State College, PA) or through direct sequencing using CEQ2000XL DNA analysis system (Beckman Coulter, Fullerton, CA).

Hardy Weinberg equilibrium (HWE) χ^2 values in the entire sample and controls only were calculated to identify possible genotyping errors. No extreme deviations ($\chi^2>50$) were observed (Table 1). Linkage disequilibrium (LD) was measured by the D' value. For each SNP, Pearson's χ^2 tests with one degree of freedom for association were performed. Odds ratios (OR), population attributable risks (PAR), and their respective confidence intervals were calculated, formula in [8].

Previous functional data lead us to focus further analyses on rs11200638 [6,7]. Joint ORs for two SNPs (rs11200638 and CFH 402, previously genotyped) were calculated using standard methods [9]. Marginal ORs and their confidence intervals for the two SNP were calculated using logistic regression with SNP CFH 402 and rs11200638 as independent variables [9]. PARs were calculated using standard methods [9]. Confidence intervals around the PARs were constructed using 999 bootstrap replicates. To control for confounding, the Mantel-Hanzel test for association with two variables was used [9]. Four genotypic models were considered (Full, Recessive, Multiplicative, and Dominant) and the Aikake information criterion (AIC) was utilized to assess the fit of each model. All R scripts used in the analysis are available upon request.

RESULTS

SNP rs11200638, approximately 6.1 kb downstream of the surrogate SNP rs10490924, resides in the promoter of the *HTRA1* serine protease gene (512 base pairs upstream of transcriptional start site). These two SNPs were in almost complete linkage disequilibrium (LD) and showed strong association with AMD in the Hong Kong study [6] and in a Caucasian population from Utah [7].

In our cohort, the two SNPs were also in complete LD, from which only two major (frequency >5%) haplotypes, one predominant in cases and one in controls, were observed. Disease association tests yielded p-values of 4.74×10^{-11} and 1.79×10^{-12} for rs10490924 and rs11200638, respectively (Table 1). Given the previous evidence of higher conservation across species [6] and the functional consequence of rs11200638 on *HTRA1* expression [6,7], additional analyses focused on this SNP.

Reanalyzing the original CFH genotype data, we found the OR covered unity (Table 2) and all interval estimates of PAR for CFH 402 variants under the four genotypic models included zero (Table 3). Of the four models, the best fit to the *HTRA1* SNP genotypic effects, as assessed by Akaike's information criterion, was the recessive model, from which the risk genotype was AA and non-risk was GG and GA (Table 3). Under the framework of recessive rs11200638 and the two observed genotypes for CFH 402, no interaction was detected between the two SNPs based on the likelihood ratio test (Table 3). Odds ratios for different genotypes of rs11200638 do not vary a great deal depending on the CFH 402 genotypes, and vice versa (Table 2). In fact, the OR curves shown in Figure 1 indicate a "removable" interaction between the two SNPs, in which the original two OR curves become parallel (i.e. no

TABLE 1. ASSOCIATION OF CHROMOSOME 10Q26 SINGLE NUCLEOTIDE POLYMORPHISMS WITH AGE-RELATED MACULAR DEGENERATION

Attribute	rs10490924 (G/T)	rs11200638 (G/A)
HWE χ^2-combined	5.4	7.6
-controls only	0.98	0.88
Risk allele	T	A
Frequency in case	0.68	0.69
Frequency in control	0.33	0.32
Allelic association χ^2 nominal p-value	4.74E-11	1.79E-12

To examine genotyping errors, Hardy Weinberg Equilibrium (HWE) χ^2 values are computed with cases and controls combined and controls alone. The age range is 51 to 90 years old with mean 74.8 and standard deviation (s.d.) 8.81 in cases, and 50 to 88 years old with mean 71.1 and s.d. 9.08 in controls.

TABLE 2. ODDS RATIOS FOR THE JOINT AND MARGINAL EFFECTS OF SINGLE NUCLEOTIDE POLYMORPHISMS COMPLEMENT FACTOR H 402 AND RS11200638 ON AGE-RELATED MACULAR DEGENERATION

	rs11200638		
CFH 402	GG/GA	AA	CFH 402 risk (adjusted for rs11200638)
TT	1	7.92	1
CT	1.11	30.52	1.41 (95% CI: 0.54,3.74)
rs11200638 risk adjusted for CFH	1	10.02; 95% CI: 4.36,23.06	

CFH indicates complement factor H. Joint odds ratios were calculated from standard formulae. Marginal odds ratios and 95% confidence intervals were calculated using logistic regression (see Methods) with each SNP was adjusted for the other.

interaction after transformation with a logarithmic function). Overall, after adjusting for the CFH 402 SNP, individuals carrying the risk homozygote AA of rs11200638 are greater than 10 times more likely to have AMD than those with the other genotypes (Table 2).

DISCUSSION

These data reconfirm the association of the *HTRA1* promoter SNP rs11200638, independent of the CFH 402 polymorphism, with wet AMD. The present study genotyped two previously identified disease associated SNPs in the chromosome 10q26 region. Both SNPs showed similar significance levels. The first SNP, rs10490924, resides in the hypothetical locus, LOC387715. Several studies have found significant association between AMD and this SNP [10-12]. So far only one transcript from this hypothetical locus has been identified in one experiment. No study has identified the transcript or protein in the retina, much less identified a change in the protein as a result of the SNP. Additionally, sequence comparisons of seventeen species presented in DeWan et al. show higher sequence

TABLE 3. TWO-WAY ANALYSES OF COMPLEMENT FACTOR H 402 AND RS11200638

					M-H test: p-value	
	PAR%		(95% CI)		LRT	AIC
Model for rs11200638	CFH 402	rs11200638	CFH 402	rs11200638	p-value	value
Full	3.4 (0, 9.7)	58.3 (50.5, 64.1)	0.07	8.30E-08	0.03	221.8
Recessive	4.6 (0, 10.7)	44 (40.5, 54.0)	0.23	6.20E-09	0.12	221.5
Multiplicative	1.7 (0, 7.8)	79.8 (73.0, 88.1)	*	*	0.02	225.7
Dominant	2.2 (0, 13.7)	58.6 (43.9, 78.9)	0.91	5.80E-04	0.1	246.9

Four genotypic models for rs11200638 are considered: Let r0, r1, and r2 be the marginal relative risks for genotypes GG, GA, and AA. Then, recessive model implies r0=r1; multiplicative model implies r1=r0r2; dominant model implies r2=r1; full model does not have any restriction on relative risks except that r0, r1, r2>0. The 95% confidence intervals (CI) of population attributable risk (PAR) were obtained via a bootstrap re-sampling method with 999 replicates. Mantel-Hanzel (M-H) tests are conducted for one SNP association adjusted for the other SNP; likelihood ratio tests (LRT) for joint single nucleotide polymorphism (SNP) association under a two-way multiplicative model: the relative risk (or OR) for any genotype pair (A, B) relative to the baseline pair (A0, B0) is the product of relative risk (or OR) of A relative to A0 and that of B relative to B0. AIC denotes the Akaike's information criterion to access goodness-of-fit for the rs11200638 model.

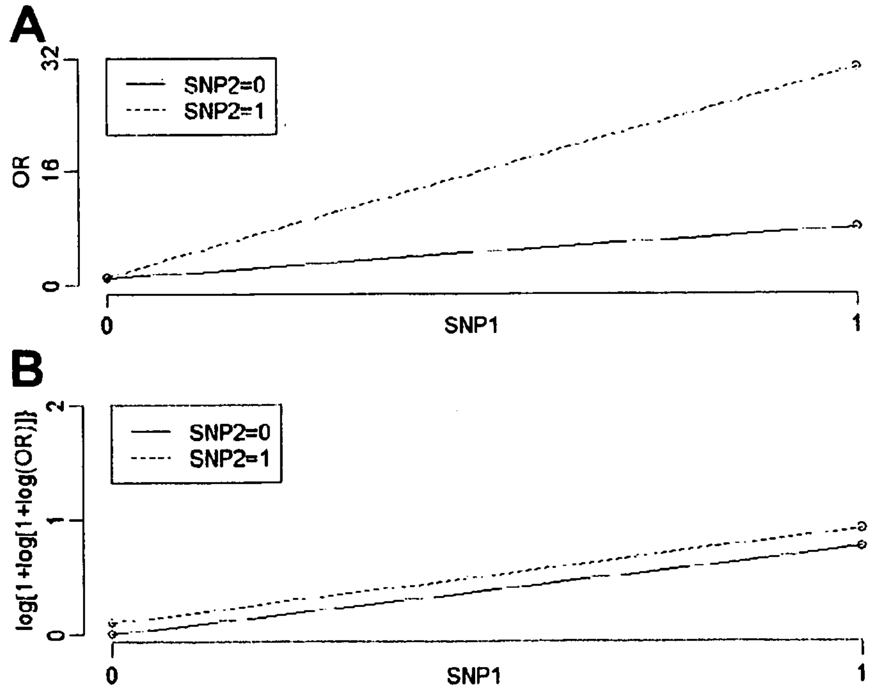


Figure 1. Odds ratio plots for two single nucleotide polymorphisms. Joint odds ratio plots for the single nucleotide polymorphisms (SNPs), complement factor H (CFH) 402, and rs11200638 before and after log transformation showing that the apparent interaction is a "removable" effect. SNP1=CFH 402: 0 is for TT and 1 is for CT; SNP2=rs11200638: 0 is for GG/GA and 1 is for AA. **A**: Original odds ratio (OR) curves: Height difference on the left is 1.11-1=0.11; height difference on the right is 30.52-7.92=22.60; slope for SNP2=0 is 7.92-1=6.92; slope for SNP2=1 is 30.52-1.11=29.41. **B**: Log(1+log(1+log)) transformation of the original OR.

conservation surrounding rs11200638 compared to that around rs10490924 [6]. *HTRA1* is expressed in the retina in humans [5] and mouse [6]. Computational analysis of the *HTRA1* promoter indicate that this SNP resides in a CpG island and may result in a change in the binding site for transcription factors AP2 and SRF [6]. Preliminary functional data suggest that individuals homozygous for the risk-allele at rs11200638 exhibit increased expression of *HTRA1* [6,7]. Therefore, given the existing functional data, it appears as if the *HTRA1* promoter polymorphism, rs11200638, is likely the underlying functional polymorphism in the 10q26 region. However, the mechanism to neovascularization is yet to be understood and will require intense investigation to uncover its link to the wet form of AMD.

ACKNOWLEDGEMENTS

This work was supported in part by grants from the National Institutes of Health and by awards from the Macular Vision Research Foundation and the Ellison Medical Foundation.

REFERENCES

1. Haddad S, Chen CA, Santangelo SL, Seddon JM. The genetics of age-related macular degeneration: a review of progress to date. Surv Ophthalmol 2006; 51:316-63.
2. Thakkinstian A, Han P, McEvoy M, Smith W, Hoh J, Magnusson K, Zhang K, Attia J. Systematic review and meta-analysis of the association between complement factor H Y402H polymorphisms and age-related macular degeneration. Hum Mol Genet 2006; 15:2784-90.
3. Okamoto H, Umeda S, Obazawa M, Minami M, Noda T, Mizota A, Honda M, Tanaka M, Koyama R, Takagi I, Sakamoto Y, Saito Y, Miyake Y, Iwata T. Complement factor H polymorphisms in Japanese population with age-related macular degeneration. Mol Vis 2006; 12:156-8.
4. Gotoh N, Yamada R, Hiratani H, Renault V, Kuroiwa S, Monet M, Toyoda S, Chida S, Mandai M, Otani A, Yoshimura N, Matsuda F. No association between complement factor H gene polymorphism and exudative age-related macular degeneration in Japanese. Hum Genet 2006; 120:139-43.
5. Tocharus J, Tsuchiya A, Kajikawa M, Ueta Y, Oka C, Kawaichi M. Developmentally regulated expression of mouse HtrA3 and its role as an inhibitor of TGF-beta signaling. Dev Growth Differ 2004; 46:257-74.
6. Dewan A, Liu M, Hartman S, Zhang SS, Liu DT, Zhao C, Tam PO, Chan WM, Lam DS, Snyder M, Barnstable C, Pang CP, Hoh J. HTRA1 promoter polymorphism in wet age-related macular degeneration. Science 2006; 314:989-92.
7. Yang Z, Camp NJ, Sun H, Tong Z, Gibbs D, Cameron DJ, Chen H, Zhao Y, Pearson E, Li X, Chien J, Dewan A, Harmon J, Bernstein PS, Shridhar V, Zabriskie NA, Hoh J, Howes K, Zhang K. A variant of the HTRA1 gene increases susceptibility to age-related macular degeneration. Science 2006; 314:992-3.
8. Armitage P. Statistical methods in medical research. New York: Wiley; 1971.
9. Scheffe, H. The Analysis of Variance. Wiley & Sons, New York, 1964.
10. Schmidt S, Hauser MA, Scott WK, Postel EA, Agarwal A, Gallins P, Wong F, Chen YS, Spencer K, Schnetz-Boutaud N, Haines JL, Pericak-Vance MA. Cigarette smoking strongly modifies the association of LOC387715 and age-related macular degeneration. Am J Hum Genet 2006; 78:852-64.
11. Rivera A, Fisher SA, Fritsche LG, Keilhauer CN, Lichtner P, Meitinger T, Weber BH. Hypothetical LOC387715 is a second major susceptibility gene for age-related macular degeneration, contributing independently of complement factor H to disease risk. Hum Mol Genet 2005; 14:3227-36.
12. Jakobsdottir J, Conley YP, Weeks DE, Mah TS, Ferrell RE, Gorin MB. Susceptibility genes for age-related maculopathy on chromosome 10q26. Am J Hum Genet 2005; 77:389-407.

The print version of this article was created on 4 Apr 2007. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.

31 Animal Models for Eye Diseases and Therapeutics

Takeshi Iwata and Stanislav Tomarev

ABSTRACT

It is believed that more than 80% of the information our brain receives comes from the visual system. Dysfunction of the visual system can significantly lower the quality of life. The most prevalent causes of visual impairment are cataracts, glaucoma, and age-related macular degeneration (AMD), which is responsible for 69% of blindness globally. In spite of the high incidence of AMD and glaucoma, a limited amount of information is available on the underlying pathological mechanisms causing these diseases. Because experimental studies of AMD and glaucoma are limited in humans, the availability of animal models is very valuable to investigate molecular mechanisms and to test new therapeutic interventions. Appropriate animal models, such as monkey, mouse, rat, and zebrafish, facilitate the identification of new genes involved in the pathology and elucidate the genetic relationships between causative and modifier genes. In this chapter the advantages and difficulties of using animal models for vision research will be discussed. Several animal models including a primate model with defined macula for AMD research and genetically modified mice models for glaucoma research will be introduced.

Key Words: Vision, Age-related macular degeneration, Retina, Macula, Drusen, Glaucoma, Retinal ganglion cells, Optic nerve.

VISUAL IMPAIRMENT AND IMPORTANCE OF ANIMAL MODELS FOR EYE DISEASES

It is believed that more than 80% of the information our brain receives comes from the visual system. Dysfunction of the visual system can alter the normal human life style and significantly lower the quality of life. The causes of visual impairments and blindness vary among ethnic groups and the global regions where they live. There are many causes of visual impairments including diabetic complications, infections, and trauma; however the most prevalent causes of visual impairment are cataracts, glaucoma, and age-related macular degeneration (AMD). According to the World Health Organization, there were more than 161 million visually impaired individuals in 2002; 124 million of this group had low vision and 37 million were blind (http://www.who.int/mediacentre/factsheets/fs282/en/index.html) (Figure 31–1).

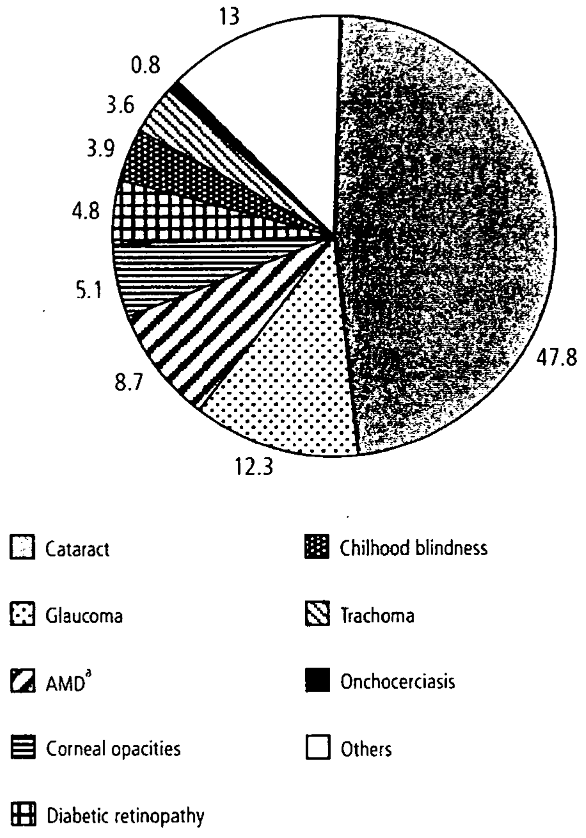
Cataract, glaucoma, and AMD are responsible for 69% of blindness globally. Although cataracts are the leading cause of blindness worldwide, recent advances in cataract surgery have significantly reduced the visual impairments caused by cataracts, especially in developed countries. Glaucoma, an optic neuropathy, is often associated with elevated intraocular pressure and is responsible for blindness in 6.7 million people across the world. Glaucoma is more common in individuals of African ancestry, and the incidence of glaucoma increases with age.

The most prevalent eye disease for elderly Europeans and Americans is AMD. This degenerative disease progresses from retinal deposits called drusen to neovascularization and retinal hemorrhages resulting in irreversible loss of central vision.

In spite of the high incidence of AMD and glaucoma, a limited amount of information is available on the underlying pathological mechanisms causing these diseases. Obtaining tissues for any disease is often difficult, and even when obtained, they may not be informative because the tissues are usually collected many hours or even days after death. Because experimental studies of AMD and glaucoma are limited in humans, the availability of animal models is very valuable because they can be used to investigate the molecular mechanisms causing these diseases and to test new therapeutic interventions. Animal models, compared to other experimental methods, e.g., cell and organ cultures or postmortem models, allow the study of different pathological factors and therapeutic treatments under *in vivo* conditions, i.e., with the visual and other systems of the body intact. Appropriate animal models, e.g., monkey, mouse, and zebrafish, also facilitate the identification of new genes involved in the pathology as well as elucidate the genetic relationships between causative and modifier genes. Equally important, specific genes can be altered in these models. Thus, it is possible to induce mutations in animals, and then search for specific phenotypes, e.g., abnormal intraocular pressure (IOP) and retinal ganglion cell degeneration. Subsequently, the affected genes can be identified by standard genetic procedures.

Many animal models of AMD and glaucoma have been developed in different mammalian and nonmammalian species. None of these models is a perfect reproduction of the human disease, and when choosing the animal model for research, the investigator should evaluate the following: (1) the similarity of the visual system of the model to that of humans, especially the eye; (2) the similarity of the time course of pathological changes in the model and in human eyes; (3) the ability to perform genetic manipulations; (4) the training required to produce affected animals; (5) the size of the eye; (6) the availability and difficulties in the

From: *Source Book of Models for Biomedical Research*
(P. M. Conn, ed.). © 2007 Humana Press Inc., Totowa, NJ.



[a]AMD = Age-related macular degeneration.

WHO 04.138

Figure 31–1. Global causes of blindness as a proportion of total blindness in the year 2002 (WHO). AMD, age-related macular degeneration.

methods of analysis; (7) the availability of animals; and (8) the cost.

AGE-RELATED MACULAR DEGENERATION

INTRODUCTION The retina is composed of nine layers of neural and glial cells that are arranged concentrically at the posterior pole of the eye. Incoming light is focused on the central area of the retina called the fovea, which is located in the center of the macular area (Figure 31–2). In humans, the size of the macula is approximately 6mm in diameter (Figure 31–3). The outer (posterior) surface of the retina is covered by a monolayer of retinal pigment epithelial (RPE) cells that forms a diffusion barrier between the neural retina and the choroidal blood supply. The RPE regulates the transport of proteins to the retina and controls the hydration and ionic composition of the subretinal space. The physiological condition of the RPE is closely associated with the pathogenesis of AMD.

AMD is a blinding disorder characterized by a marked decrease in central vision associated with RPE atrophy with or without choroidal neovascularization (CNV). Many factors, including genetic, behavioral, and environmental, are involved in this disease. AMD is characterized by the degeneration of cone photoreceptors in the foveal region of the retina resulting in a decrease of central visual acuity. The progressive impairment of the RPE cells and damage to Bruch's membrane and choriocapillaris results in retinal atrophy and photoreceptor dysfunction. In some cases, CNV develops, and the new vessels penetrate Bruch's membrane and pass into the subretinal space.

Two types of AMD are recognized: the nonneovascular type is called the dry-type AMD and includes more than 80% of the cases; the neovascular type is called the wet-type AMD and it is progressive with a higher probability of blindness. The prevalence of AMD differs considerably among the different ethnic groups, but the incidence increases with age in all groups. A lower prevalence of AMD has been reported in individuals of African ancestry than of Anglo-Saxon ancestry. Other risk factors for AMD are cigarette smoking, obesity, hypertension, and atherosclerosis.

EPIDEMIOLOGY AND GENETICS Extensive epidemiological studies have shown a genetic component for AMD. Thus, twin studies have shown a higher concordance for AMD in monozygotic twins than in dizygotic twins.[1–3] In addition, first-degree relatives of individuals with AMD have a 2- to 4-fold higher incidence of AMD than individuals without a family history of AMD. Genetic segregation studies have also shown a genetic effect that accounts for approximately 60% of AMD with a single major gene accounting for about 55% of the risk of developing

AMD. Overall, the data have suggested that the etiology of AMD has a significant genetic component. Only a small proportion of the families with AMD shows Mendelian inheritance, and the majority of the individuals inherit AMD in a complex multigene pattern.

There have been a number of attempts to identify the genes that cause AMD. With the help of the haplotype marker project (HapMap Project), genome-wide scanning has identified at least 13 loci linked to AMD on different chromosomes.[4–6]

Recently, a polymorphism of complement factor H gene (*Y402H*) was shown to be associated with an increased risk for AMD.[7–10] These results were confirmed in many of the countries with large white populations but not in Japan.[11,12] This gene is located on chromosome 1q25-31 where one of the candidate loci was identified by linkage studies. Another recent study reported that a haplotype association of tandemly located complement 2 and factor B was protective for AMD.[13]

PATHOLOGY AND BIOCHEMISTRY The early stage of the dry-type AMD is characterized by a thickening of Bruch's membrane, aggregation of pigment granules, and increasing numbers of drusen. The thickening of Bruch's membrane obstructs its function as a "barrier" between the choroid and the RPE that protects the neural retina from the choriocapillary. Drusen are small yellowish-white deposits that are composed of lipids, proteins, glycoproteins, and glycosaminoglycans. They accumulate in the extracellular space and the inner aspects of Bruch's membrane (Figure 31–3). Drusen are not directly associated with visual loss but represent a risk factor for both the dry-type and wet-type AMD. The classification of hard and soft drusen is based on their size, shape, and color; hard drusen are yellowish with diameters <50μm and are found in eyes that are less likely to progress to advanced stages of the disease, while soft drusen are darker yellow and larger in size, and are found in eyes more likely to progress to more advanced stages of AMD. A small percentage of dry-type AMD patients progress to the late stage of the wet-type AMD that is characterized by geographic atrophy or detachment of RPE and the development of CNV in the macular region. The presence of a CNV is the factor that most damages the neural retina because the newly developed vessels grow from the choriocapillaris through Bruch's membrane and extend laterally through the RPE cell layer (classic CNV) or extend between the inner Bruch's membrane and RPE (occult CNV). In advanced stages of AMD, the CNV and fluid leaked into the subretinal or intraretinal regions lead to cell death and retinal detachment.

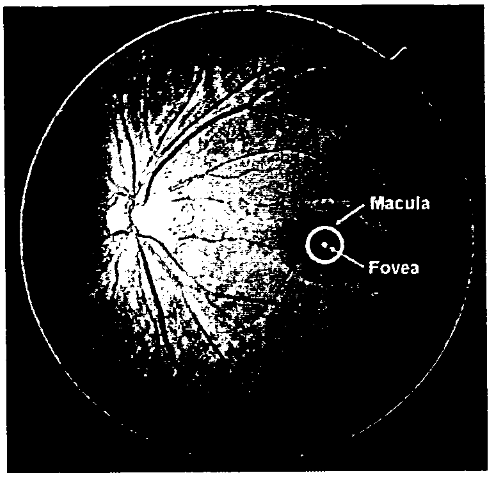


Figure 31–3. Fundus photograph of a normal human retina showing the location of the macula and the fovea.

Recent analyses of the progression of drusen have provided important clues that help understand the molecular pathology of AMD. Using both immunohistochemistry and proteomic techniques, the materials in drusen were found to be composed of molecules that mediate inflammatory and immune processes.[14,15] These molecules include components of the complement pathway and modulators of complement activation, viz. vitronectin, clusterin, membrane cofactor protein, and complement receptor-1. In addition, molecules triggering inflammation, viz. amyloid P component, α_1-antitrypsin, and apolipoprotein E, were identified in drusen. Cellular debris from macrophages, RPE cells, and choroidal dendritic cells has also been identified in drusen. On the other hand, crystallins, EEFMP1, and amyloid-β have been found at

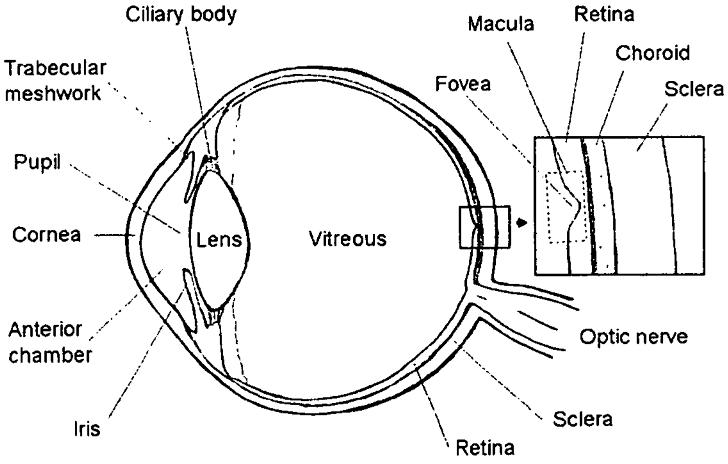


Figure 31–2. Schematic diagram of the human eye.

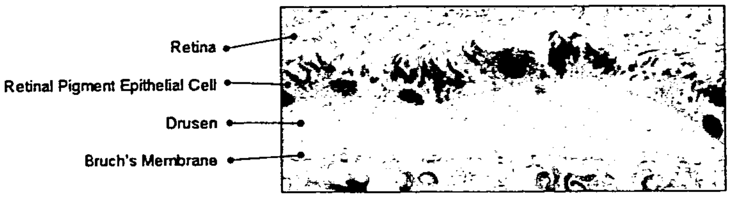


Figure 31–4. Retinal histological section showing the accumulation of drusen between the retinal pigment epithelium and Bruch's membrane in a primate model of AMD.

higher levels in drusen from individuals unaffected by AMD. The presence of immunoreactive proteins and the oxidative modifications of many proteins in drusen imply that both oxidation and immune functions are involved in the pathogenesis of AMD.

All of these findings suggest that complement activation triggers innate immune responses in the subretinal space. The codistribution of immunoglobulin G (IgG) and terminal complement complexes in drusen indicate that immune responses that directly target antigens in retinal cells might also be occurring. Antiretinal autoantibodies have been reported in a number of ocular disorders, e.g., macular degeneration in an aged monkey model.

ANIMAL MODELS Access to appropriate biological materials from affected donors at different stages of a disease is an absolute necessity for the study of mechanisms underlying the disease process. However, because it is nearly impossible to obtain retinal tissues from patients or controls, the development of animal models becomes crucial for investigating the biological pathways involved in the progression of the disease and for the development of therapeutic strategies.

Over the past few years, genetic engineering techniques have generated a number of animal models of AMD in mice, rats, rabbits, pigs, and dogs.[16] However, in mammals, a well-defined fovea is found only in primates (humans and monkeys), and a search for a monkey line affected with macular degeneration has been persistent for a long time.

A monkey with macular degeneration was first described by Stafford *et al.* in 1974. They reported that 6.6% of the elderly monkeys they examined showed pigmentary disorders and drusen-like spots.[17] El-Mofty *et al.* reported that the incidence of maculopathy was 50% in a colony of rhesus monkeys at the Caribbean Primate Research Center of the University of Puerto Rico.[18] At the Tsukuba Primate Research Center (Tsukuba City, Japan), Suzuki *et al.* found a single cynomolgus monkey (*Macaca fascicularis*) in 1986 with a large number of small drusen around the macular region (Figure 31–4).[19–21] This single affected monkey has multiplied to a large pedigree of more than 65 affected and 210 unaffected monkeys (Figure 31–5). Drusen were observed in the macular region as early as 1 year after birth, and the numbers increased and spread toward the peripheral retina throughout life. No histological abnormalities have been found in the retina, retinal vessels, or choroidal vasculatures of the eyes with drusen. Immunohistochemical and proteomic analyses of the drusen from these monkeys showed that the drusen were very similar to those in other monkeys with aged macular degeneration sporadically found in older monkeys and also with human drusen.[22,23] These observations by Umeda *et al.* have shown that the Tsukuba monkeys produce drusen that are biochemically similar to those in human AMD patients, but the development of the drusen occurs at an accelerated rate of over 25 times. Currently, 240 loci of the cynomolgus monkey are being investigated to try to identify the disease-causing gene and to understand the biological pathways leading to complement activation.

The eyes of monkey are structurally similar to human eyes, which make them extremely valuable for macular degeneration studies. However, there are limitations in using this species over other laboratory animals. Monkeys have a relatively longer life span, have a longer gestation period, have lower birth numbers resulting in a slower rate of expanding the pedigree, and are more difficult to genetically manipulate; in addition, the cost of maintenance is high. In other laboratory animals, the differences in the eye structure, lack of a fovea, and a low cone-to-rod ratio compared to humans have been considered to be a disadvantage for using them as AMD models. However, they are easier to manipulate genetically and easier and less expensive to maintain. This has made the development of a mouse model of AMD very attractive, and a number of mouse AMD models have been reported recently.

The mouse model described by Ambati *et al.* is deficient either in monocyte chemoattractant protein-1 or its cognate C–C chemokine receptor-2. These mice were found to develop the cardinal

Figure 31–5. Photographs of the fundus of two monkeys with AMD in the Tsukuba Primate Research Center showing drusen.

features of AMD including accumulation of lipofuscin in drusen beneath the RPE, photoreceptor atrophy, and CNV.[24] An impairment of macrophage recruitment allowed the accumulation of C5a and IgG, which leads to the production of vascular endothelial growth factor by the RPE cells and the development of CNVs.

Another mouse model that has three known AMD risk factors—age, high-fat cholesterol-rich diet, and expression of human apolipoprotein E (apoE2, apoE3, andapoE4)—has been developed.[25] ApoE4-deficient mice are severely affected showing diffuse subretinal pigment epithelial deposits, drusen, thickened Bruch's membrane, and atrophy, hypopigmentation, and hyperpigmentation of the RPE.

Oxidative stress has long been linked to the pathogenesis of AMD. Imamura, *et al.* reported Cu, Zn-superoxide dismutase (SOD1)-deficient mice that had features typical of AMD in humans. Senescent $Sod1^{-/-}$ mice had drusen, thickened Bruch's membrane, and choroidal neovascularization.[26] The number of drusen increased with age and also after exposure of young $Sod1^{-/-}$ mice to excess light. The retinal pigment epithelial cells of $Sod1^{-/-}$ mice showed oxidative damage, and their β-catenin-mediated cellular integrity was disrupted. These findings suggested that oxidative stress may affect the junctional proteins necessary for the barrier integrity of the RPE. These observations strongly suggested that oxidative stress may play a major role in AMD.

The complement components, C3a and C5a, are present in drusen, and were observed in Bruch's membrane of a laser-induced CNV mice model. Neutralization of C3a or C5a by antibody or by blockade of their receptors by a complement inhibitor significantly reduced the CNV. These observations revealed a role of immunological mechanisms for angiogenesis and provided evidence for future therapeutic strategies for AMD.

Although the pathology of AMD is pronounced in the macula area, it is not confined to this region. Characteristics of human AMD such as thickening of Bruch's membrane, accumulation of drusen, and CNV have been observed in mouse models. Nevertheless, the primate model will still be the choice for AMD studies, especially at the stage when new therapeutic methods are tested and evaluated for the first time. However, it would be wise and more productive to study both primate and mouse models in AMD research. This will be necessary to determine the mechanisms underlying the disease and to identify clinical and molecular markers for the early stages of AMD. The findings from these studies will provide critical information needed to develop therapies for AMD.

GLAUCOMA

OVERVIEW Glaucoma is a heterogeneous group of complex neurodegenerative disorders that is characterized by the constriction of the visual field, death of retinal ganglion cells (RGCs), and a pathognomonic deformation of the optic nerve head (ONH) known as glaucomatous cupping. Glaucomas are classified into three main types: open-angle, closed-angle, and congenital glaucoma. Each of these types is subdivided into primary and secondary types.

EPIDEMIOLOGY AND GENETICS Primary open-angle glaucoma (POAG) is the most common form of glaucoma; it occurs in about 4.5 million people worldwide and accounts for 12% of all global blindness. By the year 2020, over 11 million people will be blind from primary glaucoma.[28] POAG is often, but not always, associated with elevated IOP, which is one of the main risk factors in glaucoma. However, about a third of all patients with POAG develop the disease without an IOP elevation, and in these patients, the IOP is continuously below 21 mmHg. This form of POAG is called normal tension or low tension glaucoma (NTG). A reduction of the IOP, even in cases of NTG, is the main, clinically proven, treatment for glaucoma.

ANIMAL MODELS Among the different animal models of glaucoma, the monkey models are superior because of the anatomical similarity of the monkey eyes to human eyes and the phylogenetic similarities of these two species. At the same time, monkeys are extremely expensive and experiments on them require a highly skilled team of investigators.

Most of the existing animal models of POAG, including the monkey models, are based on the elevation of the IOP. An elevation of the IOP develops from an imbalance between aqueous humor production and outflow. Aqueous humor, a fluid produced by the ciliary body of the eye, drains out of the eye and into the blood circulatory system. The eye's outflow system consists of a series of endothelial cell-lined structures that include the trabecular meshwork (TM), Schlemm's canal (SC), which serves as a collector vessel, and the episcleral venous system (Figure 31–6). In most glaucoma models, the IOP is elevated as a result of a reduction or blockage of the aqueous humor outflow. In monkeys, an elevation of IOP is commonly induced by laser photocoagulation of the TM.[29,30] Several days after the laser treatment, the IOP increases and this elevation may last for more than a year, although more than one laser session is usually required to achieve a sustained elevation of the IOP. The IOP in treated eyes is usually between 25 and 60 mm Hg.

Other methods to elevate the IOP in monkey eyes include the anterior chamber injection of ghost red cells,[31] latex microspheres,[32] cross-linked polyacrylamide gels,[33] and enzymes.[34] Topical steroids have also been shown to elevate the IOP.[35] These latter treatments produce less consistent elevations than laser photocoagulation.[36]

Monkey glaucoma models have been shown to have changes in the optic disk, optic nerve, RGC, and nerve fiber layers similar to those observed in glaucomatous human eyes. Apoptosis was shown to be the cause of the RGCs death in a monkey photocoagulation model,[37] and apoptosis was later confirmed to be the cause in other animal models and in humans with glaucoma. Monkey glaucoma models have also been successfully used to study changes in the retinal gene expression pattern 30 days after laser photocoagulation of the TM,[35] and to test the effectiveness of new classes of hypotensive drugs.[36]

Rodent became the animal of choice when large numbers of animals were required, e.g., when examining the mechanism of RGC degeneration and neuroprotection. Several rat models of elevated pressure-induced optic nerve damage have been developed during the past decade, and they have been used to study changes in the retina and the optic nerve. Rats are easy to handle and the relatively large size of their eyes allows multiple, awake measurements of the IOP with commercially available equipment.[39] This latter is important because it is well documented that general anesthesia induces a rapid decrease in the IOP. Although there are certain differences in the structure of the rat and human eyes, all of the eye structures affected in glaucomatous human eyes exist in the rat eye.

In rat models, the IOP elevation is achieved by injection of concentrated saline solution into the episcleral veins,[40] laser

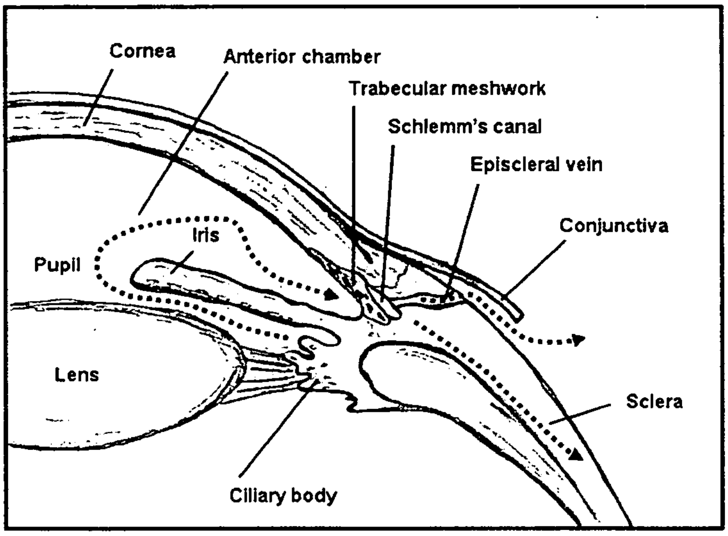


Figure 31–6. Schematic diagram of the anterior segment of the eye showing the trabecular meshwork and uveoscleral outflow pathways of aqueous humor.

photocoagulation of the TM after an injection of Indian ink into the anterior chamber,[41] laser photocoagulation of the TM.[42] and laser cauterization of episcleral veins.[43] All of these methods that lead to an elevation of the IOP require special training of the investigators.

Successful treatment of the eye leads to a rapid elevation of the IOP, although the level of elevation varies from eye to eye. Saline injection generally produces a wide range of IOP elevation from a very minimal rise to a 2-fold increase over the IOP in control eyes. The elevation of the IOP generally lasts for several weeks, and a second laser treatment is often required in the photocoagulation method to maintain an elevated IOP for more than 3 weeks.

A chronic elevation of the IOP in rats leads to apoptosis of the RGCs, degeneration of the optic nerve fibers, and remodeling of the ONH similar to those observed in human glaucomatous eyes.[40,44–45] Rat models of glaucoma have been used to study the effects of elevated IOP on the electroretinogram,[46] neuroprotective drugs,[47] and molecular changes in the retina and optic nerve using the candidate gene approach and array hybridization.[48]

A mutant rat strain was reported to have unilateral or bilateral enlargement of the eyes with an IOP ranging from 25 to 45 mm Hg. In this strain of rat, cupping of the optic nerve head was detected by funduscopic examination, and the cupping was more pronounced in older animals. The number of RGCs also declined with age.[49] Unfortunately, this strain was obtained from the Royal College of Surgeons colony that has a mutation in the receptor tyrosine kinase gene, leading to degeneration of the photoreceptors. Therefore, this strain can hardly be considered a good glaucoma model.

The construction of mouse models of glaucoma has lagged behind rat glaucoma models for a long time despite the advantages of mice over rats and other mammalian species for cost-effective genetic manipulations, availability of a wide spectrum of methods, and the existence of many genetically modified strains. However, it should be remembered that mouse and human eyes have certain important differences including the arterial blood supply to the optic nerve head and the absence of a lamina cribrosa.[50] The lamina cribrosa, a collagenous scaffold supporting the optic nerve, plays a critical role in the damage/protection of the human optic nerve.

One of the main difficulties working with mice is that their eyes are much smaller than the eyes of humans and rats, and new methods had to be developed to measure the IOPs in mice. To date, several invasive and noninvasive methods of IOP measurements have been developed for mice. The first remains one of the most reliable and accurate methods and does not depend upon the mechanical properties of the cornea. It involves the insertion of a glass microneedle connected to a pressure transducer into the anterior chamber of the eye. Using this method, it was shown that common mouse strains have different IOP between 10 and 20 mm Hg.[51] Other methods of IOP measurements in mice were later developed including noninvasive techniques. Noninvasive methods allow multiple IOP measurements to be completed in a short period of time, but the results of these measurements may depend upon mechanical properties of the cornea. To obtain reliable IOP readings, all of the described techniques require training.

Transgenic and gene-targeted knockout approaches have been used to develop several mouse models of glaucoma. The main advantage of these models is that the animals with the mutated gene provide a more uniform elevation of the IOP and damage to the retina and optic nerve similar to that found with surgically induced elevated IOPs. A large number of animals can be produced, and once a mutant mouse line is obtained, no special training is needed to produce more affected mice.

Several lines of transgenic mice have been developed that contained BAC DNAs with a mouse Tyr423His point mutation and Tyr437His point mutation of the human myocilin (Myoc) genes. The Tyr437His mutation in the Myoc gene leads to severe glaucoma in humans, and the mouse Tyr423His mutation corresponds to this human mutation. Expression of mutated mouse Myoc in the ocular drainage structures led to moderate (about 2 mm Hg during the day and 4 mm Hg at night) elevation of the

IOP and progressive degenerative changes in the peripheral RGC layer and optic nerve that resembled glaucomatous changes in human eyes.[52] In 1-year-old animals, the peripheral retina of transgenic mice had approximately 20% fewer RGCs than the peripheral retina of control littermates.

Transgenic mice with a targeted mutation in the gene for the α_1-subunit of collagen type I have also been constructed. This mutation blocks the cleavage of collagen by matrix metalloproteinase-1. Transgenic mice expressing mutated collagen had elevated IOPs. The difference in the IOP between control and transgenic mice gradually increased to a maximum of 4.8 mmHg at 36 weeks. Because these mice had progressive optic nerve axon loss with normal organization of the drainage structures, it has been suggested that they may be used as a mouse model of POAG.[53]

Recent data have demonstrated that transgenic mice expressing mutated optineurin under the control of the ubiquitous ROSA26 promoter develop optic nerve cupping and death of the RGCs without elevation of the IOP.[54] These transgenic mice may represent the first animal model of NTG.

The surgical methods used to produce rat glaucoma models have also been used in mice. However, performing surgery on the mouse eye is even more challenging than on rat eyes because of the difference in size. A significant elevation of the IOP was found in the eyes of C57BL/6J mice that had an injection of indocyanine green dye into the anterior chamber and diode laser treatment of the TM and episcleral vein region.[55] At 10 days after the surgery, the mean IOP in the operated eyes was 33.6 ± 1.5 mmHg versus 15.2 ± 0.6 mmHg in the control eyes. However, the IOP returned to normal 60 days after the surgery. Histological examination of the treated eyes 65 day after the surgery revealed anterior synechia, a decrease in the number of RGCs, thinning of all retinal layers, and damage to the optic nerve structures without evidence of prominent cupping.[55] A reduction in the function of the outer retinal layers, confirmed by electroretinographic studies, may indicate that this model produces more extensive changes in the retina compared to the glaucoma in humans.

Similar to the above model, an elevation of IOP was induced by argon laser photocoagulation of the episcleral and limbal veins in C57BL/6J mouse eyes[56] or by cauterization of three episcleral veins in CD1 mouse eyes.[57] During the first 4 weeks following laser treatment, the mean IOP in the treated eyes was about 1.5 times higher than in control eyes. The number of RGCs had decreased by 22.4 ± 7.5% of that in the controls at 4 weeks after treatment. Most of the TUNEL-positive apoptotic cells were detected in the peripheral retina.[56]

Cauterization of the episcleral veins led to a maximum IOP elevation within 2–9 days, and the IOP decreased progressively thereafter to reach more or less normal values after 24–33 days. There was a 20% decrease in the number of RGCs 2 weeks after the surgery.[57]

The DBA/2J strain has high IOP and has become a popular mouse model to study secondary angle-closure glaucoma. This mouse strain has mutations in two genes, *Tyrp1* and *Gpnmb*.[58] DBA/2J mice develop pigment dispersion, iris transillumination, iris atrophy, and anterior synechia. At the age of 9 months, the IOP was elevated in most mice and the elevation was accompanied by the death of the RGCs, optic nerve atrophy, and optic nerve cupping. Although no group of the RGCs was especially vulnerable or resistant to degeneration, fan-shaped sectors of dead or surviving RGC radiated from the ONH.[59] It has been suggested that axon damage at the ONH might be a primary lesion in this model.[59]

Several important observations were made from the studies on the DBA/2J model. It was shown that the proapoptotic protein BAX is required for the survival of RGCs but not for RGC axon degeneration, suggesting that BAX may be a candidate human glaucoma susceptibility gene.[60] Unexpectedly, a high dose of γ-irradiation accompanied by syngeneic bone marrow transfer protected the RGCs in DBA/2J mice.[61] Similar to the results obtained with rat and monkey models, genes involved in the glial activation and immune response were activated in DBA/2J retina as shown by array hybridization.[62]

Complement component, 1Q, was upregulated in the retina of several animal models of glaucoma as it is in human glaucoma with the timing suggesting that complement activation plays a significant role in the pathogenesis of glaucoma.[63]

Taken together, these findings confirm that animal models might be used to look for a molecular mechanism involved in glaucoma in humans.

The modulation of the activity of genes involved in the development of the anterior segment of the eye may lead to relatively rare developmental glaucomas that account for less than 1% of all glaucoma cases. Several genes have been implicated in congenital glaucoma and anterior segment disgenesis. They include *Cyp1b1*, *Foxc1*, *Foxc2*, *Pitx2*, *Lmx1b*, and *Pax6*. Several lines of mice with defects in these genes have been studied with glaucoma in mind (see Gould *et al.*[64] for a review). For example, mutation in the *CYP1B1* gene (cytochrome P450, family 1, subfamily b, polypeptide 1) may lead to primary congenital glaucoma (PCG) in humans. Although Cyp1b1 knockout mice did not develop elevated IOP, they had ocular abnormalities similar to the defects in humans with PCG, viz. small or absent Schlemm's canal, defects in the TM, and attachment of the iris to the TM and anterior synechia.

Mutations in the *FOXC1* gene, which encodes a transcription factor with a forkhead-winged-helix DNA binding domain, cause a range of eye abnormalities associated with glaucoma, e.g., iris hypoplasia, Axenfeld and Rieger anomaly, and Rieger syndrome. Foxc1$^{-/-}$ mice die at birth, while Foxc1$^{+/-}$ animals are viable but have defects in the eye drainage structures without changes in IOP. Similar eye defects were observed in Foxc2$^{+/-}$ mice. It has been suggested that *Foxc1*$^{+/-}$ and *Foxc2*$^{+/-}$ mice are useful models for studying anterior segment development and anomalies, and may allow the identification of genes that interact with *Foxc1* and *Foxc2* (or *FKHL7* and *FKHL14*) to produce a phenotype with elevated IOP and glaucoma.

Other animals, including rabbit, pig, and bovine, have also been used to develop animal models of glaucoma, but none of them is widely used for different reasons. Zebrafish became a powerful model for advanced genetic studies in vertebrates, especially in the case of complex diseases, and was proposed as a model for identification of modifier genes for glaucoma.[65]

CONCLUSIONS

In summary, animal models of glaucoma, including the most widely used rodent and monkey models, have already provided interesting new information about mechanisms of glaucoma in humans. However, it should be remembered that even in monkey models the time course of changes in the glaucomatous eyes may

K1

be significantly accelerated compared to that in human glaucomatous eyes. and all discussed models are indeed just models of glaucoma in humans. Results obtained with these models should not be automatically applied to human glaucoma and should be confirmed by testing in humans whenever possible. It has become clear that reaction to the same insult, e.g., elevated IOP, may be somewhat different in different animal models. Glaucoma studies in animals may help us identify the molecular mechanisms involved in the development of glaucoma in each particular model. By comparing these mechanisms, it may be possible to find some common mechanism that might be involved in glaucoma formation in humans. This will be extremely valuable for the development of new therapeutic approaches for the treatment and prevention of glaucoma in humans.

REFERENCES

1. Heiba IM, Elston RC, Klein BE. Klein R. Sibling correlations and segregation analysis of age-related maculopathy: The Beaver Dam Eye Study. *Genet Epidemiol* 1994;11:51–67.
2. Seddon JM. Ajani UA, Mitchell BD. Familial aggregation of age-related maculopathy. *Am J Ophthalmol* 1997;123:199–206.
3. Hammond CJ, Webster AR, Snieder H, Bird AC, Gilbert CE, Spector TD. Genetic influence on early age-related maculopathy: A twin study. *Ophthalmology* 2002:109:730–736.
4. Iyengar SK. Song D. Klein BE. Klein R, Schick JH, Humphrey J. Millard C. Liptak R. Russo K. Jun G, Lee KE, Fijal B, Elston RC. Dissection of genomewide-scan data in extended families reveals a major locus and oligogenic susceptibility for age-related macular degeneration. *Am J Hum Genet* 2004;74:20–39.
5. Schick JH, Iyengar SK, Klein BE, Klein R, Reading K. Liptak R, Millard C. Lee KE, Tomany SC, Moore EL, Fijal BA, Elston RC. A whole-genome screen of a quantitative trait of age-related maculopathy in sibships from the Beaver Dam Eye Study. *Am J Hum Genet* 2003;72:1412–1424.
6. Majewski J. Schultz DW. Weleber RG. Schain MB. Edwards AO. Matise TC. Acott TS, Ott J, Klein ML. Age-related macular degeneration—a genome scan in extended families. *Am J Hum Genet* 2003;73:540–550.
7. Klein RJ. Zeiss C, Chew EY. Tsai JY. Sackler RS. Haynes C, Henning AK, SanGiovanni JP, Mane SM. Mayne ST, Bracken MB, Ferris FL. Ott J, Barnstable C, Hoh J. Complement factor H polymorphism in age-related macular degeneration. *Science* 2005;308: 385–389.
8. Edwards AO, Ritter R 3rd. Abel KJ. Manning A, Panhuysen C. Farrer LA. Complement factor H polymorphism and age-related macular degeneration. *Science* 2005;308:421–424.
9. Haines JL. Hauser MA, Schmidt S, Scott WK. Olson LM. Gallins P. Spencer KL. Kwan SY, Noureddine M, Gilbert JR. Schnetz-Boutaud N, Agarwal A, Postel EA. Pericak-Vance MA. Complement factor H variant increases the risk of age-related macular degeneration. *Science* 2005;308:419–421.
10. Hageman GS, Anderson DH, Johnson LV, Hancox LS, Taiber AJ. Hardisty LI, Hageman JL. Stockman HA. Borchardt JD, Gehrs KM. Smith RJ, Silvestri G. Russell SR, Klaver CC. Barbazetto I. Chang S. Yannuzzi LA. Barile GR. Merriam JC. Smith RT. Olsh AK, Bergeron J. Zernant J, Merriam JE. Gold B, Dean M. Allikmets R. A common haplotype in the complement regulatory gene factor H (HF1/CFH) predisposes individuals to age-related macular degeneration. *Proc Natl Acad Sci USA* 2005;102:7227–7232.
11. Okamoto H, Umeda S. Obazawa M. Minami M, Noda T. Mizota A. Honda M. Tanaka M. Koyama R. Takagi I, Sakamoto Y. Saito Y. Miyake Y. Iwata T. Complement factor H polymorphisms in Japanese population with age-related macular degeneration. *Mol Vis* 2006;12:156–158.
12. Gotoh N. Yamada R. Hiratani H, Renault V, Kuroiwa S. Monet M. Toyoda S, Chida S. Mandai M, Otani A, Yoshimura N, Matsuda F. No association between complement factor H gene polymorphism and exudative age-related macular degeneration in Japanese. *Hum Genet* 2006:120:139–143.
13. Gold B, Merriam JE, Zernant J. Hancox LS, Taiber AJ. Gehrs K. Cramer K, Neel J, Bergeron J. Barile GR. Smith RT; AMD Genetics Clinical Study Group; Hageman GS, Dean M, Allikmets R. Variation in factor B (BF) and complement component 2 (C2) genes is associated with age-related macular degeneration. *Nat Genet* 2006;38: 458–462.
14. Russell SR. Mullins RF, Schneider BL, Hageman GS. Location. substructure, and composition of basal laminar drusen compared with drusen associated with aging and age-related macular degeneration. *Am J Ophthalmol* 2000;129:205–214.
15. Mullins RF, Russell SR, Anderson DH, Hageman GS. Drusen associated with aging and age-related macular degeneration contain proteins common to extracellular deposits associated with atherosclerosis, elastosis. amyloidosis, and dense deposit disease. *FASEB J* 2000;14: 835–846.
16. Chader GJ. Animal models in research on retinal degenerations: Past progress and future hope. *Vision Res* 2002;42:393–399.
17. Stafford TJ. Anness SH. Fine BS. Spontaneous degenerative maculopathy in the monkey. *Ophthalmology* 1984:91:513–521.
18. El-Mofty A, Gouras P. Eisner G. Balazs EA. Macular degeneration in rhesus monkey (Macaca mulatta). *Exp Eye Res* 1978:27: 499–502.
19. Nicolas MG. Fujiki K, Murayama K, Suzuki MT. Mineki R. Hayakawa M, Yoshikawa Y, Cho F. Kanai A. Studies on the mechanism of early onset macular degeneration in cynomolgus (Macaca fascicularis) monkeys. I. Abnormal concentrations of two proteins in the retina. *Exp Eye Res* 1996;62:211–219.
20. Nicolas MG. Fujiki K, Murayama K, Suzuki MT, Shindo N. Hotta Y, Iwata F, Fujimura T, Yoshikawa Y, Cho F. Kanai A. Studies on the mechanism of early onset macular degeneration in cynomolgus monkeys. II. Suppression of metallothionein synthesis in the retina in oxidative stress. *Exp Eye Res* 1996;62:399–408.
21. Suzuki MT. Terao K. Yoshikawa Y. Familial early onset macular degeneration in cynomolgus monkeys (*Macaca fascicularis*). *Primates* 2003;44:291–294.
22. Umeda S. Ayyagari R. Allikmets R, Suzuki MT, Karoukis AJ. Ambasudhan R. Zernant J, Okamoto H, Ono F. Terao K. Mizota A. Yoshikawa Y, Tanaka Y, Iwata T. Early-onset macular degeneration with drusen in a cynomolgus monkey (*Macaca fascicularis*) pedigree: Exclusion of 13 candidate genes and loci. *Invest Ophthalmol Vis Sci* 2005;46:683–691.
23. Umeda S, Suzuki MT. Okamoto H. Ono F, Mizota A, Terao K. Yoshikawa Y, Tanaka Y, Iwata T. Molecular composition of drusen and possible involvement of anti-retinal autoimmunity in two different forms of macular degeneration in cynomolgus monkey (Macaca fascicularis). *FASEB J* 2005:19:1683–1685.
24. Ambati J, Anand A. Fernandez S. Sakurai E. Lynn BC, Kuziel WA. Rollins BJ, Ambati BK. An animal model of age-related macular degeneration in senescent Ccl-2- or Ccr-2-deficient mice. *Nat Med* 2003:9:1390–1397.
25. Malek G. Johnson LV. Mace BE, Saloupis P. Schmechel DE. Rickman DW, Toth CA, Sullivan PM, Bowes Rickman C. Apolipoprotein E allele-dependent pathogenesis: A model for age-related retinal degeneration. *Proc Natl Acad Sci USA* 2005:102: 11900–11905.
26. Imamura Y. Noda S. Hashizume K. Shinoda K. Yamaguchi M. Uchiyama S, Shimizu T. Mizushima Y, Shirasawa T, Tsubota K. Drusen, choroidal neovascularization. and retinal pigment epithelium dysfunction in SOD1-deficient mice: A model of age-related macular degeneration. *Proc Natl Acad Sci USA* 2006;103:11282–11287.
27. Nozaki M. Raisler BJ. Sakurai E, Sarma JV. Barnum SR. Lambris JD, Chen Y. Zhang K, Ambati BK, Baffi JZ. Ambati J. Drusen complement components C3a and C5a promote choroidal neovascularization. *Proc Natl Acad Sci USA* 2006;103:2328–2333.
28. Quigley HA. Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol* 2006:90:262–267.
29. Gaasterland D. Kupfer C. Experimental glaucoma in the rhesus monkey. *Invest Ophthalmol Vis Sci* 1974;13:455–457.

30. Quigley HA. Hohman RM. Laser energy levels for trabecular meshwork damage in the primate eye. *Invest Ophthalmol Vis Sci* 1983:24:1305–1307.
31. Quigley HA. Addicks EM. Chronic experimental glaucoma in primates. I. Production of elevated intraocular pressure by anterior chamber injection of autologous ghost red blood cells. *Invest Ophthalmol Vis Sci* 1980:19:126–136.
32. Weber AJ. Zelenak D. Experimental glaucoma in the primate induced by latex microspheres. *J Neurosci Methods* 2001;111:39–48.
33. Kaufman PL. Lutjen-Drecoll E. Hubbard WC. Erickson KA. Obstruction of aqueous humor outflow by cross-linked polyacrylamide microgels in bovine. monkey. and human eyes. *Ophthalmology* 1994:101:1672–1679.
34. Zimmerman LE, De Venecia G. Hamasaki DI. Pathology of the optic nerve in experimental acute glaucoma. *Invest Ophthalmol* 1967;6:109–125.
35. Amfrmaly MF. Aqueous outflow facility in monkeys and the effect of topical corticoids. *Invest Ophthalmol* 1964:3:534–538.
36. Rasmussen CA. Kaufman PL. Primate glaucoma models. *J Glaucoma* 2005:14:311–314.
37. Quigley HA, Nickells RW. Kerrigan LA. Pease ME. Thibault DJ. Zack DJ. Retinal ganglion cell death in experimental glaucoma and after axotomy occurs by apoptosis. *Invest Ophthalmol Vis Sci* 1995;36:774–786.
38. Miyahara T. Kikuchi T. Akimoto M. Kurokawa T. Shibuki H. Yoshimura N. Gene microarray analysis of experimental glaucomatous retina from cynomologous monkey. *Invest Ophthalmol Vis Sci* 2003;44:4347–4356.
39. Moore CG, Milne ST, Morrison JC. Noninvasive measurement of rat intraocular pressure with the Tono-Pen. *Invest Ophthalmol Vis Sci* 1993;34:363–369.
40. Morrison JC. Moore CG, Deppmeier LM, Gold BG, Meshul CK. Johnson EC. A rat model of chronic pressure-induced optic nerve damage. *Exp Eye Res* 1997;64:85–96.
41. Ueda J, Sawaguchi S. Hanyu T. Yaoeda K. Fukuchi T, Abe H. *et al.* Experimental glaucoma model in the rat induced by laser trabecular photocoagulation after an intracameral injection of India ink. *Jpn J Ophthalmol* 1998;42:337–344.
42. Levkovitch-Verbin H. Quigley HA. Martin KR. Valenta D. Baumrind LA, Pease ME. Translimbal laser photocoagulation to the trabecular meshwork as a model of glaucoma in rats. *Invest Ophthalmol Vis Sci* 2002;43:402–410.
43. Shareef SR. Garcia-Valenzuela E. Salierno A, Walsh J. Sharma SC. Chronic ocular hypertension following episcleral venous occlusion in rats [letter]. *Exp Eye Res* 1995:61:379–382.
44. Garcia-Valenzuela E. Shareef S, Walsh J. Sharma SC. Programmed cell death of retinal ganglion cells during experimental glaucoma. *Exp Eye Res* 1995:61:33–44.
45. Johnson EC, Morrison JC, Farrell S. Deppmeier L, Moore CG. McGinty MR. The effect of chronically elevated intraocular pressure on the rat optic nerve head extracellular matrix. *Exp Eye Res* 1996;62:663–674.
46. Bayer AU. Danias J. Brodie S. Maag KP. Chen B, Shen F. *et al.* Electroretinographic abnormalities in a rat glaucoma model with chronic elevated intraocular pressure. *Exp Eye Res* 2001;72:667–677.
47. McKinnon SJ. Lehman DM, Tahzib NG, Ransom NL, Reitsamer HA, Liston P. *et al.* Baculoviral IAP repeat-containing-4 protects optic nerve axons in a rat glaucoma model. *Mol Ther* 2002:5:780–787.
48. Ahmed F. Brown KM. Stephan DA, Morrison JC, Johnson EC, Tomarev SI. Microarray analysis of changes in mRNA levels in the rat retina after experimental elevation of intraocular pressure. *Invest Ophthalmol Vis Sci* 2004:45:1247–1258.
49. Thanos S, Naskar R. Correlation between retinal ganglion cell death and chronically developing inherited glaucoma in a new rat mutant. *Exp Eye Res* 2004;79:119–129.
50. May CA. Lutjen-Drecoll E. Morphology of the murine optic nerve. *Invest Ophthalmol Vis Sci* 2002;43:2206–2212.
51. Savinova OV. Sugiyama F, Martin JE. Tomarev SI. Paigen BJ, Smith RS, *et al.* Intraocular pressure in genetically distinct mice: An update and strain survey. *BMC Genet* 2001;2:12.
52. Senatorov VV. Malyukova I, Fariss R, Wawrousek E, Swaminathan S, Sharan SK, Tomarev S. Expression of mutated mouse myocilin induces open-angle glaucoma in transgenic mice. *J Neurosci* 2006:26(46):11903–11914.
53. Mabuchi F. Lindsey JD. Aihara M, Mackey MR, Weinreb RN. Optic nerve damage in mice with a targeted type I collagen mutation. *Invest Ophthalmol Vis Sci* 2004;45:1841–1845.
54. Akahori M, Obazawa M, Minami M. Noda T. Noda S. Nakaya N, Tomarev S. Sasaoka M, Shimazaki A, Kawase K, Yamamoto T, Miyake Y. Iwata T. Expression of mutated optineurin leads to normal tension glaucoma and disruption of optineurin-Rab8 interaction in mice. Submitted.
55. Grozdanic SD. Betts DM, Sakaguchi DS. Allbaugh RA, Kwon YH. Kardon RH. Laser-induced mouse model of chronic ocular hypertension. *Invest Ophthalmol Vis Sci* 2003:44:4337–4346.
56. Gross RL, Ji J. Chang P. Pennesi ME. Yang Z, Zhang J, *et al.* A mouse model of elevated intraocular pressure: Retina and optic nerve findings. *Trans Am Ophthalmol Soc* 2003:101:163–169.
57. Ruiz-Ederra J. Verkman AS. Mouse model of sustained elevation in intraocular pressure produced by episcleral vein occlusion. *Exp Eye Res* 2006;82:879–884.
58. Anderson MG. Smith RS. Hawes NL, Zabaleta A. Chang B. Wiggs JL, *et al.* Mutations in genes encoding melanosomal proteins cause pigmentary glaucoma in DBA/2J mice. *Nat Genet* 2002:30: 81–85.
59. Jakobs TC. Libby RT, Ben Y, John SW, Masland RH. Retinal ganglion cell degeneration is topological but not cell type specific in DBA/2J mice. *J Cell Biol* 2005:171:313–325.
60. Libby RT, Li Y. Savinova OV, Barter J, Smith RS. Nickells RW, *et al.* Susceptibility to neurodegeneration in a glaucoma is modified by Bax gene dosage. *PLoS Genet* 2005:1:17–26.
61. Anderson MG, Libby RT. Gould DB. Smith RS, John SW. High-dose radiation with bone marrow transfer prevents neurodegeneration in an inherited glaucoma. *Proc Natl Acad Sci USA* 2005:102: 4566–4571.
62. Steele MR. Inman DM. Calkins DJ, Horner PJ, Vetter ML. Microarray analysis of retinal gene expression in the DBA/2J model of glaucoma. *Invest Ophthalmol Vis Sci* 2006:47:977–985.
63. Stasi K. Nagel D, Yang X. Wang RF. Ren L. Podos SM, *et al.* Complement component 1Q (C1Q) upregulation in retina of murine. primate. and human glaucomatous eyes. *Invest Ophthalmol Vis Sci* 2006;47:1024–1029.
64. Gould DB. Smith RS. John SW. Anterior segment development relevant to glaucoma. *Int J Dev Biol* 2004;48:1015–1029.
65. McMahon C, Semina EV. Link BA. Using zebrafish to study the complex genetics of glaucoma. *Comp Biochem Physiol C Toxicol Pharmacol* 2004;138:343–350.

Current Topics in Innate Immunity

Edited by

JOHN D. LAMBRIS
Department of Pathology & Laboratory Medicine
University of Pennsylvania
Philadelphia, Pennsylvania

Springer

Aegean Conferences

Editor:
John D. Lambris, Ph.D.
University of Pennsylvania
Philadelphia, PA 19104
www.lambris.net

Library of Congress Control Number: 2007926254

Proceedings from the 4th International Conference on Innate Immunity held in Corfu, Greece, June 4-9, 2006.

ISBN 978-0-387-71765-4 e-ISBN 978-0-387-71767-8

Springer Aegean Conferences

Printed on acid-free paper.

10 9 8 7 6 5 4 3 2 1

springer.com

Preface

Innate Immunity has long been regarded as the non-specific arm of immune response, acting immediately and in a generic way, to defend the host from infections. In the post genomic era, our knowledge of the innate immune system is enriched by findings on the specificity of innate immune reactions as well as to novel functions that do not strictly correlate with immunological defense and surveillance, immune modulation or inflammation. Several studies indicate that molecules involved in innate immunity exert functions that are either more complex than previously thought, or go well beyond the innate immune character of the system.

The advent of high-throughput platforms for genome and proteome-wide profiling, together with the enormous amount of raw genetic information that has accumulated in the databases, have stirred new expectations in biomedical research. They have led scientists to revisit established biological systems from a global and integrative perspective. Innate Immunity research is now faced with the challenge of trying to integrate isolated biochemical pathways into complex gene and protein regulatory circuits. In this respect, scientists from around the world convened at the 4th International Conference on Innate Immunity (June 4 - 9, 2006), in Corfu, Greece to discuss recent advances in this fast evolving field. This volume represents a collection of topics on natural killer cells, mast cells, phagocytes, toll like receptors, complement, host defense in plants and invertebrates, evasion strategies of microorganisms, pathophysiology, protein structures, design of therapeutics, and experimental approaches discussed during the conference.

I am grateful to the contributing authors for the time and effort they have devoted to writing, what I consider exceptionally informative chapters in a book that will have a significant impact on the Innate Immunity field. I am grateful to Rodanthi Lambris, for her assistance in formatting the text. I also gratefully acknowledge the generous help provided by Dimitrios Lambris in managing the organization of this meeting. Finally, I also thank Andrea Macaluso and Lisa Tenaglia of Springer Publishers for their supervision in this book's production.

John D. Lambris, Ph.D.

18

Complement Activation of Drusen in Primate Model (*Macaca fascicularis*) for Age-Related Macular Degeneration

Takeshi Iwata

Takeshi Iwata, National Institute of Sensory Organs, National Hospital Organization
Tokyo Medical Center, 2-5-1 Higashigaoka, Meguro-ku, Tokyo 152-8902 Japan

1 Introduction

Dysfunction of the visual system can alter normal human life style and lower quality of life. The most prevalent causes of visual impairment worldwide are cataracts, glaucoma, and age-related macular degeneration (AMD). These eye diseases are responsible for 69% of blindness globally. Although cataracts are the leading cause of blindness worldwide, recent advances in cataract surgery has significantly reduced the visual impairments caused by cataracts especially in developed countries. The most prevalent eye disease for elderly Europeans and Americans is AMD. This degenerative disease progresses from retinal deposits called drusen to neovascularization and retinal hemorrhages resulting in irreversible loss of central vision. In spite of the high incidence of AMD, a limited amount of information is available on the underlying pathological mechanisms causing these diseases. Obtaining tissues from the AMD donors is often difficult, and even when obtained, they are usually collected many hours or even days after death. Because of limitation for human tissue, the availability of animal models is becomes valuable because they can be used to investigate the molecular mechanisms of the disease and to test new therapeutic intervention.

The retina is composed of nine layers of neural and glial cells that are arranged concentrically at the posterior pole of the eye. Incoming light is focused on the central area of the retina called the fovea which is located in the center of the macula. In humans, the average size of the macula is only 6 mm in diameter. The outer surface of the retina is covered by a monolayer of retinal pigment epithelial (RPE) cells which forms a diffusion barrier between the neural retina and the choroidal blood supply. The RPE regulates the transport of proteins to the retina, and controls the hydration and ionic composition of the subretinal space. The physiological condition of the RPE is closely associated with the pathogenesis of AMD.

2 Introduction of AMD

AMD is a blinding disorder characterized by a marked decrease in central vision associated with RPE atrophy with or without choroidal neovascularization (CNV). Many factors including genetic, behavioral, and environmental, are involved in this disease. AMD is characterized by the degeneration of cone photoreceptors in the foveal region of the retina resulting in a decrease of central visual acuity. The progressive impairment of the retinal pigment epithelial (RPE) cells, and damage to Bruch's membrane and choriocapillaris results in retinal atrophy and photoreceptor dysfunction. In some cases, CNV develops, and the new vessels penetrate Bruch's membrane and pass into the subretinal space.

Two types of AMD are recognized; the non-neovascular type is called the dry-type AMD and includes more than 80% of the cases, and the neovascular type is called the wet-type AMD which is progressive with a higher probability of blindness. The prevalence of AMD differs considerably among the different ethnic groups, but the incidence increases with age in all groups. A lower prevalence of AMD has been reported in individuals of African ancestry than of Anglo-Saxon ancestry. Other risk factors for AMD are cigarette smoking, obesity, hypertension, and atherosclerosis.

3 Genetics of AMD

Epidemiological studies have shown that genetic factor play critical role for AMD. Twin studies have previously shown a higher concordance for AMD in monozygotic twins than in dizygotic twins (Heiba, Elston, Klein, and Klein 1994; Seddon, Ajani, and Mitchell 1997; Hammond, Webster, Snieder, Bird, Gilbert, and Spector 2002). In addition, first degree relatives of individuals with AMD have higher incidence of AMD over individuals without a family history of AMD. Genetic segregation studies have also shown a genetic effect that accounts for approximately 60% of AMD with a single major gene accounting for about 55% of the risk of developing AMD. Previous data have suggested that the etiology of AMD has a significant genetic component. Only a small proportion of the families with AMD show Mendelian inheritance, and the majority of the individuals inherit AMD in a complex multi-gene pattern. With the help of the haplotype marker project (HapMap Project), genome wide scanning has identified at least 13 loci linked to AMD on different chromosomes (Iyengar, Song, Klein, Klein, Schick, Humphrey, Millard, Liptak, Russo, Jun, Lee, Fijal, and Elston 2004; Schick, Iyengar, Klein, Klein, Reading, Liptak, Millard, Lee, Tomany, Moore, Fijal, and Elston 2003; Majewski, Schultz, Weleber, Schain, Edwards, Matise, Acott, Ott, and Klein 2003). Recently, a polymorphism of complement factor H (CFH) gene (*Y402H*) was shown to be associated with an increased risk for AMD (Klein, Zeiss, Chew, Tsai, Sackler, Haynes, Henning, SanGiovanni, Mane, Mayne, Bracken, Ferris, Ott, Barnstable, and Hoh 2005; Edwards, Ritter, Abel, Manning, Panhuysen, and

Farrer 2005; Haines, Hauser, Schmidt, Scott, Olson, Gallins, Spencer, Kwan, Noureddine, Gilbert, Schnetz-Boutaud, Agarwal, Postel, and Pericak-Vance 2005; Hageman, Anderson, Johnson, Hancox, Taiber, Hardisty, Hageman, Stockman, Borchardt, Gehrs, Smith, Silvestri, Russell, Klaver, Barbazetto, Chang, Yannuzzi, Barile, Merriam, Smith, Olsh, Bergeron, Zernant, Merriam, Gold, Dean, and Allikmets 2005).

These results were confirmed in many of the countries with large Caucasian populations but not in Japan (Okamoto, Umeda, Obazawa, Minami, Noda, Mizota, Honda, Tanaka, Koyama, Takagi, Sakamoto, Saito, Miyake, and Iwata 2006; Gotoh, Yamada, Hiratani, Renault, Kuroiwa, Monet, Toyoda, Chida, Mandai, Otani, Yoshimura, and Matsuda 2006). This gene is located on chromosome 1q25-31 where one of the candidate loci was identified by linkage studies. Another recent study reported that a haplotype association of tandemly located complement 2 and factor B was protective for AMD (Gold, Merriam, Zernant, Hancox, Taiber, Gehrs, Cramer, Neel, Bergeron, Barile, Smith, AMD Genetics Clinical Study Group, Hageman, Dean, Allikmets 2006). HTRA1, a serine protease 11 was recently discovered to be strongly associated with AMD. Unlike the CFH, our study shows strong association with this gene for Japanese AMD patients (Yang, Camp, Sun, Tong, Gibbs, Cameron, Chen, Zhao, Pearson, Li, Chien, Dewan, Harmon, Bernstein, Shridhar, Zabriskie, Hoh, Howes, and Zhang 2006; Dewan, Liu, Hartman, Zhang, Liu, Zhao, Tam, Chan, Lam, Snyder, Barnstable, Pang, and Hoh 2006).

4 Biochemistry of AMD

The early stage of the dry type AMD is characterized by a thickening of Bruch's membrane, aggregation of pigment granules, and increasing numbers of drusen. The thickening of Bruch's membrane obstructs its function as a 'barrier' between the choroid and the RPE that protects the neural retina from the choriocapillary. Drusen are small yellowish-white deposits that are composed of lipids, proteins, glycoproteins, and glycosaminoglycans. They accumulate in the extracellular space and the inner aspects of Bruch's membrane. Drusen are not directly associated with visual loss but represent a risk factor for both the dry-type and wet-type AMD. The classification of hard and soft drusen is based on their size, shape, and color; hard drusen are yellowish with diameters <50 µm and are found in eyes that are less likely to progress to advanced stages of the disease, while soft drusen are darker yellow and larger in size, and are found in eyes more likely to progress to more advanced stages of AMD. A small percentage of dry-type AMD patients progress to the late stage of the wet-type AMD that is characterized by geographic atrophy or detachment of RPE and the development of CNV in the macular region. The presence of a CNV is the factor that most damages the neural retina because the newly developed vessels grow from the choriocapillaris through Bruch's membrane and extend laterally through the RPE cell layer (classic CNV) or extend between the inner Bruch's membrane and RPE (occult CNV). In advanced stages of AMD, the CNV and fluid leaked into the subretinal or intraretinal regions leads to cell death and retinal detachment.

Recent analyses of the progression of drusen have provided important clues that help understand the molecular pathology of AMD. Using both immunohistochemistry and proteomic techniques, the materials in drusen were found to be composed of molecules that mediate inflammatory and immune processes (Russell, Mullins, Schneider, and Hageman 2000; Mullins, Russell, Anderson, and Hageman 2000). These molecules include components of the complement pathway and modulators of complement activation, viz., vitronectin, clusterin, membrane cofactor protein, and complement receptor-1. In addition, molecules triggering inflammation, amyloid P component, α1-antitrypsin, and apolipoprotein E, were identified in drusen. Cellular debris from macrophages, RPE cells, and choroidal dendritic cells has also been identified in drusen. Additional proteins such as crystallins, EEFMP1, and amyloid-beta have been also found in drusen. The presence of immunoreactive proteins and the oxidative modifications of many proteins in drusen imply that both oxidation and immune functions are involved in the pathogenesis of AMD. These findings suggest that complement activation triggers innate immune responses in the subretinal space. The co-distribution of IgG and terminal complement complexes in drusen indicate that immune responses that directly target antigens in retinal cells might also be occurring. Anti-retinal autoantibodies have been reported in a number of ocular disorders, e.g., macular degeneration in an aged monkey model.

5 Primate Model for AMD

Over the past few years, genetic engineering techniques have generated a number of animal models of AMD in mice, rats, rabbits, pigs, and dogs (Chader 2002). However in mammals, a well-defined fovea is found only in primates (humans and monkeys), and a search for a monkey line affected with macular degeneration has been persistent for a long time. A monkey with macular degeneration was first described by Stafford et al in 1974. They reported that 6.6 % of the elderly monkeys they examined showed pigmentary disorders and drusen-like spots (Stafford, Anness, and Fine 1984). El-Mofty et al reported that the incidence of maculopathy was 50% in a colony of rhesus monkeys at the Caribbean Primate Research Center of the University of Puerto Rico (El-Mofty, Gouras, Eisner, and Balazs 1978). At the Tsukuba Primate Research Center (Tsukuba City, Japan), Suzuki et al found a single cynomolgus monkey (*Macaca fascicularis*) (Suzuki Monkeys) in 1986 with a large number of small drusen around the macular region (Nicolas, Fujiki, Murayama, Suzuki, Mineki, Hayakawa, Yoshikawa, Cho, Kanai 1996; Nicolas, Fujiki, Murayama, Suzuki, Shindo, Hotta, Iwata, Fujimura, Yoshikawa, Cho, Kanai 1996; Suzuki, Terao, and Yoshikawa 2003). This single affected monkey has multiplied to a large pedigree of more than 65 affect and 210 unaffected monkeys. Drusen were observed in the macular region as early as one year after birth, and the numbers increased and spread toward the peripheral retina throughout life. No histological abnormalities have been found in the retina, retinal vessels, or choroidal vasculatures of the eyes with drusen. However, abnormality in electroretinogram (ERG) were observed in sever case showing dysfunction of the macula.

Immunohistochemical and proteomic analyses of the drusen from these monkeys showed that the drusen were very similar to those in other monkeys with aged