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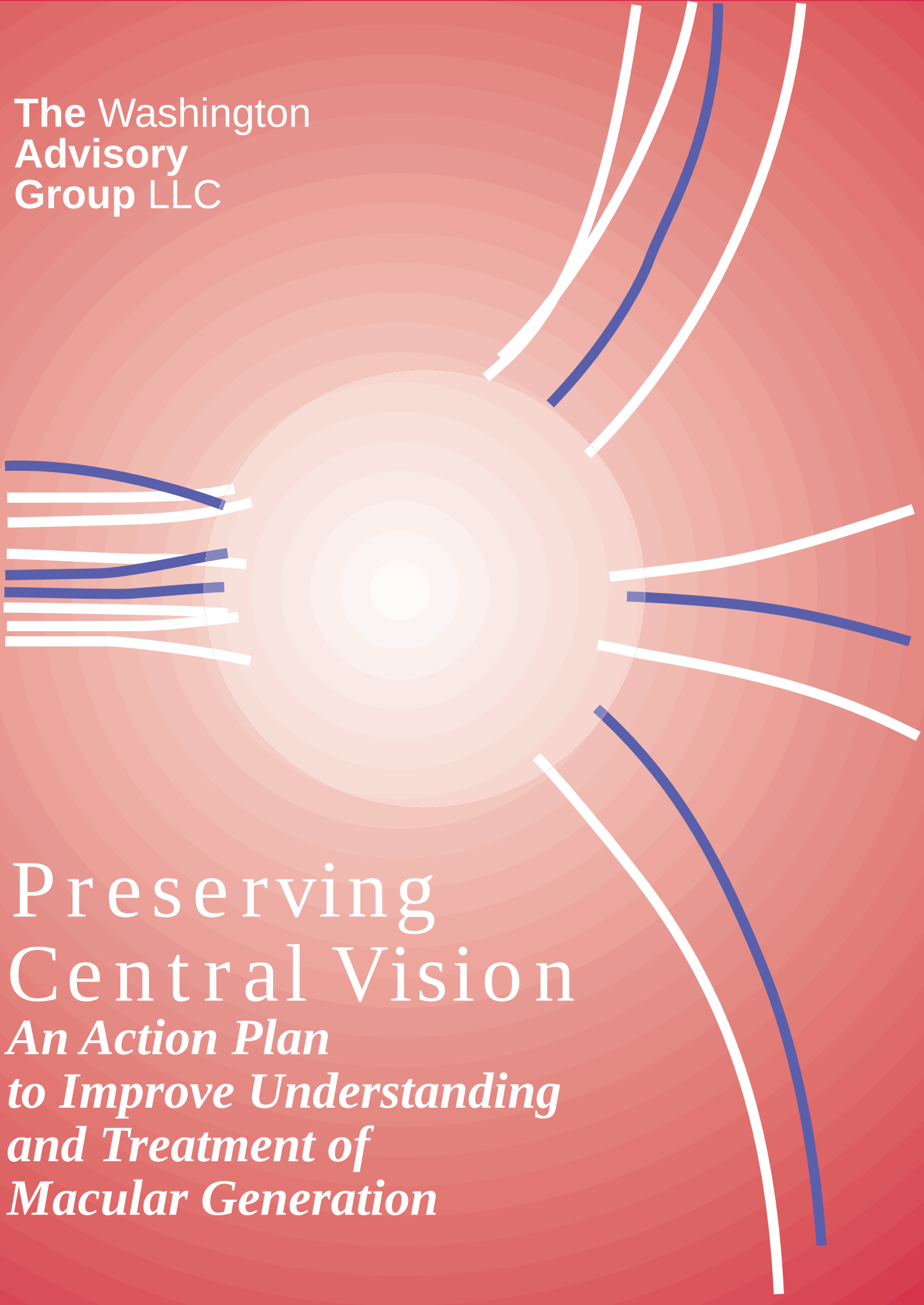

Preserving Central Vision

An Action Plan to Improve Understanding and Treatment of Macular Generation

PRESERVING CENTRAL VISION

An Action Plan to Improve Understanding and Treatment of Age-Related Macular Degeneration

Summary of a Workshop, February 25–26, 2002
Rancho Valencia, California

Workshop Co-Chairs

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Contents

Preface

A workshop on the status of research on age-related macular degeneration (AMD) was held at Rancho Valencia, near San Diego, California, on February 25–26, 2002. The workshop was funded by the Jules Stein Eye Institute Affiliates of Los Angeles, California, and organized by the Washington Advisory Group. It focused on issues in developing the fundamental knowledge about AMD needed to advance the state of medical practice, with an emphasis on improving therapeutic interventions.

The 15 participants (listed in the appendix) first heard 13 presentations on the status of AMD therapy and research. Session 1 reviewed the state of medical knowledge, including epidemiology and genetics, underlying current and proposed treatment and diagnostic capabilities. Session 2 examined in more detail the biology of the retinal structures and functions relevant to AMD disease pathways. In both sessions, the emphasis was on highlighting what remains unknown, within the context of current understanding and suggestive research results.

Session 3 focused on prospects for improved therapeutic interventions. Potential pharmaceutical interventions were discussed, as were surgical interventions to ameliorate advanced AMD. The final presentation reviewed the results of the recent clinical trial, funded by the National Eye Institute: the Age-Related Eye Diseases Study. Section 3 of this report contains summaries of the 13 presentations.

During the afternoon of the second day, the participants constructed and discussed a list of significant problems and opportunities for the AMD research community. The participants chose to present their product as an ***action plan*** for the community of investigators, funders of research (both governmental and private), and other parties interested in the goal of improved medical treatment for macular degeneration and related retinal diseases. The action plan appears in the Executive Summary in list form. The actions listed are explained and discussed more fully in Section 2.

On behalf of all the workshop participants, we thank the Jules Stein Eye Institute Affiliates for funding this endeavor. We are particularly grateful to Mr. Robert Drabkin for initiating this workshop, for his substantial efforts to ensure its success, and for his personal commitment to improving the delivery of medical knowledge to those facing the threat—or living with the reality—of lost or impaired vision from ocular diseases.

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Executive Summary

According to the National Eye Institute, age-related macular degeneration (AMD) is now the leading cause of blindness and serious vision impairment in older Americans. Nearly 1.7 million Americans have advanced forms of this disease, and 100,000 Americans are legally blind because of it. AMD is growing in significance elsewhere, as well. Although cataract remains the principal cause of age-related blindness in the developing world, AMD is taking the lead in industrialized countries, where cataract surgery is generally available. Further, as populations in industrialized countries age, prevalence of advanced AMD will increase unless effective interventions can be found.

Present treatments to lessen or avoid loss of visual function from advanced AMD remain limited. Evidence for treatments that could slow the progression of the disease at an earlier stage is just beginning to accumulate. Surgical therapies have thus far concentrated on destroying the membrane of new blood vessels that form in one of the advanced stages of the disease, but even these techniques are delaying actions rather than remedies. The genetic component of AMD, whether as the basis for conditions that initiate a disease pathway or promote its progression, is undoubtedly significant. However, no specific genes have yet been linked to it.[1]

Despite this bleak clinical and therapeutic context, the participants in a workshop on research directions formulated several principles to guide a concerted effort to improve the clinician's tools for preventing and treating this disease. First, AMD, like cancer and a number of other chronic diseases of aging, is probably a "family of diseases," which share some general clinical characteristics. Second, the genetic contribution to AMD probably differs from one form to another. Each form is likely to involve variations in a number of genes, and not necessarily the same genes for all forms. Our rapidly accumulating knowledge about the human genome, together with the techniques and technologies now available for tracing out even complex, multi-gene factors, provides new, more powerful tools for attacking the problem. Third, separate lines of inquiry and piecemeal results are beginning to link in patterns suggesting a number of pathways likely to be involved in one or more AMD forms. If a patient's particular form of AMD and that form's disease pathway are known, then key factors in the progression along that pathway can be targeted for intervention.

The workshop participants pooled their best ideas on how to apply these principles and formulated an ***action plan***, which is the subject of this report. This plan, summarized below, is explained and supported in Section 2. The technical presentations at the workshop, which stimulated the discussions leading to the plan, are summarized in Section 3.

[1] One gene locus, ARMD1 has been established. A number of genome-wide scans have been made, which may or may not point to loci.

Understanding and Treating Macular Degeneration
An Action Plan

A. Clinical Approaches

1. Classify and distinguish AMD types by criteria incorporating both genetic and clinical criteria (a dynamic genotyping/phenotyping approach) when gene mutations are known.
2. Prevention and Therapy
   - Focus on integrated intervention approaches (surgical and biochemical interventions, bio-engineered drug delivery systems).
   - Push treatment concepts from experimental results to proof of concept; establish groups for proof-of-concept clinical trials.
   - Investigate replacement/reactivation strategies for photoreceptors and retinal pigment epithelium (RPE).

B. Biology of the Macula

- Pursue detailed characterization of the macula, including foveal cone cells and RPE.
- Explore the differences between the foveal and peripheral photoreceptors and retina.
- Determine rod–cone and cone–RPE interactions in and around the macula.
- Determine factors that influence foveal cone renewal, repair, and survival.

C. Genetics of AMD

- Identify AMD-related genes and employ this genetic knowledge in AMD classification.
- Use gene transfer studies to investigate AMD pathogenesis and intervention strategies.
- Apply the tools of bioinformatics and genetic epidemiology to AMD research.

D. Pathophysiology of AMD

- Determine the major mechanisms of AMD visual loss, including rate of loss.
- Explore unresolved issues in AMD pathophysiology (composition and role of drusen, involvement of lipofuscin, antioxidants and AMD, role of macrophages in AMD, dendritic cells in the choroid, the extent to which AMD is an immune disease, etc.)
- Determine pathways in AMD with potential as intervention targets.

E. Research Resources

- Pursue an integrated approach to AMD research, perhaps through a virtual center concept.
- Seek better animal model(s) for AMD, i.e., a small foveate primate.
- Expand programs for collecting and providing access to donor eyes.
- Exploit bioinformatics as an AMD resource.

1
Introduction

Age-related macular degeneration (AMD) is a disease that impairs vision by attacking the central region of the retina needed for clear, sharp vision. The ***macula*** is an elliptically shaped area 2–5 mm in diameter, characterized by the presence of yellow pigmentation. At its center is the ***fovea***, an indented retinal area, 0.3 mm in diameter, which is specialized for high-acuity vision. The inner layers of the retina are swept aside in the foveal region (accounting for the indentation), and no blood vessels are present there. Only cone photoreceptor cells are present in the fovea, and they are the thinnest and longest photoreceptors in the retina. Underneath the entire retina is a layer of pigmented cells, the ***retinal pigment epithelium*** (RPE). In advanced AMD, abnormal material accumulates in and underneath the RPE of the central retina. Clinical manifestations of AMD, which typically appear after age 50, may include some or all of the following:

- Drusen, which are fatty deposits of varying size and morphology, and small basal deposits accumulate between the RPE and Bruch's membrane. Bruch's membrane separates the RPE from the blood vessels (the choroid) behind it. Basal deposits also form between the RPE and its basal membrane. Small, "hard" (defined borders) drusen appear to be a natural consequence of aging and are not correlated with progression to advanced AMD. Larger, "soft" (less defined borders) drusen, particularly in greater numbers, are generally considered an early precursor to advanced AMD.[2]
- Loss of patches of RPE cells and photoreceptors in the macula (called geographic atrophy) is characteristic of the form of advanced AMD called dry AMD. The functional loss (loss of visual acuity) associated with geographic atrophy depends on the extent of the atrophy and whether the fovea is affected.
- New blood vessels may develop from the choroid underneath the RPE and extend through Bruch's membrane into the space between it and the RPE. When these new vessels, called choroidal neovascular membranes, leak or rupture, the accumulation of fluid and/or blood, together with subsequent scarring, seriously impairs or destroys the photoreceptor layer. This most serious form of advanced AMD, which typically impairs central vision, is also called wet AMD or exudative AMD. Although wet AMD occurs in only 10 percent of patients diagnosed with AMD, it accounts for 80 to 90 percent of the vision loss from the disease.

AMD is now the leading cause of blindness and serious vision impairment in older Americans. A recent study estimates that nearly 1.7 million Americans have advanced AMD, defined as geographic atrophy or choroidal neovascularization (CNV) (Prevent Blindness America 2002, p. 18). The National Eye Institute estimates that 100,000 Americans are legally blind from the disease (NEI 2002). Because AMD progresses to the more advanced forms with increasing age, its incidence in an aging American population will increase unless the progression of the disease can be slowed. According to the National Eye Institute:

> As the average life span of our population increases, the number of people who develop AMD will increase dramatically in the years ahead. Unless successful means of prevention or treatment are developed, blindness from advanced AMD—and its importance as a public health problem—will increase.
>
> (NEI 2002)

In the developing countries of the world, cataract remains the principal cause of age-related blindness. But in the United States and other industrialized nations, where cataract surgery is generally available, AMD is taking the lead in age-related vision loss. For example, in the Rotterdam Study of diseases of aging, there were 32 bilaterally blind subjects and 91 visually impaired, in the study population of 8,000 over age 55 years. AMD was responsible for 55 percent of the blindness, with glaucoma second

[2] Some ophthalmologists and researchers prefer to call these early manifestations *age-related maculopathy* to distinguish them from the vision-threatening manifestations such as geographic atrophy or wet AMD (see presentation by Dr. Paulus de Jong in Section 3). This summary follows the practice of most of the workshop participants in referring to the entire range of manifestations as age-related macular degeneration, or AMD.

at just 7 percent. Cataract was still the leading cause of visual impairment, but AMD was second.[3]

Present treatments for advanced AMD remain limited, and evidence for treatments that may slow the progression of the disease is just beginning to accumulate (NEI 2002). To date, surgical therapy has concentrated on destroying the membrane of new blood vessels in wet AMD with light energy (lasers). These techniques succeed, at least for a time, if the new vessels have not reached the fovea. However, the laser treatments result in the immediate or ultimate destruction of the overlying retina. Surgical removal of the membranes from underneath the retina has so far not been successful; perhaps the simultaneous implantation of RPE cells may help, but achieving useful visual function with implants has proven difficult.[4]

The action plan for AMD research presented here resulted from a workshop of AMD research scientists and ophthalmologists held in February 2002. The workshop participants agreed that an important aspect of the AMD challenge is that it probably is many diseases, which share some commonalties in pathways and endpoints. In addition, although AMD clearly has a genetic component, the search for genetic factors thus far strongly indicates that the genetic component is multifactorial. This means that many genes are likely to be involved, whether in initiating conditions that can lead to AMD or in promoting or mitigating any number of events along one or more disease pathways.[5] Unraveling the disease pathway of a condition that develops gradually over decades, late in life (a "late-onset disease"), and that probably has diverse initiating conditions and influencing factors presents a daunting challenge to medical investigators.

Nonetheless, the participants also agreed that ***the time is ripe*** to accelerate progress, not just in understanding AMD but also in preventing or retarding the disease therapeutically. One participant compared the state of knowledge, as highlighted in the presentations summarized in Section 3, to that in the retinitis pigmentosa field 25 years ago. From the discussions of the presentations and the directions forward suggested by them, the participants constructed an ***action plan*** for a concerted, coordinated effort to achieve similar progress in understanding and treating age-related macular degeneration.

[3] Reported to the workshop by Dr. Paulus de Jong. See presentation in Section 3.
[4] See presentation by Dr. Robert Machemer, Section 3.
[5] This point is explored in the presentations by Drs. Edwin Stone and Peter Campochiaro in Section 3.

2
An Action Plan for Accelerating Progress on AMD

This section expands on the action items listed in the summary table at the end of the Executive Summary. Letters rather than numbers are used for the major headings in the table to emphasize the workshop participants' view that no one of the actions is a higher priority than the others. The synergistic interactions among them will be more important than the results from any one action undertaken in isolation. For this reason, the discussion of actions stresses interconnections among them.

A. Clinical Approaches

A.1. Classify and distinguish AMD types by criteria incorporating both genetic and clinical criteria when gene mutations are known.

A theme that arose repeatedly during the workshop discussions was the need for an AMD classification that represents what is being learned about the similarities and differences in AMD pathophysiology. If AMD really is a group of diseases—in the sense that different factors or different pathways occur in different groups of cases diagnosed as AMD—then these differences and their underlying mechanisms need to be identified and eventually understood. Under headings C and D (Genetics of AMD and Pathophysiology of AMD), many candidate factors and mechanisms are represented. The sense of the workshop was that the correct question to ask is not "Which of these is the correct explanation of AMD?" but rather "What role does each of these play, and in which cases of AMD?"

This classification must reflect both clinically observable differences in AMD cases and genetic differences that affect one or more factors in an AMD stage or pathway. The ultimate goal is to establish a definitive relationship between genetic factors (represented by distinct genotypes) and their medically significant expression in the physiology, histology, molecular biology, or biochemistry of patients with AMD (distinct AMD phenotypes). A useful classification cannot be completed in one step. It will require an iterative, reciprocal process of determining (a) which genetic factors are significant, by correlating them with phenotypes that have observable significance for AMD; and (b) which observable differences or similarities in AMD cases correlate to a genetic difference or similarity (a genotype) or to an independent factor that is not genetic.

At present, the only widely accepted classification for advanced AMD is simply as either "wet AMD" or "dry AMD" (or the synonymous technical terms for these conditions, given in the Introduction). Drs. Ronald Klein and Paulus de Jong wrote an international classification system for prevalent age-related maculopathy (ARM) that encompasses all stages of ARM including the end stage of wet or dry AMD. At the workshop, Dr. de Jong noted that the Rotterdam Study had to propose its own classification for stages in incident ARM because there was no widely accepted international classification for incident ARM or AMD. In the recent Age-Related Eye Disease Study (AREDS), stages of macular pathology similar but not identical to the Rotterdam classification were used. The workshop participants agreed that these general stages cannot be viewed as one universal pathway in AMD development.

Thus, a classification grounded in both relevant genetic differences (genotypes) and medically significant differences in physiological effect (disease phenotypes) remains an ambitious undertaking. Much of the work described under headings B through E of this section can contribute to developing a classification. But the classification is not an end in itself. It will be an essential tool for developing and applying therapeutic approaches. These approaches will recognize that AMD is a heterogeneous, multifactorial "family of diseases," which probably occur by diverse pathways. Treating it effectively will require interventions keyed to correctly identifying the particular form that threatens an individual patient.

A.2 Prevention and Therapy

Focus on integrated intervention approaches (surgical and biochemical interventions, bio-engineered drug delivery systems).

Many attempts have been made to treat AMD. No medical therapy to prevent the disease is available. Generally recognized risk factors for

AMD are age, family history of AMD, and smoking. Surgical treatments are presently addressing only a fraction of the diseased population (those with wet AMD). Surgery can eliminate a symptom or consequence of AMD, not the disease itself.

Current surgical treatments, despite their limitations, are being used because physicians need to do something now to help patients with advanced AMD. Pharmaceutical therapies are still a number of years away, at best. Photocoagulation, a surgical treatment that uses a laser to seal off and destroy the new blood vessels in wet AMD, can destroy visual function in the effort to stabilize the disease. Photodynamic therapy attempts to limit the damage to surrounding retinal tissue by absorbing the laser energy only in the new blood vessels. Yet, for both of these laser treatments, recurrence of CNV (choroidal neovascularization) is common because the factors remain that induce new blood vessels to form. Similarly, surgical removal of the neovascular membrane from underneath the fovea has been disappointing.

Surgical translocation of the retina originated from the idea of moving the macula and fovea to a healthier portion of the underlying RPE, after removing the CNV membrane. Two variants were described at the workshop. In one, the entire retina is detached and rotated (see presentation by Dr. Robert Machemer). In limited translocation, a smaller area of retina is detached, and the macula is not moved as far (see presentation by Dr. Eugene de Juan). Both techniques are appropriate only for patients who already have substantial vision loss from wet AMD or where such damage is imminent. Retinal transplants and implants were also described during the surgical treatments portion of the workshop.

A common element in all the surgical therapies is that they represent a last-ditch attempt to preserve useful vision when CNV already exists. Because AMD appears to progress to this stage gradually over several decades, earlier pharmaceutical intervention could be effective even if it only slows the progression. At this point, the heterogeneity of AMD and the likelihood that there are many pathways by which it progresses in different patients become important. Therapeutic intervention could target one or more key steps in the pathway to retinal cell damage that precedes advanced AMD. The classification described above is essential for determining which points along that patient's AMD pathway to target for intervention.

A range of potential pharmaceutical therapies may prove useful. Pharmaceutical therapy for a degenerative disease like AMD can be defined as treatment with any agent, natural or man-made, that will slow the course of the disease. Surgical therapies may still be relevant, either to treat advanced AMD, as in the techniques described above, or as a means of controlling a contributing factor or condition in a pathway to macular cell damage. Integrated intervention approaches, including bioengineered drug delivery systems, can target a disease pathway with precision. For example, a capsule of RPE cells bioengineered to overexpress a neuron-protection agent might be surgically implanted. This emerging therapy is called "encapsulated cell technology." It and other gene-transfer techniques provide potential clinical approaches to controlled delivery of a pharmaceutical agent to a precise location in the retina for an extended time.

A number of potential strategies for pharmaceutical therapies are described under heading D (Pathophysiology of AMD) below. Each strategy is premised on the importance of a particular AMD pathway, which may vary with the disease phenotype.

Push treatment concepts from experimental results to proof of concept; establish groups for proof-of-concept clinical trials.

A proven, useful classification of AMD phenotypes will make it easier to move treatment concepts from experimental results to proof-of-concept trials. Dr. Edwin Stone suggested in his workshop presentation that successful clinical trials for an AMD intervention must incorporate three principles:

1. **Heterogeneity of AMD** as a "family of diseases" with different pathways. Unless the trial groups are selected to reflect the pathway relevant to the particular intervention strategy, a positive signal from intervention is likely to be lost in the "noise" of nonresponse from cases representing other pathways (other AMD phenotypes).
2. **Sequential attack** on well-characterized variants of AMD. An early intervention effective for all AMD phenotypes is unlikely if AMD occurs by diverse pathways. Testing interventions targeted to a specific point on an established pathway of a well-characterized disease

phenotype is more likely to be fruitful in the long run, even if the phenotypes initially attacked occur in only a fraction of the total AMD population.

3. **Optimal timing** of intervention and testing for effect (Figure 1). If, as seems likely, AMD variants progress gradually and cumulatively in their early to mid stages, the difference between recognizing a positive effect of intervention versus "no effect" may depend on when the intervention occurs and the response is measured.

Each of these principles requires that the trial groups be selected on the basis of well-characterized AMD phenotypes.

Investigate replacement/reactivation strategies for photoreceptors and RPE.

Damage to photoreceptors or RPE cells appears to be important in the pathways of some, if not all, AMD phenotypes. In addition, there is no current therapy for geographic atrophy, in which patches of photoreceptors and RPE cease normal function and eventually die. Strategies for replacing and/or reactivating nonfunctioning photoreceptors and RPE cells would address both of these issues in AMD therapy. (Protecting photoreceptor and RPE cells from damage or death with neurotrophic agents is discussed further under heading D.)

B. Biology of the Macula

Pursue detailed characterization of the macula, including foveal cone cells and RPE.

Unlike the rest of the retina, the human fovea contains only cone photoreceptors, the cells responsible for color vision. Cone photoreceptor density is greater in the fovea than in the peripheral retina. The area of the macula just beyond the fovea, the perifovea, is also densely packed, but mainly with rod photoreceptors (which support black and white vision).

The outer segments of foveal cone cells are twice as long as cone cells elsewhere (Figure 2). They have a roughly constant diameter, like that of a rod outer segment (about 1 micron), rather than the tapered appearance of nonfoveal cones. The axons from foveal cones are much longer than photoreceptor axons in general. The neurons to which they connect are located further away, over the perifovea. Thus, cone cells in the fovea are anatomically distinct from cone cells elsewhere.

Our knowledge of foveal cell biology, as opposed to general rod and cone cell biology, is rudimentary. (See the presentations by Drs. John Dowling and Steven Fisher). The known

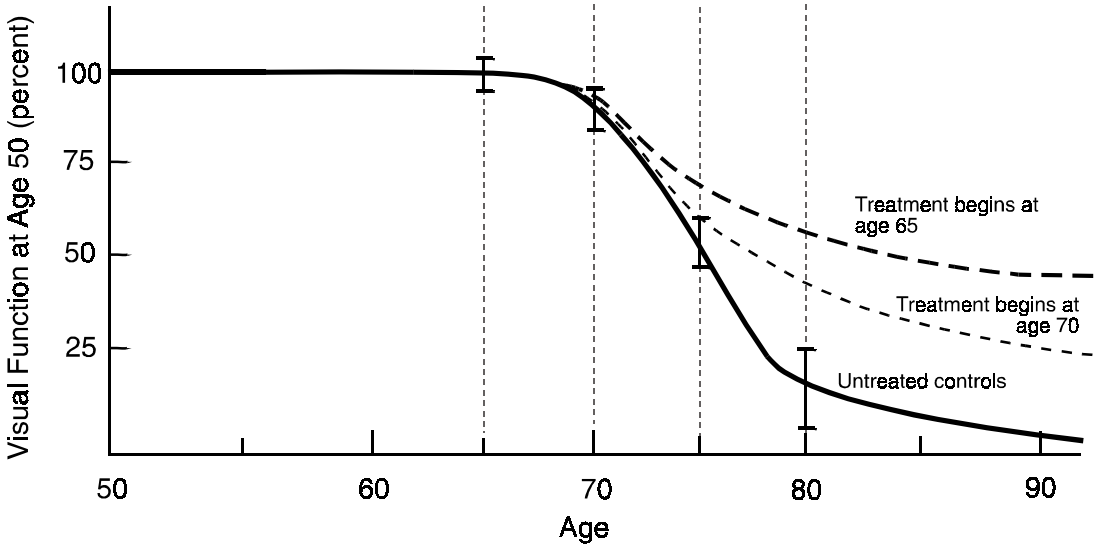

Figure 1. Concept of optimal timing in drug intervention studies of a slowly developing disease such as AMD. In this hypothetical example, if treatment begins at age 70 and effect is measured after 5 years, the difference from controls may be too small to be significant against normal population variability (represented by error bars on control curve). Even if treatment begins at age 65, measurement of effect at age 70 may not be significant against controls. In both cases, however, continued treatment significantly reduces loss of visual function in old age.

differences in foveal and macular physiology raise important questions that may shed light on initiating events or subsequent pathways that lead to AMD. For example, the length of foveal cone outer segments indicates that the underlying RPE cells may have to digest twice as many discs from the end of the continually renewed outer segment, in a given time, as do RPE cells elsewhere. The metabolic rate of photoreceptor cells is an order of magnitude higher than that of neural cells generally, yet the center of the fovea lacks the fine network of retinal blood vessels. All the oxygen and nutrient supply to, and waste product removal from, foveal cones comes from the choroidal circulation.

For these and other reasons cited in the workshop presentations, foveal and macular retinal cells may be subject to biochemical stresses that increase more as the retina ages than they do for retinal cells in the peripheral retina. These stresses may initiate or promote AMD pathways.

Explore the differences between the foveal and peripheral photoreceptors and retina.

In addition to increased metabolic stresses, other factors contributing to photoreceptor "sickness" in the macula may be differences in the molecular biology of foveal cones and macular rods, as compared to photoreceptors in the peripheral retina cells. Among the possibilities are genetic differences in enzymes involved in the cycle for regenerating the light-sensitive protein complexes (photopigments) that give photoreceptors their sensitivity to light (and thus their role in vision).

A mutation in the gene for an enzyme involved in transporting vitamin A aldehyde (retinal) between the photoreceptor and RPE cell has been identified as critical in Stargardt's disease. (Retinal serves as the chromophore [light-sensing portion] of the photopigments.) In this disease, large amounts of lipofuscin accumulate in the RPE, but the initiating defect has been traced to the photoreceptor outer segments. Stargardt's disease has been suggested as a model for AMD because a constituent of lipofuscin called A2E, a byproduct of the accumulated retinal in Stargardt's disease, has been implicated in at least some forms of AMD. Still unknown is whether this particular transporter enzyme or others in the retinal cycle are specifically involved in AMD pathways.

Large, soft drusen in the macula are associated with AMD incidence. These drusen contain lipids and proteins associated with immune-mediated pathways, similar to immune responses implicated in early stages of arterial heart disease (discussed further under heading D). Understanding the metabolic pathways by which drusen components accumulate may lead

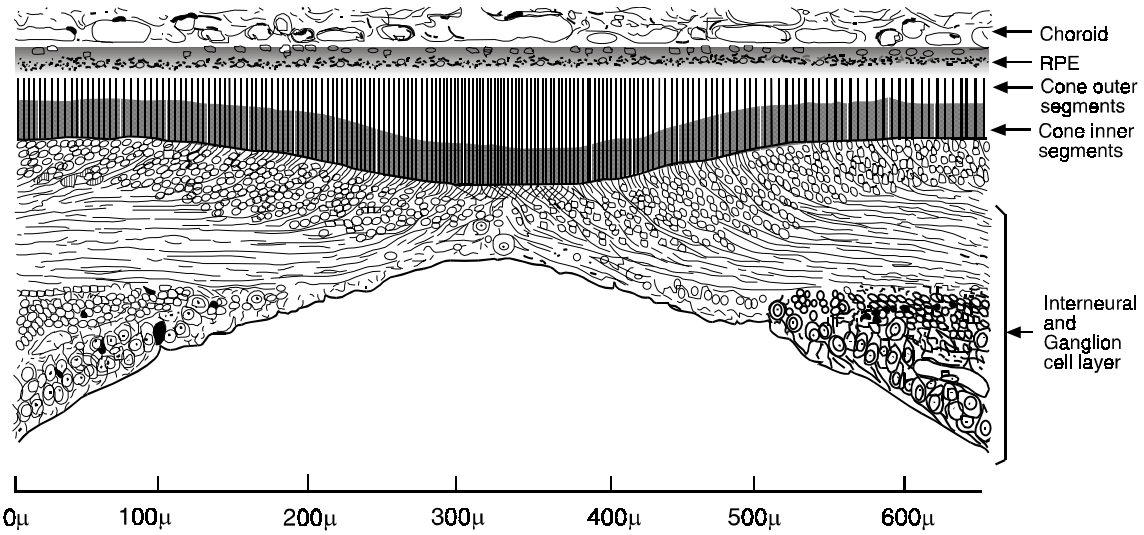

Figure 2. Central fovea of the adult human eye. The choroid and retinal pigment epithelium (RPE) are at the top of the drawing, the vitreous is at the bottom. The thin, elongated cones of the fovea can be seen above the foveal excavation (the displacement of secondary neuronal cells to the periphery of the fovea). Adapted from Polyak 1941, Figure 38. Reprinted with permission of University of Chicago Press. All rights reserved.

to the identification of other enzyme defects—and their underlying gene mutations—as factors in some AMD phenotypes.

Determine rod–cone and cone–RPE interactions in and around the macula.

The rod cells in the perifovea may be influential in some AMD pathways. There is evidence that these rods may become "sick" in a way that leads to heavier than normal accumulation of drusen and basal deposits between the RPE and Bruch's membrane. Even in diseases that are considered to be cone diseases, rods seem to be differentially affected and perhaps affected first. The interactions between the large number of rods surrounding the fovea and the cones within the fovea, perhaps mediated by rod interactions with the RPE, constitute an area about which little is known. If rods are more susceptible than cones to stresses that initiate pathways to AMD, that would be important information for both early diagnosis and potential therapeutic targets.

Three major interactions occur between photoreceptors and the RPE cells beneath them. Each RPE cell engulfs and digests (***phagocytosis***) the outermost discs of the outer segments as they are shed from the photoreceptors. This process recycles cell materials such as photopigment components and cleans up unwanted byproducts from photoreceptor activity. If processes in the photoreceptors become abnormal, the RPE cells may not be able to handle the full load of discs to be digested or byproducts may become more difficult to clean up. Unrecycled material may accumulate, and this may be the origin of drusen in their various forms. Questions that are still unanswered include: Are there differences in the phagocytosis of rod discs in the perifovea that are related to the initiation of AMD pathways in this region of the macula? Is the rate of phagocytosis for outer segments of foveal cones similar to that measured in peripheral rods? How are metabolic requirements of foveal cones affected by the need to turn over a longer outer segment than occurs in peripheral cones?

A second interaction between RPE cells and photoreceptors is the recycling of components used in photopigments by transporting them to and from the RPE cells for regeneration of the active form. There is evidence of subtle physiological differences between the outer segments of foveal and peripheral cones. These differences could become significant if, for example, a mutation affects an enzyme involved in the regeneration cycle or if the foveal cones are under stress from other factors.

A third interaction is the general role of RPE cells in the passage of nutrients and oxygen from the choroid to the photoreceptors and of metabolic waste products in the opposite direction. The lack of a retinal circulation in the region of the fovea means that its photoreceptors are completely dependent on this link to the choroid through the intervening RPE. If RPE cells under the fovea are stressed or damaged by, for example, abnormally functioning rods in the perifovea, how might that affect their role in feeding and cleaning the foveal cones? Do these interactions affect the development of AMD?

These questions illustrate just a fraction of what is not yet known about the rod–cone and photoreceptor–RPE interactions in and around the fovea and macula. There are suggestive morphological differences in the macula. The extracellular matrix around cones differs from the matrix around rods. There are also differences in the biochemical composition of the matrix around cone outer segments between cones inside and outside the fovea, as well as biochemical differences between rod and cone matrices.

Another suggestive area concerns the metabolic capacity of photoreceptors. The inner segment of all photoreceptors is packed with mitochondria (cell organelles that supply energy through aerobic oxidation). The measured metabolic rate of photoreceptors is among the highest of any cell type in the mammalian body. Cones have more mitochondria than rods, and their metabolic rate has been measured to be 15 times higher than rods. Do foveal cones have this same high metabolic rate? Are foveal cone outer segments more rod-like in structure, and perhaps in function, than has been assumed? What is the metabolic oxygen requirement for foveal cones relative to peripheral cones or rods? As will be discussed below under heading D, answers to these questions may clarify mechanisms by which AMD begins and progresses.

Determine factors that influence foveal cone renewal, repair, and survival.

Studies of retinal detachment in animals suggest that cone photoreceptors in general have a significant but limited capacity to recover from injury sustained when they are separated from the RPE. Despite these positive implications for cone recovery, which factors promote or inhibit recovery—and whether foveal cones show

similar recovery behavior—are questions still to be answered.

The renewal cycle of foveal cone outer segments is another topic of interest. Both rods and cones undergo a cycle of forming outer segment discs at the base of the outer segment and shedding discs at the tip (where the RPE cells remove them by phagocytosis). For cones and rods outside the macula, the time from a disc being formed until it is shed is about 10 days. The renewal rate for outer segments seems to be the same for cones and rods, but this has been difficult to measure precisely. Whether it is also the same for foveal cones, which have longer outer segments, is another uncertainty.

A variety of compounds can affect nerve cell growth and activity, including cell death. Collectively, these compounds are called ***neurotrophic factors***. A number of these factors are known to influence photoreceptor survival or their repair and recovery after injury or stress. (See discussion under heading D and the presentations by Drs. Gerald Chader and Peter Campochiaro.) However, much less is known about the differential effects they may have on rods and cones, or whether there are differences in effects on foveal or macular receptors versus those in the retina outside the macula.

C. Genetics of AMD

Identify AMD-related genes and employ this genetic knowledge in AMD classification.

When AMD patients are asked about AMD in other members of their family, about 23 percent know of other family members who either were diagnosed with AMD or have had AMD-like visual impairment. This strong family association indicates a genetic predisposition to AMD. The failure of the search, during the past two decades, for single-gene defects responsible for a substantial fraction of AMD cases suggests that many genes are probably involved. (See presentation by Dr. Edwin Stone.) Different combinations of genes, or different variations in the same genes, are likely to be involved in different AMD phenotypes. Is there good reason to continue the pursuit of a genetic understanding of AMD, given the multiplicity of disease pathways and complexity of genetic factors that appear to be involved?

The answer from the workshop participants was an emphatic"yes," provided that we accept the need for, and the complexity of, the task of determining phenotypes that reflect the underlying genotype (***genetically based AMD phenotypes***). Once the genotypes associated with distinct AMD phenotypes are identified (as discussed under heading A.1 above), then specific therapies can be developed to attack targets in the disease pathway relevant to a distinct phenotype. This genetic approach, which is based on building linkages between observable disease forms and their genetic basis, has the following favorable elements.

First, finding genes that play a role in AMD phenotypes can provide clues to the mechanisms at work in causing those phenotypes. "Small molecule" therapies—in which the therapeutic agent is smaller than an entire enzyme protein—are likely to come first. They will make use of the genetic knowledge about a phenotype to overcome a deficiency in, or inhibit a negative consequence of, genetically controlled mechanisms. These interventions need not eliminate the disease-related process completely, or even halt the progression of the disease entirely. To be therapeutically useful, they only need to delay vision loss from advanced AMD for a significant time; for example, from age 65 to age 85.

Second, identifying genes involved in at least some AMD phenotypes would allow medical researchers to construct useful models for conditions that represent stages or mechanisms involved in the progression toward AMD. These laboratory models may be cell or tissue cultures (in vitro models) or animals that have been altered genetically, pharmacologically, or surgically to have conditions like those found in AMD patients (animal models). Genetic alterations in test animals, using gene transfer techniques, represent a particularly powerful model for testing a suspected connection between one or more genes and an AMD stage or predisposing condition.

Third, genetically based phenotypes would be valuable for epidemiology and clinical trials. They could be used to separate an AMD population, broadly defined, into groups that are more homogeneous with respect to the mechanism causing their AMD condition (or predisposition). Results from the epidemiologic studies and clinical trials would in turn help to validate and refine the linkages between genetic composition and AMD phenotypes.

Fourth, genetically based phenotypes may be essential for designing clinical trials with an appropriate expected effect curve (what counts as a positive effect of a therapy being tested, and what does not) and with optimal timing of treatment and measurement of effects. If the

effect curve and the timing are not grounded in the underlying mechanism of the disease, then therapeutically positive results may go unnoticed.

A fifth element favoring this genetic approach is the longer-term prospect for the use of genetic screening to identify a predisposition and begin preventive therapy years, even decades, earlier than is now possible. In the current conventional diagnosis for AMD, the patient is already far down the path of disease progression before clinical observation establishes that intervention is needed.

The protein expressed by a gene may inhibit, as well as initiate or promote, a disease mechanism. Genes that code for factors that inhibit disease progression are called ***mitigator genes***. In a disease like AMD, where multiple genes are likely to be relevant to the gradual progression of the disease, mitigator genes are likely to be involved. They are important in understanding why individuals with the same predisposing conditions either never develop advanced AMD or progress toward it more slowly. They also provide "hints from nature" on therapeutic approaches that have a high likelihood of success. The genetic approach outlined above provides techniques for identifying these mitigator genes.

Use gene transfer studies to investigate AMD pathogenesis and intervention strategies.

Gene transfer studies using animal models are an important tool for testing proposed relationships between genotypes and AMD phenotypes. For example, if clinicians agree on a set of candidate AMD phenotypes, genetic screening of perhaps a hundred individuals having each phenotype might turn up a handful of prospective genes for some of the screened phenotypes. Transferring one of these candidate genes into several strains of one model species is likely to give differential expression of the phenotype-relevant condition, depending on the presence or absence of other relevant (promoting or mitigating) genes in each strain. Examining the genetic differences among the strains, including strain back-crosses, would help to identify promoter or inhibitor genes. The results from the animal models could then be used to refine the definitions of AMD phenotypes in humans and inform studies of the disease mechanisms involved in them. These results would feed into a second round of genetic screening by candidate phenotypes, and so on.

A number of animal models are needed because there are different aspects of AMD to be modeled, and these aspects may be relevant to different AMD phenotypes. At the workshop, Dr. Peter Campochiaro discussed rodent models with the following features that appear relevant to AMD phenotypes: (1) thickening of Bruch's membrane and increase in drusen-like deposits, (2) lipofuscin changes, (3) photoreceptor cell death, (4) cell death in choroidal capillaries, and (5) neovascularization coming from either the inner retinal circulation or from the choroid. Both Dr. Campochiaro and Dr. Chader discussed the relation of these models to potential AMD pathways.

An issue about appropriate animal models for gene transfer studies was raised and discussed at the workshop but not resolved. The models mentioned above are all in rodents (laboratory mouse or rat strains), which lack a macula or fovea. As noted under heading B, macular and foveal photoreceptors, RPE, and Bruch's membrane differ in details of structure and activity from their counterparts outside the macula. If any of these differences influence key events in an AMD stage or mechanism, the relevant effect may not occur, or may be more difficult to observe, in an animal without these distinctive features. Some birds (notably chickens) have a fovea-like retinal structure for acute central vision, but the only mammals known to have a human-like macula and fovea are primates. A counter-argument is that no single animal model is likely to provide a complete model for human AMD (particularly if there are actually a number of distinct AMD phenotypes). Given this constraint, gene transfer studies using rodents provide affordable partial models to study how a specific aspect of a condition or pathway relates to a known genetic difference.

Apply the tools of bioinformatics and genetic epidemiology to AMD research.

The Human Genome Project is arguably one of humankind's most significant scientific undertakings. With it, a computer search can be used to design an assay for a gene. The downside to this success story is that the amount of information available for doing such a search is vast. The tools needed to harness the power of this information about the human genome are part of the emerging field of ***bioinformatics***.[6]

[6] The National Institutes of Health defines "bioinformatics" as research, development, or application of

With these computational tools, researchers can identify a gene's locus (the gene's approximate location on a specific chromosome) and acquire the information needed for transferring the gene to an animal model.

Bioinformatics is therefore a key enabling technology for the genetic approach outlined above for developing genetically based AMD phenotypes. The workshop participants see bioinformatics being combined with differential gene expression technologies (such as animal models created through gene transfer techniques) and proteomics to address the following important issues in AMD research:

- Why do drusen form, and what determines their composition?
- Why do photoreceptor and RPE cells become sick or die at various AMD stages?
- What are the mechanisms through which factors promote photoreceptor or RPE cell survival and recovery?
- What specific insults or factors initiate new vessel formation (neovascularization)?
- What are the mechanisms by which anti-neovascular agents act?

Another tool rooted in genetics that will be necessary for differentiating medically significant AMD phenotypes is ***genetic epidemiology***, the study of the distribution of a disease within a study population or within a family. As the genetic influence in AMD progression has become more important, studies of twins and siblings are likely to be more productive than broad-based population comparisons of AMD prevalence at a given time or of AMD incidence over time. In the Rotterdam epidemiological study, if one sibling had advanced AMD, the risk ratio of advanced AMD for other siblings was 4.2 and they tended to develop the disease at a younger age. Among all families with AMD, siblings had a 50 percent lifetime risk of developing AMD, whereas controls had a 12 percent risk. For family members from families with AMD cases, the risk varied considerably, from nil to a 30 times higher risk. Although these results support the importance of a genetic role in AMD, better characterization of AMD phenotypes will be necessary to identify gene loci and candidate genes.

computational tools and approaches for expanding the use of biological, medical, behavioral or health data, including those [computational tools and approaches] to acquire, store, organize, archive, analyze, or visualize such data (NIH 2002).

D. Pathophysiology of AMD

Determine the major mechanisms of AMD visual loss, including rate of loss. Explore unresolved issues in AMD pathophysiology.

During the workshop presentations and discussions, five broad issues concerning candidate AMD disease pathways were addressed. As the summaries below indicate, these pathways and mechanisms need not be mutually exclusive.

Oxidative Damage and Lipofuscin

To do their job, the photopigments in photoreceptors must change chemically in response to light. Their high reactivity also makes them susceptible to oxidation reactions. When these side reactions occur, byproducts of the photopigment renewal cycle form. These byproducts may go on to react with other molecules, upsetting normal physiological functions. So that they do not accumulate and continue interfering with useful functions, the products of these reactions must be cleared and degraded by specialized cells. The entire chain of events from oxidation side-reactions through interference with normal functions constitutes ***oxidative damage***.

Oxidative damage to the RPE may initiate pathways to AMD. (See presentation by Dr. Marco Zarbin.) Lipofuscin appears to result from oxidation byproducts that accumulate in the discs of photoreceptor outer segments as they age.[7] If the RPE cells cannot break down all these oxidation byproducts during phagocytosis of discarded discs, a reactive residue accumulates in the RPE cells or perhaps outside them. Lipofuscin sensitizes the RPE to light, which means it makes components of the RPE cell absorb light energy more easily and become more susceptible to oxidative damage. When the concentration of lipofuscin becomes high, it

[7] A product of light absorption by photopigments is the form of retinal called all-trans retinal. All-trans retinal reacts with lipids in cell membranes to form a substance called A2E, which resists degradation into easily recycled products. A2E is certainly present in typical lipofuscin, and some researchers describe it as constituting lipofuscin, but lipofuscin, as the term is used by clinical pathologists and histologists, appears to be more than just A2E.

causes cells to function abnormally. For example, phagocytosis of discs is reduced. (This may, for example, further increase the accumulation of undigested disc material and oxidative byproducts.) Oxidation products can also damage the mitochondria and membranes of microsomes inside the RPE cells.

The environment at the tip of the outer segment has a high oxygen tension (pO_2= ~120 mm), similar to that of arterial blood, and is thus highly oxidative. Lipofuscin will form more readily in this environment and be more stable (harder to digest) than in the environment at the base of the outer segment, where the oxygen tension is similar to that of venous blood (pO_2= ~30 mm).

Lipofuscin, Drusen, and Basal Deposits

Large, soft drusen in the macula, particularly if numerous, appear to be signs of an early stage in at least some pathways to advanced AMD. However, the role of drusen is unclear. The workshop participants generally supported the view that drusen should be viewed as local biomarkers of a pathologic process that is much more global (for example, accumulating extracellular material in the macula and peripheral retina). Drusen may or may not be directly involved in the disease pathway.

- The distribution of lipofuscin in the retina correlates with the distribution of drusen. However, the role lipofuscin plays in the formation and density of drusen is not yet established.
- The Rotterdam study found that, the greater the amount of large drusen or extent of drusen at a preceding examination, the more likely a patient was to have progressed to a later disease stage. The 5-year risk of AMD varied from 0 percent for people with no ARM (age-related maculopathy) at baseline to 42 percent for an octogenarian with advanced ARM. This study also found that patients with more than 10 small hard drusen had a greater likelihood of progressing to ARM.

One hypothesis is that drusen are increased by lipofuscin production that the RPE cells cannot handle. Thus, questions about the role of drusen in AMD pathogenesis come back to issues about lipofuscin: What is it, and how may it be involved in AMD progression? Are there different types of lipofuscin?

- In some retinal abnormalities such as pattern dystrophy, a great deal of lipofuscin accumulates but visual acuity remains high. Some lipofuscin is autofluorescent (it absorbs light at one wavelength and emits it at another), while some is not. Thus, there are different types (compositions) of what investigators call "lipofuscin."
- The data indicating that lipofuscin accumulation is deleterious are specific to lipofuscin containing the oxidation product A2E, which absorbs light near the wavelengths (energy region) at which rhodopsin (the photopigment used by rod photoreceptors) absorbs.

A different role suggested for drusen is that they may cause hypoxia, or insufficient oxygen, in the environment of the photoreceptor cells. (This possibility need not exclude the possibility that drusen indicate other deleterious effects, such as accumulating basal deposits.) Dr. Steven Fisher described his work on donor eyes with high concentrations of drusen but no CNV (choroidal neovascularization) or even a diagnosis of AMD. The amount of photopigment decreased in both rods and cones in the high-drusen areas. These photoreceptors also had fewer synaptic terminals. The details suggest that drusen (or a condition occurring with them) are more toxic to photoreceptors than even the hypoxia associated with retinal detachment. The drusen may represent a physical barrier to oxygen reaching the photoreceptors, or there may be other toxic effects involved.

Photoreceptor Cell Death or Sickness?

Degeneration of photoreceptors in the macula appears to be a critical step in the pathway to CNV. An unresolved issue raised at the workshop was whether the condition that induces neovascularization is photoreceptor "sickness," which may precede cell death. If changes in the extracellular matrix and other events stress the photoreceptor cells to the point that they function abnormally for a considerable time before they die, this distressed state may trigger physiological "rescue" processes that, in the circumstances of AMD, lead to even worse damage.

Other lines of evidence suggest that stressing conditions, or even the abnormal functioning of the photoreceptors, may distress or "sicken" the RPE cells. Distressed RPE cells may no longer be able to handle the full load of disc digestion and photopigment recycling demanded of them, leading to (further) distress

in the photoreceptors they service. Signals from distressed RPE cells may also trigger immune response events that weaken Bruch's membrane as a barrier to new vessel growth from the choroid.

Bruch's Membrane as a Barrier to Choroidal Neovascularization

Bruch's membrane appears to play a critical role in pathways to wet AMD through proliferation of new blood vessels from the choroid. If there is calcification and rupturing of Bruch's membrane for any reason, the patient is at very high risk of developing CNV. Even a biochemical breach in this barrier from an abnormality in the extracellular matrix, with no physical rupture of the membrane, may allow choroidal blood vessels to grow into the space between Bruch's membrane and the RPE. (See presentation by Dr. Campochiaro on Animal Models.) The following points highlight the many observation-based findings and more speculative hypotheses discussed at the workshop concerning Bruch's membrane and its potential roles in AMD pathways:

- Whether advanced AMD progresses to CNV or to geographic atrophy (dry AMD) may depend on whether the barrier function of Bruch's membrane is compromised. (See presentation by Dr. Gregory Hageman.)
- The accumulation of basal deposits between the RPE and Bruch's membrane appears to affect the extracellular matrix of the latter in a number of ways.
- Elastin-containing membranes act as barriers to blood vessel proliferation. The elastin layer in Bruch's membrane is much thinner under the macula than it is elsewhere in the retina. At the fovea, the elastin layer is almost immeasurable, even in normal (non-AMD) eyes. There is also evidence that the elastin layer is generally thinner and more porous in donor eyes with AMD. (See presentation by Dr. Hageman.)
- Immune responses and inflammatory responses (complement cascade) occur on the choroid side and the RPE side of Bruch's membrane in cases where CNV develops. Lymphocytes or other cells activated by an inflammatory response may degrade the elastin layer in Bruch's membrane, allowing new vessels from the choroid to breach it. (See discussion below and presentation by Dr. Hageman.)
- Certain growth factors are known to promote neovascularization. When cellular production (expression) of one of these factors (vascular endothelial growth factor, VEGF) was stimulated in mice with intact Bruch's membranes, they developed neovascularization from the inner retinal circulation but not from the choroidal circulation. If Bruch's membrane was ruptured, CNV occurred. (See presentation by Dr. Campochiaro—Animal Models.)

Role of Immune Response

Drusen contain proteins, many of which are associated with processes of inflammation, coagulation, and fibrinolysis. A number of these proteins appear to be produced locally rather than circulating in the blood. (They are HLA-2 antibodies that are typically membrane bound.) Where do they come from? Dr. Dean Bok described work with cultured RPE cells indicating that RPE cells produce these and other antibodies when stimulated with gamma-interferon. Thus, antibody production could be part of the response of distressed RPE cells.

From studies of donor eyes with various stages of AMD and AMD-related conditions, Dr. Hageman has found that these and other immune response proteins constitute the bulk of the proteins in drusen. They may activate a complex sequence of immune system responses, including a complement cascade. Complement cascades are a basic physiological reaction to foreign cells, dead and sick cells, or cell fragments. Their normal function is to recognize things that should not be in the body and dispose of them. However, if activation of this inflammatory response continues for an extended period, it could cause significant local damage, such as breaching Bruch's membrane and promotion of new blood vessel growth.

- Accumulating oxidized lipoprotein by-products of photopigment cycling, such as those found in lipofuscin and drusen, could trigger a complement cascade. The mechanism would be similar to what occurs in early stages of atherosclerosis (thickening of artery walls).
- Basal deposits between the RPE and Bruch's membrane or RPE cell fragments may be involved in initiating the complement cascade.
- As part of the inflammatory response, dendritic cells originating from the

choroid may extend through Bruch's membrane into the extracellular space below the RPE. These cells seem to be responding to a more global secretion of antibody proteins, not just to drusen.

- RPE cells that have expressed proteins like those studied by Dr. Bok are capable of eliciting dendritic-cell response from the choroid. For eyes of the same age, there is more RPE cell loss in eyes with AMD than in non-AMD eyes. Thus, there is evidence for RPE cell distress as part of AMD progression. This distress could stimulate antibody expression.

The participants discussed whether the inflammatory conditions and signs of immune response described by Drs. Bok and Hageman are either intrinsic to the AMD disease pathway or represent a secondary complication, albeit one with serious consequences (e.g., wet AMD). If one views AMD as a family of diseases, which may start from different initial conditions and, in various stages, progress through alternative pathways, then this immune response mechanism appears to be a late-stage path alternative. All in all, though, it may be a major pathway for progression to AMD.

Summary Comment on AMD Pathways and Mechanisms

It is tempting to look for a single strand of causation for AMD, beginning perhaps with oxidative damage and progressing through lipofuscin accumulation to stress on photoreceptors and RPE cells. This stress, continuing over time, would cause the gradual cell distress or "sickening" that elicits an immune response, the breaching of Bruch's membrane, and finally, the new blood vessel growth from the choroid characteristic of wet AMD. However, the general sense of the workshop presentations and the consensus during the discussions was that the disease pathways in AMD are more varied than a simple linear model suggests. The initiating, promoting, and inhibiting factors that apply in individual cases appear to vary. Rather than pursuing a single line of causation, AMD research needs to unravel which strands and pathway alternatives should be marked as distinctive forms of the disease. This starting classification of AMD phenotypes must then be refined, and perhaps redefined, to reflect the genetic differences found to underlie these observable differences in disease pathway and rate of progression.

Determine pathways in AMD with potential as intervention targets.

The medical goal in studying the pathophysiology of AMD is to learn enough to do better at preventing or treating the disease. Four of the prospective AMD pathways or contributing conditions discussed above were specifically addressed in this workshop as intervention targets.

- The oxidative damage pathway may be targeted with antioxidants.
- Growth of new blood vessels, as in CNV, may be inhibited with antineovascular agents.
- The stress on photoreceptors and RPE cells, leading to their sickness or death, may be countered using neurotrophic agents.
- Immune response mechanisms may be targeted with anti-inflammatory drugs.

These intervention targets and the modes of intervention discussed only suggest the range of possibilities that will open, once the genetics, basic biology, and pathophysiology of AMD are understood. Of course, use of any path-specific intervention requires first establishing that the targeted pathway is relevant to an individual patient's form of AMD or propensity to develop it (that is, the patient's genetically based AMD phenotype).

Antioxidants to Prevent or Reduce Oxidative Damage

Potential evidence that antioxidants may inhibit AMD progression comes from AREDS (Age-Related Eye Disease Study), a set of clinical trials funded by the National Eye Institute. There were four treatment groups. Group 1 received three antioxidant supplements: vitamin C (500 mg), vitamin E (400 IU), and beta carotene (15 mg). Group 2 received 80 mg of zinc (as zinc oxide), plus a copper supplement to prevent anemia from the zinc. Group 3 received both the antioxidant and zinc supplements, while Group 4 received a placebo.

The major results of the trial were reported as reductions in probability, for treatment versus placebo, that participants progressed to advanced AMD or to significant vision loss over the five years of treatment. For participants in all categories of AMD progression, the reduction in odds was statistically significant only for Group 3. For the highest-risk participants, treatment with zinc alone or zinc plus antioxidants reduced the odds of progressing to advanced AMD with

statistical significance.

There was disagreement, both at the workshop and in the literature, on the extent to which the data from AREDS demonstrate that antioxidants as a dietary supplement are a therapeutic intervention for AMD progression. (The workshop discussion is summarized in Section 3, under "The AREDS Trial for AMD Progression.") The AREDS study design does not address the issue of whether specific phenotypes of the disease might be more susceptible to intervention with antioxidant supplements than the AMD population in general. Nor does it address whether more targeted delivery of antioxidants to the eye or the retina would inhibit oxidative damage as a factor in AMD initiation or progression.

Antineovascular agents

Antineovascular agents may counteract other factors that promote new blood vessel development (pro-angiogenic factors), or they may promote atrophy and removal of new vessels. Their utility in AMD intervention would be to inhibit CNV. If the switch to new blood vessel formation in AMD depends on a shift in the balance between factors promoting and inhibiting blood vessel formation, then antineovascular agents may be able to prevent a shift to CNV or shift the balance back toward CNV inhibition. Although neovascularization is a late stage in AMD progression, and can even be considered a complication of the underlying retinal degeneration, it is the stage responsible for most of the serious vision loss from AMD.

Because neovascularization is also an issue in cancer intervention (tumor growth depends on a rapidly growing blood supply), a number of antineovascular agents are available, and some are already in clinical trial for AMD intervention. Research is also underway on factors that inhibit one or more specific pro-angiogenic factors (such as VEGF) that appear to initiate or promote CNV. However, neovascularization in different contexts responds differently to a particular antineovascular agent. For example, many inhibitors of neovascularization in the cornea are ineffective against CNV. (See the presentations by Drs. Chader and Campochiaro.)

Neurotrophic Agents

Agents that help neuronal cells survive may intervene in AMD pathways that weaken or lead to the death of photoreceptor cells, RPE cells, and other cell types in the retina (e.g., Müller glial cells). These ***neurotrophic agents*** might inhibit progression to geographic atrophy (dry AMD), as well as intervening in retinal cell atrophy that precedes CNV.

Various growth factors, as well as antioxidants such as lutein, are candidates for neurotrophic agents in AMD intervention. One suggestion was to investigate neurotrophic agents that are effective in retinitis pigmentosa or other diseases with pathways involving neuronal cell death. (See the presentation by Dr. Chader for further details.)

Targeted Delivery of Pharmaceutical Agents

An issue for therapies using either antineovascular or neurotrophic agents is targeting delivery of the agent to the retina. Direct injection into the eye is probably not therapeutically acceptable, particularly if repeated administration is required. Options for targeted delivery that were discussed at the workshop include pharmaceutical gene therapy, encapsulated cell delivery, and trans-scleral delivery.

- In pharmaceutical gene therapy, a gene for producing the pharmaceutical agent is inserted into cells of the retina (or near it), typically by use of modified viruses (vectors) in a process called transfection.
- As noted under heading A.2, encapsulated cell delivery involves implanting genetically modified cells, isolated in a protective capsule, in the vitreous chamber of the eye. These cells contain genes that produce the pharmaceutical agent, which is small enough to diffuse through tiny pores in the capsule wall to their target cells in the retina.
- Trans-scleral delivery involves various modes of application of the pharmaceutical agent to the sclera, the white outer layer of the eyeball, from which it ultimately diffuses into the retina.

Targeted delivery of pharmaceutical agents was addressed in the presentations by Dr. Chader and by Dr. Campochiaro (Clinical Applications).

Anti-Inflammatory Drugs and the Immune Response Pathway

If immune response proteins and the complement cascade are involved in the pathway leading to CNV, known anti-inflammatory agents may be useful interventions in appropriate cases. For example, they might prevent the degradation of Bruch's membrane as a barrier to

new vessel proliferation. As with antineovascular agents, this intervention approach would target the development of CNV but would not address whatever events of macular stress or degradation lead to it. Targeted delivery of the anti-inflammatory agent could be an issue, if high concentrations in the choroid–RPE region must be maintained for an extended treatment period.

E. Research Resources

Pursue an integrated approach to AMD research, perhaps through a virtual center concept.

Most research on AMD in the United States is funded through individual investigator grants, such as the RO1 grants from the National Eye Institute. As one participant noted, this funding approach emphasizes specific achievements and competition to produce significant results. However, it also fosters short-term research aims and short time courses for the research undertaken. The research often must be designed to yield publishable results within about 18 months.

In addition to the National Eye Institute, which is by far the largest supporter of research on AMD, a number of private philanthropic foundations and grant-making organizations provide funding for research or dissemination of information about AMD. Unfortunately, this multiplicity of efforts, all with the best of intentions, is largely uncoordinated. The total research effort thus remains diffuse, whereas a concerted attack on the several promising lines of inquiry into AMD pathogenesis and its genetic components, as outlined in this action plan, would improve the chances of earlier breakthroughs for intervention.

The participants agreed that a more coordinated, integrated research effort, which should complement rather than replace individual investigator research, could make significant advances in pursuing many of the objectives presented in this action plan. The participants agreed that mechanisms are needed to encourage investigators to work together. Continuity of funding—to maintain a program that is more than a series of individual research projects—is another priority. The objective would be to develop a consortium of investigators whose work collectively articulates larger aims in AMD research than any one single-investigator grant could.

Rather than funding for physically localized research centers, which in the past have been problematic in sustaining high-quality research, the approach favored at the workshop was to establish "virtual centers" or "centers without walls." These centers would emphasize multidisciplinary, integrated programs focused on objectives like those in the action plan. The participating investigators would not need to work at a single physical location. Examples include the multidisciplinary research centers within the University of California system, which encourage and even require cooperation among researchers at different campuses. The resource base for virtual centers could be amplified and stabilized, if the various entities with resources to contribute—the National Eye Institute, the private grant-making foundations, and the pharmaceutical companies—coordinated their roles in supporting the centers.

Another resource issue that could be addressed through a virtual center approach is the need to attract young scientists to work on AMD. Virtual centers would provide visibility for AMD as a significant area of research, with opportunities for multidisciplinary and collaborative research of interest to young investigators. Centers would also offer stability and avenues for career development through programs with continuity beyond that of the typical individual grant.

Seek better animal models for AMD, i.e., a small foveate primate.

As noted in the discussion of gene transfer studies (heading C), animal models are a critical tool for developing a mechanistic understanding of AMD and a genetically based classification of AMD phenotypes. This tool also enables more-advanced research on methods of intervention, such as pharmaceutical gene transfer. At present, a number of animal models are available that represent portions of a pathway or suspected mechanism in AMD progression. Current animal models are predominantly mouse and rat strains, including a number of variants that have been genetically altered (transgenic models) to model a specific factor or defect of relevance to AMD. To the extent that AMD comprises a number of distinguishable disease phenotype/genotypes, a single animal model for the entire disease may not be attainable. Different models may be needed for each of the major phenotypes of AMD, or even for individual events thought to play a role in the disease process. For example, there is not even an animal model for drusen formation or one in which the effects of drusen on the retina can be studied.

However, a fundamental limitation of rodent

models is that these species lack a macula and fovea. If the indications summarized under heading B prove correct, physiological and biochemical features of the macula and fovea will be significant for AMD disease pathways, as opposed to pathologies of retinal degeneration not specific to these structures. There may also be AMD-significant differences between Bruch's membrane in rodents and humans, especially for pathways leading to CNV. Chickens and some other bird species have a fovea-like structure for central visual acuity, but only among primates are there a human-like macula and fovea. A search for a small foveate primate that could be readily bred in captivity should be a priority.

Expand programs for collecting and providing research access to donor eyes.

The participants agreed on the importance to AMD research of a repository for donor eyes like the program Dr. Hageman has built over the past 12 years at the University of Iowa Center for Macular Degeneration. Specimens are gathered while the tissue is still fresh enough to distinguish damage due to disease rather than to post-mortem atrophy. For genetic matching using microarrays, expression analysis, and other techniques of modern genetic research, the tissue must be fresh. This program is described more fully in the presentation by Dr. Hageman.

Exploit bioinformatics as an AMD resource.

The importance of bioinformatics as a tool for a genetic approach to classifying and understanding AMD is discussed above (heading C).

3
Workshop Presentations

The thirteen workshop presentations were divided into three sessions. The Monday morning session included a general review of what is known clinically and from pathobiological studies about macular degeneration and related conditions (Dr. Marco Zarbin), a comparison of epidemiological studies of macular degeneration with an emphasis on the Rotterdam study of age related disease (Dr. Paulus de Jong), the role of genetics and genetic studies in macular degeneration (Dr. Edwin Stone), and animal models of macular degeneration (Dr. Peter Campochiaro).

The Tuesday afternoon session consisted of four presentations on the state of biological knowledge about macular degeneration and promising trends and opportunities for understanding the biological conditions responsible for initiation and progression of AMD. The presenters were Drs. John Dowling, Dean Bok, Steven Fisher, and Gregory Hageman. The Wednesday morning session focused on current and potential therapeutic interventions, including pharmaceutical therapies (Drs. Gerald Chader and Peter Campochiaro), surgical interventions (Drs. Robert Machemer and Eugene de Juan), and dietary supplements (Dr. Peter Dudley). The current positions and academic degrees of all twelve presenters are included in the list of workshop participants in the Appendix.

The summaries below are intended to convey just the highlights and key themes of each presentation. To do justice to the depth of material in each presentation in a constrained space, the summaries employ more technical terminology than do the expositions in Sections 1 and 2.

Session 1. AMD Overview, Epidemiology and Genetics of AMD, Animal Models

Marco Zarbin: Pathobiology of AMD

In addition to opening the workshop with a broad background review, Dr. Zarbin sought to raise some unresolved issues and points of controversy, to stimulate the group's discussions. AMD can be distinguished from other diseases that share some of its features by defining it as a disease characterized by accumulation of abnormal material on both sides of the RPE basal membrane and associated with manifestations of drusen, atrophy, choroidal new vessels, and onset after age 50. Dr. Zarbin believes dysfunction in the RPE is the first manifestation of AMD. As an hypothesis for discussion, he suggested that this dysfunction results from oxidative damage. The RPE dysfunction causes the abnormal accumulations in the extracellular matrix and associated changes in Bruch's membrane. These changes in turn lead to the manifestations of advanced AMD: CNV or geographic atrophy.

Dr. Zarbin presented five lines of evidence for oxidative damage as initiating damage to the RPE. First, RPE enzymes that help prevent oxidative damage (by scavenging free radicals) decrease with age. Second, a decrease in macular pigments, which protect the RPE from oxidative damage, correlates with risk factors for AMD and with advanced AMD. Third, an argument can be made that three risk factors for AMD—age, cigarette smoking, and white race—are related to potential for oxidative damage to the RPE. Fourth, the AREDS clinical trial found that increased dietary supplements of antioxidant vitamins and minerals decreases the likelihood of progression to advanced AMD for certain high-risk groups. Fifth, the lipofuscin in drusen appears to be a product of oxidative damage. Lipofuscin sensitizes the RPE to light, causes abnormal cell functions when it reaches high concentrations, and is correlated with drusen density.

A number of factors, such as poly-unsaturated lipids in outer segment membranes and the high level of oxygen in the RPE environment, increase the susceptibility of the RPE to oxidative damage. Also, there are a large number of potential oxidation intermediates in the RPE environment. Dr. Zarbin described evidence that extracellular deposits between the RPE and Bruch's membrane (basal linear deposits) are products of oxidative damage and may cause what we refer to as drusen. Drusen may, therefore, be a focal manifestation of a more generalized problem, such as the accumulation of basal linear deposits. Changes in Bruch's membrane appear to be associated with increasing basal linear deposits, and he believes that some of these, such as decreased

permeability, alter the nutrient flow to the RPE and removal of toxic byproducts. The thickening of Bruch's membrane in Sorsby fundus dystrophy, for example, suggests that decreased permeability stresses the RPE. Two studies have shown that high dose vitamin A therapy in patients with Sorsby's fundus dystrophy results in amelioration in dark adaptation curves and lessening of symptoms of nyctalopia (Jacobson et al. 1995, Weber et al. 1994). These results imply that the ~30 µm thick abnormal extracellular deposit (which resembles basal linear deposit) between the RPE and choriocapillaris can alter the diffusion of essential nutrients from the choroid to the RPE-retina.

The basal linear deposits appear to act as antigens, stimulating the immune response system. In animal models of excess material accumulating in the extracellular matrix, this antigenic response leads to neovascularization from the choroid only if Bruch's membrane is damaged. Otherwise, the neovascularization develops from the circulation in the inner retina. Dr. Zarbin thinks that atrophy of the choriocapillaris may create hypoxia in the RPE environment, which in turn elicits CNV. If the linkage can be verified from thickening of Bruch's membrane to hypoxia in the RPE, and from RPE hypoxia to CNV, this linkage could become a target for therapeutic intervention. Another factor in promoting CNV appears to be overproduction of VEGF (vascular endothelial growth factor) by the RPE. It appears that Bruch's membrane has to be compromised before overproduction (overexpression) of VEGF by the RPE results in choroidal growth. If Bruch's membrane is not damaged, the new vessels remain in the choroidal space.

In closing, Dr. Zarbin described AMD as a disease with a variable natural history (not a linear progression through the same stages in every case). It would be a mistake to attempt to treat it by targeting a single stage. A spectrum of treatments is needed to target different factors in AMD progression. An area that needs further exploration is the permeability change in Bruch's membrane with increasing basal linear deposits. His suggested model for AMD progression does not answer the question of how oxidative damage can lead to CNV in some patients but leads to geographic atrophy in others.

During the discussion of Dr. Zarbin's presentation, there was agreement with the point that drusen are local biomarkers for a pathologic process that is more ubiquitous in the macula than the drusen themselves. Dr. Hageman noted that, when dendritic cells from the choroid invade the space between Bruch's membrane and the RPE, they appear to be responding to a more global secretion of antibody proteins, not just to local drusen accumulation. Another comment led to general discussion and consensus on the point that AMD should be acknowledged to be a group of diseases. Even the oxidative damage hypothesis advanced by Dr. Zarbin should be viewed as one candidate for initiating pathways to later AMD stages.

Paulus de Jong: Epidemiology of Macular Degeneration

Dr. de Jong described himself as an ophthalmologist who, thirty years ago, was unsettled by his inability to help patients diagnosed with macular degeneration. His group started by analyzing 179 post-mortem eyes, in which they observed thickening of Bruch's membrane and calcification beginning when the patients were in their twenties. Not until ages 60, 70, and 80 did large drusen typically appear, along with the further complications classified as macular degeneration (van der Schaft et al. 1992). In Dr. de Jong's view, AMD is a disorder that has already been developing for 20 years or more before accepted clinical manifestations appear.

The Rotterdam epidemiologic study, which began in 1990, offered an opportunity to find determinants and risk factors for the disease. This understanding would, he hoped, aid in forming better hypotheses about how the disease develops and ways to intervene. The study is following a cohort of 8,000 people age 55 and older. The ophthalmology group, one of four investigator groups looking at diseases of aging in this cohort, selected glaucoma and AMD for detailed analysis because they were the two major eye disorders of the elderly about which the least was known.

Because there was no accepted international classification for stages or forms of ARM, the investigators proposed a system for classifying a wider range of conditions than just degeneration of the macula, which they classified as the two late stages of the disease (corresponding to wet or dry AMD). For all stages, they adopted the term "age-related maculopathy" or ARM, which encompasses as its end stage AMD.

Population-based epidemiology on ARM is difficult. In the study population of 8,000, the Rotterdam investigators initially found only 32 subjects who were blind in both eyes and 91 with visual impairment. AMD was responsible for 55

percent of the blindness, with the second most prevalent cause, glaucoma, accounting for only 7 percent. After cataract, AMD was the second most prevalent cause of visual impairment.

Despite the importance of AMD (or ARM) among eye diseases of aging, there are many confounding factors. The fundus photographs from the beginning of the study show no evidence of drusen in 40 percent of the 150 Rotterdam subjects with AMD now. The need to study a large population to get a small number of AMD cases creates a continuous tension between recruitment bias and statistical power. For example, although other studies have reported that gender (being female) may be a risk factor, the Rotterdam investigators found no gender difference after correcting for the higher life expectancy of women and other age-related confounding factors. When risk factors reported in nine epidemiologic studies are compared, the only risk factor that appears uniformly is smoking. If the data from the three most comparable studies (Rotterdam, Beaver Dam in the United States, and Blue Mountain in Australia) are pooled—yielding a combined study population of 14,752 with 241 AMD cases—the only statistically significant risk factors are smoking and age.

Statistical associations with AMD in prevalence studies often vary. For example, density of macular pigment correlates positively with AMD prevalence in some studies and negatively in others. Dr. de Jong suspects these results and other variable associations indicate that multiple factors are involved. If AMD is indeed a "family of diseases," prevalence associations are a weak guide to causal relationships.

The Rotterdam study is also accumulating incidence data on ARM and AMD (occurrence of the disorders in a study population over time). The classification scheme uses clinically observable forms (stages) of ARM with predictive value for disease progression to AMD. Stage 0 is small hard drusen; stage 1 is small soft drusen; stage 2 is large soft drusen. Stages 4a and 4b are geographic atrophy and CNV, respectively. Progress through these stages corresponds to increased risk of developing late-stage ARM (stage 3 or 4). For example, the greater the amount of large drusen or the extent of drusen at a preceding examination, the greater the likelihood of higher stage ARM at the next examination. Among signs in fundus photographs that were predictive of ARM progression, the highest risk was with more than 10 percent of the grid area covered by drusen. Other predictors of disease progression were presence of soft, indistinct drusen or more than 10 large drusen. An unexpected result was that greater than 10 small drusen in the grid area was also a positive risk factor for progression. No significant associations (using regression analysis) have been found in the Rotterdam data between incident ARM and a variety of prescription drugs and drugs that affect the RPE. A substudy of cumulative use of prescription drugs and ARM incidence is underway. There may be significant associations of ARM incidence with dietary fat intake and HDL cholesterol.

In conclusion Dr. de Jong said that, in his view, the epidemiologic studies of the past 15 years have been disappointing in generating useful hypotheses for better treatment of ARM, although progress has been made in understanding the disease. Many studies report conflicting results. This might be due to the relatively low prevalence of ARM compared with more common diseases of aging, such as systemic hypertension. Thus, one is either hampered by low power in population-based studies or selection bias in case-control studies. Incidence data are more valuable than prevalence data for discovering risk factors for a disease like ARM, but based on his experience, the funding required to continue the followup work is difficult to sustain. He believes that genetic epidemiology will be a more productive approach. A major theme of the discussion after this presentation was the interpretation and design of epidemiologic studies for a disease that is heterogeneous with respect to pathogenic pathways and genetic variations.

Edwin Stone: Genetics of Macular Degeneration and Related Conditions

To convey his general view of how genetic studies may be relevant to treating or preventing macular degeneration, Dr. Stone described his vision of how the disease might be treated in the future. During a visit to her physician, a 31-year-old patient reports that a relative with AMD has suffered significant vision loss and wants to know what her risk is and whether she can do anything about it. Her physician determines her AMD phenotype and performs a battery of tests that identify her genotype as one known to strongly dispose the individual to developing advanced AMD. Because this genotype has been known for some time, a transgenic animal model has been available for use in pharmaceutical testing, which identified a class of compounds that inhibit her form of the disease. The patient

starts taking the medicine, which slows down but does not eliminate the disease. As a result, the patient becomes legally blind at 85 rather than at 65.

Dr. Stone sees this scenario of genetically based treatment with small-molecule therapy as important in the future, but it will not be the only way that AMD is treated. Other intervention modalities such as surgery or implants will be needed, either because of the form of the disease a patient has or because the patient is old enough that the disease has progressed to a stage threatening vision loss. The point is to use preventive approaches to delay visual impairment as long as feasible.

Asking whether AMD has a strong genetic component is different from asking if there is one gene responsible for the disease. Family relationship is a risk factor for AMD that shows up repeatedly in the literature. When asked whether AMD has occurred in their family, 23 percent of AMD patients say yes. That makes genetic influence the biggest causal "signal" we have found for AMD. However, this does not mean that only a few genes are involved, or that the same genes are involved in every form of the disease. For the past decade or so, investigators looked at a number of genetic diseases linked to mutations in a single gene—including Stargardt's disease, Sorsby's fundus dystrophy, and Best's disease—as candidates for the genetic component of AMD. The results always showed as much occurrence of the candidate genetic differences in the controls as in the AMD population. In short, there was no statistical signal that mutations in these genes were involved in AMD.

However, testing these "candidate disease" hypotheses did provide insights into mechanisms that might be involved in one or more AMD phenotypes (see heading D in Section 2). Dr. Stone believes the next decade of research on the genetic component of AMD will begin to uncover the multiplicity of genes involved and their relevance to specific AMD phenotypes. A polymorphism or mutation in any one of these relevant genes may provide the genetic code for an initiating condition, a promoter for one disease pathway, or an inhibitor that protects individuals with that form of the gene from rapid AMD progression. Genes in this last category, *mitigator genes*, are particularly important for the understanding they provide of disease pathways and potential intervention targets. We now have a much richer toolbox of genetic research methods, including the mapping of the human genome and genetic modeling in animals, to find these mitigator genes.

When one takes this genetics-informed view of AMD, three principles can be formulated that should guide any clinical trial of an AMD intervention. First, because AMD is really a number of diseases, no single therapy is likely to work in every case. A therapy that is in fact highly effective for a specific disease pathway may not show enough of an effect to be of interest, when the study population includes multiple disease pathways (phenotypes). For a disease with a strong genetic component, the phenotypes need to reflect genetic differences and dispositions, as well as clinically observable differences.

Second, studying a form of the disease that has a low prevalence but is well characterized may yield positive results for intervention sooner than seeking one global treatment for the multiple forms of the disease. This principle of sequential attack allows a complex disease to be segmented into achievable therapeutic tasks.

Third, the timing of both the experimental intervention and the point at which potential effects are measured is important. Looking for the effect of treatment too soon may yield an insignificant improvement over controls simply because the effect has not shown up yet. Interventions that inhibit development of a slowly progressive disease like AMD are even harder to time optimally and are likely to require more time to see the inhibition relative to controls. The experimental design must take into account the *effect curve* of treatment versus controls, as a function of time. (Optimal timing relative to the effect curve is illustrated in Figure 1; see Section 2.) Genetically based phenotypes are important in distinguishing the effect curve for different forms of a heterogeneous disease like AMD.

Identifying the key genes involved in even some of the AMD phenotypes will enable useful in vitro and animal models to be developed for the predisposing conditions at different stages in AMD progression. These models will, in turn, further elucidate the disease mechanisms and pathways involved in specific forms of the disease, thereby refining our classification of AMD phenotypes. Further along, there is the possibility to use genetic screening in ways suggested by Dr. Stone's scenario of future treatment of patients long before the disease causes visual impairment. Today, patients are already well along the path of disease progression before clinical observation can

establish that intervention is or will be needed. Genetic screening for well-characterized phenotypes of the disease would allow physicians to begin treatment years and even decades sooner. For some phenotypes, depending on the effect curve of the intervention, this earlier intervention will make a critical difference in inhibiting the disease progression.

During the discussion, Dr. Stone was asked about the best strategy for finding genetic abnormalities relevant to AMD phenotypes. He outlined a "sequential candidate gene approach" that would work at finding genes that play a role in a small percentage of specific, clinically well-characterized AMD phenotypes. These genes would then be expressed in a number of strains of an animal model. The differences in expression in the strains and their back-crosses could be used to find additional genes relevant to a specific phenotype. This genetic information would then help to refine the classification of clinically relevant phenotypes. Through this and later discussions, the workshop formulated the iterative approach to classifying AMD phenotypes/genotypes, as recommended in Section 2.

Peter Campochiaro: Animal Models of Macular Degeneration

Dr. Campochiaro began by reviewing the range of existing animal models for specific features that appear to be important in a majority of AMD phenotypes. There are several animal models for changes in Bruch's membrane and formation of drusen-like deposits, at least one model for lipofuscin changes, several models for photoreceptor cell death and cell death in choroidal capillaries, and a number of models for aspects of retinal and choroidal neovascularization.

Mice that have been genetically altered to express variations of an enzyme called cathepsin develop autofluorescent deposits in the RPE, similar to lipofuscin. Bruch's membrane is also thickened, and the altered mice lose photoreceptors faster than normal mice. This model supports the hypothesis that a change in the enzymes critical for digesting photoreceptor cell products can produce consequences similar to conditions observed in human macular degeneration.

In comparisons between other mouse strains with accelerated signs of aging and strains that are resistant to the same signs of senescence, the effects of aging include changes in Bruch's membrane, accumulation of deposits beneath the RPE, and changes in the RPE. However, neither photoreceptor degeneration nor invasion of Bruch's membrane accompanies these signs of aging.

Apolipoprotein-E (Apo-E) is a protein that transports cholesterol in the blood. In Apo-E knockout mice (mice in which the Apo-E gene is altered or removed, so that Apo-E is not produced), thickening of Bruch's membrane and accumulation of deposits occur at an early age. This model suggests that high blood lipids may play a role in accumulation of RPE-altering deposits and changes in Bruch's membrane.

In mice that are genetically altered to lack entirely the ability to make a protein linked to Stargardt's disease, the oxidation byproduct of all-trans retinal called A2E and its two progenitors increase if the mice are reared in light. (The gene for this protein, ABCR(ABCA4), is commonly referred to as the "ABCR gene," and the transgenic mice are called "homozygous ABCR knockout mice"). There is no increase in these compounds, which are linked to lipofuscin accumulation, if the ABCR knockout mice are reared in the dark. This model supports the view that defects in the processes needed to maintain photoreceptor cell activity in the presence of light can lead to disease-promoting accumulations in the retina. Discussion of these ABCR-knockout models (homozygous and heterozygous), in which photoreceptor cell death has not been observed, led to a second point. One gene defect, such as this one in the ABCR gene, may initiate a change that requires other genetic variations or defects to produce subsequent events in the complex pathway leading to photoreceptor cell death and retinal degeneration.

When wild type mice are placed in a high-oxygen environment for an extended time, photoreceptor cells in the central retina degenerate. Dr. Campochiaro attributes this effect to increased oxidative damage due to higher blood oxygen levels in the choroid, as choroidal blood flow is greater under the central retina. This damage does not occur in mice that are genetically altered to express fibroblast growth factor-2 (FGF-2) in their photoreceptors. If oxidative damage is an important initiating condition for AMD phenotypes, Dr. Campochiaro added, FGF-2 or related growth factors may be useful for therapeutic intervention. (The presentations by Drs. Chader and Campochiaro in Session 3 also discuss these growth factors.)

The current animal models for neovascularization in the retina are complex, and Dr. Campochiaro noted that no animal model will

have all the aspects found in human macular degeneration and CNV. However, in these models any action that disrupts Bruch's membrane leads to CNV. If Bruch's membrane is disrupted in wild type mice, they develop CNV. If Bruch's membrane is disrupted in mice genetically altered to express VEGF, a stimulator of blood vessel growth, the CNV is far greater, covering almost the entire eye. If Bruch's membrane is not breached, the transgenic VEGF mice develop retinal neovascularization, rather than CNV.

From these and other studies of mouse and rat models, Dr. Campochiaro concludes that photoreceptor degeneration is critical in pathways to CNV, as well as events in the extracellular matrix and Bruch's membrane. There may be five or ten phenotypes involved in the full pathway to human macular degeneration and CNV. He believes the route to unraveling them includes both molecular genetics (finding the gene defects through genetic epidemiology, for example, and modeling them) and working backward from animal models with AMD-like conditions to the genes that can cause these conditions. He does not think there will ever be one single animal model for AMD because of the disease's heterogeneity and the complexity of the disease pathways. There will be a host of different models for different aspects of AMD, but not one single, all-purpose model.

In response to a question during the discussion, Dr. Campochiaro noted that disruption of Bruch's membrane sufficient to allow CNV does not require a physical rupture. A biochemical breach—for example, one resulting from accumulation of antigenic material in the sub-RPE space—could sufficiently disrupt the membrane. Other issues discussed included whether animal models with a fovea and/or macula were needed for some aspects of AMD and whether CNV in AMD should be viewed as a serious but secondary complication (if Bruch's membrane is breached) of a retinal degenerative disease that in itself (without the complication) would be relatively mild. If the latter view is taken, then perhaps interventions that target the neovascularization mechanisms would be sufficiently effective.

Session 2. The Biology of Macular Degeneration

John Dowling: Review of Macular Biology and Degeneration

During his review of current scientific knowledge about the biology of the macula in both its normal and degenerative conditions, Dr. Dowling emphasized issues that remain unresolved but could be critical in understanding AMD. These issues are highlighted here.

From a medical standpoint, the critical characteristic of AMD is the loss or impairment of central vision. Thus, the macula and fovea may be distinct in key respects from the rest of the retina. Yet, Dr. Dowling noted, the scientific knowledge of the fovea, as opposed to knowledge about retinal photoreceptor biology in general, is rudimentary. After reviewing some of the distinctive anatomical features of foveal cone cells, particularly with respect to their outer segments, he stated that foveal cones are very different from cones in the peripheral retina. The difference in blood supply to the fovea (it lacks the inner retinal circulation found elsewhere) means that these cones are entirely dependent on the choroidal circulation for feeding (nutrients and oxygen) and waste product removal.

Just as the cone density in the fovea is very high relative to average photoreceptor density, the density of rods in the perifovea is equally high. Rods and cones interact, and an unresolved issue is whether degeneration of these nearby rods in the perifovea affects the foveal cones. Because the carotenoid pigments found in the macula and fovea are antioxidants and protect the foveal cones from higher intensity light and higher energy (blue) light, decreases in amounts of macular pigments may also play a role in AMD.

The role of RPE cells in phagocytosis of outer segment discs and the turnover rate for discs has been well studied in rod cells, but details of these processes for foveal cones are still unknown. The load on foveal or perifoveal RPE cells relative to general phagocytosis rates in the retina could be relevant to AMD progression. Similarly, the cycle for renewing retinoids by transporting them from the outer segment discs to the RPE cell and back has been studied for rods and cones generally. Although the basic cycle appears to be the same in rods and cones, there are different enzymes in the two types of photoreceptors. We do not know if there are differences between the foveal and peripheral

cones in this complex process. To illustrate the types of subtle differences that could occur, Dr. Dowling described the work of Sun and Nathans (2001) on accumulation of all-trans retinal as a source of photo-oxidative damage in the discs, leading to increased formation of A2E and lipofuscin. For a long time, the ABCR transporter enzyme for all-trans retinal—the enzyme that is missing in Stargardt's disease—was thought to be present only in rods. There is now compelling evidence that this enzyme is present in cones as well.

The oxygen requirements for foveal cones, relative to other cones and to rods, are also unknown. We do not know how the increase in oxygen tension from the base of the outer segment to the tip may affect the tendency for oxidative damage in foveal cones (or perifoveal rods) relative to photoreceptors outside the macula.

During the discussion, Dr. Campochiaro described research indicating that loss of rods occurs before cone loss in AMD phenotypes and other macular diseases. The cones appear to be more resistant to damage. This may support a disease pathway, for at least some AMD phenotypes, in which damage occurs first in the perifoveal rods, with the foveal cones affected only at a later stage. Dr. Dowling agreed that the trophic influence of rods on cones, and particularly of the perifoveal rods on foveal cones, was an important question. The structural differences of the fovea and perifovea may be different enough from the peripheral retina that animal models with an all-cone fovea may be necessary to understand mechanisms specific to AMD, compared with retinal diseases generally. Another suggestion discussed by the participants was that an initiating condition for AMD may be that the perifoveal rods are "sick" but survive, rather than dying quickly. For lipofuscin to accumulate in abnormal amounts (and possibly to contain more reactive or antigenic components), it may be necessary that the rods remain alive but function abnormally.

Dean Bok: Biology of the Retina

Dr. Bok began by comparing the organization of the layers of the normal, healthy retina with the situation in AMD, where the RPE and photoreceptor cells undulate over large drusen and basal deposits in the extracellular matrix. His presentation centered on the questions, "What are the mechanisms underlying these changes? What are some useful models for understanding how they occur and how they affect retinal function?"

Dr. Bok has used cultured RPE cells in an in vitro model for lipofuscin formation and the role of A2E. This reactive molecule, which forms in the acidic environment of a lysosome from the condensation product of all-trans-retinal with a specific phospholipid (phosphytidyl ethanolamine), can potentially leak from the lysosome. It also absorbs blue light and can damage cultured RPE exposed to blue light, leading to RPE cell death. This result sheds light on experiments with transgenic mouse models for Stargardt's disease (ABCR knockout mice) in which the precursor to A2E is formed. Reducing the precursor for A2E could reduce the damage and preserve vision. Although the ABCR gene has not been directly implicated in AMD, the animal models in which this gene is modified provide useful ways to study conditions like those leading to lipofuscin accumulation.

A spontaneous mutant mouse strain, called *rds*, lacks a protein that holds together the edges of a disc in photoreceptor outer segments. The heterozygous *rds* mouse has much shorter outer segments and discs that are abnormally large in diameter. Thus, the RPE cells must ingest and digest large round boluses of shed disc. The morphological consequences include greatly elevated lipofuscin and basal laminar deposits. Dr Bok believes this work and other evidence indicate that lipofuscin accumulates when the physical state of the outer segment or its oxygen environment is altered.

Dr. Bok described evidence that RPE cells can produce some of the molecules of the immune response that Dr. Hageman has identified in drusen. He has used RPE cell cultures to study HLA-2 antigen production. Cells stimulated with gamma-interferon have more HLA-2 proteins and other immune response molecules on their apical side (the side normally facing the retinal photoreceptors). After showing up first on the apical side, the HLA-2 proteins appear to be transported within the cell to the basal side (the side toward Bruch's membrane), where they are deposited in the culture substrate, which acts like Bruch's membrane in an intact retina. This immune response activity has not yet been demonstrated in the human retina, but Dr. Bok noted that this cell culture model has paralleled human retina activity for every function his group has examined.

In closing, Dr. Bok summarized the available methods for isolating and studying some of the RPE processes that occur in the

visual pigment cycle and lipofuscin formation. Among the RPE processes that can be modeled are (1) uptake and processing of retinol (vitamin A); (2) phagocytosis of outer segment discs; and (3) attraction, processing, and presentation of antigen.

One of the issues discussed after this presentation was whether all lipofuscin is the same and is equally deleterious. There are several lines of evidence that lipofuscin varies significantly in composition. Sometimes it is autofluorescent and sometimes not, and the amount of A2E varies.

Steven Fisher: Cone Cell Biology

Dr. Fisher's presentation focused on cone biology, comparisons between rods and cones, and work he has done on degeneration of rods and cones. The accepted view for why humans need two types of photoreceptors is to provide useful vision over a wide range of light intensities. In humans, about 5 percent of retinal photoreceptors are cones, but the fovea contains only cones. The renewal cycle of outer segments is similar in rods and cones. In both, discs are formed at the base and shed at the tip, with about 10 days between disc formation and the time it is shed (and removed by RPE phagocytosis).

Dr. Fisher reviewed the basic morphological differences between rods and cones in the bulk of the primate retina outside the fovea, including differences in their structural relationship with the RPE cells below them. Unlike other cones, foveal cones have the tips of their outer segments buried in the apical surface of their RPE cell, like the rods elsewhere in the retina. The outer segments of foveal cones have no discernible taper, unlike other cones. The physiological significance of these morphological differences between foveal and extrafoveal cones is not yet known but should be studied further. The differences may provide clues to the increased susceptibility of the fovea to AMD.

An important consideration for transport of photopigment components and other functionally significant molecules is the extent to which the plasma membrane of a photoreceptor cell is continuous with the discs in the outer segment. Continuities are thought to make it easier for molecules to move between the plasma membrane and the discs. The extent of continuity in cones varies with species. In mammals, continuities are clearly present at the base of the outer segment, but it is unclear whether continuities occur all the way to the tip. Whether foveal cones are similar to other cones in this respect is unknown, as is the physiological significance of the extent and location of membrane–disc continuities.

The extracellular matrix around cones differs biochemically from the matrix around rods, and the matrix around foveal cones differs from that around extrafoveal cones. Extrafoveal cones have more mitochondria than rods have, and the metabolic rate of extrafoveal cones has been measured to be 15 times greater than that of rods. Although foveal cones have more mitochondria than rods, the difference in metabolic rates has not been measured. Another physiological difference between mammalian cones and rods generally is in the complexity of their synapses. Whereas rod synapses have only one or two points of contact with post-synaptic cells, cone synapses have between 8 and 40 points of contact.

Dr. Fisher and his coworkers have compared the responses of rods and cones to retinal detachments of varying lengths of time. If the feline retina is detached for 3 to 7 days, there are immense changes in the morphology of RPE cells. The outer segments of photoreceptors degenerate, and opsin molecules move to the plasma membrane. In general, cone physiology degenerates quickly after 7 days of detachment, while rods appear to retain more functionality (rhodopsin and other rod-specific molecules continue to be expressed in rods; analogous molecules disappear from the cones). The cone cells are still there, but the specific markers for important biochemical activity are nearly gone.

If the retina is reattached after one day of detachment, many of the markers for cone activity reappear after three days. However, the morphological relation between cones and RPE cells never fully recovers. With 3 days of detachment, there is still some recovery, but patches of cones do not recover at all. Rods appear better able to regain functionality after longer periods of detachment. There are important cellular events that occur when the retina is detached for a period of 1–3 days. This may be of significance to further development of surgical procedures, such as macular translocation, that are used to treat AMD because the retina is detached during this procedure and probably remains detached for 1 to 3 days.

Dr. Fisher's hypothesis about the pathological consequences of retinal detachment is that detachment causes hypoxia and hypoglycemia in the detached photoreceptors. Among results that support this hypothesis are experiments in which maintaining a high-oxygen environment (hyper-

oxia) around the photoreceptors during a period of detachment limits the functional loss during that time and may enhance recovery after reattachment.

These results led to an hypothesis that drusen (particularly large drusen) might cause hypoxia as one mechanism of photoreceptor damage in AMD. In collaboration with scientists at the Center for the Study of Macular Degeneration at the University of California Santa Barbara, Dr. Fisher has begun to study donor eyes with high concentrations of drusen but no CNV or even a diagnosis of AMD. The opsins disappear from both rods and cones over drusen, and photoreceptor synaptic terminals decrease in number. An interesting difference from the retinal detachment studies is that the rhodopsin in rods disappears; it does not just redistribute to the plasma membrane. Synaptic contacts also disappear. He has revised his working hypothesis to investigate whether drusen may be toxic to photoreceptors in some way, rather than just being a physical barrier.

Gregory Hageman: Biology of Macular Degeneration

The major theme of Dr. Hageman's presentation was the use of a strategy to compare human donor eyes with AMD to eyes of similar age without AMD. He has sought general pathways that may be associated with macular degeneration, in the hope of finding a pathway common to a number of AMD phenotypes and genotypes of the disease. If such a pathway were identified, it could be targeted for therapeutic intervention. The pathways on which he focused in this presentation involve the region between the choriocapillaris and the RPE. He and coworkers have observed markers of immune and inflammatory responses, specific to eyes with AMD, in processes occurring in this region. If these responses are indeed factors contributing to late-stage AMD (for example, CNV), they may be important targets for intervention aimed at preventing the more devastating consequences of the disease.

A central tool in this research has been a repository of human donor eyes developed over the past 12 years. It includes eyes of different ages and ethnicity, with and without AMD. To date, 2,700 pairs of eyes have been collected, all within 4 hours of death. This rapid collection is critical for minimizing post-mortem protein and RNA degradation. For more than 90 percent of the eye pairs, extensive medical and ophthalmological histories are available, as well as blood and serum samples. Between 25 and 30 percent of the samples are from individuals who were clinically diagnosed as having macular degeneration. The investigators are establishing an extensive characterization baseline, ranging from gene expression analysis to rigorous morphological analysis, for a sample of 200 donors. Half of these donors were diagnosed with AMD; half were diagnosed as not having AMD.

The work Dr. Hageman described began with investigating the composition of drusen. Many of the drusen-related processes he found occur throughout Bruch's membrane in eyes with AMD. Over the past five years, he and his collaborators have found that the bulk of the proteins identified in drusen are related to the immune response system. Dendritic cells, which are very specific and powerful antigen-presenting cells (another immune response component), are also associated with drusen in AMD eyes. Not only the drusen but also Bruch's membrane and the space between it and the RPE contain a great deal of activated immune response complement. It thus appears that a complement cascade—an archaic pathway in the body's immune response involving about 30 different interacting proteins—is occurring in the region between the choriocapillaris and RPE.

In an acute inflammatory response, the complement cascade performs the beneficial function of cleaning up local injury (whether an invasion by foreign cells or removal of damaged cells), and then it dissipates. In AMD eyes, there appears to have been chronic activation of the complement cascade continuing over decades. All the components of the cascade have been identified in the choroid–RPE region. Most of these components appear to be made locally by RPE or photoreceptor cells. Indeed, the entire system of complement cascade components, as well as promoters and inhibitors of steps in the cascade, appears to be expressed locally by cells in this region from the choroid to the RPE. This finding was unexpected because most complement components were thought to be produced only in the liver, from where they would travel to the site of an inflammatory response via the circulatory system. Gene expression analyses show differential expression of some complement components in tissues from AMD eyes. In addition, complex activation appears to be much more robust in the macula than in the peripheral region of the retina. The data suggest that there may be differences between the macula and periphery with respect

to complement activation. However, it is also possible that the observed difference in activity is an artifact of the stage in this chronic inflammation at which eyes are diagnosed as having macular degeneration.

The classical pathway for complement activation is formation of an antigen–antibody complex, which stimulates the first round of the complement cascade. Dr. Hageman suspects, however, that alternative, lectin pathways may play the principal role in AMD. Drusen contain cholesterol and cholesterol esters, so the trigger in AMD may be similar to complement activation in the early stages of atherosclerosis. Oxidized low-density lipoproteins in the choroid-to-RPE region may be the trigger, or it may be the basal laminar deposits that accumulate between Bruch's membrane and the RPE. An unanswered question is whether these materials, which originate from RPE cells, result from complement attack on RPE cell membranes or from oxidative damage, a gene defect, or some other initiating pathway.

In the set of 200 baseline eye-pairs, the damage to the RPE associated with AMD is evident. Over a period of nine decades (the age of the oldest donors in the baseline set), the RPE in AMD eyes declines by about 40 percent, as opposed to a 15 percent decline in eyes without AMD. As Dr. Bok noted in his presentation, HLA type 2 proteins and other immune response proteins increase in RPE cells under certain stress conditions. Dr. Hageman thinks these proteins and others may attract dendritic cells, promoting the extension of their cell processes through Bruch's membrane to reach the antibodies presented on the basal side of the RPE or in deposits in the extracellular space between the RPE and Bruch's membrane. These dendritic cells appear to be recruiting T cells (the next stage in the complement cascade), thereby amplifying the inflammatory response.

The presence of specific cascade inhibitors is also suggestive. In eyes with AMD, a membrane-bound inhibitor is prevalent on the basal membrane of RPE cells. The RPE cells may be trying to protect themselves from attack. The effectiveness and level of expression of various inhibitors and regulators of complement activity may reflect genetic variability, thereby providing a genetic link to differences in severity of the damage from chronic inflammation.

A link may exist between this chronic complement activation and other work on CNV (such as the animal models described by Drs. Campochiaro and Zarbin), which indicates that a breach in Bruch's membrane is necessary for new blood vessels to proliferate from the choroid into the space on the basal side of the RPE. Elastin forms an elastic layer within Bruch's membrane (and elsewhere in the body) that acts as a barrier to vessel growth through the membrane. Normally, elastin protein is produced during the third trimester of life only in the case of injury or other damage to elastin structures. If elastin peptides are present, indicating that existing elastin is being degraded, the elastin gene resumes expressing the protein. The signs of robust elastin synthesis in AMD eyes may indicate that the elastin layer in Bruch's membrane is being degraded—perhaps by lymphocytes from the choroid breaching the elastin layer in response to signals from distressed RPE cells.

The elastin layer is naturally more porous and about 25 percent thinner in the macular region than in the peripheral layer. At the fovea, it is almost immeasurable. Porosity appears to vary inversely with thickening of the entire membrane. (Disorganization of the normal layered structure of the membrane may account for both thickening of the membrane and increased porosity.)

Dr. Hageman's hypothesis is that many of the proposed AMD-initiating or promoting pathways, such as oxidative damage, formation of drusen and basal deposits, and even complement activation, may be occurring over a wider region of the retina than just the macula. Degradation of the elastin layer, whatever the mechanism, may breach Bruch's membrane first in the macula, where the layer is inherently thinner. This evidence suggests that, in AMD, chronic inflammation processes are causing cumulative damage to Bruch's membrane. The outer collagenous layer of Bruch's membrane, which contributes to its barrier function, is also degraded in eyes with AMD. Thus, complement activation in response to conditions that stress the RPE, continuing over decades, may result in degradation of Bruch's membrane in the more vulnerable macular region, and the eventual development of CNV. The inflammatory condition may in fact be a secondary response to the primary pathways causing RPE stress and sickness. If Dr. Hageman's hypothesis is confirmed, anti-inflammatory therapies targeted to the retina may be able to prevent or slow the degradation of Bruch's membrane leading to CNV.

A major issue raised during the discussion was whether the inflammatory responses Dr.

Hageman has observed are intrinsic to the disease pathway leading to advanced AMD. Dr. Hageman replied that the immune response and inflammatory response markers he described occur in 90 percent of the eyes with AMD and not in the control eyes (those diagnosed as not having AMD). The general sense of the discussion was that chronic, immune-system regulated processes seem to be important candidates for drusen formation and secondary-stage progression to CNV for at least some AMD phenotypes.

Session 3. Strategies for Therapeutic Intervention

Gerald Chader: Neurotrophic and Antineovascular Agents for AMD: Basic Considerations for Pharmaceutical Therapy

The theme of Dr. Chader's presentation was that two classes of biologically active compounds may have value in at least slowing the progression of AMD. Neuron-protective agents (neurotrophic factors) may be therapeutically useful in early-stage AMD. Antineovascular agents may have value in slowing the progression to wet AMD.

In principle, any agent that promotes neuron survival in the retina or the brain may be a candidate for preserving the photoreceptors and RPE in dry AMD and may delay wet AMD. The probable mechanism of protection for these neurotrophic factors is to intervene in processes leading to the death of photoreceptor cells, RPE cells, or supporting retinal cells such as Müller cells. Pharmaceutical companies could examine whether neurotrophic agents already found to be effective in retinitis pigmentosa or other diseases with neural death pathways were also beneficial for AMD.

The first neurotrophic factors found to delay retinal cell death were in the fibroblastic growth factor (FGF) family. Several forms of FGF have now shown effectiveness in animal models in which untreated controls have early photoreceptor cell loss because of a specific genetic defect. Ciliary neurotrophic factor (CNTF) is the agent found to be most effective in delaying photoreceptor degeneration in animal models for retinitis pigmentosa. However, side effects (herpes virus activation) have delayed a clinical trial of one form of this neurotrophic agent until an encapsulated cell delivery technique is available to target the retina. Dr. Chader thinks CNTF may be useful against geographic atrophy in late-state AMD. Lens epithelium–derived growth factor (LEDGF), which protects neurons against programmed cell death (apoptosis), is another candidate. Still other candidates for protecting retinal neurons include a variety of antioxidants, such as the carotenoid lutein; docosahexaenoic acid (DHA), which is being tested in two clinical trials for efficacy with retinitis pigmentosa; interleukins; pigment epithelium–derived growth factor (PEDF); and other compounds that have shown some degree of retinal protection in rodent models with genetic retinal defects.

Whereas the potential use of neurotrophic agents to prevent retinal neuronal cell damage involved in dry AMD is still a largely untested hypothesis, known antineovascular agents are already being tested, or are planned for testing, for efficacy with wet AMD. Most of the research and clinical trials being funded by pharmaceutical companies is directed at inhibitors of, or antibodies to, pro-angiogenic factors. This strategy rests on the principle that neovascularization in AMD or similar disease progressions results from a shift in the balance, in the local environment, between negative and positive regulators of new blood vessel development. Pro-angiogenic factors that could be targeted to shift this balance away from neovascularization include VEGF, FGFs, placental growth factor, platelet-derived growth factor, tumor growth factors, angiogenin, and others. For example, anti-VEGF aptamers[8] could neutralize VEGF by binding to it, blocking it from binding to receptor sites for stimulating blood vessel growth. Proteolytic fragments or cryptic domains of some proteins can also inhibit angiogenesis effectively. In addition to its neurotrophic effects, PEDF has been shown to be a potent antineovascular agent in both the cornea and retina. It does not seem to affect normal blood vessels, but it appears to induce apoptosis of endothelial cells in developing vessels (Volpert et al. 2002).

A major obstacle to practical therapy with any of the candidate neurotrophic or antineovascular agents is targeting delivery of the agent to the retina, or at least to the eye. Most animal studies of these candidates have used injection into the eye. For human therapy, intra-

[8] An aptamer is a chemically synthesized short strand of ribonucleic acid that is tailored to bind to a specific molecule, preventing it from binding to functional receptor sites. The binding affinity of aptamers can equal or exceed that of antibodies specific to the target molecule.

ocular injection is undesirable for reasons of safety and patient acceptability, particularly when repeat dosing is necessary. Oral delivery avoids these obstacles, but for many of these agents there are likely to be conflicts between systemic side effects and doses large enough to provide an effective concentration at the retina. Better options, Dr. Chader suggested, are likely to be found among such methods as pharmaceutical gene therapy, encapsulated cell delivery, or trans-scleral delivery. (Definitions from Dr. Chader are in Section 2; see "Targeted Delivery of Pharmaceutical Agents.")

In summary, there are many candidate neurotrophic agents available for studies on controlling dry AMD (geographic atrophy). The neuron protection approach, however, is still largely a theoretical suggestion that has not yet attracted support leading to clinical testing. For antineovascular agents, some of the many available candidates are already in clinical trial. Dr. Chader recommended that cancer trials could lead the way in testing the safety of antineovascular agents, after which their efficacy for wet AMD could be evaluated. Even with the long list of candidates in both classes, Dr. Chader stressed in closing, agents more specific to AMD are needed that do not require invasive administration and have long-term efficacy.

The discussion of Dr. Chader's presentation raised the issue of the extent to which an agent with established antineovascular effects in one tissue would be effective in another. Although there may be variations in effectiveness even between different parts of the eye (neovascularization in the anterior segment versus CNV), agents that have been effective in one area are at least reasonable candidates for testing in AMD. The participants also discussed recent work on the receptor sites and mechanisms of action for various neurotrophic and antineovascular agents. In addition to stimulating activity in RPE cells or photoreceptors, some of the neuroprotective agents may affect the Müller glial cells directly, with indirect effects on the photoreceptors. Because many of these factors have systemic roles, such as the roles of growth factors in embryonic development or the endocrine response to steroids, potential negative side effects are major issues. These complexities in the dose–response relationship for growth factors introduce an added dimension of subtlety into clinical testing for safety and efficacy.

Peter Campochiaro: Clinical Implications of Testing Promoters and Inhibitors of Angiogenesis in Animal Models

In this second presentation, Dr. Campochiaro focused on experiments with antineovascular agents in animal models for CNV. As Dr. Chader mentioned, FGFs have been found to promote neovascularization in animal tests. When Dr. Campochiaro studied transgenic mice in which the normal FGF gene had been replaced (FGF knockout mice), vascular development was normal. If the FGF gene was overexpressed, there also was no neovascularization. Dr. Campochiaro's explanation is that the primary function of FGF has nothing to do with blood vessels and neovascularization. FGF is normally secreted within a cell and acts there. The angiogenic effects appear to be a pathological consequence when FGF is present outside cells, as occurs in exogenous injection or cell disruption, and the normal mechanisms to control it are swamped.

The angiogenic factor VEGF, unlike the FGFs, appears to be critical to neovascularization. For example, the VEGF knockout mouse embryo has no vascular development. Dr. Campochiaro's group tested kinase inhibitors as candidate VEGF inhibitors in a mouse model with CNV induced by rupturing Bruch's membrane. One of these was able to inhibit CNV completely in this CNV model. A clinical trial of this kinase inhibitor is being completed now, but the trial is for diabetic macular edema. If this trial is successful, testing will probably be expanded to other conditions with neovascularization, such as AMD. Dr. Campochiaro expects that VEGF inhibitors will be important in the future as antineovascular agents, but the effects of VEGF inhibition on other body systems and conditions, for example in coronary heart disease, are still unknown. Because of the potential for side effects from systemic intake, targeted delivery to the eye will probably be essential.

One limitation of VEGF inhibition is that it is time-sensitive. Once new blood vessels are established (after about two weeks), they are insensitive to VEGF inhibition.

An alternative strategy that Dr. Campochiaro's group has explored is endogenous inhibition of neovascularization. In this work, genetic manipulation of the animal model produces local or systemic overexpression of a candidate inhibitor without the complications of exogenous injection. They found important

differences, depending on the circumstances inducing neovascularization and the location. Tumor neovascularization, for instance, differs from ocular varieties in response to transfection of a gene for an inhibitor, and corneal neovascularization differs from CNV.

In one case, the gene for endostatin was transfected into the liver of a mouse model for CNV. The genetically altered livers produced endostatin at high serum concentrations, and there was marked inhibition of CNV. The degree of inhibition correlated with serum endostatin concentration. The group also used an adenovirus vector to introduce the gene for an antineovascular agent into the eye. The results confirmed that local delivery (through endogenous production from a transfected gene) can inhibit neovascularization in an animal model. In gene transfer experiments with PEDF, retinal injection of an adenovirus vector for the PEDF gene gave stronger expression in RPE and glial cells than did intravitreous injection of the vector. RPE expression of PEDF occurred only in the vicinity of the injection bleb. PEDF delivery by gene transfer not only inhibited new vessel development but also caused existing CNV to regress. There was marked apoptosis of CNV blood vessels but not of established blood vessels. Injection near, but not into the eye (periocular injection) of adenoviral vectors for genes of antineovascular agents has also been effective in inhibiting neovascularization inside the eye, even though the gene transfection and expression occur outside the eye.

Dr. Campochiaro sees gene therapy using vectors coding for agents such as PEDF as a means of achieving systemic delivery of a therapeutic agent. It opens opportunities to limit side effects, while achieving long-term high concentrations of endogenous inhibitors.

Robert Machemer: Surgical Approaches to AMD

In AMD, the surgeon tries to eliminate a symptom, but not the causes of the disease. The objective is to preserve or improve vision. The surgeon must act before scar tissue has formed underneath the retina, for very little can be accomplished surgically once scarring occurs.

The presently used laser surgery techniques continue to have inherent limitations. In attempting to stabilize the growth of new blood vessels from the choroid, photocoagulation can destroy visual function. Photodynamic therapy attempts to eliminate the new blood vessels with minimal damage to adjacent tissue. When either technique is used, however, the physiological signal stimulating new vessels remains, and repeat treatments are needed. Even in photodynamic therapy, the roughly 3 to 6 month cycle of treatment has a cumulative effect on retinal tissue, and therefore on visual function, similar to that of photocoagulation.

Several alternative surgical approaches have been tried and abandoned. Surgery beneath the retina (submacular surgery) pulled out too much RPE along with the neovascular membrane. RPE transplantation has not worked to date. Nor has thermal therapy underneath the retina to stop neovascularization. The studies of antineovascular agents described by Drs. Chader and Campochiaro are undoubtedly promising for the future, but the physician needs something that can be done now for patients threatened with imminent vision loss.

Dr. Machemer's technique for translocating the retina began by considering cases in prematurely born children in which the fovea is in an abnormal location (displaced fovea). These cases suggested that the fovea can be in a different location and still perform its function of providing high-acuity central vision. The idea of purposefully moving the fovea to a healthier area of RPE had been addressed as early as 1983. Early on, the techniques were difficult, and limited positive results in these animal experiments were difficult to repeat. Experience gained in treating patients and further rounds of animal experiments helped his team at Duke University improve the techniques. The procedure requires removing the lens and vitreous, then infusing fluid into the subretinal space to detach the entire retina. The retina is cut circumferentially in the periphery, rotated 30 to 45 degrees around the optic nerve, and then reattached with the macula in a different position. Reattachment is analogous to the procedure used in reattaching a giant retinal tear. Reconnection between the photoreceptors and the RPE is not perfectly normal, but the outer retina recovers.

In the first three attempts to use the technique on human patients, one case was ideal for a proof-of-principle test. A 77-year old man, whose other eye had already been lost to AMD, had been able to read only three days before the surgery, but his vision had degenerated to 1/200. A large subretinal hemorrhage covered the area of central vision. After the retina was detached, the coagulated blood was removed mechanically and the retina was rotated around the optic nerve head, placing the fovea over an area of healthier

RPE. After reattaching the retina, photocoagulation was used at the periphery to form adhesions. This translocation improved the patient's vision from 1/200 to 20/80.

The full detachment and translocation procedure initially required 6½ hours of difficult surgery, and was complicated by heavy intraocular scar tissue formation (PVR). Because of the relatively large angle of rotation, muscle surgery to rotate the eyeball is needed to correct for the changed position of the fovea. The technique has been adopted and modified by other surgeons, including Dr. de Juan (see below), who developed a less-invasive procedure with minimal translocation. Today the surgery is done in 2½ hours with a much reduced complication rate. The success rate in improving visual function with the full rotation of the retina is now 77 percent, which, Dr. Machemer noted, compares well with either photocoagulation or photodynamic therapy. The technique may prove useful for dry AMD, as well as for CNV. However, Dr. Machemer cautioned that the clinical indications for applying it successfully to dry AMD have not yet been formulated.

Dr. Machemer believes several steps are needed to make the full detachment and translocation procedure a practical surgical technique. First, more pilot studies are needed to improve the technique and establish indications. Second, if results from these pilot studies are convincingly positive, then it may not be essential to run a large clinical trial to compare its efficacy with other techniques. However, if the results are promising but mixed, then a trial for comparison with other techniques will be required.

With respect to followup on initially successful cases, Dr. Machemer said there have been no instances of CNV at the new location of the fovea. There has been neovascularization at the old site of the fovea, but it can be treated with photocoagulation without loss of visual function. One constraint is that the followup time is limited because of the advanced age of the patients.

During discussion, one question raised was whether loss of RPE would breach the blood–retina barrier, reducing the efficacy of the technique. The response from Dr. Zarbin was that the RPE can repropagate to restore the barrier, although the new region of RPE does not always function well with respect to the photoreceptors above it.

Eugene de Juan: Limited Retinal Translocation, Retinal Transplantation, and Electronic Implants

Dr. de Juan described his own work in developing a variant of Dr. Machemer's retinal translocation surgery. He also summarized other experimental approaches to restoring central vision, such as retinal transplants and electronic implants.

Limited retinal translocation does not require removal of the lens and vitreous. The detachment is only partial, and the surgery can be performed in less than an hour under local anesthesia. After removal of the neovascular membrane and partial detachment of the retina around the macula, a fold (buckle) is made in the sclera to move the fovea over a different region of RPE. Muscle surgery is not required to reposition the eye to correct for double vision, as the movement of the fovea is small enough that the brain can compensate for the change.

Of the first 270 cases in which limited translocation was performed, 76 had post-surgical complications, which typically occur within 3 months. Dr. de Juan added that, with 500 translocations performed now, the complication rate is decreasing. For 76 cases with at least a year of followup, about half showed improvement from no better than 20/200 (patient legally blind) to at least 20/50 (patient able to read and drive). The CNV recurrence rate after 1 year is about 35 percent, comparable to that reported in the Macular Photocoagulation Study.

When CNV recurs, it does so on the side of the old CNV site (i.e., the previous location of the fovea) toward the new fovea location. The same effect occurs with full detachment and translocation, even though the movement of the fovea is in opposite directions in the two procedures. Dr. de Juan sees this result as evidence that the factors driving CNV are influenced by a signal from the foveal retina. The results from both translocation techniques also show that the fovea does not have to be located over foveal RPE to maintain useful central vision.

For the past two years, Dr. de Juan has been investigating presurgery indications of success or failure for limited translocation in restoring visual function. He has found that central fixation is a better predictor of post-surgical success than even visual acuity before surgery.

In his review of experimental work on retinal transplants, Dr. de Juan described

transplants as more appropriate for severe retinal degeneration, where the patient has no vision, than they are for loss of visual acuity, as is common in AMD. His group has been experimenting with direct replacement of photoreceptors in cases of geographic atrophy or CNV. Transplanted retina or RPE tissue will survive if placed under the retina, and the tissue remains organized in normal layers. If fetal tissue is transplanted, it develops into layers of photoreceptors. What has yet to be demonstrated is functional integration of the transplanted tissue with the imaging capability of the entire retina/brain system. Formation of functional synapses between graft and host neurons has not yet been clearly demonstrated. Similarly, transmission of a robust, functional signal from the graft neurons to the host brain has yet to be established.

At least two research groups are attempting to implant retinal prostheses in patients. One group, which places a photodiode array under the retina (subretinal implant), has reported that patients are seeing significantly better. Dr. de Juan's group has used an external camera attached to an array at the vitreal–retinal surface, which stimulates the neural network with the light pattern detected by the camera (epiretinal implant). He compared the future for the latter approach with the progress made with cochlear implants to provide auditory function for the hearing-impaired. Just as it took 25 years to progress from using cochlear implants for the deaf to using them to help the hearing-impaired, Dr. de Juan thinks it possible that, in 20 years, implants may be able to help the visually impaired, not just patients who are totally blind.

The AREDS Trial for Antioxidants in AMD Progression

Dr. Peter Dudley of the National Eye Institute reviewed the AREDS trial for AMD and led a discussion of its implications, including some controversy concerning the interpretation of the results. The study is summarized in Section 2. Interested readers should consult the published AREDS report (AREDS Investigators 2001) and responses to it (Seigel 2002; Ferris et al. 2002).

The workshop discussion raised several issues. First, some participants stressed that the results showed that the zinc or zinc-plus-antioxidant supplements only showed a positive effect in the high-risk AMD categories, not in the category with early signs of AMD. Other participants saw the results as another indication of the heterogeneity of AMD. If the study population could have been divided by genetically relevant phenotypes, there may have been a much larger beneficial effect in some phenotypes (where the supplements might be protective for a particular disease pathway) and no effect for others (where the supplements would have no influence on an active pathway). A third point was that, given the multifactorial, multigenic nature of AMD, seeing even a small effect with a simple treatment is an important positive result.

Participants also discussed the metabolic role that zinc might play in reducing the risk of disease progression. The results appear to imply a zinc deficiency in at least part of the study population, although there may also be some unknown metabolic or protective factor that zinc affects. Particularly puzzling was the lack of a significant reduction in risk with antioxidants alone, whereas the zinc supplement alone was effective for the high-risk group in reducing the rate of progression to wet AMD.

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Acronyms

AMD	Age-related macular degeneration
AREDS	Age-Related Eye Disease Study (sponsored by the National Eye Institute)
ARM	Age-related maculopathy
CNTF	Ciliary neurotrophic factor
CNV	Choroidal neovascularization
DHA	Docosahexaenoic acid
FGF	Fibroblast growth factor
LEDGF	Lens epithelium–derived growth factor
PEDF	Pigment epithelium–derived growth factor
RPE	Retinal pigment epithelium
VEGF	Vascular endothelial growth factor

Appendix
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