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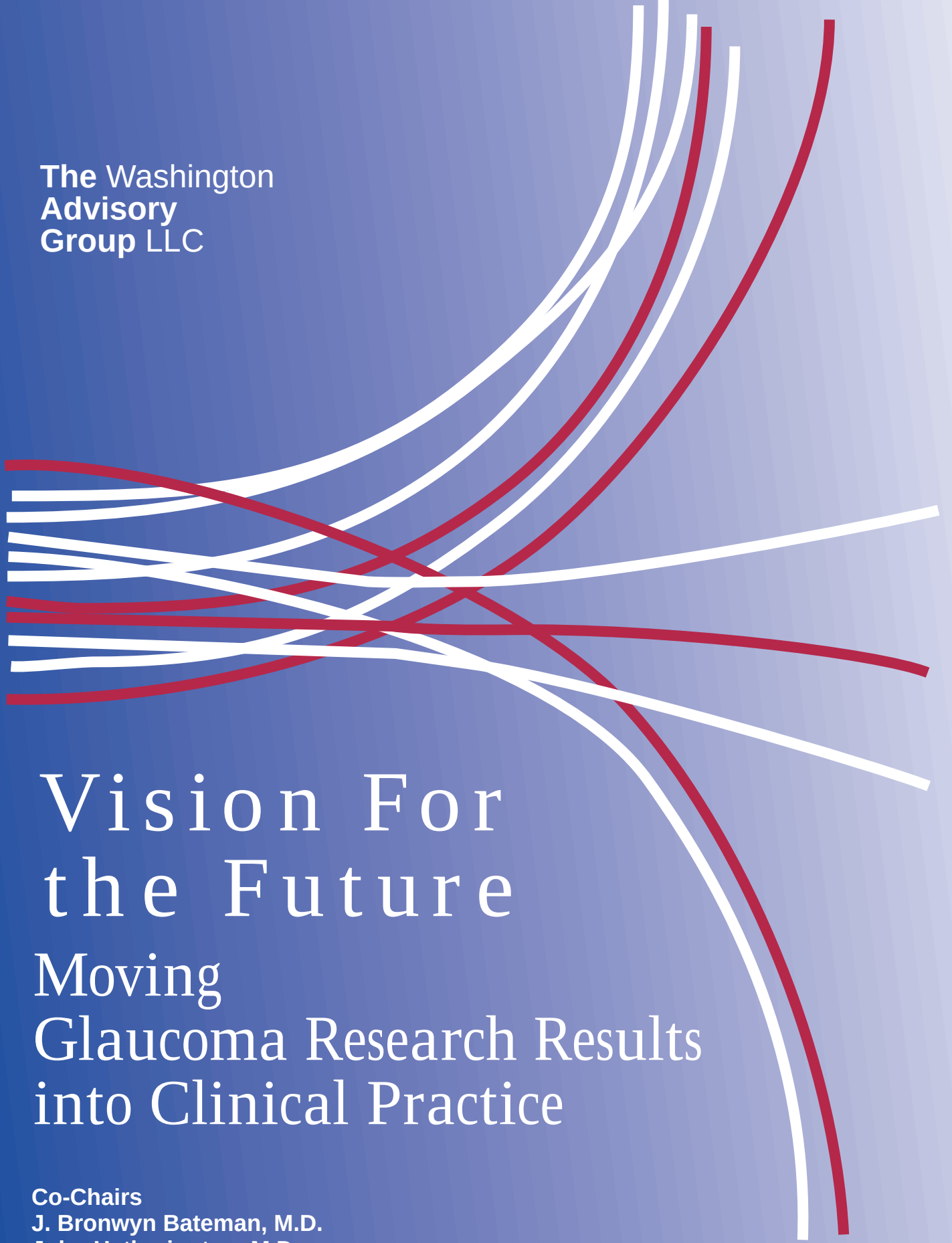

Vision For the Future

Moving Glaucoma Research Results into Clinical Practice

Co-Chairs
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John Hetherington, M.D.
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VISION FOR THE FUTURE

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Summary of a Workshop, June 26-27, 2000
Rancho Valencia, California

Workshop Co-Chairs

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The Principal who led this workshop project on behalf of the Washington Advisory Group was James B. Wyngaarden, M.D. Dr. Wyngaarden also served as a co-chair for the workshop.

Contents

Preface

A workshop on the status of glaucoma research was held at Rancho Valencia, near San Diego, California, on June 26–27, 2000. 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 and opportunities in accelerating the pace of research and the movement of research results into clinical practice. The ten participants (listed in the appendix) first heard eight presentations on the status of glaucoma research. The workshop context was set by first presenting a medical practitioner's view of what might come from glaucoma genetics research and a practitioner's concern for better approaches to protecting the optic nerve. The next four presentations emphasized genetic aspects of glaucoma and the status of research on the genetics of the various types of glaucoma. The final three presentations addressed the status of work on the pathophysiology of glaucomas and potential new targets for therapeutic intervention. Chapter 3 contains summaries of the eight presentations.

During the afternoon of the first day, the participants were asked to construct a list of significant issues and opportunities confronting the glaucoma research community. This list was then discussed and refined during the remainder of the workshop, with the aim of presenting a consensus summation of the most significant issues and opportunities. As the participants discussed the list items and shared views on how to accelerate the research effort, the notion of Glaucoma Research Centers emerged as the most effective way to accomplish a number of important objectives. As the workshop concluded, the participants reorganized the list one more time to emphasize the importance of this central theme. The final version is the Executive Summary list of opportunities and issues for glaucoma research and application.

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 with glaucoma or at risk of developing it.

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Executive Summary

Glaucoma is the second most common cause of blindness in the United States and the leading cause of blindness among African Americans. Three million Americans are probably affected already, and the incidence of glaucoma is expected to increase as the average age of the population increases. Nevertheless, the current state of understanding, as well as diagnostic and therapeutic capability, is far from optimal. Substantial research efforts have produced significant, but not yet sufficient, additions to our medical and scientific knowledge about glaucoma. Progress in diagnosis and therapy for those suffering from glaucoma or at risk of developing the disease has similarly been significant but not yet sufficient.

Glaucoma is a complex, heterogeneous disease with a significant genetic component. Cross-disciplinary interaction on a continuing basis is key to accelerating progress in understanding how glaucoma occurs and using that understanding to prevent or treat it. The participants in the workshop described here agreed that support for one or more *multidisciplinary centers focused on glaucoma research* is essential to sustain the requisite degree of interaction among clinical practitioners and biomedical scientists. Funding institutions and other organizations with an interest in the fight against glaucoma should consider the following key points.

Opportunities and Issues for Glaucoma Research and Its Application

A. Establish One or More Academic Centers for Glaucoma Research

1. **Cross-disciplinary interactions are key to substantive progress.** Glaucoma is a complex, heterogeneous disease. A research context is needed in which those investigating genetic factors, pathogenesis, and therapeutic targets and approaches have frequent interactions and ample opportunity for collaboration.

2. **Center grants** from federal or philanthropic sources, or both, may be essential for establishing Glaucoma Research Centers where multidisciplinary work would be emphasized. Although the National Eye Institute has in the past supported center grants, it has not done so recently.

3. **The time is right for a dedicated sequencing facility focused on identifying glaucoma susceptibility genes**. A substantial list of candidate gene loci for glaucoma-related factors is being developed. A centralized facility is needed to identify the genes and sequence variations in each potential glaucoma locus.

B. Use Genetic Methodology from Work on Other Complex Common Diseases.

4. **Adopt/adapt the best methods used in studying the genetics of other complex (heterogeneous) diseases.** Four general methods that can be pursued are sibling pair analyses, pedigree analyses in large families, analyses of DNA variants, and animal models.

5. **An iterative approach is essential** for defining glaucoma genotypes and refining the phenotypes, for both common and uncommon forms of glaucoma. Organized efforts are needed to iterate the process of using genotypes to refine phenotypic characterization, while also using phenotype data to support genotype delineation.

6. **Refining the phenotypes of glaucoma.** Precise and quantifiable information for phenotyping is needed from clinical practitioners and from existing or new clinical prospective trials.

7. **Cross-disciplinary collaboration** is essential to this iterative approach. More collaboration across disciplines (population and molecular genetics; clinical, histologic, and biochemical characterization; cell biology; and others) is needed to support the iterative process of defining glaucoma genotypes and refining phenotypes.

8. **Data Mining.** A major issue for increasing the effectiveness of genetic research on glaucoma is the ability to get adequate characterization and supporting data from clinical practice records and/or from the large prospective, publicly supported clinical trials.

9. **Use of Existing Clinical Trials for Glaucoma**. A large pool of well-characterized glaucoma patients already exists in recently completed or ongoing clinical trials. However, there are a number of obstacles to using this substantial data resource in the iterative process of defining genotypes and refining phenotypes of glaucomas.

10. **Use of Ethnic Subpopulations for Genetic Screening**. Because glaucoma is a heterogeneous disease, careful definition of the research objective and study design is essential in population studies.

C. Expand Knowledge of Glaucoma Pathogenesis and Potential Therapeutic Targets

11. **The Pathophysiology of the Anterior Chamber.** The pathophysiology of aqueous humor outflow must be better understood, both to develop the genetic basis for increased intraocular pressure and to identify potential targets for therapeutic intervention.

12. **Retinal Ganglion Cell Death.** The initiating events and subsequent stages by which cells die should be explored for their potential role in glaucoma pathogenesis.

13. **Moving Pharmacologic Intervention from the Laboratory to the Patient.** Moving potential pharmacologic treatments for glaucoma from research to clinical use requires Food and Drug Administration (FDA) approval. Only the pharmaceutical industry has the resources to conduct the phase II and III trials required by FDA. But the industry has been slow to support new pharmacologic approaches.

14. **Wound Healing and Surgical Technique Improvement.** Large clinical studies that involved surgical intervention may be useful for gathering information needed to establish the genetic basis for variable response to surgery.

1
Introduction

Glaucoma is an eye disease defined by a characteristic acquired loss of cells in the retina and degeneration of the optic nerve. There are different types of glaucoma, which are distinguished by differences in the conditions that seem to initiate or influence the degeneration of the optic nerve. In the United States, glaucoma of all types is the second most common cause of legal blindness, and its incidence is expected to increase as the U.S. population ages (AAO, 2000, p. 5). Glaucoma is already the leading cause of blindness among African Americans. The National Advisory Eye Council recently estimated that 3 million Americans have glaucoma (NAEC, 1998, p. 77).

In the United States, the National Eye Institute supports research on glaucoma through its Glaucoma Program, and several private institutions also support research on glaucoma. Given the significance of this disease and its potential for damage in terms of both personal well-being and societal losses, where should research efforts be directed, and how can they best be developed, to improve timely diagnosis and treatment of glaucoma? What can be done to encourage productive research and accelerate the transfer of research results into clinical practice? These questions were the focus of the workshop held in June 2000 at Rancho Valencia, California.

A Disease of Complex Origins

Glaucoma is defined by its consequences: damage to the optic nerve, specifically through loss of retinal ganglion cells, with consequent functional deficiencies in the visual field progressing to blindness. To understand the barriers to more rapid progress in fighting glaucoma, it helps to view glaucoma as a *common functional outcome* that can result from a variety of underlying conditions. These conditions can be expressed by diverse physiological pathways leading to damage to the retinal ganglion cells.

A link between chronic elevated *intraocular pressure* (IOP) and progressive damage to the optic nerve has been known for a long time. Knowledge of this link, together with development of objective quantitative methods to measure IOP in a physician's office, has led to common acceptance of elevated IOP as the condition initiating glaucoma. However, many people whose IOP is above the normal range (ocular hypertension) do not develop glaucoma, while a small but significant fraction of glaucoma patients have IOP in the normal range (*normal pressure glaucoma*).

The most common type of glaucoma in the western industrialized countries, including the United States, is *primary open angle glaucoma* (POAG). This type of glaucoma, which typically is associated with elevated IOP, is principally an adult-onset disease. It often develops in middle or old age. However, there are several rare congenital or developmental glaucomas that appear related to defective development of the embryonic eye.

For a type of juvenile-onset glaucoma that is rarer than adult-onset POAG, occurrence of the disease is strongly correlated with mutations in the TIGR/myocilin gene. This gene codes for a protein named trabecular meshwork glucocorticoid response protein (TIGR) or myocilin. Although TIGR/myocilin mutations have been found in a small fraction of patients with POAG (1 to 2 percent), the major genetic factors for susceptibility to POAG are not yet known.

In POAG, and in the juvenile-onset glaucoma associated with the TIGR/myocilin gene, elevated IOP results from decreased outflow of aqueous humor through a complex structure in the front of the eye, called the *trabecular meshwork*. In these glaucoma types, the *angle* of the eye, where the trabecular meshwork is located, remains open (hence the descriptor "open angle" glaucoma). Many nonwestern and developing countries have higher incidences than occur in the United States of glaucoma types in which IOP is elevated because the angle is closed in a way that blocks outflow of aqueous humor via the trabecular meshwork.

Accelerating Progress

An important starting point is the realization that glaucoma is a neural pathology that can result from diverse initiating conditions. Its geneses can be influenced by diverse factors.

With this acknowledged complexity, and significant unknowns about even the most commonly diagnosed types of glaucoma, the workshop discussions focused on ways to accelerate progress in three areas:

1. Diagnosing the preconditions of neural atrophy
2. Predicting the probable progression of effects when these preconditions are identified
3. Preventing, ameliorating, or even reversing the eventual damage to the optic nerve.

2
The Value of a Multidisciplinary Center Focused on Glaucoma Research

As the workshop progressed, the discussion returned with increasing frequency to a common theme. During the closing session, the participants identified this theme as the key issue confronting glaucoma research and clinical care. Because glaucoma is a complex, heterogeneous disease, cross-disciplinary interactions will be critical in accelerating the pace of research and the translation of research results into clinical practice. Different tools and approaches are needed for different issues and problems that arise in dealing with the different types of glaucoma and the full range of factors that may affect the course of the disease. Mechanisms for organization among researchers across disciplines are needed. The participants agreed that *one or more multidisciplinary research centers focusing on glaucoma* was the most practical and effective approach for meeting these needs.

Rationale for Glaucoma Research Centers

A multidisciplinary approach has proven effective in dealing with other complex diseases with strong genetic components, such as cardiovascular disease, hypertension, and Alzheimer's disease. Dr. Anne Spence and other participants noted that, where genetics research is making real headway in exploring the genetic basis of a common complex disease, the research community has taken a multidisciplinary approach. An example of this need for multidisciplinary approaches emerged particularly strongly during the presentations. Refinement of glaucoma phenotypes will require ongoing working relationships between those who study the tissue structure of the eye and glaucoma geneticists who are planning population studies. Although some individuals from these communities interact intermittently, frequent interaction at a working level is needed.

The workshop participants agreed that a Glaucoma Research Center could increase medical practitioners' interest in genetic issues and in supporting the genotype/phenotype research cycle with adequate patient data. Interaction between practitioners and research scientists is also essential for studying the special or rare types of glaucoma. These studies require collecting data on morphology, blood work for genetics, and clinical observation.

Although there are currently no U.S. research centers for glaucoma on the scale envisioned by this workshop, there are precedents for such a center. Other institutes of the National Institutes of Health have supported research centers aimed at a cross-disciplinary approach to a significant, complex disease or closely related conditions. Research centers that attract medical scientists from diverse disciplines are also common in Europe. During the workshop, Dr. Lütjen-Drecoll described the development and multidisciplinary attractions of the glaucoma research center at the Friedrich-Alexander-University of Erlangen-Nuremberg, where she works.[1] There are a number of multidisciplinary groups based in ophthalmology departments at U.S. universities, as well, although these are currently much smaller than the kind of center envisioned. Thus, successful models exist for the type of focused research center advocated at the workshop.

A Glaucoma Research Center would be especially valuable as the base for performing large-scale sequencing of the genetic loci identified as potentially relevant to the onset or course of glaucoma-inducing conditions. The geneticists among the participants emphasized that large-scale sequencing has now become cost-effective for analysis of genetic variants, rather than the older and more conventional approaches to genotyping. After a genetic researcher identifies relevant regions on chromosomes, one or more candidate genes of interest in that region still need to be identified and screened for correlation with the type of glaucoma under scrutiny. These candidate genes could be screened by determining the deoxyribonucleic acid (DNA) sequence of gene variants in individuals carrying the

[1] For more information on this center, see the Internet website at <www.rrze.uni-erlangen. de/ sfb-539>.

genetic markers for a glaucoma phenotype or for glaucoma-relevant risk factors and protective factors. In addition to mapping base pair differences in the protein-encoding portion of a candidate gene, sequencing should include the promoter region in front of the gene, as genetic variation in regulation of a gene may be important.

The multidisciplinary resources of a Glaucoma Research Center would also provide the tools needed to study types of glaucoma other than adult-onset POAG. Methods are needed to acquire useful organ and tissue samples for characterizing the histology, biochemistry, and molecular biology of the various rare types of glaucoma, such as developmental glaucomas and juvenile-onset glaucoma. Adult-onset glaucomas other than POAG, such as angle closure glaucoma, could also be investigated using these multidisciplinary resources.

The workshop participants voiced concern that there are not enough scientists doing basic research, ranging from physiology to genetics, on glaucoma-related conditions. One explanation offered was that the complexity of the phenomena underlying the more common types of glaucoma, coupled with the difficulty of accessing suitable sources of material for investigation (e.g., tissue samples) may discourage young scientists. A Glaucoma Research Center would provide resources and a community of cross-disciplinary colleagues that would help to attract and support young investigators.

The likelihood that existing drugs being used clinically or being tested for other diseases could be effective therapeutically for one or more types of glaucoma was another topic of discussion. A Glaucoma Research Center would improve the capacity to observe and act on these *serendipitous drug effects* on glaucoma-related factors such as IOP or retinal cell death. Collaborations of glaucoma clinical specialists and researchers with those involved in clinical study and basic research on other diseases will aid in identifying and making use of these serendipitous effects.

Building Support for Centers

As the importance of Glaucoma Research Centers emerged during the workshop's closing session, questions arose of how to implement them. First, the community of glaucoma clinical practitioners and biomedical scientists must articulate more clearly and forcefully to potential funding sources the crucial needs for multidisciplinary collaborations and focused resources for major new tools such as gene sequencing and molecular biology. Second, mechanisms for communicating this message must be sought, such as genetics and cell biology conferences with a focus on glaucoma.

The remainder of this chapter, corresponding to sections B and C of the list of opportunities and issues in the Executive Summary, extends the arguments highlighted above for a multidisciplinary approach to glaucoma research and the value of a research center focused on glaucoma. The presentation focuses first on genetic research and then on pathophysiology. However, a cross-cutting theme throughout is that a range of valuable interactions across these and other specialties, along with clinical practice in treating glaucomas, must be tapped if substantial progress against glaucoma is to occur.

Methods of Genetic Research

If a major Glaucoma Research Center were established in the United States, progress in understanding the genetic factors underlying susceptibility to glaucoma would certainly be part of its mission. Section B of the list in the Executive Summary outlines the principal barriers and opportunities in pursuing this understanding, as identified and discussed during the workshop.

Adopt and Adapt the Best Methods Used in Studying Other Complex Diseases

Four general methods of genetic research that have been used successfully with other common, heterogeneous diseases such as hypertension, cardiovascular disease, and cancer are sibling pair analyses, pedigree analyses in large families, analyses of DNA variants, and animal models. Applicability of these methods to glaucoma genetics is discussed below.

Sibling Pair Analyses and Pedigree Analyses. Pedigree analysis using large families is less applicable for POAG than other common diseases because increased IOP—the principal measurable marker for susceptibility to the common types of glaucoma—often does not increase in the POAG patient until middle age. This late onset makes it difficult to establish a POAG phenotype across generations (vertically) in a pedigree tree. Sibling pairs that have the

disease therefore become important for identifying glaucoma susceptibility loci. But a genetic analysis requires many sibling pairs to identify candidate glaucoma loci. Genetic analysis using sibling pairs becomes even more difficult if phenotypes that may be genetically distinct cannot be differentiated in the study.

The genetics investigators at the workshop noted that it remains very difficult to get the data needed to characterize early stages of the common glaucomas objectively and to differentiate types of glaucoma that may well be genetically distinct. Better clinical data are needed to define and diagnose glaucoma phenotypes.

Analyses of DNA Variants. A gene-sequencing facility focusing on genetic loci relevant to glaucoma risk factors would be a crucial tool for performing analysis of DNA variants to define glaucoma genotypes. All glaucoma-related genes that have been identified so far are for early-onset glaucomas. With the exception of the TIGR/myocilin gene, all code for transcription factors that regulate development and seem to be related to abnormal development of the eye. Genetic research hasn't yet identified the genetic factors for susceptibility to the more common types of glaucoma, including adult-onset POAG.

Finding genes that have a secondary role may provide clues to how changes in IOP or other conditions initiate optic nerve atrophy. Genes that may be involved in the progression of the disease include those that affect aqueous humor dynamics (e.g., the trabecular meshwork) and those that affect the stability of retinal ganglion cells.

Animal Models. Differences in gene expression in animal models for glaucomatous conditions can be used as a tool for genomic localization. The workshop participants, particularly during the presentation by Dr. Arthur H. Neufeld, discussed inducing elevated IOP experimentally in animals to study retinal ganglion cell neuropathy, response to intervention, and other aspects of glaucoma pathophysiology. These investigations of differences in gene expression can be extended through studies of genetically altered animals having a "knockout" gene (a gene that contains a specific mutation that prevents it from expressing a functional protein). Gene expression differences in animal models are an excellent example of the value gained from greater interaction among geneticists and pathophysiologists, in areas such as cell biology, histology, and biochemistry, toward identifying candidate genes.

Iterative Process of Defining Glaucoma Genotypes and Refining Phenotypes

Multiple genotypes are involved in the genetic basis of glaucoma. This principle can already be surmised from the heterogeneous nature of glaucoma: the multiplicity of distinct physiological antecedents to progressive ganglion cell degeneration, the number of genomic loci for specific glaucomas, and the still-undeciphered genetic complexity underlying susceptibility to adult-onset POAG. Furthermore, the underlying genetic conditions for POAG probably involve complex interactions among DNA sequence variants occurring in both genes and regulator regions, rather than being linked to one or a few specific gene mutations.

As mentioned above in discussing sibling pair analysis and pedigree analysis in large families, identifying genetic markers for adult-onset POAG is complicated by uncertainties in the definitions and working clinical criteria for different types of glaucoma or pre-glaucomatous conditions. Yet these often subtle or difficult to observe differences in physiological conditions are key to differentiating the *phenotypes* of glaucoma, on which genetic analysis depends.

A recurring theme at the workshop was the interdependence between successful genetic analysis to *define glaucoma genotypes* and the validation of candidate genetic factors through physiological observations to establish corresponding phenotypic variations. In this situation, an iterative approach becomes essential. More complete, standardized, and objective diagnosis of glaucoma-relevant physiology and pathology is needed to guide the genetic analysis. The results from these genetic studies can guide research on the multiple factors and candidate pathways involved in initiating or sustaining glaucoma-causing physiological conditions. Glaucoma Research Centers appealed to the participants as the best way to ensure the frequency and quality of working interactions among specialists that will be necessary to drive this iterative process forward.

Refining the Phenotypes of Glaucoma and Cross-Disciplinary Collaboration

The human geneticists at the workshop noted that more accurate diagnosis is needed for

their work, including better documentation and objective characterization of the evidence for a diagnosis of glaucoma or pre-glaucoma conditions. A recurring problem is insufficiently quantitative measures of effect, such as changes in the visual field or damage to the optic nerve. The need for more complete and documented quantitative diagnostic data is great enough that some mechanism may be needed to compensate clinicians for the time and cost of collecting the data.

The problem of adequate diagnostic information led to discussion of the choice of descriptors for phenotyping. More rigorous and objective markers at the level of cellular changes or tissue structure, or at the biomolecular level, may be needed to define candidate phenotypes. These physiologically based phenotypes could then be correlated with candidate genotypes identified through methods such as sibling pair analyses and gene sequencing of individuals characterized according to these refined phenotypes.

The workshop participants agreed that phenotyping simply by IOP levels is not adequate to support genetic research. Although glaucoma diagnosis began with measuring IOP and observing whether the angle was open or closed, methods of phenotyping are progressing. Dr. Lütjen-Drecoll, for example, includes tissue structures in her work on differentiating types of glaucoma. The process of refining phenotypes must also include testing whether a candidate classification or reclassification works for clinical identification and diagnosis, as well as for genetic classification. It will have to be an iterative and interactive process, involving clinical practitioners and geneticists, as well as physiologists in a range of disciplines (histology, cell biology, molecular biology, and biochemistry).

Data Mining and the Use of Existing Clinical Trials for Glaucoma

An essential source of the data needed for defining glaucoma genotypes and refining phenotypes is the documentation of diagnoses by clinical practitioners. The Preferred Practice Patterns developed by the American Academy of Ophthalmology for physician diagnosis and treatment of glaucoma patients appear adequate for this purpose. However, concerted effort is needed to ensure that these guidelines are followed. To mine these clinical practice records for useful data and create uniform records suitable for human genetics studies, trained document reviewers may be needed.

The current large clinical trials supported by the National Eye Institute are potentially a rich source of high-quality data needed for the iterative process of defining genotypes and refining phenotypes. It will be a daunting task to add or change any data acquisition or sample collection requirements that would involve altering an approved study protocol. A second consent from participants may be needed for blood sampling and genetic testing in all but one of the current large trials. Even access to study data may be difficult to arrange. Nevertheless, the history of changes to large cancer trials and other major trials supported by the National Institutes of Health shows that these obstacles can be surmounted. A first step is to assess which trials would give the most significant increase in information needed for an iterative approach to genetic research. The next step would be to develop and voice support from the research, clinical practice, and patient communities to bring about any changes needed.

Use of Ethnic Subpopulations for Genetic Screening

The various types of glaucoma have different incidence rates in different ethnic groups. This fact and the recognition of genetic factors in susceptibility to glaucoma have fostered interest in genetic screening studies of ethnic subpopulations. Proposals for such studies require careful consideration of scientific, medical, and ethical issues, which are not always clearly presented and discussed prior to beginning the research.

Screening studies that target ethnic subpopulations necessitate careful design to ensure that they can answer useful questions about glaucoma phenotypes and genotypes. Often, not enough thought is given to questions such as:

- What kinds of questions is a given subpopulation ideally suited to answer?
- What hypothesis is to be answered with a population?
- How should a target population be sampled to get a reliable, statistically significant, and generally credible answer?

Pathogenesis of Glaucoma and Therapeutic Targets

A Glaucoma Research Center should also include programs for research on the pathophysiology of glaucoma and investigations of therapeutic approaches. Both of these areas of inquiry will contribute to and learn from the work on glaucoma genetics. Indeed, the iterative process discussed above for accelerating the understanding of the genetic bases for the various types of glaucoma requires the continuing involvement of specialists in physiological and pharmacologic disciplines. In addition, to understand and treat the common glaucomas, it is essential to understand changes in the trabecular meshwork related to elevated IOP and the linkages of IOP to effects on tissue structure and cell function in the rear of the eye and the optic nerve head.

In human patients it is difficult to quantify objective, direct measures of disease progression such as retinal cell loss. Animal models can be used to investigate risk factors for retinal ganglion cell death and establish indirect markers of retinal cell loss that can be transferred to clinical diagnosis. Animal models can also be used to test and refine hypotheses about the mechanisms underlying different types of glaucoma, as in Dr. Neufeld's work demonstrating retinal ganglion cell protection in the context of elevated IOP. (See presentation in Chapter 3.)

The workshop participants discussed several key directions for research in understanding the pathophysiology of the anterior chamber as it relates to glaucoma, as well as issues and opportunities in research on retinal ganglion cell death. For the anterior chamber, the pathophysiology of aqueous fluid outflow was highlighted in several presentations. More research is needed on the initial changes in the optic nerve head that cause damage to retinal ganglion cells, including changes in supporting cells and structures around the axons of the ganglion cells.

Pathophysiology of the Anterior Chamber

Much work is still needed to sort out the pathogenesis of the multiple, heterogeneous forms of POAG. As Dr. Lütjen-Drecoll noted at the workshop (see Chapter 3), there are pronounced differences in the histologic changes associated with different types of glaucoma. Understanding these differences, how they originate, and how they link to eventual nerve cell damage, will be critical for refining glaucoma phenotypes, predicting or following the course of a particular type of glaucoma, and providing effective preventive or therapeutic treatments. As in the work on genetics of glaucomas, cross-disciplinary collaboration is key to increasing the rate of progress in understanding the pathophysiology and etiology of obstructed aqueous humor outflow.

Illustrative of the unresolved issues and major opportunities that can be pursued are the following examples from the workshop presentations:

- The outflow pathways from the anterior chamber can be manipulated in multiple ways, thereby reducing IOP. Identifying new potential targets for intervention to reduce IOP was discussed by Dr. Paul Kaufman.
- Although a strong correlation exists between optic nerve damage and glaucoma-associated changes in the extracellular material of the trabecular meshwork, various causal mechanisms could account for this correlation. The favored view is that the extracellular changes *lead to* increased IOP, which then leads to nerve damage in the retina and optic nerve. However, it is also possible that common factors induce both changes. Further work is needed to establish and explain this mechanism or to uncover and confirm one of the logically possible alternatives.
- When a factor associated with structural changes in glaucomatous eyes is identified (such as the TGF-β_2 increase studied by Dr. Lütjen-Drecoll), careful investigation is needed to assess whether that factor is involved in causing the damaging changes or is a response to ameliorate damage arising from other factors.

Retinal Ganglion Cell Death

In seeking preventive and therapeutic approaches to glaucoma treatment, the potential advantages of directly protecting the nerve from damage should not be overlooked. If the nerve can be protected (preventing retinal ganglion cell loss), then the antecedent conditions and factors affecting glaucoma susceptibility will be less damaging. Although this approach represents a

radical shift from the traditional emphasis on reducing IOP to treat POAG, there are precedents.

Dr. Neufeld's presentation on optic nerve protection focused on preventing damage to the retinal cell axon. More research emphasis is needed on understanding the initial changes in the optic nerve head that are the proximate cause of damage to the axon of retinal ganglion cells. Dr. Neufeld believes that glial cells surrounding the axons, particularly the astrocytes, play a key role in this aspect of glaucoma pathophysiology. Other supportive tissue or cell types may also be involved. Factors to be explored for potential roles in initiating events in the nerve head and subsequent stages of neural damage include the effects of glutamate, nitric oxide, growth factors, and modulation of apoptotic events.

Moving Pharmacologic Intervention from the Laboratory to the Patient

To move potential pharmacologic treatments for glaucoma, whether by lowering IOP or by other routes for protecting the optic nerve, from research to clinical use, Food and Drug Administration (FDA) approval is essential. The Good Laboratory Practice and Good Manufacturing Process standards required by the FDA are typically not met in academic laboratories and require substantial financial resources.

To date, only the pharmaceutical industry has the resources to meet these requirements and conduct clinical trials on the scale required for Phase II and Phase III FDA approval. However, because of the substantial investments required and the low rate of successful commercialization for candidate drugs moving through the clinical trial process, for-profit companies reasonably prefer established approaches to more innovative but riskier opportunities.

In response to political pressure from patient communities (notably, the AIDS and cancer communities), the FDA and the pharmaceutical industry have worked to accelerate the trial and review process in some circumstances. Glaucoma is a costly and debilitating disease, and the incidence of the adult-onset types is likely to increase as the American population ages. Similar mobilization of patient and care-provider communities for glaucoma may help to encourage innovative approaches to testing and approving potentially useful therapies.

Glaucoma Research Centers could provide stable research and development environments, supplementing and helping to bridge the gap between existing academic research laboratories and industry's development facilities. With such centers to focus the demand for progress and provide a mechanism for interim development, innovative approaches to satisfying regulatory approval requirements may emerge more easily.

Wound Healing and Improving Surgical Techniques

For POAG patients with severe IOP elevation and poor response to pressure-lowering drugs, or for angle closure glaucomas, surgical intervention can be used to increase the aqueous outflow. However, the response to surgical opening of an outflow pathway differs markedly among patients. Some healing of the wound after surgery is needed, but if healing is too rapid or too complete, it can close the pathway, with consequent return of elevated IOP. In some patients, particularly in infants and children or members of some ethnic groups, excessive scarring may occur. The potential for such scarring affects the decision on whether a surgical treatment should be attempted.

Glaucoma practitioners need to know how to control the healing response after surgery and how to predict the likely response of a patient to a surgical technique. Understanding the genetic factors in differential healing responses would help, as would a better understanding of the physiology of healing in response to different surgical techniques and improved pharmaceutical approaches to control the healing response.

3
Workshop Presentations

The eight workshop presentations were divided into two sessions. The first focused on issues and opportunities in research on the genetic basis for glaucoma. Common themes included approaches to dealing with the genetic complexity of common types of glaucoma, the relative dearth of understanding of the genetic model for adult-onset POAG, and the importance of multidisciplinary efforts among clinicians, physiology-oriented researchers, and geneticists to overcome the obstacles to progress. The second session focused on pathophysiology of the anterior chamber and the optic nerve. Principal themes of the second session were a physiological perspective on how the pressure regulation system in the anterior chamber works and the cell biology of glaucoma in both the anterior chamber and the optic nerve.

The current positions and academic degrees of the eight presenters are included in the list of workshop participants in the appendix.

John Hetherington

Dr. Hetherington spoke from the perspective of a clinician on what clinical practitioners are expecting from genetics research on glaucoma. He also addressed the importance of neuroprotection as a guiding principle for treatment.

Going back to the beginning of the twentieth century, there were a variety of attempts to identify predictors of susceptibility to glaucoma. Many of these early attempts focused on looking for susceptibility to pressure rise in the anterior chamber due to environmental factors. Now the search for reliable screening tests has focused on genetic factors. Current research on treatment methods includes searching for neuroprotection agents. The established risk factors for glaucoma include, for example, family history, IOP, race, and age. Less certain risk factors, on which some controversy exists, include myopia, diabetes, and hypertension.

A key issue is finding the genetic factors involved in the common types of open angle glaucoma. Clinicians are aware that there are many genes being investigated for links to glaucoma. In reviewing the list of genetic loci or genes identified to date, Dr. Hetherington noted that all are related to rare types of glaucoma, such as the developmental glaucomas or the juvenile-onset glaucoma related to the TIGR/myocilin gene. He illustrated their rarity with the statistic that a clinician in practice might see an average of one case of congenital glaucoma every five years.

From a clinician's view, the value of genetic testing is to provide a progression of clinical applications. The earliest of these is likely to be screening to assess the tendency to develop a glaucoma. Dr. Hetherington sees this application as very near for juvenile-onset glaucoma linked to the TIGR/myocilin gene. Subsequent applications would include early diagnosis, aid to the practitioner in selecting the most appropriate therapy, and eventually gene-designed therapy. Medication based on genetics appears to Dr. Hetherington to be ten to twenty years away.

With respect to directions in genetic screening, Dr. Hetherington sees value in screening for TIGR/myocilin gene mutations only in the first-degree relatives of patients with juvenile-onset glaucoma. Chronic open angle glaucoma occurs in only a few percent of the general population, depending on the population study. Only 2 to 3 percent of glaucoma patients in this population test positive for a mutation of the TIGR/myocilin gene, making the test less rewarding.

Medications that might eventually result from genetic research include those based on gene transfer techniques. Dr. Hetherington is uncertain, however, whether such techniques, if they were to require periodic intraocular injections, would be an acceptable therapy to ophthalmologists and patients. (The participants' discussion of this point noted that acceptance would likely depend on the required frequency of injections; injections once a year or less frequent might be acceptable.)

Dr. Hetherington suggested that foreign clinicians who train in the United States and return to their own countries to practice could provide a resource base for data collection. They could, for instance, be useful for identifying family-related histories of glaucoma types that are specific to, or that occur more frequently in those differing populations.

In closing, Dr. Hetherington gave a clinician's perspective on some of the obstacles to use of genetic methods. First, large pedigree trees are limited because common glaucomas are primarily late-onset diseases. Second, if adult-onset POAG and other common types are heterogeneous diseases involving conditions in the trabecular meshwork, collagen tissue, astrocytes, etc, then multiple genes are likely to be involved.

During the discussion, Dr. Wiggs returned to the point that all the genes and genetic loci identified so far are tied to early-onset glaucomas. With the exception of the TIGR/myocilin gene, all code for transcription factors that regulate development and seem to cause abnormal development of the eye. The current hypothesis is that this abnormal development causes high IOP and consequent optic nerve disease. The function of the TIGR/myocilin protein and its role in juvenile-onset glaucoma are still in debate. Other participants raised the possibility that genes coding for transcription factors involved in ocular development may also have an as yet unknown role in later life in tissue repair. Dr. Wiggs agreed with this and added that the genes involved in stability of retinal ganglion cells also have yet to be identified. So clinicians as well as those involved in glaucoma research need to understand that multiple genetically based factors are likely to play a role in POAG and other glaucomas.

Bronwyn Bateman

Dr. Bateman highlighted the diversity of the genetic factors in glaucoma with slides showing the differences in glaucomatous eyes. Cases of congenital glaucoma are very rare in the United States, but they occur more frequently in the Middle East and among some ethnic groups. All of the juvenile and developmental glaucomas involve elevated IOP.

The etiology of common, adult-onset glaucomas is very complicated. In individual cases, there may be environmental factors (for example, injury to the eye in childhood) as well as genetic factors. She iterated the point that genetic research has not yet touched on the genetic bases for the more common types of glaucoma.

Next, Dr. Bateman questioned the clinical definition and characterizations of glaucoma. What is glaucoma, from a diagnostic perspective, and at what point is an individual affected by late-onset glaucoma? These and related issues in drawing boundaries make population-based genetic analysis even more difficult. In closing, she noted that genetic analysis and testing raise ethical questions that will have to be addressed at some point.

M. Anne Spence

Dr. Spence described herself as a population geneticist or statistical geneticist. Her research includes joint efforts with biomedical scientists studying other diseases, such as autism, as well as genetics research on glaucoma. She began by characterizing the value and the limitations of the Human Genome Project (HGP) as a genetics resource. The HGP provides partial sequences of the nucleotide bases forming the DNA strands of the human genome. The usefulness of these sequences to the geneticist depends on additional factors, such as having well-annotated gene maps that show where a DNA sequence fits in the chromosome. For many of the published DNA sequences, the existing maps are rather poor, in Dr. Spence's view, as are the annotations to indicate where the genes are and what is known about them. To use the sequence information provided by the HGP, the geneticist needs to know where to look. Dr. Spence estimates that another two to five years of intensive work will be needed to provide scientists with a "user-friendly" gene map. The work of annotating this map will never be finished.

Dr. Spence believes that glaucoma-related genetic research should now be focusing on establishing genotypes by large-scale sequencing of genes such as the TIGR/myocilin gene. Sequencing a gene or a genetic region (sometimes 10 or more genes) identified as potentially relevant to glaucoma should be carried out for multiple individuals, to establish the genetic variants. This sequencing should include promoter regions, as well as the sequence defining the protein expressed by a gene. The discussion of this suggestion led the participants to the concept of a large-scale gene-sequencing facility as an important component of a Glaucoma Research Center.

However, Dr. Spence added, researchers cannot make progress with genotyping unless they know the glaucoma phenotypes. This raises the problem, noted by other presenters, of glaucoma definitions and characterization of different types of glaucoma. A major problem in genetics research on common disorders, Dr. Spence added, has been clarifying the question, "What are you studying?" Brainstorming on

definitions can be important to understanding the genetic basis and rethinking the dimensionality of phenotypes. As an example, she cited changes in the concept of hypertension.

In her presentation and throughout the discussions, Dr. Spence stressed the importance of taking a multidisciplinary approach. She remarked that the genetics research community tends to focus on one tool for studying populations. Different tools and approaches are needed for different issues and problems. Where genetics research is making real headway in exploring the genetic basis of other common, complex diseases, the research community has taken a multidisciplinary approach.

In genetics research on glaucoma, geneticists who are planning population studies need to refine their working phenotypes in terms of physiological differences found by those who study the tissue structure, cell biology, biochemistry, etc., of the glaucomatous eye. All these disciplines are not currently talking enough to each other as communities, although some individuals within them do interact. For comparison, Dr. Spence listed the disciplines of her colleagues on a genetics study of autism. In addition to statistical genetics (population genetics) and molecular genetics, the team has specialists in pediatric neurology, clinical psychology, sociology, and cell biology.

Dr. Spence also stressed the need for careful design of population studies and definition of population samples. Often, insufficient thought is given to questions such as what hypothesis is to be answered with a population, what kinds of questions a given subpopulation is ideally suited to answer, and how to sample that population to get a good answer.

During the discussion, Dr. Wiggs commented that one problem in refining glaucoma phenotypes is insufficiently quantitative measures of effect, such as visual field changes or damage to the optic nerve. This opened a general discussion among the participants on refining the characterizations on which phenotypes are based. Dr. Spence added that the effort at correlating genotypes to refined phenotypes must include testing whether the refined classifications work for diagnosis and recognition in clinical practice, as well as for genetic classification. Rather than a one-way influence, there must be an iterative and interactive process crossing over disciplinary and specialty boundaries.

To expand the effort in glaucoma genetics, Dr. Spence suggested several steps. Key players in the glaucoma research community should enlist the aid of statistical geneticists in their work. She also advocated that, to "spread the word," glaucoma specialists should attend other professional meetings than those dedicated to glaucoma. A third step would be to establish a shared database to coordinate information on annotations for genetic sequences.

Janey Wiggs

Dr. Wiggs began with a list of major tenets in her perspective on glaucoma. First, glaucoma is genetic, or at least there are genetic susceptibilities for all types of glaucoma. No environmental factor has yet been identified. Second, to do genetic analysis for glaucoma, large families (pedigrees) are not necessary. Rather, better approaches are needed to studying the genetics of adult onset glaucomas. Third, glaucoma in her view is unlikely to be an inevitable consequence of aging. Therefore, there is significant medical value in learning enough about the genetic basis of the different types of the disease to provide pre-onset screening and preventive therapy, as well as genetically informed intervention as initiating events occur. Fourth, there are quantitative aspects that can be measured to "tease out" the glaucoma phenotypes. Establishing objectively differentiated phenotypes is important because glaucoma is a heterogeneous disease. Success in genetic studies will require focusing in on a smaller number of genes relevant to a particular phenotype, rather than trying to identify the genetic factors involved in many types of glaucoma at once. Finally, Dr. Wiggs agrees with Dr. Spence on using multiple approaches in glaucoma genetics; the community's efforts should not be limited to a single approach.

The defining pathology of glaucoma is that the ganglion cells in the optic nerve die. The question to answer is "Why do they die?" There is an association with elevated IOP. If the IOP becomes high enough, the nerve will die, but why elevated IOP leads to ganglion cell death is not known. Little about the process of nerve damage is established, except that it appears to be apoptotic. It also seems likely that glutamate and nitric oxide metabolism are somehow involved.

On the role of protecting the optic nerve, Dr. Wiggs noted that, if the nerve can be protected, then none of the antecedent factors matter. She believes that one link in the pathogenesis of glaucomas is genetic susceptibility to ganglion cell death. Another link is genetic susceptibility

to increased IOP. For POAG, the latter susceptibility appears related to reduced outflow from the anterior chamber (for example, problems in the trabecular meshwork), rather than to increased inflow. Science has not yet found these links. Thus, her work has focused on identifying genes connected with susceptibility to ganglion cell death, susceptibility to increased IOP, or changes in the trabecular meshwork.

Dr. Wiggs anticipates that identifying genes for susceptibility to initiating events and stages in glaucoma processes will aid in providing a prognosis for an individual with a family history of glaucoma. Ideally, a physician would be able to answer at least some of the following questions: Will this individual be likely to develop an elevated IOP? When will it happen? Will the elevated IOP respond to medication, and to which ones? Will the individual develop nerve damage if the IOP is untreated? Can nerve damage be prevented or treated in this individual?

In an ideal world, identification of glaucoma-related genes will provide DNA-based diagnosis that will lead to identification of risk factors. Presence of specific gene variants will be a prognostic indicator of both increased IOP and likelihood of ganglion cell death. The genetic information also could indicate the likely response to specific therapeutic and ameliorative interventions, whether pharmacologic or surgical. Ideally, knowledge of the proteins expressed by the genes will lead to new treatments based on knowing the roles of those proteins in glaucoma initiation and progress.

The only gene found so far that is not related to abnormal ocular development is the TIGR/myocilin gene. Mutations in this gene are associated with a severe, juvenile-onset open-angle type of glaucoma. Rarely, mutations in TIGR/myocilin contribute to adult-onset POAG.[2] No genes have been discovered that commonly contribute to adult-onset POAG.

The developmental and juvenile-onset glaucomas all involve effects on the trabecular meshwork, leading to high IOP as the cause of ganglion cell death, irrespective of ganglion cell genetic defects. These types show straightforward Mendelian heredity. But adult onset glaucoma appears to combine effects on the trabecular meshwork and susceptibility to ganglion cell death. Although the same gene could be involved, it is more likely that multiple genes and gene combinations are involved.

Dr. Wiggs discussed the role of one gene linked with congenital-defect glaucoma. This type of glaucoma is associated with an abnormal angle and is treated by surgery. The gene is induced by exposure to dioxin, so it is somehow involved in metabolism of toxins. How defects in the expressed protein, a cytochrome P-450 protein, apparently cause only a developmental eye disease is not yet understood.

For Dr. Wiggs' genome screening study of adult POAG, the selection criteria required evidence of visual field defects, indicating damage to the optic nerve, as well as elevated IOP. For siblings to be included in the screening, there had to be evidence in each sibling of optical nerve damage and visual field dysfunction. These stringent selection criteria made it difficult to find enough sibling pairs. Finding even 182 pairs that met the criteria was very difficult. The screening study identified seven new glaucoma susceptibility loci for adult-onset POAG. Sequencing of candidate genes located in these regions is continuing.

Dr. Wiggs described the process by which regions containing genetic markers are further analyzed to identify and screen candidate genes. After the regions of interest on chromosomes are identified, one or more candidate genes of interest in each region need to be identified and sequenced. Reiterating Dr. Spence's point, Dr. Wiggs noted that the HGP does not help find these regions. The researcher still needs the patients and the genetic analysis to locate the chromosome regions with candidate genes. Once the chromosome regions that correlate with individuals having the conditions under study have been fairly well defined, the DNA sequences from the HGP are very helpful in identifying the genes in those regions. Finding the genes once the regions are localized is therefore not a problem. The next issue is determining the priority in which genes in the chromosome regions will be sequenced for individual variants. In a typical localized region of 1 to 2 centimorgans in extent, there are often ten or more genes.

Commenting more broadly on the difficulties of glaucoma genetic research, Dr. Wiggs said that the current health care system provides inadequate incentives for attending physicians to perform the careful clinical testing and detailed reporting of diagnostic observations required to distinguish potentially important phenotypic

[2] Other workshop participants noted that mutations in the promoter region for the TIGR gene may show a stronger correlation with POAG.

differences. She favors a mechanism to compensate the practicing clinician for providing the detailed information needed for genetic analysis. In addition, once candidate genes are identified, it will be necessary to relate defects in those genes back to specific, clinically recognizable phenotypes.

Dr. Wiggs closed with a view of the future. Once genes related to adult-onset POAG are found and the mutations or polymorphisms that are tied to glaucoma susceptibility are identified, appropriate clinical information will need to be collected on individuals with the phenotypic characteristics related to these genotypes. Dr. Wiggs predicts that this information will lead to identifying a number of disease-relevant genetic variations (polymorphisms and mutations) and a range in severity of defects, which interact in various ways to create susceptibility to glaucoma. She stressed the importance of ensuring that the general clinical community collects the information needed to characterize the phenotypes correlated with susceptibility-inducing genotypes.

During the follow-up discussion, Dr. Spence and Dr. Wiggs described approaches to prioritizing the genes to be sequenced and conducting the sequencing for individual variants in the genomes of study subjects. One conventional approach is to use information about the proteins expressed by the candidate genes to prioritize the sequencing effort. Dr. Spence noted, however, that the difficult step of gathering or developing sufficient information on expression could now be bypassed in favor of a large-scale sequencing effort. This direct approach is now feasible because of the techniques and equipment available for rapid sequencing.

Henri-Jean Garchon

Dr. Garchon began by saying that there is not yet an underlying genetic model for adult POAG. It could be a polygenic disease, with multiple genes affecting the phenotypes. These phenotypes can be viewed as risk factors for developing POAG. Using this meaning for a "risk factor," the development of glaucoma in a given individual is a function of the expression of genetic-based risk factors and the period over which these risk factors are expressed in that individual. In this framework for a glaucoma genetic model, a combination of risk factors leads to the disease.

A major point of Dr. Garchon's model is that these genetic risk factors are determined by common genetic variants (polymorphisms), not by specific mutations. Although this is an assumption, there is evidence supporting it. These common genetic variants often involve a change in a single base pair in the gene sequence. A variant may also alter the regulation of gene expression [e.g., base pair variants in the promoter region], rather than altering the structure of the protein expressed.

Glaucoma as a disease is the result of optic nerve damage. What we can observe and evaluate easily in patients is the IOP. Field defects are more difficult to observe and evaluate objectively, but it can be done. For other significant risk factors, such as vascularization, changes in the optic nerve head, and ganglion cell stability, observation and clinical evaluation in the living patient are even more difficult. In addition to questions about the risk factors that lead to glaucoma, other questions to be answered include which factors make glaucoma severe, and which factors make it responsive to treatment.

To explore these questions about underlying genetic factors, Dr. Garchon has been looking at variability in the age of onset of juvenile glaucoma associated with mutations in the TIGR/myocilin gene. (Age of onset was determined by detection of both elevated IOP and changes in the visual field.) The initial hypothesis is that occurrence of this disease in an individual follows simple Mendelian patterns of heritability of the gene mutation. In some of the families studied, both adult-onset and juvenile-onset glaucomas occur. The mechanisms for the observed variability in time of onset could be genetic—the consequence of specific mutations or variants in other genes—or they could be environmental. The clinical value of exploring the basis of the variability lies in the potential to anticipate onset of the disease and provide follow-up and treatment. Furthermore, if genetic variants that protect against early onset can be found, this information could lead to new therapeutic approaches.

Dr. Garchon presented data on the variability of onset of glaucoma in several families having carriers of TIGR/myocilin mutations. In all, there were 86 individuals who carried a glaucoma-linked mutation. Within each family, all carriers can be traced to the same founder. The severity of glaucoma correlated with the age of onset. Several of the correlation graphs presented by Dr. Garchon appear bimodal, indicating the presence of other genes modifying the effect of the TIGR/myocilin mutation. He noted that work

is continuing to identify the modifying gene or genes. In his analysis, he used a model of susceptibility with age of onset and presence of a mutant gene as parameters. He has narrowed the genetic screening of his study families to four regions, on four different chromosomes, which may be loci of relevant genes.

Genetic screening approaches that have been used for identifying genes relevant to Alzheimer's disease can be a useful model for genetic screening for adult-onset glaucoma because both diseases involve a late-onset neuropathy. This genomic screening was undertaken because Dr. Garchon and his associates were interested in a candidate gene associated with late-onset progressive neuropathy, Apo-E. Age of onset was chosen as the characteristic on which to screen because they found significant variability in age of onset. The patterns found in the TIGR/myocilin glaucoma families were analogous to the pattern in a family with a history of Alzheimer's disease; family members with one allele of the Apo-E gene have earlier onset than those with another allele.

Dr. Garchon agreed with the point made by Dr. Wiggs that the mechanism is not yet known by which a mutation in the TIGR/myocilin gene initiates changes leading to glaucoma. Differences in regulation of this gene are associated with changes in the trabecular meshwork, but it is not clear which change is cause and which is consequence. It is possible that a promoter affecting TIGR/myocilin expression plays a role. Dr. Garchon noted that many polymorphisms have been identified in the vicinity of the TIGR/myocilin gene, and among these may be some involved in differential regulation of that gene.

In a retrospective study of 142 glaucoma patients, Dr. Garchon and his coworkers looked for a particular variant that occurs within a hundred base pairs of the TIGR/myocilin gene and therefore may be involved in regulation of the gene's expression. In this study, they found a significant correlation between effectiveness of treatment (in reducing IOP) and presence of the variant in the promoter region.

During the follow-up discussion, Dr. Paul Kaufman noted that the incidence of TIGR/myocilin mutation in adult-onset POAG is low. He asked if it were possible that a higher correlation might exist between variants in the promoter region of the gene and , adult-onset POAG. Dr. Garchon said this was possible, but added that he had looked only at fairly common polymorphisms in the promoter region, rather than rare mutations. Discussion continued on how variations in TIGR/myocilin expression might be related to differences in the development and response to treatment of elevated IOP, as well as possible effects in the trabecular meshwork. Dr. Spence remarked that this discussion exemplified the interactive process of refining phenotypes of glaucoma while learning about genotypes (such as variants in the TIGR/myocilin promoter region) that may correlate to the newly formulated phenotypes.

Paul Kaufman

Dr. Kaufman began with a summary of some of his general views on glaucoma etiology and therapy. He views elevated IOP as a causal risk factor for glaucoma at every level of intraocular pressure. He acknowledged this position might be considered "rather dogmatic," but it can be supported from recently published results from the Advanced Glaucoma Intervention Study (AGIS 2000). With respect to gene therapy, Dr. Kaufman sees the fundamental issue in all pharmacologic intervention as getting a drug delivered to the point of effective operation in appropriate concentration, without side effects on other parts of the body or functions. All drugs that aim at affecting the signal pathways among cells act on some protein or enzyme that ultimately is expressed by a genetic mechanism. Why not, then, use a gene product (e.g., a structural protein or enzyme) that affects the pathway? Taking this approach, one would look for ways to affect the regulation and expression of that genetic mechanism to get the desired effect on the concentration of the structural protein or enzyme of interest. In principle, an appropriate degree of up-regulation or down-regulation could be produced that would last for an extended period. Many issues still need to be addressed, such as tissue specificity, getting a gene into the right cells, and regulating it.

The main theme of Dr. Kaufman's presentation was an exploration of potential physiological targets for therapeutic approaches, based on enhancing fluid outflow from the anterior chamber to lower IOP. Any point along the sequence of structures controlling outflow, or of elements regulating these structures, is a potential target, even if that structure or regulatory element is not involved in causing the elevated IOP. These potential targets for manipulating outflow involve one or the other of

two outflow pathways: the trabecular meshwork or the secondary outflow around bundles of the ciliate muscle.

The trabecular meshwork is a latticework of connective tissue beams with endothelial-like cells surrounding each layer of the lattice. Closer to the outflow canal, endothelial cells are simply swimming in the extracellular matrix and are not attached to a supporting structure. Dr. Kaufman's hypothesis is that most of the resistance to outflow, in eyes with either normal or elevated IOP, is in the region without the supporting structure. However, this region can also be affected by the geometry of the latticed region, so that the tissue may function as an integrated whole. The cell junction complexes in both regions are very dynamic. They probably vary in number, location, and tightness, depending on the history and state of the cell's environment. The junctions can be affected by drugs, hormones, and physical stresses such as pressure and shear stress.

Dr. Kaufman stressed the complexity of the junctional structures. They involve dozens of structural proteins and signal transduction proteins. The signal transduction proteins regulate relationships between the structural proteins and the activity of the cell junctions.

One of the many ways of manipulating this complex system to affect outflow is to use cytochalasins to disrupt microfilaments. Injecting a cytochalasin into the anterior chamber of monkey eyes dramatically increases outflow. The effect is relatively short lived and highly reversible. Cytochalasins cause visible expansion of the trabecular meshwork. Dr. Kaufman believes this expansion results from a weakening of the junctional complexes, which relaxes the meshwork, facilitating fluid movement across it. This explanation suggests manipulating the cytoskeletons of adhesion complexes in the meshwork as a potential therapeutic approach.

New compounds are being studied that change the cell junctions by truncating the microfilament structures. These *latrunculins*, which are extracted from an ocean-dwelling sponge, bind to free actin in cells. Decreasing the availability of free actin slows the assembly of the microfilaments, thereby gradually weakening the actomyosin structure. This degradation causes secondary changes in the junctional complexes. Adding a latrunculin to cells in culture leads to gradual separation of the cells. The effect is highly reversible.

Another drug that affects the lattice structure of the trabecular meshwork is a protein kinase inhibitor called H-7. It probably inhibits the myosin light chain kinase pathway, in effect decoupling actin from myosin in the cell, with consequent loss of cellular contractility. This leads to lowering of IOP in normal-pressure monkeys by increasing the outflow from the anterior chamber several-fold. Both latrunculins and H-7 decrease IOP, whether applied as drops or injected into the anterior chamber.

In the normal monkey eye, outflow, as visualized using tracers, is through discrete pathways. Relaxants open up many more pathways through the trabecular meshwork because the whole system is more relaxed.

In general, prostaglandins affect the uveoscleral outflow pathway, not the trabecular meshwork. $PGF_{2\alpha}$, the parent compound of a drug used clinically, affects the extracellular matrix. Treatment of the eye with this compound removes collagen connective tissue from the extracellular matrix of the ciliary muscle, even in the sclera overlying the ciliary muscle.

Dr. Kaufman described some recent research in transfecting genes into cells that are relevant to glaucoma, using replication-defective adenoviruses or herpes viruses. At present, this work does not involve therapeutic genes, just reporter genes. Genes could be transfected into cells involved in producing the aqueous fluid, cells in the trabecular meshwork, and cells relevant to affecting the uveoscleral pathway. Significant issues now are duration of expression, site specificity, and checking for deleterious side effects. Dr. Kaufman believes this transfection methodology is ready now for use in research on what a particular protein, such as TIGR/myocilin, does in the eye, although it is not yet ready for clinical development as a therapy for elevated IOP.

Elke Lütjen-Drecoll

Dr. Lütjen-Drecoll discussed structures and functions in the front of the eye related to glaucoma. She noted that pronounced histologic differences are associated with different types of glaucoma. Elevated IOP is present in all these types of glaucoma, but it is associated with very different structural changes, which are characteristic of the particular type of glaucoma. Thus, she has concluded that elevated IOP cannot be the *cause* of the changes observable in extracellular structures.

The changes in the trabecular meshwork observed in POAG cases are much like age-related changes but are more extensive.

However, Dr. Lütjen-Drecoll thinks there is something in addition to aging that is involved in the glaucoma-related changes. The age-related changes are necessary but not sufficient to cause the increase in IOP associated with POAG.

The structural changes associated with steroid-induced glaucoma are entirely different from the changes observed in POAG. Juvenile-onset glaucoma looks like a cross between those two types, but Dr. Lütjen-Drecoll has been able to study only a few (about nine) cases of juvenile-onset glaucoma.

She has observed a strong correlation between optic nerve damage and glaucoma-associated changes in the extracellular material of the trabecular meshwork. This comment led to a general discussion among the participants on possible causal relationships between optic nerve fiber degeneration and extracellular changes in the anterior chamber. Possibilities that were mentioned include parallel changes, as well as the conventional view that extracellular changes lead to increased IOP, which then leads to nerve damage in the retina and optic nerve. The results obtained in eyes with pseudoexfoliation glaucoma indicate that the latter might be true for these cases, whereas in POAG eyes it seems that common factors are causative for both anterior and posterior eye segment changes.

To search for such factors Dr. Lütjen-Drecoll has further identified the changes observed in the trabecular meshwork of glaucomatous eyes. In particular, in eyes with POAG she found increases in fibrous material such as collagen VI and fibronectin, decreases in cellularity and increases in stress-related proteins and TIGR/myocilin. She has then treated trabecular cells in culture or perfused anterior segments of human eyes with different factors to see which of these factors induce structural changes in the trabecular meshwork comparable to those seen in eyes with POAG. One of the factors that induces such changes is a form of Transforming Growth Factor β, called TGF-β_2

TGF-β_2 is a factor increased in the aqueous humor in about half of the POAG eyes that Dr. Lütjen-Drecoll has investigated. TGF-β is associated with response to injury; which raises the question whether something happening in the trabecular meshwork is associated with a general physiological response to injury. The participants discussed whether this factor might be associated with the response to ameliorating the condition causing elevated IOP or might instead be involved in the changes that induce elevated IOP. Is it possible that the TGF-β_2 is produced in trying to heal something but only makes the overall condition worse by elevating IOP? Dr. Lütjen-Drecoll noted that TGF-β in vitro induces increases in fibrous material and the enzyme tissue transglutaminase, which crosslinks extracellular matrix components in a way that prevents them from being digested by metalloproteinases. Such a mechanism could lead to the "plaque formation" typically seen in eyes with POAG. Treatment of cells with TGF-β also induces increase in expression of αB-crystallin and TIGR/myocilin in trabecular cells.

As a consequence of these studies, she is now also investigating ciliary epithelium in cell and organ cultures. She assumes that changes in, for example, TGF-β or other factors in aqueous humor can be caused by impaired secretion from the ciliary epithelium. From her perspective, additional work is particularly important in the following areas:

1. Better classification of glaucomatous changes. Such classifications will help to find pathogenetic factors (gene defects or factors in the aqueous humor) causing glaucomatous changes in the anterior and posterior eye segments.
2. Identifying other factors in the aqueous humor that change in glaucomatous eyes.

Dr. Lütjen-Drecoll thinks transgenic mice will be an important animal model for glaucoma research. In transgenic mice with overexpression or knockout of various genes, she looks for the histologic and biochemical changes in the anterior and posterior eye segment and compares them with those found in glaucomatous eyes. She is currently studying mice that overproduce αB-crystallin, to see if this stress protein specifically protects against changes in the trabecular meshwork and optic nerve.

She is also studying transgenic mice that carry a knockout variant of the gene for glutathione peroxidase, which scavenges free radicals. In these animals, effects of oxidative damage to the anterior and posterior eye segments can be studied.

Arthur H. Neufeld

Dr. Neufeld's research interest is in pharmacologic therapy. With respect to getting a new

therapy into clinical use, he suggested the following comparison of classical pharmacology with gene therapy: Classical methods introduce "small molecules" into the system to intervene in disease processes. Gene therapy can be viewed as "pharmacology using large molecules," but it has to go through the same [FDA] approval steps as pharmacology using small molecules.[3]

Dr. Neufeld sees the principal practical difference between the approaches as the time required to move from research results to clinical applicability. In his view, the classical approach will produce results, and affect clinical practice, sooner than gene therapy. In addition, Dr. Neufeld thinks that, after 120 years of treating glaucoma by reducing IOP, it is unlikely that classical pharmacology will greatly improve our ability to lower IOP. He has focused on the search for neuroprotective drugs, with the aim of directly preventing or treating optic neuropathy.

The progressive, chronic nature of glaucoma means that not all ganglion cells are sick or dying at the same time. This implies that nerve cell damage, whether continuous or intermittent, occurs over an extended time. Dr. Neufeld's work has focused on protecting the healthy cells and perhaps reversing the decline of "sick" cells.

An important question is which cells to affect. Dr. Neufeld believes attention to the glial cells, which are 10 times more numerous than neural cells, may be critical. Whereas the nerve cells conduct electrical impulses, the glial cells perform a range of essential supporting functions. They regulate blood flow, synthesize everything, supply nutrients, and remove metabolites. They regulate the ionic environment and kill anything that invades the nervous system. Thus, Dr. Neufeld views the glial cells as the "action cells" of the optic nerve and retina. He believes that many critical events in disease processes affecting the optic nerve occur in the glial cells. Two types of glial cells are associated with the optic nerve at the retina: astrocytes and microglia. Dr. Neufeld has focused on astrocytes in the human optic nerve and in animal models.

There are four pharmacologic approaches to neuroprotection being pursued by different research groups.

1. Supply missing neurotrophic factors. (Hypothesis: in glaucoma the essential neurotrophic factors needed to keep nerve cells alive are not getting there, because of compression of the optic nerve.)
2. Block glutamate excitotoxicity. (Hypothesis: excess glutamate is produced, which is known to be toxic to nerve cells. Some evidence exists that glutamate is elevated in glaucoma patients.)
3. Stop the apoptotic cascade. (Dr. Neufeld thinks this is too late in the process to be effective over a long time.)
4. Inhibit nitric oxide (NO) neurotoxicity.

The first three approaches aim at protecting the retinal ganglion cell body. Dr. Neufeld sees this strategy as coming too late, if the neural axon is already damaged. Instead, his group is working on the fourth approach, particularly on NO inhibition at the optic nerve head. The guiding hypothesis is that NO is damaging the cell axons in the nerve head. NO in the extracellular environment forms a free radical, peroxynitrite, which is a very reactive and destructive free radical. If this is the disease mechanism and the axon is already damaged by peroxynitrite exposure, then stabilizing ganglion cell bodies will not protect the optic nerve from loss of function.

Dr. Neufeld works from a general picture that begins with stresses on the optic nerve head and retina resulting from elevated pressure in the anterior chamber or perhaps other initiating factors. When this retinal cell system is under stress, the astrocytes increase production of glial fibrillary acidic protein (GFAP) and become reactive astrocytes.

In astrocytes of patients with glaucoma, Dr. Neufeld's group has found an enzyme that produces excessive NO, called NOS-2. This enzyme is not seen in subjects without glaucoma. To explore why NOS-2 appears in stressed astrocytes, they looked for the presence of cytokines. In the human glaucomatous optic nerve head, they found tumor necrosis factor alpha (TNF-α). This cytokine, which occurs in damaged or stressed tissue, makes cells destructive by increasing inflammation-related reactions. Human astrocytes that are exposed to TNF-α produce NOS-2, and Dr. Neufeld has shown that TNF-α can induce NOS-2 directly.

Dr. Neufeld and his coworkers have investigated whether the increase in NOS-2 is a direct effect of pressure or an indirect effect of the tissue being modified. Astrocytes were cultured under ambient pressure and under elevated hydrostatic pressure for 12 to 48 hours. Those

[3] Other participants noted that gene therapy may be much longer lasting than the drugs used in classical pharmacology.

cultured at elevated pressure had significantly greater levels of NOS-2. Thus, pressure can directly induce NOS-2 production *in vitro*.

In summary, they have established the presence *in vivo* of a cytokine that can produce NOS-2. And, *in vitro*, NOS-2 production is induced by elevated pressure. Dr. Neufeld discussed possible approaches to pharmacologic intervention, given these conditions.

One approach is to inhibit NOS-2 with a drug that can be used in animal models. Dr. Neufeld's group produced rats with chronic moderately elevated IOP to mimic human glaucoma for this purpose. They used three-vessel cautery to increase IOP in one eye of each rat. The NOS-2 inhibitor was aminoguanidine. First they showed that optic nerve cupping occurs in rats with elevated IOP, and they found that NOS-2 in eyes increased within 4 days of elevated IOP. They also treated rats pharmacologically to inhibit NOS-2 and examine the effect on retinal ganglion cell death. The animals with elevated IOP were divided into two groups: untreated controls and those treated with aminoguanidine administered in their drinking water. For 6 months, IOP and body weight were measured, then the animals were sacrificed and retinal ganglion cells were counted in both eyes.

The data, which have been published, demonstrate that aminoguanidine decreased ganglion cell death (Neufeld et al. 1999). Untreated animals lost 40 percent of the retinal ganglion cells in the high-IOP eye. Loss of retinal ganglion cells in animals treated with aminoguanidine was less than 10 percent, despite the elevated IOP. There was no effect of treatment on IOP or body weight. With this treatment protocol, they demonstrated pharmacologic neuroprotection in the context of elevated IOP.

In a second treatment protocol using aminoguanidine, Dr. Neufeld and coworkers assessed the efficacy of pharmacologic intervention *after* a period of untreated elevated IOP. Half of the treatment group in this protocol did not begin aminoguanidine treatment until 3 months after cauterization to raise the IOP in one eye. The results showed that, even after retinal ganglion cell loss had begun, intervention to inhibit NOS-2 halted further damage to retinal ganglion cells. The untreated control group lost 36 percent of retinal ganglion cells. Animals treated with aminoguanidine for the entire time after cautery lost about 9 percent (comparable to the first protocol). Animals treated with aminoguanidine beginning 3 months after cautery showed no further loss of retinal ganglion cells.

Based on this work, a pharmaceutical company has agreed to perform follow-up pharmacologic studies with glaucomatous monkeys, as a preliminary to a clinical trial. However, Dr. Neufeld noted that several years of determined effort, after the initial pharmacologic studies, were required to gain interest from a company in pursuing it.

Dr. Neufeld also described work by his group in using animal models to explore risk factors in glaucoma. The research question was how to model the effects of a risk factor on loss of retinal ganglion cells in experimental animals. For a preparatory short-term study, they used retinal ischemia/reperfusion in rats. One week after the reperfusion, the retina was excised and examined for loss of retinal ganglion cells.

One potential risk factor for retinal cell death they investigated using this model was the age of the animal. They found that, following retinal ischemia, young animals lose about 20 percent of their retinal ganglion cells, whereas old animals lose about 38 percent. When they looked at diabetes as a potential risk factor, they found greater loss of ganglion cells in the rats with induced diabetes.

They also investigated strain difference as a factor by comparing albino Wistar rats with brown Norway rats. In Wistar rats, most of the loss of retinal ganglion cells was in the retinal periphery, not in the central retina. In Norway rats, there was marked loss in the central retina. So different strains of rat had different spatial distributions of ganglion cell loss.

For most markers of age-related diseases in rats, caloric restriction throughout life is a protective factor. When Dr. Neufeld's group used caloric restriction in this animal model of retinal ischemia, they found no difference in the central retina, but in the periphery caloric restriction reduced ganglion cell loss. Dr. Neufeld plans to extend this work in several directions, including studies in glaucomatous rats. A key point that has already been established by the work to date is that risk factors for human glaucoma can be studied successfully in an animal model.

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Acronym List

AIDS	Acquired immune deficiency syndrome
DNA	Deoxyribonucleic acid
FDA	U.S. Food and Drug Administration
HGP	Human Genome Project
IOP	Intraocular pressure
NO	Nitric oxide
POAG	Primary open angle glaucoma
TGF	Transforming Growth Factor
TIGR	Trabecular meshwork glucocorticoid response protein

Appendix
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