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Eye of the beholder: Rare Eye Diseases in Focus

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A rare disease affects less than 1 in 2,000 individuals, with the majority of rare diseases affecting children. Gracing the cover of this issue, Helen Keller (1880 – 1968), blinded as a baby, made it her life-long mission to alleviate all forms of human disabilities and became a luminary figure for her work on blindness. This special issue on *Rare Diseases of the Eye* articulates through reviews and original research articles where we are currently in our understanding of basic research in rare eye disorders. This collection is intended to bring focus to new advances in our understanding of the genetic, molecular and cellular underpinnings of rare eye diseases, by providing us, in some instances, with new insights into novel candidate targets and cellular pathways of ocular pathology. It is also my hope that an appreciation of the understanding of a cellular/molecular mechanism(s) for a rare disease not be viewed as being limited solely to that condition, as there are numerous instances where a mechanism is shared with a major eye disease. And hence, studies focusing on rare eye disorders are not just restricted silos of research endeavors, as rare disease research is making it possible to envision opportunities that could translate to other common eye conditions as well. This is important to point out, because developing a treatment for a rare disease is very expensive. Despite these challenges, the future of research is ripe full of possibilities. And thus, I would like to cite one of Helen Keller's own inspiring quotes, "*Although the world is full of suffering, it is also full of the overcoming of it.*"

Many rare diseases of the eye can be traced to abnormalities in neural crest development. Understanding that proper development of the cranial neural crest, from which, neural crest cells derive and give rise to structures in the eye, in their review *Relationship between neural crest cell specification and rare ocular diseases*, Akula, Park and West-Mays describe the etiology of several rare ocular diseases. Fate mapping studies have become all the more important in understanding eye development, and have afforded insight into the pathways that could hold important clues for these diseases. Notably, multiple transcription factors are found to play a role in the development of the cranial neural crest as highlighted by the number of diseases linked to these regulators. From the anterior eye segment, abnormalities including Axenfeld-Rieger syndrome, Peters anomaly, and aniridia, to posterior eye abnormalities such as BOFS and CHARGE syndrome, mouse models for many of these conditions have been developed. These important tools are invaluable in the molecular dissection of cellular and molecular mechanisms.

Taking a different turn in emphasis, the research article in *Molecular dissection of cone photoreceptor-enriched genes encoding transmembrane and secretory proteins* by Papal, Monti, Tennison and Swaroop focuses on understanding how cones degenerate in retinitis pigmentosa (RP). RP is caused by mutations in rod specific genes that lead to rod cell

degeneration, but interestingly, this disease progressively also causes cone degeneration, expanding the severity of blindness. The neural retina luciferase zipper gene (Nrl) that governs rod fate determination has been in the focus recently, as it is believed that Nrl inactivation induces cone cell genes in rods. In fact, Nrl inactivation in mouse models of RP was found to rescue vision loss, but a clear understanding of how this happens remains elusive. Hypothesizing that the genes expressed in ‘cone-like’ cells of Nrl-deficient mice might help explain events involved in the rescue phenotype, a comparative analysis of transcriptome from developing S-cone-like photoreceptors in the Nrl-deficient mouse was compared with transcriptome of rod cells. From a short list of plasma membrane and secreted proteins, the Ephrin type receptor 10 (Eph10) was selected for further study. Knockdown of Eph10 gene in the Nrl-deficient retina caused photoreceptor cells to become mislocalized in the retina. These findings suggest that Eph10 might be involved in cell positioning and mediates a mechanism of intercellular communication between cones and other cells of the disease-affected retina. This opens up a new line of enquiry in this enigmatic rod-driven disease.

The retina is also host to tumors that can cause blindness. A common retinal tumor is retinoblastoma, which occurs early in childhood. This type of cancer has been widely studied as a model system for CNS tumors because the retinal tumors can be detected readily by imaging through the transparent cornea and lens at the anterior pole. Dimaras and Corson, in *Retinoblastoma, the visible CNS tumor: A review*, describe several clinical vignettes of retinoblastomas, detailing their genetic origins, clinical presentation and pathology. Retinoblastoma is caused by mutations of the retinoblastoma (Rb1) gene with presentation as unilateral as well as bilateral tumors. Patients with inherited germline mutations can succumb to a ‘second hit’ that causes the bilateral tumors, whereas unilateral tumors are often the result of genetic inactivation of both alleles. Although most of what we know about retinoblastomas come from knowledge of lesions in the Rb1 gene, mutations in the transcriptional co-repressor BCOR also contribute to this benign tumor. For these tumors to become malignant, genetic changes in other targets are necessary, as found in the involvement of MDM4 and KLF14 for metastasis to occur. Gene amplification, gene loss and methylation are several of the known causative mechanisms of retinoblastoma. Finally, the inherited trilateral retinoblastoma also features an intracranial tumor, which is often found to grow in the pineal gland. These tumors are more challenging to treat than their retinal counterparts.

Moving to another childhood cancer, one that is relatively common for a rare disease, neurofibromatosis 1 (NF1) occurs at the rate of 1 in 3,000 individuals. Freret and Gutmann review the molecular pathobiology and genetics of this disease in *Insights into optic pathway glioma vision loss from mouse models of neurofibromatosis type 1*. NF1 occurs in people who have inherited mutations in the gene for neurofibromin-1, which is a suppressor of the RAS oncogene. This autosomal dominant cancerous condition is caused by the loss-of-function of NF1, causing tumors of the optic nerve, optic tracts and optic radiations in the brain leading to gliomas termed optic pathway gliomas (OPGs). NF1 patients are born with a loss-of-function NF1 allele and later develop a somatic inactivation of the remaining NF1 allele following the ‘two-hit’ model, akin to that we encountered in retinoblastoma. The second hit can be either a genetic mutation or epigenetic modification of the NF1 locus. Transgenic mouse models have illuminated the cellular and molecular determinants of these

OPGs. The diagnosis of NF1-related vision loss at a very young age is complicated because 75–85% of OPGs harbor other CNS comorbidities as well, such as attention deficits. Even though these young patients can be treated with chemotherapy, such treatments typically do not reverse their vision loss. A wide range of genetically engineered mice have now been developed to model NF1. Mice with *Nf1* deficiency develop optic gliomas and show retinal ganglion cell death with nerve fiber thinning, similar to that found in humans. However, these mice lack Rosenthal fibers, eosinophilic granular bodies, or optic tract and radiation deficits, which are hallmark facets of the human disease. Despite this, much has emerged regarding the mechanisms that regulate cell proliferation governed by NF1 loss. The RAS-MEK-ERK, PI3-AKT-mTOR and cAMP signaling pathways in NF1-defective cells and transgenic animal models have provided many druggable targets resulting in small molecule inhibitors being developed for NF1 that are being tested in clinical trials.

Thavikulwat, Edward, AiDarrab and Vajarant review the literature on visual dysfunction due to glaucoma in *Pathophysiology and management of glaucoma associated with phakomatoses*. Phakomatoses is a broad term for the neuro-oculo-cutaneous disorders arising in structures from the embryonic ectoderm. Glaucoma is a disease of complex etiology that principally affects the optic nerve, where the initiating pathology as well as the associated clinical syndromes associating with glaucoma are quite diverse. Several rare diseases, including NF1, Von Hippel-Lindau disease and tuberous sclerosis, to mention a few, have ocular manifestations that can cause glaucoma. NF1 patients develop glaucoma in the same eye afflicted by the neurofibroma that is visibly evident as it typically develops in the eye lid. In NF1, the anterior eye chamber is infiltrated by neurofibromas that cause angle closure and fibrovascularization. As increased intraocular pressure (IOP) is a common end point in all these diverse conditions, treatment is initiated essentially with topical IOP-lowering glaucoma drugs, and in more severe cases where anatomical structures of the eye grow abnormal, surgical management to decrease IOP is also employed. Mouse models for NF1 deficiency do not show the anterior eye segment abnormalities, but a recent finding made in a new transgenic line may help explain why some human NF1 patients can also develop neurofibromas of the cornea. In a recent study investigating overexpression of mitogen-activated protein kinase kinase 1 (MEK1) in Schwann cells it was found that these transgenic mice develop corneal neurofibromas due to chronic overactivation of extracellular signal-regulated kinases 1, 2 (ERK1/2), defining this downstream target of the RAS-MEK-ERK pathway as being a critical regulator of corneal neurofibromas (Bargagna-Mohan et al., 2017). Other mechanisms that prevent ERK1/2 activation in the anterior segment in NF1 loss-of-function transgenic mouse models (as well as humans) may be operational that prevent corneal neurofibromas from developing in NF1 deficiency. More needs to be done to delineate what these other modifiers are so that one could develop more selective means to arrest cell growth in NF1.

Could inflammation be at the heart of glaucoma? Wei, Cho, Thee, Jager and Chen argue for a change in our view in *Neuroinflammation and microglia in glaucoma: time for a paradigm shift*. Stepping away from the usual culprits associated with glaucoma, such as elevated IOP, these authors envision a role for inflammation in neuronal loss in glaucoma, citing that this disease can have quite a range of etiologies, including a form of glaucoma where patients have normal IOPs. A common thread among different forms of glaucoma is the activation of

microglia and macroglial cells (astrocytes and Muller glia), which are cells that are broadly responsive to infections, disease and injury. Given retinal glial cells can respond to diverse types of stressors, including changes in IOP, by secreting cytokines these cells help to amplify inflammatory processes that exacerbate neuronal damage in the retina. The correlation between microglial activation and neuronal loss in the DBA2J genetic model of glaucoma has also been shown, which then begs the question what comes first as neural damage can also induce microglial responses. Defining these microglial responses further by their M1-like and M2-like phenotypes (based on the convention for the M1 and M2 phenotypes for macrophages), it appears that in experimental glaucoma the M1-like phenotype has a role in protection and is triggered early. These M1-like cells produce pro-inflammatory cytokines initially but convert to M2-like cells (producing anti-inflammatory factors) before entering a resting state. It is believed that chronic retention of the M1-like state can be neurotoxic. This scenario is amplified by astrocytes also changing their reactive response (reactive gliosis) by exhibiting an A1 -like and A2-like phenotype with similar functions as microglia. Since both of these glial responses occur almost coincidentally, defining their individual roles in neurodegenerative processes has been a challenge. Interestingly, multiple toll-like receptors appear to be involved with the key pro-inflammatory transcription factor, nuclear factor kappa B, being activated in the DBA2J mouse model of glaucoma. Work still remains as to which targets of inflammation will afford tangible outcomes in this condition.

Albinism is a rare condition that afflicts 1 in 20,000 people. Ocular cutaneous albinism (OCA) is attributed to decreased melanin synthesis, which results in lower levels of pigment in the retinal pigment epithelium. A spectrum of genetic mutations, including ones in the tyrosinase gene contribute to OCA. Interestingly, visual disorders are the key pathological outcome of OCA and are attributed to abnormal retinal development just prior to or after birth. These conditions range from foveal hypoplasia, to strabismus and photophobia to name a few, although one cannot certainly pair the severity of tissue/cellular malformation with extent of visual function. Ocular albinism (OA) is another form of pigment-related visual disorder that is caused by mutations in GPR143. Shining light on the melanosome, the organelle that provides pigments to the retinal pigment epithelium, McKay reviews these pigment-related ocular disorders in *Pigmentation and vision: Is GPR143 in control?* In OA1 caused by mutations in *GPR143* the visual disorder is not due to reduced melanin synthesis but absence of GPR143 signaling. Leveraging this observation that pigment reduction is not the primary cause of visual dysfunction in OCA, but the tissue impacted by this disease is the retinal pigment epithelium (RPE), one then recognizes that a paracrine factor produced by the RPE must affect neuronal function as these neural cells do not express the albinism genes. Discovery of L-DOPA as the ligand of GPR143 has helped unravel the relationship between changes in melanin biosynthesis and L-DOPA. L-DOPA results from the conversion of tyrosine by the enzyme tyrosinase. In albinism there is a reduction of L-DOPA, and hence, prospective clinical trials were initiated to supplement L-DOPA in patients with albinism. Although the results showed no therapeutic benefit, it should be kept in mind that albinism is a developmental disorder and patients may benefit from L-DOPA therapy being started very early in childhood. Reduction in melanin also occurs with aging, in particular in the macula, which is the precise region afflicted by age-related macular degeneration

(AMD). Interestingly, as the fovea (located within the macula) is also centric to melanin deficits in albinism, McKay has explored the idea whether L-DOPA supplementation would protect AMD patients. The answer to this inquiry came from a survey of humans being treated with L-DOPA for Parkinson's disorders that showed they were either protected from AMD or showed delayed onset. It is known that people of Caucasian descent are most susceptible to AMD possibly because they have lower melanin levels than those of other races. Further study of this insightful hypothesis remains to be done in double-blind clinical trials.

The pathological similarities between Sorsby Fundus dystrophy (SFD) and AMD is yet another clear example of a rare condition and common disease having similar retinal co-morbidities. SFD is caused by mutations in the gene encoding tissue inhibitor of metalloproteinase-3 (TIMP-3), which like the more common condition AMD, includes the presence of drusen deposits underlying the RPE, disruption of Bruch's membrane and choroidal neovascularization. In *SORSBY FUNDUS DYSTROPHY: Insights from the past and looking to the future*, Anand-Apte, Chao, Singh and Stoehr review the literature on SFD. TIMP-3 is a multifunctional extracellular protein that is synthesized by the RPE. In SFD and AMD patients, TIMP-3 accumulates as subretinal deposits, provoking interest in understanding the molecular mechanisms by which this protein becomes dysregulated in these diseases. This launched investigations into in vitro studies, TIMP-3 knock-in and knock-out mice as well as studies performed using human induced pluripotent stem cells. While a lot has been learned from molecular biology studies on the activity of TIMP-3 mutants, it still remains unclear whether SFD is a gain-of-function or loss-of-function disease as the challenges are complex due to differences where the TIMP-3 gene is mutated. As treatments developed for neovascular AMD have proven successful in blocking choroidal neovascularization, SFD patients have benefitted from these anti-angiogenic drugs. Because SFD can be detected by genetic testing, following patients through fundus imaging is key to catching their retinal disease at an early stage when they also would benefit the most with these treatments.

Biomarkers give retinal diseases their clinical characteristics, and over the past decade, we have witnessed the development of numerous noninvasive technologies to image the retina for disease biomarkers. Paavom Lee, Allikmets, Tsang and Sparrow focus our attention on a class of fluorescent biomarkers derived as byproducts from vitamin A in the article *Photoreceptor cells as a source of fundus autofluorescence in recessive Stargardt disease*. These authors provide very interesting data showing that the autofluorescence from the fundus is not due to melanin loss in RPE cells, but the accumulation of autofluorescent bisretinoid molecules that are produced from photoreceptor cells. Bisretinoids form through non-enzymatic reactions when retinaldehydes chemically react with each other and result in a fluorescent molecule. These are typically shed from photoreceptors and removed by the RPE. In Stargardt's disease that is caused by mutations in the gene for the ATP-binding cassette transporter 4 (ABCA4), a deficiency of this protein results in bisretinoids accumulating to toxic levels in the retina. Blue light at 488 nm helps to visualize these fluorescent deposits called flecks in the retinas. Interestingly, these flecks were associated with regions of RPE cell death using multiple imaging techniques, where the 488 nm short wavelength autofluorescence, infrared and near infrared light imaging showed overlapping

sites of RPE deficits. What is exciting is that each of these flecks could be localized to the RPE layer using another imaging technique known as swept-source optical coherence tomography that provides a histological view of the retinal layers for registration. This type of high-resolution imaging could potentially be exploited to follow the course of disease over time or patient's response to therapy. One foresees that similar applications of multimodal imaging could be applied to AMD in the future as these bisretinoids are also found in this disease.

In summary, human genetics, in vivo biomarker imaging, and development of genetically engineered mouse models are helping unravel insights into molecular mechanisms of rare diseases of the eye. We live in an exciting time when the molecular underpinnings of a rare disorder can help spur drug discovery and quite possibly also benefit more common conditions such as AMD as well. We can anticipate the next decade to be the era of powerful possibilities for treating rare diseases of the eye.

References

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Helen Keller at age 8 with her tutor Anne Sullivan in July 1888 on Cape Cod, Massachusetts.

Photo by Family member of Thaxter P. Spencer, now part of the R. Stanton Avery Special Collections, at the New England Historic Genealogical Society.