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MUTATIONS IN KNOWN GENES ACCOUNT FOR 58% OF AUTOSOMAL DOMINANT RETINITIS PIGMENTOSA (adRP)

Stephen P. Daiger[1,2], **Lori S. Sullivan**[1], **Anisa I. Gire**[1], **David G. Birch**[3], **John R. Heckenlively**[4], and **Sara J. Bowne**[1]

1*Human Genetics Center, School of Public Health, The Univ. of Texas, Houston, TX, USA*

2*Dept. of Ophthalmology, The Univ. of Texas, Houston, TX, USA*

3*Anderson Research Center, Retina Foundation of the Southwest, Dallas, TX, USA*

4*Kellogg Eye Center, Univ. of Michigan, Ann Arbor, MI, USA*

1. INTRODUCTION

Inherited retinal diseases such as autosomal dominant retinitis pigmentosa (adRP) are strikingly complex, with mutations in many different genes causing the same disease, with many different mutations in each gene, and with different clinical consequences resulting from the same mutation, even within the same family. For example, mutations in sixteen genes are known to cause adRP and an additional two adRP genes have been mapped but not identified yet (Table 1). This raises two questions: what fraction of adRP cases are accounted for by mutations in known genes, and what accounts for the remaining cases?

To answer these questions we applied a step-wise screening process to a cohort of well-characterized adRP families, now numbering 215.[1] Methods included sequencing of known genes, detection of deletions using MLPA (multiplex ligation-dependent probe amplification),[2] linkage mapping against known loci, and genome-wide linkage mapping. By this combination of approaches we detected mutations in 58% of the families (largely Americans of European origin). Approximately 3% of these families have large deletions that cannot be detected by conventional PCR-based methods, and linkage testing against known loci revealed several additional mutations that were not detected earlier. Thus some of the remaining families are likely to have large deletions or other "hidden" mutations in known genes. However, linkage testing also confirms the existence of new adRP genes.

Being able to find the cause of adRP in all or nearly all affected individuals is a difficult but achievable goal, perhaps within the next decade.[3] This information is of immediate value to patients and families, and is a necessary precursor to gene and mutation-specific therapies.

2. METHODS

We tested a panel of affected individuals from 215 adRP families for mutations in most of the known dominant RP genes (Table 1) [Sullivan *et al.*, 2006[1] and unpublished]. To be included in the study a family had to have a diagnosis of adRP by a knowledgeable clinical specialist, and either a) three affected generations with affected females, or b) two affected generations with male-to-male transmission. The latter requirement was to reduce the likelihood of including families with X-linked RP. This possibility arises because some mutations in the X-

Corresponding author: S. P. Daiger, E-mail: stephen.p.daiger@uth.tmc.edu..

linked gene RPGR affect female "carriers", thus the disease in these families may be misinterpreted as adRP.[4-6]

The cohort of adRP patients was screened (largely by DNA sequencing) for mutations in the protein coding regions and intron-exon junctions of all adRP genes or gene regions causing at least 1% of cases. ORF15, the "hot spot" for mutations in RPGR, was also tested in families without male-to-male transmission. Determining whether a novel, rare variant is pathogenic was done using several computational and genetic tools.[8-11]

In subsequent studies, we tested several of the remaining families for linkage to genetic markers within or close to the known adRP genes and to RPGR [Sullivan *et al*.,[2] 2006 and unpublished]. This was done to uncover mutations that might have been missed by sequencing or to locate genes that have been mapped but not identified yet. In one large family we found linkage to the PRPF31 gene, even though careful re-sequencing failed to disclose a DNA change. Further testing revealed that affected members of the family have a complex deletion and insertion in PRPF31. This rearrangement was not detected earlier because only the non-deleted, homologous chromosome was sequenced, that is, the deletion is "invisible" to sequencing. We then tested the remaining families for deletions in PRPF31 using multiplex ligation-dependent probe amplification (MLPA).[2,12]

Finally, two large families without mutations in known genes were tested for genome-wide linkage using the ABI 5 cM microsatellite panel (n = 811) [Sullivan *et al*., 2005[13] and unpublished]. Multipoint linkage analysis was done using the LINKAGE package.[14]

3. RESULTS

3.1. Mutations in known dominant RP genes account for 58.6% of adRP families

To determine the genes and mutations causing retinopathy in the 215 families in the adRP cohort we tested affected probands for mutations predicted to cause at least 1% of adRP cases. Subsequently, families without mutations detected by sequencing were subjected to linkage testing against STRP (short tandem repeat polymorphism) marker sets within or contiguous to known adRP loci, if sufficient family members were present.[2] In those cases were linkage testing indicated a known gene, that gene was tested more extensively in affected family members.

By these approaches, we identified single-nucleotide substitutions and small indels as the likely cause of adRP in 55.8% of the cohort families (Table 2 and Figure 1). Pathogenicity of novel variants was confirmed by segregation within families and bioinformatic analyses [Sullivan *et al*., 2006[1] and unpublished].

In addition, linkage mapping and SNP (single-nucleotide polymorphism) exclusion in one large adRP family revealed a complex chromosomal rearrangement in the PRPF31 (RP11) gene not detectable by sequencing. Based on this finding we designed MLPA probe sets spanning the PRPF31 locus and tested for deletions and copy number variants in other families in the cohort. In total, we identified deletions and rearrangements in thePRPF31 gene in six (2.8%) additional families (Figure 2) [Sullivan *et al*., 2006[2] and unpublished].

3.2. Several adRP genes are rare causes of adRP or are misidentified

We found no mutations in four of the genes, CA4, FSCN2, NRL and RP9 (PAP1). Based on published evidence, mutations in CA4, FSCN2 and NRL are real but rare causes of adRP. In contrast, we believe that the gene associated with the RP9 locus, PAP1, is not the cause of this disease. We and others failed to find mutations; in addition, we discovered that one of the reported disease-causing mutations is probably a paralogus variant, that is, the result of PCR

amplification of two nearly identical gene copies. For this reason, we believe that the gene at the RP9 locus has not been identified yet.

3.3. Family history and phenotypes are useful for prioritizing genes to test

We considered whether the pedigree and phenotype are useful predictors of the underlying gene. In three circumstances they are. First, families in which females are consistently less severely affected than males, and without male-to-male transmission, are more likely to have an RPGR mutation. Second, skipped generations are more common with mutations in PRPF31 than in other adRP genes. Third, symptoms of RDS mutations are much more varied than mutations in other genes, ranging from RP, to choroidal atrophy, to complex maculopathies.[15-17] Otherwise, there are numerous phenotypic differences among individuals with different mutations, but there is so much clinical variability that these differences are not pathognomonic.

3.4 Linkage mapping indicates additional adRP loci

Of the 215 adRP families enrolled in these studies, we identified disease-causing mutations in 126 using a variety of methods (Figure 3). Of the remaining families, two are large enough for genome-wide linkage mapping and were tested for linkage to the ABI 5 cM STR marker set. Linkage mapping in these families suggests the existence of novel adRP loci [Sullivan *et al.*, 2005[13] and unpublished].

4. CONCLUSIONS

Mutations in known adRP genes account for at least 58% of adRP cases. “Common” mutations among the total account for at least 35% of cases, but novel mutations are found in the remainder. Thus screening adRP patients for known mutations and for mutations in selected regions of adRP genes can detect a large fraction of disease-causing variants, but additional methods, including MPLA and linkage, are also required. These prevalences are based largely on Americans of European origin and Europeans; other populations have different “common” mutations and different prevalences.

Deletions and copy number variants in PRPF31, not detectable by sequencing, account for 2.8 % of cases. Some of these deletions are very large, encompassing flanking genes. Deletions in other genes may also cause adRP.

Digenic RDS-ROM1 and X-linked dominant mutations in RPGR affect 0.5% and 1.5%, respectively, of “dominant” RP families. For diagnostic and counseling purposes it is very important to consider alternate modes of inheritance in adRP families.

Identifying the underlying disease-causing mutation in families with adRP is an essential step in diagnosis, counseling and, eventually, treatment.[3]

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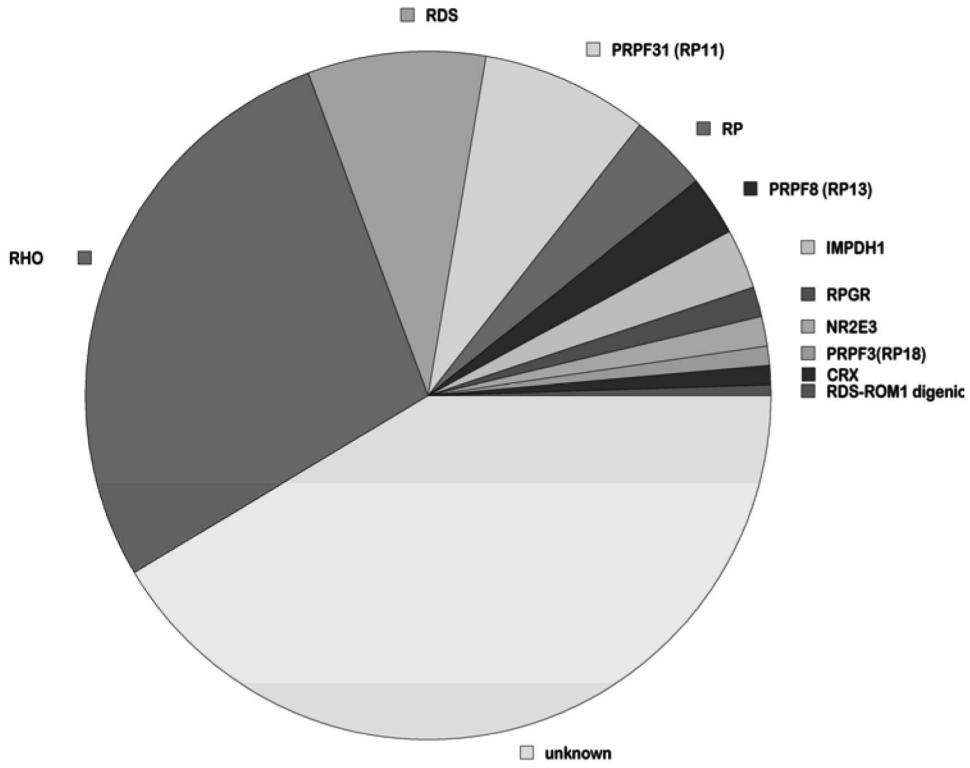

Figure 1.
Percent of mutations per gene in adRP families.

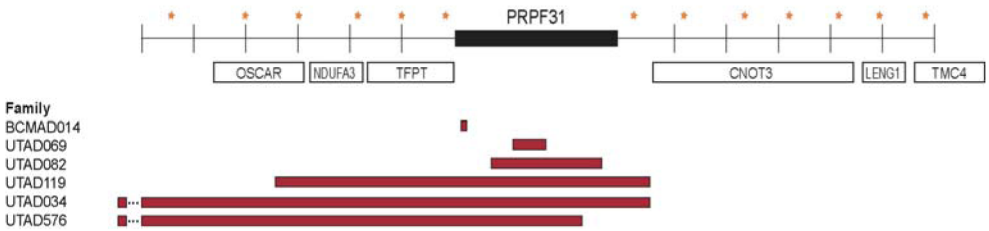

Figure 2.
PRPF31 deletions in six adRP families. Stars indicate SNPs, bars indicate deleted regions.

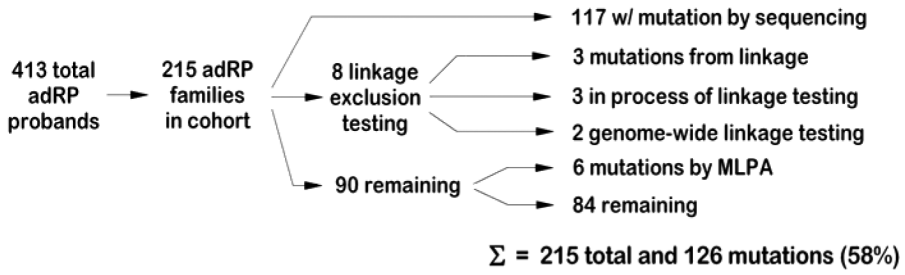

Figure 3.
Summary of results of genetic testing of probands and families with adRP.

Table 1

Genes known to cause dominant retinitis pigmentosa (in chromosomal order) [7]

	Symbol	Protein	Location
	a. Known adRP genes		
1.	PRPF3 (RP18)	pre-mRNA splicing factor 3	1q21.2
2.	SEMA4A	semaphorin 4A	1q22
3.	RHO	rhodopsin	3q22.1
4.	GUCA1B	guanylate cyclase activating protein 1B	6p21.1
5.	RDS	peripherin 2	6p21.2
6.	RP9 (PAP1)	pim-1 kinase associated protein	7p14.3
7.	IMPDH1 (RP10)	inosine monophosphate dehydrogenase 1	7q32.1
8.	RP1	RP1 protein	8q12.1
9.	ROM1	rod outer membrane protein 1	11q12.3
10.	NRL	neural retina leucine zipper	14q11.2
11.	NR2E3	nuclear receptor subfamily 2 group E3	15q23
12.	PRPF8 (RP13)	pre-mRNA splicing factor 8	17p13.3
13.	CA4 (RP17)	carbonic anhydrase 4	17q23.2
14.	FSCN2	retinal fascin homolog 2	17q25
15.	CRX	cone-rod homeobox transcription factor	19q13.32
16.	PRPF31 (RP11)	pre-mRNA splicing factor 31	19q13.42
	b. Mapped autosomal genes		
1.	RP33		2cen-q12.1
2.	RP31		9p22-p13
	c. Dominant-acting X-linked gene		
1.	RPGR (RP3)	retinitis pigmentosa GTPase regulator	Xp11.4

Table 2

Mutations in the adRP cohort, N = 215 [Sullivan 2006[1,2] and unpublished].

Gene	No. families	% total	Gene	No. families	% total
CA4	0	0.0	PRPF31 (RP11)	17	7.9
CRX	2	0.9	RDS	18	8.4
FSCN2	0	0.0	RDS-ROM1 digenic	1	0.5
IMPDH1	6	2.8	RHO	60	27.9
NR2E3	3	1.4	ROM1	0	0.0
NRL	0	0.0	RP1	8	3.7
PRPF3 (RP18)	2	0.9	RP9	0	0.0
PRPF8 (RP13)	6	2.8	RPGR	3	1.4
			TOTALS	126	58.6