Genetic Testing Strategies in Inherited Retinal Diseases: Practical Aspects for Ophthalmologists

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ABSTRACT

Rapidly developing genetic testing methods has transformed the diagnostic process for inherited retinal diseases (IRDs). Besides, management options for IRDs are increasing including gene and stem cell therapies. In contrast to these rapidly evolving options for diagnosis or management, number of ophthalmologists specialized in genetic diseases or number of geneticists specialized in ophthalmological diseases are limited. Therefore, ophthalmologists should have a better understanding of genetic testing algorithms for proper management of patients with IRDs. This review provides the basic aspects of genetic testing strategies for ophthalmologists.

Keywords: Genetic counseling, genetic testing, inherited retinal diseases, next-generation sequencing, Sanger sequencing

INTRODUCTION

Inherited retinal diseases (IRDs) involve retinal degenerations resulting from the disease-causing variations in structure, function, or level of proteins important for retinal integrity or function. IRDs can lead to significant vision loss and are estimated to effect 1 in 1000 individuals[1]. Currently, there are more than 270 genes listed as the cause of IRDs in RetNet (Retinal Information Network, https://sph.uth.edu/retnet/)[2]. The diagnosis and management of IRDs were more challenging in the past. However, current genetic testing technologies provide clinicians better options for diagnosis of IRDs. Additionally, the progress in the diagnosis and management opportunities of IRDs cause higher need for more specialist in ophthalmic genetics field and broader awareness among the ophthalmologists who encounter patients with IRDs in their clinics.

Genetic tests have been recommended for all individuals with suspected or presumed IRDs by several associations worldwide[3,4]. Genetic testing process starts with the suspected diagnosis of IRD as a result of clinical evaluation and then continues with the molecular investigation of DNA samples from the patient with one or more available techniques. Those techniques aimed to find the differences in the DNA sequence of the patient in comparison to the reference sequence. The results are analyzed and interpreted in the context of relevant public databases, published reports in literature and the clinical findings. The process is finalized with the counselling of the patient with the results and their implications[4]. Genetic testing can confirm the clinical diagnosis or define the accurate diagnosis when the clinic is indeterminate. Additionally, genetic testing may improve management by providing information for prognosis, follow-up plan, treatment decision and genetic counselling to estimate recurrence risk, to guide family planning and reproductive decisions. In syndromic patients with associated IRDs, genetic testing may allow life-saving interventions by early detection, monitoring, and preventive measures. The first approved retinal gene therapy for *RPE65* mutation-associated retinal dystrophy Luxturna (voretigene neparvovec-rzyl) has been the most attractive example for emphasizing the importance of genetic testing among clinicians and patients with IRDs. There have been several active gene therapy clinical studies for IRDs[5,6]. The genetic testing can provide access to available treatments and clinical studies.

In this review the genetic testing strategies will be discussed with the aim of the practical aspects of genetic tests for all ophthalmologists who have a role in the management process of IRDs.

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Received: 07.09. 2022
Accepted: 08.09.2022

Ret-Vit 2022; 31: 193-197

DOİ:10.37845/ret.vit.2022.31.34

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Types of genetic tests relevant to the applied technique

There are several genetic tests options: targeted or comprehensive, revealing short sequence variants or large structural variants. The optimal method of testing is not obvious and should be decided according to the clinical findings, inheritance pattern, suspected clinical diagnosis and the cost, coverage, availability of the test.

The types of genetic tests relevant to the applied technique could be summarized in three groups:

1. Targeted genetic tests

Sanger sequencing (dideoxy or chain termination) technique is the mostly used targeted genetic test since early 1990[7]. This technique could determine the sequence of a nearly 1000 base-pair DNA segment with the highest per-base accuracy. As a result of this highest accuracy, Sanger sequencing is also used for confirmation of the presence of disease-causing variants detected by next generation sequencing (NGS) techniques in clinical genetic diagnostic laboratories. The yield of Sanger sequencing is low, and the usage has become restricted since NGS became cheaper and more accessible. However, it is still preferred in a smaller number of indications because it provides more accurate results for the tested genes with no or few secondary findings. Targeted genetic test types were listed below:

Single variant analysis: For some IRDs, there has been a unique variant as the underlying genetic cause of the disease or a hot spot variant with higher frequency among patients. For example, in Doyne honeycomb retinal dystrophy (DHRD) there is a single reported variant (c.1033 C>T, p. Arg345Trp) in *EFEMP1* [8] or in Late-onset retinal degeneration (LORD) there is a founder variant (c. 489 C>A, p. Ser163Arg) in *C1QTNF5* [9]. In those scenarios, single variant analysis with Sanger sequencing or restriction fragment length polymorphism (RFLP) techniques can provide more cost-effective and rapid results.

Single exon analysis: For some IRDs, a region (one or more exons) of a disease-causing gene has been reported to carry all or nearly all disease-causing variants. For example, in Sorsby fundus dystrophy (SFD) most of the reported variants located in the last exon of *TIMP3*[10]. Therefore, sanger sequencing should be the preferred genetic test with patients with a clinical diagnosis of SFD.

Single gene analysis: For some IRDs, a single gene or a few number of genes were reported to be responsible. For example, *CHM* for choroideremia[11]; *RS1* for X-linked retinoschisis[12], *KCNV2* for KCNV2 retinopathy which has a pathognomonic electroretinogram (ERG) finding[13]. In scenarios like those single gene analysis with Sanger sequencing should be the preferred genetic testing method.

2. Broader (Parallel or Bundled) genetic tests

Many IRDs are genetically heterogenous which means there are several causative genes defined for the same disease. Retinitis pigmentosa (RP) is one of the striking examples, more than 100 genes defined as causative for RP[14,15]. Testing multiple genes in a single assay has become possible at first by arrayed primer extension microarray technology (APEX)[16] then by massively parallel sequencing (next-generation sequencing-NGS) approaches[17]. NGS technology sequences millions of DNA fragments in parallel, then those sequence reads are mapped and compared to the reference sequences by alignment and variant calling software tools respectively[18].

Panel-based genetic tests: These tests include targeted analysis of a set of genes. Panel-based genetic testing could be possible by hybridization as in APEX or by massively parallel sequencing as in NGS. In APEX microarray technology, there are chips carrying immobilized DNA segments associated with one of the known genetic variants to be tested[7]. This technology allows detection of previously known variants in patients but is not suitable for diseases with a high novel mutation rate such as Familial exudative retinopathy (FEVR)[19]. Gene-panel tests using NGS technology allow detection of both known and novel variants and there have been comprehensive panels for several conditions such as vitreoretinopathy panel, retinal dystrophy panel, cataract panel used for genetic diagnosis in clinics. The variant detection rate in those panels could be variable depending on the disease, content of the panel and the study population[7]. The diagnostic yield of panel testing could be as high as %82[14].

Whole exome sequencing (WES): This testing technique uses NGS technology as panel-based genetic tests but captures all protein-coding genes of the human genome (1% of the genome). WES has higher sensitivity and specificity for IRDs; therefore, clinicians may prefer WES as a primary diagnostic tool for retinal dystrophies instead of panel-based tests[7]. One important drawback of WES is the huge amount of data produced which need more time and effort to catch and interpret the disease-causing variants. This step is the most challenging part and generally needs a multidisciplinary approach for accurate evaluation. The role of ophthalmologist in this team is to provide detailed clinical findings and actively participating this evaluation process when needed. Another drawback of WES is the higher rate of secondary findings. Secondary findings are the test results that are unrelated to the primary purpose of testing[20]. American College of Medical Genetics (ACMG)

recommended to report secondary findings including pathogenic or likely pathogenic variants of 73 genes that are associated with several diseases such as cancer, heart disease, etc[21]. Those genes were selected because they could provide the possibility of early diagnosis, prevention, or treatment. However, approach to secondary findings differs in different countries because news of an unexpected disease condition may lead to discrimination risk and additional stress for individuals, family members. To minimize the detection of secondary findings and decrease the amount of data analyzed for reaching the primary finding, a recent approach is to mask non-IRD causing genes in WES data and create virtual panels (also named as exome slices or exome-based panels.) This approach enables update of gene content without additional sequencing[22].

Whole genome sequencing (WGS): This technique involves the sequencing of all bases in a genome (Three billion base-pairs per human genome). WGS can provide data for structural variants (copy number variants including larger deletions or insertions in the genome) or can detect variants in non-coding regions which could affect splicing or gene regulatory regions and may cause disease. Currently, WGS is the last choice in clinical settings and used when other tests failed to define the disease-causing variants. WGS is generally used by research laboratories because interpretation of WGS data is much more challenging and requires high-throughput evaluation tools and bioinformatic experts[7].

3. Structural variant analysis

Structural variants involve larger DNA segments (>1000 bp) and can include insertions, deletions, inversions, and balanced translocations. Structural variants resulting in genomic imbalances are called as copy number variants (CNV). Those variants are mostly missed with gene panel tests or WES. For detection of structural variants multiplex-ligation dependent probe amplification (MLPA), array comparative genomic hybridization (aCGH) and SNP array techniques could be used. Also there exist computational tools for analyzing NGS data to detect structural variants. For this, WGS has greater power than gene panels or WES[23]. Structural variant analysis is used as a part of routine genetic testing of IRDs nowadays. However, it can be preferred only in case of unknown diagnosis, diseases affecting more than one organ system or when other genetic tests failed to define the causative variant.

Types of genetic tests relevant to the aim

Aim of genetic tests differs and should be important to consider especially for the context of genetic counselling[24].

Diagnostic testing aims to detect the underlying genetic cause of the disease of concern, and it includes a wide range of test types from single gene test to WGS. **Segregation** testing involves the testing of family members after the proband to confirm a diagnostic result. **Carrier** testing aims to define if an identified pathogenic variant in a family member with an autosomal recessive or X-linked recessive disease is absent or present in the other family members. This test should be used by medical genetics specialists and not by ophthalmologists alone. **Predictive** testing aims to identify genetic variants that cause an inherited disease before the signs and symptoms appear in an individual. This test could be used for adult-onset conditions. Predictive tests in childhood have ethical implications and are controversial. **Reproductive** testing aims to identify people who have an increased risk for having a child affected with a genetic disorder or identify an affected embryo or fetus. Reproductive testing includes carrier, invasive or noninvasive prenatal and preimplantation testing. Legal frameworks and available options differ between countries. **Research** testing could be offered to patients when diagnostic tests fail to give a result. The context of research testing should be explained to the patient clearly.

Interpretation of the genetic testing results

Genetic testing process includes the comparison of the tested DNA sequence with the reference sequence and listing the differences as variants. Those identified variants are analyzed to determine if they are associated with the disease of concern or not. A variant classification system recommended by ACMG has been used to report detected variants in genetic tests[25]. This classification system takes into consideration the variant frequency in general population and in disease population, type of the variant (protein truncating or protein altering), functional data, segregation data in family members, location of the variant, previous reports about the variant in databases and literature, computational evidence from multiple in silico tools. According to the ACMG classification system variants are classified as benign, likely benign, variant of uncertain significance (VUS), likely pathogenic or pathogenic. When a variant has contradictory evidence for both benign and pathogenic criteria or cannot fulfill the criteria of a benign or pathogenic variant, it is classified as VUS. When the genetic test result included VUS, it will be told to the patient with caution because additional testing or future re-evaluation could change the class of this variant. The diagnostic reports generally include pathogenic, likely pathogenic variants or VUS related with the disease genes. Additionally, likely pathogenic or pathogenic variants in mandatory disclosure genes may be included in the reports.

Important notes for genetic testing

- Detailed ocular and systemic phenotyping plus detailed history are very important before choosing the diagnostic genetic test. This pretest evaluation could help the clinician to define the more cost-effective and highly accurate option for the patient. Besides, detailed phenotyping supports the variant interpretation step of testing process.
- Pretest and posttest genetic counseling are necessary steps in the management process of patients with IRDs. The aim of the test, the test options, the possibility of inconclusive or negative test result, the possibility of secondary findings, the timing and insurance coverage of the test, the impacts of the test results for the patient and for the other family members should be discussed and written consent should be taken from the patient.
- There is not a miracle genetic test for the diagnosis of IRDs, each patient should be evaluated individually before test decision. The best genetic test is not the WES or WGS for every patient. Do not forget that targeted tests may be the best option for some patients.
- VUS in the genetic test report means that the test result should be re-evaluated in future as the increasing genetic information may change the class of the variant or further genetic testing such as segregation analysis could help to change the class of the variant and reach a conclusive test result.
- A negative test result does not mean that the patient does not have an underlying genetic cause. Further or future genetic testing may find the underlying cause.
- Especially in undiagnosed cases, clinician should be aware if CNVs are evaluated during testing process, if mitochondrial genome is covered to decide for further testing.
- Epigenetics and environmental factors could be the reason of normal WES or WGS.
- It is recommended to avoid genetic testing for genetically complex disorders like age-related macular degeneration as a routine clinical work-up[4].
- Continuous communication and multidisciplinary approach are the best options for the management of IRDs.

CONCLUSION

During the management of patients with IRDs, genetic testing has become a routine part of the clinical evaluation. Since all the patients do not have a chance to be consulted with an ophthalmic genetics' specialist or medical genetics specialist, ophthalmologists encounter the questions of the patients about the testing process and the test results. Additionally, when ophthalmologists and genetics specialist have an opportunity to collaborate for IRDs, it is better if they can use a common language about the genetic tests. As a result, today's ophthalmologists should have a basic understanding of the genetic testing process for proper management of patients with IRDs.

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