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REVIEW
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Genetic testing in translational ophthalmology

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Abstract

Inherited eye diseases are a group of conditions with genetic and phenotypic heterogeneity. Advances in ocular genetic research have provided insights into the genetic basis of many eye diseases. Genetic and technological progress is improving the management and care of patients with inherited eye diseases. Diagnostic laboratories continue to develop strategies with high specificity and sensitivity that reduce the costs and time required for genetic testing. The introduction of *next generation sequencing* technologies has significantly advanced the identification of new gene candidates and has expanded the scope of genetic testing. Gene therapy offers an important opportunity to target causative genetic mutations. There are clinical trials of treatments involving vector-based eye gene therapies, and a significant number of loci and genes now have a role in the diagnosis and treatment of human eye diseases. Applied genetic technology heralds the development of individualized treatments, ushering ophthalmology into the field of personalized medicine. Many therapeutic strategies have demonstrated efficacy in preclinical studies and have entered the clinical trial phase.

In this paper we review the topic of genetic testing in inherited eye diseases. We provide some background information about genetic counseling and genetic testing in ophthalmology and discuss how genetic testing can be helpful to patients and families with inherited eye diseases.

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Introduction

Genetic eye diseases include those that are the principal causes of blindness among infants (Leber congenital amaurosis, coloboma), children (early onset retinitis pigmentosa, Mendelian glaucoma, Mendelian cataract) and adults (pattern dystrophies, retinitis pigmentosa, late onset glaucoma and cataract). Hereditary ophthalmological conditions vary considerably in their symptoms/signs and severity. Genetic testing can be useful to confirm their genetic nature and etiology.

The main inherited eye diseases can be classified as:

- Retinal disorders: achromatopsia, ocular and oculocutaneous albinism, Leber congenital amaurosis, congenital stationary night blindness, choroideremia, pattern dystrophies, best vitelliform macular dystrophy, central areolar choroidal dystrophy, cone rod dystrophies, Sorsby's fundus dystrophy, Stargardt macular dystrophy, Doyne honeycomb retinal dystrophy, Norrie disease, Refsum disease, retinitis pigmentosa, retinitis punctata albescens, fundus albipunctatus, x-linked juvenile retinoschisis, enhanced s-cone syndrome, Bardet-Biedl syndrome, Senior-Loken syndrome, Usher syndrome, familial exudative vitreoretinopathy, color vision deficiency, Bietti crystalline dystrophy
- Lens disorders: Mendelian cataract
- Optic nerve disorders: optic atrophy, Mendelian glaucoma
- Corneal disorders: corneal dystrophies and other Mendelian corneal diseases
- Eye movement disorders: infantile nystagmus, Mendelian strabismus
- Refraction disorders: Mendelian myopia
- Malformations: coloboma

Inherited eye diseases have a genetic component that can be identified by appropriate genetic tests. The variety of genetic tests has increased over the years.

The ACCE (analytical validity, clinical validity, clinical utility and ethical, legal and social implications) evaluation process for genetic testing is well established. When evaluating the use of genetic tests, it is necessary to consider their analytical validity, clinical validity and clinical utility. Analytical validity is the accuracy with which a particular genetic characteristic is identified in a given laboratory test. Clinical validity is the accuracy with which a genetic test identifies a clinical status. Clinical utility refers to the risks and benefits resulting from test use. Important variables in evaluating evidence about clinical validity and clinical utility are the study population selection criteria, the clinical outcomes measured, and the comparability of cases and controls. The gold standard of clinical utility is the evaluation of results from prospective trials with randomized subjects in order to compare different genetically informed treatments (1,2). Ethical, legal, social and psychosocial issues contribute to the net risk/benefit balance of a genetic test for tested individuals, their families, and the population at large. Improvements in health outcomes (morbidity, mortality and disability) should be primary endpoints in assessing the utility of genetic testing (3).

"Utility gene test" cards are disease-specific guidelines regarding the clinical utility of genetic testing. These cards provide quick guidance to clinicians, geneticists, referrers, service providers and patients. The "utility gene test" cards are written by an expert team. Ideally, each card consists of the following parts: Description of the disease including susceptible genes (https://www.omim.org/), aims of testing, description of test, possible results of the test, risk for progeny, possible unexpected results from the test, possible limits of the test, analytical specificity and sensitivity, diagnostic specificity and sensitivity, prescription appropriateness, clinical utility and references. The "Utility gene test" card is important for confirming that the specific genetic test for a disease is useful for diagnosis, differential diagnosis, couple risk assessment and access to clinical trials. These cards cover all the elements needed to assess risks and benefits of genetic test application. It is important that the requirements for a test be defined in the context of their impact on the clinical setting. The laboratory genetic test is one of the components of an overall evaluation (4).

Impact of genetic testing on ophthalmological medicine

Molecular genetic testing in eye patients is very important for a number of reasons. First because it is critical for an accurate diagnosis of certain eye diseases. While a number of eye patients can be diagnosed on the basis of clinical findings, others cannot be confidently diagnosed without molecular genetic testing. For example, it is impossible to diagnose optic atrophy on a purely clinical basis in individuals with a negative family history for the disease; diagnosis is only possible by molecular genetic testing (5). In other cases, molecular genetic testing can confirm the diagnosis in a situation in which the clinical findings suggest more than one disease. For example, a child may have symptoms suggesting autosomal dominant familial exudative vitreoretinopathy (FEVR) caused by variations in the frizzled-4 gene (6). It is very difficult to distinguish FEVR from other diseases, such as persistent fetal vasculature and retinopathy of prematurity, on a solely clinical basis; molecular genetics may be necessary to establish the diagnosis (7). It is also possible to diagnose clinically similar diseases by genetic testing. For example, retinitis pigmentosa, Leber congenital amaurosis and Bardet-Biedl syndrome can be caused by mutations in different genes (8). Genetic tests have diagnostic as well as prognostic value for patients and their familiars. By way of illustration, genetic testing has prognostic value in retinoblastoma. Retinoblastoma is caused by mutations in both alleles of the *RB1* gene and it is the commonest childhood malignant eye tumor. Genetic testing for *RB1* mutations is indicated in affected individuals with newly diagnosed retinoblastoma and to differentiate hereditary from sporadic cases; it also provides indications regarding prognosis, intensity of eye monitoring and risk of disease developing in other members of the family. The genetic test is efficient in 92% of cases. Monitoring and diagnosis of high-risk infants improves prognosis by enabling use of less-intensive treatments (9).

An accurate molecular genetics diagnosis can be important for gene therapy. Viral-mediated gene therapy is useful for one subtype of Leber congenital amaurosis with mutations in the *RPE65* gene (10). Gene therapy can also be used for drug delivery, as many retinal diseases can benefit from local production of a specific RNA or protein (11).

Genetic counseling

Genetic testing is more likely to be informative after ocular genetic consultation (12). Ocular genetic consultation should include explaining the disease concept to the referring physician and the family. However, as more affected patients are diagnosed by gene mutation identification, it will be possible for relatives to request presymptomatic diagnosis and carrier identification by DNA testing. It is necessary to provide information about molecular biology, inheritance patterns, recurrence risks, genetic testing and research initiatives. The utility of genetic testing should be discussed with the patient, who may refuse testing if he/she feels that the likelihood of a positive result is extremely remote (13). However, genetic counselors should make sure that patients or parents (in the case of minors) understand the advantages, disadvantages and risk/benefit ratio of a certain genetic test. The genetic information content of the counseling session is accessible at Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov) or GeneTests reviews (http://www.genetests.org) and the Orphanet Journal of Rare Diseases (https://ojrd.biomedcentral.com). The American Academy of Ophthalmology (AAO) has published new recommendations for genetic testing. The genetic test should be pre-

ceded by gathering of clinical data, test results and pedigree. The pattern of inheritance should be considered together with eligibility criteria for genetic testing in relation to pathology. Pre-test counseling and informed consent are also necessary, and the post-test counseling appointment can be made. At post-test counseling, the test results are discussed. Information about trial and experimental therapies should also be provided, and the patient should be informed about the option of estimating risk of recurrence and extending examination to other family members (14). Counseling with a multidisciplinary care team in compliance with privacy and anonymity is an efficient system for patients (15).

Techniques for identifying genetic etiology of inherited eye diseases

The quality and utility of genetic tests depend on their reliability, validity, sensitivity, specificity, positive predictive value and negative predictive value. Sequencing methods can enhance diagnostic sensitivity. Laboratory techniques to analyze chromosomes and genes are used for the diagnosis of inherited eye diseases. Cytogenetic tests allow analysis of the number and morphology of chromosomes and detection of chromosomal duplications, deletions, insertions and translocations. They include karyotype analysis, FISH analysis and Sky test. Gene screening tests are based on analysis of the nucleotide sequence and include SSDGE or SSCP, DGGE, RFLPs, DNA microarrays and DNA sequencing (16). Not all laboratories test all genes. Techniques include panels of all known mutations, targeted mutation analysis, complete sequencing of a specific gene and simultaneous sequencing of many genes. Pooling of samples and use of automated instruments are a few of the approaches that are currently employed to optimize genetic diagnosis. The changes brought about by *next generation sequencing* (NGS) technologies have revolutionized ophthalmology (17). NGS can generate information about carrier states in autosomal and X-linked recessive conditions, which vary in severity and penetrance (18). In particular, NGS technologies are important for eye diseases characterized by genetic and phenotypic heterogeneity, such as congenital cataract, inherited retinal diseases and inherited optic nerve disorders. NGS technology can use a comprehensive panel of genes yielding a higher diagnostic rate. NGS gene panels are appropriate options for patients with a relatively certain clinical diagnosis that has genetic heterogeneity. For example, using a more comprehensive panel that comprises all 254 known candidate genes for retinal dystrophy gives a diagnostic yield of 51% (19) and NGS target enrichment for the 115 genes associated with congenital cataract has been developed with success (20). Next generation sequencing does have some limitations. Many pathogenic variants in inherited eye diseases are not likely to be large gene deletions. Some genes, such as *PRPF31*, which is associated with autosomal-dominant retinitis pigmentosa, have a higher percentage of deletions that would be missed with sequencing panels (21). There are gene-targeted comparative genomic hybridization arrays, MLPA arrays and deletion and duplication panels to complement the sequencing panels (22). The most comprehensive testing performed by laboratories is exome sequencing. This approach can be considered for patients without a clear clinical diagnosis, patients with high clinical heterogeneity and patients who previously had negative genetic testing or whose affected relatives previously had negative comprehensive testing. Analysis options include proband-only or patient plus parents. Proband plus parents testing can increase the detection rate of exome sequencing (23). Use of this technical approach establishes a more efficient and cost-effective clinical care algorithm for patients and increases the known mutational basis and understanding of the epidemiology of the inherited eye disease. A personalized approach enables treatment based on genetic profile, which encompasses optimized genetic counseling and trials of new molecular therapies (24). Biomedical research has evolved towards translation with a focus on personalized approaches. Personalized ophthalmology requires retrospective analysis, prospective studies and randomized-controlled trials. The creation of patient and mutation databases and global interdisciplinary projects are an aspect of diagnostic progress in ophthalmology (25). While laboratories like MAGI (http://www.magi-group.eu/) in Italy provide a non-profit service and accept samples for a large variety of diseases, others have formed consortia in which samples are handled by a central service, such as the National Eye Institute (NEI) in Bethesda, USA.

Application of genetic testing in clinical ophthalmological practice

While a positive test can help establish the diagnosis after estimating the probability of the change being pathogenic, a negative test does not exclude a genetic eye disorder since there are several different patterns of inheritance and different genes involved, many of which are little known or unknown (26). An example is the genetic test for glaucoma in which different genes, different patterns of inheritance and different clinical presentations are involved (27):

- infants with congenital glaucoma (*CYP1B1* and *LTBP2*)
- children and teens with early onset glaucoma (*PITX2, FOXC1, PAX6, LMX1B,* and mutations in the *MYOC* gene if there is a positive family history)
- young adults (<50 years) with glaucoma and a strong positive family history of mutations in the *MYOC* gene
- patients with optic nerve disease and a positive family history of normal tension glaucoma but no personal history of elevated intraocular pressure generally related to mutations in the *OPTN* gene.

In most cases, the genetic test results do not alter treatment but are helpful for genetic counseling and for starting treatment early to prevent or slow down progression. Another reason why genetic testing is important is that it allows other affected family members and carriers to be identified.

Unlike early-onset glaucoma, where mutations can only be identified in about 10-20% of patients, in forms of retinal de-

generation, more causative genes are known, making it possible to identify mutations in about 50% of patients (28,29), some of whom may be eligible to participate in clinical trials. Patients and their family members can obtain information about trials anywhere in the world at *www.clinicaltrials.gov*.

A major institution involved in such trials is *The National Eye Institute NEI* (https://www.nei.nih.gov), with which MAGI collaborates. This institute has a program called "Eye Gene" that accepts samples from patients with inherited eye disorders. Eligible individuals gain access to the diagnostic testing performed by the eyeGENE Network and have the option to participate in research studies related to their disease. A network in *NEI* named *The National Ophthalmic Disease Genotyping and Phenotyping Network (eyeGENE®)* links researchers and clinicians who are actively developing gene-based therapies to treat inherited eye diseases that were once considered untreatable. This network currently includes a coordinating center at *NEI*, a patient registry, a controlled-access centralized biorepository for DNA, and a curated de-identified genotype/phenotype database.

Many parents of minors at risk for developing symptoms of an inherited eye disease request genetic testing before the disease becomes clinically manifest. Except where there is preventive treatment for the condition, examination should be confined to clinical and electrophysiological testing, only pursuing molecular testing after clear signs of the disease appear. Confirming clinical diagnoses through eyeGENE® molecular testing has helped establish correlations between cone abnormalities and vision loss in Stargardt disease and X-linked retinoschisis. The eyeGENE Network also provides meaningful results for patients.

Testing female relatives of patients with X-linked diseases for the family's disease-causing allele is one of the most powerful uses of molecular testing in clinical practice. When an X-linked disease is diagnosed in a patient, there are often several female relatives of child-bearing age at significant risk of having an affected child. When they are distant relatives of the affected individual (e.g. female cousins whose mother is the proband's aunt) they are often unaware that their future children might be affected with a genetic eye disease (30).

Conclusions

The molecular diagnosis of genetic eye diseases has proven to be fundamental because of the prognostic and therapeutic value of detecting the underlying genetic mutation. According to J.L.Wiggs, patients with various types of disease may benefit from genetic testing, including those with eye movement disorders, optic neuropathies and corneal dystrophies. Patients who do not want a definitive diagnosis should not undergo genetic testing. Patients who do not wish to know their prognosis or carrier status should not be tested.

Fast and relatively inexpensive genetic tests with high sensitivity and specificity are available for inherited eye diseases. The bottleneck to more widespread use of this technology is shifting from the laboratory to the clinic. The challenge of developing personalized ophthalmology will require global collaborative efforts in which academic institutions, patient and clinical networks, regulatory authorities and commercial companies, including the pharmaceutical industry, work together. As inherited eye diseases are genetically heterogeneous, high-throughput next generation sequencing yields high diagnostic rates.

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