Research Opportunities

Corneal Dystrophies: Molecular Genetics to Therapeutic Intervention—Fifth ARVO/Pfizer Ophthalmics Research Institute Conference

Jayne S. Weiss1,2,3 and the Fifth ARVO/Pfizer Ophthalmics Research Institute Conference Working Group4

The fifth annual conference of the ARVO/Pfizer Ophthalmics Research Institute was held on Friday and Saturday, May 1 and 2, 2009, in Fort Lauderdale, Florida. The conference, which was funded by the ARVO Foundation for Eye Research through a grant from Pfizer Ophthalmics, facilitated the gathering of experts from within and without the field of ophthalmology and vision research, to encourage discussion and strategizing and to further understand and eventually develop preventive measures and treatments for the corneal dystrophies.

The meeting comprised a working group of 29 participants including ophthalmologists, vision scientists, molecular geneticists, veterinary ophthalmologists, and plant biologists, as well as 26 observers from ARVO/Pfizer, clinicians, and clinical and basic science researchers.

The four sessions were as follows:

Session I: Corneal Dystrophies, The Basic Clinical Framework—Defining the Problem
Session II: Molecular Genetics of the Corneal Dystrophies
Session III: Next Steps, Corneal Dystrophies—Interventional Strategies
Session IV: Schnyder Corneal Dystrophy as a Model of Challenges and Opportunities


During the remainder of each session, participants gave brief talks, followed by discussion of pertinent and sometimes controversial issues, identification of unanswered questions, and formulation of research goals for the future.

SESSION I: CORNEAL DYSTROPHIES, THE BASIC CLINICAL FRAMEWORK—DEFINING THE PROBLEM

Session I was moderated by Alan Sugar, MD, and Eduardo Alfonso, MD. David C. Musch, PhD, MPH, spoke about the scarcity of information on the overall prevalence of corneal dystrophies and the financial impact of these disease entities. He indicated that estimates of the relative frequency of corneal dystrophies have relied primarily on corneal transplantation registries and large case series from corneal transplantation surgeons. The Eye Bank Association of America’s Statistical Report on Eye Banking Activity for 20082 stated that Fuchs dystrophy represented 8% (n = 2,273) of the 29,515 corneal transplantations reported in 2008 by 77 U.S. Eye Banks. In a notable reflection of changes in the standard of care, of the 14,451 Descemet’s stripping endothelial keratoplasties (DSEKs) performed in 2008, 50% (n = 7,231) were for Fuchs dystrophy. By comparison, the Australian Corneal Graft Registry Report of 20073 showed that corneal dystrophies were the fourth most frequent indication for penetrating keratoplasty (PKP) after keratoconus, bullous keratopathies, and failed corneal grafts. In the Australian report, the corneal dystrophies were further divided into Fuchs dystrophy (83.8%), granular dystrophy (4%), lattice dystrophy (2.6%), posterior polymorphous dystrophy (PPCD; 1.9%), crystalline dystrophy (0.9%), juvenile dystrophy (0.6%), anterior dystrophy (0.4%), and unspecified (3.7%). According to the French National Waiting List,4 dystrophies were the third most common indication for PKP and were divided into Fuchs dystrophy (65%), lattice dystrophy (10%), granular dystrophy (4%), macular dystrophy (2%), and other (20%).

However, Dr. Musch pointed out that these sources actually document only the tip of the iceberg of the prevalence of corneal dystrophy. To get a more accurate idea of the prevalence, he examined a data set (the i3 InVision Data Mart Dataset; Ingenix, Inc., Eden, Prairie, MN) of insurance claims by 40 million covered lives in the United States. The database contains detailed, fully de-identified records of all beneficiaries in a large managed-care network in the United States. We had access to data for beneficiaries in the Data Mart database who had any form of eye care from January 1, 2001, through December 31, 2007. In this data set, there were 8.3 million patients with eye care diagnostic codes; 26,768 had endothelial dystrophy (prevalence [P]: 540/106 covered lives [CL]), 8,658 had anterior dystrophy (P: 108/106 CL), 531 had macular dystrophy (P: 7/106 CL), 485 had lattice dystrophy (P: 6/106 CL), and 433 had granular dystrophy (P: 5/106 CL). He noted that the prevalence of specific corneal dystrophies varies geographi-
Fifth ARVO/Pfizer Research Institute Working Group

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Similarly because of differences in genetic pools. For example,
macular dystrophy has been reported to account for one third
of the total number of PKPs performed in Iceland, whereas
PPCD is one of the most prevalent corneal dystrophies in the
Czech Republic. William Dupps, MD, PhD, discussed the impact of corneal
dystrophy diagnosis from the perspective of the affected
patient. He shared experiences of those members of his own
family with Schnyder corneal dystrophy (SCD). He explained
that inherited diseases such as the corneal dystrophies not only
affect the patient, but also have effects that reach beyond the
patient’s lifetime to innumerable offspring. Consequently, a
variety of emotions are experienced by patients on learning
that they have a genetic disease. These emotions included
anticipatory grief, which occurs before an impending loss;
ambiguous loss, which is without immediate closure, because the
end point is remote or ill defined; and disenfranchised
grief, a socially unsanctioned grief without typical grieving
“scripts” or mourning rituals. He suggested that ophthalmol-
ogists should be conscious of these psychologic implications
when informing the patient of a diagnosis of genetic disease
and should legitimize the grieving process in their interactions
and through serious consideration of additional aids, such as
genetic counselors. Ultimately, research is critically important
to those who have the corneal dystrophies, because it offers
the hope of resolving ambiguity through better diagnosis, of
changing the future of innocent offspring by developing im-
proved treatments, or even of prevention.

Hans U. Møller, MD, PhD, discussed the diagnostic chal-
enges presented by pediatric corneal dystrophy. He noted that
despite the numerous publications over the past century in the
field of corneal dystrophies, most of the clinical descriptions
and photographs of corneal dystrophies have been in adults.
Consequently, there is a dearth of information about the ap-
pearance of the dystrophy in children, in whom the signs may
be more subtle and the examination more difficult to perform.
He suggested that understanding what to look for in the pedi-
atric patient would shed light on which dystrophies are due to
increasing deposition of material and which are due to struc-
tural changes in the corneal tissues. The speed of progression
and course of dystrophy may reveal information about patho-
genesis. Consequently, the basic scientist may be able to learn
something from the clinical course of the disease, to determine
whether the information fits with the proposed biochemistry
of the suspected gene product. He urged participants to share
photographs of pediatric cases of dystrophy and to publish
case reports of dystrophies in the pediatric population.
I led a discussion on the new nomenclature of corneal dystrophies published in 2008 by the International Committee for Classification of Corneal Dystrophies (IC3D), a committee that I organized and chaired. The historical background driving the nomenclature revision was as follows. As advances in genotyping are made, we have discovered that mutations in different genes can result in a similar phenotype, such as Thiel-Behnke corneal dystrophy, and mutations in a single gene (TGFBI) can result in different allelic dystrophy phenotypes (Reis-Bückler’s, Thiel-Behnke, granular types 1 and 2, and lattice type 1). Consequently, the phenotypic classification system of corneal dystrophies had become archaic with the emergence of newer genetic information. As corneal dystrophy research entered the 21st century, I knew it was imperative to begin the effort by revising the nomenclature to reflect our current genetic, clinical, and histopathologic knowledge. With the support of the Cornea Society and its then President, Michael Belin, MD, I organized the IC3D in 2005 by recruiting international experts in genetics, ophthalmology, and pathology. The committee critically reviewed the corneal dystrophy literature to exclude inaccurate information from the revised nomenclature. Then, a template was created for each dystrophy, summarizing the genetic, clinical, and pathologic information (Table 1). Each dystrophy was assigned to one of the following four categories, to indicate the level of evidence supporting the existence of a given dystrophy:

- **Category 1**: A well-defined corneal dystrophy in which the gene has been mapped and identified and specific mutations are known.
- **Category 2**: A well-defined corneal dystrophy that has been mapped to one or more specific chromosomal loci, with the gene(s) not yet identified.
- **Category 3**: A well-defined clinical corneal dystrophy in which the disorder has not yet been mapped to a chromosomal locus.
- **Category 4**: A suspected new or previously documented corneal dystrophy for which the evidence that it is a distinct entity is not yet convincing.

As knowledge advances, the progress in mapping and gene identification will be reflected in the higher or lower category assigned to a specific corneal dystrophy, with all valid corneal dystrophies eventually attaining the classification of category 1. The organization of the corneal dystrophies remained anatomic (Table 1). The use of a common nomenclature that reflects our understanding of the underlying genetics should facilitate research and diagnosis. The nomenclature can be accessed through the website of the Cornea Society (www.corneasociety.org).

**Stephen C. Kaufman, MD, PhD, reviewed imaging technologies including in vivo confocal microscopy, optical coherence tomography, and ultrasonic biomicroscopy, which**

**Table 1. The IC3D Corneal Dystrophy Classifications**

<table>
<thead>
<tr>
<th><strong>Epithelial and Subepithelial Dystrophies</strong></th>
<th><strong>MIM</strong></th>
<th><strong>IC3D Abbreviation</strong></th>
<th><strong>IC3D Abbreviation</strong></th>
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</table>

have been used to image corneal dystrophies. Measurement obtained with these devices may aid in determining advancement of the dystrophy and in the future may be helpful in determining the success of genetic or pharmacologic treatments to prevent or treat progression.

J. Fielding Hejtmancik, MD, PhD, discussed the EyeGENE Project. This National Institutes of Health (NIH)-sponsored program supports the genetic testing of corneal dystrophy patients by offering free DNA analysis (the cost of obtaining and shipping the blood sample is the responsibility of the referring clinician and patient). To create a research database of clinical, phenotypic information coupled to patient genotyping results that will be available to the entire vision research community, EyeGENE requires the referring clinician or his/her staff to answer several standardized questions online about the patient’s clinical presentation. In phase II of this project, registered vision researchers will be able to view the de-identified phenotype and genotype of patients with a wide range of inherited eye diseases (including the corneal dystrophies). In addition, researchers will be able to submit proposals to request an aliquot of a patient’s DNA for research and/or to inform patients of a clinical study for which they may qualify. Consequently, EyeGENE will facilitate further genetic characterization of the individual patient while creating a DNA repository and patient registry of prospective, clinical data coupled to genotypic data from individuals with a broad range of inherited eye diseases, including inherited corneal dystrophies. Clinicians interested in participating in EyeGENE should e-mail the EyeGENE coordinating center at eyeGENEinfor@nei.nih.gov or access the website http://www.nei.nih.gov/resources/eyegene/professionals.asp.

The indications for DNA-based diagnosis were discussed. Reasons for testing included the academic reason of finding the gold standard of diagnosis, assisting with genetic counseling, and addressing the desire of some patients to have their diagnoses confirmed by objective laboratory testing, even if the information did not alter treatment or prognosis, especially if there may be benefit to others through research. In addition, our knowledge of phenotype-genotype correlations has progressed sufficiently to make some important clinical distinctions based on molecular testing. For example, in granular corneal dystrophy type 2 (GCD2), also known as granulattice dystrophy or Avellino corneal dystrophy, there can be a dramatic worsening of corneal opacification in the affected patient if LASIK is performed. Consequently, LASIK is contraindicated in those homozygous and those heterozygous for GCD2. However, unlike homozygous patients, who have obvious findings on slit lamp examination, even early in childhood, heterozygotes may have few to no symptoms, with only subtle findings on examination. Genetic screening is beneficial, because it can identify those patients who would not be otherwise identified as heterozygous for GCD2 and can serve to warn them to avoid LASIK surgery, because it could markedly worsen their vision.

A lively discussion followed regarding the role of genetic testing in the routine workup of the corneal dystrophy patient. Some participants thought that the success of PKP in corneal dystrophy treatment makes genetic testing unnecessary. They argued that PKP is highly successful in restoring excellent vision through replacement of the diseased cornea. The corneal dystrophies usually result in minimal visual disability when compared with other inherited diseases of the retina or optic nerve. Consequently, the participants thought that research dollars were best spent on more visually disabling diseases and the genetic testing in dystrophy patients is not warranted.

Another viewpoint was that PKP and other, newer corneal replacement procedures such as DSEK, although highly successful, are still fraught with problems. PKP is not a definitive cure for the dystrophy patient. The dystrophy can recur in the new corneal tissue, corneal transplants can undergo rejection, and frequently, other co-morbidities such as glaucoma or cataract develop after PKP. Even if the PKP graft remains clear, other interventions to treat the ophthalmic complications are often necessary. In addition, 5-year PKP graft survival ranged from 56% to 79% in the Newcastle Corneal Transplant Registry (Sellevoll HB, et al. IOVS 2009;50:E Abstract 2200), and so in the course of a patient’s lifetime, repeat surgery was often necessary. As all surgical procedures have inherent risk, development of preventive treatments is still important, in the attempt to lessen the potential of vision loss. In reference to Dr. Dupps’ comments on the impact of the dystrophy diagnosis on the genetic counseling of the dystrophy patient deserves the hope that research may prevent his or her progeny from developing the disease and losing vision.

Using EyeGENE and developing a more scientific approach to the corneal dystrophy patient by obtaining confirmatory genetic testing would facilitate the creation of a DNA repository of the corneal dystrophies and foster the research necessary to develop better treatments or preventive therapy. In addition, confirmatory genetic testing would provide confirmation or refute phenotypic diagnosis of these entities.
types of MCD has yet to be found, and not all cases of MCD are accompanied by a mutation in CHST6.27

Brian P. Brooks, MD, PhD, discussed how new genetic technologies can aid in the diagnosis and treatment of inherited corneal diseases. The tools of classic Mendelian genetics, such as microsatellite mapping combined with logical candidate genes, have been fruitful in the discovery of new genes in inherited corneal disease (e.g., the 5q corneal dystrophies).28 These methods require careful clinical phenotyping and family information, high-quality DNA in sufficient quantity, and many PCR and sequencing reactions.29 Although these methods of gene discovery are useful and viable when applied to a large family with a highly penetrant Mendelian disease, they become more cumbersome when phenotypes and diseases with complex inheritance are studied.30

Increasing attention has been paid to the use of single-nucleotide polymorphisms (SNPs) in the mapping of genetic loci. Modern microarray technology enables us now to assay literally hundreds of thousands of SNPs simultaneously across the genome of an individual.31 These arrays can also be used to detect copy number variations of genetic information (deletions and duplications), which are increasingly being recognized as being associated with human health and disease.32,33 Microarray technology still requires the skilled clinician to provide accurate phenotype and family information and high-quality DNA; it also requires specific instrumentation. Although microarray technology can be used to map genes for Mendelian disorders in a relatively short period, they are increasingly being used in studies of diseases with complex inheritance via genomewide association analyses. Although both traditional mapping with microsatellites and SNP microarray assay require expert data analysis, the latter should involve a statistical geneticist, both in the planning and analysis stages of experimentation.34

Once a gene for a condition has been found, sequencing can be used in a clinical setting to diagnose and counsel patients. Traditionally, this is done by PCR amplification of the exons and exon-intron boundaries of the responsible gene, followed by deoxynucleotide sequencing.35 In cases in which only one or a few genes are responsible for a condition, this remains perhaps the most cost-effective and rapid one by which sequence variants can be ascertained. However, when multiple genes are known to be responsible for a given disease or phenotype (e.g., in Leber congenital amaurosis or autosomal dominant retinitis pigmentosa), such traditional methods can become lengthy and involved. Hierarchical sequencing strategies have been developed for several conditions to help alleviate this situation.35 However, alternative approaches, such as DNA mutation detection microarrays and DNA sequencing arrays, are being explored as possible rapid, cost-effective approaches to genetic heterogeneity.36,37 Finally, next-generation sequencing technologies are increasingly being used to obtain large amounts of DNA sequence information in a relatively short period.38 These techniques have been used to great benefit in the research arena. Their use for clinical sequencing is only now being explored. For now, the Sanger method is the gold standard for determining DNA sequence variants in patients.

Anthony Aldave, MD, led a discussion entitled “Remaining Questions on Molecular Genetics of the TGFB1 Dystrophies.” In 1997, mutations were identified in the TGFB1 gene28 in affected individuals from families with four different corneal dystrophies. The finding that the TGFB1 gene is a common causative in these dystrophies, which have distinct phenotypic and histopathologic features, was the first in the identification of several disease genes in the past decade in individuals with a variety of inherited corneal dystrophies. Further investigations have demonstrated that the dystrophic deposits noted clinically and on histopathologic examination consist of the mutated form of the protein product of the TGFB1 gene, transforming growth factor-β-induced protein (TGFBlp). Ironically, even though the TGFBl dystrophies are classified as Bowman layer and stromal dystrophies, most of the TGFBlp is produced by the corneal epithelial cells. As the name implies, the production of the constitutively expressed TGFBlp is inducible by TGFB1, which is the likely mechanism by which the rate of TGFBlp production by activated keratocytes significantly increases after corneal injury or surgery.

These insights have led to the development of novel treatment strategies for the TGFB1 dystrophies, including the use of antimetabolites, methylated peptides (meptides) and RNA interference, to prevent the formation of recurrent dystrophic deposits after surgical intervention. Hologenic TGFB1p deposits must be answered before we are able to develop truly effective, targeted treatments to prevent and/or eliminate the dystrophic deposits that characterize the TGFB1 dystrophies. Three major questions are:

1. What are the functions of TGFBlp in the cornea, and what are the expected consequences of a therapeutic intervention, such as nontargeted RNA interference, that produces a knockdown of both mutant and wild-type TGFBlp?
2. Why are the dystrophic deposits that are associated with TGFB1 mutations confined to the cornea?
3. What is the mechanism of pathologic TGFBlp deposition? Is it secondary to conformational misfolding, abnormal interactions with other components of the corneal stroma, accumulation of dysregulated TGFBlp, or another cause?

Our ability to obtain answers to these questions about the structure and function of TGFBlp will lead to a greater understanding of the nature of pathologic TGFBlp deposition and will facilitate development of novel treatment strategies. These novel therapeutic interventions must initially be shown to be safe and effective in suitable in vivo and animal models of the TGFB1 dystrophies, and these animal models have yet to be developed.

Rajiv Basaiawmoit, PhD, led a discussion on recombinant TGFBlp and the molecular approaches that have been developed to determine the basis for deposit formation in the cornea. The TGFB1-linked corneal dystrophies result from abnormal accumulation of protein deposits in the cornea, predominantly consisting of the extracellular matrix protein, TGFBlp, and fragments of the full-length protein. Distinct phenotypes result from different mutations in the fourth FAS1 domain of TGFBlp, making this domain an interesting one to study. Research on the three variants of the highly mutagenic fourth domain has demonstrated that the stability, folding, and aggregation behavior of three FAS4 mutants (A546T, R555W, and R555Q), representative of different corneal dystrophies, correlate with the pathologic features of the intact protein. A stability series for the studied mutants correlated with their aggregation propensities in vitro and distinguished amyloid (in lattice corneal dystrophy in the A546T mutant) from nonamyloid (as in granular corneal dystrophy in the A546T mutant) from nonamyloid (as in granular corneal dystrophy in the A546T mutant) behavior. The intriguing observation was that the behavior of the FAS4 mutant fragments correlates with the behavior of the corresponding full-length TGFBlp mutants. Small-angle x-ray scattering (SAXS), with complementary biophysical and biochemical methods, demonstrates a low-resolution structure of wild-type (WT) TGFBlp and corneal dystrophy mutant R124H that reveals no major structural or shape differences, which suggests that the mutation that causes the clinical pathology is not induced by any major structural change in the protein as an effect of the mutation, but rather by a change
in the environment around the mutation that creates or breaks links with cornea-specific binding partners. In addition, both structures, along with additional complementary data also showed higher order multimer formation. Multimerization properties for full-length TGFβp are probably linked to the multifunctional nature of the protein.40 Such analysis facilitates further understanding of the dystrophies on a molecular level.

Jonathan Lass, MD, and Sudha Iyengar, PhD, led the discussion concerning the challenges in finding the gene associated with Fuchs endothelial corneal dystrophy (FECD). FECD has long been recognized as having a familial pattern manifested by the onset of guttae and progressing to corneal edema, necessitating PKP or, more recently, Descemet stripping automated endothelial keratoplasty (DSAEK). Most have a late onset with a female predominance. However, an uncommon form of the dystrophy, noted before the age of 50, has an equal male-female occurrence and has been associated with various mutations in the COL8A2 gene.41, this gene mutation has not been found in the late-onset disease.42 More recently, there have been reports of the association of mutations in the SLC4A11,43 ZEB1,44 and PITX2 genes in several families with FECD, but these still represent a small sample of the larger FECD, and predominantly Caucasian, population in the United States. Finally, five regions with linkage signals on chromosomes 1, 7, 15, 17, and X have been reported in late-onset disease, but in a very small number of affected individuals.45 Genetic studies of the disease are complicated by its late onset, the difficulty in assembling families, and the potential loss of information in younger family members who cannot be differentiated from normal control subjects, but who may be affected although the disease is not yet apparent.

In light of these efforts, Drs. Lass and Iyengar organized the FECD Genetics Multicenter Study to help elucidate the major genetic components underlying FECD (Lass JH, et al. IOVS 2005;46:ARVO E-Abstract 4924). The study involved 33 academic and private practice sites and the collection of family information, grading of the phenotype, and gathering of DNA specimens from affected subjects, their families, and unaffected control subjects. Phenotypic grading using a modified scale by Krachmer from grades 0 (no guttae) to 6 (confluent guttae greater than 5 mm in diameter with stromal and/or epithelial edema) was used. Pathologic confirmation of the proband was made in most of the probands. Since individual effects of these genetic risk variants may be difficult to detect in small, family-based studies, as have been conducted in the past, the study design combines two approaches: a familial study arm and a case-control component. (See Note Added in Proof at end of article.)

To date, more than 500 families have been enrolled, including more than 1000 participants, 650 sibling pairs, and 360 affected sibling pairs. An age, sex, and ethnically matched unrelated control group with normal corneas is also being assembled for a case-control analysis. Candidate gene screening is now under way for COL8A2, SLC4A11, ZEB1, and PITX2 variants, and the most efficacious design will be used to identify genes associated with late-onset FECD, as performed in other suspected complex trait disorders.47-48 Through these efforts, novel insights into the genetic pathogenesis of FECD may be obtained and may provide new targets for subsequent therapies.

John Gottsch, MD, continued the discussion by elaborating on an investigation of the pathogenesis and molecular genetics of FECD, which affects 4% of the population, with a substantial fraction of these cases consistent with autosomal dominant inheritance. He confirmed a connection with early-onset FECD in a large kindred in which the disease mapped to COL8A2.49 Other large families have been linked to 15p15.1-13q21.15 (FCD1),50 18q12.2-q12.13 (FCD2),51 and 5q35.2-q35.2 (FCD3).52 The age-severity relationships among patients with the FCD1, FCD2, or FCD3 genotype demonstrate that FECD3 has a lower rate of disease development, whereas FCD1 has a higher rate. Consequently, identification of the genotype provides prognostic information regarding disease progression for the individual with that genotype.

Yaron Rabinowitz, MD, led the discussion about challenges in finding the genetic basis of keratoconus,49 53-55 which is the commonest cause of corneal transplantation in most Western countries and costs $50 million in health care resources annually. He reviewed the clinical and topographic signs and the differential diagnosis. There are multiple challenges to determining the causative gene(s) in this disease. One challenge is that keratoconus is associated with other conditions such as Down syndrome, atopy, and eye rubbing. When attempting to define the genetic basis for keratoconus, it is critical to exclude these associations and study only clean keratoconus cases with no other associations. Also, when performing genetic analysis it is critical to be able to identify all individuals who are and are not affected. Determining the diagnosis may present challenges because keratoconus can vary from the forme fruste stage, with only topographic abnormalities; to the early stage, which also has positive retroillumination; to clinically obvious keratoconus, in which slit lamp abnormalities including corneal thinning and Vogt stria may be obvious. Studies have shown that genes play a major part in the pathogenesis of the disease, although environmental factors may be modulatory. One approach to isolating genes causative of keratoconus is the use of gene expression analysis that identifies the genes present in the cornea.

Another potential causative association with keratoconus is the level of aquaporin 5,56 which appears to be suppressed in the cornea, plays a role in corneal wound healing, and is localized to the corneal epithelium. This observation suggests that wound healing and water transport across the human corneal epithelium in keratoconus are defective.

Albert Jun, MD, PhD, led the discussion about keratoconus pathogenesis and the search for the gene. As in virtually every disease, the onset and progression of keratoconus involves genetic and environmental risk factors. The genetic risk factors for keratoconus have not been studied extensively, and multiple chromosomal loci have been reported. Identification of specific genes conferring a major risk for keratoconus in a substantial proportion of patients remains elusive.

The genetics of keratoconus was reviewed and discussed by Dr. Rabinowitz, and the environmental risk factors including eye rubbing, inflammation, and oxidative stress were discussed by Dr. Jun. The role of eye rubbing in the pathogenesis of keratoconus has been extensively documented and may involve direct biomechanical stretching, release of inflammatory mediators from the corneal epithelium, or keratocyte apoptosis. In addition to the long-standing association between atopy and keratoconus, multiple reports support a role for inflammation in the onset and/or progression of this disease. Keratoconic corneas have been found to have increased MMP-956,58,59 and reduction of IL1-α mRNA levels.59 In particular, increased IL-6, TGF-α, and MMP-9 stimulate abnormalities of tear film cytokines60 in keratoconus patients, consistent with an imbalance between upregulated proinflammatory mediators and downregulated anti-inflammatory mediators. Alterations in oxidative stress61 defense have been associated with keratoconus. Thus, increased oxidative stress could lead to keratoconus via mechanisms involving inflammation, extracellular matrix remodeling, or keratocyte apoptosis. The roles of eye rubbing, inflammation, and oxidative stress in keratoconus also suggest potential preventive approaches, including behavioral modification and anti-inflammatory medications and the...
diligent wearing of sunglasses to reduce ultraviolet (UV)-induced oxidative stress on the cornea.

The basic pathogenic schema for keratoconus involves the interplay of genetic and environmental risk factors leading to cellular and tissue level abnormalities that progress to clinical disease. Future study of keratoconus pathogenesis could benefit from global profiling approaches, including gene expression microarrays and proteomics of epithelium and/or stroma. In addition, animal models such as the NC/Nga atopic dermattis mouse model, which shows corneal changes strikingly similar those in to keratoconus, may be helpful in future studies of the pathogenesis of keratoconus. In particular, global profiling and animal models may facilitate studies of the complex interplay between genetic and environmental risk factors that contribute to disease onset and progression. These tools also may provide approaches to evaluate future therapeutic strategies for keratoconus.

Winston Kao, PhD, led the discussion about the use of transgenic/gene knockout models in corneal dystrophies. One challenge in corneal dystrophy research, repeated by more than one of the discussants during the symposium, is the relative scarcity of animal models for most of the corneal dystrophies. The few mouse lines exhibiting corneal dystrophy include Krt12+/− mice with Meissmann corneal dystrophy, Lum+/− mice with cloudy and thin corneal stroma, Kera+/− mice with clear and thin corneal stroma, Chst5+/− mice with clear cornea and thin stroma, and Zeb1+/− mice with PCCD.6,4–6,8 It is unclear why many of genetic mutations in mice do not exhibit clinical manifestations similar to those observed in humans. It worth noting the major anatomic differences between human and mouse eyes: The mouse cornea is about one-third the size of the mouse cornea may account for the difference observed between the human cornea plana in Kera mutation and keratocan-knockout mice, in that the mutation of Kera (keratanic gene) in human cornea plana causes flat and cloudy corneas, whereas keratan-null mice (Ker−/−) exhibit subtle corneal anomalies (e.g., thin and clear corneas with a narrower corneal-iridal angle that resembles a narrow corneal-iridal angle in the human cornea plana).

The bidirectional mesenchyme–epithelium interactions via growth factors are essential for morphogenesis during development, wound healing, and homeostasis in adults. Growth factors play pivotal roles in modulating functions of mesenchymal cells of neural crest origin and differentiation of peridermal epithelial cells of ocular surface ectoderm during eye morphogenesis. Dr. Kao shared the results of his research in which mouse lines were created that overexpress transgenes and/or ablate genes of interest in a cornea-specific manner with techniques of transgenesis and gene-targeting, to elucidate the molecular and cellular mechanisms that govern the morphogenesis of ocular surface tissues. He demonstrated that mice lacking Tgfb2 by the conventional gene-targeting technique exhibit defects resembling human Axenfeld/Peters anomaly, but the mechanism by which TGF-β2 signaling maintains corneal homeostasis and corneal wound healing in adults remains unknown. It is known, however, that different signaling pathways triggered by the binding of TGF-β to its receptors are mediated by canonical Smads and p38 MAPK during the healing of different types of corneal injuries (i.e., keratectomy, alkali burn, and epithelium debridement). It is anticipated that blocking the Smad signaling pathways may be beneficial to the healing of alkali-burned corneas. His group’s results showed that healing of alkali-burned corneas was greatly improved by the administration of the Adeno-Smad7 virus. In another series of experiments, overexpression of FGF-7, driven by a crystalline promoter, changed the corneal epithelium into lacrimal glands. In contrast, excess FGF-7 in the corneal epithelium of K12-tTA/tet-O-FFG-7 bitransgenic mice, provided by doxycycline induction, leads to ocular surface lesions that resemble those in human Ocular Surface Neoplasia (OSSN). Excess FGF-7 during embryonic development and the neonatal stage causes nuclear translocation of β-catenin, which may account for the pathogenesis observed in the experimental animals.

The efficacy of stem cell transplantation in curing corneal genetic defects was examined in human umbilical mesenchymal stem cells (MSCs) by intrastromal transplantation of the cells into corneas of Lum−/− mice, which manifest thin and cloudy corneas. After Frank Larkin, MD, FRCP, FRGOM, the corneal stroma, assumes a dendritic morphology, synthesis lumican, and show enhanced keratocan and aldehyde dehydrogenase 3A expression. HRT II examination revealed that MSC transplantation reduced corneal stromal haze. In summary, increased work with genetically modified animals via transgenesis and gene-targeting techniques is critical, as it will facilitate creation of the most effective experimental models for development of gene therapy strategies and cell therapy to treat the corneal diseases caused by altered gene functions in experimental mice.

### Session III: Next Steps, Corneal Dystrophies—Interventional Strategies

The third session, regarding the development of interventional strategies for the corneal dystrophies, was moderated by Shigeru Kinoshita, MD, PhD, and Irene Maumenee, MD. The keynote lecture, “Developing Gene Therapies for Corneal Dystrophies,” was given by Frank Larkin, MD, FRCP, FRGOM.

Dr. Larkin began the discussion by indicating that, at present, there are no gene-based approaches to treatment of the corneal dystrophies. The research advances in the field of corneal dystrophies certainly lags behind those in other ophthalmic subspecialties. For example, gene therapies for Leber congenital amaurosis have been developed and are already in trial. In contrast, for more than 15 years, there have been techniques of gene transfer to the corneal epithelium, stroma, and/or endothelium that continue to remain in development. Studies of the feasibility of gene transfer and kinetics of transgene expression have been undertaken ex vivo and in vivo, by using physical, viral vector, and nonviral vector techniques of gene transfer. Functional gene transfer research has been developed for applications such as antiangiogenesis, prevention of allotransplant rejection, modulation of stromal wound healing, and endothelial cell cycle control. Gene therapy for corneal dystrophies is likely, at least in the first instance, to be virus vector-mediated. Opportunities in the field of gene therapy of genetic corneal diseases lies in newer designs of low-immunogenicity vectors, longer duration of transgene expression, and application in combination with stem cell transplantation.

There are several reasons that progress in genetic interventional strategies has not yet been made for the field of corneal dystrophies. Dr. Larkin reiterated the sentiments of prior speakers that the absence of in vivo animal models is an impediment to the development of gene therapy for the corneal dystrophies. He also restated aspects of a prior discussion in expressing that he agreed that the availability of a relatively effective treatment, PKP, is an impediment to obtaining priority for research funding, because it could suggest that the corneal dystrophies are less visually debilitating than inherited ocular diseases for which no treatment exists.

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Investigators in inherited retinal disorders have clear advantages in making progress. First there is the development of some animal models for these diseases. Some in the group suggested that research funding will prioritize the development of genetic intervention for visually disabling diseases without any known treatment, such as those that affect the retina. In summary, more detailed information on the molecular genetics of inherited diseases and animal models is necessary for the development of effective genetic treatments for corneal dystrophies. Available technologies are probably safe and effective, at least in the short and possibly medium term, with little further development.

Eung Kweon Kim, MD, PhD, discussed the use of mitomycin after PRK in GCD2 as a model for the use of antimetabolites to inhibit recurrence. LASIK and LASEK (laser-assisted epithelial keratectomy) are contraindicated in patients with GCD2, because the procedures can cause exacerbation of the dystrophy, with increased opacification. Mitomycin C 0.02% has been tried in conjunction with phototherapeutic keratectomy (PTK) to delay recurrence of GCD2, although opacification still recurs after this treatment. The long-term follow-up results with PRK or LASEK on GCD2 corneas did not show that the use of mitomycin C decreases the postlaser recurrence of GCD2.

Irene Maumenee, MD, discussed the use of cystinosin as a model in the development of pharmacologic interventional agents for the corneal dystrophies. The impact of ocular genetic diseases is substantial, considering that one third of the 5554 entries in OMIM have ocular involvement; 7000 genes lead to identifiable metabolic disease, and approximately 2300 of those have ocular involvement. Fifty percent of pediatric blindness is genetic.

Cystinosin affects 1 in 100,000 to 200,000 individuals, with a high frequency of occurrence in Northern France. The disease is characterized by early severe renal failure requiring renal transplantation; poor growth; failure of thyroid, endocrine, and exocrine pancreatic function; and reduced pigmentation of skin and hair. The deposition of conjunctival and corneal cystine crystals can be associated with photophobia, recurrent erosions, and corneal scarring. Affected individuals may also demonstrate progressive retinal pigment epithelial mottling, reduction of ERG response, and progressive loss of visual field. With discovery of the enzymatic defect, topical and oral cysteamine have been used to treat the ocular disease. Dr. Maumenee described treatment with oral cysteamine, with reports of moderate success. Dr. Brooks indicated that his patients with ocular findings are treated with topical cysteamine.

Mutations in the cystinosin gene affect the lysosomal membrane proteins involved in cystine transport. Fifty percent of patients are homozygous for a 65-kb deletion. The importance of genetic counseling was stressed as being preventive of this chronic debilitating disease. Other lysosomal storage diseases include mucopolysaccharidoses and mucolipidoses, which may have ocular involvement. In Fabry disease, a rare X-linked disorder affecting males and, more mildly, females, there is a deficiency of α-galactosidase (ceramide trihexosidase) that results in the accumulation of glycosphingolipids and disease of the blood vessels of the heart, kidney, brain, skin, and eye. Ocular findings include verticillate keratopathy. Agalsidase-β (Fabrazyme; Genzyme, Cambridge, MA) can be given as an infusion therapy every 2 weeks to treat the disease, but at a high cost. The treatment is a prototype for other lysosomal storage diseases such as Gaucher disease, Hurler syndrome, Hunter syndrome, and Pompe disease. Discussion ensued of the high cost of pharmacologic treatment of cystinosin, with mention of the controversial subject of the implications for genetic counseling if health care dollars become less available for disease treatment.

Shigeru Kinoshita, MD, PhD, discussed using gelatinous droplike corneal dystrophy (GDDL) as a model for treating abnormal corneal epithelial permeability. GDDL is most commonly seen in ethnic Japanese, with abnormal deposition of amyloid in the subepithelial and superficial cornea, leading to corneal vascularization and involvement of the underlying cornea. Mutations in TACSTD2 (tumor-associated calcium signal transducer 2; M1S1) have been confirmed to result in GDDL. Severe impairment of the corneal epithelial barrier function in GDDL is also associated with lactoferrin deposition within the amyloid. Phenotypically, the pathogenesis of amyloid deposits in the corneal stroma may be related to penetration of constituents like lactoferrin. Understanding of the pathogenesis of the disease has been beneficial in devising therapeutic interventions. For example, continuous wearing of soft contact lens after PRK is effective in preventing amyloid deposit formation. Dr. Kinoshita hypothesized that lactoferrin deposits in a subepithelial corneal stroma are due to traumatic corneal epithelium and lactoferrin abnormality, decreasing epithelial permeability.

Farhad Hafezi, MD, PhD, discussed the use of primary (keratoconus) and secondary (iatrogenic) keratoclastia as a model for collagen cross-linking riboflavin/ultraviolet A therapy. He described the technique of mechanical removal of the corneal epithelium, application of riboflavin (vitamin B2) to saturate the corneal stroma and anterior chamber, and application of ultraviolet A light at a wavelength of 365 nm to induce additional cross-links within and between collagen fibers in the anterior 270 to 330 μm of the corneal stroma. Current results in more than 200 eyes with clinical follow-up from 6 months to 6 years are encouraging and suggest that the progression of keratoclastia can be arrested. Results of longer term follow-up and detailed analysis of side effects are needed before this method becomes a routine procedure.

**SESSION IV: SCHNYDER CORNEAL DYSTROPHY AS A MODEL OF CHALLENGES AND OPPORTUNITIES**

I moderated the final session of the symposium, with Howard Kruth, MD, on SCD as a model of challenges and opportunities. I have been involved in research on SCD for the past two decades, from my early work defining the clinical changes, to subsequently identifying the chromosomal abnormality, reporting long-term prognosis, and identifying the causative gene. My discussion was entitled, “Challenges to Genetic Mapping: Making of the Clinical Diagnosis of SCD.”

In 1992, I identified and studied four pedigrees with 18 SCD patients. This effort led to my reporting the previously unknown observation that only 50% of affected individuals with the disease, previously called Schnyder crystalline corneal dystrophy, actually had evidence of corneal crystalline deposits. Subsequently, I continued to see patients who had SCD that had not been diagnosed because of the absence of crystalline deposits. Clinicians assumed that, in the absence of the deposits, the patients could not have SCD. More than a decade ago, I suggested an alternative name for SCD with no deposits, SCD sine crystals, to emphasize that crystalline deposition is not integral to making the diagnosis of SCD.

Subsequently, the confusion caused by the nomenclature was a motivating force for me to organize the International Committee for Classification of the Corneal Dystrophies (IC3D), to revise the entire corneal dystrophy nomenclature. Under the nomenclature revision published in 2008, Schnyder corneal dystrophy is now the recommended name in preference to Schnyder crystalline corneal dystrophy. Progres-
sive corneal opacification from abnormal lipid corneal depo-
osition, with or without crystalline deposits, is the hallmark find-
ing in this disease. Whereas crystalline SCD can be diagnosed as early as 17 months of age, diagnosis of acrystalline disease may be delayed until the fourth decade, because the signs are more subtle.\textsuperscript{101} Identifying criteria for making an accurate clinical diagnosis of SCD is critical to the performance of genetic linkage studies. It is hoped that the revision of the nomenclature will facilitate diagnosis of this entity.

Sheila Crispin, MA, VetMB, BSc, PhD, DVA, DVOphthal, DipECVO, FRCVS, gave the keynote lecture, discussing SCD and other lipid keratopathies in the dog. There are several differences between dogs and humans in relation to lipid metabolism and corneal lipid deposition. In the dog, as in the human, there are three types of corneal lipid deposition, and all three have potential association with hyperlipoproteinemia. Central, mainly subepithelial, crystalline corneal deposits,\textsuperscript{102} similar to those in SCD, are the commonest form of lipid deposition. Small dogs such as the Cavalier King Charles Spaniel are most frequently affected; there is no common association with hyperlipoproteinemia. Lipid keratopathy is the next most common type in the dog, and both local ocular factors and systemic abnormalities of lipoproteins are of relevance.\textsuperscript{103} In both these canine conditions, the affected corneal keratocytes accumulate excessive amounts of lipid, in droplet form, and die. The resulting metrical lipidic debris is rich in cholesterol and phospholipid. Corneal arcus,\textsuperscript{104} the least common corneal lipid deposition in the dog, is not associated with aging but always occurs in association with hyperlipoproteinemia — the reverse of the situation in humans, where corneal arcus occurs much more frequently than the crystalline corneal opacities of SCD. High-density lipoprotein (HDL) is the major cholesterol carrier\textsuperscript{41,102,104 –107} in dogs, whereas low-density lipoprotein (LDL) is the predominant lipoprotein and major cholesterol carrier in humans.

The significance of the central location of corneal crystalline deposits in dogs and humans was discussed. HDL particles are smaller and are likely to diffuse more easily to the central cornea, unlike larger LDL particles. Dr. Kruth and I have demonstrated that levels of HDL-associated apolipoproteins, such as apolipoprotein A1, are increased in SCD corneas.\textsuperscript{108} Certainly, it is logical to expect that the reversal of predominant lipoprotein particle type in the blood, HDL versus LDL, between dog and human may well explain the similar reversal of frequency of corneal findings between the two species. Common to both species is that the temperature of the central cornea is approximately 36.6°C below core temperature, and so the possibility of a temperature-dependent enzyme defect in keratocytes should be investigated further, as it could be argued that keratocytes in the cooler, central area of the cornea would be most susceptible. Further understanding of the underlying metabolic defect in the two species should shed light on the differences between incidences of metabolic and corneal lipid findings.

Dr. Kruth led a discussion entitled, “SCD Lipid Metabolism and Link to Atherosclerosis — What the Dystrophies Can Teach Us about Systemic Disease.” SCD is characterized by accumulation of lipids and lipid particles in the cornea similar to those lipid particles that accumulate in human atherosclerotic plaques.\textsuperscript{108} Cholesterol and phospholipid accumulate in the extracellular connective tissue space in both diseases in the form of crystals, liposomes, and droplets.\textsuperscript{109} Thus, there may be some pathogenic similarities between the diseases that involve lipid accumulation. Cholesterol accumulates because of an imbalance in deposition and removal. Some genetic disorders affecting HDL function (lecithin cholesterol acyltransferase [LCAT], ATP-binding cassette transporter A1 [ABCA1], and apolipoproteins A1 [ApoA1] deficiencies) also result in corneal opacification, as well as lipid abnormalities of the blood. The various HDL deficiency syndromes are typified by defective lipid clearance and demonstrate the critical role of HDL in effecting removal of cholesterol from cells.

Further understanding of lipid abnormalities in SCD may shed light on the metabolic abnormality in this disease that leads to lipid deposition in the cornea with progressive opacification. One possible mechanism is decreased cholesterol removal. Is there a connection between HDL and \textit{UBIAD1} HDL components, ApoA1 and ApoE, show increased accumulation in SCD corneas, suggesting that SCD is a disease of HDL metabolism. In addition, \textit{UBIAD1}, the defective gene in SCD, interacts with the C-terminal portion of ApoE.\textsuperscript{110} This finding suggests that the underlying abnormality resulting in abnormal accumulation of corneal cholesterol results from faulty cholesterol removal, as HDL functions to remove cholesterol from tissues.

Another possible mechanism of cholesterol accumulation in the cornea is increased cholesterol deposition. The cholesterol could be derived from plasma lipoproteins (HDL more likely than LDL) or could be produced by the corneal cells. \textit{UBIAD1} also shows similarities to genes involved in regulation of the cholesterol synthesis pathway. It is a potential prenyltransferase, and other prenyltransferases regulate cholesterol synthesis. Thus, abnormal increased synthesis of cholesterol could also be a factor in SCD cholesterol accumulation.

Michael Nickerson, MS, led the discussion on “Snyder Corneal Dystrophy: What Molecular Genetics Has Taught Us about \textit{UBIAD1}.” In 2007, two groups, Orr et al.\textsuperscript{111} and Weiss et al.,\textsuperscript{112} independently identified mutations in the \textit{UBIAD1} (\textit{UbiA} prenyltransferase domain containing 1) gene as causative of SCD. The gene is named after an enzyme in \textit{E. coli}, UbiA, that plays a critical role in the synthesis of ubiquinone, an electron carrier coenzyme in oxidative phosphorylation. All mutations characterized to date affect amino acids in a two-exon transcript that encodes a protein containing a prenyltransferase domain. Genetic analysis of 30 SCD families that I observed over time characterized 13 different mutations. In a significant finding, of the 20 distinct SCD mutations published to date, Asn102Ser, which is now considered to be a potential mutation hot spot, was identified in 17 (41%) of 41 families.\textsuperscript{113–115} All mutations characterized to date alter amino acids that are highly conserved across species in the prenyltransferase domain. Protein modeling has indicated that the protein has eight membrane-spanning helices, and mutations cluster in three regions of the protein that are predicted to reside on one side of the membrane. Disease-causing alterations so far do not conclusively allow determination of disease pathogenesis.

Ludger Wessjohann, PhD, led the discussion on “UbiA, A First Structural Insight into Membrane-Bound Prenyltransferases: Can We Learn Something for \textit{UBIAD1}?”. In \textit{E. coli}, the UbiA-transferase, which is membrane bound, consists of 290 amino acids and is a key enzyme in the biosynthesis of ubiquinone. The enzyme is difficult to study, because no purification possible, and no crystallization for x-ray analysis has been possible so far. Molecular modeling has been used instead in conjunction with mutational studies to contradict the earlier notion that there are two independent active sites in the enzyme; in fact, they form one active site only. Protein homology modeling has limits, because more than 30% amino-acid identity is necessary to produce a modeled structure with a resolution equivalent to that of a medium-resolution x-ray protein structure. Threading is another tool for folding analysis that searches for homologous proteins based on secondary structure prediction only. A protein model based on threading and three-dimensional analysis allows predictions of the active site of UbiA. The model of \textit{E. coli} UbiA with the information...
about known mutations in \textit{UBIAD1} that result in SCD was used in constructing a three-dimensional model of the UBIAD1 protein. All detrimental mutations are localized on regions of protein lying on one side of the membrane. The S75F polymorphism, seen in 3\% of normal healthy individuals without SCD,\textsuperscript{111,112} is predicted to be on the other side.

**Summary**

Many challenges remain before effective preventive and therapeutic interventions can be developed for the corneal dystrophies. For some corneal dystrophies, such as FED, the major genetic components must still be elucidated. For other corneal dystrophies, such as SCD, a causative gene has been identified, but the pathogenesis must still be unraveled. Although this research may be facilitated by the use of a keratocyte culture of diseased cells or protein modeling, effective animal models are not available. More animal models are needed to aid in developing novel therapeutic modalities, including gene therapy.

An understanding of the pathogenesis of the corneal dystrophies is needed to develop rational treatments, but may be less important than research in the more visually disabling diseases. Nevertheless, development of a preventive treatment for the affected patient is critical, to avoid visual loss and surgical intervention. Furthermore, understanding of dystrophy’s pathogenesis may have broader significance and enhance understanding of other disease entities. For example, my interest in SCD over the decades has been fueled by the potential connection to atherosclerosis. Perhaps, the cornea could serve as a model for lipid-binding agents that can be used in other diseased parts of the body, such as atherosclerotic vessels.\textsuperscript{114}

In the 21st century, we must adopt a more scientific approach to corneal dystrophy. The use of the IC3D nomenclature system, which emphasizes the genetic basis of the disease, should facilitate investigation of entities on the basis of genetic abnormalities. Objective genetic testing will not only confirm or refute our phenotypic diagnosis but also will facilitate the development of a DNA repository and research efforts.\textsuperscript{114} Research is integral to determining the etiology and pathogenesis of the individual corneal dystrophies and ultimately to developing effective preventive and treatment strategies for these patients.

**Note Added in Proof**

The transcription factor 4 gene (TCF4) encoding a member of the E-protein family (E2-2) was recently found to be associated with typical FCD. \textit{NEJM}. August 25, 2010 (10.1056/NEJMoa1007064).

**References**


