In December 2017, the U.S. Food and Drug Administration approved Luxturna (voretigene neparvovec-rzyl; Spark Therapeutics, Philadelphia, PA) as a new gene therapy to treat children and adult patients with RPE65 mutations resulting in vision loss. The availability of this landmark treatment has brought attention to the need for genetic testing by retina specialists.

For this column, Kimberly Drenser, MD, PhD, and Edward H. Wood, MD, from William Beaumont Hospital in Royal Oak, MI, provide us with an overview of this important topic. They will begin with a historical perspective, dating back to 1953 during the days of Watson and Crick. They will then review genetic sequencing, provide an overview of the importance of genetic testing in retina, and conclude with American Academy of Ophthalmology guidelines for genetic testing.

We are grateful to Drs. Drenser and Wood for generously sharing their extensive knowledge on this topic with our community. Given the numerous gene therapies currently in clinical trials, I anticipate that genetic testing will be routinely offered to our patients in our practices. Therefore, this piece will likely be of interest to many of us in the retina community.

**A BRIEF HISTORY OF GENETIC SEQUENCING**

The discovery of DNA\(^1\) in 1953 and the development of the central dogma\(^2\) (DNA makes RNA, RNA makes protein) in 1970 marks a paradigm shift. The discovery of DNA cleaving enzymes, type 2 restriction enzymes,\(^2,3\) in 1970 followed by the ability to clone DNA via molecules called plasmids\(^4\) (1973) allowed for targeted DNA cutting, isolation, and mapping, thus setting the stage for genetic sequencing. Frederic Sanger’s development of “chain termination” method of sequencing\(^5\) at Oxford (1977) and polymerase chain reaction (PCR) by Kary Mullis (1985)\(^6\) revolutionized genetic sequencing. Sanger sequencing was used by the Human Genome Project\(^7\) (1990-2003) and Craig Venter’s group at Celera\(^8\) to map the first human genome and remains the gold-standard methodology (with error rates less than 1%). However, Sanger sequencing is slow and requires large DNA fragments to be cloned, which contributed to the Human Genome Project costing $2.7 billion.\(^9\) These disadvantages inspired scientists to create a second generation of genetic sequencing methods. Next-generation sequencing (NGS) methods allow sequencing of huge numbers of samples at one time, so called “massively parallel sequencing.” This improvement makes it possible to sequence an individual’s entire genome (with the “genome” consisting of 3x10\(^9\) bases) in “whole genome sequencing”
(WGS), or the protein-coding regions (“exome” consisting of $3 \times 10^7$ bases accounting for 1% of the entire genome but encompassing roughly 85% of disease-causing mutations) in “whole exome sequencing,” (WES). Software analytics compare these findings to reference genomes and identify variants.

WHY SHOULD RETINA SPECIALISTS CARE ABOUT GENETIC TESTING?

Patients Want Genetic Testing

An interview of 48 patients with retinitis pigmentosa (RP) showed that 92% of participants desired genetic counseling and 86.5% desired genetic testing. Although patients with multifactorial disorders such as age-related macular degeneration (AMD) may also desire genetic testing, the American Academy of Ophthalmology (AAO) task force currently recommends against routine genetic testing for these conditions: “Currently, clinicians should avoid routine genetic testing for genetically complex/multifactorial diseases, such as AMD; instead, genetic testing should be used in Mendelian (monogenic) disease, such as RP or Stargardt disease.”

Clinical Testing Rarely Predicts Genetic Diagnosis

This is because one retinal phenotype may be caused by mutations in different genes, or “genetic heterogeneity.” For example, more than 100 different genes are associated with RP, and 23 are associated with LCA. Conversely, one gene can be associated with several different phenotypes, or “phenotypic heterogeneity.” For example, CRB1 mutations may cause Leber congenital amaurosis (LCA), RP, and cone-rod dystrophy. Many retinal diseases display “allelic heterogeneity” in which many different phenotypes are a result of various mutations within a single gene. Approximately 250 disease-causing genes have been identified and catalogued in the Retinal Information Network (http://www.sph.uth.tmc.edu/RetNet/). Genetic testing serves as a diagnostic tool for many patients with suspected Mendelian disorders, carrying significant impact for patients and their families. Benefits include prognostication, guidance of further testing, referral for syndromes, and management of pathology.

Genetic Interventions Are Only Available to Patients With a Genetic Diagnosis

In essence, gene therapy replaces defective DNA with a normal (wild-type) DNA sequence of a target gene, often using a recombinant viral vector to infect the host cell, express the normal (wild-type) protein, and restore cellular function. Gene therapy is currently available for those with RPE65-associated LCA (the U.S. Food and Drug Administration approved voretigene neparvovec-rzyl [Luxturna; Spark Therapeutics, Philadelphia, PA] on December 19, 2017). In addition, phase 1/2 gene therapy clinical trials are currently underway (Figure 1; https://clinicaltrials.gov/ct2/home).
and many others are being explored in preclinical animal studies. In addition, gene editing with clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated systems (CAS) holds significant promise for treating genetic diseases by allowing for targeted cleavage and insertion of DNA down to a single base pair. Editas Medicine (Cambridge, MA) plans to have three early stage clinical trials and two late-stage clinical trials using CRISPR/CAS underway by 2022, including targeting CEP290-associated LCA. With one gene therapy treatment currently available and many others in the pipeline, the potential significance of genetic testing for patients with retinal disorders has never been higher.

**Genetic Testing of Unique Patient Populations Can Generate Novel Discoveries and New Therapeutic Approaches**

In our group’s experience, genetic sequencing of a pediatric population with retinal vascular disease has been illuminating and impacted the way we care for patients with Wnt-associated vitreoretinopathies (WAVR), such as familial exudative vitreoretinopathy, Norrie disease, pseudoglioma osteoporosis syndrome, and retinopathy of prematurity. Genetic testing for individuals with WAVR allows us to properly diagnose and treat the patient as well as counsel family members, which is important in these progressive diseases. Our increased understanding of the genetic basis for these diseases (centering around wnt/B-catenin signaling pathways) has inspired the generation of a therapeutic compound to target this pathway. One can imagine a similar thread beginning with genetic diagnosis and ending in targeted therapy for other disorders affecting unique patient populations.

**HOW MAY GENETIC TESTING BE PERFORMED?**

Routine genetic testing in the clinic still remains elusive. It is advisable to work in coordination with a geneticist to guide the appropriate testing. Patients with high suspicion for a specific monogenic disease may begin with targeted Sanger sequencing. Syndromic conditions with possible chromosomal alteration may be approached using chromosomal single nucleotide polymorphism microarrays. When a mitochondrial disorder is suspected, mitochondrial genome sequencing is appropriate. Often querying a panel of genes employing NGS (validated for ophthalmic disease) is chosen. A variety of companies and organizations provide NGS services for ophthalmic patients (Figure 2). These companies will work with you to provide sample collection kits and often bill directly to insurances. NGS panels for inherited retinal diseases have high diagnostic rates ranging from 50% to 57%. When NGS panels do not result in a hit, one may choose to proceed onto WES or WGS. This tiered approach, with increasing levels of sequencing minimizes cost, time, and unnecessary data acquisition.

**CONCLUSION**

Retina specialists have the incredible privilege of being involved in gene therapy and shaping the future to come. The AAO task force on genetic testing defines a genetic test as the sum of five parts: (1) the clinical determination that a genetic eye disease is likely to be present, (2) the molecular investigation of genomic DNA samples from one or more individuals, (3) the analysis of the resulting molecular data in the context of relevant published literature and public databases using appropriate statistical methods, (4) the interpretation of the data in the context...
of the clinical findings, and (5) the counseling of the patient about the interpreted findings and their implications.” When performing genetic testing, the retina specialist should consider all of these components in order to maximize the positive impact of genetic testing.

REFERENCES


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Disclosures: Drs. Wood and Dresner report no relevant financial disclosures. Dr. Haririprasad is a consultant or on the speakers bureau for Alcon, Allergan, Bayer, OD-OS, Clearside Biomedical, Ocular Therapeutix, Alimera Sciences, Leica, Spark, and Regeneron.