2023 ANA Symposium: PAX6, Aniridia, and Beyond

September 29 - October 1

Working to Solve the Aniridia Puzzle

A Patient-Focused Summary of Presentations

Author
Janelle Collins
ANA Board Member at Large

Editor
Shari Krantz
ANA Board Secretary

Reviewers
Robert Grainger, PhD
James Lauderdale, PhD
Peter Netland, PhD, MD
Kelly Trout, BSN, RN

Published April 2024

Introduction

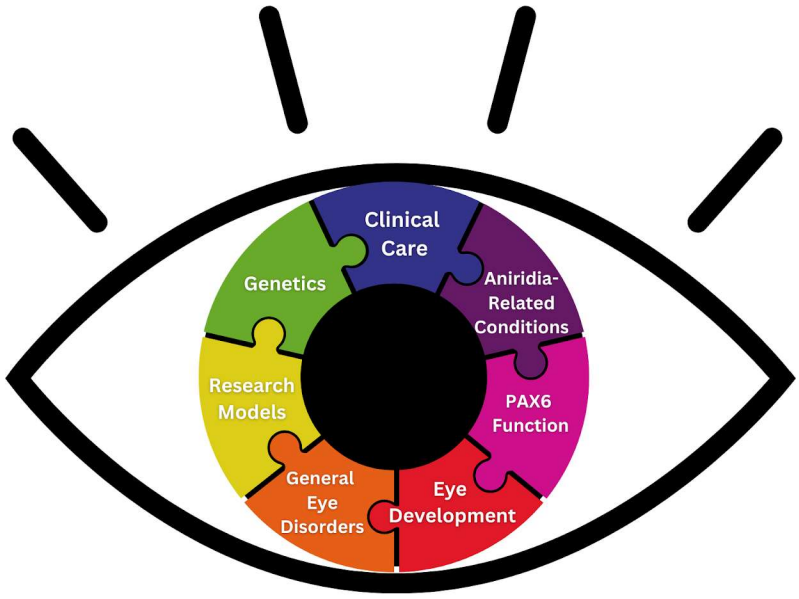
The *2023 ANA Symposium: PAX6, Aniridia, and Beyond* was held at the University of Virginia in Charlottesville, September 29-October 1. The meeting was attended by scientists, clinicians, and representatives of patient advocacy organizations.

This scientific meeting provided the most in-depth assessment to date of the role of *PAX6* and other eye genes involved in aniridia and related congenital eye disorders. Presenters at the meeting are among the world's leading experts in these areas.

Much like a group working together to assemble a jigsaw puzzle, progress in understanding and treating aniridia is a collective effort, with each person contributing unique perspectives and skills. During this meeting, each speaker contributed a piece to reveal unexpected features of the aniridia puzzle.

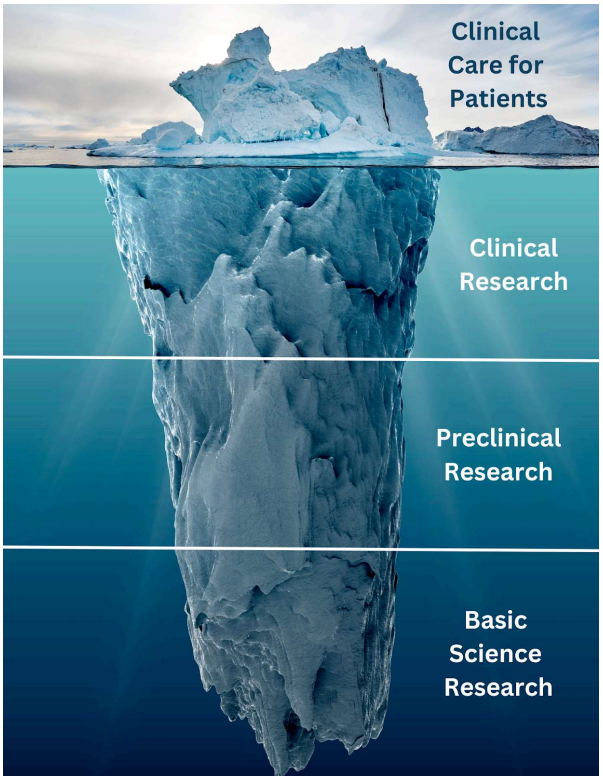
As a result, exciting new ideas about treating aniridia and related genetic-based eye disorders were considered, especially concerning the cornea, lens, and glaucoma. Equally important were the new collaborations developed between attendees.

This document will explain how each speaker's presentation fits into the aniridia puzzle, as well as describe important takeaways in patient-friendly terms.



Solving the Aniridia Puzzle

How Science Becomes Medicine



The path that is followed for scientific investigations to become standard medical practice is well established but not always well understood outside of the research community.

Think of this process as an iceberg. The clinical care that patients experience is like the tip of the iceberg. This is the only part that is visible to patients most of the time. However, without the foundational layers beneath, clinical care would not exist.

The complex process required for any science to be translated into medicine involves the following steps:

Basic Science Research
The journey from science to medicine usually begins with basic scientific research. Basic research means scientists in a lab are trying to learn how living things work (like cells, animals, and people), how bodies develop, and what can cause problems like diseases or injuries.

Harvard scientists actually took a careful look at the discovery of new drugs, and they discovered that curiosity-driven, fundamental research programs are the "best route to the generation of powerful new medicines".[1] This means that the complex science done in labs is absolutely essential for future medical breakthroughs, even if it is perhaps least understandable to patients.

> ***"That got us to thinking, what if…?"***
>
> - Tom Glaser, uttered some of the most important words used in science during his presentation

Preclinical Research
Preclinical research builds upon discoveries found through basic science. Once a promising target or approach is identified, preclinical studies are conducted. This phase involves experiments in cell cultures, animal models, and sometimes human tissues to test the safety and efficacy of potential therapies. Researchers work to validate their findings and determine if a treatment strategy has the potential to be effective in humans.

[1] Spector, J. M., Harrison, R. S., & Fishman, M. C. (2018). Fundamental science behind today's important medicines. *Science translational medicine*, *10*(438), eaaq1787. https://doi.org/10.1126/scitranslmed.aaq1787

It has often been said that you must "cure the mouse before you can cure the human." Preclinical research is where scientists determine whether, indeed, the mouse (zebrafish, frog, etc.) has been cured.

Clinical Research

If promising results were obtained in preclinical testing, the next step is clinical research, which is testing in humans. Clinical trials are set up that test safety and efficacy of new therapies, starting with small numbers of people, and gradually increasing as safety is proven.

Clinical research can also include retrospective studies of existing groups of patients, examining existing procedures for best practices, and comparing genotypes (i.e., an organism's genetic configuration) with phenotypes (i.e., an organism's observable traits) in populations.

Clinical Care

If a clinical trial is successful, the therapy/treatment may be approved by a regulatory agency for use in the general population.

Just like climbing an iceberg, this process does not easily or quickly go up in a straight line. There are times where the path is smooth and progress seems fast, but then there are times where progress is impeded or actually slides backwards. It takes trial and error, time, and skill, but eventually the process can create breakthroughs to the next level.

Throughout this process, collaboration between scientists, clinicians, regulatory agencies, pharmaceutical companies, and patient communities is essential. Part of ANA's mission is to facilitate this collaboration within the aniridia landscape, thus improving outcomes for people with aniridia.

Clinical Care

Multiple presenters contributed to the clinical care puzzle. These presentations fall into both the clinical research and clinical care levels of the science iceberg.

Peter Netland, ophthalmologist and leading expert in glaucoma in aniridia, was a symposium host. He has worked with aniridia patients for more than 20 years, is Chair of the ANA Board of Directors, and was a principal investigator for the Ataluren clinical trial in aniridia.

Peter Netland *(University of Virginia, USA)*

There are many different options for surgical procedures for glaucoma in aniridia. How does a surgeon choose the best option? To help answer this question, Dr. Netland presented newly completed research comparing the variety of surgical procedures that can be used for glaucoma in aniridia and their various historical success rates. He also discussed the factors that impact surgical decision making.

Key Takeaway

When this study is published, it will provide an excellent standard of care for surgeons performing glaucoma surgeries in aniridia patients.

Additional Discussion Points

- Aniridia is a pro-fibrotic syndrome, so a conservative approach to surgery is very important.
- Infantile glaucoma in aniridia can be treated with different (more standard) surgeries compared to childhood or adult-onset glaucoma in aniridia.
- Reminder: Of those with aniridia that have glaucoma, 93% have open angle glaucoma. The remaining 7% have closed angle glaucoma and all had prior surgeries.[2]
- There was agreement among ophthalmologists in attendance that preventative goniotomy is not recommended in aniridia patients.
- Consensus in the group was that glaucoma drainage implants are helpful in patients with aniridia and glaucoma.

Dr. Freeman shared this interesting picture of the cells of the cornea.

John Freeman *(MECA Eye & Laser Center, USA)*

Dr. Freeman gave an overview of clinical findings, current management, and potential therapies for aniridia-related

[2] Bajwa A, Burstein E, Grainger RM, Netland PA. Anterior chamber angle in aniridia with and without glaucoma. *Clin Ophthalmol*. 2019;13:1469-1473 https://doi.org/10.2147/OPTH.S217930

keratopathy (ARK). Since ARK is a disease of the cornea, and the cornea is the clear front part of the eye, ARK is literally "right in front of our very eyes."

Key Takeaway

The cornea should be self-healing and self-maintaining, but ARK is a failure of that corneal homeostasis. Currently, there is no guaranteed prevention, and the existing treatments leave much to be desired. However, scientific understanding of *PAX6* function and control is creating great potential for therapeutic intervention that will allow future therapies to improve clinical outcomes.[3]

Points for Patients

- Humidity seems to be good for aniridic corneas
- Use of preservative-free artificial tears and autologous serum drops is positive
- Antihistamines like Benadryl should be used with caution because they dry out the eye

Additional Discussion Points

- Although we have no guaranteed way to prevent progression of keratopathy, common sense treatments like autologous serum drops and preservative-free tears seem to be worthwhile.
- Current treatments are imperfect
    - Limbal Stem Cell Transplants have up to 40% failure rate and involve immunosuppressant medications which can have unpleasant effects. However, this procedure can be repeated, and if it fails, it is non-destructive to the eye.
    - Boston Keratoprosthesis (KPro) has a long-term failure rate of approximately 20%. If it works, it gives long-term success. If it fails, it is destructive to the eye.
- Potential early treatments being studied include
    - Nonsense suppression drugs such as Ataluren[4] and Amlexanox;[5]

[3] L. Latta, F.C. Figueiredo, R. Ashery-Padan, J.M. Collinson, J. Daniels, S. Ferrari, N. Szentmáry, S. Solá, R. Shalom-Feuerstein, M. Lako, S. Xapelli, D. Aberdam, N. Lagali, Pathophysiology of aniridia-associated keratopathy: Developmental aspects and unanswered questions, *The Ocular Surface*, Volume 22, 2021, Pages 245-266, ISSN 1542-0124, https://doi.org/10.1016/j.jtos.2021.09.001.

[4] Djayet, C., Bremond-Gignac, D., Touchard, J., Secretan, P. H., Vidal, F., Robert, M. P., Daruich, A., Cisternino, S., & Schlatter, J. (2020). Formulation and Stability of Ataluren Eye Drop Oily Solution for Aniridia. *Pharmaceutics*, *13*(1), 7. https://doi.org/10.3390/pharmaceutics13010007.

[5] Lima Cunha, D., Sarkar, H., Eintracht, J., Harding, P., Zhou, J. H., & Moosajee, M. (2023). Restoration of functional PAX6 in aniridia patient iPSC-derived ocular tissue models using repurposed nonsense suppression drugs. *Molecular therapy. Nucleic acids*, *33*, 240–253. https://doi.org/10.1016/j.omtn.2023.06.016

  - Other repurposed drugs that would increase *PAX6* expression, such as MEK inhibitors,[6] ritanserin,[7] and duloxetine;[8] and
  - Using nerve growth factors like Oxervate
- Potential late treatments being studied include
  - Gene restoration therapies
  - Newer surgical approaches
  - Additional topical agents and pharmacological approaches, such as anti-VEGF compounds.[9]
- Challenges in translating these avenues into successful treatments for patients include
  - *PAX6* is dosage and timing sensitive, which makes it tricky to get correct levels
  - Translating from animal models and cell models to humans can be difficult and time consuming due to high threshold of safety required
  - Early keratopathy holds the greatest opportunity for correction, but it is difficult to measure the slowing of disease progression, which makes it hard to provide meaningful study endpoints that require significant time

John Freeman (right) is an ophthalmologist who has worked with aniridia patients for many years, first during his fellowship under Ed Holland, then in Memphis with Peter Netland (left). During this time, he was the first to characterize aniridic fibrosis syndrome. John is now in private practice specializing in cataract surgery and cornea disease.

Ken Nischal *(University of Pittsburgh, USA)*

While aniridia caused by the *PAX6* gene usually doesn't involve corneal opacities at birth, aniridia due to other genetic causes sometimes does. In his presentation, Dr. Nischal discussed cases of congenital corneal opacities resulting from the *FOXC1*, *PITX2*, *CYP1B1*, and *FOXE3* genes that had aniridia-like presentation.

---

[6] Rabiee, B., Anwar, K. N., Shen, X., Putra, I., Liu, M., Jung, R., Afsharkhamseh, N., Rosenblatt, M. I., Fishman, G. A., Liu, X., Ghassemi, M., & Djalilian, A. R. (2020). Gene dosage manipulation alleviates manifestations of hereditary *PAX6* haploinsufficiency in mice. *Science translational medicine*, *12*(573), eaaz4894. https://doi.org/10.1126/scitranslmed.aaz4894

[7] Oved, K., Zennaro, L., Dorot, O., Zerbib, J., Frank, E., Roux, L. N., Bremond-Gignac, D., Pichinuk, E., & Aberdam, D. (2021). Ritanserin, a potent serotonin 2A receptor antagonist, represses MEK/ERK signalling pathway to restore PAX6 production and function in aniridia-like cellular model. *Biochemical and biophysical research communications*, *582*, 100–104. https://doi.org/10.1016/j.bbrc.2021.10.036

[8] Dorot, O., Roux, L. N., Zennaro, L., Oved, K., Bremond-Gignac, D., Pichinuk, E., & Aberdam, D. (2022). The antipsychotropic drug Duloxetine rescues PAX6 haploinsufficiency of mutant limbal stem cells through inhibition of the MEK/ERK signaling pathway. *The ocular surface*, *23*, 140–142. https://doi.org/10.1016/j.jtos.2021.12.003

[9] van Velthoven, A. J. H., Utheim, T. P., Notara, M., Bremond-Gignac, D., Figueiredo, F. C., Skottman, H., Aberdam, D., Daniels, J. T., Ferrari, G., Grupcheva, C., Koppen, C., Parekh, M., Ritter, T., Romano, V., Ferrari, S., Cursiefen, C., Lagali, N., LaPointe, V. L. S., & Dickman, M. M. (2023). Future directions in managing aniridia-associated keratopathy. *Survey of ophthalmology*, *68*(5), 940–956. https://doi.org/10.1016/j.survophthal.2023.04.003.

Key Takeaway
To ensure that the patient receives the correct diagnosis, molecular genetics is incredibly important in cases of congenital aniridia with corneal opacities present at birth.

Additional Discussion Point
When there are congenital corneal opacities, it can be difficult to look inside the eye at remaining structures to determine whether there are any abnormalities. High frequency ultrasound, often available in a NICU, can be used to determine the status of the iris and the lens.

Ken Nischal (shown speaking virtually) is an ophthalmologist and a pioneer of pediatric corneal transplants. He is often sent difficult cases involving children with corneal opacities at birth. He has published widely on the topics of pediatric cataract, glaucoma, and cornea.

Kevin Gregory-Evans *(University of British Columbia, Canada)*

Using the example of the eye condition retinitis pigmentosa, Dr. Kevin Gregory-Evans discussed how easy it is to misdiagnose a genetic eye disease. He gave many examples where something looked like retinitis pigmentosa at first glance, but once thoroughly investigated turned out to be something else.

Kevin Gregory-Evans is an ophthalmologist specializing in genetic eye diseases, including aniridia. He was a principal investigator in the Ataluren clinical trial.

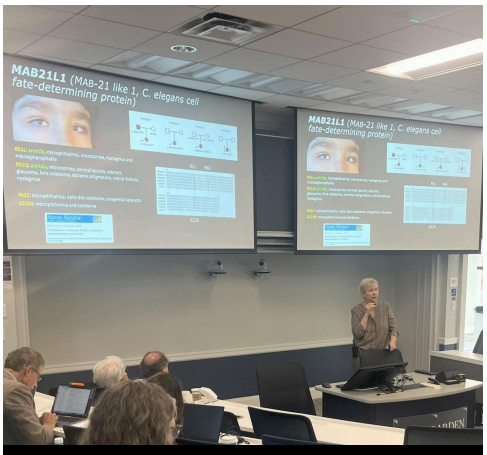
Key Takeaway
Because medical diagnostics is an imperfect science, misdiagnoses do occur. Molecular genetics is particularly important for confirming diagnoses, but when genetics testing cannot find the cause, it is especially important to thoroughly investigate the situation before pronouncing a diagnosis.

Caution for Clinicians
Patients tend to become attached to their diagnosis, so if it changes, it can be very jarring. It is therefore important to do thorough investigations when cases are not cut and dry.

Points for Patients
- Remember that the medical sciences are imperfect.
- Until a diagnosis is confirmed with genetics, be cautious about fully embracing that diagnosis.

Genetics

New information was discussed related to the genetics puzzle piece of aniridia. Genetics research can fall into multiple levels of the science iceberg, including basic science, preclinical, and clinical research.



Elena Semina (speaking about MAB21L1) researches the genetics of eye disorders. She has published more than 100 articles related to ocular disorder genetics and has been studying this topic for 30 years. Through this meeting, the relationship between the Semina Lab and the aniridia patient community was strengthened.

Elena Semina *(Medical College of Wisconsin, USA)*

Substantial progress has been made in identifying the genetic factors responsible for aniridia and other developmental disorders related to the front of the eye (collectively called Anterior Segment Disorders, or ASD). Overall, the diagnostic success rate is presently about 90% for typical aniridia resulting from a *PAX6* anomaly. However, only approximately 50% of other ASD phenotypes are successfully diagnosed genetically. Dr. Semina's lab specializes in investigating difficult cases that haven't been diagnosed elsewhere.

Dr. Semina's presentation discussed the latest genetic data related to various forms of ASD, with a focus on aniridia and aniridia-like phenotypes. The Semina Lab investigated 150 individuals from 126 families and identified the genetic cause for 98 out of the 126 families. In the process, they found new genes that were previously not associated with aniridia, such as *MAB21L1,*[10] as well as new variants of known genes *PAX6* and *FOXC1*.

Points for Patients

- Early genetic diagnosis can improve management of patients
- If a definitive mutation for *PAX6* or any gene has already been found, repeating the genetic testing will not change the diagnosis.
- Repeat testing is important if no definitive mutation was identified, if the last testing was many years ago, or if the results returned were a "Variant of Uncertain Significance" or VOUS.
- When a result was a VOUS, the Semina Lab may have more information on the meaning of that variant and can update genetic testing information with those results.
- Families that are enrolled in the study at the Semina Lab will receive their genetics results if discovered, typically at no cost to the family.

Key Takeaway

Since there is significant overlap between the expression of many anterior segment genes, it is

[10] Seese, S. E., Reis, L. M., Deml, B., Griffith, C., Reich, A., Jamieson, R. V., & Semina, E. V. (2021). Identification of missense MAB21L1 variants in microphthalmia and aniridia. *Human mutation*, *42*(7), 877–890. https://doi.org/10.1002/humu.24218.

recommended that broad genetic testing or exome analysis be performed in most cases of aniridia, with copy number variation analysis included.

Additional Discussion Points

The gene that is mutated in aniridia is associated with different systemic features that may be encountered in a patient.

- *PAX6* mutations are most frequently the cause of aniridia. However, *PAX6* mutations can also result in non-aniridia phenotypes, such as microphthalmia, microcornea, and Peter's anomaly.
- *FOXC1* mutations often involve congenital glaucoma, heart anomalies, hearing loss, skeletal abnormalities, and more.
- *PITX2* mutations typically involve developmental glaucoma, dental/craniofacial anomalies, gastrointestinal and umbilical issues, and more.
- Knowledge of *MAB21L1* in aniridia is still relatively new. Currently it appears that microphthalmia, microcornea, cataracts and more are associated, but no systemic features.

Tom Glaser researches the genetic basis of eye development in mammals and congenital eye malformations. Working with David Walton in 1992, Tom was the first to discover that aniridia in humans resulted from mutations in the PAX6 gene.

Tom Glaser (*University of California, Davis, USA*)[11]

Whenever researchers discover a set of malformations caused by a previously unknown site on the human genome, it is considered an important breakthrough in basic science. Dr. Glaser reported on one such recent result discovered in his lab. This study reported a new set of eye and brain malformations that resulted from a master regulator gene that directs the development of particular body segments or structures.

Key Takeaway

Dr. Glaser's presentation revealed an unexpected mechanism of genetic disease. While it is not specifically known to be related to aniridia at this time, it is essential information moving forward.

Additional Discussion Points

Given that this is a new and unpublished discovery, it is possible that there are other adult-onset diseases or conditions that are caused by insertions or deletions at this particular location in the genome–we do not know yet. Researchers are beginning to look into these possibilities.

- This study provides important context for the presentations given by Janey Wiggs and Ruth Ashery-Padan.

[11] Glaser, T., Walton, D. S., & Maas, R. L. (1992). Genomic structure, evolutionary conservation and aniridia mutations in the human PAX6 gene. *Nature genetics*, *2*(3), 232–239. https://doi.org/10.1038/ng1192-232

Cheryl Gregory-Evans[12] *(University of British Columbia, Canada)*

Now that new gene therapy treatments are becoming available for developmental/early-onset ocular diseases, it is increasingly important to have a confirmed genetic diagnosis to receive proper treatment.

There is a backlog of outstanding testing requests in Medical Genetic departments, which is affecting ocular disease patients in Canada. Dr. Gregory-Evans presented data assessing the success and timeliness of genetic testing in one specialty ocular genetics clinic in Canada.

Key Takeaway
A specialist ocular genetics clinic can provide faster service in Canada as opposed to a standard Medical Genetics department.

Caution for Clinicians
It is helpful to perform clinical correlation with genetics results before releasing the results to patients.

Point for Patients
This presentation provided attendees with a better understanding of Canada's medical system.

Cheryl Gregory-Evans focuses on understanding the molecular basis of human eye and brain diseases, using zebrafish, mouse and lizard model systems. Cheryl was responsible for the pre-clinical research done in mice that led to the Ataluren clinical trial in humans with aniridia.

Janey Wiggs *(Harvard Medical School, USA)*

It is clinically important to understand the genes that cause or contribute to glaucoma susceptibility. This allows clinicians to identify people at high risk for glaucoma before there are symptoms, thus making it easier to preserve vision.

Many genes that cause childhood glaucoma, including those responsible for aniridia, show very different disease courses in different individuals. Even family members with the exact same mutation can have different presentations of glaucoma. Identifying factors that influence these variable outcomes related to disease-causing gene mutations could help provide more precise information and better insight into why certain individuals develop glaucoma and others do not.

[12] Wang, X., Gregory-Evans, K., Wasan, K. M., Sivak, O., Shan, X., & Gregory-Evans, C. Y. (2017). Efficacy of Postnatal In Vivo Nonsense Suppression Therapy in a Pax6 Mouse Model of Aniridia. *Molecular therapy. Nucleic acids*, *7*, 417–428. https://doi.org/10.1016/j.omtn.2017.05.002.

Dr. Wiggs described a study in which they investigated the impact of a gene burden score (a polygenic risk score[13] or PRS) on the risk of developing glaucoma in people who carry a gene mutation known to be associated with early-onset disease.[14] Among the mutation carriers, they found that people who have a high genetic burden, or high PRS score, were more likely to develop glaucoma compared to people with a low score, suggesting that including the PRS score in the genetic test would be useful.

Key Takeaway

Future research in this area may show that PRS scores are useful in predicting which people with aniridia are most at risk of developing glaucoma.

Janey Wiggs (right) is known internationally as an expert in complex and advanced glaucoma and the genetics that cause it. Her research during the past 20+ years has provided critical information regarding the biology of the disease.

[13] Craig, J. E., Han, X., Qassim, A., Hassall, M., Cooke Bailey, J. N., Kinzy, T. G., Khawaja, A. P., An, J., Marshall, H., Gharahkhani, P., Igo, R. P., Jr, Graham, S. L., Healey, P. R., Ong, J. S., Zhou, T., Siggs, O., Law, M. H., Souzeau, E., Ridge, B., Hysi, P. G., … MacGregor, S. (2020). Multitrait analysis of glaucoma identifies new risk loci and enables polygenic prediction of disease susceptibility and progression. *Nature genetics*, *52*(2), 160–166. https://doi.org/10.1038/s41588-019-0556-y

[14] Victor Anthony de Vries, Akiko Hanyuda, Joëlle Vergroesen, Ron Do, David S Friedman, Peter Kraft, Yuyang Luo, Jessica Tran, Sze H Wong, Nazlee Zebardast, Caroline C W Klaver, Janey L Wiggs, Ayellet V. Segre, Jae H Kang, Wishal Ramdas, Louis R Pasquale; The clinical utility of a glaucoma polygenic risk score in four European-ancestry cohorts. *Invest. Ophthalmol. Vis. Sci.* 2023;64(8):99.

Aniridia-Related Conditions

Although the name "aniridia" focuses on the iris, the condition is panocular, which means that it can affect the entire eye. Complications can occur in other parts of the eye anatomy, including the cornea, lens, anterior chamber, retina, tear film, and optic nerve. There can also be systemic complications related to aniridia. Clinical and Pre-clinical research is ongoing in all of these different conditions.

Dominique Bremond-Gignac *(Université de Paris, France)*

PAX6-related congenital aniridia is characterized by a hypoplastic or absent iris and foveal hypoplasia (underdevelopment of the fovea, which is the part of the retina responsible for the most detailed vision). It has been recently shown that, in patients harboring *PAX6* mutations, foveal hypoplasia is more frequently encountered than complete absence of iris.

Pr. Bremond-Gignac described two separate studies related to aniridia. The first characterized retinal disorders that occur in congenital aniridia, and correlated the degree of foveal hypoplasia with best corrected visual acuity, *PAX6* mutation, and degree of iris hypoplasia.[15] The second study characterized optic disc hypoplasia in aniridia and investigated the relationship between optic disc hypoplasia and foveal hypoplasia.

Dominique Bremond-Gignac (left), with John Morris, Chair, IWSA Board of Directors, is an ophthalmologist involved in phenotype/genotype studies on rare diseases, especially aniridia. Dominique is also Chair of the Scientific Committee for Aniridia Europe and the Geniris Association in France.

Key Takeaway

Foveal hypoplasia is the most reliable sign to confirm a congenital aniridia diagnosis–even more reliable than iris hypoplasia.[16]

Additional Discussion Points

- Genes have different regions that serve different purposes. At the end of the *PAX6* gene is a region called the 3' regulatory region. This region is like the end part of a recipe that

[15] Daruich, A., Robert, M. P., Leroy, C., DE Vergnes, N., Beugnet, C., Malan, V., Valleix, S., & Bremond-Gignac, D. (2022). Foveal Hypoplasia Grading in 95 Cases of Congenital Aniridia: Correlation to Phenotype and PAX6 Genotype. *American journal of ophthalmology*, *237*, 122–129. https://doi.org/10.1016/j.ajo.2021.12.007

[16] Daruich, A., Duncan, M., Robert, M. P., Lagali, N., Semina, E. V., Aberdam, D., Ferrari, S., Romano, V., des Roziers, C. B., Benkortebi, R., De Vergnes, N., Polak, M., Chiambaretta, F., Nischal, K. K., Behar-Cohen, F., Valleix, S., & Bremond-Gignac, D. (2023). Congenital aniridia beyond black eyes: From phenotype and novel genetic mechanisms to innovative therapeutic approaches. *Progress in retinal and eye research*, *95*, 101133. https://doi.org/10.1016/j.preteyeres.2022.101133

tells you when to stop, how to adjust the cooking speed, and how to make sure the recipe turns out just right. Deletions restricted to this 3' regulatory *PAX6* region are the only *PAX6* variants that are not associated with severe (Grade 3 or 4) Foveal Hypoplasia.

- The grade of foveal hypoplasia is correlated to low vision and can help in deciding whether further treatments or investigations are necessary to improve a patient's visual acuity.
- The amount of blood vessels (i.e., the vasculature) in the fovea and parafoveal areas are significantly different in aniridia vs controls.

Ali Djalilian *(University of Illinois at Chicago, USA)*

Building on the topic of aniridia-related keratopathy presented by John Freeman, Ali Djalilian provided updates on Clinical and Pre-Clinical Research being done regarding treatments for ARK, including multiple methods that his lab has been investigating.

Ali Djalilian is an ophthalmologist and leading expert in corneal wound healing and management of complex corneal diseases. He is editor in chief of the highly respected journal *The Ocular Surface* and has been studying the cornea for more than 20 years.

The first method discussed was repurposing MEK inhibitors–a class of medicines that helps control cell growth and survival–to increase PAX6 expression, which produced wonderful results in mouse corneas.[17] Dr. Djalilian discussed the challenges and next steps regarding that particular line of research, including the need to determine appropriate animal models for testing, the long-term safety of MEK inhibitors applied topically, and the optimal stage for intervention.

The second method discussed was the use of Mesenchymal Stromal Cells (MSCs). These MSCs are a type of cell that give rise to most tissues in the body, such as skin, blood, and bone. MSCs are multipotent, which means they can develop into multiple different types of cells. They are found in many adult tissues and are critical to tissue maintenance and repair. Due to these qualities, MSCs are being investigated in many different areas as a way to create cell-based therapies. In the cornea, they have been shown to have anti-inflammatory and anti-scarring effects.[18] Dr. Djalilian's lab conducted a small

[17] Rabiee, B., Anwar, K. N., Shen, X., Putra, I., Liu, M., Jung, R., Afsharkhamseh, N., Rosenblatt, M. I., Fishman, G. A., Liu, X., Ghassemi, M., & Djalilian, A. R. (2020). Gene dosage manipulation alleviates manifestations of hereditary *PAX6* haploinsufficiency in mice. *Science translational medicine*, *12*(573), eaaz4894. https://doi.org/10.1126/scitranslmed.aaz4894

[18] Putra, I., Shen, X., Anwar, K. N., Rabiee, B., Samaeekia, R., Almazyad, E., Giri, P., Jabbehdari, S., Hayat, M. R., Elhusseiny, A. M., Ghassemi, M., Mahmud, N., Edward, D. P., Joslin, C. E., Rosenblatt, M. I., Dana, R., Eslani, M., Hematti, P., & Djalilian, A. R. (2021). Preclinical Evaluation of the Safety and Efficacy of Cryopreserved Bone Marrow Mesenchymal Stromal Cells for Corneal Repair. *Translational vision science & technology*, *10*(10), 3. https://doi.org/10.1167/tvst.10.10.3.

trial using an injectable form of MSCs on humans with non-healing ocular wounds to determine safety levels.[19] The next phase of clinical trials will begin soon, which will involve a multicenter trial with placebo to determine if these MSCs are actually helpful.

The third method discussed was the use of an eyedrop containing a conditioned media known as a Secretome. This conditioned media is like a broth, which–unlike the MSC injections discussed above–does not contain the actual stem cells, but instead contains factors produced by those cells. Secretome has been shown to have healing effects for eye injuries in mice.[20] A Phase I Clinical Trial, primarily testing for safety, began in late 2023 for this Secretome, and includes some patients with aniridia who have ARK.

Key Takeaway
Multiple methods of treating ARK and healing corneal injuries are being investigated, including current clinical trials. One clinical trial involves patients with aniridia.

Point for Patients
The Secretome Phase I Clinical Trial is enrolling patients at this time. Some aniridia patients with mild to moderate ARK may qualify.

Melinda Duncan researches ocular wound healing, particularly in relation to how the eye responds to surgeries and how cataracts form. She is one of the foremost researchers on aniridic fibrosis syndrome.

Melinda Duncan *(University of Delaware, USA)*

Lenses are clear eye structures that help focus incoming light so that we can see objects clearly. People with aniridia are usually born with clear lenses that look normal to doctors; however, in late childhood/early adulthood, most people with aniridia develop cataracts, a condition where the lens becomes cloudy, blocking the ability to see clearly.

While this is a common consequence of aniridia, we really don't understand how it occurs. Since it is not possible to obtain lenses from young people with aniridia as they develop cataracts, we know very little about why PAX6 mutations result in early onset cataracts in individuals with aniridia.

However, we know of mice with *Pax6* mutations that are similar to those with humans with aniridia, and these mice also develop early onset cataracts. Dr. Duncan described

[19] Arthur Yukuang Chang, Mohammad Soleimani, Rebecca Jung, Grace Tu, Reza Dana, Bennie H Jeng, Peiman Hematti, Nadim Mahmud, Elmer Y. Tu, Charlotte E. Joslin, Ali R Djalilian; Phase I Study on the Safety of Locally Delivered Allogeneic Mesenchymal Stem Cells for Promoting Corneal Repair. *Invest. Ophthalmol. Vis. Sci.* 2023;64(8):3136.
[20] Amirjamshidi H, Milani BY, Sagha HM, Movahedan A, Shafiq MA, Lavker RM, Yue BY, Djalilian AR. Limbal fibroblast conditioned media: a non-invasive treatment for limbal stem cell deficiency. Mol Vis. 2011 Mar 8;17:658-66. PMID: 21403854; PMCID: PMC3056128.

her lab's work in such mice to understand why *Pax6* mutations result in cataracts in animals, which will provide insight into why humans need two working copies of the *PAX6* gene to maintain lens health.[21]

Key Takeaway

The correct amount of PAX6 is essential for lens maintenance. When the proper amount of PAX6 isn't available, lens cells act like they are chronically wounded. This sensitizes the rest of the eye to form scar tissue, even parts of the eye that do not directly need PAX6 for their functions.

Additional Discussion Points

- Lens size in people with aniridia is smaller than in those without aniridia, which is consistent with what is seen in mice, rats, frogs, and zebrafish.[22]
- There is a significant inflammatory response created by the lens cells themselves.
- Lens fibers produced in adulthood in aniridia show defects in protein production, which leads to cataracts.

Kelly Trout *(International WAGR Syndrome Association & Aniridia North America, USA)*

Aniridia is a rare genetic condition, with an incidence of about 1 of every 40,000 to 100,000 people, and therefore it is difficult for doctors and scientists to study. Kelly Trout discussed the importance of creating a database of aniridia patients. Aniridia North America has created this database, named the *EYEris Aniridia Registry*, and will launch it in 2024.

Kelly Trout (left), with John Morris (IWSA Board Chair) and Shari Krantz (IWSA Executive Director and ANA Secretary), is a founding member of ANA and the IWSA. She is the Director of Research and Medical Advocacy for the IWSA and led the team that developed the WAGR Syndrome Patient Registry.

Developing a database of aniridia patients and the complications they experience can help provide researchers with valuable information and help clinicians to better understand and treat these conditions. The Registry will include information like patient age, medical history, and even genetic testing results.

Some positive things about developing this Registry include:

[21] Voskresenskaya, A., Pozdeyeva, N., Batkov, Y., Vasilyeva, T., Marakhonov, A., West, R. A., Caplan, J. L., Cvekl, A., Wang, Y., & Duncan, M. K. (2021). Morphometric analysis of the lens in human aniridia and mouse Small eye. *Experimental eye research*, *203*, 108371. https://doi.org/10.1016/j.exer.2020.108371.

[22] Duncan, M. K., Daruich, A., Valleix, S., & Bremond-Gignac, D. (2024). Reduction of lens size in PAX6-related aniridia. *Experimental eye research*, *238*, 109746. https://doi.org/10.1016/j.exer.2023.109746

- Helping Science: It provides scientists access to standardized information and data sets to study. They can look for patterns and learn more about what causes Aniridia and how to treat it. They can also see how conditions associated with aniridia change over time.
- Better Care: By knowing more about aniridia, doctors can provide more individualized patient care, hopefully improving quality of life.
- Working Together: When knowledge is shared, it makes it easier to find answers. Scientists, doctors, and aniridia patients can work together and share what they know.
- New Medications and Therapies: Companies that develop new therapies and produce medications can access the Registry to identify patients who may be interested in new treatments, thus accelerating their development and applications.

Researchers, doctors, and aniridia patients and their families can all take part in the *EYEris Aniridia Patient Registry*. By working together, we can learn more about aniridia and make life better for those who have it.

Points for Patients

- Participation in the EYEris Aniridia Registry will be free and will consist of filling out a questionnaire.
- Participating in the Registry is a way for patients to directly contribute to research into aniridia.
- In a rare disease, information from every single person matters!

Key Takeaway

During the last decade, rare disease registries have undergone significant transformation. In the interest of creating an aniridia registry that meets current practices, including standardized questions, Aniridia North America will be launching the *EYEris Aniridia Registry* in 2024.

Additional Discussion Point

The International WAGR Syndrome Association created and launched a registry similar to the *EYEris Aniridia Registry* in 2015. Research conducted using data from the *WAGR Syndrome Patient Registry* resulted in the first-ever clinical care guidelines for WAGR syndrome, a change in name from WAGR syndrome to WAGR Spectrum Disorder, and documentation of conditions that the medical community previously did not associate with WAGR syndrome.[23] Knowledge and experience gained from the design and implementation of the WAGR registry was applied to create the *EYEris Aniridia Registry,* which will launch in 2024.

[23] Duffy, K. A., Trout, K. L., Gunckle, J. M., Krantz, S. M., Morris, J., & Kalish, J. M. (2021). Results From the WAGR Syndrome Patient Registry: Characterization of WAGR Spectrum and Recommendations for Care Management. *Frontiers in pediatrics*, *9*, 733018. https://doi.org/10.3389/fped.2021.733018

Jim Lauderdale *(University of Georgia, USA)*

While John Freeman covered the topic of ARK at a Clinical Care level, and Ali Djalilian covered ARK at Pre-Clinical and Clinical Research levels, Dr. Lauderdale presented information learned in his lab from Basic and Translational research regarding the cornea, limbal stem cells (stem cells that form the border between the cornea and the sclera), and ARK.

Jim Lauderdale (left) is a member of the ANA Board of Directors and co-organizer of this Symposium. He researches the genes and pathways that control how the eye is made and how visual information is processed in the brain. He has spent much of his career studying aniridia and the PAX6 gene.

Developing new therapeutic approaches for ARK is aided by better understanding of changes in the cornea and why these changes occur in aniridia patients.[24] Research using different approaches–including animal models, new technologies for working with human cells, and advanced technologies for cataloging all of the genes expressed in single cells–is expected to provide an understanding that will lead to new therapies to treat or prevent ARK.

Key Takeaway

It has long been thought that the primary driver of ARK was limbal stem cell deficiency (LSCD); however, the LSCD hypothesis cannot explain changes in the cornea that occur while the ocular surface and limbus appear to be mostly normal. Recent research suggests that there are other significant factors at play. Identifying the pathways and mechanisms involved in ARK is needed to develop new therapies.

Additional Discussion Points

- There is evidence of a change in the signaling environment (the network of cells, pathways, and processes that allow vision) of the aniridic mouse cornea that precedes clinical signs of keratopathy.
- In an aniridic mouse model, there is a reordering and partial loss of nerves in the aniridic cornea that precedes keratopathy.
- In an aniridic mouse model, blood vessels are retained in the stroma (the middle layer of the cornea) and increase in numbers simultaneously with keratopathy.
- In both mice and humans, there is evidence of an inflammatory response in the cornea preceding ARK.

[24] Lagali, N., Wowra, B., Fries, F. N., Latta, L., Moslemani, K., Utheim, T. P., Wylegala, E., Seitz, B., & Käsmann-Kellner, B. (2020). Early phenotypic features of aniridia-associated keratopathy and association with PAX6 coding mutations. *The ocular surface*, *18*(1), 130–140. https://doi.org/10.1016/j.jtos.2019.11.002

PAX6 Function

Most cases of aniridia result from a mutation or deletion in the *PAX6* gene, so it is essential to understand how the *PAX6* gene functions correctly as well as what happens when something goes wrong. Without this understanding, it is difficult to create effective treatments. The presentations in this section are all in the Basic Science Research section of the science iceberg, and are therefore more technical.

David Price (left) is a neurobiologist whose research focuses on how the brain develops, and specifically, the crucial role PAX6 plays in brain development. He is pictured here with fellow scientist Justin Kumar (right).

David Price *(University of Edinburgh, UK)*
The development of an organism is like a very complicated chain reaction. The meticulous choreography of the complex chemical reactions involved in this chain reaction is essential to ensure the generation of a successful adult organism.

Due to this complexity, there is a very real risk that chance variations will derail development unless there are mechanisms to stop this from happening. The *PAX6* gene is known to be heavily expressed in the cerebral cortex of the human developing brain. Dr. Price's research sought to discover exactly what *PAX6* does in cortical development, and discovered that *PAX6* plays a protective role in the embryonic cerebral cortex of mammals, including humans.[25]

When *PAX6* was completely removed from the developing mouse and human cerebral cortical cells that usually contain it, some of them started turning into nerve cells of the wrong type. However, not all cells turned into the wrong type, which was an interesting result. Upon further investigation, results seemed to indicate that all the cells from which *PAX6* was removed were at risk of going wrong, but the risk only became a reality for those whose immediate environment contained enough morphogens (certain molecules that direct cell fate decisions during embryonic development).

[25] Manuel, M., Tan, K. B., Kozic, Z., Molinek, M., Marcos, T. S., Razak, M. F. A., Dobolyi, D., Dobie, R., Henderson, B. E. P., Henderson, N. C., Chan, W. K., Daw, M. I., Mason, J. O., & Price, D. J. (2022). Pax6 limits the competence of developing cerebral cortical cells to respond to inductive intercellular signals. *PLoS biology*, *20*(9), e3001563. https://doi.org/10.1371/journal.pbio.3001563

Key Takeaway

The cerebral cortex in the human brain plays a key role in memory, thinking, learning, reasoning, problem-solving, emotions, consciousness, and functions related to your senses. A proper balance between inhibitory and excitatory neurons is essential to carry out its functions. Dr. Price's research seems to show that *PAX6* reduces the risk of early brain cells making the wrong type of nerve cells (inhibitory) instead of the right type (excitatory), thus having a protective effect.

Additional Discussion Points

- We don't know if this protective effect is still lost if only one copy of *PAX6* is missing, as in aniridia.
- In scientific literature, the balance between excitation and inhibition is often considered a potentially important factor underlying a range of neurodivergent conditions.[26] [27] However, additional research is needed to make any firm predictions about how these findings functionally impact aniridia patients.
- Hypothetically, these findings may help explain the variation in effects seen between people with the same *PAX6* mutation. This preliminary research should be interpreted cautiously.

Ales Cvekl *(Albert Einstein College of Medicine, USA)*

As discussed in Melinda Duncan's presentation, lens abnormalities, including cataracts, are found in aniridia patients. During the past decade, Dr. Cvekl's lab pioneered the generation of primitive lenses and cone-enriched retinas using stem cells. These can be used to model human eye diseases like aniridia.

To fully harness the power of these systems, multiple unbiased state-of-the-art methods were implemented to analyze parts of individual ocular tissues using human and mouse models.[28] These methods allowed new analysis of the early stages of lens formation.[29] They also allow Dr.

Ales Cvekl is a leading expert in the development of the lens of the eye. He has been researching PAX6 and lens development for more than 30 years.

[26] Uzunova, G., Pallanti, S., & Hollander, E. (2016). Excitatory/inhibitory imbalance in autism spectrum disorders: Implications for interventions and therapeutics. *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry*, *17*(3), 174–186. https://doi.org/10.3109/15622975.2015.1085597

[27] Richter, M. A., de Jesus, D. R., Hoppenbrouwers, S., Daigle, M., Deluce, J., Ravindran, L. N., Fitzgerald, P. B., & Daskalakis, Z. J. (2012). Evidence for cortical inhibitory and excitatory dysfunction in obsessive compulsive disorder. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *37*(5), 1144–1151. https://doi.org/10.1038/npp.2011.300

[28] Cvekl, A. (2024), Multi - omics studies of eye and lens development. Acta Ophthalmol, 102:. https://doi.org/10.1111/aos.16484

[29] Cecília G. De Magalhães, Ales Cvekl, Ruy G. Jaeger, C. Y. Irene Yan, bioRxiv 2023.11.30.569417; doi: https://doi.org/10.1101/2023.11.30.569417

Cvekl's lab to systematically probe the effects of individual human *PAX6* mutations to identify precise molecular mechanisms of normal and mutated PAX6 proteins.[30] This research will pave the road for individual therapies applying the concept of personalized medicine.

Key Takeaway

We have learned an immense amount about *PAX6* during the 32+ years since its discovery. However, there's still a lot more to learn. Dr. Cvekl's lab is working to understand how *PAX6* controls every stage of lens development, and in the process, they have created multiple new tools that others can use to push research forward. All of these things together will hopefully lead to better individual therapies in the future.

Additional Discussion Points

- PAX6 is a transcription factor, which means that it helps determine whether other genes are turned "on" or "off".
- While there are about 1600 transcription factors in the body, only about 12 of them play as important a role as PAX6 does. *PAX6* is a very important gene in the body.

Justin Kumar is a developmental biologist who uses fruit fly models to study how a cell becomes one kind of tissue (like an eye) and not another (like a wing or an antenna). He has been studying Pax6 and eye development for more than 25 years.

Justin Kumar *(Indiana University, USA)*

The PAX6 transcription factor is required in all seeing animals that have been examined to date, and is often considered the "master control gene" of eye development. The compound eye of the fruit fly (Drosophila) provides an excellent model for studying the processes required for eye development. In Drosophila, there are two *PAX6* genes, called *Eyeless* and *Twin of Eyeless*, which control development of the eye.

It has been shown that *PAX6* is integral for the "retinal determination network", which is the network of genes responsible for the development of the retina.[31] However, despite intense interest in this topic, much of what *PAX6* does during retinal development has not been discovered.

Dr. Kumar's presentation discussed new functions of *PAX6* in eye development that his lab has recently

[30] Camerino M, Chang W, Cvekl A. Analysis of long-range chromatin contacts, compartments and looping between mouse embryonic stem cells, lens epithelium and lens fibers. Epigenetics Chromatin. 2024 Apr 20;17(1):10. doi: 10.1186/s13072-024-00533-x. PMID: 38643244; PMCID: PMC11031936.
[31] Kumar JP. Retinal determination the beginning of eye development. Curr Top Dev Biol. 2010;93:1-28. doi: 10.1016/B978-0-12-385044-7.00001-1. PMID: 20959161; PMCID: PMC5830122.

discovered, using Drosophila (fruit fly) as a research model.

Key Takeaway
PAX6 acts in an "officiating" role during eye development. Think of *PAX6* as a referee who makes sure that everybody playing the game is following the rules. When mutated, *PAX6* cannot officiate as effectively, which is why problems develop.

Additional Discussion Points

- Because *PAX6* is similar across species, when taken from one species it can functionally substitute for its loss in another. For instance, you can take the mouse version of *Pax6*, put it in a fruit fly, and get an ectopic eye (an eye that develops out of place or in an unusual position).
- One new function discovered for *PAX6* is its role in maintaining symmetry of eye size across the body axis.
- A new gene has potentially been discovered that *PAX6* officiates in the patterning of the eye during development.

Robert Grainger *(University of Virginia, USA)*

How cell determination occurs during development of an embryo is not well understood. However, the eye of the frog, *Xenopus Tropicalis*, is useful for studying this process because of its accessibility on the surface of the embryo.

Dr. Grainger's presentation shared how his lab has investigated this lens and retina formation using new, highly sensitive technology. This new technology, called single cell RNA-Sequencing (or scRNA-seq), has been used to look at specific gene activities at the level of single cells.

Rob Grainger (front middle), pictured here with the ANA Board of Directors, researches how the eye is formed during embryonic development. Dr. Grainger was a co-organizer of this Symposium.

Aniridic frogs are available with a phenotype very similar to humans with aniridia. Using this new technology (scRNA-seq) in these frogs, the Grainger Lab is studying the changes associated with aniridia in all eye tissues (lens, retina, cornea, and iris) with unprecedented resolving power, allowing them to recognize previously unidentifiable genetic changes associated with aniridia.

Key Takeaway

Recent studies revealed approximately 30-40 early response genes that are missing in the aniridic frog. These genes are essential to lens development and regulated by *PAX6*. Similar research into early response genes in the retina and brain is ongoing, with important findings emerging.

Additional Discussion Points

As discussed in other presentations, *PAX6* is an essential regulatory gene, and is stably transcribed throughout development to preserve its important functions throughout life. This is accomplished through a stable "feedback loop" to maintain its expression.

New evidence from the single-cell data indicates that the lens feedback loop is a "positive" one, where *PAX6* stimulates its own production to maintain the correct levels. In the lens, therefore, if *PAX6* is removed due to a mutation, the level of the gene gradually decreases.

However, early results in the retina and brain seem to indicate a "negative" feedback loop, where *PAX6* prevents its own overproduction. In the retina and brain, therefore, if *PAX6* is removed due to a mutation, the level of the gene gradually increases to levels higher than normal.

This data suggests that there is a fundamental difference in how *PAX6* works in different tissues of the embryo. These differences must be sorted out if we are to understand how this amazing gene operates.

Zbynek Kozmik is a molecular biologist who researches the genes required for development, such as PAX6. He has published more than 175 articles and uses a whole zoo of animals for research, including mice, fish, annelid worms, and jellyfish.

Zbynek Kozmik *(Czech Academy of Sciences, Czech Republic)*

It is well established by years of scientific inquiry that *PAX6* represents an essential gene for eye development in mice and humans. Other presentations, such as those by Justin Kumar, Rob Grainger, and Ales Cvekl, discussed some of the ways that *PAX6* officiates and guides other genes to do what they are supposed to do. These genes that *PAX6* regulates are referred to as "downstream" of *PAX6*.

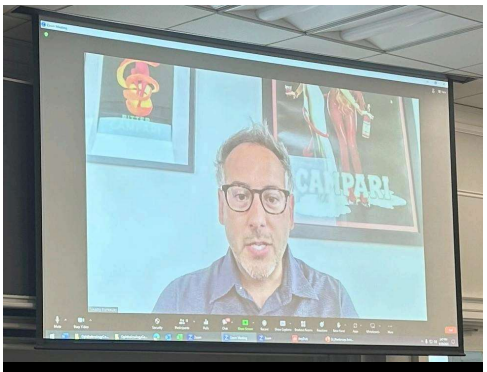
In his presentation, Zybnek Kozmik turned the attention to genes that must be regulating *PAX6*. These genes are referred to as "upstream" of *PAX6*. Historically, it was clear that there must be genes upstream of *PAX6* regulating developmental processes, but precisely how has been poorly understood.

Dr. Kozmik's lab identified two redundant genes, *Meis1* and *Meis 2*, that are critical regulators of *PAX6* and provided evidence of their role in development of lens, retina, and retina pigment epithelium. They also performed genetic tests of selected elements of the Pax6 gene regulatory network to see how important they are for *PAX6* gene function.

Key Takeaway
Just like *PAX6* is a referee officiating development, there are also genes that regulate *PAX6*. Dr. Kozmik's lab recently identified two such genes, *Meis1* and *Meis2*.[32] This information, along with work presented by Ales Cvekl and Rob Grainger, provided significant new insights into how *PAX6* works.

Timothy Blenkinsop *(Icahn School of Medicine at Mt. Sinai, USA)*

In most cases of aniridia, the mutation in the *PAX6* gene is a loss-of-function mutation. This means that the PAX6 protein is either not produced or is not functional. As a result, the eye does not develop properly, and aniridia is the result.



Tim Blenkinsop, shown here presenting virtually, studies the adult human eye in both normal and abnormal states. His lab's approach is to create models of eye diseases using cells isolated from human donor tissues, in the effort to more closely mimic human eye disorders and learn more about them.

Stem cells are cells that can develop into all the cell types of the body, enabling scientists to study how the human body develops. Scientists can now create stem cells with the same mutations that humans have, which allows them to study the underlying mechanisms of disease. These studies provide clues into how to improve disease outcomes.

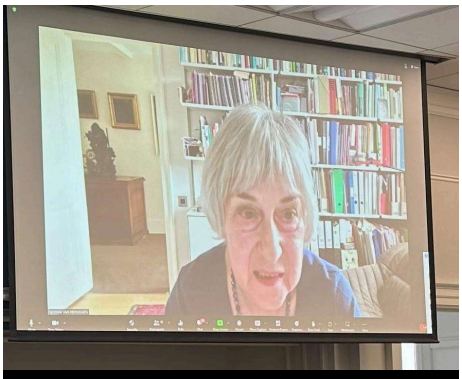
Dr. Blenkinsop described his work using stem cells to develop eye organoids, which are cultures of cells that possess characteristics of the human eye. These organoids were developed to study mutations that lead to aniridia. Using this approach, iris muscle cell specification was altered which may provide insight into the altered developmental trajectory of aniridia patients..

Key Takeaway
We still have gaps in understanding about aniridia, because symptom severity is difficult to predict based on genetics alone. Clinical expression varies widely within individual patients and families, and missense phenotypes are difficult to predict. To investigate this further, Dr. Blenkinsop proposes creating a stem cell bank, where the stem cells are provided by aniridia patients. This approach would allow creation of stem cells that match the genetics of a specific patient, to determine if they can reproduce the patient's symptom severity.

[32] Dupacova, N., Antosova, B., Paces, J., & Kozmik, Z. (2021). Meis homeobox genes control progenitor competence in the retina. *Proceedings of the National Academy of Sciences of the United States of America*, *118*(12), e2013136118. https://doi.org/10.1073/pnas.2013136118

Eye Development

Although *PAX6* is absolutely essential to the development of the eye, there are many other important factors at play during eye development. Understanding the full scope of eye development is vital in order to create new clinical treatments. The presentations in this section are all in the Basic Science Research section of the science iceberg, and are therefore highly technical.



Veronica van Heyningen, shown here presenting virtually, is widely credited with the discovery of the *PAX6* gene. A legend in the genetics world, she dedicated her life's work to the understanding of *PAX6* and aniridia, and contributed immeasurably to our understanding of *PAX6* function.

Veronica van Heyningen[33] *(University College London, UK)*

The fact that the detailed way people develop and function from conception to death is influenced by the intertwined actions of genetics and environment is frequently discussed. Less often mentioned is that random (stochastic) or chance occurrences also influence many biological events.

Random fluctuations are a part of most biological systems and are necessary for some key events to be triggered. For example, in early development we start from a single fertilized cell that then divides to produce many copies of itself. However, to make a complex being requires different cell types to develop in an orderly manner. Perhaps surprisingly, this process is initiated by random fluctuations of cell components. In this way cells able to fulfill different functions can emerge.

The way grandparental genes are passed to the next generation is also a partially random event, so siblings inherit different combinations of genetic features. Further along the developmental pathway other differences also arise through random events. Dr. van Heyningen discussed several differences that arise through random events, including some affecting the development of the eye.[34]

> **"*It's amazing that development ever works–not that it sometimes goes wrong.*"**
>
> - Veronica van Heyningen

[33] van Heyningen V. (2022). A Journey Through Genetics to Biology. *Annual review of genomics and human genetics*, *23*, 1–27. https://doi.org/10.1146/annurev-genom-010622-095109

[34] van Heyningen V. (2024). Stochasticity in genetics and gene regulation. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, *379*(1900), 20230476. https://doi.org/10.1098/rstb.2023.0476

For example: the red, green and blue light-sensing function of the cone-cells in the retina is assigned randomly, so there is a random mosaic carpet of these receptors. The way nerve connections are made from the retina to the brain in early infancy is also through random neural firing followed by selection of strong connections.

Key Takeaway
Random events have a significant effect on biological processes and development.

Seth Blackshaw (center) with Ruth Ashery-Padan (left) and Justin Kumar (right) is a leading expert in the development of the retina and the hypothalamus. He has been studying the development of the retina for more than 25 years, has contributed to more than 190 publications, and holds several patents and copyrights.

Seth Blackshaw *(Johns Hopkins University, USA)*

The central nervous system is incredibly complex. The retina is ultimately a stripped down component of the central nervous system. Therefore, studying the retina is a great way to figure out how neural development happens.

Dr. Blackshaw presented his work related to how retinal cells develop, how they are kept alive, and how they may be repaired or replaced.

Key Takeaway
Using the new technology single cell RNA-sequencing (scRNA-seq) in mice, Dr. Blackshaw's lab has been able to determine which genes are expressed, where they are expressed, and when they are expressed in the development of the retina. They continue to learn more about the specific function of each of these genes.[35]

Additional Discussion Points

- Zebrafish have an amazing ability to regenerate retinal cells after an injury.[36] Birds and mammals do not have this ability. Dr. Blackshaw's lab is studying this remarkable ability to learn how to make regeneration of the retina work. This

> ***"Single cell RNA Sequencing has had a similar effect to what the Hubble Telescope has had on astronomy. Instead of looking at blobs, we're looking at individual stars."***
>
> - Seth Blackshaw

[35] Zhang, X., Leavey, P., Appel, H., Makrides, N., & Blackshaw, S. (2023). Molecular mechanisms controlling vertebrate retinal patterning, neurogenesis, and cell fate specification. *Trends in genetics : TIG*, *39*(10), 736–757. https://doi.org/10.1016/j.tig.2023.06.002

[36] Lyu, P., Iribarne, M., Serjanov, D., Zhai, Y., Hoang, T., Campbell, L. J., Boyd, P., Palazzo, I., Nagashima, M., Silva, N. J., Hitchcock, P. F., Qian, J., Hyde, D. R., & Blackshaw, S. (2023). Common and divergent gene regulatory networks control injury-induced and developmental neurogenesis in zebrafish retina. *bioRxiv : the preprint server for biology*, 2023.08.08.552451. https://doi.org/10.1101/2023.08.08.552451

information is important in thinking about how to repair a damaged retina.

- Mice do not see in color very well due to having a high number of rods vs cones in their retina and are therefore a poor system for studying the retina. However, the 13-lined ground squirrel has a high number of cones versus rods, and is therefore a better animal model for studying the retina.

Nadean Brown *(University of California Davis, USA)*

As discussed in other presentations, understanding is incomplete regarding what initiates, maintains, and shuts off biological processes involved in eye development. Although transcription factors like PAX6 are required for this process, cell signaling pathways are also critical for early eye formation.

Nadean Brown (right), pictured with Tom Glaser, investigates the genetic pathways underlying mammalian eye formation. This research will contribute to a better understanding of congenital eye diseases and ultimately inform stem cell therapies to correct vision loss.

Cell signaling pathways are networks that enable cells to sense and respond to their environmental cues. Dr. Brown presented new data from a study regarding the important signaling pathway known as Sonic Hedgehog.

Key Takeaway

The data presented provided an “ah ha!” moment for other scientists regarding how cells communicate during the earliest stages of eye development. Extending previous work done by Seth Blackshaw and others, Dr. Brown’s work provides important new insights into how to regenerate or repair the retina in the future.

Ruth Ashery-Padan *(Tel-Aviv University, Israel)*

When a baby is growing inside its mother, certain proteins, such as PAX6, work together to help different parts of its body develop properly. Dr. Ashery-Padan's lab is trying to figure out these special groups of proteins, their roles in causing diseases, and the way these proteins maintain tissue function throughout life. They are focused on the cells in the eye called retinal pigmented epithelium (RPE). These cells are important for good vision and can be affected by many eye problems.

In order to look closely at how genes are turned on and off in the RPE, they created a cellular model to study human RPE development and to model inherited human retinal diseases. In the process, they identified new genes that play a role in human RPE and also linked these newly identified genes to the genetics of age-related macular degeneration.

Ruth Ashery-Padan researches the gene networks required for eye development. She has had about 80 articles published during a research career that has spanned nearly 30 years.

Key Takeaway

This study exemplifies how studying these proteins and their effects on genes can help us understand and treat genetic diseases of the eye.

General Eye Disorders

It is important to understand and research eye disorders in general to help solve the aniridia puzzle. Frequently, information learned researching other eye disorders can be applied to creating new treatments for aniridia. This presentation belongs in the Pre-clinical Research section of the science iceberg.

Brian Brooks is Clinical Director of the National Eye Institute at the National Institutes of Health in Maryland. He oversees clinical research and patient care. His research interests focus on inherited eye diseases that affect children.

Brian Brooks *(National Eye Institute, National Institutes of Health, USA)*

Coloboma is a congenital ocular malformation syndrome that, like aniridia, can cause severe vision loss in children. Unlike aniridia, it has many genetic causes, many of which have not yet been discovered. Even when we understand the genetics, the mechanisms of disease (and therefore the means of preventing them) are poorly understood.

A rare human form of syndromic coloboma is RERE syndrome, also known as NEDBEH syndrome (Neurodevelopmental disorder with or without anomalies of the brain, eye, or heart). Dr. Brooks described his work that involved a detailed analysis of a NEDBEH syndrome fish model, showing that it has coloboma accompanied by a curious overgrowth of the early optic nerve.

He went on to show that these abnormalities are mediated by a well-known developmental signaling pathway, Sonic Hedgehog (SHH), and that they can partially rescue these abnormalities using a drug that inhibits SHH. We think these findings shed light on the pathogenesis of disease in people with NEDBEH syndrome.[37]

Key Takeaway

A partial cure for a rare type of syndromic coloboma was tested in zebrafish, with some success. This may help us to understand how this rare disease develops and how it may someday be treated.

[37] George, A., Lee, J., Liu, J., Kim, S., & Brooks, B. P. (2023). Zebrafish model of RERE syndrome recapitulates key ophthalmic defects that are rescued by small molecule inhibitor of shh signaling. *Developmental dynamics : an official publication of the American Association of Anatomists*, *252*(4), 495–509. https://doi.org/10.1002/dvdy.561

Research Models

Research models are essential tools for scientists to investigate how living things work, understand diseases, and develop new treatments. These can include animal models and models created in a petri dish, known as "in vitro" models. Common animal models in aniridia include mice, frogs, and zebrafish, although other models are being explored. Scientists choose the model that works best for their particular area of study.

Elizabeth Simpson (pictured front with Ruth Ashery-Padan) has an overall research goal to develop gene-based therapies for disorders of the brain and the eye. Her short-term goal is to cure the mouse model of aniridia to lay the foundation upon which human gene therapy for aniridia can be designed.

Elizabeth M. Simpson *(University of British Columbia, Canada)*
As discussed in presentations by John Freeman, Ali Djalilian, and Jim Lauderdale, there is no reliable long-term vision-saving therapy for aniridia. One exciting potential approach is to use CRISPR to permanently correct the variants in the *PAX6* gene that cause aniridia.

CRISPR is like a pair of molecular scissors able to cut out and replace the unwanted variant. Elizabeth Simpson presented two studies related to her quest to develop a gene therapy that could cure the mouse model of aniridia.[38] [39]

There are many different kinds of CRISPR "scissors", or enzymes. The first study she presented involved determining the best CRISPR enzyme to use.

In order to test the best CRISPR enzyme, Dr. Simpson's lab first developed a new mouse model and new cell lines. To do this, they introduced human DNA into the mouse model and the stem cell lines. This humanization makes it easier to translate therapies developed back to humans in the future. They then tested five different enzymes in these humanized cell lines and mice. They found that one enzyme would fix the variant chromosome in about 75% of the mouse cell lines without messing up the non-variant chromosome.

---

[38] Mirjalili Mohanna, S. Z., Hickmott, J. W., Lam, S. L., Chiu, N. Y., Lengyell, T. C., Tam, B. M., Moritz, O. L., & Simpson, E. M. (2020). Germline CRISPR/Cas9-Mediated Gene Editing Prevents Vision Loss in a Novel Mouse Model of Aniridia. *Molecular therapy. Methods & clinical development*, *17*, 478–490. https://doi.org/10.1016/j.omtm.2020.03.002

[39] Adair, B. A., Korecki, A. J., Djaksigulova, D., Wagner, P. K., Chiu, N. Y., Lam, S. L., Lengyell, T. C., Leavitt, B. R., & Simpson, E. M. (2023). ABE8e Corrects Pax6-Aniridic Variant in Humanized Mouse ESCs and via LNPs in Ex Vivo Cortical Neurons. *Ophthalmology and therapy*, *12*(4), 2049–2068. https://doi.org/10.1007/s40123-023-00729-6

Now that they had identified a good enzyme to use, they needed to determine the best delivery method. They tested five injection methods and two delivery materials. They discovered that one particular injection method (called "intrastromal injection") paired with a specific delivery material (called "recombinant adeno-associated viruses" or rAAVs) provided wide-spread delivery to all three layers of the cornea. They were also able to demonstrate that this delivery method worked in the adult aniridic mouse cornea.[40]

Key Takeaway

New mouse models and stem cell lines have been created, which helped determine the best enzyme, injection method, and delivery material to use for a CRISPR-based strategy for curing aniridia-related keratopathy.

Additional Discussion Points

- In addition to humanizing the mouse DNA, they have created a mouse model where the keratopathy develops more slowly like it does in humans.
- There is still a lot of biology to be done to determine how effective the results will be and how long the results might last.

Andrew Wegerski is a pre-doctoral fellow working in Brian Brooks' Lab at the National Eye Institute. As an experienced aquarist and herpetologist, he is an expert in rearing and maintaining diverse fish, invertebrates,and reptiles for the biotechnology and aquarium industries. Working with genetics and vision science researchers at the NEI allows him to use his knowledge of reptiles and fish to study vision.

Andrew Wegerski *(National Eye Institute, National Institutes of Health, USA)*

Macular diseases are the leading cause of blindness in Americans over the age of 60 and represent significant healthcare burdens worldwide. While age-related macular degeneration is the most common example of macular disease, there are numerous genetic disorders, notably albinism and aniridia, that similarly affect vision from early developmental stages. Mutations affecting specific genes (*TYR* and *PAX6*) associated with these disorders often produce foveal hypoplasia, an irreversible loss of central vision in which a specialized area of the retina, the fovea, fails to fully develop. This region appears as a retinal pit and is responsible for high acuity, color vision.

A major challenge to studying macular and foveal disorders is that commonly available animal models, such as zebrafish, chicks, and mice, do not have foveae. The few animal models that do have fovea, such as monkeys or birds, require a lot of space and resources and are difficult to reproduce in high volumes in a laboratory setting. However,

[40] Mirjalili Mohanna, S. Z., Korecki, A. J., & Simpson, E. M. (2023). rAAV-PHP.B escapes the mouse eye and causes lethality whereas rAAV9 can transduce aniridic corneal limbal stem cells without lethality. *Gene therapy*, *30*(9), 670–684. https://doi.org/10.1038/s41434-023-00400-6

certain reptiles like day geckos have human-like, foveated retinas and many traits desirable for modeling retinal disorders.

Mr. Wegerski presented his work using these geckos as a model system for understanding the genetic roots of foveal development. He is studying how the gecko's fovea grows, comparing it to how the human fovea develops, and finding out if they use the same genes and pathways. If they do, then he will use CRISPR to attempt to change those genes in geckos to see if it is possible to stop their foveae from forming.

Key Takeaway

Since geckos have a similar fovea to humans, using geckos as new animal models will provide a basis during embryonic development in which to study the development of the fovea. It will also provide new clinical insights into rare genetic eye disorders like aniridia, which are difficult to study in traditional animal models.

Additional Discussion Points

- This work builds upon Ashley Rasys' work with the brown anole lizard and the successful surgical techniques using CRISPR that were used to create the first genetically-modified lizard.
- Multiple researchers offered to share information and techniques with Andrew, illustrating how collaboration can help drive research forward.

Ashley Rasys *(National Eye Institute, National Institutes of Health, USA)*

As Andrew Wegerski discussed, a current area lacking in the field of ophthalmology and vision research is a robust preclinical animal model to assist in the development of novel treatments for individuals with foveal and macula disorders of the eye, such as aniridia.

Ashley Rasys is a post-doctoral fellow working at the National Eye Institute. She previously worked in Jim Lauderdale's Lab at the University of Georgia, where she was involved in creating the first genetically engineered lizard.

An attractive vertebrate to develop as a foveated animal model is the *Anolis sagrei* lizard, which actually has two foveae. This lizard has both a central and a temporal pit in its retina. This species also has a short development time, reproduces in high numbers, its genome has been sequenced, and it is amenable to gene-editing technologies.[41] Together these attributes make them an exciting model to rapidly test potential pathways involved in fovea formation and implement novel therapeutic strategies in engineered lizards.

[41] Geneva, A. J., Park, S., Bock, D. G., de Mello, P. L. H., Sarigol, F., Tollis, M., Donihue, C. M., Reynolds, R. G., Feiner, N., Rasys, A. M., Lauderdale, J. D., Minchey, S. G., Alcala, A. J., Infante, C. R., Kolbe, J. J., Schluter, D., Menke, D. B., & Losos, J. B. (2022). Chromosome-scale genome assembly of the brown anole (Anolis sagrei), an emerging model species. *Communications biology*, *5*(1), 1126. https://doi.org/10.1038/s42003-022-04074-5

Dr. Rasys presented additional ways clinical researchers can use lizards. In particular, she highlighted how the anole's permeable eggshell allows the passive transfer of drugs to developing embryos. Their preliminary work testing the drug, propylthiouracil (PTU), demonstrates the usefulness of this approach to identify critical periods in fovea development, pinpoints ideal times to implement treatments to rescue mutant phenotypes, and illustrates the potential impact lizards can provide as a preclinical animal model to advance research.

Key Takeaway

The brown anole is becoming an excellent preclinical animal model for studying the development of the fovea. Dr. Rasys' work highlights the ability to genetically modify these lizards, as well as to identify the ideal treatment times to prevent foveal hypoplasia.

Marta Grannonico is a post-doctoral fellow in Xiaorong Liu's Lab. She was recently awarded a grant to investigate retinal developmental issues in aniridia, with the goal of discovering new markers of retinal disease. This work will have a profound impact on the treatment of aniridia and pediatric eye diseases in general.

Marta Grannonico *(University of Virginia, USA)*

Optical coherence tomography (OCT) is a type of medical imaging commonly used for diagnosing retinal damage in a variety of eye diseases. Dr. Grannonico discussed a new imaging method, the visible light optical coherence tomography fibergraphy (called "vis-OCTF"), which provides clearer and more detailed retinal images compared to the standard near-infrared (NIR) OCT systems that are commercially available.

The vis-OCTF system was first tested in mice. However, mouse models have limitations because mouse eyes are different from human eyes. To overcome these limitations, tree shrews were explored as a new animal model. Tree shrews are small mammals with eyes that closely resemble human eyes in many ways. Dr. Liu's Lab then compared the retinal structures in mice and tree shrews using both imaging of live animals and preserved samples. This comparison provided essential insights and laid the groundwork for using tree shrews as a model to study retinal diseases.

Finally, they directly compared the performance of the new vis-OCTF with the standard NIR-OCT by taking images of the same patients using both techniques. The side-by-side comparison revealed that vis-OCTF can generate high-resolution images, allowing for accurate characterization of the retina's structures. This advancement holds great promise for improving the diagnosis of retinal damage in different eye diseases.

Key Takeaway

A new imaging tool, called vis-OCTF, provides higher resolution images of the retina than ever before. This technology is being developed for clinical use in a multicenter trial to enable improved imaging of patient eyes.

Acknowledgements

As a result of this symposium, researchers collectively gained a new view of how *PAX6* and related eye genes work. This new knowledge puts research on a better path forward towards the development of new clinical approaches that will work for aniridia.

ANA is grateful for the professionals who attended the Symposium and presented their findings, as well as the patient representatives who provided valuable insight during the meeting.

Seated: left to right: Susan Wolfe, Shari Krantz, Suzanne Chinn, Ruth Ashery-Padan, Nadean Brown, David Price, Grayson Chinn

Middle row, left to right: Robert Grainger, Marta Grannonico, Seth Blackshaw, Janey Wiggs, Justin Kumar, Melinda Duncan, Bobbi Schain, Michael Schain, Kelly Trout

Standing rear, left to right: Arjun Dirghangi, James Lauderdale, John Morris, Janelle Collins, Ales Cvekl, Elena Semina, Elizabeth Simpson, Dominique Bremond-Gignac, Cheryl Gregory-Evans, Kevin Gregory-Evans, John Freeman, Peter Netland, Zbynek Kozmik, Matthew Wolfe, Tom Glaser

This meeting was made possible by the generous support of the University of Virginia, the Vision for Tomorrow Foundation, the University of Georgia, Delta Gamma's Service For Sight Grant, Miranda's Mission, the International WAGR Syndrome Association, the William and Anita Newman Foundation, and the Schain family.