From the Laboratory to the Clinic: Molecular Genetic Testing in Pediatric Ophthalmology

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PURPOSE: To review the current state of molecular genetic testing as it relates to pediatric ophthalmology and to discuss its uses.

DESIGN: Review and evaluation of available molecular genetic testing.

METHODS: Literature review and discussion of testing in practice based on the authors’ clinical and laboratory experience.

RESULTS: Fee-for-service testing for many genetic eye diseases now is available. A report is always generated for fee-for-service testing. Detection of DNA variants in genes known to cause eye disease must be interpreted taking into account the variability of the human genome, the presence of benign variants (polymorphisms), and the carrier frequency of recessive alleles. Negative results in genetic testing are helpful in some disorders for which most of the causative genes are known and many disease-causing variants have already been reported, but are less helpful in those that currently have many undiscovered causative genes or novel mutations. Research-based testing also is available, but does not always yield a result. Patients with RPE65-associated Leber congenital amaurosis may be eligible for the current gene therapy trial. Patients with a variety of disorders may benefit from improved surveillance if their genetic diagnosis is known.

CONCLUSIONS: Entry into the genetic testing system often is via the patient’s ophthalmologist. Collaboration with geneticists and genetic counselors, use of web sites to keep up with the ever-changing availability and detection rates, and knowledge of clinical trials, when combined with excellent clinical diagnosis, can improve diagnosis and allow eligible patients to participate in treatment trials. (Am J Ophthalmol 2010;149:10–17. © 2010 by Elsevier Inc. All rights reserved.)

TYPES OF GENETIC TESTING AVAILABLE

EVER SINCE THE FIRST GENES FOR GENETIC EYE DISEASES were discovered, DNA testing for diagnostic purposes has been available to some patients. In 1989, a method was reported for laboratory differentiation between germline and nonheritable retinoblastoma, and soon after, rhodopsin mutations were noted to be the cause of some cases of autosomal dominant retinitis pigmentosa. Seemingly overnight, a new era began in ophthalmology. A specialty that formerly was based largely on clinical diagnosis and often on subtleties of appearance developed into a field where retinopathies that appeared to be identical with the ophthalmoscope were found to be caused by different mutations in the same gene, or even by mutations in different genes altogether. In addition, some ocular disorders that seemed to be completely different from each other clinically have been found to be caused by mutations in the same gene.

Although a clinical diagnosis would have sufficed for most patients a generation ago, the explosion of molecular discoveries during the past 2 decades has fueled many patients’ desires for a rapid and accurate molecular diagnosis of their disease. As a result, clinicians will need to acquire a new level of understanding about how molecular biology is used to understand and ultimately to impact the pathophysiologic features of these diseases. Like much of ophthalmology, this is a constantly changing landscape. This review will discuss why genetic testing is important, and how active clinicians can incorporate it into their practice in a practical and effective manner.

In the past, many dedicated ophthalmologists sent their patients’ blood samples to research laboratories only to find that they rarely received any results from these investigations. Although some patients did receive a genetic diagnosis, the physician often was required to field many requests for updates on the status of their testing with the answer, “I don’t know.” Patients and their doctors had an unspoken fee-for-service expectation of performance from the research labs, whereas in fact the research labs had insufficient personnel, instrumentation, communication strategies, or a combination thereof to deliver test results with this degree of customer service. Fortunately, clini-
cians and patients are gaining a greater appreciation for the complexity of genetic testing at the same time that research and commercial laboratories are gaining a greater understanding of their collaborators’ and clients’ needs. Thus, the expectation gap is closing for many genetic tests for eye diseases.

All fee-for-service testing, and some research testing, is performed using validated laboratory procedures specified by the Clinical Laboratory Improvement Act (CLIA). Currently available fee-for-service testing for inherited eye disease is very different from the research testing clinicians may have experienced in the past. First, there is a fee for the testing that is payable either by the patient or by their insurance carrier. More importantly, there is a written report, of either positive or negative results, that is generated and sent to the referring physician or patient, or both, in every case. Because many different genes can cause some inherited eye diseases, a report of negative results usually is not nearly as helpful in patient management as one reporting positive results. In addition, because genes are still actively being found and certain alleles are observed only in certain populations, even positive results require more validation and qualification than a more routine laboratory test like a white blood cell count or liver enzymes analysis. This will be discussed more fully below. Here, it is sufficient to emphasize the point that for a test to be offered in a fee-for-service manner, that test must be validated and performed in a laboratory that is certified to meet CLIA standards.

Today when a clinician suspects a genetic eye disease based on examination and testing in the office, an excellent first resource is the web site www.genetests.org, managed by Roberta Pagon, MD, at the University of Washington, Seattle. This site is user friendly and allows the physician to query for testing for various diseases. There are links to laboratories at which fee-for-service testing for each disorder is performed, as well as detailed information on how to send a sample. The site states what type of testing is offered and whether carrier and prenatal testing is offered at each laboratory. Labs offering fee-for-service testing must be CLIA certified. Information on laboratories that offer research-based testing also is available on this site.

For patients with insurance, printing the forms for the laboratory of choice, having the physician fill out the appropriate fields, and taking the forms to a phlebotomy lab, often are all that is required. However, many insurance carriers will not pay for testing without precertification. Patients, physician office staff, or both should call the carrier before having blood drawn to avoid surprise bills. In addition, as with all bills sent for payment to insurance carriers, each insurer has their own policies about which tests will be covered and which denied. In our experience, with appropriate attention to precertification, insurance companies will cover genetic testing for inherited eye disease approximately 80% of the time. However, it still behooves the physician to tell patients that each insurance plan is different, and that it is always safest to get a precertification number even before having the blood drawn. Because of the recent proliferation of fee for service genetic tests for all types of genetic disorders, many certified genetic counselors and genetic nurse specialists have become expert at getting precertification and coverage for genetic testing. For many clinicians, the most efficient way to obtain testing for a given patient is to refer them to a general genetics clinic with their suspected ocular diagnosis and paperwork for a laboratory offering testing in hand. This collaboration between ophthalmologist and geneticist or genetic counselor is ideal because the ophthalmologist can provide the clinical diagnosis, then the genetics clinic can organize DNA testing, interpret the results, and offer genetic counseling. In many academic medical centers, genetic eye disease clinics are being formed that include an ophthalmologist with a specialty in genetic eye disease, in collaboration with a geneticist or genetic counselor. These combination clinics can provide the best of both worlds in just 1 visit for the patient.

Fee-for-service laboratories generally send the results to the ordering physician so he or she can discuss those results with the patient. The ordering physician must have a plan in advance for who will discuss and interpret the results with the patient. Many ophthalmologists prefer to work with a geneticist who will meet with the patient after the results of testing are obtained, because the discussion may be very complex. Recurrence risk in offspring, risk to other family members, reproductive options, and how to interpret both positive and negative results are outside the scope of many ophthalmologists’ realm of practice. It is important to remember that essentially all genetic eye disease is heritable, but not all genetic eye disease is inherited. Spontaneous new mutations are not uncommon. In these patients, there is no family history, but the genetic mutations may be passed on to subsequent generations. Some diseases have protean manifestations that only a very careful family history or examination of additional family members can elicit.

What the ophthalmologist can and should do is identify patients with likely genetic eye disease and make a tentative clinical diagnosis to direct genetic testing. Other members of the team can perform the genetic test, can help explain it to the patient, and can help to counsel the patient and his or her family. Thus, a good working relationship between the patient, ophthalmologist, the laboratory, and the counselor is key to taking maximum advantage of the genetic tests that currently are available.

**WHY IS MOLECULAR GENETIC TESTING IMPORTANT IN PEDIATRIC OPHTHALMOLOGY?**

MOLECULAR GENETIC TESTING IS IMPORTANT FOR CHILDREN WITH OCULAR DISEASES FOR A NUMBER OF REASONS. FIRST, IT IS CRITICAL FOR ACCURATELY DIAGNOISING CERTAIN DISEASES. ALTHOUGH A NUMBER OF OCULAR DISEASES IN CHILDREN CAN BE
diagnosed based solely on clinical findings, there are certain diseases that cannot be diagnosed with confidence without molecular genetics. For example, in children without a family history of optic atrophy, it is possible to diagnosis Kjer dominant optic atrophy definitely only using molecular genetics. For other diseases, molecular genetic testing can confirm the diagnosis in a situation in which the clinical findings are suggestive of one disease, but there is some overlap with other diseases. For example, a child may have findings suggestive of autosomal dominant familial exudative vitreoretinopathy caused by the frizzled-4 gene, but because of the overlap with other diseases such as persistent fetal vasculature and retinopathy of prematurity, molecular genetics may be necessary to establish the diagnosis definitively. Although positive test results may be diagnostic, negative test results are not helpful in this disorder because there are several different genes and different methods of inheritance; it is estimated that only approximately 20% of index cases of familial exudative vitreoretinopathy are caused by currently detectable frizzled-4 gene mutations, whereas another 20% are caused by mutations in LRP5, making the genetic diagnosis rate approximately 40% overall. Furthermore, molecular genetic testing often can establish more accurately the risk of the disease occurring in future pregnancies as well as in the offspring of the child than can clinical diagnostics alone.

Obtaining a molecular genetics diagnosis also is important in terms of enrolling patients in clinical trials. Mutations in more than 14 different genes have been shown to cause Leber congenital amaurosis (LCA), and many of these affect retinal function in very different ways. It is not usually possible to distinguish between these different genetic subtypes on the basis of clinical findings alone. Approximately 8% of patients with LCA have mutations in the retinal pigment epithelium-specific 65-kDa protein gene (RPE65). This gene encodes a protein that is required to isomerize all-trans-retinyl esters into 11-cis-retinal. Patients with the RPE65 genotype have been shown to have a modest improvement in retinal function after the subretinal injection of adeno-associated virus carrying wild-type RPE65 complementary DNA.

Because there is a treatment available for at least 1 form of LCA, it seems incumbent on ophthalmologists to determine if a patient with LCA has RPE65-associated disease. For patients whose LCA is not caused by mutations in RPE65, accurately establishing the correct molecular cause may give them the opportunity to participate in new treatment trials as they become available for other genetic subtypes of the disease.

Molecular genetic testing also can be helpful in predicting future problems. If a child is known to be at risk for certain ocular or systemic diseases, the intervals for screening can be adjusted accordingly. For example, one of the authors recently was referred a 2-year-old child with the 6p deletion syndrome. Because the deletion involved the FOXC1 gene, which is one of the genes that has been shown to be associated with Axenfeld-Rieger anomaly, he was referred for an ocular examination. On examination, he was found to have posterior embryotoxon and subtle corectopia consistent with Axenfeld-Rieger anomaly. Although he did not having any findings suggestive of glaucoma at that time, given that glaucoma develops in as many as 50% of patients with Axenfeld-Rieger anomaly, he will be screened at more frequent intervals.

Molecular genetic testing also can be invaluable in assigning a risk to a patient with an ocular disease for developing certain systemic diseases. An example of this is sporadic aniridia. Autosomal dominant aniridia is caused by inactivation of 1 copy of the PAX6 gene at 11p13. Most patients with aniridia have an intragenic mutation, but as many as 18% of patients with sporadic aniridia have a deletion involving the Wilms tumor suppressor gene, WT1, which is located near the PAX6 gene. Although Wilms tumor develops in only 1 in 10 000 typical children, the risk of Wilms tumor developing increases 67-fold in patients with sporadic aniridia. Like retinoblastoma and the RB1 tumor suppressor gene, Wilms tumor is believed to arise from a loss of function of both WT1 tumor suppressor genes (i.e., the so-called 2-hit hypothesis). Abnormalities in the WT1 gene can be identified by chromosome analysis of 11p13 using fluorescence in situ hybridization, and by strategic mutation analysis. Therefore, children with sporadic aniridia benefit from referral to a geneticist for DNA testing and should be screened on a regular basis for Wilms tumor if confirmed by genetic testing.

Another example of medical surveillance being guided by a specific genetic subtype is CEP290-associated LCA. We now know that different mutations of the CEP290 gene can cause isolated LCA, Joubert syndrome, Senior Loken syndrome, or Meckel Gruber syndrome. Children diagnosed with CEP290-associated LCA therefore should have a screening renal ultrasound and regular urinalysis, and brain magnetic resonance imaging should be considered at diagnosis.

Since the advent of genetic testing, it has become apparent that many diseases exhibit genetic heterogeneity. This means that a single phenotype (clinical presentation) can be caused by many different genes or different mutations in the same gene, or both. Conversely, there can be phenotypic heterogeneity. This means the same gene, and in some cases even the same mutation, can cause different clinical appearances in different people. Genetic heterogeneity initially was a surprise, because ophthalmic diseases had been divided and subdivided by the best means possible at the time: ophthalmoscopic or slit-lamp appearance. However, as our understanding of these diseases grows, it is clear that genetic heterogeneity makes sense. Different gene malfunctions easily can lead to the same final common pathway of photoreceptor cell dysfunction and death, to the characteristic pigmentary changes seen in retinal degeneration, or to the loss of corneal or
crystalline lens cell clarity resulting from abnormal cells in these tissues. For example, mutations in the ABCA4 and the RDS genes can cause very similar fundus changes that most clinicians would call retinitis pigmentosa. Yet other mutations in the ABCA4 and RDS genes cause only maculopathy, which clinicians would call Stargardt disease or pattern dystrophy.

Bardet Biedl syndrome is a multisystem disorder characterized by the unusual constellation of extra digits, obesity, renal disorders, reproductive disorders, developmental delays, and a progressive severe early onset retinal dystrophy. With such an unusual combination of features, one might think there would be only one gene in the human body that could cause this. Yet Bardet Biedl syndrome is now known to be caused by at least 12 genes. How can this be? A recent discovery shows that most of these genes contribute to a protein complex called the BBSome, which is important in cilia throughout the body. Now it makes perfect sense: if any one of the very different genes that contribute a piece to this BBSome complex is not working, the result is the same no matter which one of them it is: a cilia disorder. And many organs with cilia—such as brain, retina, gonads, kidney, olfactory receptors, hair cells of the ear—may be affected, linking the seemingly unrelated aspects of the disease. Of interest, CEP290 is also important in cilia.

In cases in which the same mutation causes different clinical presentations, it is likely that other modifying genes are involved that have not yet been discovered, or that there are environmental factors that play a role in how the gene is expressed. Two examples of this are the finding that variations in the Rpe65 gene modulate light damage susceptibility in mice and influence the progression of retinal degeneration in transgenic mice with retinitis pigmentosa, and that at least 2 loci on the X chromosome seem to influence how the 11778 mitochondrial mutation causes Leber hereditary optic neuropathy.

## FOR WHICH OCULAR DISORDERS IS FEE-FOR-SERVICE TESTING AVAILABLE?

BECAUSE THE LIST OF DISEASES FOR WHICH FEE-FOR-SERVICE testing is available lengthens daily, ophthalmologists should become familiar with [www.genetests.org](http://www.genetests.org) to determine which testing is available. For certain disorders, there may be both research and fee-for-service testing options. At [www.retinoblastomasolutions.org](http://www.retinoblastomasolutions.org), nonprofit fee-for-service testing is offered for retinoblastoma. Bilateral and unilateral retinoblastoma patients and their families can be tested. Another nonprofit genetic testing laboratory can be accessed at [www.carverlab.org](http://www.carverlab.org). Physicians can order fee-for-service testing for LCA, as well as many other disorders. Patients and families with LCA also can enroll in Project 3000, an initiative to genotype all 3000 people estimated to be living with LCA in the United States. Patients may opt to be contacted regarding research studies for this disorder as they become available and can stay up to date with research progress. Some disorders seen frequently in pediatric ophthalmology practice for which fee-for-service genetic testing currently is very useful are listed in the Table. This list is not comprehensive.

### LIMITATIONS OF CURRENTLY AVAILABLE GENETIC TESTING

ARE THERE SOME PATIENTS FOR WHOM GENETIC TESTING is not recommended? Patients who do not want a definitive diagnosis should not undergo genetic testing. Patients who do not wish to know their prognosis or who do not want to know their carrier status should not have testing. An estimate of the patient’s chance of obtaining a useful result from each test should be part of the discussion with the patient when one is deciding whether to order a genetic test, and if the patient believes the chance of a positive result is too low, they may opt against testing. At [www.genetests.org](http://www.genetests.org), most diseases for which testing is of-

### TABLE. Some Pediatric Genetic Eye Disorders for Which Fee-for-Service Testing Is Currently Available

<table>
<thead>
<tr>
<th>Disorder</th>
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<tbody>
<tr>
<td>Leber congenital amaurosis</td>
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<tr>
<td>Achromatopsia</td>
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<tr>
<td>Autosomal dominant retinitis pigmentosa</td>
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<tr>
<td>Stargardt macular dystrophy</td>
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<td>Best disease</td>
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<tr>
<td>Bardet Biedl syndrome</td>
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<td>Usher syndrome</td>
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<tr>
<td>Retinoblastoma</td>
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<tr>
<td>Tuberous sclerosis</td>
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<tr>
<td>Albinism (OCA1, OCA2, X-linked)</td>
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<tr>
<td>Neurofibromatosis (NF1, NF2)</td>
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<tr>
<td>Anterior segment dysgenesis (PAX 6)</td>
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<tr>
<td>Reiger syndrome</td>
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<tr>
<td>Aniridia/Wilm tumor</td>
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NF = neurofibromatosis; OCA = oculocutaneous albinism; PAX 6 = Paired Box 6.
ferred have a GeneReview section that discusses the current detection rates for different types of testing. For example, according to information on genetest.org at this writing, more than 90% of patients with oculocutaneous albinism type 1 will have at least one allele found with current testing, whereas the rate of detection for oculocutaneous albinism type 2 is difficult to calculate because of the very large number of polymorphisms in this gene. The detection rate for neurofibromatosis type 1 depends on the technique used to screen this very large gene; a multistep mutation detection protocol identifies approximately 95% of pathologic mutations. For neurofibromatosis type 2, where deletions as well as point mutations are fairly common, the detection rate is more than 90% for familial cases but 72% for singleton patients. For LCA, the detection rate is 50% to 60%. This information changes rapidly and is updated frequently. The chance of obtaining a definitive result is improving steadily for most tests, and if one has not ordered a specific test for several months, it is wise to review this before talking with a patient.

In our experience, most patients want a definitive diagnosis and are interested in testing if there is at least a 50% chance of obtaining a meaningful result. Each patient is different, so careful explanation and counseling about the state of genetic testing must be individualized for each person’s disorder. Some disorders for which genetic testing is available may not have a high enough detection rate to make testing useful for most patients. Many disorders require advanced knowledge of the clinical characteristics of the disorder, the inheritance pattern, and the known molecular causes to interpret the results correctly. For example, in autosomal recessive diseases, 2 copies of the abnormal gene must be found to be certain that the variations discovered are disease causing. However, some common recessive disorders, such as Stargardt disease and autosomal recessive albinism, have a very high rate of finding only 1 of the 2 mutations with current technology. Now that many convincingly disease-causing mutations are known for both diseases, meaningful reports can be generated in some cases based on the finding of only a single allele. In most other autosomal recessive disorders, the finding of a single allele would be interpreted as equivocal, or not involved with the disease in question at all. Most genes will be found to contain multiple nondisease variations when they are evaluated in a large number of individuals in the population. It requires constant vigilance by the laboratory to distinguish variations that actually cause disease from those that are incidental findings, and physicians always should be a bit skeptical of a novel molecular result in a patient whose clinical findings are atypical.

Another complexity is that every individual harbors several autosomal recessive alleles in the heterozygous, or carrier, state. During testing for a genetically heterogeneous disorder, one may discover the carrier state of an allele in one gene, whereas the true disease-causing mutations for that patient actually lie in another gene. This fact underscores the desirability of identifying both disease alleles in a patient before issuing a positive report and the additional desirability of demonstrating those alleles to segregate as expected in additional family members.

Some disorders that are almost always inherited in an autosomal recessive manner do have rare forms that are dominant. For example, most cases of Stargardt disease are inherited in an autosomal recessive manner (caused by mutations in the ABCA4 gene), but a few percent are inherited in a dominant fashion and are caused by mutations in ELOVL4. Similarly, a few percent of cases of LCA are inherited in a dominant fashion (most caused by mutations in CRX), whereas the recessive form of the disease is caused by mutations in at least 13 other genes.

Isolated congenital cataracts are estimated to be autosomal dominant in 50% of cases in Western countries, and many genes have been found that, when mutated, cause this disorder. But at present, no fee-for-service testing exists to allow us routinely to diagnose children and their families.

Congenital glaucoma can be inherited in an autosomal recessive fashion in some families. Mutations in the CYP1B1 gene have been found to cause up to 50% of primary congenital glaucoma worldwide, but the rate varies widely based on ethnicity. Close to 100% of congenital glaucoma cases in patients from Saudi Arabia and some Gypsy groups have been reported to be the result of CYP1B1 mutations, but only 17% to 30% of cases in Chinese, German, and Spanish populations are the result of this gene. Because of this ethnic variability, some families have a higher chance of obtaining a diagnostic result than others. In addition, even if CYP1B1 testing demonstrates negative results, this does not rule out autosomal recessive inheritance, because there are as-yet undiscovered genes. A commercial test is available to test CYP1B1, but families must be counseled carefully both before and after testing. If the test results are positive, very precise genetic counseling can be given, family members can be tested, and preimplantation genetic testing can be offered. However if the test results are negative, the recurrence risk to the parents must still be assumed to be 2%, and yet preimplantation testing would not be possible in these cases.

Usher syndrome is the most common cause of combined deafness and blindness. It is autosomal recessive and is caused by at least 9 genes. Because children are born deaf or hard of hearing and only later does retinitis pigmentosa start to develop, many parents and patients do not know the child will have multiple sensory issues until later in life. Knowing that this is a deaf–blind syndrome may affect important decisions, such as whether to perform cochlear implantation early in life, which is controversial in the deaf community, as well as for family planning and educational planning. In addition, many Usher patients usually do not understand why they are having difficulty...
with certain activities for a long time before diagnosis, which causes added anxiety. For all of these reasons, genetic testing for children born deaf or hard of hearing would be a positive development. Testing for Usher syndrome is available and currently detects approximately 50% to 80% of mutations, depending on which gene is involved and the exact method of testing.\textsuperscript{35}

**THE MECHANICS OF GENETIC TESTING**

**CLINICIANS WHO ORDER AND INTERPRET GENETIC TESTS** quickly learn that all genetic tests are not alike. Some tests are designed to examine rapidly multiple genes for mutations that previously were identified in other patients (allele-specific tests), whereas other tests are designed to examine the entire coding sequence of 1 or more genes in search of disease-causing mutations that previously might never have been observed. There are strengths and weaknesses with both strategies. Allele-specific tests often are less expensive and have a shorter turnaround time than similarly complex tests involving DNA sequencing. In addition, mutations that have been observed to segregate properly with the disease in many different families are more likely to be clinically meaningful than missense mutations observed for the first time in an isolated patient. However, some disease-causing genes have a relatively high rate of novel mutations identified even many years after their initial discovery. Some laboratories attempt to provide patients the best of both worlds by offering an allele-specific test for a given disease as an initial step with the idea that more extensive DNA sequencing approaches can be used subsequently if the allele-specific test is only partially revealing or does not detect any mutations.

One situation in which this tiered testing approach is particularly effective is for genetically heterogeneous autosomal recessive disorders like Bardet Biedl syndrome, Usher syndrome, LCA, and recessive retinitis pigmentosa. In extensively outbred populations such as those of North America and Europe, most patients affected with an autosomal recessive disease will be compound heterozygotes—that is, they will inherit different disease-causing alleles in the same gene from their mother and their father. By definition, one of these mutations will be more common than the other and therefore is more likely to be represented on an existing allele-specific test for that disease. When this more common allele is detected by a rapid and inexpensive allele-specific test, it allows the laboratory to focus the remaining testing effort (to find the other less common allele) on a single gene. For many genetically heterogeneous recessive diseases, this combination of approaches often will reach a final answer more rapidly and less expensively than either testing method alone.

Regardless of the specific method used to identify disease-causing mutations in a given proband, it is almost always a good idea to also obtain samples from additional family members to help with the proper interpretation of the findings. The observation of 2 novel, plausible, disease-causing mutations in a patient with a recessive disease is a much weaker molecular result than the same observation coupled with evidence that one or both of his parents are heterozygous for one of the patient’s alleles. A surprising number of plausibly disease-causing variants turn out to lie on the same chromosome when family members are examined to determine the segregation of the alleles. For recessive disorders, 2 mutations on the same allele will not cause disease; both alleles must have at least 1 mutation. A side benefit to such segregation testing is that it dramatically lessens the likelihood that an error has occurred anywhere in the chain of sample handling.

Prenatal diagnosis is in theory possible for all diseases that can be diagnosed by direct analysis of DNA. However, many laboratories that offer genetic testing for eye diseases are not set up to provide results with sufficient speed to make prenatal testing practical. If a patient is considering prenatal testing for an eye disease as part of a family planning strategy, a laboratory should be engaged before the pregnancy occurs to make sure that all of the necessary reagents and personnel are in place to perform the test in the compressed time scale necessary for this type of testing.

Preimplantation genetic testing also is possible for virtually every disease that can be diagnosed by direct analysis of DNA. With this approach, a couple pursues in vitro fertilization much as one would in an infertility setting. Then, single cells are removed from the resulting embryos and tested for the presence of the family’s disease-causing mutation(s), and these testing data are used to choose which embryos are transferred to the mother’s uterus.

Testing of the female relatives of patients with X-linked diseases for the presence or absence of the family’s disease-causing allele is one of the most powerful uses of molecular testing in clinical practice. When one makes the diagnosis of an X-linked disease in a patient, there are often several women of child-bearing age who are at significant risk of having an affected child. Moreover, when these women are distantly related to the affected individual (e.g., female cousins whose mother is the proband’s aunt) they often are unaware that their future children may be affected with an eye disease. When a disease-causing mutation is known in the proband, carrier testing is relatively inexpensive to perform. Informing the proband (or his parents if he is a minor) of the availability of such testing is an important part of counseling regarding X-linked disease.

Many parents of minor children at risk for an inherited eye disease will seek genetic testing for the children before any evidence of the disease is manifest. In almost all cases, unless a preventive treatment for the condition is known, one should confine one’s examination to clinical and electrophysiologic methods and should pursue molecular testing only after clear signs of the disease are evident. There are many reasons for avoiding presymptomatic
testing of minors. Most diseases exhibit some degree of variable expressivity such that a disease genotype does not definitively predict a disease phenotype. For example, in 5% to 10% of patients who harbor true disease-causing mutations in bestrophin, a visually significant Best disease macular lesion never develops.36,37 Thus, in these patients, ophthalmoscopy actually is more predictive of clinical outcome than molecular testing. When a patient reaches 18 years of age and the disease still is not evident clinically, he or she may choose to undergo presymptomatic testing both to understand better the risk of future disease and also to plan his or her own families. Performing such testing earlier robs them of their right to choose not to know, if indeed that would be their choice as an adult.

Perhaps the most common question asked by doctors who order and interpret genetic tests of those who perform them in the laboratory is why it takes so long to complete and report tests. Patients and doctors are accustomed to routine tests which are reported within days or even minutes. Unfortunately, the human genome is a very large and noisy place with millions of non–disease-causing variations competing for a geneticist’s attention with the 1 or 2 that are truly responsible for a patient’s disease. Perhaps the greatest difficulty is the relative coarseness of our current diagnostic nomenclature compared with high-resolution reality in the genome. As just one example, the clinical finding of retinitis pigmentosa can be caused by more than 50 genes that collectively span millions of base pairs of genomic sequence. The solution lies partially in the continuous recognition and teaching of genotype–phenotype correlations so that doctors can focus their laboratories’ energies into relatively high-yield activities.

This, coupled with tiered testing (with interim reports given at the end of each tier) will lessen the sense that testing is not progressing and will convey better (to the doctor and the patient) the true complexity of the laboratory task. At the same time, molecular methods and bioinformatic strategies are becoming more powerful so that the costs and turnaround times for even the most complex tests should continue to decrease.

CONCLUSIONS

GENETIC TESTS WITH REASONABLY HIGH SENSITIVITY, high specificity, moderate cost, and a turnaround time often measured in weeks are now available for dozens of inherited eye diseases. Insurance companies are increasingly willing to pay for them. In the coming few years, these tests steadily will improve and will become more numerous. The bottleneck to more widespread use of this technology is gradually shifting from the laboratory to the clinic. Where once no tests were available, now there are tests but an insufficient number of doctors who are knowledgeable enough to order them and interpret them. Ophthalmologists should see this as a tremendous opportunity to convert the negative messages of past decades, which essentially were that nothing could be done, into a message of realistic hope: with a blood test the exact cause of a child’s vision problem may be diagnosed, and eligibility for the current treatment trials can be determined. Doing so not only will contribute to the discovery of additional disease-causing genes, it also will make possible the clinical trial of many exciting new therapies.

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