Prevalence of Disease-Causing Mutations in Families with Autosomal Dominant Retinitis Pigmentosa: A Screen of Known Genes in 200 Families

Lori S. Sullivan,[1] Sara J. Bowne,[1] David G. Birch,[2] Dianna Hughbanks-Wheaton,[2] John R. Heckenlively,[3] Richard Alan Lewis,[4] Charles A. Garcia,[5] Richard S. Ruiz,[5] Susan H. Blanton,[6] Hope Northrup,[7] Anisa I. Gire,[1] Robyn Seaman,[1] Hatice Duzkale,[1] Catherine J. Spellicy,[1] Jingya Zhu,[1] Suma P. Shankar,[1] and Stephen P. Daiger[1,5]

Purpose. To survey families with clinical evidence of autosomal dominant retinitis pigmentosa (adRP) for mutations in genes known to cause adRP.

Methods. Two hundred adRP families, drawn from a cohort of more than 400 potential families, were selected by analysis of pedigrees. Minimum criteria for inclusion in the adRP cohort included either evidence of at least three generations of affected individuals or two generations with evidence of male-to-male transmission. Probands from each family were screened for mutations in 13 genes known to cause adRP: *CA4*, *CRX*, *FSCN2*, *IMPDH1*, *NRL*, *PRPF3* (*RP18*), *PRPF8* (*RP13*), *PRPF31* (*RP11*), *RDS*, *RHO*, *ROM1*, *RP1*, and *RP9*. Families without mutations in autosomal genes and in which an X-linked mode of inheritance could not be excluded were tested for mutations in ORF 15 of X-linked RPGR. Potentially pathogenic variants were evaluated based on a variety of genetic and computational criteria, to confirm or exclude pathogenicity.

Results. A total of 82 distinct, rare (nonpolymorphic) variants were detected among the genes tested. Of these, 57 are clearly pathogenic based on multiple criteria, 10 are probably pathogenic, and 15 are probably benign. In the cohort of 200 families, 94 (47%) have one of the clearly pathogenic variants and 10 (5%) have one of the probably pathogenic variants. One family (0.5%) has digenic RDS-ROM1 mutations. Two families (1%) have a pathogenic RPGR mutation, indicating that families with apparent autosomal transmission of RP may actually have X-linked genetic disease. Thus, 107 families (53.5%) have mutations in known genes, leaving 93 whose underlying cause is still unknown.

Conclusions. Together, the known adRP genes account for retinal disease in approximately half of the families in this survey, mostly Americans of European origin. Among the adRP genes, *IMPDH1*, *PRPF8*, *PRPF31*, *RDS*, *RHO*, and *RP1* each accounts for more than 2% of the total; *CRX*, *PRPF3*, and *RPGR* each accounts for roughly 1%. Disease-causing mutations were not found in *CA4*, *FSCN2*, *NRL*, or *RP9*. Because some mutations are frequent and some regions are more likely to harbor mutations than others, more than two thirds of the detected mutations can be found by screening less than 10% of the total gene sequences. Among the remaining families, mutations may lie in regions of known genes that were not tested, mutations may not be detectable by PCR-based sequencing, or other loci may be involved. (*Invest Ophthalmol Vis Sci.* 2006;47:3052–3064) DOI:10.1167/iovs.05-1443

Retinitis pigmentosa (RP) is the most common form of inherited retinopathy, with a prevalence of approximately 1 in 3500.[1] From linkage mapping, positional cloning, and candidate gene screening, at least 35 unique loci have been identified for nonsyndromic forms of RP. The underlying genes for 26 of these loci have been reported (RetNet; http://sph.uth.tmc.edu/RetNet/ provided in the public domain by the University of Texas Houston Health Science Center, Houston, TX). For autosomal dominant RP (adRP), which accounts for 20% to 40% of all cases, 14 genes have been identified (Table 1). Population surveys of subsets of these genes suggest that the known adRP genes may account for between one fourth to one half of cases or families (Table 1).

The Laboratory for Molecular Diagnosis of Inherited Eye Diseases was established in 1994 to determine the disease-causing genes and mutations in patients and families with RP. The Diagnostic Laboratory is a joint project of the School of Public Health and the Hermann Eye Center at the University of Texas Health Science Center in Houston. To date, more than 400 adRP families or probands have been ascertained by clinicians submitting samples to the Diagnostic Laboratory. From these families, we selected a set of 200 with a diagnosis of adRP and pedigree evidence to support this mode of inheritance. In this report, we summarize a systematic screen of these families for mutations in genes known to cause adRP.

We screened the 200 families for mutations in 13 of the 14 currently known adRP genes (Table 1). The thirteen genes have an expected prevalence of 2% or greater or an unknown prevalence. We did not screen for mutations in *GUCA1B* because previous surveys suggest that it accounts for much less than 1% of adRP in white subjects.[7] Also, for three genes,

From the [1]Human Genetics Center, School of Public Health, [5]Department of Ophthalmology and Visual Science, and [7]Division of Medical Genetics, Pediatrics Department, the University of Texas Health Science Center, Houston, Texas; the [2]Retina Foundation of the Southwest, Dallas, Texas; the [3]Kellogg Eye Center, University of Michigan, Ann Arbor, Michigan; the [4]Cullen Eye Institute, Department of Ophthalmology, Baylor College of Medicine, Houston, Texas; and the [6]Department of Pediatrics, University of Virginia, Charlottesville, Virginia.

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Corresponding author: Lori S. Sullivan, School of Public Health, the University of Texas HSC, 1200 Herman Pressler Drive, Houston, TX 77030; lori.s.sullivan@uth.tmc.edu.

PRPF3, *PRPF8*, and *RP1*, sequencing was limited to one exon each because previous surveys failed to find mutations outside of these exons[15,16,32] (De Erkenez AC, et al. *IOVS* 2002;43: ARVO E-Abstract 791).

In addition, we tested selected families for mutations in open reading frame (ORF) 15 of the X-linked RP gene *RPGR*. Although X-linked RP usually affects males only, some *RPGR* mutations, particularly in ORF 15, can cause disease in "carrier" females with a simulation of autosomal dominant inheritance.[28,33,34] Females are usually more mildly affected than males in these families, but the pedigree may be misconstrued as adRP. Thus some "adRP" families without mutations in known adRP genes may, in fact, have X-linked RP.

A major difficulty in screening for mutations in dominant-acting genes is to distinguish rare, benign sequence changes from pathogenic mutations.[35] Often, the number of affected individuals available for testing is not large enough to establish pathogenicity by segregation alone. Ideally, functional studies could distinguish benign from pathogenic protein changes. However, the genes implicated in dominant RP are a heterogeneous group with widely differing functions. Many are predominantly or exclusively photoreceptor-specific. Rhodopsin and GUCA1B are the only known adRP-associated proteins directly involved in phototransduction; but RDS and ROM1 maintain disc structure in photoreceptors, CRX and NRL are retinal transcription factors, and RP1 and RPGR localize to the connecting cilium of photoreceptors. In contrast, CA4 and IMPDH1 code for widely expressed "housekeeping" enzymes, and three other adRP-associated proteins—PRPF3, PRPF8, and PRPF31—are highly conserved, ubiquitously expressed components of RNA-splicing complexes. Mechanisms by which mutations lead to retinal disease are also highly varied (see Table 1 and RetNet).

Because of this functional heterogeneity, there is no general method for evaluating genetic variation within each gene and assessing pathogenicity of rare variants. To establish pathogenicity in this survey we used a combination of segregation information, general computational approaches and individualized evaluation based on the unique characteristics of each gene and protein.

METHODS

Patients, Families, and Control Subjects

Among the approximately 650 families or probands enrolled in studies at the Laboratory for Molecular Diagnosis of Inherited Eye Diseases, over 400 have a diagnosis of dominant RP. From these, we selected 200 families with a diagnosis of adRP at submission and pedigree evidence of autosomal dominant transmission. Our criteria were either the presence of three or more generations, with both males and females among all affected family members, or at least two affected generations with male-to-male transmission. The requirements for three generations, including females, or for male-to-male transmission, reduced the likelihood of including X-linked families.

Probands and other family members were ascertained primarily in Texas, California, and Michigan as part of studies in collaboration with investigators at (1) the Anderson Vision Research Center, Retina Foundation of the Southwest (Dallas, TX; DGB), (2) the Cullen Eye Institute, Baylor College of Medicine (Houston, TX; RAL), (3) the Hermann Eye Center, the University of Texas Health Science Center at Houston (CAG, RSR), (4) the Jules Stein Eye Institute, UCLA School of Medicine (Los Angeles, CA; JRH), and (5) the Kellogg Eye Center, University of Michigan (Ann Arbor, MI; JRH). Additional clinicians who submitted samples are listed in the Acknowledgments. Diagnostic testing included a complete ophthalmic and fundus examination; measurement of visual acuity, visual fields and dark adaptation; and electroretinography. Pedigrees were constructed based on patient interviews. Ethnicity is that reported by the proband.

Pedigrees were analyzed to determine the likelihood of autosomal dominant inheritance relative to autosomal recessive or X-linked inheritance. Each pedigree was coded into LINKAGE format with an invariant genotype at a dummy locus for each individual.[36] The likelihood at 0% recombination to the dummy locus was then determined for each family based on three models: autosomal dominant inheritance with 95% penetrance, autosomal recessive inheritance with 99% penetrance in homozygotes, and X-linked inheritance with 10% penetrance in heterozygous females and 99% penetrance in males. The disease allele frequency for each model was set to 0.001 and the a priori probability of each mode was assumed to be equal. Likelihood ratios for autosomal dominant versus recessive, and autosomal dominant versus X-linked, were then calculated.

White control DNAs were from 50 unrelated CEPH (Centre d'Etude du Polymorphisme Humain) parent pairs.[37] DNAs from Caribbean controls ($n = 47$) were kindly provided by Edwin Stone (University of Iowa, Iowa City, Iowa).

Blood and DNA samples were provided by our clinical collaborators under approved protocols. If blood was provided, DNA was prepared with a commercial extraction kit (PureGene; Gentra, Minneapolis, MN).[3,32]

The study was performed in accordance with the Declaration of Helsinki and informed consent was obtained from all participants. The research was approved by the Committee for Protection of Human Subjects, University of Texas HSC, Houston, and by the respective human subjects' review boards at each participating academic institution.

Mutation Testing

DNA testing of *CA4*, *FSCN2*, *NRL*, *PRPF3*, *PRPF8*, *PRPF31*, *ROM1*, and *RP9* was entirely by cycle sequencing. Mutation screening of *CRX*, *IMPDH1*, *RDS*, *RHO*, and *RP1* in earlier years was done by single-strand conformational analysis (SSCA).[3,9,32,38] Variants detected by SSCA were sequenced to determine the underlying nucleotide change. All recent testing of these genes was performed by direct sequencing.

PCR primers, annealing temperatures, and amplimer-specific details are listed in the Supplementary Tables, available online at http://www.iovs.org/cgi/content/full/47/7/3052/DC1. In general, 30–50 ng of genomic DNA was amplified with Taq polymerase (AmpliTaq Gold; Applied Biosystems [ABI], Foster City, CA, or HotStarTaq; Qiagen, Valencia, CA) in a 12.5 μL reaction volume for 35 cycles.

SSCA was conducted by incorporating 1 μCi of [^{32}P]dCTP during PCR amplification. Radiolabeled fragments were separated by electrophoresis overnight on acrylamide gels (0.6 × MDE; FMC Bioproducts, Cambrex BioSciences, Walkersville, MD). Dried gels were visualized by autoradiography. (See Bowne et al.,[32] Bowne et al.,[9] Sohocki et al.,[38] and Sohocki et al.[3])

DNA sequencing was performed in forward and reverse directions as described previously.[9] PCR products were treated with exonuclease I and shrimp alkaline phosphatase (ExoSapIt; USB, Cleveland, OH) and sequenced bidirectionally with fluorescent dye-terminators (BigDye v1.1; ABI) using primers listed in the Supplementary Tables, available online at http://www.iovs.org/cgi/content/full/47/7/3052/DC1. Sequence reactions were purified with sephadex columns (Princeton Separations, Adelphia, NJ) and run on an automated capillary sequencer (either an ABI 310 or ABI 3100 Avant Genetic Analyzer). Sequence analysis was done using commercial software (either AutoAssembler or SeqScape software; ABI). (See Bowne et al.,[32] Bowne et al.,[9] and Sohocki et al.[3])

Families in which a definitely pathogenic mutation was found were not routinely tested for further mutations. Thus, not all genes were sequenced in all families.

IMPDH1 Haplotyping

Published SNPs within 250,000 bp of the *IMPDH1* gene were amplified by PCR and typed by DNA sequencing.[39] Haplotypes for the region

Table 1. Autosomal and X-Linked Genes Known to Cause Dominant Retinitis Pigmentosa

Symbol	Protein	Location	Prevalence	Population	References
CA4 (*RP17*)	Carbonic anhydrase 4	17q23.2	—		2
CRX	Cone-rod homeobox transcription factor	19q13.32	2/206 (1%) 0/148 (0%)	American Spanish	3, 4
FSCN2	Fascin 2	17q25.3	4/120 (3%)	Japanese	5, 6
GUCA1B	Guanylate cyclase activating protein 1B	6p21.1	0/200 (0%) 3/63 (5%)	British Japanese	7, 8
IMPDH1 (*RP10*)	Inosine monophosphate dehydrogenase 1	7q32.1	2/60 (3%) 7/183 (4%) 2/96 (2%) 6/190 (2%)	American American Japanese North American	9–11*
NRL	Neural retina leucine zipper	14q11.2	4/189 (2%) 3/200 (2%) 0/96 (0%) 1/148 (1%)	American British Japanese Spanish	4, 12–14†
PRPF3 (*RP18*)	Pre-mRNA processing factor 3	1q21.2	1/96 (1%) 1/150 (1%)	Japanese Spanish	15, 16†
PRPF8 (*RP13*)	Pre-mRNA splicing factor C8	17p13.3	4/190 (2%) 0/96 (0%) 5/150 (3%)	American Japanese Spanish	16, 17†,‡
PRPF31 (*RP11*)	Pre-mRNA splicing factor 31	19q13.42	4/96 (4%) 3/150 (2%)	Japanese Spanish	16‡
RDS	Peripherin 2	6p21.2	17/206 (8%) 5/96 (5%) 2/148 (1%)	American Japanese Spanish	3, 4, 18†
RHO	Rhodopsin	3q22.1	53/206 (26%) 2/96 (2%) 19/90 (20%) 29/148 (20%)	American Japanese North American Spanish	3, 4, 19, 20†
ROM1	Rod outer membrane protein 1	11q12.3	0/173 (0%) 2/224 (1%)	American North American	21–23
RP1	RP1 protein	8q12.1	8/206 (4%) 21/266 (8%) 1/96 (1%) 5/148 (3%)	American British Japanese Spanish	3, 4, 24–26†
RP9 (*PAP1*)	Pim-1 associated protein	7p14.3	—		27
RPGR	Retinitis pigmentosa GTPase regulator	Xp11.4	—		28, 29

Gene symbols are those approved by the HUGO Nomenclature Committee (http://www.gene.ucl.ac.uk/nomenclature/) followed by the alternate symbol, if any, in parenthesis. Genes are listed alphabetically by symbol here and in the text. Alternate symbols are not used further, except to distinguish the pre-mRNA splicing proteins. Map locations are based on the assembled human genome (May 2004 build) using the UCSC Genome Browser (http://genome.ucsc.edu/). Affected/total (percent) is unrelated adRP probands or families, with probable or definite pathogenic mutations, based on surveys of more than 50 individuals, as can best be deduced from the publication. Note: additional prevalence values are reported by Ramesar et al.[30] and Ziviello et al.[31]

* Wada Y, et al. *IOVS* 2003; 44: ARVO E-Abstract 2306.

† Wada Y et al. *IOVS* 2004; 45: ARVO E-Abstract 2456.

‡ De Erkenez AC et al. *IOVS* 2002;43: ARVO E-Abstract 791.

were reconstructed after typing multiple family members carrying the *IMPDH1* Asp226Asn mutation.

Analysis of Variants

All nonpolymorphic variants were sequenced at least twice in proband samples for confirmation. When available, additional family members were also sequenced to determine the presence or absence of the variant.

Several Web-based analysis programs were used to determine the likelihood that specific variants are pathogenic. Splice-site variants were analyzed using the prediction program NNSPLICE 0.9 (http://www.fruitfly.org/seq_tools/splice.html).[40] The Web-based program PolyPhen (Polymorphism Phenotyping, http://www.bork.embl-heidelberg.de/-PolyPhen/ provided in the public domain by the European Molecular Biology Laboratory, Heidelberg, Germany)[41] was the starting point for analysis of amino acid substitutions. In addition, we used SIFT (Sorting Intolerant from Tolerant: http://blocks.fhcrc.org/sift/SIFT.html/ provided in the public domain by the Fred Hutchinson Cancer Research Center, Seattle, WA)[42] as a measure of sequence conservation and Grantham scores as a measure of the chemical change in amino acids.[43]

The first step of the PolyPhen analysis was locating the sequences in the current protein databases to identify relevant annotation of protein structure and function. Individual amino acid changes were queried and, if the amino acid change was at an annotated site (e.g., a disulfide bond or a binding site), this information was used in the subsequent analysis. If the amino acid change was within a defined site, appropriate subprograms determined whether the amino acid change would affect the site (e.g., PHAT to characterize transmembrane domain changes or COILS to predict secondary structure).

Next, via BLAST, an alignment of homologous sequences was constructed to assess the level of sequence conservation at the site of the change. The PSIC (position-specific independent counts) subprogram calculated the "profile matrix" for the specific set of sequences and this matrix evaluated the amino acid change in question. The difference in profile scores for the two amino acids is a measure of how often (if ever) that substitution has occurred in the protein family.

The last stage of the PolyPhen analysis involved mapping the site of the amino acid change to the known three dimensional (3-D) structure

of the protein by a BLAST search of the protein structure databases (PDB or PQS) and analyses of matches. Amino acid changes were assessed for their effect on the hydrophobic core of the protein, possible interactions with ligands, and other known features of the protein structure. The results of all these analyses were merged to produce a prediction of how damaging the amino acid substitution is likely to be. Variants evaluated by PolyPhen were classified as probable, possible, benign, or unknown.

RESULTS

Probands and Families

The demographic and pedigree characteristics of the 200 adRP families included in this study are summarized in Table 2 (details in Supplementary Tables, http://www.iovs.org/cgi/content/full/47/7/3052/DC1). Ninety-three percent of the families had three or more affected generations, and 25% had five or more. Nearly 70% were known to have five or more affected family members (living or dead), and male-to-male transmission was observed in more than half. Skipped generations were reported in 24% of families. The likelihood ratio for autosomal dominant versus autosomal recessive inheritance was 10^3 or greater for each family, with only one exception—a small family with a skipped generation (data in Supplementary Tables). The likelihood ratio of autosomal dominant versus X-linked inheritance was at least 10^3 for more than 70% of families, and more than 15% had suggestive evidence for dominant inheritance (odds of 10–10^2). However, 12% of families had a likelihood ratio that does not exclude X-linkage or actually favors X-linked inheritance (odds of 1 to $<10^{-2}$). Ethnicity was at least 73% white, but inclusion of Hispanics and some of the "unknown" families would bring the proportion of "Americans of European origin" close to 90%.

DNA samples from four or more affected family members were available from one-third of the families. Conversely, another third of the families were represented by a DNA sample from a single affected proband only. However, the "proband only" families are not demographically different from families with multiple samples.

TABLE 2. Summary Characteristics of Families

	Number (%)
Generations of known affected	
2	13 (6.5)
3	72 (36.0)
4	65 (32.5)
5 or more	50 (25.5)
Unknown	0
Number of known affected	
2–4	64 (32.0)
5–10	88 (44.0)
11–20	37 (18.5)
>20	11 (5.5)
Unknown	0
Male-to-male transmission	
No	91 (45.5)
Yes	108 (54.0)
Unknown	1 (0.5)
Skipped generations	
0	150 (75.0)
1–2	47 (23.5)
>2	2 (1.0)
Unknown	1 (0.5)
Odds adRP v. XIRP	
$\geq 10^3$	143 (71.5)
10–10^2	33 (16.5)
1–10^{-1}	21 (10.5)
$\leq 10^{-2}$	3 (1.5)
Unknown	0
Ethnicity	
Asian	7 (3.5)
African American	6 (3.0)
White	146 (73.0)
Hispanic	14 (7.0)
Other	4 (2.0)
Unknown	23 (11.5)

N = 200.

Mutation Screening

Summary. The potential disease-causing variants found in the 13 adRP genes and ORF 15 of *RPGR* in the 200 families are listed in Table 3. Coded family numbers, specific mutations, and related data are in the Supplementary Tables, http://www.iovs.org/cgi/content/full/47/7/3052/DC1. Variants are ranked as pathogenic, probably pathogenic, or benign based on criteria described in the next section. The table includes polymorphic variants if they were not known to us before observation in an affected proband. However, the table does not include a systematic listing of the many known polymorphic variants found in these genes among the individuals screened.

A total of 82 distinct, rare (nonpolymorphic) variants were detected. Of these, 57 are clearly pathogenic based on multiple criteria, 10 are probably pathogenic, and 15 are benign. In the cohort of 200 families, 94 (47%) have one of the clearly pathogenic variants and 10 (5%) have one of the probably pathogenic variants. One family (0.5%) has digenic RDS-ROM1 mutations. Two families (1%) have a pathogenic *RPGR* mutation, indicating that families with apparent autosomal transmission may actually have X-linked RP.

Determining Pathogenicity. Assessing whether a novel variant in a known disease-causing gene is pathogenic is often challenging. The first considerations are whether the variant segregates with disease, has been found in other affected individuals or families, and is absent from appropriate control subjects. Segregation may not be definitive, though, because in many cases only a few family members are available for testing, and an unaffected individual with a pathogenic mutation may represent incomplete penetrance rather than failure to segregate. A mutation that introduces a radical change in the message or protein is probably pathogenic, but the extreme situation—a null allele—may not be pathogenic by itself. Finally, missense mutations are particularly problematic because the background variation in human proteins is so great.

We assessed each potentially pathogenic mutation by several criteria, with results summarized in Table 3. The table shows whether the variant segregates with disease, has been reported previously, or is present in control subjects. Also indicated is whether a mutation introduces a radical change in the message or protein. Finally, a scoring system was used to evaluate missense changes.

The scoring system used in Table 3 is based on several techniques to assess whether a particular amino acid substitution is pathogenic (see the Methods section and the legend to Table 3). The techniques range from a simple assessment of "chemical conservation" based on structural and biochemical properties of individual amino acids,[43] to methods that consider context-dependent properties of an amino acid within a particular protein. The most advanced methods use several sequential analyses: alignment of related sequences to determine evolutionary conservation, database searching to determine the location of critical positions such as active sites or disulfide bonds, and analysis of 3-D models.[41] These analyses

TABLE 3. Potentially Pathogenic Variants Identified in the adRP Cohort

Type Variant	Mutation: Protein	Mutation: Nucleotide	Families (*n*)	Scores: Seg.	Scores: Prior	Scores: Struct.	Scores: SIFT	Scores: Funct	Scores: PolyP.	Scores: Gran.	Comment
CA4—carbonic anhydrase 4											
Pathogenic	None										
Probably pathogenic	None										
Benign	Thr198Met	593 C→T	1	No	No	—	No	—	Possib.	81	.96/.04 in African control subjects
	Val234Ile	700 G→A	1	No	No	—	No	—	Benign	29	In one control
	Val237Leu	709 G→T	1	No	No	—	No	—	Benign	32	.98/.02 in African control subjects
	Arg239Gln	716 G→A	1	No	No	—	No	—	Benign	43	.95/.05 in African control subjects
CRX—cone rod homeobox protein											
Pathogenic	Arg41Gln	122 G→A	1	5	Yes[38,44]	—	Yes	—	Prob.	43	
	Leu146_Pro149del	436_447del	1	15	Yes[38]	—	NA	—	NA	NA	
Probably pathogenic	None										
Benign	None										
FSCN2—retinal fascin											
Pathogenic	None										
Probably pathogenic	None										
Benign	Val17Ile	49 G→A	1	No	No	—	No	—	Benign	29	In one white control subjects
	Gly165Arg	493 G→A	1	No	No	—	No	—	Benign	125	
	Pro231Ser	691 C→T	1	No	No	—	No	—	Benign	74	
	Ala323Thr	967 G→A	1	No	Yes[45]	—	Yes	—	Possib.	58	.96/.04 in white control subjects
	Leu489Val	1465 C→G	1	No	No	—	No	—	Benign	32	.98/.02 in white control subjects
IMPDH1 (RP10)—inosine monophosphate dehydrogenase											
Pathogenic	Asp226Asn	676 G→A	5	20+	Yes[9]	—	Yes	Yes	Prob.	23	Affects CBS domain[9]
Probably pathogenic	None										
Benign	None										
NRL—neural retinal leucine zipper											
Pathogenic	None										
Probably pathogenic	None										
Benign	Arg147Arg	441 G→A	2	Prbn.	No	—	—	—	—	—	Silent substitution
	Gln174Arg	521 A→G	1	No	No	—	Yes	—	Prob.	43	
PRPF3 (RP18)—pre-mRNA splicing factor 3											
Pathogenic	Pro493Ser	1477 C→T	1	Prbn.	Yes[15]	—	Yes	—	Prob.	74	
	Thr494Met	1481 C→T	1	Prbn.	Yes[15]	—	Yes	—	Prob.	81	
Probably pathogenic	None										
Benign	None										
PRPF8 (RP13)—pre-mRNA splicing factor 8											
Pathogenic	Phe2304Leu	6912 C→G	1	2	Yes[17]	—	Yes	—	Prob.	22	
	Arg2310Gly	6928 A→G	1	2	Yes[17]	—	Yes	—	Prob.	125	
	Glu2331fs/ter 2358	6991delG	2	2	No	fs	NA	NA	NA	NA	
Probably pathogenic	IVS 41/exon 42 junction	IVS41-4 G→A	1	Prbn.	No	as	NA	NA	NA	NA	
	Ala2328Val	6983 C→T	1	Prbn.	No	—	No	—	Benign	64	
Benign	None										

TABLE 3. (*continued*). Potentially Pathogenic Variants Identified in the adRP Cohort

Type Variant	Mutation		Families (*n*)	Scores							Comment
	Protein	Nucleotide		Seg.	Prior	Struct.	SIFT	Funct	PolyP.	Gran.	
PRPF31 (RP11)—pre-mRNA splicing factor 31											
Pathogenic	Exon 2/IVS 2 junction	IVS2+1 G→A	1	Prbn.	No	as	NA	NA	NA	NA	
	Gln74ter	220 C→T	1	Prbn.	No	ps	NA	NA	NA	NA	
	Asn131fs/ter197	390delC	1	3	No	fs	NA	NA	NA	NA	
	Glu141ter	421 G→T	1	Prbn.	No	ps	NA	NA	NA	NA	
	Met212fs/ter238	636delG	1	2	No	fs	NA	NA	NA	NA	
	Gly253fs/ter317	758_767del	1	Prbn.	No	fs	NA	NA	NA	NA	
	Exon 10/IVS 10 junction	1049_IVS10+20del/insCCCCT	1	Prbn.	No	as	NA	NA	NA	NA	
	Exon 10/IVS 10 junction	IVS10+1 G→A	1	5	No	as	NA	NA	NA	NA	
	Glu325ter	973 G→T	1	Prbn.	No	ps	NA	NA	NA	NA	
Probably pathogenic	Ala291Pro	871 G→C	1	Prbn.	No	—	NA	NA	Possib.	27	
	Cys299Arg	895 T→C	1	4	No	—	NA	NA	Prob.	180	1 Nonpenetrant if pathogenic
Benign	Gly272Val	815 G→T	1	No	No	—	NA	NA	Prob.	109	Chinese variant
RDS—peripherin 2											
Pathogenic	Val206_Val209del	616_627del	1	4	No	Yes	NA	NA	NA	NA	4 Amino acids lost
	Exon 2/IVS 2 junction	IVS2+3 A→T	4	20+	Yes[3]	as	NA	NA	NA	NA	Was "1068+3 A→T"
	Arg46ter	136 C→T	1	Prbn.	Yes[46]	ps	NA	NA	NA	NA	
	Leu126Arg	377 T→G	1	Prbn.	Yes*	—	Yes	—	Prob.	102	
	Arg172Gln	516 G→T	1	Prbn.	Yes[47]	—	No	—	Benign	91	
	Digenic Leu185Pro	554 T→C	1	3	Yes[48]	—	Yes	—	Prob.	98	Digenic with ROM1 Ala114fs
	Pro210Arg	629 C→G	1	Prbn.	Yes[49]	—	Yes	—	Prob.	103	
	Pro210Leu	629 C→T	1	Prbn.	Yes[50]	—	Yes	—	Prob.	98	
	Pro216Ser	646 C→T	1	Prbn.	Yes[51]	—	Yes	—	Prob.	74	
	Pro216Leu	647 C→T	2	6	Yes[18]	—	Yes	—	Prob.	98	
	Gly266Asp	797 G→A	2	7	Yes*	—	Yes	Yes	Possib.	94	Transmembrane site
Probably pathogenic	Tyr141Cys	422 A→G	1	4	No	—	Yes	—	Prob.	149	Other mutation at 141
	Ser198Arg	594 C→G	1	Prbn.	No	—	Yes	—	Prob.	110	
	Pro216Arg	647 C→G	1	Prbn.	No	—	Yes	—	Prob.	103	Other mutations at 216
Benign	Gly137Ser	409 G→A	1	No	No	—	No	—	Benign	56	Family has another pathogenic mutation
	Leu45Phe	133 C→T	1	No	Yes[35]	—	Yes	—	Possib.	22	Common Caribbean variant
RHO-rhodopsin											
Pathogenic	Thr17Met	50 C→T	1	Prbn.	Yes[52]	—	Yes	—	Prob.	81	
	Pro23His	68 C→A	20	20+	Yes[53]	—	Yes	—	Prob.	77	
	Leu57Arg	170 C→T	1	Prbn.	Yes[54]	—	Yes	Yes	Possib.	102	Transmembrane site
	Thr58Arg	173 C→G	1	Prbn.	Yes[55]	—	Yes	Yes	Possib.	71	Transmembrane site
	Gly106Arg	318 G→A	1	8	Yes[54]	—	Yes	—	Prob.	125	
	Gly106Trp	316 G→T	2	2	Yes[56]	—	Yes	—	Prob.	184	
	Cys110Phe	329 G→T	1	Prbn.	Yes[57]	—	Yes	Yes	Prob.	205	Disulfide bond
	Arg135Trp	403 C→T	4	8	Yes[56]	—	Yes	Prob.	—	101	
	Arg135Leu	404 G→T+405 G→T	3	20+	Yes[56]	—	Yes	Prob.	—	102	
	Ala164Val	491 C→T	1	Prbn.	Yes[57]	—	Yes	Yes	Benign	64	Transmembrane site

(*continues*)

TABLE 3. (*continued*). Potentially Pathogenic Variants Identified in the adRP Cohort

Type Variant	Mutation		Families (*n*)	Scores							Comment
	Protein	Nucleotide		Seg.	Prior	Struct.	SIFT	Funct	PolyP.	Gran.	
RHO-rhodopsin (continued)											
	Pro170Arg	509 C→G	1	7	Yes[3]	—	Yes	Yes	Possib.	103	Transmembrane site
	Pro171Ser	511 C→T	1	Prbn.	Yes[58]	—	Yes	Yes	Possib.	74	Transmembrane site
	Pro171Gln	512 C→A	1	Prbn.	Yes[59]	—	Yes	Yes	Possib.	76	Transmembrane site
	Glu181Lys	541 G→A	1	Prbn.	Yes[55]	—	Yes	Prob.	Prob.	56	
	Cys187Tyr	560 G→A	1	3	Yes[60]	—	Yes	Yes	Prob.	194	Disulfide bond
	Asp190Asn	568 G→A	2	2	Yes[61]	—	No	No	Benign	23	
	His211Arg	632 A→G	1	Prbn.	Yes[62]	—	Yes	Yes	Possib.	29	Transmembrane site
	Pro267Leu	800 C→T	1	Prbn.	Yes[52]	—	Yes	Yes	Possib.	98	Transmembrane site
	IVS 4/exon 5 junction	IVS4-1 G→A	1	7	Yes[63]	as	NA	NA	NA	NA	
	Val345Met	1033 G→A	1	Prbn.	Yes†	—	Yes	Yes	Prob.	21	BNG binding site
	Pro347Ala	1039 C→G	1	Prbn.	Yes[62]	—	Yes	Yes	Prob.	27	BNG binding site
	Pro347Thr	1039 C→A	1	11	Yes[19]	—	Yes	Yes	Prob.	38	BNG binding site
	Pro347Leu	1040 C→T	2	2	Yes[55]	—	Yes	Yes	Prob.	98	BNG binding site
Probably pathogenic	Leu46Arg	137 T→G	1	4	No	—	Yes	Yes	Possib.	102	Transmembrane site
	Ser270Arg	810 C→A	1	Prbn.	No	—	Yes	Yes	Possib.	110	Transmembrane site
	ter349Gln	1045 T→C	1	2	No	ap	NA	Yes	NA	NA	58 Amino acids added
Benign	Thr70Met	209 C→T	1	No	No	—	Yes	—	Benign	81	
ROM1—rod outer membrane protein											
Pathogenic	Digenic Leu114fs/ter131	339insG	1	3	No	NA	NA	NA	NA	NA	Digenic with RDS Leu185Pro
Probably pathogenic	None										
Benign	Arg229His	686 G→A	1	Prbn.	Yes[64]	—	No	—	Benign	29	Rare population variant
	Tyr234Tyr	702 C→T	1	Prbn.	No	NA	NA	NA	NA	NA	Silent substitution
	Met271Thr	812 T→C	1	Prbn.	Yes[64]	—	No	—	Benign	81	Rare population variant
	Arg287Trp	859 C→T	1	No	No	—	Yes	—	Prob.	101	
RP1—RP1 protein											
Pathogenic	Leu762fs/ter777	2285_2289del	3	4	Yes[26]	fs	NA	NA	NA	NA	
	Arg677ter	2029 C→T	3	20+	Yes[32]	ps	NA	NA	NA	NA	
	Gly723ter	2167 G→T	1	2	Yes‡	ps	NA	NA	NA	NA	
Probably pathogenic	None										
Benign	His1034Arg	3101 A→G	1	No	No	—	NA§	—	NA§	29	.99/.01 in CEPH
	Leu1808Pro	5423 T→C	1	Prbn.	Yes[32]	—	NA§	—	NA§	98	Family has another pathogenic mutation
RP9 (PAP1)-Pim-1 associated protein											
Pathogenic	None										
Probably pathogenic	None										
Benign	Lys210Arg	629 A→G	NA	No	No	—	NA§	NA§	NA§	NA§	.73/.23 in white control subjects

TABLE 3. (*continued*). Potentially Pathogenic Variants Identified in the adRP Cohort

Type Variant	Mutation		Families (n)	Scores							Comment
	Protein	Nucleotide		Seg.	Prior	Struct.	SIFT	Funct	PolyP.	Gran.	
RPGR-X-linked retinitis pigmentosa GTPase regulator											
Pathogenic	Arg195fs/ter229	ORF15+558del	1	6	Yes[65]	fs	NA	—	NA	NA	
	Glu256fs/ter492	ORF15+764_765del	1	11	No	fs	NA	—	NA	NA	
Probably pathogenic	None										
Benign	None										

Families, number of families in the cohort with this mutation; Scores, evidence used to evaluate pathogenicity: (1) Seg., segregating in family; No, discordant segregation in family and/or present in control subjects; Prbn., observed in proband only; numbers represent affected with variant if more than proband; (2) Prior, prior publications with independently ascertained families, if known. (3) Struct.: predicted consequences to protein structure; as, abnormal splicing; ap, abnormal protein; fs, frame shift; ps, premature stop; -, no evidence. (4) SIFT, sorting intolerant from tolerant (http://blocks.fhcrc.org/sift/SIFT.html)[42] as a measure of sequence conservation; No, not conserved or conserved in a few species only; Yes, conserved in several or all species tested; NA, not applicable; (5) Funct., amino acid change in a biologically relevant functional site; -, no evidence; NA, not applicable; (6) PolyP., PolyPhen (polymorphism phenotyping, http://www.bork.embl-heidelberg.de/-PolyPhen)[41] ratings for amino acid substitutions; Prob., probably pathogenic; Possib., possibly pathogenic; Benign, no evidence of pathogenicity; NA, not applicable; (7) Gran., Grantham score[43] for chemical change of amino acid substitutions; NA, not applicable. To measure the severity of amino acid substitutions we used the chemical difference matrix of Grantham, which estimates the distance between amino acids based on side-chain composition, polarity, and molecular volume. Benign amino acid substitutions tend to have distance scores <70, whereas disease-causing substitutions are more likely to have scores >70.[66] For disease-causing substitutions in rhodopsin, Grantham distances average >91.[67]

* Kajiwara K, et al. *IOVS* 1992; 33:ARVO Abstract 1396.
† Stone EM, et al. *IOVS* 1993; 34:ARVO Abstract:1149.
‡ Grimsby JL, et al. *IOVS* 2000; 41:ARVO Abstract:192.
§ NA, no structures available for PolyPhen and/or SIFT comparative analysis (RP1) or sequence too repetitive for analysis (PAP1).

are then combined with other data to predict pathogenicity.[35,67]

Gene-Specific Findings

CA4. Four missense changes were found in the *CA4* gene, three in African-American or African-Caribbean families. Of the three African variants, each was polymorphic in African-Caribbean control subjects, and benign or "possible" by PolyPhen analysis. The remaining variant, in a white family, was also found in white control subjects and is benign by analysis. Consistent with other reports (Ciccodicola A, et al. *IOVS* 2005; 46:ARVO E-Abstract 1817), the prevalence of mutations in CA4 causing adRP in this population was less than 1% (95% confidence interval [CI] 0%–1.5%).

CRX. One missense mutation and one deletion, both reported earlier,[3,38] were found in the *CRX* gene. Both segregated with disease in multiple affected individuals and both were predicted to be pathogenic. Affected members of both families had diminished rod and cone ERGs, but did not present with a diagnosis of cone–rod dystrophy, which was the original phenotype associated with CRX mutations.[44,68] Affected members of the family with the CRX deletion had early-onset, rapid-progression adRP.[38] No other nonpolymorphic variants were found in the cohort.

FSCN2. Five missense variants were found in the *FSCN2* gene. Each of the five was found in controls and/or did not segregate with disease. Computational analysis indicates that they are benign or "possible." Thus, no pathogenic variants were observed in this population and the prevalence of mutations was less than 1%. This contrasts with the higher prevalence found in Japanese adRP families—in part, the result of a single mutation.[5]

***IMPDH1* (*RP10*).** Five families (2.5%) of the cohort have disease-causing mutations in *IMPDH1*. All the families have the same mutation, an aspartic acid to asparagine substitution at codon 226, described previously.[9,32,39] To determine whether the observed mutation has arisen more than once or if all the families share a common ancestor, haplotype analysis of the *IMPDH1* region was performed in four families in which multiple affected individuals were available. SNPs flanking the *IMPDH1* gene were typed in affected family members and haplotypes were reconstructed (data not shown). Two distinct haplotypes were found in the four families, suggesting the Asp226Asn mutation has arisen independently more than once.

***NRL*.** Two nonpolymorphic variants were observed in NRL. One, Arg147Arg, is a silent substitution. The other variant, Gln174Arg, was found in an unaffected family member, has a low Grantham score and is probably benign. Thus, the prevalence of NRL mutations in this population was less than 1%.

***PRPF3* (*RP18*).** Two families (1%) in the cohort had nonpolymorphic missense changes in PRPF3. Both substitutions have been reported in other adRP families,[15] and both are probably pathogenic by PolyPhen analysis.

***PRPF8* (*RP13*).** Six families in the cohort have five different potential disease-causing variants in *PRPF8*. Three are amino acid substitutions, one is a deletion leading to a frameshift, and the other is a splice-site mutation. Two of the amino acid substitutions, Phe2304Leu and Arg2310Gly, have been reported before and are known to be pathogenic.[17] The previously unreported substitution, Ala2328Val, is predicted to be benign by PolyPhen analysis and has only a modest Grantham score based on the small chemical distance between alanine and valine. The splice-site variant is more likely to be pathogenic, based on sequence conservation, but splice-site prediction programs suggest that the variant sequence may be a *better* splice signal than the wild-type sequence. For each of these variants, only a single affected individual was available

for testing. We conclude that four families have definitely pathogenic mutations and two have probably pathogenic mutations; however, evidence for pathogenicity of the two novel mutations is equivocal.

***PRPF31* (*RP11*).** Twelve families in the cohort have possible disease-causing variants in PRPF31. One variant, Gly272Val, does not cosegregate with disease in the family. Nine variants are nonsense mutations, frameshift mutations, or alterations to splice-site consensus sequences, and are highly likely to be pathogenic. The two remaining variants, Ala291Pro and Cys299Arg, are predicted to be pathogenic, but the first was observed in a single affected individual only. The second was found in four affected members of one family. The Cys299Arg variant was also found in one at-risk, unaffected family member, but this is consistent with the high incidence of nonpenetrance for *PRPF31* mutations. None of the 12 variants was found in control subjects. We conclude that 11 families (5.5%) have disease-causing mutations in PRPF31.

RDS and Digenic RDS-ROM1. Twenty families of the cohort have 15 different, potential disease-causing variants in peripherin/RDS. Most variants are amino acid substitutions, but one causes a premature termination after amino acid 45, another is a 12-bp deletion that causes an in-frame deletion of amino acids 206 to 209, and a third affects an intron–exon junction (see Table 3). We also found a benign amino acid substitution in RDS: Leu45Phe, which was reported previously as a common variant in Caribbean populations.[35]

Of the 15 potentially pathogenic variants, 11 have been reported previously in other families with adRP. One of these, Leu185Pro, was originally reported as a cause of adRP but was later shown to cause disease only when inherited along with a null mutation in *ROM1*, that is, to cause digenic RP.[18,48] After finding the Leu185Pro mutation in three affected members of one family in the cohort, we sequenced the *ROM1* gene in these individuals. All three have a 1-bp insertion in exon 1 of *ROM1*, causing a frameshift and premature termination at codon 131. This *ROM1* mutation has been observed in another family with digenic RP.[48]

Four RDS variants have not been reported previously. One, a Gly137Ser missense change, did not segregate with disease in the proband's family and is apparently benign. The remaining three, Tyr141Cys, Ser198Arg, and Pro216Arg, are probably pathogenic. Each is at a conserved site in the protein and the amino acid changes are significant by PolyPhen analysis and Grantham score.

Thus, 18 families in the cohort (9%) have pathogenic mutations in RDS, and an additional family (0.5%) has mutations in both *RDS* and *ROM1* and thus digenic (or diallelic) disease.

***RHO*.** Fifty-four families of the cohort have 27 different, potential disease-causing variants in rhodopsin. Of these, 23 have been reported previously in other families with adRP. The remaining four were found in only a single proband each within the cohort. Most variants are amino acid substitutions, but one is a change at an intron/exon splice site, and another changes the termination codon to glutamine and adds an additional 58 amino acids to the protein.

Analysis of the four novel variants observed in our cohort suggests that three are potentially disease causing. Both the Leu46Arg and the Ser270Arg variants change amino acids in the transmembrane regions of the protein and both changes are likely to be disruptive. The ter349Gln variant is highly likely to be pathogenic, producing a protein that is 58 amino acids longer than the normal protein. A similar mutation, ter349Glu, is known to be one of the most severe rhodopsin mutations.[69] The fourth novel variant, Thr70Met, was not found in subsequent testing of the affected father and affected brother of the proband and, therefore, must be benign.

Thus, 53 families (26.5%) in this cohort have a pathogenic rhodopsin mutation, by far the largest fraction of the total.

ROM1. Except for the digenic allele described in the RDS section, no pathogenic variants in *ROM1* were observed in the cohort. Three missense changes and a silent substitution were found. Two of the missense changes, Arg229His and Met271Thr, have been reported previously in unaffected control subjects,[64] and the third, Arg287Trp, was present in an unaffected family member. The prevalence of nondigenic, dominant-acting *ROM1* mutations in this population is less than 1%.

RP1. Seven families (3.5%) of the cohort have three different disease-causing mutations in RP1. All have been reported previously in multiple adRP families. Two, Arg677ter and Gly273ter, are nonsense mutations that should cause premature protein termination; the other is a 5-bp deletion that should cause a frameshift and premature termination. We also detected two amino acid substitutions that can be classified as benign, His1034Arg and Leu1808Pro. The first is novel but was also found in two unaffected control subjects. The second, Leu1808Pro, was reported previously as possibly pathogenic,[32] but subsequent testing revealed an *IMPDH1* mutation in the same family; the *IMPDH1* mutation is certainly the cause of disease in this family. To date, no missense mutations in RP1 are known to be pathogenic.[70]

RP9. No potentially pathogenic mutations were observed in *PAP1*, the putative *RP9* gene.[27] In the early stages of designing and optimizing primers for sequencing this gene, we observed a heterozygous His137Leu variant (ACA→ACT), identical with one of the two reported pathogenic mutations.[27] Subsequent sequencing and analysis showed this to be a "paralogous variant"—that is, the result of simultaneous amplification of two highly similar sequences: *PAP1* at 7p14.2 and a *PAP1*-like gene 20 kb distal to *PAP1*. By redesigning primers, we were able to eliminate this artifact. Both lack of *PAP1* mutations in this cohort and our experience with the paralogous variant raise the possibility that PAP1 is not, in fact, the RP9-causing gene.

RPGR. Two families predicted to have adRP actually have X-linked RP (1%). Mutations in ORF 15 of *RPGR* were found in each family. Both mutations were small deletions, causing a frameshift and premature termination. One family had at least five known, affected females. Both families have more than four generations of affected individuals, but no male-to-male transmission.

Discussion

Prevalence of Mutations in adRP Genes

The mutations found in this survey are summarized in Table 4A. The findings are applicable largely to Americans of Western European origin. We identified definitely pathogenic or probably pathogenic changes in 107 families, that is, in 53.5% of the cohort (95% CI = 50.0%–57.0%). Of these, rhodopsin mutations are the most common cause of disease, representing 26.5% (23.4%–29.6%) of the total. *RDS* mutations account for the second largest group, 9% (7%–11%), and *PRPF31* accounts for the third largest group, 5.5% (3.9%–7.1%). Among the other genes, *IMPDH1*, *PRPF8*, and *RP1* account for 1% to 5% each, and *CRX*, *PRPF3*, and *RPGR* account for roughly 1% each. Digenic inheritance of *RDS-ROM1* mutations was observed in one family. No mutations were found in *CA4*, *FSCN2*, *NRL*, or *ROM1* (other than digenic). These latter genes are, most likely, rare causes of adRP in whites of Western European origin (Fig. 1).

TABLE 4. Mutations in the adRP Cohort

A. Summary

Gene	Probands (*n*)	% of Total
CA4	0	0.0
CRX	2	1.0
FSCN2	0	0.0
IMPDH1	5	2.5
NRL	0	0.0
PRPF3 (*RP18*)	2	1.0
PRPF8 (*RP13*)	6	3.0
PRPF31 (*RP11*)	11	5.5
RDS	18	9.0
RDS-ROM1 digenic	1	0.5
RHO	53	26.5
ROM1	0	0.0
RP1	7	3.5
RP9	0	0.0
RPGR	2	1.0
Total	107	53.5

B. Frequent Mutations

Gene	Mutation	% of Total
IMPDH1	Asp226Asn	2.5
PRPF3 (*RP18*)	Thr494Met	1.0*
PRPF8 (*RP13*)	Glu2331fs/ter 2358	1.0
RDS	IVS2+3 A→T	2.0
	Pro216Leu	1.0
	Gly266Asp	1.0
RHO	Pro23His	10.0
	Gly106Trp	1.0
	Arg135Leu	1.5
	Arg135Trp	2.0
	Asp190Asn	1.0
	Pro347Leu	1.0
RP1	Arg677ter	1.5
	Leu762fs/ter 777	1.5
Total		28.0

C. Frequent Exons or Regions

Gene	Region	% of Total
IMPDH1 (*RP10*)	Exon 7	2.5
PRPF8 (*RP13*)	Exon 42	1.0
RDS	Exon 2	7.0
RHO	Exon 1	14.5
	Exon 2	5.5
	Exon 5	4.0
RP1	Nt 1948–2338	3.5
Total		38.0

* One per 200 in this study but 1% in Chakarova et al.[15]

Do Mutations in *PAP1* Cause the RP9 Form of adRP?

We also failed to find mutations in *PAP1*, which has been proposed as the *RP9* gene.[27] Other groups have also failed to find PAP1 mutations in adRP families.[31] One of the *PAP1* "mutations" we detected in the cohort turned out to be a paralogous variant, that is, the result of PCR amplification of two nearly identical *PAP1* gene copies. This variant is the same as one of the purported disease-causing mutations.[27] These findings cast doubt on whether *PAP1* is, in fact, the *RP9* gene.

Common Mutations and Screening Strategies

Several "common" mutations account for a substantial fraction of the total in this population. Fourteen mutations (Table 4B) were each found in 1% or more of families. In aggregate, they account for 28% of the total, that is, for at least 5% of *all* RP cases, if the percent of adRP among all cases is 20% (a conservative estimate). Further, the common mutations and many of the remainder are clustered within just seven exons of *IMPDH1*, *PRPF8*, *RDS*, *RHO*, and *RP1*, accounting for 38% of the total (Table 4C). Thus, sequencing seven gene regions, spanned by 14 PCR primer sets (listed in Supplementary Tables, http://www.iovs.org/cgi/content/full/47/7/3052/DC1), will detect a large fraction of the total, making this a useful initial screening strategy for diagnostic testing. Note, though, that this conclusion applies to this ethnic–geographic group only, and that screening the remaining gene regions will potentially detect an additional 16% of mutations, most rare or unique.

The fraction of adRP cases caused by the genes tested in this cohort differs considerably from observations in other ethnic–geographic populations, as summarized in Table 1. One reason is that some of the common mutations are frequent as a result of founder effect and, thus, likely to be rare or absent from other populations. For example, the *RDS* IVS2+3 and *RHO* Pro23His mutations each descend from a common white ancestor and have not been reported in other ethnic groups[19] (Shankar SP, et al. *IOVS* 2004;45:ARVO E-Abstract 3719). In contrast, several common mutations arise from independent, recurrent events, among them the *IMPDH1* Asp226Asn, *RHO* Arg135Leu, *RHO* Arg135Trp, and *RP1* Arg677ter mutations[9,71] (and this publication). Whether these will be recurrent mutations in other populations has not been established.

Does Evaluation of Pedigrees and Clinical Findings Help to Prioritize Screening?

Before testing, we categorized the 200 adRP families by seveal criteria (Table 1 and Supplementary Tables, http://www.iovs.org/cgi/content/full/47/7/3052/DC1). Further, we have clinical data on affected members of most families, so we can compare phenotypes with genotypes. Is either the pedigree structure or the clinical picture a useful guide to the underlying gene and mutation?

Uncommon Retinal Findings in RP Families

Secondary retinal findings may be useful in prioritizing gene testing. For example, families with both dominant RP and complex macular disease are much more likely to have RDS mutations than predicted by prevalence alone.[3,72,73] Thus, RDS screening should be a high priority in families with macular findings in some (not necessarily all) affected individuals. Likewise, *CRX* mutations are more likely in families with cone–rod dystrophy, but because *CRX* mutations are rare among adRP families, this is not particularly helpful in setting priorities.

***PRPF31* in adRP Families with Skipped Generations.** Families with multiple skipped generations, but otherwise typical adRP, are more likely to have mutations in *PRPF31* (*RP11*).[74,75] Of the 11 families in the cohort with *PRPF31* mutations, 5 (45%) have pedigree evidence of one or more skipped generations, which is significantly different from the overall fraction, 24%. Although other adRP genes, such as RP1,[24] may show nonpenetrance, it is a more common phenomenon with PRPF31, making this a high-priority candidate in such cases.

X-Linkage in Autosomal Dominant Families. Families without male-to-male transmission but with affected females may have mutations in ORF 15 of *RPGR*.[28,33,34] Thus, some large, multigeneration families with affected females may have

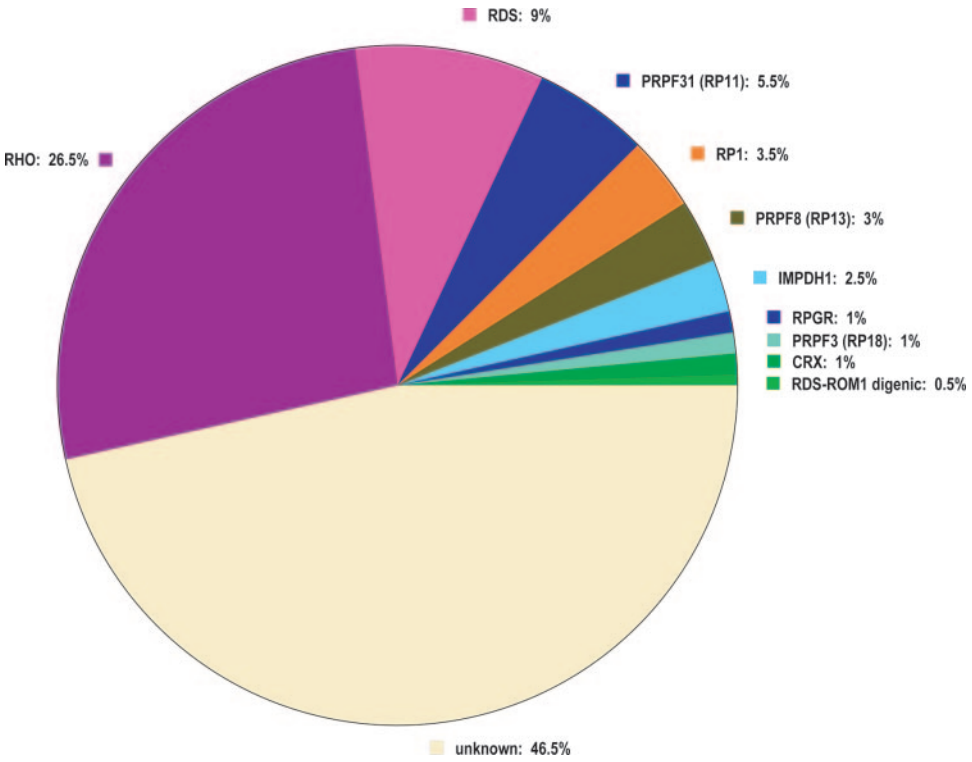

FIGURE 1. Fraction of dominant retinitis pigmentosa caused by mutations in known genes and in the remaining unknown genes.

X-linked RP masquerading as autosomal dominant RP. For example, the two families with ORF 15 mutations in the cohort have five and seven affected females, respectively, and both were calculated to have odds favoring autosomal dominant inheritance of 10^3 or greater. However, in all cases, females are more mildly affected than males, which should be a strong indicator of X-linked inheritance.

Detectable Mutations in Families with a High Probability of Autosomal Dominant Inheritance. Finally, among the cohort of 200 families, 121 had a calculated odds ratio of 10^4 or greater favoring autosomal dominant over X-linked inheritance. Of these, 76 (63%) had detectable mutations. Conversely, 24 families had a calculated odds ratio of 1 or less—that is, either both modes are equally likely, or X-linkage is favored. Of the second set of families, 5 (21%) have detectable mutations, a highly significant difference. Thus, the probability calculations confirm the intuitive expectation: The more certain it is that a family has autosomal dominant inheritance, the more likely a mutation will be found.

Strengths and Limitations of Computational Analysis of Missense and Splice-Site Mutations

Establishing pathogenicity of novel, rare variants, particularly missense mutations and intronic variants, is challenging. We used a staged, systematic approach that incorporates segregation information (including prior reports), cross-species comparisons, and several computational tools (Table 3). However, it is possible that a few variants labeled "pathogenic" or "probably pathogenic" are not pathogenic; and that an occasional predicted "benign" variant is not benign. This problem has been addressed by others.[35] Several mutations listed in Table 3 as "probably pathogenic," based on all available information, are reported in pubic databases as definitely pathogenic. Thus, as a caution, where the evidence for pathogenicity is limited or ambiguous, these doubts should be incorporated into counseling of patients and families.

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