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Which Leber congenital amaurosis patients are eligible for gene therapy trials?

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Abstract

Background—In 2007, clinical trials began for gene-replacement therapy for *RPE65*-associated Leber congenital amaurosis (LCA). To enroll, subjects must have both disease-causing *RPE65* alleles identified. Determining which patients have true disease-causing mutations requires a multistep approach.

Methods—This study is a retrospective case series using the estimate of pathogenic probability (EPP) algorithm and genotyping of family members to establish phase.

Results—Five probands and their families were studied. Patient 1 had genetic testing elsewhere and was reported to have 2 disease-causing *AIPL1* mutations. The family received incorrect prenatal counseling based on this result. We found both variations to be benign ethnic polymorphisms (EPP = 0). Case 2 had possible disease-causing mutations in *RPE65*, *RPGRIP1*, and *CRB1*; however, screening of family members revealed that only *CRB1* variations were disease causing and the *RPE65* change was a polymorphism found in 11% of African Americans. Case 3 had a diagnosis of *CRB1* associated LCA, but this mutation had an EPP = 0; a true homozygous disease-causing mutation was later found in *RDH12*. Patient 4 had 3 mutations found in *RPE65*, but only 2 were disease causing. Patient 5 had a homozygous mutation in *RPE65*. Only patients 4 and 5 would be eligible for clinical trials of *RPE65* gene replacement.

Conclusions—To be eligible for participation in current *RPE65* gene therapy trials, patients' DNA must contain 2 correctly segregating alleles with an EPP = 2 or 3. Interpretation of DNA variants is complex; genetic misdiagnosis may lead to ineffective treatment in some patients and lack of treatment in others.

Introduction

Leber congenital amaurosis (LCA) is a congenital retinal dystrophy present in approximately 1/80,000 births.¹ The typical presentation is an infant with nystagmus and very poor vision. The electroretinogram is severely reduced or nonrecordable. It is estimated that about 3,000 people in the United States are living with LCA. Leber congenital amaurosis and related early-onset retinal degenerations are caused by mutations in at least 15 genes: *GUCY2D*, *RPE65*, *CRX*, *AIPL1*, *CRB1*, *RPGRIP1*, *RDH12*, *IMPDH1*, *TULP1*, *CEP290*, *LCA5*, *SPATA7*, *RD3*, *LRAT*, and *MERTK*.² Most cases are inherited in an

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autosomal recessive manner.² However, LCA caused by mutations in the *CRX* gene is inherited as an autosomal dominant disorder, often caused by a new mutation,³ and *IMPDH1* causes early-onset autosomal dominant retinitis pigmentosa (RP).⁴

In 2001 a dog model of *RPE65* LCA was treated with subretinal injection of viral vector bearing a normal copy of the *RPE65* gene.⁵ This trial was successful in restoring some vision and a recordable ERG in the dog, and these effects have been long lasting. In 2008 three human gene therapy trials were reported using a similar technique. Most patients had some improvement in visual function, and there were no serious complications.^{6–8}

With the advent of a clinical treatment trial, patients, parents and physicians have a renewed interest in obtaining a genetic diagnosis to determine whether or not they are eligible for the current, or perhaps future, gene therapy trials. Genetic testing is much more widely available than ever before, but it is still not as simple as ordering more routine tests, such as a complete blood count or liver function studies. Genetic testing is designed to detect changes from the norm in the genetic code. However, only some of these changes, or mutations, are disease causing. The rest, called benign polymorphisms, are natural variations in the genome that do not cause disease. In ordering and interpreting genetic testing for patients with LCA, it is vitally important that a multistep approach be in place to ensure correct interpretation of variations found in the genome of patients. Patients with benign polymorphisms in the *RPE65* gene (and therefore true disease-causing mutations in some other, unidentified gene) who are erroneously enrolled in the *RPE65* gene replacement trial get no benefit, and their results falsely lower the successful outcome rate of the trial. Similarly, if a polymorphism is found in another LCA gene, making patients believe themselves ineligible for the *RPE65* trial, the patients, and the clinical trial, miss opportunities.

The Carver Nonprofit Genetic Testing Laboratory at the University of Iowa has detected or confirmed the mutations in the patients currently enrolled in U.S. *RPE65* clinical trials using a multistep testing strategy that employs an allele-specific screening test to detect known mutations, followed by confirmatory sequencing and/or sequencing of known LCA genes. The order of testing is determined by the mutation detection probability distribution (MDPD), an algorithm that predicts which genes and which areas of the genes are most likely to harbor disease-causing variants.⁹ Using the MDPD increases the rapidity of finding the disease-causing gene. The resulting mutations are assigned an estimate of pathogenic probability (EPP) from 0 (benign polymorphism) to 3 (probable disease-causing mutation).⁹

Methods

This study was a retrospective, selected case series. Probands were screened using an allele-specific SNplex (ABI) assay as described previously.¹ In brief, an allele-specific assay was devised for all known LCA-causing gene mutations reported in at least 2 individuals either in our laboratory or in the published literature. Variants detected with this assay were confirmed by automated DNA sequencing. If the SNplex assay was negative, sequencing of the known LCA genes was performed in a manner consistent with the known MDPD, meaning that the areas sequenced first were those most likely to harbor mutations. Mutations were scored using the EPP algorithm: EPP = 0 means that the change in DNA does not cause a change in an amino acid and/or is very unlikely to cause disease; EPP = 3 means that the change is highly probable to be disease causing; and EPP = 1 or 2 are intermediate.⁹ Family members then received genetic testing for phase, that is, for an autosomal recessive disease each parent should carry one of the child's mutant alleles. Similarly, each of the child's two mutations should lie on a different parental allele, confirming that there is no normal copy of the gene in the affected person. Two variations inherited on the same chromosome from one parent with a normal allele inherited from the other does not meet the

criteria for phase consistent with autosomal recessive transmission. Normal controls were matched for each patient's ethnicity. Appropriate University of Iowa Institutional Review Board (IRB) approval was obtained and the study conformed to requirements of the Health Insurance Portability and Accountability Act.

Case Reports

Patient 1

A Hispanic family presented for genetic testing for LCA. Their first child was born with nystagmus and blindness. Genetic testing elsewhere revealed 2 mutations in the *AIPL1* gene, which is known to be associated with LCA.¹⁰ The parents subsequently had prenatal testing for their second child to look for these 2 variations. The *AIPL1* variations were not found on the prenatal testing, and the parents were reassured. However the second child was born with LCA.

Examination of a large cohort of North American LCA patients¹ revealed that the *AIPL1* changes in this family are ethnic-specific polymorphisms in the Hispanic population with an EPP = 0. Thus this child does not have *AIPL1* associated LCA. Since the *AIPL1* polymorphisms are not contributing to the disease in this family, their absence in the fetus was not clinically relevant. This child's DNA can now be tested to discover the true disease-causing gene and to determine eligibility for a gene therapy trial.

Patient 2

An African American family presented with 3 affected boys and 1 unaffected sister. All 3 boys were found to harbor plausible disease-causing mutations in *RPE65*, *CRB1*, and *RPGRIP1*.

Analysis of the unaffected mother's DNA revealed that the 2 *RPGRIP1* variants were on the same allele so were unlikely to be disease causing. She was homozygous for the *RPE65* variant Ala434Val. Subsequent screening of African American controls showed that Ala434Val is present in 11% of the African American population; this is too common for the carrier frequency. The mother is homozygous and unaffected; thus it is likely a polymorphism. The mother and unaffected sister each carry 1 of the *CRB1* variants, and the affected brothers have 2 *CRB1* variants, each on a different allele. This is consistent with disease-causing autosomal recessive inheritance. The 2 *CRB1* variants had an EPP = 2 and 3. This result strongly supports *CRB1* being the causative gene in this family. These children are not eligible for the *RPE65* gene therapy clinical trial.

Patient 3

A family presented with one child diagnosed elsewhere with a *CRB1* variation that was reported to the family as being disease causing. However, only one allele was found.

In our lab, the *CRB1* change was confirmed but was a known benign polymorphism with an EPP = 0. Sequencing was performed in other LCA genes and a novel homozygous disease-causing mutation in *RDH12* was found. Thus this patient would not be a candidate for the *RPE65* gene replacement clinical trial, nor would the patient be a candidate for any *CRB1* specific trials in the future, though the patient might be a candidate for future therapies designed for *RDH12*-associated LCA.

Patient 4

A female patient with LCA was followed since infancy by one of the authors, and genetic testing was offered as soon as it became available. Three mutations were found in *RPE65*.

Two mutations were found to be on the same allele; one of these was found to be a benign polymorphism with an EPP = 0. The other 2 mutations were in correct phase, 1 from each parent, each with an EPP = 3. This patient is eligible for the *RPE65* gene therapy trial.

Patient 5

A child with nystagmus was suspected to have LCA. A homozygous variation in the *RPE65* gene was discovered with an EPP = 3. His parents were genotyped, and each carried the same allele. This child may be eligible for the gene therapy trial.

Discussion

Leber congenital amaurosis is a blinding disorder for which there are now clinical trials of gene replacement therapy. Gene therapy, in which a normal copy of the patient's defective gene is introduced under the retina with a viral or other vector, is absolutely specific to the dysfunctional gene in a given patient. That is, placing a normal copy of the *RPE65* gene under the retina of an LCA patient with a defective *CRB1* gene will not improve vision and may cause harm. For this reason the utmost care must be taken to confirm genetic diagnoses before proceeding with gene replacement therapy. This is not always a straightforward matter: much is still unknown about genetic disorders. Only about 65% of LCA patients have a mutation that can be found with current technology.¹ Many disease-causing mutations are novel when they are observed and must be investigated in detail to determine their pathogenicity. There are many non-disease-causing variations in the genome that must be differentiated from disease-causing mutations by a rational multistep approach, which usually includes genotyping family members to be sure that mutations are on different alleles. As our list of definite disease-causing mutations grows, and our understanding of modifier genes and other factors becomes deeper, genetic diagnosis will become more streamlined.

In conclusion, gene therapy for patients with *RPE65* associated LCA is now in clinical trials. Patients and families can be offered genetic testing, but results are complex and must be interpreted by practitioners with experience in both clinical and molecular genetics to avoid either ineffective treatment or lack of treatment due to an erroneous genetic diagnosis.

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