JUVENILE RETINOSCHISIS: A MODEL FOR MOLECULAR DIAGNOSTIC TESTING OF X-LINKED OPHTHALMIC DISEASE*

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ABSTRACT

Background and Purpose: X-linked juvenile retinoschisis (RS) provides a starting point to define clinical paradigms and understand the limitations of diagnostic molecular testing. The RS phenotype is specific, but the broad severity range is clinically confusing. Molecular diagnostic testing obviates unnecessary examinations for boys at-risk and identifies carrier females who otherwise show no clinical signs.

Methods: The XLRS1 gene has 6 exons of 26-196 base-pair size. Each exon is amplified by a single polymerase chain reaction and then sequenced, starting with exons 4 through 6, which contain mutation "hot spots."

Results: The 6 XLRS1 exons are sequenced serially. If alterations are found, they are compared with mutations in our >120 XLRS families and with the >300 mutations reported worldwide. Point mutations, small deletions, or rearrangements are identified in nearly 90% of males with a clinical diagnosis of RS. XLRS1 has very few sequence polymorphisms. Carrier-state testing produces 1 of 3 results: (1) positive, in which the woman has the same mutation as an affected male relative or known in other RS families; (2) negative, in which she lacks the mutation of her affected male relative; and (3) uninformative, in which no known mutation is identified or no information exists about the familial mutation.

Conclusions: Molecular RS screening is an effective diagnostic tool that complements the clinician's skills for early detection of at-risk males.

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Useful outcomes of carrier testing depend on several factors: (1) a male relative with a clear clinical diagnosis; (2) a well-defined inheritance pattern; (3) high disease penetrance; (4) size and organization of the gene; and (5) the types of disease-associated mutations. Ethical questions include molecular diagnostic testing of young at-risk females before the age of consent, the impact of this information on the emotional health of the patient and family, and issues of employability and insurance coverage.

INTRODUCTION

X-linked juvenile retinoschisis (XLRS) is one of the more common causes of juvenile macular degeneration affecting boys. However, all of the retinal and macular dystrophies are relatively uncommon in a general ophthalmology practice, and distinguishing among these many conditions presents a diagnostic challenge. In the case of XLRS, the suspicion is increased upon finding a family inheritance pattern with only males affected and related through females, which is consistent with a classic X-chromosomal disorder.

Generally, two kinds of questions are raised by patients with these retinal dystrophies. The first concerns the expectation for vision in future years, and the second involves the inheritance risk for offspring and relatives of the affected male. The laws of inheritance extend the impact of these diseases to multiple members of the family beyond the patient himself. The risks for males include developing the condition and for females, carrying the disease-causing gene. Often the risk status cannot be ascertained definitively by ophthalmic evaluation alone. The advent of molecular gene testing provides a uniquely accurate diagnostic tool that complements currently available clinical tools and expands the technical expertise of the clinician. It is within this context that we have developed a molecular diagnostic clinical service for X-linked retinal dystrophies and other conditions. This paper highlights some of the caveats inherent in providing diagnostic information to families at risk for X-linked retinal dystrophies.

Vision loss in XLRS is usually discovered in affected males in early grade school and is slowly progressive. XLRS causes splitting through the superficial layers of the retina (schisis) within the macula and in the retinal periphery and results in an absolute scotoma in the involved area.[1] There is considerable phenotypic variability in XLRS, both in the age at onset and the size and location of the lesions. The macula typically shows linear intraretinal cystic cavities that radiate from the fovea for about 1 to 1.5 disc diameters in a spoke-wheel pattern. The foveal schisis in XLRS is nearly always bilateral, but the pattern may be quite asymmetric. Some

cases simply have multiple discrete cysts scattered across the macula. In the periphery, splitting occurs through the nerve fiber/ganglion cell layer that lies at the inner surface of the retina. When the schisis is limited to the peripheral retina, reasonably good vision can be conserved for several decades. Visual acuity typically stabilizes during late grade school and teenage years and can remain constant into middle age. In older age, these cysts may coalesce and result in macular atrophy not dissimilar from age-related macular degeneration.

In males, visualization of the fundus and the finding of an "electronegative" electroretinographic (ERG) response can be diagnostic.[1] XLRS is highly penetrant with upwards of 95% of affected males showing some form of foveal schisis. There is also a wide range of intrafamilial variability in affected males. It is not possible to predict the course of the disease within a family on the basis of the clinical course of other affected family members. All of the daughters of affected males are obligate carriers. The male children of these daughters are at a 50% risk for developing XLRS, and the female children are at a 50% risk of being carriers. Female carriers of the XLRS1 gene, however, rarely exhibit fundus changes or abnormalities, and ERG testing cannot identify the female carriers. Thus, with currently available clinical methods, it is not possible to refine a risk estimate for a potential carrier female beyond that defined by a traditional pedigree analysis.

METHODS

Blood samples were obtained from affected and at-risk males and potential female carriers. Informed consent was obtained from all participating individuals, and the studies were approved by the institutional review board. DNA was isolated from lymphocytes by standard procedures. Each of the 6 exons of the XLRS1 gene were amplified by PCR using primers described by Sauer and associates,[2] and the products were sequenced using a cycle sequencing reaction kit (Amersham, Arlington Heights, Ill), with the same primers as used for amplification.

Currently, the mutation analysis of the XLRS1 gene in our laboratory includes only the exons and the intron-exon junctions. Sequence alterations in other parts of the gene, including the promoter region or within the intronic sequences, cannot be detected by this method. We sequence the exons serially beginning with exons 4 or 6, which have the highest probability of mutation, and then on through the remaining exons until a mutation is identified or the entire coding sequence has been screened. One hundred normal chromosomes from unrelated individuals were screened to identify polymorphisms (natural sequence variations) that

occur in the normal population but do not cause the disease.

To optimize the efficiency of screening in potential carrier females, and to identify the causative mutation in the family, a blood sample from an affected male in the family is screened first. Once a mutation is identified, then the potential carrier female can be screened for the specific mutation.

RESULTS

The XLRS gene maps to the short arm of the X-chromosome (Xp22.1-22.3). This gene was cloned in the fall of 1997 and named XLRS1.[2] The gene contains 6 exons (size range. 26-196 base pairs [bp]) and codes for a predicted protein of 224 amino acids in length (Fig 1). The feasibility of diagnostic testing of this gene is considerably enhanced by the small number of exons, each of which is sufficiently small (<200 bp) that it can be

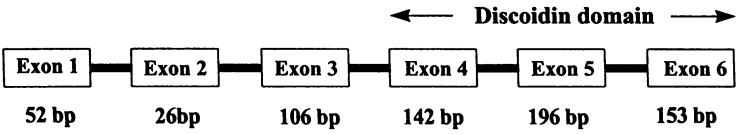

FIGURE 1
Molecular organization of XLRS1. Cartoon of XLRS1 gene shows base pair length of the 6 exons and the dicoidin domain that stretches across exons 4 through 6. The introns are represented as black bars between exons. (Not drawn to scale.)

amplified in a single PCR reaction. More than 90% of XLRS cases studied thus far have mutations in XLRS1, and no genetic heterogeneity is known for this disease. The X-linked pattern of inheritance of XLRS, the genetic homogeneity, and the high penetrance, combined with the molecular organization of the gene, establish the utility of mutation screening of the XLRS1 gene as a molecular diagnostic probe that can identify at-risk males and carrier females.

The initial screening of the XLRS1 gene by the International Retinoschisis Consortium identified 82 different mutations in 214 families with 234 cases studied, for a true positive rate of 91%.[3] Thirty-one of these mutations were present in more than one family, with the 214G→A mutation found in 34 apparently unrelated families. Although mutations were found in each of the 6 exons, the majority occurred in exons 4, 5, and 6. This region of the predicted protein encodes a discoidin domain that is believed to convey the biologic activity of the XLRS1 protein (Fig 1).

Table I summarizes 87 independent mutations that have been found to date (http://www.dmd.nl/rs/rs.html). Several types of changes were found:

- *Missense* mutations change a single amino acid due to the substitution of a nucleotide in the coding sequence.
- *Nonsense* mutations generate a stop codon and lead to a truncated protein.
- *Frame shift* mutations are caused by nucleotide deletions or insertions and alter the protein product.
- *Splice site* mutations affect the transcription of DNA to RNA by altering the exon-intron boundaries of the gene.

For XLRS, we have found that the majority of the mutations in exons 4 through 6 are missense and nonsense changes, whereas the changes in exons 1 through 3 are frame shift or splice site mutations.

TABLE I. MUTATIONS IN XLRS1 GENE

EXON	NUCLEOTIDE SUBSTITUTION (CODING SEQUENCE)	NUCLEOTIDE SUBSTITUTION (SPLICE SITES)	INSERTIONS/ DELETIONS	NUMBER OF PATIENTS WITH MUTATIONS
1	2	2	6	12
2	1	0	1	2
3	2	3	1	15
4	15	2	3	97
5	18	1	3	36
6	21	1	5	66
Total	59	9	19	228

Several pieces of evidence suggest that the mutations in the XLRS1 gene reported in Table I result in juvenile retinoschisis. First, none of these mutations have been detected in 100 X chromosomes from normal, unrelated individuals. Second, in families where additional members were available for study, the XLRS1 mutations cosegregated with the disease.[3] Finally, very few polymorphisms (sequence changes that are not associated with the disease) have been identified. To date, only 6 sequence polymorphisms have been reported in the XLRS1 gene (http://www.dmd.nl/rs/rs.html and listed in Table II).

Thus far, we have not noted a highly significant correlation between disease severity and mutation type or location. The most extensive RS

TABLE II. POLYMORPHISMS IN XLRS1 GENE

EXON	NUCLEOTIDE CHANGE	AMINOACID
5	T330C	Cys 110 Cys
5	T426C	Cys 142 Cys
5	G472A	Asp 158 Asn
6	C660T	Ile 220 Ile
6	G666C	Lys 222 Asn
6	C678T	3'UTR

disease in our patient population occurred in 1 family in which 5 of 6 affected males have had 1 or more retinal detachments in one or both eyes. This family has a large deletion that totally eliminates exons 2 and 3 (data not shown) and is likely to result in the formation of a highly truncated protein or null product.

The following cases illustrate several outcomes of molecular screening for XLRS.

Case 1

A 27-year-old man complained of progressive night blindness over the past decade. His fundus showed an unusual picture of diffuse retinal pigment epithelium (RPE), with many patches of pigmentary disturbance in the mid and far periphery, which could reflect either a congenital abnormality or an acquired inflammatory or infectious process (Fig 2A). The peripheral retina had several regions of extensive retinoschisis without retinal detachment, but it was difficult to determine whether this was through the proximal retinal layers or lay deeper in the outer plexiform layer. A fluorescein angiogram showed extensive RPE involvement even across the macula, with hyperfluorescence (Fig 2B). There was no family history of other affected male or female relatives in the current or previous generations. The electroretinogram showed an "electronegative" response (Fig 3).

From this clinical presentation, it was difficult to know with certainty whether this was juvenile X-linked retinoschisis. Night blindness is not typical for XLRS. The diagnosis was further hindered by a compete lack of other affected relatives (which might indicate an X-linked recessive inheritance pattern), the extensive involvement of the outer retina, and the presence of multiple foci of RPE pigmentary disturbance consistent with possible inflammatory episodes. Sequencing of the XLRS1 gene found a Gly70Ser mutation (G→A change of bp 208) in exon 4. With this definitive molecular diagnosis, one can proceed with targeted medical care for the patient's retinal disease and counsel family members about their disease risks.

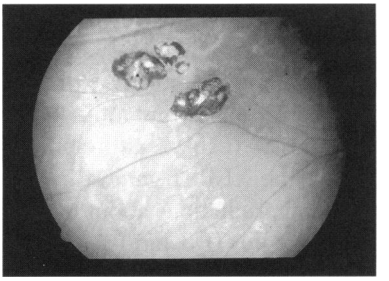

FIGURE 2A
Fundus photograph of nasal retina left eye shows three foci of RPE pigmentary disturbance and retinoschisis.

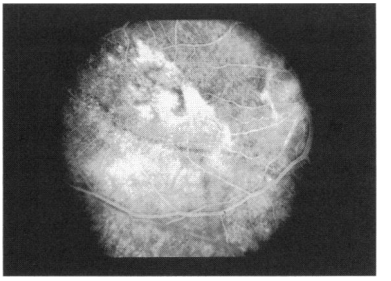

FIGURE 2B
Fluorescein angiogram of the right eye macula shows extensive RPE disturbance causing diffuse hyperfluorescence even across the fovea.

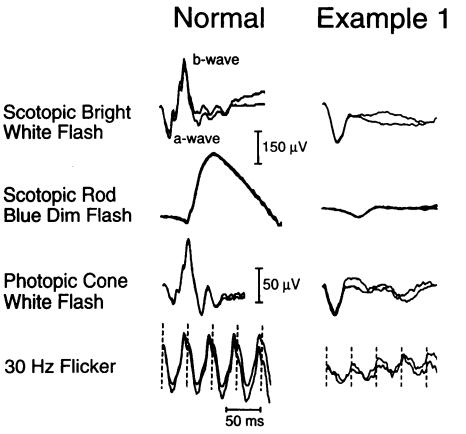

FIGURE 3
Electroretinogram showing "electronegative response" resulting from b-wave loss but a-wave preservation.

Case 2

The family in Fig 4 came to our attention because of a 6-month-old boy (III-3) who had esotropia and leukocoria. Examination with the patient under anesthesia revealed a total retinal detachment in one eye, and parafoveal spoke-wheel radiating cysts in the fellow eye. A diagnosis of juvenile X-linked retinoschisis was considered. The clinical course was very difficult, and multiple surgical retinal reattachment attempts for the

right eye had failed. The family was surprised by the provisional diagnosis of juvenile retinoschisis, because there was no family history of other affected males across 3 generations. Sisters of this boy's mother expressed considerable concern that they might be harboring a mutation in the XLRS1 gene, which would put their sons at risk. If this child's ophthalmologic problems were due to a mutation in the XLRS1 gene, then the rules of X-linked inheritance make very specific predictions about the risk to other members of the family. Specifically, females II-3 and I-2 are obligate carriers, while I-4, I-6, II-1, II-2, II-5, and II-6 are at risk for being carriers, and their children are also at risk (Fig 4A, indicated with asterisks). The parents questioned the clinical diagnosis, and they requested molecular testing.

Molecular screening of the affected baby boy III-3 found a missense mutation at nucleotide 598 in exon 6. This mutation had been previously reported in a number of XLRS families.[3] This finding established the diagnosis. Molecular screening for this mutation confirmed the carrier status in the maternal grandmother (I-2) and mother (II-3). The grandfather did not have this mutation (Fig 4B). Additional at-risk members of this fami-

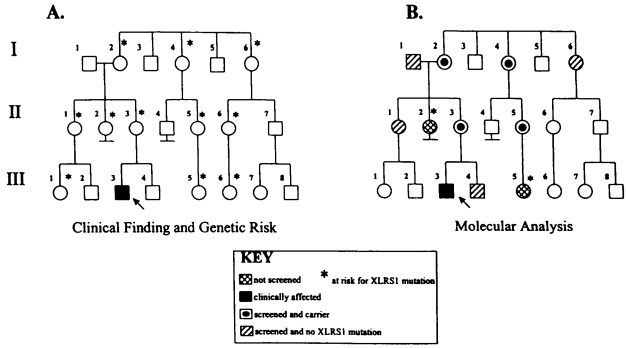

FIGURE 4A AND 4B

Pedigree of case 2. A, If male III-3 has juvenile retinoschisis, then female relatives indicated with asterisks are at risk for being carriers. B, Molecular screening identified an XLRS1 mutation in boy (III-3). Results of screening of at-risk females showed that some were carriers (e.g. II-3) and others were not (e.g. II-1). Some were at risk but chose not to be tested (e.g. II-2). This allows complete genetic counseling for the family.

ly also requested screening. Two additional females were carriers (I-4 and II-5), and 2 females were found to be noncarriers (I-6 and II-1). The parents of the proband were also concerned about the risk status for their 4-year-old son (III-4) and requested molecular screening. Molecular

screening found that he had not inherited the maternal XLRS1 gene that contains a mutation; he is not affected.

Case 3

Pedigree UMRS-1 shows a well-established XLRS pedigree with classic X-

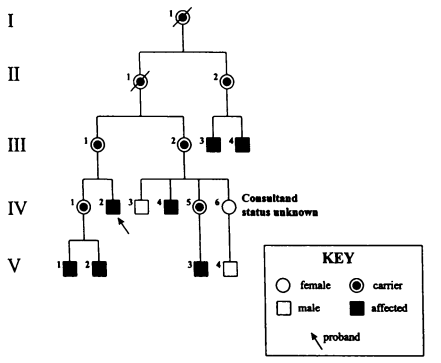

FIGURE 5

Pedigree UMRS-1 with juvenile retinoschisis. Woman IV-6 wants to know her carrier status.

linked recessive inheritance (Fig 5). Woman IV-6 is at 50% risk of being a carrier, and she requested molecular testing to learn her specific status. We have examined a number of her affected male relatives, and they have clinically certain juvenile X-linked retinoschisis, including the classic parafoveal "spoke-wheel" cystic cavities, peripheral retinal inner layer schisis, and an "electronegative" dark-adapted ERG with loss of the b-wave but minimal a-wave change. We previously published this pedigree as part of our linkage and mapping efforts toward cloning the juvenile retinoschisis gene.[4] Two individuals in this pedigree had recombination events that were important in defining the XLRS1 genetic interval. This family gave a LOD score of >2.0, which is further evidence that the disease-causing gene lies within the XLRS1 interval.[5] Despite this, when we subsequently sequenced the entire XLRS1 gene, we found no sequence changes in the family. This family is an example of a failure of our current methods of molecular screening for XLRS. Consequently, the result is uninformative for genotyping, and we cannot offer carrier testing for the at-risk female (IV-6) by sequencing the XLRS1 gene at this time. An alternative approach for this woman (IV-6) would be to perform haplotype analysis. Haplotype analysis is a much more extensive procedure and consequently is more expensive, and the result may be less definitive than tracking the specific mutation.

DISCUSSION

Molecular screening of the XLRS1 gene is an effective diagnostic tool that confirms and compliments the clinician's skill. It provides early detection of at-risk males. Often, young mothers with sons at risk for this disease are highly anxious, even when examination under anesthesia reveals no indication of disease. In some cases, these boys undergo repeated examinations under anesthesia, with cumulative medical risk from the multiple examinations alone. Providing specific molecular diagnosis of the presence or absence of the disease gene in these boys minimizes recurrent examinations of unaffected males. In contrast, the definitive molecular diagnosis of the XLRS disease gene in a young boy provides a specific indication for examination under anesthesia to determine possible involvement of the peripheral retina.

In the case of juvenile retinoschisis, the power of molecular screening arises from the fact that the test has a high degree of sensitivity, specificity, reliability, and informativeness. These terms indicate the following:

- *Sensitivity* is the frequency with which the test yields a positive result when the disease is present. This refers to the ability of the test to detect affected individuals.
- *Specificity* is the frequency with which the test yields a negative result when the disease is absent. This refers to the ability of the test to exclude those who are unaffected.
- Reliability means having a high degree of precision (the results of repeated testing on the same specimen are identical) and a high degree of accuracy (the test correctly identifies positive and negative results).
- Informativeness means the test results provide medical information that is relevant to the care of the patient.

Sequencing is a technique for mutation detection that can yield a result with a high degree of sensitivity, specificity, and reliability. The utility of this technique, however, depends on the gene that is being screened. Some genes are not amenable to diagnostic sequencing owing to the large size. For example, screening the Stargardt's ABCR gene with 50 exons presents a formidable technical challenge.[6] The molecular organization of the XLRS1 gene with a small number of exons, each of which is less than 200 bp, allows the use of sequencing as a reliable and cost-effective screening method. In addition, the presence of only a few sequence polymorphisms in the XLRS1 gene improves the informativeness of the screen. By contrast, the BRCA1/BRCA breast cancer genes are highly polymorphic, and it can be difficult to associate sequence changes

observed in currently unaffected women with the risk for developing overt disease.[7]

In case 1, juvenile X-linked retinoschisis was suspected, but the certainty of this clinical diagnosis was obscured by the atypical clinical presentation and the lack of relevant family history. Finding a mutation in the XLRS1 gene, and knowing that this is a common mutation in other XLRS families, pins down the diagnosis and allows for the best-directed medical care for the individual and genetic counseling for relatives. This demonstrates the sensitivity of XLRS1 molecular diagnosis.

Even though XLRS1 screening is a highly sensitive test, it does not work for all families. Case 3 points out the limitations in the sequencing methodology for mutation detection. Linkage analysis has localized the disease in this family to the XLRS1 with a high degree of certainty.[4] However, we have been unable to find a mutation within the coding sequence of the XLRS1 gene in this family. This limitation of gene screening is not uncommon. For example, one of our Best's families that maps to the Bestrophin gene does not yet have an identifiable mutation.[5]

Additional limitations include the following types of molecular changes: Sequence analysis in affected males with large deletions of either a part or the complete exon, or a sequence alteration in the region where primers hybridize for PCR amplification, cannot produce a PCR product. Negative results generated by these sequence alterations are uninterpretable and not totally trustworthy. In carrier females, these types of sequence changes exist in a heterozygous state and are also undetectable by PCR and sequencing. Large deletions can be analyzed with certainty by amplifying with primers to the sequences flanking the deletion boundaries. However, these boundaries are rarely known. While characterization of the deletion boundaries is theoretically possible, it requires significant effort owing to the limitations in the current technology. Sequence changes that involve a splice site should be used for diagnostic purpose with caution, because not all splice site alterations will affect the protein product. Clearer, but still cautious, diagnosis can be made if the segregation of these sequence changes is followed in the family.

Testing of at-risk females for their carrier status has three possible outcomes:

- Positive: The woman has a mutation that has already been found in her affected male relative, or that is known to occur in other XLRS families, and consequently is highly certain to cause the disease. Thus, she is a carrier. The informativeness of this result requires having current knowledge about which mutations are known to be associated with the disease. An updated list of XLRS1 mutations is published on the

Internet at www.dmd.nl/rs/rs.html. For other genetic diseases, a starting point to search for mutation information is through "Online Mendelian Inheritance in Man" (OMIM at www.ncbi.nlm.nih.gov/Omim/).

- Negative: This means the specific finding of the absence of a mutation that occurs in a woman's affected male relative. Thus, this woman is not a carrier. This implies that molecular screening of carriers must be performed with knowledge of the causative gene in a woman's affected male relative. The strength of the conclusion for these noncarrier females arises from the high specificity and reliability of the test.
- Uninformative: Sequencing of the XLRS1 gene in a woman yields no known mutation, and no information exists about the causative mutation in a male relative. The family in case 3 is a demonstration of an "uninformative result." This family has clinically certain juvenile X-linked retinoschisis, and linkage analysis indicates that the disease-causing gene in this family resides in the location of the XLRS1 gene. Consequently, the disease in this family is highly likely to involve the XLRS1 gene, but we have found no mutation in this family to date. As a consequence, testing of women who are at risk for the carrier state in this family will be uninformative until the causative genetic event is revealed in an affected male.

In case 2, the molecular analysis confirmed the diagnosis and provided precise risk estimates to both males and females in this family. Those females who are not carriers are free from the stress associated with uncertain genetic status, while male children who were previously believed to be at risk are also freed from concerns about developing the disease at a later date. Women who are found to be carriers can use this information to plan for the future with knowledge rather than fear. The benefits of a definitive diagnosis for males, refined risk analysis for females, and psychological relief are obvious. However, it is important to consider the associated risks. While this molecular screening has relieved some of the genetic burden and provides reassurance for some individuals in the family, it has also revealed novel information about other members who may not have otherwise considered themselves at risk.

There are unanswered questions about the impact of this information on the emotional health of the individual, on family relationships, on employability, and on health and life insurance coverage. It is important to consider molecular testing as more than a simple laboratory analysis; it needs to include pretest education and counseling, provision of informed consent, accurate interpretation of the test results and their implications, follow-up conveyance of the test results and their implications, and

posttest education, management, and support.[9-12] This is especially true for testing that determines the carrier status of a woman with otherwise undetermined status.

Finally, caution should be used in offering molecular diagnostic screening to young females who are at risk for the carrier state but who have not reached the age of consent. Clinical and ERG testing of these young at-risk girls in retinoschisis families is unrevealing, with very rare exception. Consequently, the only available diagnostic means is through gene testing. While one can strongly advocate that these women eventually need to learn their carrier status, there currently are a number of societal and personal medical issues involved in molecular testing before the age of consent.[13]

SUMMARY

Juvenile X-linked retinoschisis provides an interesting disease to consider as a model for molecular diagnostic testing of X-linked ophthalmic diseases. Among the factors to consider before embarking on testing are the probability that molecular screening of the gene will yield a true outcome, either true-positive or true-negative. Among the factors important for this outcome are the ability to make a clinically certain diagnosis in an affected male relative, to understand the pattern of inheritance, and to have this well defined in the family at hand. In addition, it is critical to know the degree of penetrance of the disease when a gene mutation is involved, the forms of disease-associated mutations, and the size and organization of the gene, a key determinant of the feasibility of laboratory testing. In the years ahead, rapid advances in molecular diagnostic testing for ophthalmic diseases can be expected, with the identification of additional disease-causing genes and the development of new automated gene scanning and sequencing methods.

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DISCUSSION

DR JOHN R. HECKENLIVELY. Members and guests, Dr Sieving has been a leader in X-linked juvenile retinoschisis research starting in 1990 with his efforts to better identify the specific site on the X chromosome of the gene[1] as well as his participation in the cloning effort.[2] His article focuses on the usefulness of molecular diagnostic techniques in confirming difficult cases of juvenile retinoschisis. While the diagnosis of X-linked juvenile retinoschisis (XLRS) in males is relatively easy in most instances, there are atypical presentations, such as a missing macular schisis,[3] severe schisis, or development of pigmentary retinopathy,[4] where molecular confirmation is very useful.

The carrier status of XLRS is impossible to detect by retinal examination, but a carefully constructed pedigree often identifies obligate carriers, that is, daughters of affected males. However, the daughters of an X-linked carrier have a 50% chance of inheriting the XLRS gene, and again there is no clinical test that detects the carrier status. Molecular testing is useful in determining carriers for XLRS and other X-linked diseases where the gene is known. The usefulness of having the knowledge of the carrier status, with today's level of technology and available health care for

genetic diseases, is perhaps less useful.

What are the choices for X-linked carriers today? They can avoid giving birth to male offspring given the 50% risk of passing the disease, but this poses a difficult ethical and personal quandary for most patients and many health providers. One technological advance that is expensive and not readily available is a method of laboratory fertilization where the father's sperm and mother's eggs are co-mixed, giving multiple early embryos. At about 3 days, one or two cells can be taken from each embryo and tested with polymerase chain reaction techniques for the known mutation. All nondiseased embryos can be identified, some used for implantation and others cryopreserved for later use. This technique has been performed successfully with cystic fibrosis parents. [5,6]

I would like to look at the issue of molecular diagnosis from a broader prospective. Medicine is essentially in the beginning of a new era in which molecular approaches are allowing us to identify chromosomal sites of genes and to then identify the actual gene and its products. Once the gene

TABLE I. SEQUENCE OF EVENTS FOR SOLVING AND TREATING HEREDITARY RETINAL DISEASES

1. Identify the gene locus
2. Isolate (clone) the gene
3. Identify the gene product and its interactions
4. Identify the key pathologic events
5. Design treatment strategies

product and the pathways involved in the process are found, the key pathologic events can be studied so that research approaches can be designed to counteract the abnormal gene action (Table I).

We are in a difficult period because we can diagnosis many hereditary ocular conditions on a molecular level, but in general we still do not have precise treatments available. The future clearly holds great promise for many different approaches for therapeutic intervention in inherited diseases, including gene and RNA therapies, or anti-apoptotic treatment with medications such as growth factors. Knowing the exact mutation that is causing the patient's disease will be essential for most therapeutic interventions currently under research.

Over the next decade, it will become increasingly important that patients obtain an accurate molecular diagnosis so that when molecular treatments commence, eligible patients will be easily identified, and the patients themselves also can assume the responsibility to learn if a treatment has become available for their disease. Patients are much better

informed today than in the past; it is not unusual for patients to search medical databases on the Internet and to e-mail questions to experts in the field asking for information about their disease. Patients who know their gene diagnosis will be able to follow the treatment status for their disorder.

Currently, many patients with inherited ocular diseases have nonspecific clinical diagnoses. When feasible, patients with hereditary ocular diseases should be referred to a specialist who can help them obtain a gene diagnosis by DNA analysis or by appropriate clinical studies. Unfortunately, the current tendency of HMOs to refuse to pay for testing and diagnosis for hereditary ocular diseases on the grounds "nothing can be done". This statement is no longer true, and the medical profession needs to put pressure on administrators to pay for these tests. Molecular testing is now at the forefront of the standard of care, and it already provides critical life shaping information to the patient. This will also lead to therapeutic interventions in the near future.

I think the importance of this talk is that it gives good insight into the role of molecular genetics into the diagnosis of hereditary retinal diseases. We now need to move forward with the use of these techniques for patient care. The problem is that the health care system is not yet fully ready for the use of these techniques. Insurance companies are reluctant to pay for it, and there is not yet a general recognition of the value of the techniques, in part because an understanding of these techniques and training in their use has not yet been given to most physicians who are practicing. We have a great challenge to try and move forward into this new era.

Dr Sieving has given us a preview of the usefulness of molecular testing using X-linked juvenile retinoschisis patients to illustrate the importance of establishing a complete diagnosis by defining the mutation in the XLRS gene. I would like to congratulate him on bringing these issues to the attention of the American Ophthalmological Society and for his leadership in the field of hereditary retinal diseases.

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DR KENT W. SMALL. I would like to congratulate Dr Sieving and his group. Positional gene cloning for disease genes is an extremely arduous task. Dr Sieving helped to lay the groundwork that allowed Christian Sauer in Germany to clone the gene. These tasks over the past decade have basically paralleled work of the the human genome project, and the actual cloning of this gene occurred because the Sanger Center in the UK was doing brute force sequencing of the X-chromosome in the exact region of the XLRS gene, at which point Christian Sauer pulled the sequences out of the database and was first to identify mutations in XLRS. I congratulate Dr Sieving on an excellent talk about utilizing genotyping of X-linked juvenile retinoschisis for clinical diagnosis.

DR DEVRON H. CHAR. It has become interesting to study the genomic alterations in tumor suppresser genes and find that many mutations are similiar to those Dr Sieving pointed out for juvenile retinoschisis, with many different missense point mutations and other types of mutations. The obvious import of the tumor gene work is to better understand where the protein is altered and thereby get to genetic therapy. I am wondering whether we can take that a step further in juvenile retinoschisis also.

DR WILLIAM S. TASMAN. I have one question for Dr Sieving. You mentioned that the petaloid pattern of macular cysts is reported to be present in about 95% of cases of males with juvenile retinoschisis, and yet you showed us a very nice example of a patient that could be confused with Stargardt's disease. I wonder whether you think that the 95% figure reported in the literature is too high?

DR PAUL A. SIEVING. Thank you for these insightful comments. We are in a period of medicine in which basic research is being translated into clinical practice, and it is very interesting right now to be a clinician in the area of hereditary eye diseases. The new complexity brought on by genetic information is nearly overwhelming. Already more than 40 genes have been identified as associated with various forma of retinal degeneration. As I mentioned on the last slide, on the X chromosome alone, the seemingly simple condition of X-linked retinistis pigmentosa now has been genetically mapped to at least 5 loci on the X chromosome, indicating that

there are at least 5 genetically different forms of X-linked RP. Eventually, the task facing each of us as ophthalmic physicians will be to diognose the precise genetic disease in each patient, in order to provide the patient with precise information about the nature and course of the disease, and ultimately to direct appropriate therapeutic intervention.

Dr Small gave a very brief, interesting history of the arduous work involved in gene cloning. Although disease gene cloning has only been going on for only a little more than a decade; it has now evolved to a point where nearly every issue of *Science* and *Nature* reports a new gene, and many of these are involved in ocular diseases. It is a molecular era in which we clinicians should be guided by the ultimate needs of our patients for precise diagnosis and development of treatment.

Dr Char raises an interesting point. Careful attention to the nature of the mutations in a gene, and the distribution of the mutation along the length of the gene, and the relationship of the precise mutation to disease severity, provides important clues to understand the role of the gene product in the disease. This, in turn, will help us devise appropriate future therapies.

A related issue is the relationship of mutations, or sequence changes, to disease. One needs to sort out the differences between real mutations and simple non-disease-causing sequence changes. As an example, this complexity hits home to many families concerning the breast cancer gene, the BRCA1 gene, which is one of several breast cancer genes. The relationship between a woman having a mutation in the BRCA1 gene and the event of developing breast cancer has been quite difficult to establish. Some women with mutation in the BRCA1 gene develop breast cancer whereas other women do not. This means that a clinician cannot rely on strict genetic determinism for our patient to develop disease. In other words, although gene sequencing has, in theory, great power for clinical diagnosis, the unknown frontier of information is about how genes control proteins and how the proteins effect disease. That is the area to be worked out in the years ahead.

Dr Tasman raises the interesting question as to the accuracy of the number in the literature, that 95% of case of juvenile retinoschisis show a petaloid pattern of cysts in the macula. We need to think about the origin of this number, because of the self-fulfilling way in which we clinicians function. In clinical diagnosis of a patient in our office, we start with the patient's state as a clinical unknown and interpolate toward a known category. We are appropriately reluctant to go beyond the bounds of what is known clinically. Consequently, we apply the diagnosis of juvenile retinoschisis to those cases that fit the established pattern, and the descriptions become self-fulfilling. One important aspect of the advent of molec-

ular diagnosis is to break out of the self-fulfilling of current clinical descriptors.

Specifically concerning the clinical task of diagnosing retinoschisis, I am most comfortable when I see this petaloid pattern. Of the 100 or so cases that I have looked at, I have set aside about twenty of those and given only a tentative diagnosis of retinoschisis. When we subsequently looked for mutations in the XLRS1 gene in these 20 cases, we found mutations in many of them even though the clinical phenotype had been at the extremes of the textbook disease spectrum. So, the new era of molecular descriptions of ophthalmic diseases is going to give us clinical verificaton and feedback that will modify our textbook descriptions. Ultimately this will help us understand how better to make these diagnoses and what the clinical range is for the manifestation of disease in these diagnoses. As some of our retinoschisis cases illustrated, surprises are always in store when we have new ways to look at disease.

Thank you.