPE65 in the physiology of vision and, perhaps, the visual cycle, a targeted disruption of the mouse Rpe65 gene was generated in order to more directly assess its function [34]. No effects of the knockout on viability were noted. In -/- mice, RPE65 mRNA was not detectable by RT-PCR. No major changes in RPE morphology were noted in 7-week old +/- or -/- animals, nor was there loss of photoreceptor nuclei. However, subtle changes were seen by LM in photoreceptor outer segments (OS) of the -/-, manifesting as lighter staining of OS. By EM, the discs of the -/- were disorganized and also not as tightly packed as in the +/+ and +/-. In contrast, the ERGs of -/- mice were severely abnormal compared to +/- and +/+ littermates. The b-wave response elicited from -/- animals was strongly depressed. The threshold stimulus intensity required to elicit this response was much higher than for +/+ or +/-. Furthermore, rhodopsin difference spectrum analysis of +/+, +/-, and -/- retinas revealed that -/- retinae had no rhodopsin. Western blot analysis showed that opsin apoprotein was present in -/- retinas, though levels were decreased compared to +/+ and +/-. The absence of rhodopsin holoprotein, even though the apoprotein is present, indicates severe disruption of 11-cis-retinal supply to the photoreceptors. This knockout model will provide important insights into retinoid metabolism in the outer retina, as well as pigment synthesis and regeneration. It will also provide a useful model for LCAII and related arCSRDs and to study possible therapies for these conditions.

In conclusion, RPE65 null mutations in both human and mouse show severe changes in visual function. These mutations appear to cause disruption of the vitamin A visual cycle of the RPE, resulting in interruption of the supply of the 11-cis-retinal chromophore. The precise nature of this disruption is under investigation. Though the human and mouse mutations described to date relate to early onset blindness, it is possible that mutations with less severe effects on RPE65 expression/function exist and manifest themselves in late onset disease. In light of this, further analysis of this gene with respect to later onset diseases such as AMD is warranted.

Other retinal diseases caused by genes that are preferentially expressed in the RPE have also been identified. Mutations in the gene for cellular retinaldehyde-binding protein (CRALBP), which is expressed predominantly in RPE and Mueller cells, have been identified in patients with in autosomal recessive retinitis pigmentosa [35]. Recently, the gene that causes Best disease, Bestrophin, was identified and found to be expressed predominantly in the RPE [36]. Although so far there is no hint about the function of Bestrophin, given that Best disease is a macular degeneration and has some similarities to AMD, it seems reasonable to hope that studies on the function of Bestrophin will provide help in understanding AMD.

In addition to genes that are preferentially expressed in photoreceptor and/or RPE cells, widely and even ubiquitously expressed genes can also cause specific retinal degeneration. David Valle discussed how general metabolic diseases can preferentially affect individual tissues or cell types. Gyrate atrophy of the retina, an autosomal recessive disease, is caused by deficiency of the widely expressed enzyme ornithine-delta-aminotransferase (OAT) [37]. Patients demonstrate systemic

elevation of serum ornithine levels, yet the major phenotype is a localized chorioretinal degeneration. Missense, nonsense, and frameshift mutations in the OAT gene have been identified [38,39]. Promoter mutations have also been suspected, but not definitively identified.

In order to develop an animal model for gyrate atrophy, an OAT-deficient mouse was generated by gene targeting. [40,41]. The mice demonstrate many similarities to the human disease. Anatomically, there is a slowly progressive retinal degeneration that involves first the RPE (large phagosomes and crystalloid inclusions), and then the photoreceptors (disorganized and short rod outer segments followed by actual photoreceptor loss). During this period there is also progressive loss of electroretinogram (ERG) amplitude. Once difference between the mouse model and the human disease is that in the mice the retinal degeneration is primarily central, whereas in humans it is more peripheral.

The OAT-deficient mouse is also a valuable resource for testing potential therapies for gyrate atrophy. Based on the biochemistry of the disease and limited clinical trials, it has long been suspected that long term reduction of ornithine with an arginine-restricted diet slows down progression of the localized chorioretinal degeneration. However, partially due to substantial difficulty in maintaining an arginine-restricted diet, this hypothesis has been difficult to prove. Through use of the mouse model, it is now clear that arginine restriction does indeed slow down disease progression. Ongoing studies are now exploring the possible combined use of dietary restriction and gene therapy approaches.

Bernhard H. F. Weber summarized recent studies on the pathogenesis of Sorsby's fundus dystrophy (SFD). SFD is an autosomal dominantly inherited macular disorder with late onset usually in the third or fourth decade of life. Abnormal deposits of confluent lipid containing material in the inner portion of Bruch's membrane have been recognized as an early manifestation of the disease [42]. Later in life, sudden loss of central vision may occur either due to choroidal neovascularization with subretinal hemorrhage and the development of a disciform scar or, in the minority of patients, due to atrophic changes.

Choroidal neovascularization is also a hallmark of the exudative form of age-related macular degeneration (AMD). Similar to SFD, it is characterized by a submacular ingrowth of choroidal vessels through a damaged Bruch's membrane. Therefore, a greater understanding of the primary causes of the complex AMD may be gained by elucidating the molecular pathology of SFD.

Following the rationale of the positional candidate approach, mutations in the gene encoding the tissue inhibitor of metalloproteinases-3 (TIMP3) were shown to be associated with the SFD phenotype [43]. TIMP3 represents one member of a growing family of distinct inhibitors of matrix metalloproteinases (MMPs). Together or in concert with other enzymes the MMPs can degrade most components of the extracellular matrix (ECM). Several studies have shown that TIMP3 indeed is an extracellular matrix component of Bruch's membrane thus suggesting a direct role of mutant TIMP3 in Bruch's membrane associated pathology such as the formation of extracellular deposits as well as the ingrowth of choroidal vessels [44,45].

To date, all mutations identified in SFD result in a substitution of an amino acid by cysteine at the C-terminus of the 188 amino acid TIMP3 glycoprotein. The tertiary structure of the normal protein is thought to be conferred by 12 conserved cystein residues which form intramolecular disulfide bonds along the entire polypeptide. The introduction of an additional cysteine thiol-group should be expected to result in a TIMP3 protein with altered or impaired functional properties. To further understand the molecular mechanisms underlying the specific mutations in SFD, a knock-in Ser156Cys as well as a Ser156Met knock-in mutation were generated via a gene targeting approach. The Ser to Cys alteration at amino acid residue 156 was found to be associated with an unusual early onset of SFD [46,47]. In contrast, the Ser156Met mutation is not known to cause a phenotype in human but may be crucial in gaining additional insight into the basic mechanism underlying the cysteine mutations. The mutated ES cells were injected into C57Bl/6 blastocysts and chimeric mice were obtained from all injected ES cells. So far, germ line transmission of the Ser156Cys mutation has been achieved. Preliminary analysis of the retinas of two month old mice reveal overall normal morphology with a suggestion of thickening of Bruch's membrane and possible RPE mottling. No evidence of neovascularization has been observed. Ongoing studies will more fully define the phenotype of these mutant mice.

Over the next few years, one can be confident that an increasing number of genes that when mutated can cause photoreceptor loss will be identified. One can also be confident that the combined use of biochemical, cell biological, and transgenic and knock-out studies will provide important information about the functions of these genes, and about the mechanisms by which alteration of the function can lead to retinal disease. Hopefully, this new information will also provide insights about the etiology and pathogenesis of AMD. However, along with this hope, one must also keep in mind some of the questions raised by these studies of "other retinal degenerations," and the limitations of some of the methods that have been used to study them. Among these questions are: How can such similar phenotypes be caused by mutations in such different genes? And how can mutations in the same gene cause such different ocular phenotypes? What is the role of modifier genes and the environment? Why is it so difficult for retinal disease to define reliable genotype-phenotype correlations? Since the mouse does not contain a macula, will it be possible to develop a mouse transgenic or knock-out (knock-in) model of AMD?

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