Prevalence of Mutations in eyeGENE Probands With a Diagnosis of Autosomal Dominant Retinitis Pigmentosa

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PURPOSE. To screen samples from patients with presumed autosomal dominant retinitis pigmentosa (adRP) for mutations in 12 disease genes as a contribution to the research and treatment goals of the National Ophthalmic Disease Genotyping and Phenotyping Network (eyeGENE).

METHODS. DNA samples were obtained from eyeGENE. A total of 170 probands with an intake diagnosis of adRP were tested through enrollment in eyeGENE. The 10 most common genes causing adRP (*IMPDH1*, *KLHL7*, *NR2E3*, *PRPF3/RP18*, *PRPF31/RP11*, *PRPF8/RP13*, *PRPH2/RDS*, *RHO*, *RP1*, and *TOPORS*) were chosen for PCR-based dideoxy sequencing, along with the two X-linked RP genes, *RPGR* and *RP2*. *RHO*, *PRPH2*, *PRPF31*, *RPGR*, and *RP2* were completely sequenced, while only mutation hotspots in the other genes were analyzed.

RESULTS. Disease-causing mutations were identified in 52% of the probands. The frequencies of disease-causing mutations in the 12 genes were consistent with previous studies.

CONCLUSIONS. The Laboratory for Molecular Diagnosis of Inherited Eye Disease at the University of Texas in Houston has thus far received DNA samples from 170 families with a diagnosis of adRP from the eyeGENE Network. Disease-causing mutations in autosomal genes were identified in 48% (81/170) of these families while mutations in X-linked genes accounted for an additional 4% (7/170). Of the 55 distinct mutations detected, 19 (33%) have not been previously reported. All diagnostic results were returned by eyeGENE to participating patients via their referring clinician. These genotyped samples along with their corresponding phenotypic information are also available to researchers who may request access to them for further study of these ophthalmic disorders. (ClinicalTrials.gov number, NCT00378742.)

Keywords: retinitis pigmentosa, eyeGENE, DNA sequencing, inherited retinal diseases, mutation prevalences

The pace of gene discovery for disease-causing mutations responsible for inherited eye diseases has accelerated over the past several decades. Disease-causing mutations have been found to cause cataracts, corneal dystrophies, glaucoma, strabismus, retinal degenerations, and many other ocular diseases. These diseases are responsible for vision impairment in approximately 40 million Americans. Over 500 genes have been identified as playing a role in inherited vision disorders (provided in the public domain at <http://www.ncbi.nlm.nih.gov/omim>).

This wealth of genetic information on ophthalmic diseases is paving the way for development of gene-based therapies.¹ Thirty years ago, clinicians could do little more than concede to individuals with an inherited eye disease that nothing could be done for them, but time has brought the field new genetic information and technologies. Novel gene-based treatments may soon be available for diseases that were once considered untreatable. As the field continues to move towards clinical trials for genetic therapies, there is a need to identify well-characterized patient populations to fulfill eligibility requirements for these trials. At the same time, it is essential to continue to study the effect of disease-causing mutations on the

fundamental biology of the visual system. The National Eye Institute (NEI), a part of the National Institutes of Health (NIH), established the National Ophthalmic Disease Genotyping and Phenotyping Network (eyeGENE, a registered trademark of the National Institutes of Health) in response to these needs and the potential opportunities for gene-based therapies and treatments.

The eyeGENE Network is a multidirectional genetics initiative whose primary purpose is to promote research into the genetic causes of rare eye diseases.^{2,3} This is accomplished by expanding patient access to genetic diagnostic testing, and by creating and maintaining a research database, DNA biorepository and patient registry of individuals and families referred from clinical organizations throughout the United States and Canada. The National Ophthalmic Disease Genotyping and Phenotyping Network is a component of the NEI intramural research program and is a model public/private partnership between the federal government, eye health care providers, molecular diagnostic laboratories certified under Clinical Laboratory Improvement Amendments (CLIA) standards, private industry, and extramural scientists supporting a broad research constituency. The eyeGENE Network currently

TABLE 1. adRP Genes and Regions Screened

Gene	Reference mRNA	Reference Protein	Region Screened
<i>IMPDH1</i>	NM_000883.3	NP_000874.2	c.875-1074
<i>KLHL7</i>	NM_001031710.2	NP_001026880.2	c.443-618
<i>NR2E3</i>	NM_014249.2	NP_055064.1	c.150-210
<i>PRPF3 (RP18)</i>	NM_004698.2	NP_004689.1	c.1427-1526
<i>PRPF31 (RP11)</i>	NM_015629.3	NP_056444.3	c.1-1500 (plus UTR exon 1)
<i>PRPF8 (RP13)</i>	NM_006445.3	NP_006436.3	c.6854-7180
<i>PRPH2 (RDS)</i>	NM_00322.4	NP_000313.2	c.1-1041
<i>RHO</i>	NM_000539.3	NP_000530.1	c.1-1047
<i>RP1</i>	NM_006269.1	NP_006260.1	c.1500-3200
<i>TOPORS</i>	NM_005802.3	NP_005793.2	c.1975-2820
<i>RP2</i>	NM_006915.2	NP_008846.2	c.1-1053
<i>RPGR</i> w/ORF15	NM_001034853.1	NP_001030025.1	c.1-3459
<i>RPGR</i> exons 16-19	NM_000328.2	NP_000319.1	c.1754-2448
<i>RPGR</i> exon 9A	NT_079573.4 (genomic)	None	g.1011927-1011634

includes eight CLIA diagnostic laboratory partners, more than 500 registered users from over 270 registered clinical organizations, and is now testing more than 100 genes for over 35 inherited eye disease categories. To date, eyeGENE has received over 4500 samples from individuals with rare, inherited eye diseases. In addition to offering diagnostic testing and supporting a corresponding DNA biorepository, the eyeGENE database allows for continued analysis of patient populations through genotype/phenotype correlations as well as helping to establish the prevalence of disease-causing mutations in the eyeGENE patient population.

The Laboratory for the Molecular Diagnosis of Inherited Eye Disease (LMDIED) at the University of Texas Health Science Center at Houston has been an eyeGENE partner since the inception of the initiative. Intake of samples involves patients seen at clinical centers throughout the United States and Canada. Blood samples from these patients are sent to the eyeGENE Coordinating Center CLIA Laboratory in Bethesda, MD. Once DNA is extracted, a portion is sent for diagnostic testing while another is stored in the eyeGENE biorepository for use in eyeGENE-approved research studies. After clinical data is vetted by the eyeGENE coordinating center, DNA samples from those participants with apparent dominant retinopathies are sent to LMDIED for genetic testing. Since 2007, over 450 individual eyeGENE samples have been tested through the Houston facility, representing both families with autosomal dominant retinitis pigmentosa (adRP) as well as an array of dominant maculopathies.

Here, we report findings from a cohort of families suspected of having adRP as determined by information provided by the referring clinician and the eyeGENE coordinating center. Pathogenic mutations were identified in nearly one-half of these samples, consistent with previous population surveys.

METHODS

Classification of Families for Testing

Individuals with an inherited eye disease eligible for testing through eyeGENE participated by referral through an eyeGENE-approved certified eye care specialist or genetics professional. Clinical details based on minimum criteria developed by eyeGENE were provided by referring clinicians and entered into the eyeGENE database. The clinical information and family history were reviewed by the eyeGENE coordinating center to confirm that a patient's phenotype was consistent with the diagnosis of retinitis pigmentosa (RP).

Dominant inheritance was presumed with evidence of affected individuals present in more than one generation, whether or not consecutive. Additionally, in the absence of male-to-male transmission, genes causing X-linked retinitis pigmentosa (XLRP) were requested as a follow-up test, once adRP-associated mutations were excluded.

This study was performed in accordance with tenets of the Declaration of Helsinki, and informed consent was obtained from each individual tested or from parents or guardians for individuals under age 18. The study was approved by the Committee for the Protection of Human Subjects of the University of Texas Health Science Center at Houston and by the Combined Neuroscience Institutional Review Board (CNS IRB) at the NIH, protocol 06-EI-0236.

DNA Extraction/Quantitation Protocols

For each individual enrolled in eyeGENE, a blood sample was collected in K2 EDTA tubes (Becton, Dickinson and Company, Franklin Lakes, NJ) and shipped to the eyeGENE coordinating center CLIA laboratory. Blood samples were received and processed according to CLIA regulations and the eyeGENE protocol.² Signed patient consent forms were also documented. After the receiving process was completed, whole blood was extracted by either manual or automated (Autopure LS; Qiagen, Valencia, CA) DNA extraction methods using the Gentra Puregene (Qiagen) chemistry. DNA concentration was measured using a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE). The majority of the extracted DNA was stored in the eyeGENE biorepository, while an additional de-identified portion was sent to the Houston facility for diagnostic testing.

PCR and Sequencing

All probands with a preliminary diagnosis of adRP were tested for mutations in the set of 10 genes that are the most frequent known causes of adRP (Table 1). All tests were validated by prior publications from this laboratory.⁴⁻⁸ In some cases (*RHO*, *PRPH2*, and *PRPF31*) the entire gene was screened. In the remaining genes, only regions considered mutation hotspots were screened. From 10 to 50 bp of intronic splice-donor and splice-recipient sequences were analyzed for each coding exon tested. Rhodopsin, the most common cause of adRP was tested first. Once a pathogenic mutation was identified, genes not yet screened were not tested further. If a variant was not clearly pathogenic, the entire panel of genes was tested until a mutation was determined or until the panel of genes was

TABLE 2. Patient Characteristics

Race	Ethnicity	Number of Probands in adRP Cohort	Number With Mutations	Percent of Category With Mutation Found
Hispanic	Unknown	11	4	36%
Not Hispanic	Asian	10	5	50%
Not Hispanic	Black or African American	9	6	66%
Not Hispanic	White	130	70	54%
Not Hispanic	Native Hawaiian or Pacific Islander	1	0	0%
Not Hispanic	American Indian or Alaska Native	0	NA	NA
Not Hispanic	More than one	3	2	66%
Unknown	Unknown	6	1	16%
Total		170	88	52%

exhausted. Families in which no pathogenic mutations were identified in the adRP panel and in which XLRP was a possibility (i.e., with no male-to-male transmission of disease) were tested for mutations in the X-linked genes *RPGR* and *RP2*.

All amplimers except ORF15 of *RPGR* were sequenced completely in both directions. For technical reasons, ORF15 can only be sequenced in one direction and a series of internal sequencing primers were used to provide complete, overlapping coverage.⁵ In general, genomic DNA was amplified for 35 cycles with AmpliTaq Gold 360 Master Mix (Applied Biosystems, Foster City, CA) and the appropriate M13-tailed primers (Supplementary Table S1). For *RPGR* ORF15, amplification was performed for 40 cycles and sequencing was done using additional internal primers (Supplementary Table S2). PCR product was treated with ExoSAP-IT (Affymetrix, Santa Clara, CA) and sequenced using BigDye v1.1 (Applied Biosystems). Sequencing reactions were purified using BigDye Xterminator (Applied Biosystems), run on a 3500 Genetic Analyzer (Applied Biosystems), and analyzed using SeqScape v2.7 (Applied Biosystems).

Analysis of Novel Variants

Variants identified by sequencing were analyzed to determine the likelihood of pathogenicity. First, existing databases of disease-causing mutations (e.g., the Human Gene Mutation Database Professional 2013.1, provided in the public domain at <http://www.hgmd.org>) were searched for previous reports of the variant. Additionally, databases of benign variation such as dbSNP (provided in the public domain at <http://www.ncbi.nlm.nih.gov/snp/>), the 1000 Genomes database⁹ (provided in the public domain at <http://browser.1000genomes.org/index.html>), and the NHLBI Exome Sequencing Project database (provided in the public domain at <http://evs.gs.washington.edu/EVS2>; Exome Variant Server; NHLBI GO Exome Sequencing Project [ESP], Seattle, WA [provided in the public domain at: <http://evs.gs.washington.edu/EVS>]) were also examined to determine if the variant was found in healthy controls. If a variant was not previously reported as either pathogenic or benign, standard tools such as SIFT,¹⁰ PolyPhen-2,¹¹ and MutationTaster¹² were used to assess the potential pathogenicity of the observed variant.

Reporting

Genetic testing reports were returned to the eyeGENE coordinating center from LMDIED and genetic testing results were entered online in the secure eyeGENE database. The eyeGENE coordinating center linked each coded, de-identified LMDIED report back to the patient and, as part of its quality assurance procedures, reviewed it for accuracy and consistency with standardized network methods of reporting. Once

approved, the results were returned to the referring clinician who would share and explain the results to the patient, and provide genetic counseling or assist the patient in seeking genetic counseling.

RESULTS

The adRP Cohort

The 170 probands tested for mutations in the adRP gene panel originated from families with between one and five generations of affected individuals. For 14 families (8%) with affected individuals in a single generation only, the hypothesis that the family had a dominant form of RP was weak. In several cases, clinicians had only anecdotal evidence of additional family members that would support a dominant form of inheritance. In other families, the clinicians suspected a particular gene to be the cause of disease, based on the clinical phenotype and, if true, the disease would be classified as dominant. In 9 of the 14 probands there was little hard evidence of a dominant mode of transmission.

Families with the strongest likelihood of truly having an autosomal dominant cause of disease based on family history were much more likely to have a pathogenic mutation in one of the genes in the adRP panel. While mutations were found in only 14% of the single-generation probands, 63% of the four-generation families had detectable mutations.

The ethnic makeup of the adRP cohort is shown in Table 2. The majority of samples were from patients whose referring clinicians identified their patients as "White," although all major ethnic groups were represented to some extent.

Clinical Categories and Prevalence

Clinical descriptions were provided by the referring clinician at the time of enrollment. All 170 families in the adRP cohort had a primary diagnosis of retinitis pigmentosa. Pathogenic mutations were identified in 52% of these families. Asian, African American, and White probands were most likely to have a pathogenic mutation in a gene from the adRP panel, while no mutations were found in the probands who described their background as Native Hawaiian or Pacific Islander.

Table 3 is a comprehensive list of the 55 different mutations found in the eyeGENE adRP cohort.

Novel Mutations

Nineteen mutations were found that have not been reported previously. Two novel *RHO* mutations were identified (p.Leu328Trpfs*32 and p.Glu341Glyfs*13), both of which are frameshifts in exon 5 that are predicted to produce abnormally long proteins. Other pathogenic frameshifts in exon 5 have

TABLE 3. Likely Pathogenic Mutations

DNA Change	Protein Change	Number of Occurrences in Cohort	Codon Changed	Reference
<i>RHO</i> NM_000539.3	<i>RHO</i> NP_000530.1			
c.44A>G	p.Asn15Ser	2	AAT-AGT	13
c.50C>T	p.Thr17Met	3	ACG-ATG	14
c.68C>A	p.Pro23H	17	CCC-CAC	15
c.152G>T	p.Gly51Val	2	GGG-GTC	16
c.173C>G	p.Thr58Arg	1	ACG-AGG	17
c.190C>T	p.Gln64X	1	CAG-TAG	18
c.392T>C	p.Leu131Pro	1	CTG-CCG	19
c.403C>T	p.Arg135Trp	3	CGG-TGG	20
c.404G>T	p.Arg135Leu	3	CGG-CTG	21
c.512C>T	p.Pro171Leu	1	CCA-CTA	16
c.541G>A	p.Glu181Lys	1	GAG-AAG	16
c.563G>A	p.Gly188Glu	1	GGA-GAA	18
c.647T>A	p.Met216Leu	3	ATG-AAG	22
c.982delC	p.Leu328Trpfs*32	1	CTG-TGG	This study
c.1021dupG	p.Glu341Glyfs*13	1	GGA-GGG	This study
c.1021G>A	p.Glu341Lys	1	GAG-AAG	23
c.1040C>G	p.Pro347Arg	2	CCG-CGG	24
c.1039C>T	p.Pro347Ser	1	CCG-TCG	17
<i>PRPH2</i> NM_00322.4	<i>PRPH2</i> NP_000313.2			
c.828+3A>T	p.?	2	NA	25
c.389T>C	p.Leu130Pro	1	CTG-CCG	This study
c.424C>T	p.Arg142Trp	1	CGG-TGG	Hoyng CB. <i>IOVS</i> 1995;36: ARVO Abstract S825
c.634A>G	p.Ser212Gly	1	AGC-GGC	26
c.646C>T	p.Pro216Ser	1	CCT-TCT	27
c.647C>T	p.Pro216Leu	1	CCT-CTT	28
<i>IMPDH1</i> NM_000883.3	<i>IMPDH1</i> NP_000874.2			
c.931G>A	p.Asp311Asn	2	GAC-AAC	29
<i>RPI</i> NM_006269.1	<i>RPI</i> NP_006260.1			
c.2029C>T	p.Arg677X	2	CGA-TGA	30
c.2167G>T	p.Gly723X	1	GGA-TGA	25
c.2172_2185del	p.Ile725Argfs*6	1	ATA-AGA	31
c.2180_2181delinsAA	p.Cys727X	1	TGT-TAA	This study
c.2181T>A	p.Cys727X	1	TGT-TGA	This study
c.2194C>T	p.Gln732X	1	CAG-TAG	This study
c.2285_2289del	p.Leu762Tyrfs*17	2	TTA-TAC	32
c.2585C>G	p.Ser862X	1	TCA-TGA	This study
<i>PRPF31</i> NM_015629.3	<i>PRPF31</i> NP_056444.3			
c.-3_7del	p.?	1	NA	This study
c.19_20insA	p.Leu7Hisfs*4	1	CTC-CAT	This study
c.267delA	p.Glu89Aspfs*11	1	GAA-GAT	This study
c.528-39_531del	p.?	1	NA	This study
c.808_809insC	p.His270Profs*8	1	CAC-CCA	This study
c.915_916insTGT	p.Val305_Asp306insCys	1	-/TGT	This study
c.1060C>T	p.Arg354X	1	CGA-TGA	This study
c.1084delA	p.Met362X	1	ATG-TGA	This study
<i>PRPF8</i> NM_006445.3	<i>PRPF8</i> NP_006436.3			
c.6901C>T	p.Pro2301Ser	1	CCC-TCC	33
c.6912C>G	p.Phe2304Leu	1	TTC-TTG	34
c.6991delG	p.Glu2331Argfs*28	3	GAG-AGG	De Erkenez AC. <i>IOVS</i> 2002;43: ARVO E-Abstract e791
<i>NR2E3</i> NM_014249.2	<i>NR2E3</i> NP_055064.1			
c.166G>A	p.Gly56Arg	1	GGG-AGG	35
<i>TOPORS</i> NM_005802.3	<i>TOPORS</i> NP_005793.2			
c.2554_2557del	p.Glu852Glnfs*13	1	GAG-CAA	This study
c.2556_2557delGA	p.Glu852Aspfs*20	1	GAG-GAC	36

TABLE 3. Continued

DNA Change	Protein Change	Number of Occurrences in Cohort	Codon Changed	Reference
<i>KLHL7</i> NM_001031710.2 c.457G>A	<i>KLHL7</i> NP_001026880.2 p.Ala153Thr	1	GCG-ACG	6
<i>RPGR</i> NM_001034853 (w/ORF15) c.360dupG c.1243_1244delAG c.1507-27_2729delins103 c.176G>T c.2309delA c.2323_2324delAG c.2405_2406delAG	<i>RPGR</i> NP_001030025.1 (w/ORF15) p.Leu121Alafs*3 p.Arg415Glyfs*37 p.? p.Glu726X p.Lys770Argfs*45 p.Arg775Glufs*59 p.Glu802Glyfs*32	1 1 1 1 1 1 1	GAG-TAG	This study 37 This study 38 39 38 40

been reported before,^{25,41} so it is likely that these new frameshifts are pathogenic as well. Of the eight unique mutations in *RP1*, four are novel and cause premature termination at three different positions in exon 4 (p.Cys727X, p.Gln732X, and p.Ser862X). All occur in the mutation hotspot of exon 4 and are likely to be pathogenic.

All of the eight *PRPF31* mutations are novel, and pathogenicity appears to be highly likely for each. Five of the eight are small insertions or deletions, three of which result in frameshifts (p.Leu7Hisfs*4, p.Glu89Aspfs*11 and p.His270-Profss*8); one creates an immediate stop codon (p.Met362X); and the last inserts an additional amino acid without changing the reading frame (p.Val305_Asp306insCys). Protein length is conserved in *PRPF31* and this insertion may disrupt an α -helix, so the Val305_Asp306insCys mutation is likely pathogenic. Two *PRPF31* mutations are deletions across coding/noncoding junctions. One starts in the 5'UTR and deletes the first two codons (c.-3_7del), while another deletes the intron/exon junction at the beginning of exon 7 (c.528-39_531del). In both cases, a normally functioning protein is not possible.

The single novel *PRPH2* mutation (c.389T>C, p.Leu130-Pro) is predicted to be pathogenic (PolyPhen-2 score = 1, SIFT score = 0, Grantham distance⁴² = 98) and has not been observed in healthy controls. Both new *RPGR* mutations are likely to be pathogenic, with one causing a frameshift in exon 5 (p.Leu121Alafs*3) and the other deleting exons 13, 14, and part of 15 (c.1507-27_2729delins103).

The only variant for which pathogenicity is uncertain is the novel amino acid substitution in the *TOPORS* gene (c.2264A>G, p.Asn755Ser) (Table 4). Bioinformatic analyses suggest that it is benign, as it occurs at a relatively non-conserved region of the protein and the two amino acids have similar biochemical characteristics (Grantham distance = 46). However, this variant is not found in any of the large datasets of healthy genome variation (1000 Genomes,⁹ NHLBI exome sequencing project). Only a single affected individual from the family was available for testing so there was no opportunity to look for segregation. The other novel *TOPORS* mutation is a frameshift (p.Glu852Glnfs*13) and is likely to be pathogenic.

DISCUSSION

Summary of Findings

The eyeGENE Network, by engaging private clinical practices in addition to larger academic institutions, has generated a pool of patient data and corresponding DNA samples to allow for identification of disease-causing mutations and novel variants in genes known to cause inherited eye diseases such as adRP.

In this study of 170 families, 55 mutations were found collectively in 88 probands, giving an overall detection rate of 52% (88/170). Unsurprisingly, mutations in *RHO* are the most common cause of adRP in this cohort. However, the rhodopsin fraction is inflated by the Pro23His mutation, which is a founder mutation largely unique to Americans of European origin.¹⁶ Forty-five probands had a total of 18 different pathogenic *RHO* mutations for an overall frequency of 26% (45/170), with the common p.Pro23His mutation accounting for 38% of *RHO* mutations (17/45). Mutations in *RP1* account for 6% (10/170) of the adRP cohort while mutations in *PRPF31/PRP11* have the next highest frequency, accounting for 5% (8/170). *PRPH2* (*RDS*) and *RPGR* mutations were each found in 4% of the cohort (7/170) while the remainder of pathogenic mutations were found in *PRPF8* (5/170, 3%), *IMPDH1* (2/170, 1%), *TOPORS* (2/170, 1%), *KLHL7* (1/170, 0.5%), and *NR2E3* (1/170, 0.5%) (Fig.).

Relation to Other Mutation Screening Projects

Mutation frequencies in the individual adRP genes examined in this cohort are consistent with those found in previous studies using Sanger sequencing^{4-8,43} while the overall success rate of finding a causative mutation was slightly lower.^{8,44} This is likely due both to inclusion of families that have very weak evidence for being dominant as well as screening only the most common subset of adRP genes and gene regions. In a nonoverlapping cohort of 258 adRP families studied previously in LMDIED, which were tested for all known adRP genes using multiple techniques, the overall success rate was 72%⁵ when dominant (or possibly X-linked) inheritance was clearly documented by family history.

TABLE 4. Variants of Unknown Significance

DNA Change	Protein Change	Number of Occurrences in Cohort	Codon Changed	Reference
<i>TOPORS</i> NM_005802.3 c.2264A>G	<i>TOPORS</i> NP_005793.2 p.Asn755Ser	1	AAT-AGT	This study

FIGURE. Fraction of mutations identified in the adRP cohort.

The effect of inclusion of more broadly classified patients is seen in the results from eyeGENE families in which only a single affected individual or generation was reported. In those probands, causative mutations were only found 16% of the time, this yield suggests a need to use more stringent inheritance criteria when a targeted adRP panel is used for diagnostic testing. However, stringent inheritance criteria will eventually become less relevant for diagnostic testing, though not for research, as next generation and exome sequencing become the norm.

Relation to Overall eyeGENE Goals

The data obtained in these studies contribute to the goals of eyeGENE in a number of ways. Individuals with inherited eye disease are provided increased options for genetic testing as well as the potential molecular confirmation of a clinical diagnosis. In addition, these individuals gain access to the larger clinical and vision research communities through their participation. In these probands, confirmed adRP or XLRP disease-causing mutations found through genetic testing provide patients with results they can use to enroll in gene-specific clinical trials. Patients also have the option to be recontacted by eyeGENE to participate in trials for which they may be eligible. A more immediate benefit of genotype data is facilitation of genetic counseling for these families. This includes clarifying patterns of inheritance and recurrence risks, and offers the possibility of predictive testing in at-risk relatives.

Furthermore, identification of novel mutations in adRP and XLRP genes yields additional insight into genotype/phenotype correlations as well as the normal functioning and cellular properties of causative genes.

Participation of CLIA-certified diagnostic laboratories in the eyeGENE Network allows these laboratories to access samples from individuals that may have otherwise been inaccessible. Many Network CLIA laboratories, such as LMDIED, have ongoing research studies on diseases whose genes they test. For example, in this study, individuals with no identified mutations in the adRP or XLRP genes or gene regions tested may participate in ongoing research studies at research institutions interested in gene discovery.

To further the main goal of eyeGENE, which is to facilitate research into inherited eye diseases, vision researchers are allowed to request access to patients, clinical data, and/or DNA samples from patients enrolled in the Network. The National Ophthalmic Disease Genotyping and Phenotyping Network provides molecular testing of ocular disorders while also building a controlled-access database, patient registry, and biospecimen collection. The eyeGENE Database contains information on family history, ophthalmic examination results, and additional clinical data making this population useful for gene discovery. Hundreds of samples are available from

patients in whom no mutations were found through testing or for whom no test was available through the program. This population lends itself to microarray-based mutation detection technologies, and next-generation whole exome and whole genome sequencing, being developed by eyeGENE Network participants and others.^{45–47} One goal of eyeGENE is to eventually genotype all samples in the repository and provide participants with a molecular understanding of their ocular condition.

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