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ORIGINAL ARTICLES

A Report on Molecular Diagnostic Testing for Inherited Retinal Dystrophies by Targeted Genetic Analyses

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Aim: To test the utility of targeted sequencing as a method of clinical molecular testing in patients diagnosed with inherited retinal degeneration (IRD).

Methods: After genetic counseling, peripheral blood was drawn from 188 probands and 36 carriers of IRD. Single gene testing was performed on each patient in a Clinical Laboratory Improvement Amendment (CLIA) certified laboratory. DNA was isolated, and all exons in the gene of interest were analyzed along with 20 base pairs of flanking intronic sequence. Genetic testing was most often performed on *ABCA4, CTRP5, ELOV4, BEST1, CRB1,* and *PRPH2.* Pathogenicity of novel sequence changes was predicted by PolyPhen2 and sorting intolerant from tolerant (SIFT).

Results: Of the 225 genetic tests performed, 150 were for recessive IRD, and 75 were for dominant IRD. A positive molecular diagnosis was made in 70 (59%) of probands with recessive IRD and 19 (26%) probands with dominant IRD. Analysis confirmed 12 (34%) of individuals as carriers of familial mutations associated with IRD. Thirty-two novel variants were identified; among these, 17 sequence changes in four genes were predicted to be possibly or probably damaging including: *ABCA4* (14), *BEST1* (2), *PRPH2* (1), and *TIMP3* (1).

Conclusions: Targeted analysis of clinically suspected genes in 225 subjects resulted in a positive molecular diagnosis in 26% of patients with dominant IRD and 59% of patients with recessive IRD. Novel damaging mutations were identified in four genes. Single gene screening is not an ideal method for diagnostic testing given the phenotypic and genetic heterogeneity among IRD cases. High-throughput sequencing of all genes associated with retinal degeneration may be more efficient for molecular diagnosis.

Keywords: inherited retinal degeneration, targeted genetic testing, retinal dystrophy, molecular diagnosis, DNA testing, retinitis pigmentosa

Introduction

Retinal dystrophies are a group of inherited retinal degenerations (IRDs) with phenotypic and genetic heterogeneity (den Hollander *et al.*, 2010). Over 250 genes have been implicated in causing IRD (RetNet) (Daiger *et al.*, 1998). Genetic testing has the potential to accurately diagnose and predict disease occurrence in early and late-onset IRD (Chiang *et al.*, 2015; Tajiguli *et al.*, 2016; Weisschuh *et al.*, 2016). Finding the genetic basis of IRDs is the first step of recruitment into gene mutation-based clinical trials (Wiggs and Pierce, 2013; Chiang and Trzupek, 2015; Lee and Garg, 2015; Nash *et al.*, 2015). Sensitive and specific genetic tests are currently available at moderate cost for dozens of inherited eye diseases to genotype patients (Chiang and Trzupek, 2015; Chiang *et al.*, 2015; Consugar *et al.*, 2015;

Lee and Garg, 2015). Compared with whole exome or genome sequencing with challenging data analysis, targeted genetic testing may improve cost efficiency and decrease turnaround time (Stone, 2003). We designed this observational study to evaluate the probability of finding a causative genetic mutation with single gene testing in patients clinically diagnosed with an IRD. We report the likelihood of finding a causative mutation for IRD in unrelated probands and carriers from families with a diagnosis of dominant and recessive IRDs.

Materials and Methods

Patient sample collection

This study was performed in accordance with tenets of the Declaration of Helsinki and the Institutional Review Board at

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the University of California, San Diego (UCSD). Genetic consultation regarding the possibility of obtaining a molecular diagnosis was discussed with the family before genetic testing. All included patients were deemed to have an IRD by clinical exam or be a potential carrier of the disease. Potential carriers included obligate carriers and members at risk to inherit causative mutations (parents and siblings of probands). A test request form, including patient name, date of birth, gender, ethnic background, gene test requested, indication for referral, and family history in pedigree format was submitted along with the blood sample to the Ophthalmic

Molecular Diagnostic Laboratory at UCSD between 2009 and 2011 with input from the clinician and/or genetic counselor. Deidentified requisition forms were retrospectively reviewed for clinical information.

Amplification and sequencing

DNA was isolated from blood samples using standard protocols, and 17 retinal genes were screened for mutations by polymerase chain reaction amplification of all exons with at least 20 base pairs of flanking intronic sequence (Table 1)

TABLE 1. RETINAL DISEASE GENES SCREENED FOR MUTATIONS AND THE ASSOCIATED PHENOTYPES AND INHERITANCE PATTERN

No.	Gene	Cytogenetic location	OMIM entry	No. of exons	Retinal disease	Mode of inheritance
1	ABCA4	1p22.1	601691	50	Cone–rod dystrophy	Autosomal recessive
					Fundus flavimaculatus	Autosomal recessive
					Stargardt macular dystrophy	Autosomal recessive
					Early onset severe retinal dystrophy	Autosomal recessive
					Retinitis pigmentosa	Autosomal recessive
2	ARL6	3q11.2	608845	9	Bardet–Biedl syndrome	Autosomal recessive
					Retinitis pigmentosa	Autosomal recessive
3	BEST1/VMDM2	11q12.3	607854	11	Vitelliform macular dystrophy	Autosomal dominant
					Bestrophinopathy	Autosomal recessive
					Vitreoretinochoroidopathy	Autosomal dominant
					Retinitis pigmentosa	Autosomal dominant
					Cone–rod dystrophy with posterior staphyloma, microcornea, and cataract	Autosomal dominant
4	C1QTNF5/CTRP5	11q23.3	608752	3	Late onset retinal degeneration	Autosomal dominant
5	CDHR1	10q23.1	609502	17	Cone–rod dystrophy	Autosomal recessive
					Retinitis pigmentosa	Autosomal recessive
6	CERKL	2q31.3	608381	13	Retinitis pigmentosa	Autosomal recessive
7	CRB1	1q31.1	604210	11	Leber congenital amaurosis	Autosomal recessive
					Retinitis pigmentosa	Autosomal recessive
					Pigmented paravenous chorioretinal atrophy	Autosomal dominant
8	CRX	19q13.33	602225	4	Cone–rod dystrophy	Autosomal dominant
					Leber congenital amaurosis	Autosomal dominant
9	DHDDS	1p36.11	608172	9	Retinitis pigmentosa	Autosomal recessive
10	EFEMP1	2p16.1	601548	12	Doyne honeycomb degeneration of retina	Autosomal dominant
11	ELOVL4	6q14.1	605512	6	Stargardt-like macular dystrophy	Autosomal dominant
12	FAM161A	2p15	613596	7	Retinitis pigmentosa	Autosomal recessive
13	PDE6B	4p16.3	180072	22	Retinitis pigmentosa	Autosomal recessive
					Congenital stationary night blindness	Autosomal dominant
14	PRPH2	6p21.1	179605	3	Macular dystrophy retinitis pigmentosa	Autosomal dominant
					Retinitis punctata albescens	Autosomal dominant or recessive
					Foveomacular dystrophy with choroidal neovascularization	Autosomal dominant
					Central areolar choroidal dystrophy	Autosomal dominant
15	Rhodopsin	3q22.1	180380	5	Retinitis pigmentosa	Autosomal dominant or recessive
					Retinitis punctata albescens	Autosomal dominant or recessive
					Congenital stationary night blindnesss	Autosomal dominant
16	RPE65	1p31.3–31.2	180069	14	Leber congenital amaurosis	Autosomal recessive
					Retinitis pigmentosa	Autosomal recessive
17	TIMP3	22q12.3	188826	5	Sorsby's fundus dystrophy	Autosomal dominant

OMIM, Online Mendelian Inheritance in Man.

followed by dideoxy sequencing and analysis using ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA) as previously described (Alapati *et al.*, 2014). Mutations, including substitutions, deletions, and insertions, involving a few base pairs in the coding region and splice junctions can be detected with our methodology. Large exonic deletions or insertions and mutations in introns, however, may not be identified.

Analysis of novel variants

PolyPhen-2 (Adzhubei *et al.*, 2010) and sorting intolerant from tolerant (SIFT) (Sim *et al.*, 2012) prediction were used to assess the potential pathogenicity of novel variants detected. Frequency of alleles was obtained from the ExAC database (Lek *et al.*, 2016).

Reporting

A written report, including the laboratory methods, test results, interpretation, known published detection rates, and references, was provided to the referring clinician.

Results

We describe the results of genetic testing performed on 225 patients (190 probands and 35 individuals at risk to inherit causative mutations or obligate carriers). No mutations were found in 124 (55%) cases (Table 2). The causative mutations were found in 19 (26%) patients with dominant IRD and 70 (59%) patients with recessive IRD. Single heterozygous familial mutations or variants of unknown significance (VUS) were found in 15 (45%) of at-risk and obligate carriers from families with IRD. The majority of patients tested had no known family history of an inherited retinal dystrophy. All patients in this study that have a positive or negative family history of IRD were referred for diagnostic testing independently. Ethnicity information, while requested in all reports, was seldom provided.

ABCA4 was the most often tested gene (113), followed by *C1QTNF5* (21) (Table 2). At the time of diagnostic testing, multiple novel sequence changes were observed in *ABCA4* (27), *BEST1* (2), *EFEMP1* (1), *PRPH2* (1), and *TIMP3* (1) (Table 3). Subsequently, some of these have been reported (Table 3).

The patients' phenotype belonged to one of the three major categories of retinal diseases: cone–rod dystrophies, macular dystrophies, and retinitis pigmentosa (RP). Hereditary cone–rod dystrophy (CRD) is characterized by central vision loss with the primary loss of cones and secondary loss of rods (Michaelides *et al.*, 2006) with more than 20 implicated causative genes. In our study, molecular diagnostic testing for CRD was carried out by screening the *ABCA4*, *CRX*, and *CDHR1* genes. The second category of patients had macular degeneration, which can segregate as a dominant or recessive trait. The *ABCA4* gene was screened in patients with recessive Stargardt macular degeneration (STGD1) or recessive macular degeneration, whereas *PRPH2*, *ELOVL4*, *EFEMP1*, *CTRP5*, *TIMP3*, and *BEST1/VMD2* were analyzed in patients with dominant macular degeneration. *BEST1/VMD2* mutations are associated with both dominant and recessive vitelliform macular dystrophies. The third category, RP, is the most common and genetically heterogeneous IRD, and all RP patients in the study had recessive disease. More than 35 genes have been implicated in recessive RP. In this study, one of six genes (*Rhodopsin*, *CERKL*, *RPE65*, *CRB1*, *FAM161A*, and

TABLE 2. RESULTS OF CLINICAL MOLECULAR TESTING OF 17 RETINAL GENES

	No. of patients tested			Cases in which causative mutation found		
	Proband	*Carrier*	*Total*	*Proband*	*Carrier*	*Total*
Recessive IRD genes						
ABCA4	88	25	113	52	8	60
ARL6	1	1	2	1	0	1
BEST1/VMD2	1	1	2	1	1	2
DHDDS	3	2	5	3	1	4
CDHR1	3	1	4	3	0	3
CERKL	2	0	2	2	0	2
CRB1	12	0	12	3	0	3
FAM161A	3	0	3	3	0	3
PDE6B	1	0	1	1	0	1
RPE65	4	2	6	1	2	3
Totals	118	32	150	70	12	82
Dominant IRD genes						
BEST1/VMD2	15	0	15	9	0	9
CRX	3	0	3	0	0	0
C1QTNF5/CTRP5	19	2	21	1	0	1
EFEMP1	6	0	6	4	0	4
ELOVL4	14	0	14	1	0	1
PRPH2	9	1	10	2	0	2
TIMP3	4	0	4	1	0	1
Rhodopsin	1	0	1	0	0	0
Totals	72	3	75	19	0	19

IRD, inherited retinal degeneration.

TABLE 3. NOVEL SEQUENCE CHANGES DETECTED IN THIS STUDY AND PREDICTION OF THEIR IMPACT ON THE PROTEIN

Gene	Nucleotide change	Amino acid change	Zygosity	Exon no.	rs ID (ExAC frequency)	PolyPhen2 Prediction	PolyPhen2 Score	SIFT Prediction	SIFT Score
ABCA4	c.294C>G	p.Asn98Lys	Het	3	rs145133167 (0.0001)	Benign	0.016	Tolerated	0.1
ABCA4	c.564delA	p.Glu189SerfsX12	Het	5	NL (NL)	Frameshift mutation	N/A	Tolerated	0.43
ABCA4	c.3395T>C	p.Ile1132Thr	Het	23	NL (NL)	Probably damaging	1	Damaging	0
ABCA4	c.1237A>T	p.Asn413Tyr	Het	9	NL (NL)	Benign	0.005	Damaging	0.01
ABCA4	c.677G>T	p.Arg226Leu	Het	6	rs144310835 (0.0003)	Possibly damaging	0.759	Tolerated	0.12
ABCA4	c.3547G>T	p.Gly1183Cys	Het	24	rs75267647 (0.0012)	Benign	0.001	Tolerated	0.06
ABCA4	c.5137C>A	p.Gln1713Lys	Het	36	rs374343397 (0.00004)	Probably damaging	1	Damaging	0.01
ABCA4	c.5137A>G	p.Gln1713Arg	Het	36	rs374343397 (0.00002)	Probably damaging	1	Damaging	0
ABCA4	c.3279C>A	p.Asp1093Glu	Hom	22	rs61752418 (NL)	Probably damaging	1	Damaging	0
ABCA4	c.4504T>G	p.Cys1502Gly	Het	30	rs61750149 (0.00003)	Probably damaging	0.996	Damaging	0.02
ABCA4	c.5447T>C	p.Phe1816Ser	Het	38	NL (NL)	Probably damaging	1	Damaging	0
ABCA4	c.853C>T	p.Gln285Ter	Het	7	NL (NL)	Nonsense change	N/A	Nonsense change	N/A
ABCA4	c.1677G>C	p.Trp559Cys	Het	12	NL (NL)	Possibly damaging	0.944	Damaging	0.05
ABCA4	c.1861A>G	p.Arg621Gly	Hom	13	rs141122703 (0.0003)	Benign	0.001	Damaging	0.02
ABCA4	c.2852T>C	p.Ile951Thr	Hom	19	NL (NL)	Possibly damaging	0.689	Damaging	0
ABCA4	c.4556C>T	p.Thr1519Met	Hom	31	rs115859773 (0.00007)	Benign	0.262	Damaging	0.03
ABCA4	c.5391T>C	p.Cys1797Cys	Het	38	rs147187030 (0.0001)	Synonymous change	N/A	Tolerated	1
ABCA4	c.3871C>T	p.Gln1291Ter	Het	27	NL (0.000009)	Nonsense change	N/A	Nonsense change	N/A
ABCA4	c.3211T>C	p.Ser1071Pro	Het	22	NL (0.00002)	Probably damaging	0.999	Damaging	0.08
ABCA4	c.4326C>A	p.Asn1442Lys	Het	29	NL (0.00002)	Possibly damaging	0.565	Tolerated	0.01
ABCA4	c.5176insG	p.Thr1726AspfsX61	Het	36	NL (NL)	Frameshift mutation	N/A	Damaging	N/A
ABCA4	c.302+26A>G	Intronic	Het	3	NL (NL)	VUS	N/A	VUS	N/A
ABCA4	c.4216delC	p.H1406TfsX29	Het	28	NL (NL)	Frameshift mutation	N/A	Damaging	0.17
ABCA4	c.3017G>T	p.Gly1006Val	Het	20	NL (NL)	Probably damaging	1	Damaging	0
ABCA4	c.655A>T	p.Arg219Ter	Het	6	NL (NL)	Nonsense change	N/A	Nonsense change	N/A
ABCA4	c.2971G>C	p.Gly991Arg	Het	20	rs61749455 (0.0007)	Probably damaging	1	Damaging	0
ABCA4	c.1529T>C	p.Leu510Pro	Het	11	NL (NL)	Probably damaging	0.988	Damaging	0.03
BEST1	c.332C>T	p.Ser111Leu	Het	4	NL (NL)	Probably damaging	1	Damaging	0.01
BEST1	c.824C>G	p.Pro274Arg	Het	7	rs148326372 (0.00007)	Probably damaging	1	Damaging	0
EFEMP1	c.1321-14C>T	Intronic	Het	10	NL (NL)	VUS	N/A	VUS	N/A
PRPH2	c.4G>T	p.Ala2Ser	Het	1	NL (NL)	Probably damaging	0.999	Damaging	0.01
TIMP3	c.628G>T	p.Asp210Tyr	Het	5	NL (NL)	Probably damaging	0.999	Damaging	0

N/A, not applicable; NL, rs ID is not listed in dbSNP and/or ExAC database; SIFT, sorting intolerant from tolerant; VUS, variants of unknown significance.

PDE6B) was tested. These genes were selected based on research findings or the phenotype and ethnicity of the patients.

Recessively inherited diseases

ABCA4. *ABCA4* mutations are implicated in autosomal recessive STGD1, fundus flavimaculatus, CRD, and RP (Maugeri *et al.*, 2000). Diagnostic tests for *ABCA4* were ordered in 113 patients (88 probands and 25 potential carriers) suspected with CRD, STGD1, fundus flavimaculatus, and/or RP. We identified two or more pathogenic changes in 52 (59%) probands and single heterozygous pathogenic changes in 8 (32%) carriers. Of the 25 carriers tested, 24 were obligate carriers. In addition, multiple previously described polymorphic variants were detected in these individuals, ranging from 0 to 11 per sample.

ARL6. Mutations in *ARL6* have been implicated in Bardet–Biedl syndrome and autosomal recessive RP (Khan *et al.*, 2013). Diagnostic tests for *ARL6* were ordered in one proband based on research findings, and a homozygous mutation c.362G>A, p.Arg121His was found in exon 6. The potential carrier tested was the wife of the proband with no family history, and no mutations or single nucleotide polymorphisms (SNPs) were found.

BEST1. Mutations in *BEST1* have been associated with Best vitelliform macular dystrophy, adult onset vitelliform dystrophy, RP, and multifocal vitelliform dystrophy with subretinal fluid known as autosomal recessive bestrophinopathy due to biallelic *BEST1* mutations (Burgess *et al.*, 2008; Fung *et al.*, 2015). Diagnostic testing for autosomal recessive bestrophinopathy was ordered in one proband and one obligate carrier, the mother of the proband. In the proband, the novel damaging compound heterozygous changes c.332C>T, p.Ser111Leu (exon 4) and c.824C>G, p.Pro274Arg (exon 7) were identified. In the related carrier, c.824C>G, p.Pro274Arg was identified in exon 7.

DHDDS. Mutations in *DHDDS* have been implicated in autosomal recessive RP in Ashkenazi Jewish patients (Zelinger *et al.*, 2011). Four probands and one carrier were tested for *DHDDS* mutations based on Ashkenazi Jewish ancestry and a phenotype consistent with autosomal recessive RP. The two obligate carriers tested were the mother and father of the probands. A set of compound heterozygous mutations, c.124A>G, p.Lys42Glu and c.616A>G, p.Thr206Ala, were found in one proband. A homozygous c.124A>G, p.Lys42Glu mutation was found in two probands, whereas this change was observed in the heterozygous state in a carrier.

CDHR1. *CDHR1* mutations are a less common cause of autosomal recessive CRD (Cohen *et al.*, 2012). Based on research findings, molecular diagnostic testing for four patients from a single family was ordered (Duncan *et al.*, 2012). The potential carrier tested was a sibling of the proband. A causative mutation confirmed the clinical diagnosis in three patients. All samples had the same previously reported homozygous nonsense variant c.1381C>T, p.Gln461X in *CDHR1* resulting in a premature truncation of the protein. The potential carrier was found to be a heterozygous carrier for the same mutation (Table 2).

CERKL. *CERKL* is associated with autosomal recessive RP at locus RP26. Both patients were identified as having a homozygous frameshift mutation c.967delAT, p.Met323-Valfs20 (rsID 750151209) (Table 2).

CRB1. *CRB1* has been associated with autosomal recessive retinal dystrophies, including RP with preserved para-arteriolar retinal pigment epithelium (PPRPE), early onset RP without PPRPE, and Leber congenital amaurosis (LCA) (Bujakowska *et al.*, 2012). We identified compound heterozygous mutations in three carriers and no mutations in nine probands. The mutations included previously reported c.2843G>A, p.Cys948Tyr, as well as c.3880T>C, p.Cys1294Arg changes (Table 2).

FAM161A. *FAM161A* mutations cause an autosomal recessive form of RP. In one family, three members screened for mutations in this gene were observed to carry a homozygous nonsense mutation p.Arg335X. Genetic testing for these patients was ordered based on research findings (Duncan *et al.*, 2016) (Table 2).

PDE6B. The third most common cause of autosomal recessive (RP) is due to *PDE6B* mutations (Dvir *et al.*, 2010). We identified the previously reported compound heterozygous mutations c.1954C>T, p.Gln652X and c.2116A>T, p.Lys706X in the one proband tested (Table 2).

RPE65. Mutations in *RPE65* are associated with autosomal recessive RP, LCA, and autosomal dominant retinal degeneration (RD) (Gu *et al.*, 1997). Of the six patients (four probands and two obligate carriers) referred for molecular diagnosis, causative mutations were identified in three patients (one proband and two carriers). The two carriers were the parents of the probands. The observed mutations included the previously reported nonsense mutation c.370C>T, p.Arg124X and a missense mutation c.311G>T, p.Gly104Val. The proband had a compound heterozygous mutation, and carriers had heterozygous c.370C>T, p.Arg124X or c.311G>T, p.Gly104Val mutations (Table 2).

Dominantly inherited diseases

BEST1/VMD2. Of the 15 probands referred for dominant *BEST1/VMD2* gene testing, heterozygous mutations were observed in 9 patients (60% of probands). Novel heterozygous sequence changes c.250T>G, p.Phe84Val and c.880C>T, p.Leu294Phe were discovered along with the previously reported c.741G>A, p.Arg218Cys, c.72G>T p.Trp24Cys, and c.727G>A, p.Ala243Thr heterozygous mutations. Three family members and an unrelated patient had the same c.741G>A, p.Arg218His heterozygous mutation.

CRX. Mutations in *CRX* account for 5–10% of autosomal dominant CRD (Hamel, 2007). No mutations were identified in the three probands diagnosed with CRD (Table 2).

C1QTNF5/CTRP5. Late-onset retinal macular degeneration is caused by a heterozygous c.489C>G, p.Ser163Arg mutation in the *C1QTNF5/CTRP5* gene. Of the 21 patients referred for *C1QTNF5/CTRP5* gene testing, 19 were probands and 2 were asymptomatic family members at risk

of inheriting the disease. We identified a heterozygous c.489C>G, p.Ser163Arg mutation in one proband (Table 2).

EFEMP1. A c.1033C>T, p.Arg345Trp mutation in *EFEMP1* causes Malattia Leventinese/Doyne Honeycomb Retinal Dystrophy. Of the nine patients (all probands) tested for *EFEMP1* mutations, three had the heterozygous c.1033C>T, p.Arg345Trp mutation, whereas a novel *IVS10-14C>T* VUS was identified in the heterozygous state in one patient (Table 2).

ELOV4. *ELOVL4* has been implicated in dominant Stargardt-like macular dystrophy (Vasireddy *et al.*, 2010). Among the 14 probands referred for *ELOVL4* gene testing based on the clinical diagnosis, a causative heterozygous mutation c.797delAACTT was discovered in one patient (Table 2).

PRPH2. Mutations in *PRPH2* or *RDS* have been identified in 20% of patients with adult vitelliform macular dystrophy (Felbor *et al.*, 1997). *PRPH2* mutations are also associated with various retinal and macular dystrophies (Renner *et al.*, 2009; Coco *et al.*, 2010). Of the 10 patients (9 probands) referred for gene testing, a previously reported heterozygous mutation c.514C>T, p.Arg172Trp (Weleber *et al.*, 1993) was identified in 1 proband with dominant macular dystrophy, and a novel heterozygous mutation c.4G>T, p.Ala2Ser was detected in another male proband (Tables 2 and 3).

TIMP3. Heterozygous mutations in *TIMP3* result in Sorsby's fundus dystrophy, a rare autosomal dominant late-onset retinal dystrophy (Li *et al.*, 2005). We identified a novel probably damaging c.628G>T, p.Asp210Tyr variant in the heterozygous state in one of the four probands tested (Tables 2 and 3).

Rhodopsin. *Rhodopsin* mutations are the most commonly reported cause of autosomal dominant RP with a prevalence of 20–25% of cases (Van Soest *et al.*, 1999). No mutation was discovered in the one proband tested.

Novel potentially pathogenic variants

Sequence changes that were possibly or probably damaging and novel at the time of genetic testing were detected in *ABCA4* (14), *BEST1* (2), *PRPH2* (1), and *TIMP3* (1) (Table 3). Based on physical and evolutionary comparative considerations of the effects of the mutation on the structure and function of the corresponding gene, the novel mutations are determined to be damaging and potentially pathogenic by PolyPhen2 and SIFT. An additional two novel changes with unknown significance were identified (Table 3).

Discussion

In this study, we report the probability of finding a mutation in patients and carriers of IRD by targeted gene testing in 225 cases. The underlying cause of disease was determined in 89 (47%) of probands, and mutations were observed in 12 (34%) of suspected carriers. Among the variants detected, 32 were novel, 17 are possibly or probably damaging missense changes, 3 are frameshift mutations, and 8 are variants located in intronic regions or in coding regions determined to be benign. This knowledge expands the genotype spectrum

and will provide molecular diagnoses to patients with similar mutations and IRD. Furthermore, these data reflect the expected outcomes of targeted genetic testing performed in a single diagnostic laboratory.

When a diagnostic test is performed, the outcomes may include three categories: (1) detection of the causative mutation, (2) absence of mutations in genes screened, and (3) finding VUS. In the current cohort of patients analyzed, the overall rate of detection of causative mutation(s) in a proband was 47% (26% for dominant diseases and 59% for recessive diseases). The rate of detection of a carrier mutation was 38% for recessive diseases. Our proband mutation detection rate is similar to our previous analysis (Downs *et al.*, 2007), where we reported a 51% detection rate. In another study of patients with recessive Stargardt disease, the causative mutation detection rate using similar methods was 44% (Briggs *et al.*, 2001). In a study of patients with autosomal dominant RP, the disease-causing mutation was found in 52% of probands with complete sequencing of 12 genes (Sullivan *et al.*, 2013). Mutations in known genes are estimated to contribute to IRD pathology in about 50% of dominant cases (Daiger, 2004). Whereas, in recessive conditions, mutations in known genes contribute to 40–50% of cases (Riveiro-Alvarez *et al.*, 2013). The rate of detection of causative mutations in our study conducted between 2009 and 2011 is consistent with earlier reported findings.

Over 35 genes have been implicated in causing nonsyndromic recessive IRD. The overlap in the phenotype of recessive IRDs and genetic heterogeneity make selection of genes for genetic testing challenging. The overall lower detection rate of mutations in our recessive IRD cohort could be due to screening only some of the many genes associated with this condition (Table 1), the significant overlap in the phenotype, and the genetic heterogeneity of recessive IRDs. Furthermore, failure to detect certain mutation types due to methodology limitations may also contribute to the low detection rate (Amano *et al.*, 2009; Lee and Garg, 2015). Clinically significant intronic mutations have been described in patients with IRDs (den Hollander *et al.*, 2006; Braun *et al.*, 2013). Similarly, copy number variations have also been observed in IRD patients (Schrider and Hahn, 2010). As our sample analysis is focused on exonic regions, mutations in noncoding regions will not be detected. These limitations may result in a low mutation detection rate.

Mutation detection rates vary widely depending on the gene of interest and type of retinal disease. Five of the eight genes tested in our study, *CDHR1, FAM161A, DHDDS, ARL6*, and *PDE6B* were screened in one to four probands based on research findings. Whereas, molecular diagnostic testing for *ABCA4*-associated diseases was requested for a majority (113 of 150) of patients. The rate of detection of causative mutations in these patients varies between 50% to 80%, depending on testing methods and patient ethnicity (Braun *et al.*, 2013; Fujinami *et al.*, 2015). In a previous study, we observed causative mutations in 48% of patients diagnosed with *ABCA4*-associated disease, whereas mutations were found in 59% of the probands in the current study by screening the coding region of the *ABCA4* gene (Downs *et al.*, 2007). The causative mutation detection rate in patients diagnosed with *ABCA4*-associated disease is low. This could be due to phenotypic heterogeneity and their overlap with several other common IRDs. The underlying disease-causing

mutations in some of the patients with a single heterozygous or no *ABCA4* mutation may lie in other genes associated with overlapping phenotypes. Contrary to *ABCA4* gene testing results, Best's macular degeneration has a higher success rate in finding causative mutations. This is likely due to the relatively homogeneous and unique phenotype of vitelliform macular dystrophy. Reporting these mutations in the context of patients with IRD will have a significant clinical impact if the same mutations are found in other patients.

The genetic diagnosis is influenced by the number of genes tested, methodology used, and the extent of contribution of known gene mutations in explaining the underlying cause of IRD. The findings of our study in combination with previous studies make a strong case for the need for efficient next-generation sequencing technologies for molecular diagnosis of IRD. Furthermore, identification of new IRD genes may improve molecular diagnoses. These methodologies may also reveal causative mutations in multiple genes. Screening selected genes for mutations may be efficient for genetically homogeneous phenotypes with little phenotypic overlap with other diseases. However, for diseases such as *ABCA4*-related diseases and RP with wide genetic heterogeneity and phenotypic overlap with other conditions, screening all related genes or the entire exome or genome may yield a higher mutation detection rate. This may improve the chances of finding the causative mutation(s) and making a molecular diagnosis.

The necessity for a molecular diagnosis is significant in the current era of gene-based and personalized medicine. Causative mutations or modifiers may be found in more than one gene, influencing eligibility and prognosis in clinical trials. It is possible that more high-throughput genetic screening methodologies will identify mutations and sequence rearrangements involving exonic, intronic, and intergenic regions. Based on our experience with targeted gene screening and exome capture methodologies, we plan to perform whole genome sequencing and evaluate its mutation detection efficacy in IRD patients. Better knowledge of IRD mutations and improved diagnostic technology will directly influence patient care and access to gene mutation-based clinical trials.

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